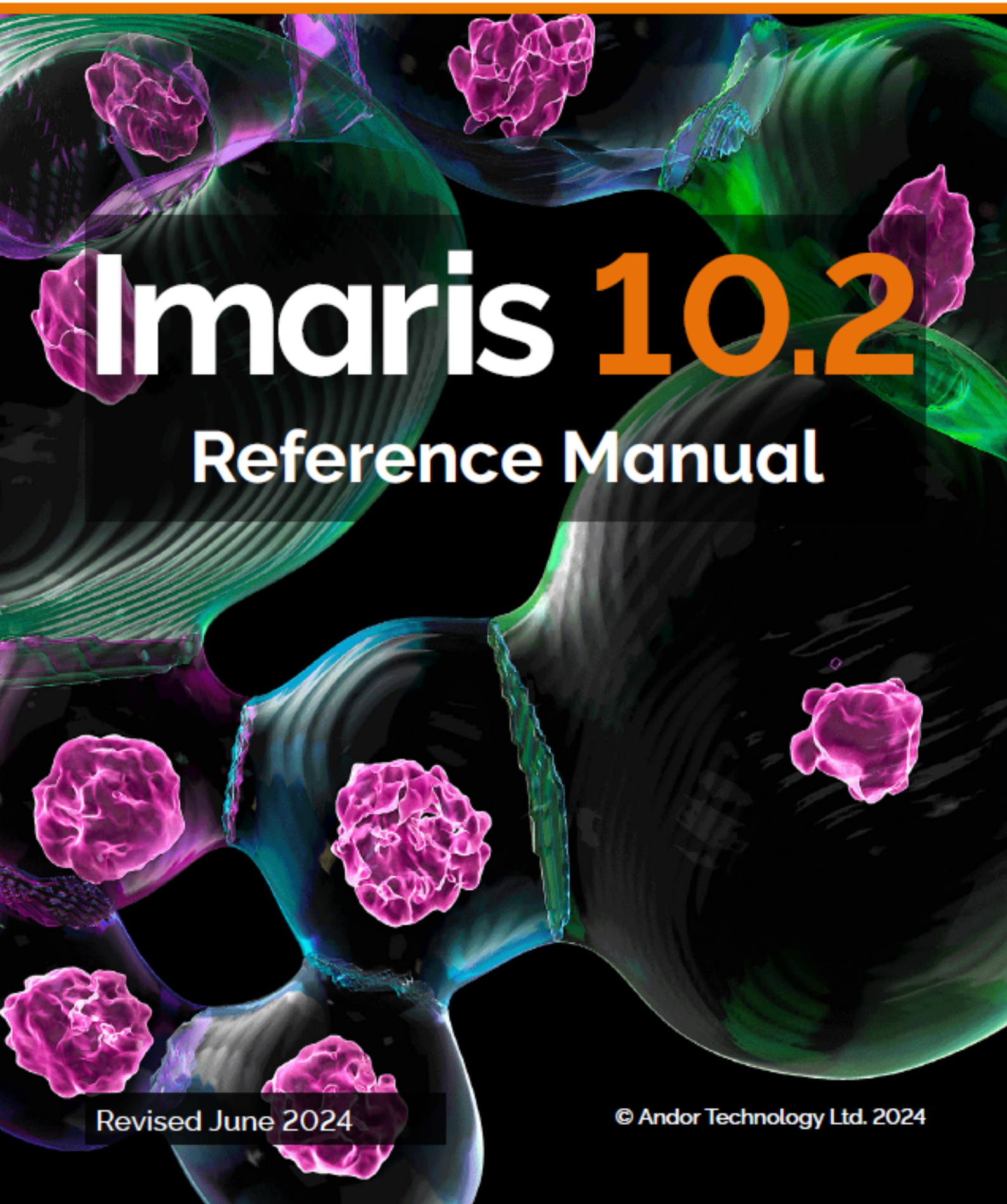
OXFORD INSTRUMENTS IMARIS



Imaris 10.2

Reference Manual

Revised June 2024

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Section 1 Preface - Getting Started

Welcome to the Reference Manual. This document describes all the functionality of Imaris. Thank you for using Imaris Software.

Getting Started in Imaris Viewer

Imaris Viewer is a free program for visualizing multidimensional microscopy images. To download a copy of Imaris Viewer please visit www.imaris.com/viewer



For existing users of Imaris, files previously analyzed in Imaris can be viewed from any workstation or laptop within Imaris Viewer. Inspection of raw files is possible and objects created in Imaris are preserved within Imaris Viewer.

If you are new to Imaris we encourage you to use Imaris Viewer as a free viewer for your microscopy images. The image browser, Arena, is useful for viewing thumbnails and organizing your images and our 3D/4D visualization tools are included within Surpass view.

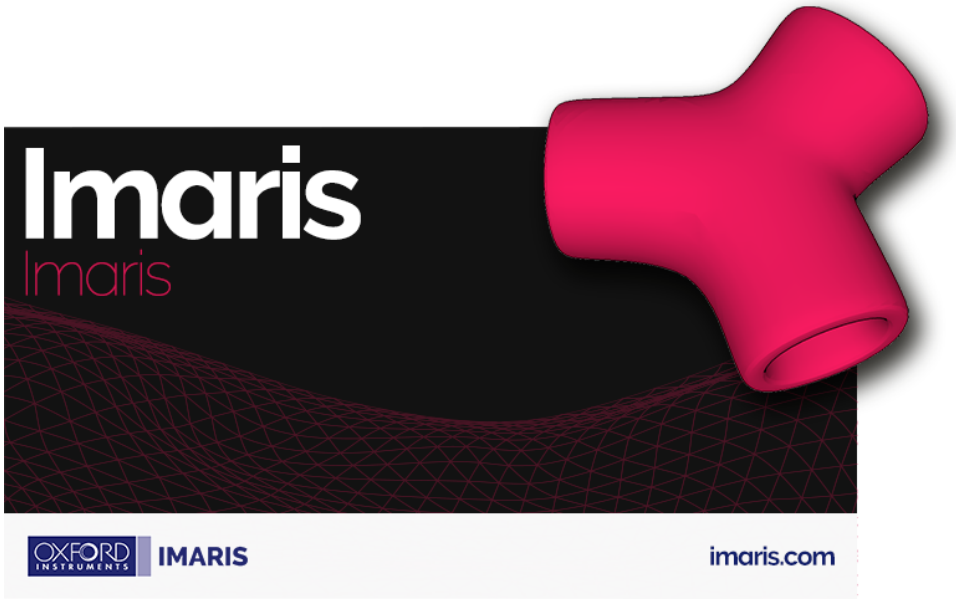
The supplied documentation for Imaris Viewer provides extensive guidance of the available features of the program. Please note that some features described in the documentation are only available with the full version of Imaris.

Getting Started in Imaris

Imaris is a full program for visualizing and analyzing multidimensional microscopy images. Imaris is available with an appropriate license.

Today, optical microscopes can record several channels simultaneously to produce multi-channel images. Imaris is an application designed to visualize such complex microscopic data. Imaris uses a special file format to describe images with parameters and can incorporate image files for all major microscopes and image acquisition systems. The images can be viewed in several different ways and processed to provide the optimum

amount of information from 2D or 3D still images, time series, and animations.



Once a data set has been loaded into Imaris, individual parameters such as channel colors, geometrical settings or voxel sizes can be adjusted. Imaris has a variety of tools available, such as cropping, threshold cutting and filters for processing the images to bring out the required details.

An Overview of the Viewing Mode Options

Imaris provides different viewing options for visualization and production of high quality images for presentations, data visualization and storage. This section provides a brief overview of these modes found in the top menu bar, and how they may be used. Each mode is explained in detail in later sections of this manual.



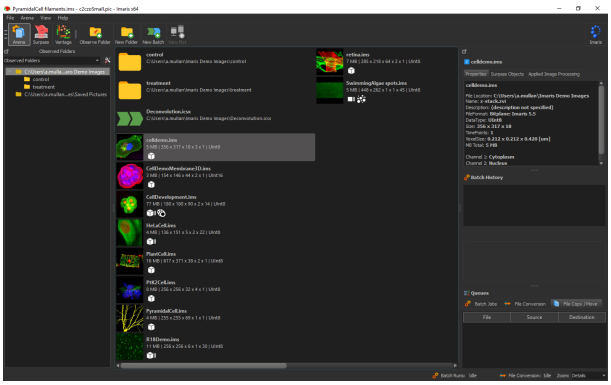
Arena

The Arena is an easy-to-use image management system to manage your image data files. Arena has the ability to batch process through a group of images and produce a statistical data-visualization reports, providing an ideal solution for all your image processing and analysis requirements.

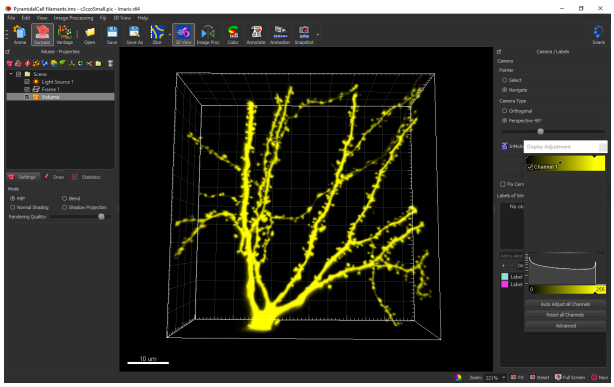


Surpass View

Surpass view offers powerful tools for data preparation, presentation and manipulation of different types of image data. This is presented in a large viewing area (default 3D view) with numerous tools and creation wizards for processing your image data effectively.



Refer to the Arena section for further information.

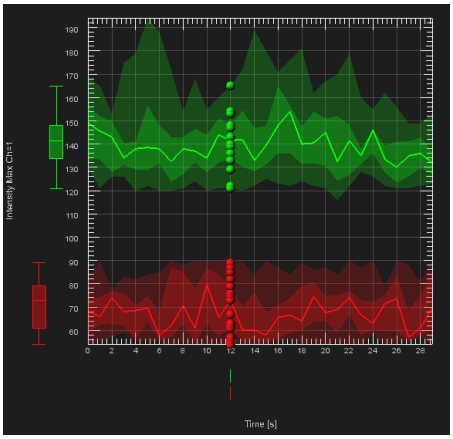


Refer to the Surpass section for further information.



Vantage View

Vantage view is a statistical data-visualization module. This provides a range of tools to create high-dimensional color plots and permits analysis of multi-object images e.g. after processing in Surpass. Its interactive visualization methods help you to enhance the understanding of complex statistical data by creating plots using actual segmented objects.

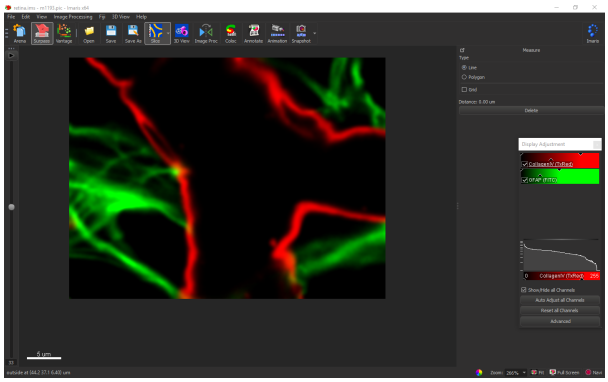


Refer to the Vantage section for further information.



Slice View

Slice view allows you to view each slice of the image.

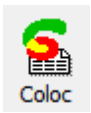


Refer to the Slice section for further information.



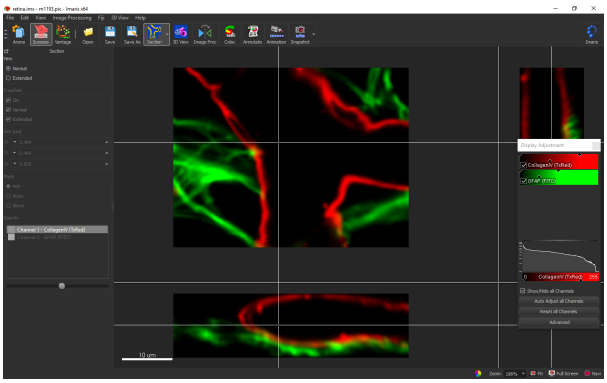
Section View

The Section view option enables simultaneous viewing along three coordinate axes (cross-sectional view).

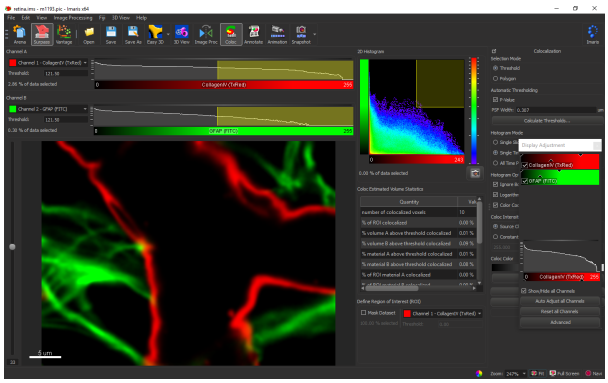


Colocalization View

This mode is for Colocalization computations.



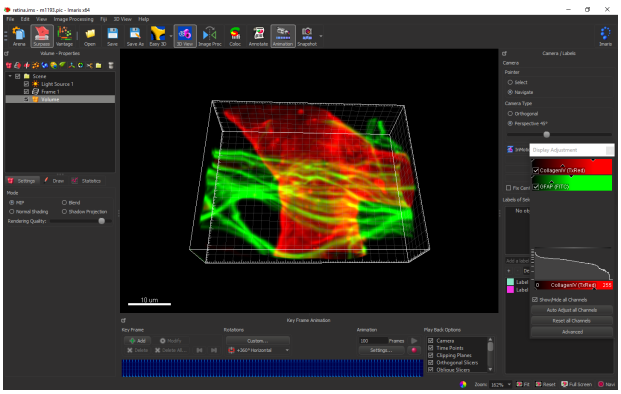
Refer to the Section section for further information.



Refer to the Colocalization section for further information.

Animation

An Animation can be created from the Slice and 3D modes, or with the key frame animator in Surpass.



Refer to the Animation section for further information.

Snapshot

Use snapshot to copy the current image view. You can adjust the settings e.g. file format, image size etc. making working with multiple images, quick, easy and consistent.

Refer to the Snapshot section for further information.

Labkit Segmentation

Labkit is an open source machine learning pixel classification plugin and is available from Imaris.
See Labkit Segmentation for further information.

Easy Navigation

Navigate within the Imaris modules using frequently used toolbars, menus, and interactive controls that remain the same, and can all be operated with the mouse buttons.

View-specific menus

Configure and define the appearance of image data and access processing methods with options specific to the selected view. Toolbars and options update in context with selected options.

Help
Hover the mouse over an object for a tool-tip. Right-click any any object to go directly to the relevant section of this Imaris reference manual.

1.1 Installation and License Information

Imaris software is delivered electronically or can be downloaded from the Imaris Customer Portal. The electronic copy includes a folder containing the necessary technical documentation.

To find the latest information about minimum and recommended hardware requirements please visit our system requirements webpage.

Installation

To install the software, please follow our Installation Guide.

Licensing

To run Imaris, the appropriate licenses for the required modules, such as Imaris base, ImarisTime, ImarisColoc or Imaris MeasurementPro are required. Without licenses, Imaris can only be run in a restricted mode. In case of any license problems or queries, please refer to the FAQ support information on our website imaris.oxinst.com/support/faqs for detailed information in the License and Installation section.

Starting Imaris

Imaris can be started by one of the following methods:

- Double-click on the Imaris icon (we recommend copying the icon to the desktop).
- Drag the icon of an image or a file to the Imaris program icon.

The software opens with the main screen.

1.2 Supported File Formats

File Formats

Imaris can read the following file formats, i.e. it can read the image and the parameters.

	Format Name	Extension	Remarks
Imarisbase			
Bitplane	Imaris 5.5	*.ims	
Bitplane	Imaris 3.0	*.ims	
Bitplane	Imaris 2.7	*.ims	
Bitplane	Imaris Scene File	*.imx	
Andor	iQ Image	*.kinetic	
Andor	Multi-TIFF	*.tif,*.tiff	

	TIFF-Series	*.tif,*.tiff	
	BMP-Series	*.bmp	
Big Data Viewer		*.xml, *.h5	
lmarisreaderaperio			
Aperio (SVS, TIF)	Single-file pyramidal tiled TIFF, with non-standard metadata and compression	*.svs, *.tif	
lmarisreaderdeltavision			
Applied Precision	DeltaVision	*.r3d,*.dv	
lmarisreaderbiorad			
Bio-Rad	MRC	*.pic	
lmarisreaderiplab [BioVision purchased IPLab in 2006]			
BioVision	IPLab IPM	*.ipm	Mac
BioVision	IPLab IPL	*.ipl	Windows
lmarisreaderdicom			
DICOM file format (series)		*.dcm	
lmarisreadergatan			
Gatan	DigitalMicrograph	*.dm3	
lmarisreaderge			
GE	GE InCell 6000	*.tiff, *.tiff, *.xdce	
lmarisreaderhamamatsu			
Hamamatsu Compix	SimplePCI	*.cxd	
Hamamatsu (NDPI)	Multi-file JPEG/NGR with proprietary metadata and index file formats and single-file TIFF-like format with proprietary metadata	*.ndpi	
lmarisreaderiii			
Intelligent Imaging Innovations	SlideBook	*.sld	
lmarisreaderimagej			
TIFF ImageJ (adjustable file series)		*.tif, *.tiff	
lmarisreaderimod			
IMOD	MRC	*.mrc,*.st,*.rec	
lmarisreaderleica			
Leica	Image File Format LIF	*.lif	
Leica	Image File Format LOF	*.lof	
Leica	Experiment Structure in Multiple Files XLEF	*.xlef	
Leica	TCS-NT	*.tif,*.tiff	
Leica	Series	*.tif,*.tiff,*.inf,*.info	
Leica	Vista LCS	*.tif,*.tiff,*.lei,*.raw	
lmarisreadermicromanager			
Micro-Manager	Image5D	*.tif,*.tiff,*.txt	

Imarisreaderuniversalimaging [Molecular Devices acquired Universal Imaging in 2002]		
Molecular Devices	MetaMorph STK	*.stk
Molecular Devices	MetaMorph ND	*.nd
Imarisreadernikon		
Nikon	Image Cytometry Standard ICS	*.ics,*.ids
Nikon	ND2	*.nd2
Imarisreaderolympus		
Olympus	cellR	*.tif,*.tiff
Olympus	FluoView OIB	*.oib
Olympus	FluoView OIF	*.oif
Olympus	FluoView OIR	*.oir
Olympus	FluoView TIFF	*.tif,*.tiff
Olympus	VSI	*.vsi
Imarisreaderome		
Open Microscopy Environment	XML	*.ome
Open Microscopy Environment	TIFF	*.tif,*.tiff
Imarisreaderperkinelmerinc		
PerkinElmer	Improvision Openlab LIFF	*.liff
PerkinElmer	Improvision Openlab RAW	*.raw
PerkinElmer	UltraView	*.tim,*.zpo
Imarisreaderprairie		
Prairie Technologies	Prairie View	*.xml,*.cfg,*.tif,*.tiff
Imarisreadertill		
TILL Photonics	TILLvisION	*.rbinf
Imarisreaderzeiss		
Zeiss	AxioVision	*.zvi
Zeiss	LSM 710, LSM 510	*.lsm
Zeiss	LSM 410, LSM 310	*.tif,*.tiff
Zeiss	CZI	*.czi
BMP (adjustable file series)		*.bmp
TIFF (adjustable file series)		*.tif,*.tiff

1.3 About this manual

Information in this manual is subject to change without notice and does not represent a commitment on the part of Oxford Instruments Andor. Oxford Instruments Andor is not liable for errors contained in this manual or for incidental or consequential damages in connection with the use of this software.

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For further questions or suggestions please visit our website at:

imaris.oxinst.com

or contact our support teams:

eusupport_imaris@andor.com
ussupport_imaris@andor.com
asiasupport_imaris@andor.com
imariscn.support@oxinst.com

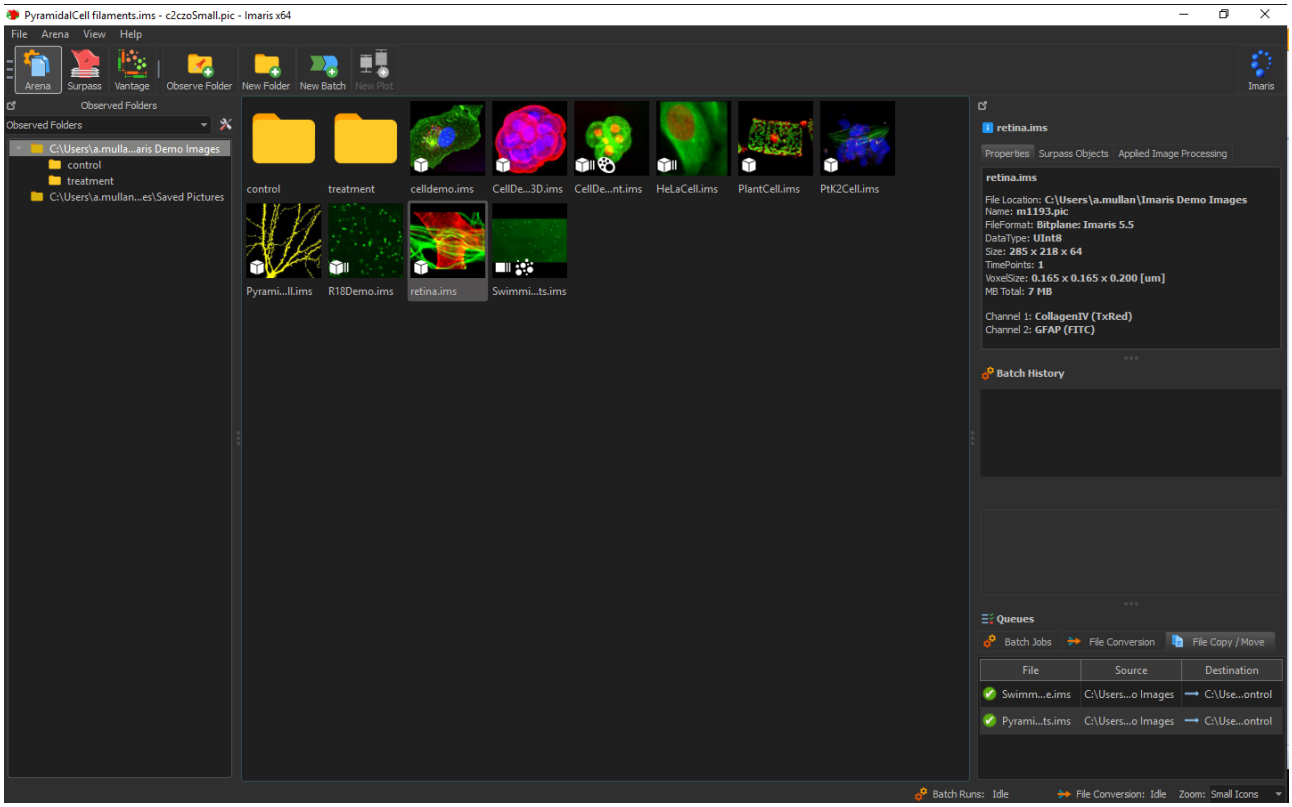
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Imaris Reference Manual developed to support Imaris release 10.2

Section 2 Arena View

The **Arena View** provides an easy-to-use image management interface. Image analysis often requires the application of the same creation parameters to any number of images. Arena lets you speed up this process by enabling "batch processing" to be performed on multiple images.



The Arena is your central working area in Imaris and an interface for visualizing, processing, analyzing and interpreting all your images. This is where you add images and creation parameters, create and execute batch runs, and configure all your statistical data-visualization settings.

The Arena view consists of a number of elements which can be grouped as follows:

- **Observed Folders** is a new feature introduced for Imaris 9.3 that lets you select and browse file locations used for your images i.e. you can select folders to "observe" for quick access.
- The **File Image viewing area** provides an area to view contents of an observed folder. The different options for how images are displayed is described in the following paragraphs.
- The **Image information** panel provides information for the selected image file in three tabs: covering the properties of the file, information about the Surpass objects applied and what Image processing has been applied.
- The **Batch History** panel shows information related to batch processing that has been applied (if applicable).
- The **Queues** panel shows the status and other information for any **Batch Jobs**, **File Conversion** details and **File Copy / Move** options.

These options are described in more detail in the following sections of this manual.

At the bottom right hand side of the Arena view a status bar provides some further information such as **Batch Runs** status and **File Conversion** status. It also provide an option for how the files in the file viewing area are displayed called **Zoom**.

Zoom can be used to change how the images are displayed in Arena. You can select from: **Compact** for a summary view, **Details** that provides a summary of the image files, or **Small**, **Medium** or **Large** Icons, these are shown in order from left to right below. Zoom can help you navigate and find the required image, if you have many, or similar stored images.



Above: From left to right - Compact, Details, Small Icon, Medium Icon, Large Icon

Arena Items

Each Arena item has a number of different properties associated with it. Properties information affects how items are visualized or processed. Some properties are fixed and others are user-defined. A summary is displayed in the viewing window depending on the selected viewing options.

Depending on the selected item, a right-click displays different options. The type of item determines what options and tools are available.

Using Imaris Without Arena

Imaris has been developed so that the Arena provides the most effective means of accessing and managing your images, and image data. It is however also possible to configure Imaris (version 8.3.0 onwards) so that Arena View is not used. During installation, an option is provided to not use Arena. Using Arena is recommended- if you want to install Imaris without Arena, please contact support for further information.

2.1 Arena Toolbar

In All Views


Arena


Surpass


Vantage



What's New


Newsletter


Imaris

In Arena View



Observe Folder



New Folder



New Batch



New Plot

2.2 Observed Folders Panel

Observed Folders is a system (introduced in Imaris 9.3) for organizing image files and the metadata that is attached or associated to them. It provides an interface that will be familiar to that used by file explorer or similar applications on your computer, making it easy to find and manage your image files from multiple locations.

You can navigate through the nested folder structure and the image files within each location will be displayed in the image viewing area.

To Add a new image file location to the Observed Folder list

Click **Observe Folder** and navigate to and select the required folder location.

Add a New folder

The **New Folder** option will add a new subfolder within the currently selected folder.

1. Click new folder
2. Name the folder
3. The new sub-folder will be created

Remove an Observed Folder

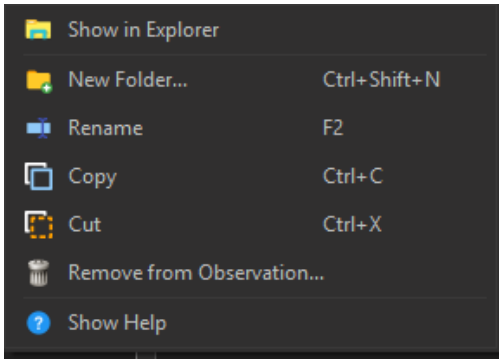
1. Select the folder in the **Observed Folder List**
2. Right Click the folder
3. Select **Remove from Observation...**

Notes

- **Removing an observed folder just removes the observed folder from the list displayed in Imaris - the original folder and its image contents will be unaffected.**
- **Please note that when you drag and drop an item to a new location within the Arena, a copy of that item is created in the new location, the item is not moved from the original location.**
- **If you modify the copied image created from the drag and drop process, the original image is not modified.**
- **Folders that you cannot access are highlighted in red.**

2.3 Observed Folder Options

Right mouse-click a folder under observation to bring up the options menu:



Show in Explorer
This will locate the selected folder in the file explorer window.

New Folder
Create a new folder within the selected folder. The new folder will be created in Arena and on your file system.

Rename
Select to rename the current folder

Copy
Copy the folder and its contents.

Cut
Cut the folder and its contents (so it may be removed, or pasted elsewhere).

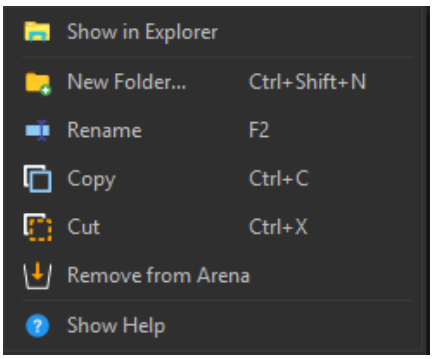
Remove from Observation
Remove the folder from Observation - this does not delete the folder, just removes it from observation.

2.4 New Folder

Create a new folder using the New Folder  button in the Arena Toolbar.

2.5 Folder Options

Right mouse-click a folder to bring up the options menu for the folder:



Show in Explorer
This will locate the selected folder in the file explorer window.

New Folder
Create a new folder within the selected folder. The new folder will be created in Arena and on your file system.

Rename
Select to rename the current folder

Copy
Copy the folder and its contents.

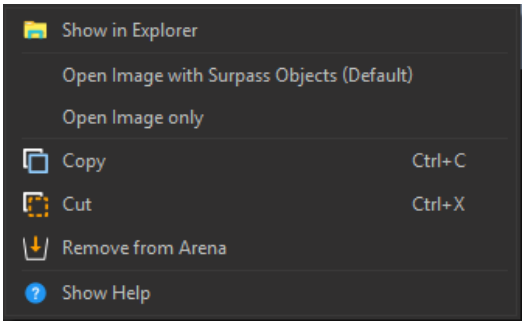
Cut
Cut the folder and its contents (so it may be removed, or pasted elsewhere).

Remove from Arena
Remove the folder from Arena - this does not delete the folder, just removes it from being displayed in Arena.

2.6 Image File Options

How to Copy/Move and delete items from Arena

Right mouse-click an item to bring up the options menu for the item:



This menu provides options for the following:

Show in Explorer
This will locate the files in the selected folder in an file explorer window. Note that if files have been processed or modified the original files can also be viewed in this location.

Open Image with Surpass Objects (default)
Open the image in Surpass.

Open Image only
Open the image in Surpass without image processing steps displayed.

Copy
Copy the image file.

Cut
Cut the image file (so it may be removed, or pasted elsewhere).

Remove from Arena
Remove the file from Arena- this does not delete the file, just removes it from being displayed in Arena.

Revert to Previous Image
Return the image file to a previous state.

Revert to Original Image
Return an image to the original state.

2D Images Only

For 2D images the additional options are available:

Open as 3D Image with Surpass Objects (default)
Open the image in Surpass.

Open as 3D Image only
Open the image in Surpass without image processing steps displayed.

2.7 Image Properties Panel

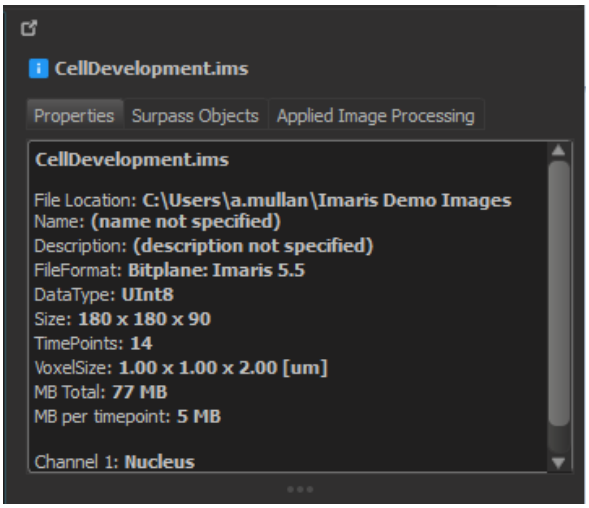
The image properties panels provides useful information about the item that is currently selected in three tabs:

- Properties
- Surpass Objects
- Applied Image Processing

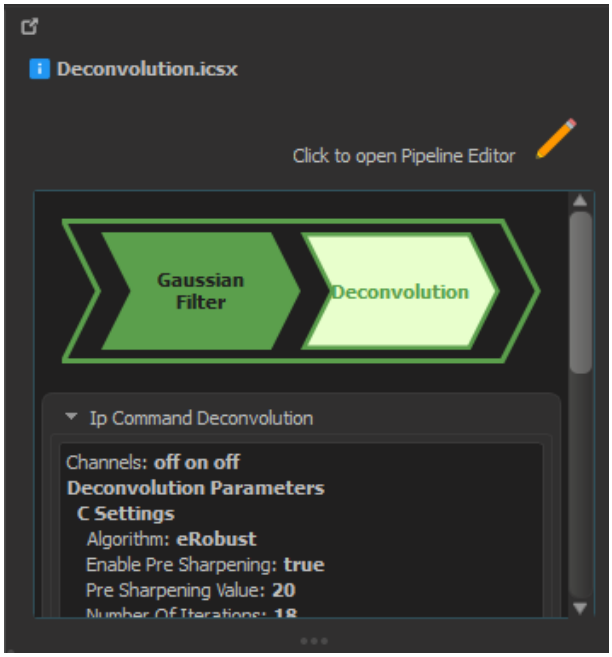
2.7.1 Properties Tab

Properties tab

The properties tab displays the file metadata associated with the selected image file. This includes file format, size information and channels.



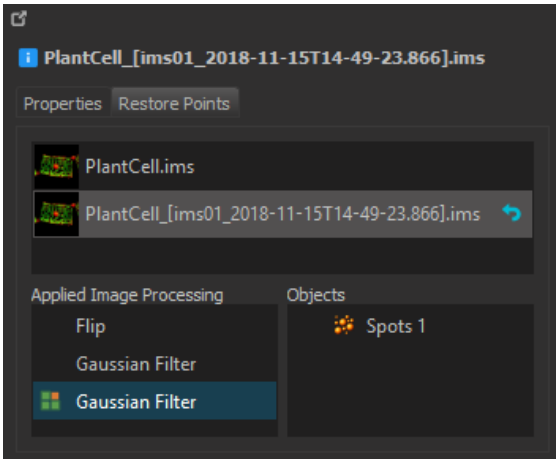
If the item is a batch item the properties will be displayed. A drop down box can be selected to expand the information for the step in the batch process.



Click the **Click to open Pipeline Editor** to view the batch pipeline in more detail and edit as required.

2.7.2 Restore Points

The restore points tab provides information on available restore points. If an image has been subject to image processing steps it may be reverted to a prior state.



Applied Image Processing tab

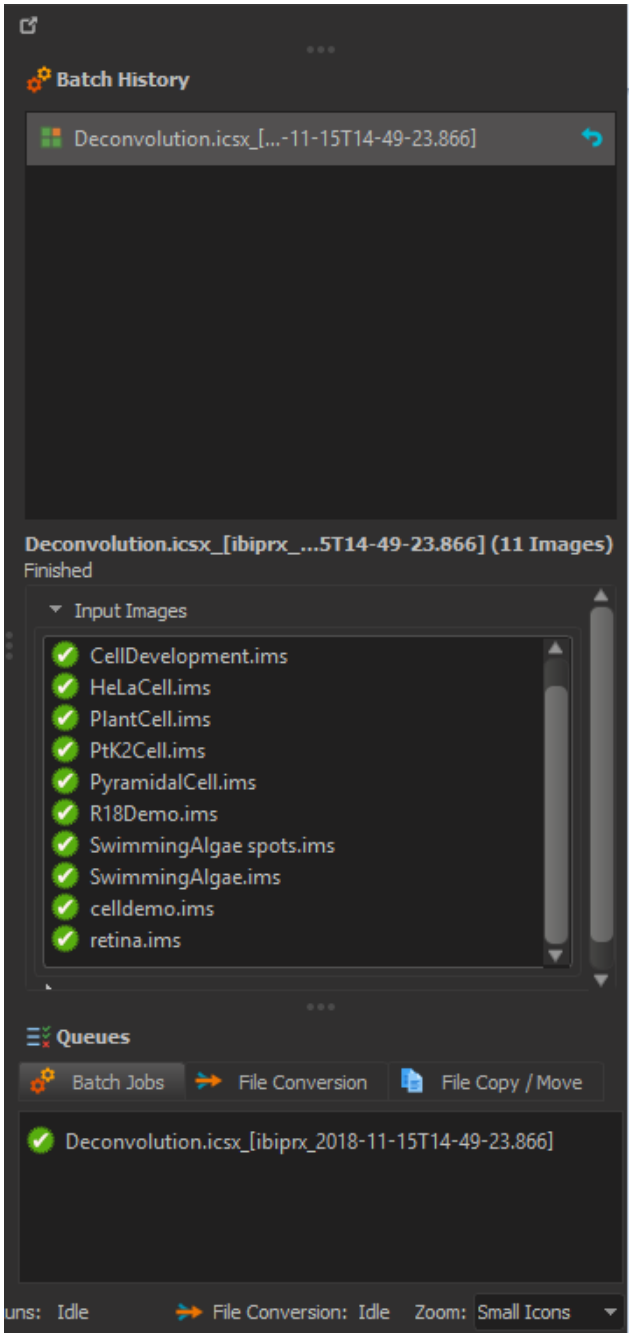
If any image processing information is available for the selected image they will be displayed here.

Objects tab

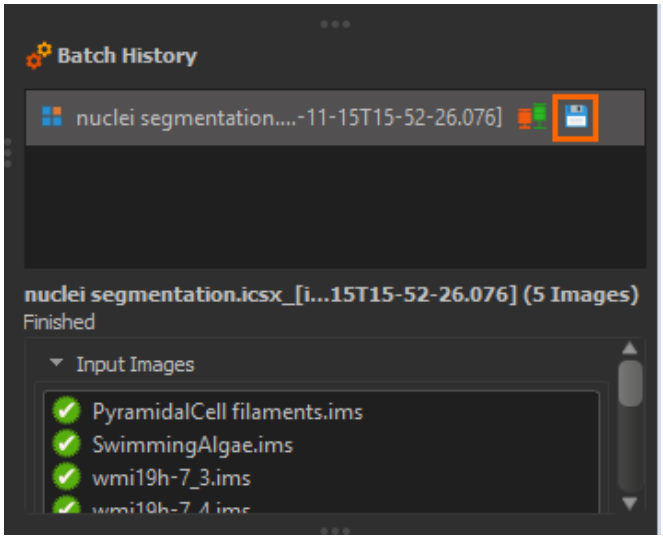
If the image has any surpass objects such as cells, filaments or spots they will be displayed here. Note also that each thumbnail image of the file will show a small surpass icon if any are present for the image.

2.8 Batch History Panel

Any batch information for the currently selected item will be displayed in the Batch History panel.



If a batch has been completed additional icons will be displayed:



This gives the options to view the Vantage plot for the data  or export the data  .

View vantage Plot

View the data in graphical format. Refer to the Vantage section for further information

Export Statistics

For some further statistical analysis you can export the data either as a Comma Separated Values file (.csv), or directly as an Excel file (.xml or xls).
Click on the selected export button and select the file type.
All generated statistical data are exported to Excel, which starts automatically, and sorted in different sheets.

2.9 Queues

The Global Queues window displays the status of batch, copying and conversion processes- for a description of batch processing refer to the batch processing section.The batch queue table lists the current job(s) as well as a list of all completed batch jobs.
For each job a short description is displayed: name, the current state of the job, the time at which the job has been submitted and finished as well as the progress percentage.

The Global Queues panel is organized into three tabs:

- Batch Jobs - shows the status and information for any batch jobs applied.
- File Conversion - shows details any file conversion
- File Copy / Move - shows the file location information

The keyboard shortcut to open the Batch Queue window is Ctrl+Shift+B.

2.10 Batch Processing

Image analysis often requires the application of the same creation parameters to any number of grouped images. Batch protocols are the most powerful and efficient way of segmenting and analyzing images. The ability of Imaris to batch process a group of images to produce a statistical data-visualization report is a key part of Imaris Arena view.
Batch processing lets you apply the same creation parameters to all images within the selected Group, sparing you the repetitive work.

Create a new batch pipeline using the New Batch  button in the Arena toolbar.

The following section lists the components of the batch processing framework in Imaris and provides an overview of the steps you have to follow to create a creation parameters item, a batch run and analyze the results of the batch.

Before you start analyzing your images there are some aspects to be confirmed and a few steps to be completed before pressing "**Run Batch**" on any batch run.

The batch process has the following stages:

- **Batch Setup Step 1 - Image Processing** (apply image processing algorithms to the image)
- **Batch Setup Step 2 - Objects** (add object detection to the batch pipeline).

Note that it is possible to select either of these processes- you do not have to do both steps.

The creation parameters are automatically applied to all images within a group and simultaneously, a new Arena item, the Batch Collection item is created.
Once the Batch job is completed you can analyze the results within Vantage view.

2.10.1 Batch Setup Step 1 - Image Processing

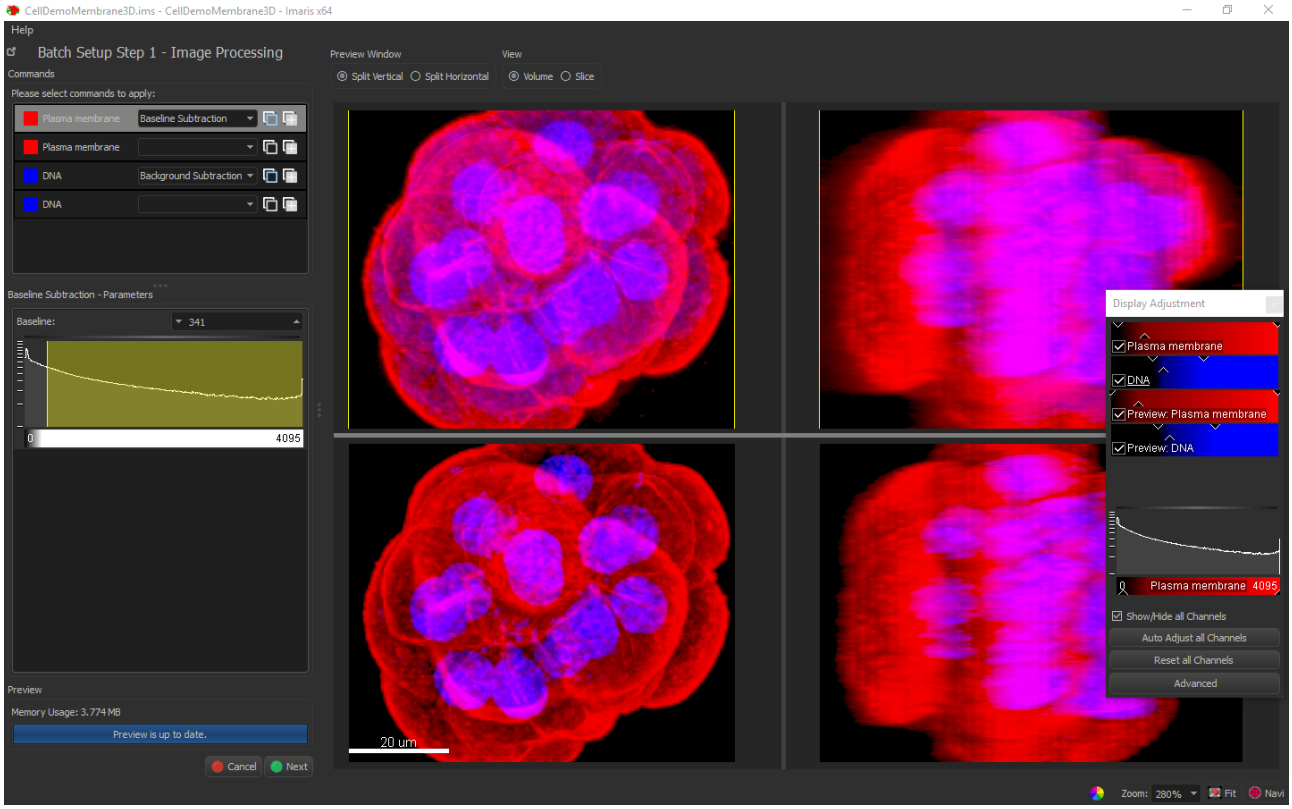
In step 1, image processing steps can be applied to the image for each channel as you require.

For more information on image processing please see the Image Processing Dialog section of the reference manual.

Commands

Commands can be selected from the drop down list. The individual parameters for the selected channel and its effect are shown in the parameters panel below. These parameters will vary depending on the command selected.

The copy and paste icons can be used to copy and paste commands and their parameters across into other channels. This is useful for commands such as deconvolution that have multiple field parameters, or for example, baseline subtraction which uses histograms settings.



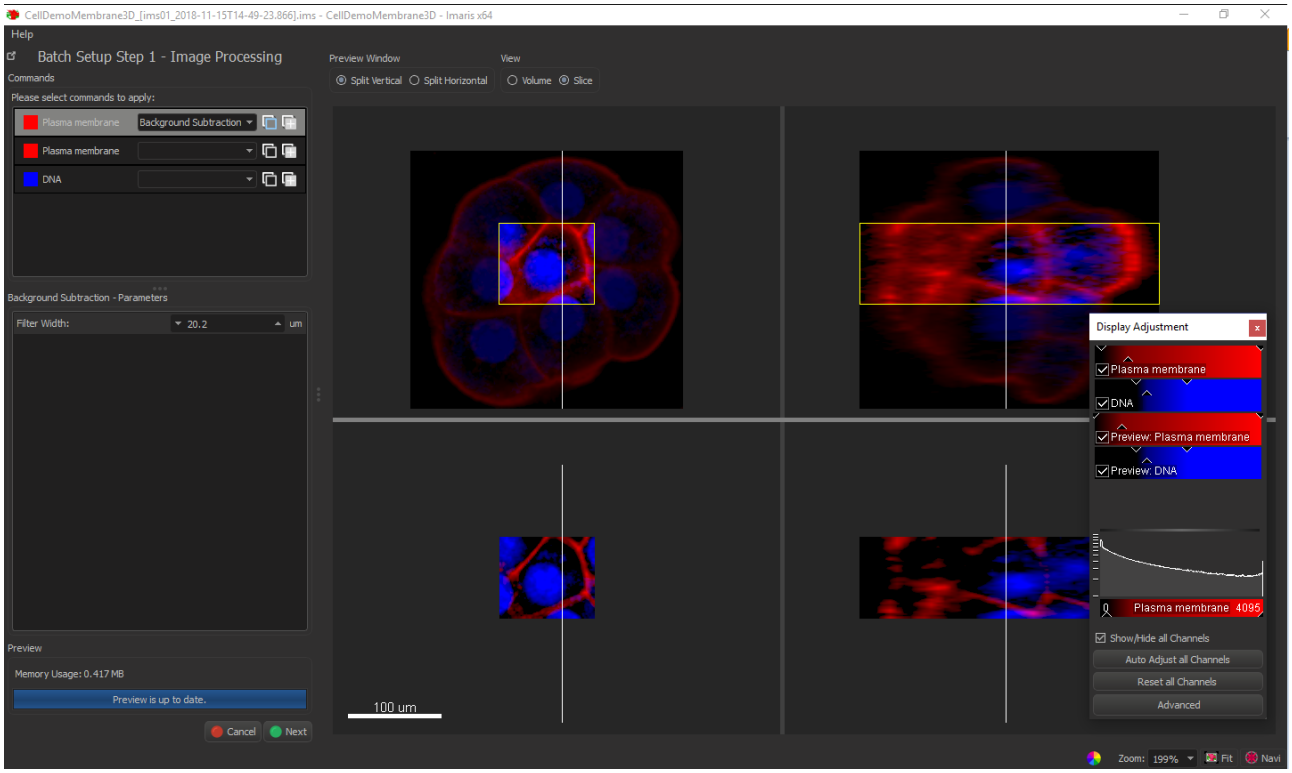
Preview Window

A preview window lets you compare the image in volume or slice mode before and after the effect is applied in either a vertical, or horizontal split.

The system will automatically set a default region of interest (ROI) to view the effects in the viewing window.The memory requirements for the selected ROI are shown in the bottom left hand corner.

To resize the ROI:
- Position the mouse pointer at a corner and click the left mouse button to stretch or reduce the ROI size.
- Position the mouse pointer at a side and click the left mouse button to increase or decrease the width/height or the ROI.
- Position the mouse pointer near the edges of the ROI and click the left mouse button to move the ROI position across the image.

In Slice View, a line indicates the slice through the image:



Click the line and drag the line as required.

Select **Next** to proceed to the next step, or **Cancel** to exit the Batch Setup process.

2.10.2 Batch Setup Step 2 - Objects

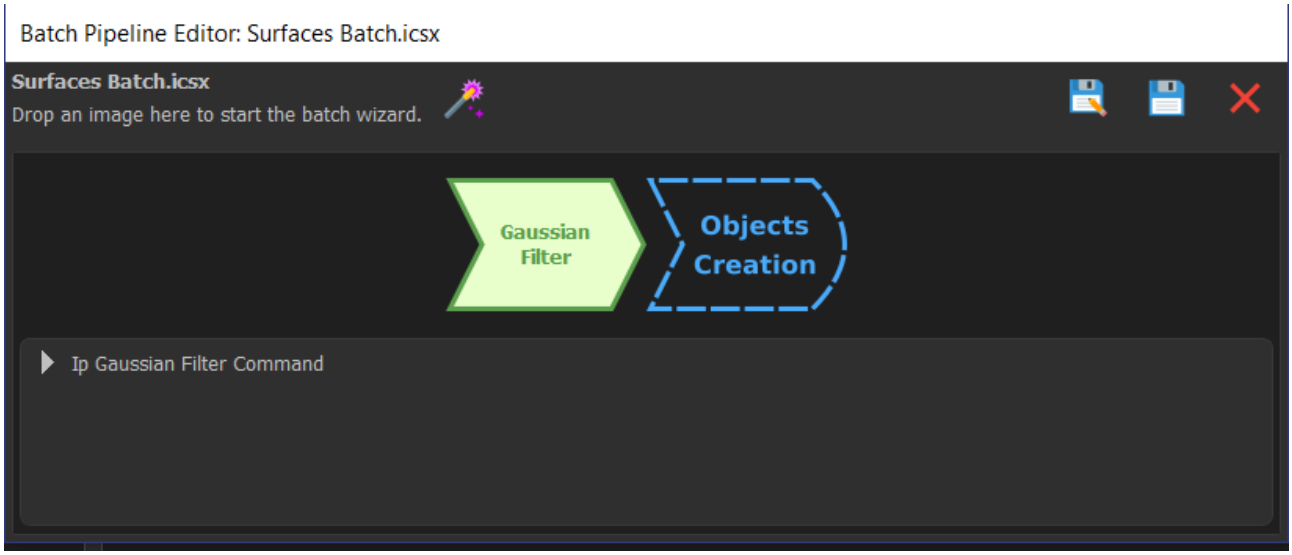
In step 2, you can add object detection to the batch pipeline. The object creation windows appears and the wizard can be followed to complete the process. Refer to the creation wizard sections in image processing for information on the respective object type.

- Complete the wizard as required and click **OK** when done, **Back** to return to the previous step, or **Cancel** to exit the batch creation process.
- After pressing OK provide a name for the new batch file.
- Once the batch has been created it has not yet run on any images files.
- To run the batch on files you need to use the Run Batch option. To do this click the batch file and right mouse click it to run batch on all image files within the folder.

2.10.3 Batch Pipeline Editor

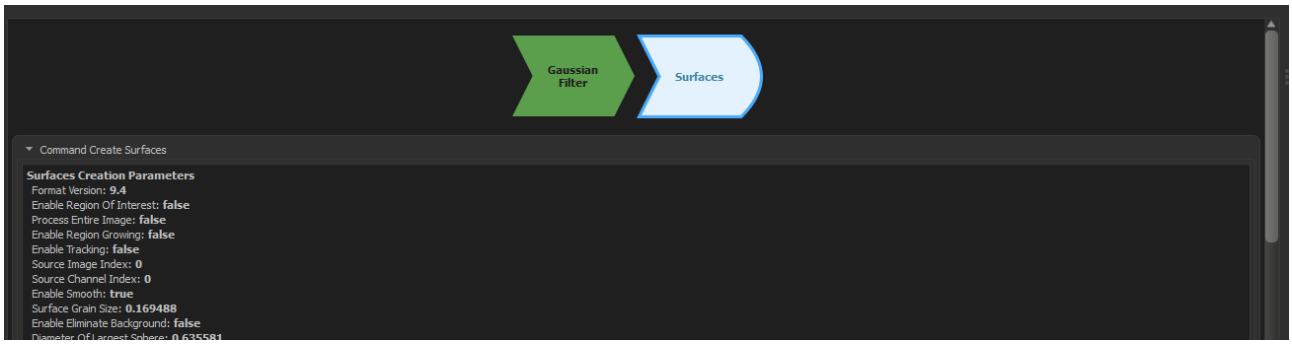
Once a batch process has been created the pipeline editor provides several functions:

1. You can drag and drop images to start the batch wizard process or click on the batch wizard icon 
2. You can view the summary pipeline view
3. You can close the editor and save/and or rename the batch setup
4. You can view the batch settings details.

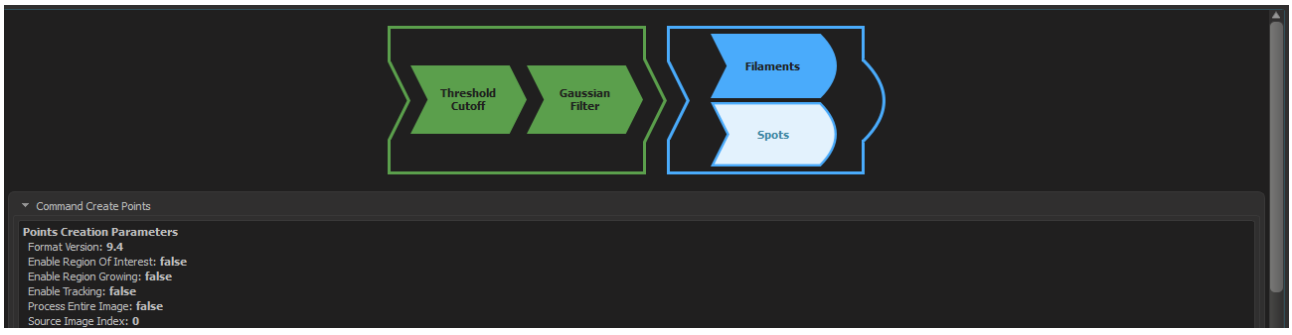


2.10.4 Batch Pipeline Steps

The batch pipeline steps are displayed to summarize the image processing commands and objects that are contained within the batch processing creation parameter. In the batch pipeline summary below Gaussian Filter image processing is applied, then a Surface object is created. In this example, the Surface object is selected and the Surfaces Creation Parameters information is displayed when the drop down is selected.



Multiple image processing steps and objects can be included in the batch creation parameter as shown in the example below.



2.10.5 Batch File Options

Right clicking the batch file brings up the following options - including the option to run the batch on the image files.

Show in Explorer
This will up the files in the selected folder in an file explorer window. Note that if files have been processed or modified the original files can also be viewed in this location.

Run Batch
This option will run the image processing and objection detection parameters you have set for batch processing.

Run Batch including Sub-folders

This option recursively applies the creation parameters to all the images of the chosen image and all its subfolders.

Rename
Rename the batch file.

Copy
Copy the batch file.

Cut
Cut the batch file (so it may be removed, or pasted elsewhere).

Remove from Arena
Remove the file from Arena- this does not delete the file, just remove it from being displayed in Arena.

Delete from Disk
To delete the file completely- use the delete from disk option.

2.10.6 Run Batch

As soon as you press the Run Batch Job option Imaris will run the batch according to the settings you have entered on a selected image file, or all image files within the folder location.

2.10.7 Click to open Batch Pipeline Editor

If a batch file is selected the Click to open Batch Pipeline Editor option is displayed in the Image properties panel.

Refer to the topic Batch Pipeline Editor.

2.11 Vantage plot

Vantage plots are the most informative and flexible way to visualize data. They facilitate the analysis of results and illustrate patterns and trends that are not immediately apparent by just looking at the raw or segmented data. Within the Vantage view there are many different plot types and a selection of various plot dimensions are available so the appearance of results can be easily configured.

To create a Vantage Plot item, select an image file within the Arena, then select the New Plot icon in the Main toolbar. A Vantage Plot, is created. On saving the Vantage plot you will be prompted to enter a name for the Plot item, once saved the plot item will appear in the arena.

A Vantage Plot item can also be used in the Comparison of Batch Results in Vantage.

Double-click on the Vantage plot items in the arena to open the Vantage view.

Right-click options

Right-clicking on a Vantage plot item provides a specific context sensitive menu offering you various actions.

Open in Vantage

Select this option to visualize the plot item within the Vantage view.

Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item, and then select the rename option and in an editable field change the name.

Delete

Select the item(s) to be removed and select the Delete option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.

2.11.1 Comparison of Batch Processing Results in Vantage

A Vantage Plot item generates a separate plot component on a per object basis and allows for the meaningful visualization and analysis of results. It helps you find relationships among several statistical variables, determine their relative importance and therefore draw a comparison between Batch Collection results more clearly.

How to compare Batch results

To see if two or more images with objects created using Batch Processing have scientifically relevant differences between them and assess the degree of variation from one group to the next, create a Vantage plot item.
To create a Vantage plot item, select the images you would like to compare and then select the New Plot icon in the Main toolbar. When saving the new plot item enter a descriptive name and a new Vantage Plot item is created within the arena.

Within the Vantage view, the comparison Vantage plot shows an arrangement of Batch Collections results combined in one plot.

The Batch results are overlaid, which helps you to determine how 'different' these image groups are. Comparisons can be drawn between any selected statistical variables that you are interested in.

2.11.2 Arena Vantage view

In Vantage view, in the Plot Input area, a Vantage tree displays all source objects. In the Vantage Properties Area you can choose the plot type and which variables are to be assigned to (X, Y, Z, Colour or scale) plot dimensions. For more information on Vantage view and instructions on how to select a plot type and assign plot dimensions, please refer to the Vantage view Section.

Save

Selecting the Save option stores the Vantage plot data and created Vantage plot item within the selected Arena Folder. Within the Arena tree, a subgroup Vantage appears (within the tree) and is named accordingly.
Selecting the Save option leads a dialog to appear prompting you for a descriptive name for the item.
In the Name field enter a descriptive name for your Vantage Plot item and click the **OK** button. This is the name that will appear within the Arena view. Within the folder, at the bottom of the selected group the newly created Vantage plot item will appear.

Save as

Selecting the Save as option allows you to modify the already created Vantage plot item and creates a new Vantage plot item within the selected Arena Folder.

Section 3 Surpass View

The Surpass view provides extended functions for visualization, including:

- A large viewing area with numerous tools for data preparation, presentation and manipulation.
- A selection of different types of data display as well as any combination of them.
- The possibility of loading additional External objects for comparison.
- Surpass Grouping functions.

How to Open the Surpass View?

- Double click on the image in the Arena
- Click on the Surpass icon in the Main toolbar

Save and Load Surpass Configuration

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Scene and can be stored in a Scene file with the extension *.imx. The Scene can be loaded again to the same data set or to another data set. For details please refer to Section Surpass View - Overview - Scene File Concept.

See also:
Ortho Slice
Cells
Clipping Plane
Filament
Reference Frame
Frame
Surpass Group
Measurement Point
Oblique Slicer
Ortho Slicer
Light Source
Volume
Surfaces
Spots

3.1 Surpass Toolbar

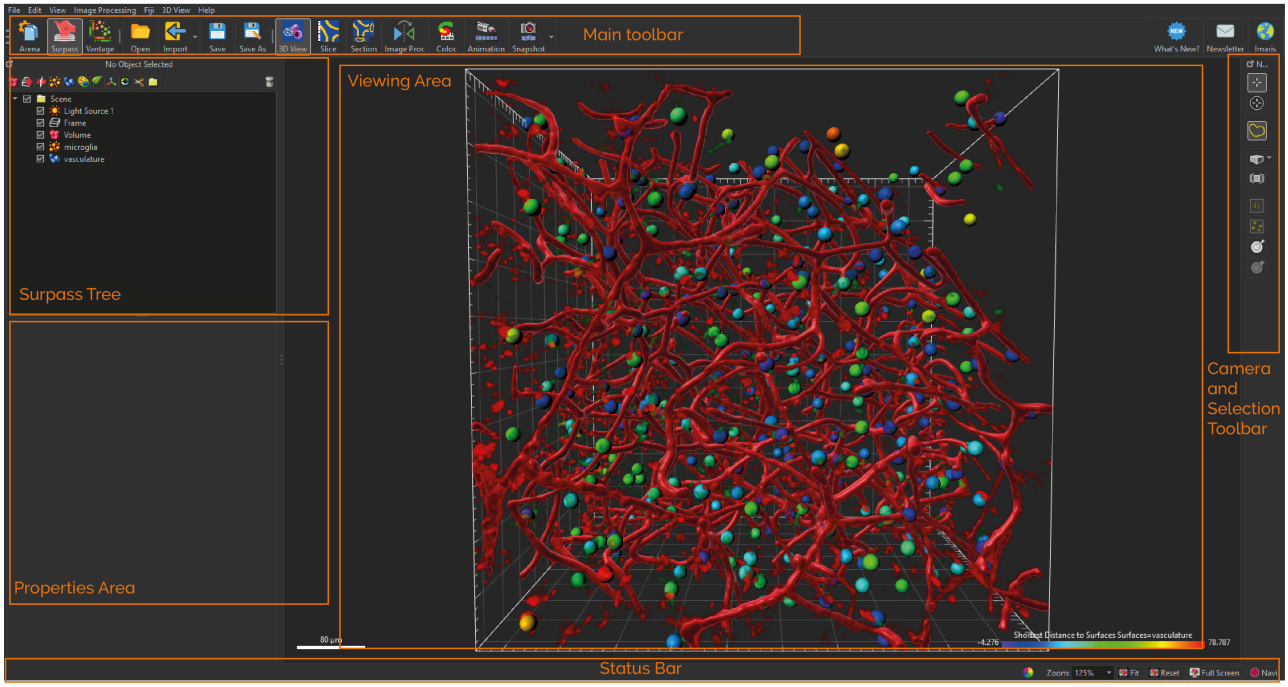
The Surpass main toolbar provides quick access to frequently used items:

In All Views	
	Arena
	Surpass

	Vantage	
	What's New	
	Newsletter	
	Imaris	
	In Surpass View	
	Open	
	Import (drop down)	Import Segmentation/Label Import Scene Import Spots or Tracks Add Image/Volume
	Save	
	Save As	
	2D View	
	3D View	
	Slice	
	Section	
	Image Processing	
	Coloc	
	Animation	
	Snapshot	

3.2 Overview

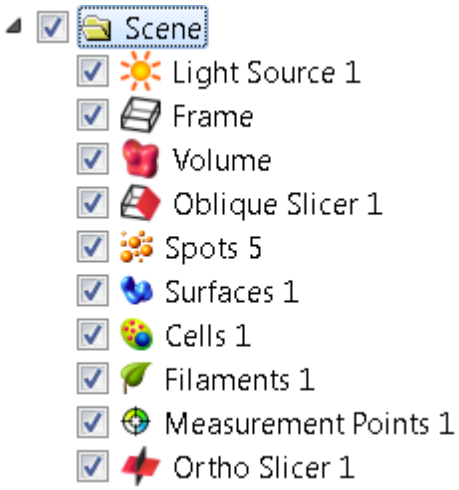
The Surpass view main screen consists of six different areas:



- At the top is the Main Toolbar.
- On the left hand side you find the Surpass Tree and the Properties Area. In the Properties Area you find all available parameters for the selected Surpass Tree Item. The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.
- The Viewing Area displays all objects that are added to the Surpass Tree and are checked visible.
- The **Camera Toolbars** area contains the Camera and Selection Toolbar which features a number of tools for data preparation, presentation and manipulation, most of which are displayed as buttons.
- On the bottom you find the Status Bar. Align to option is available when multi-image mode is applicable.

3.2.1 Surpass Tree

The Tree displays a tree list of all Surpass Tree Items that were added to the viewing area.



Structure

The tree list is automatically generated and updated when a Surpass Tree Item is added or deleted. The first added object generates a **Surpass Group - Scene**. All following new objects are stored in this **Surpass**

Group. A name is generated automatically for each Surpass Tree Item. To change the name, double-click on the item and enter a new name. Move objects or Surpass Groups from one to another by dragging and dropping them with the left mouse button.

How to Add a new Surpass Tree Item?

All available Surpass Tree Items are available in the menu Surpass. To add new Surpass tree item click on the icon.



You find a list of all Items in the Section Surpass View - Overview - Properties Area.

Display

Each Surpass Tree Item includes a check-box. Check the box to make the object visible in the viewing area. Uncheck the box to make the object invisible in the viewing area. The currently active object in the viewing area is highlighted in the Surpass Tree.

Surpass Groups

You can group objects into so-called component groups. Functions applied to the component group apply to all of its members. This facilitates the application of colors or the deletion of objects.

Please note: If Group folder is checked invisible, all Items in the folder are invisible.

Multiple Selection

You may select more than one listed Item at a time for an operation. The selection functions in Surpass correspond to the Windows functions:

- Consecutive: Press and hold the Shift-key down and select the first, then the last entry to be selected from the list. All entries in between the two are also selected.
- Selective: Press and hold the Ctrl-key down and select any required entries from the list.

All selected entries are highlighted and commands or operations apply to all of them.

Objects Toolbar

In the Objects toolbar you find a selection of Surpass Tree Items. To customize the Objects toolbar please refer to Section Menu Edit - Preferences... - 3D View (Object Creation Buttons).

Button **Delete...**

To delete Surpass Tree Item, highlight the Item in the Surpass Tree and click the button **Delete...** . The Delete selection window with a confirmation question is displayed.

Naming Conventions

Objects are automatically named by Surpass as follows:

Cells	Cells n
Clipping Plane	Clipping Plane n
Contour Surface	Surface
External Object	External Object n
Filament	Filament n
Reference Frame	Reference Frame n
Frame	Frame
Surpass Group	Surpass Group n
Surfaces	Surface n
Light Source	Light Source n
Measurement Point	Measurement Points n
Oblique Slicer	Oblique Slicer
Ortho Slicer	Ortho Slicer n
Spots	Spots n
Volume	Volume (only one volume can be created)

Surpass Tree Item Properties

Each Surpass Tree Item has its own set of adjustable parameters. They are displayed in the properties area.

Surpass Tree Item Tabs

Each Surpass Tree Item has its own set of adjustable parameters. They are grouped in different Tabs.

Save and Load Surpass Tree Configuration

The actual Imaris configuration (including Surpass Tree and all existing items) in the Surpass view is called Scene and can be stored in a scene file with the extension *.imx. The Scene can be loaded again to the same data set or to another data set. For details please refer to Section Surpass View - Overview - Scene File Concept.

See also:
Menu Edit - Preferences... - 3D View (Object Creation Buttons)
Menu Surpass - Delete Selected Objects...
Surpass View - Overview - Properties Area
Surpass View - Surpass Group

3.2.2 Scene File Concept

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Scene and can be stored in a scene file with the extension *.imx. The Scene can be loaded again to the same data set or to another data set.

Surpass View - Surpass Group

3.2.3 Properties Area

The Properties Area displays all available parameters for the selected Surpass Tree Item.

Surpass Tree Item - Properties

The name of the heading is a combination of the selected Surpass Tree Item, followed by "- Properties". If you select another Surpass Tree Item the heading changes accordingly.

Tab X
The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.

List of available Tabs:

Surpass Tree Item	Parameter Tab
Cells	Tab Rebuild see 3D View - Cells Creation Wizard Tab Settings Tab Rebuild Tab Edit Tab Filter Tab Statistics Tab Color
Clipping Plane	Tab Settings
External Object	Tab Settings Tab Color
Filament	Tab Rebuild see 3D View - Filament Creation Wizard Tab Settings Tab Recompute Tab Draw Tab Edit Tab Filter Tab Statistics Tab Color
Frame	Tab Settings Tab Color
Surpass Group	Tab Settings Tab Color Tab Statistics
Light Source	Tab Settings Tab Color
Measurement Point	Tab Settings Tab Edit

	Tab Intensity Tab Statistics Tab Color
Ortho Slicer	Tab Settings
Oblique Slicer	Tab Settings
Spots	Tab Rebuild see 3D View - Spots Creation Wizard Tab Settings Tab Rebuild Tab Edit Tab Filter Tab Statistics Tab Color
Surfaces	Tab Rebuild see 3D View - Surfaces - Creation Wizard Tab Settings Tab Rebuild Tab Draw Tab Edit Tab Filter Tab Statistics Tab Color
Volume	Tab Settings Tab Draw Tab Statistics

3.2.4 Creation Wizards

A Creation Wizard is a user interface element where you are guided through a sequence of dialogs. In a Creation Wizard you are prompted to perform a task in a specific sequence.

Examples for Creation Wizards in Imaris are the Cells Creation Wizard, the Filament Creation Wizard, the Spots Creation Wizard, and the Surfaces Creation Wizard. Once you insert a new Item: Filament, Spots, Cell or Surfaces, you are automatically on the Tab Rebuild in the Properties Area.

See also:
3D View - Cells - Creation Wizard
3D View - Filament - Creation Wizard
3D View - Spots - Creation Wizard
3D View - Surfaces - Creation Wizard

3.2.5 Viewing Area

Pan

To move the image within the Surpass view (pan the object) choose the mouse pointer mode Navigate. Click and hold the right mouse button while dragging the mouse. Release right mouse button to place the image.

On a PC or with a three-button mouse or on a Mac:

Right-click & drag	Pan image

On a Mac with a one-button mouse:

ctrl + click & drag	Pan image

Rotate

Rotating an image allows to change the viewing angle on a three-dimensional object.

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while moving the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Be sure to hold the left mouse button down during the whole rotation. Stop moving the mouse and release the left mouse button to stop the rotation.

On a PC or with a three-button mouse or on a Mac:

Left-click & drag	Rotate image

On a Mac with a one-button mouse:

Click & drag	Rotate image

How to Keep the Image Continuously Rotated

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while you move the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Release the left mouse button while still dragging the mouse. The result is a continued rotation (speed of the rotation according to prior mouse motion). To stop the continued rotation re-click in the image area.

Zoom

In the Surpass view you zoom the image either by using the mouse or by selecting one of the buttons in the Status Bar at the bottom of the screen.

Using the Mouse

Choose the mouse pointer mode Navigate. To zoom in on the image hold the middle mouse button and drag it towards you. To zoom out from the image hold the middle mouse button and drag it away from you.

On a PC or with a three-button mouse or on a Mac:

Middle-click & drag	Move up: zoom out Move down: zoom in

On a Mac with a one-button mouse:

Shift + ctrl + click & drag	Move up: zoom out Move down: zoom in

Using the Buttons in the Status Bar

Zoom %
Imaris lets you enlarge or reduce the viewing size of your data set as is required with the Zoom function. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value. At 100% zoom factor one pixel on screen represents one voxel.

Button **Fit**

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button **Reset**

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

Button **Full Screen**

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window re-click on the button **Full Screen** in the lower right corner.

Viewing options for Zoom and rotate can be adjusted in Preferences for 3D View - please refer to 3D View Preferences.

See also:
View
Toolbars - Status Bar
Surpass View - Overview - Camera Toolbar (Pointer Navigate)

3.2.6 Camera and Selection Toolbar

The toolbar to the right hand side provides the controls for the Camera and Selection functions.



Pointer Select Mode
Use the Pointer Select Mode to select an object in the image, e.g. to set Measurement Points on the object surface. Hold control to select multiple objects.

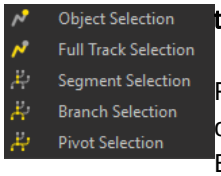


Circle Selection Mode
Use the Circle Selection Mode to select multiple objects simultaneously. Hold control and use the scroll function on the mouse to adjust the size of the circle, this will allow for the simultaneous selection of a smaller or greater number of objects as desired.

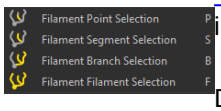
The shortcut keys 1 and 2 let you quickly switch between Pointer Select Mode and Circle Selection mode respectively.

Navigate
Once either the Pointer Select Mode or the Circle Selection Mode have been selected, by dragging the cursor across the screen, the cursor becomes two turning arrows and you can use either pointer to navigate, move, rotate or zoom the image in the viewing area.

Select Object/Track Options (Object/Track selection option is only available with tracks, i.e. if you have a time series dataset or with Filaments)



For **tracks of time series data**, the selection tool allows the user to chose between the options shown in the image: Object Selection, Full Track Selection, Segment Selection, Branch Selection and Pivot Selection. Object Selection and Full Track Selection are likely to be frequently used, whereas Segment Selection, Branch Selection and Pivot Selection are likely to be used only when split tracks are present.
Please refer to the track editor for the specific object of interest, Cells Tab Edit Tracks, Filaments Tab Edit Tracks, Spots Tab Edit Tracks and Surface Tab Edit Tracks for more information.



During **Filament object creation or during editing** choose between **Filament Point Selection, Filament Segment Selection, Filament Branch Selection or Filament Filament Selection.**



Orthogonal Camera
Orthogonal display using parallel lines.



Perspective Camera
Perspective projection is a type of drawing, or rendering, that graphically approximates on a planar (two-dimensional) surface.



InMotion is described in the section InMotion



Center viewing area to center of selected objects to move the selection in the center of the viewing area.



Zoom and center viewing area to fit selected objects to move the selection in the center of the viewing area.

Set Center of rotation to center of scene
Select the **Set Center button...** then select an object, in 3D view, to define a new center of rotation.



Rotation centers can be set on Surfaces, Contour Surface, Ortho Slicer and External objects Additional drawing and stereo view options may be enabled- please refer to 3D View

Fix Current Rotation Center
To set the center of the 3D cursor position so it will rotate about this point even if the image object is moved within the image viewing window, select the following.

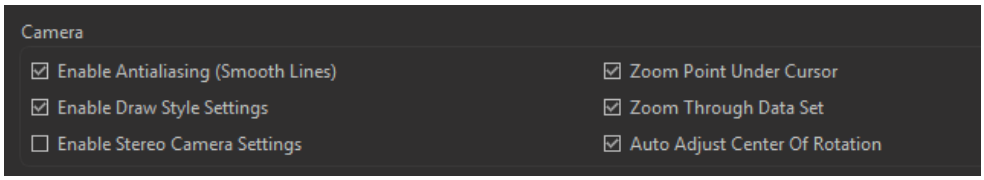


You can press the shortcut key F6 when the 3D cursor is visible to perform this function (the Fix Center option in the Camera panel gets checked). This shortcut key

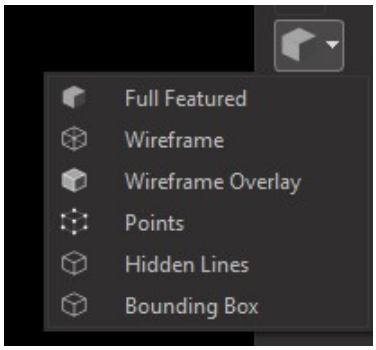
will have no effect when the 3D cursor is not visible.

Draw Style (Enable Draw Style Settings in Preferences)

To enable the Draw Style Settings option, go to File/Preferences and select 3D view from the menu on the left hand side. Under the Camera section Enable Draw Style settings by checking the tick box. Select OK at the bottom of the preferences dialog to save this change.



Once enabled a new icon with drop down is avaliable in the camera toolbar:



Full Featured
Shows all objects as they are.

Wireframe
Show the objects as a simple wireframe only.
Please note that this will only show the points and edges of the object, without hidden triangle surfaces.
Draws objects; Surfaces, Spots, Filaments, Cells and Measurement Points as red colored wireframe models.

Wireframe Overlay
Lays a red wireframe model over objects; Surfaces, Spots, Filaments, Cells and Measurement Points.

Points
Draws objects as a point model.

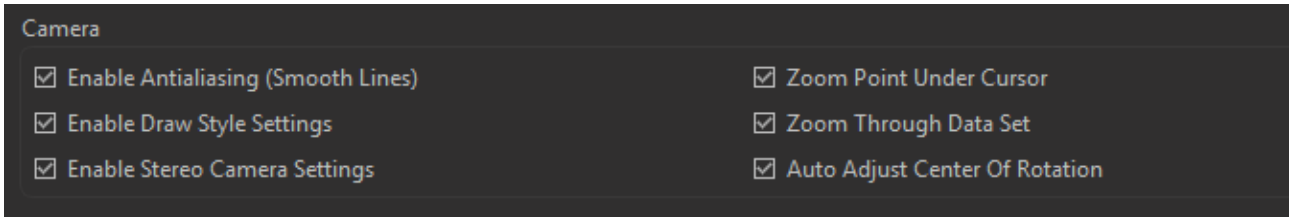
Hidden Lines
Draws objects as wireframe models and hides all background lines.

Set Volume and OrthoSlicer objects invisible before selecting Hidden Lines.

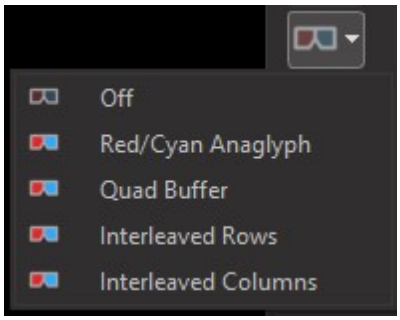
Bounding Box
Shows only the boxes surrounding the objects.

Stereo (Enable Stereo Camera Settings in Preferences)

To enable this option, go to File/Preferences and select 3D view from the menu on the left hand side. Under the Camera section select the Enable Stereo Camera settings by checking the tick box. Select OK at the bottom of the preferences dialog to save this change.



Once enabled a new icon with drop down is avaliable in the camera toolbar:



Off
No stereo display in the viewing area.

Red/Cyan Anaglyph
This display mode requires colored glasses.

Quad Buffer
This display mode requires shutter glasses.

Interleaved Rows
This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

Interleaved Columns
This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

3.2.7 Mouse & Keyboard PC

General Mouse & Keyboard Functions in the Surpass View

Ctrl + 1	Activates the 3D View
Esc	Switch mouse mode pointer or circle selection mode
1	Pointer selection mode
2	Circle selection mode
Ctrl + middle scroll	Changes the size of the circle in circle selection mode
Drag	Navigate
O	for object selection mode
T	for track selection mode

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Selection Modes (Pointer or Circle)

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Left-click	Select object
Ctrl + left-click	Add object(s) to selection/remove object from selection
Left-click & drag	Rotate image (scene)
Right-click & drag	Pan image
Middle-click & drag	Move up: zoom out Move down: zoom in

See also:
Addendum - Mouse & Keyboard PC

3.2.8 Mouse & Keyboard Mac

General Mouse & Keyboard Functions in the Surpass View

Command + 1	Activates the 3D View
Esc	Switch mouse mode Navigate/Select
1	Pointer selection mode
2	Circle selection mode
Ctrl + middle scroll	Changes the size of the circle in circle selection mode
Drag	Navigate
O	for object selection mode
T	for track selection mode

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Selection Modes (Pointer or Circle)

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Click	Select object
Command-click	Add object to selection/remove object from selection

With a one-button mouse:

It is recommended to use a 3 button mouse for Imaris on Mac.

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Left-click & drag	Rotate image

See also:
Addendum - Mouse & Keyboard Mac

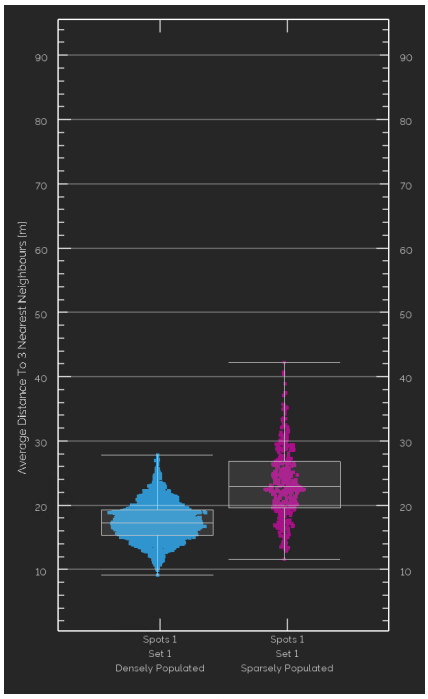
Section 4 Vantage View

Vantage 1D View, 2D View, Spatial View, Time View and Objects View are statistical data-visualization modules that allow the user to plot and visualize numerical values. The interactive visualization methods improve understanding of complex statistical data by uncovering hidden relationships between objects and identifying patterns and trends.

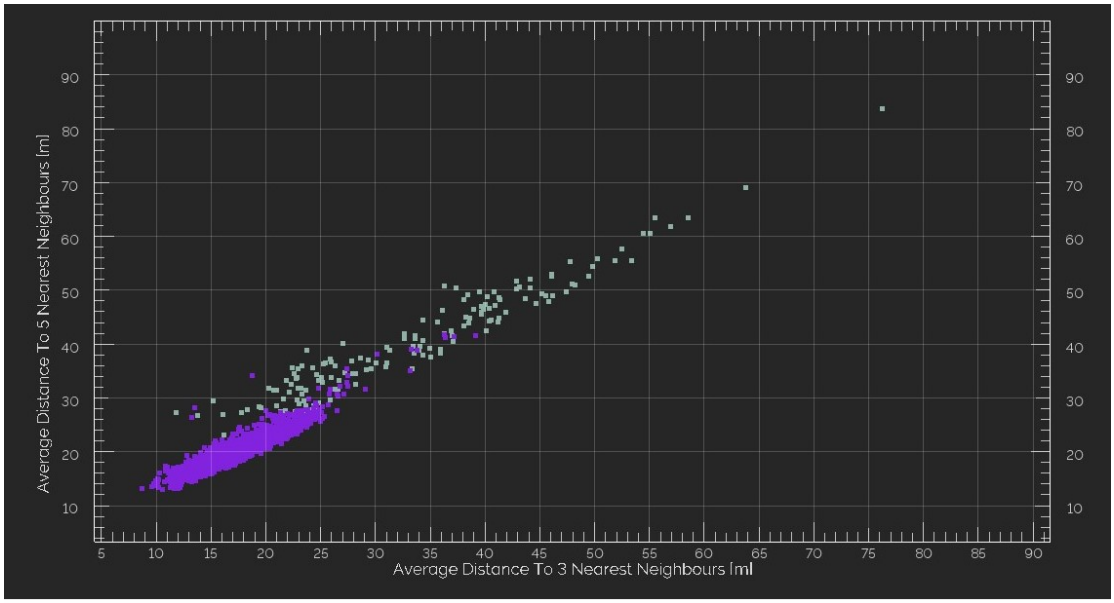
To effectively visualize and analyze the relevant statistical data, vantage can be used to create clear, concise and colorful plots in multiple dimensions. The created plots are interactive, simply select the object or data point of interest either in Objects, 1D or 2D View or in the Surpass View for further analysis.

Time View allows the user to examine time series data. Spatial View allows the user to examine the relationship between spots and surfaces to determine whether the position of spots relative to the surface are randomly distributed. Additionally spatial statistics can be used to examine the image intensity with respect to the surface object.

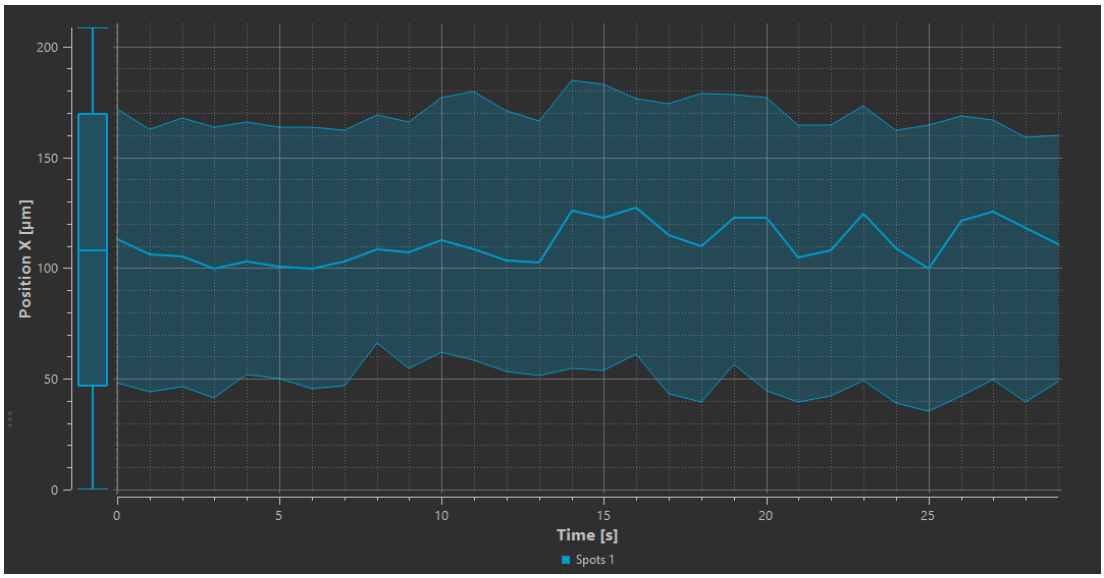
Imaris Vantage allows the user to detect meaningful underlying relationships and to explain observed phenomena between objects.



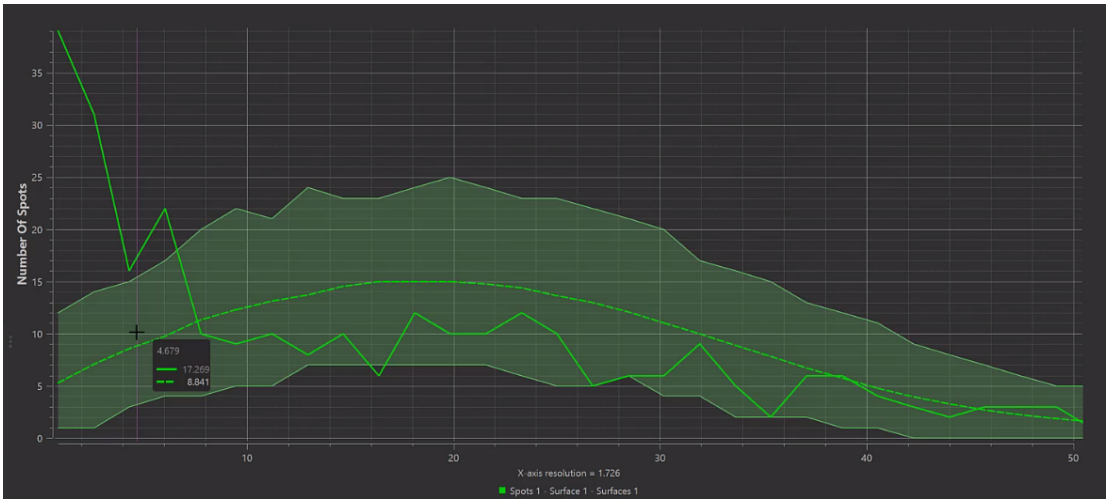
1D View



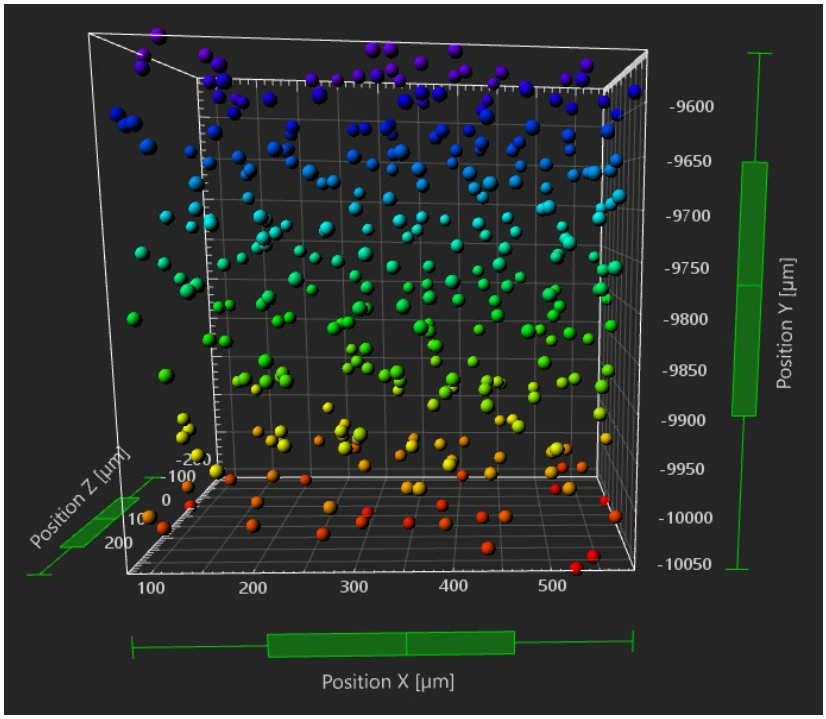
2D View



Time View



Spatial View



Objects View

4.1 Vantage Main Toolbar

The Vantage View main toolbar provides quick access to frequently used items.

In All Views


Arena


Surpass


Vantage



What's New


Newsletter


Imaris

In Vantage View


Save


Save As


1D View

 2D View

 Time View

 Spatial View

 Objects View

 Snapshot

4.1.1 Save Vantage view

To save an existing data set click **Save** in the menu or the Save icon in the Main toolbar.

Selecting this option saves the Vantage plot data within the selected Arena Group. The Vantage plot item is created if none existed before.

Please note: Save functionality is implemented at maximum speed. This functionality overwrites the existing file without warning and only writes changed or new data without deleting obsolete ones. As a result, the resulting Saved file could actually be larger than the original.

4.1.2 Save as Vantage View

To save the changes made to the Vantage plot in a different Vantage plot item whilst maintaining the original plot click on the Save as icon

This option enables you to modify the Vantage plot and its name and creates a new Vantage plot item within the Arena view.

Enter the name for the file to be saved or confirm the suggestion. Click **OK**.

4.2 Vantage Menu

Please refer to the following sections:
Save
Save as
View
Vantage Plot
Preferences...
Help

4.2.1 Vantage Plot

Vantage Plot
To insert a new Vantage Objects View plot, click the **Objects View** icon.

Delete Selected Objects...
Select this option and in the Vantage Tree all highlighted items will be deleted.

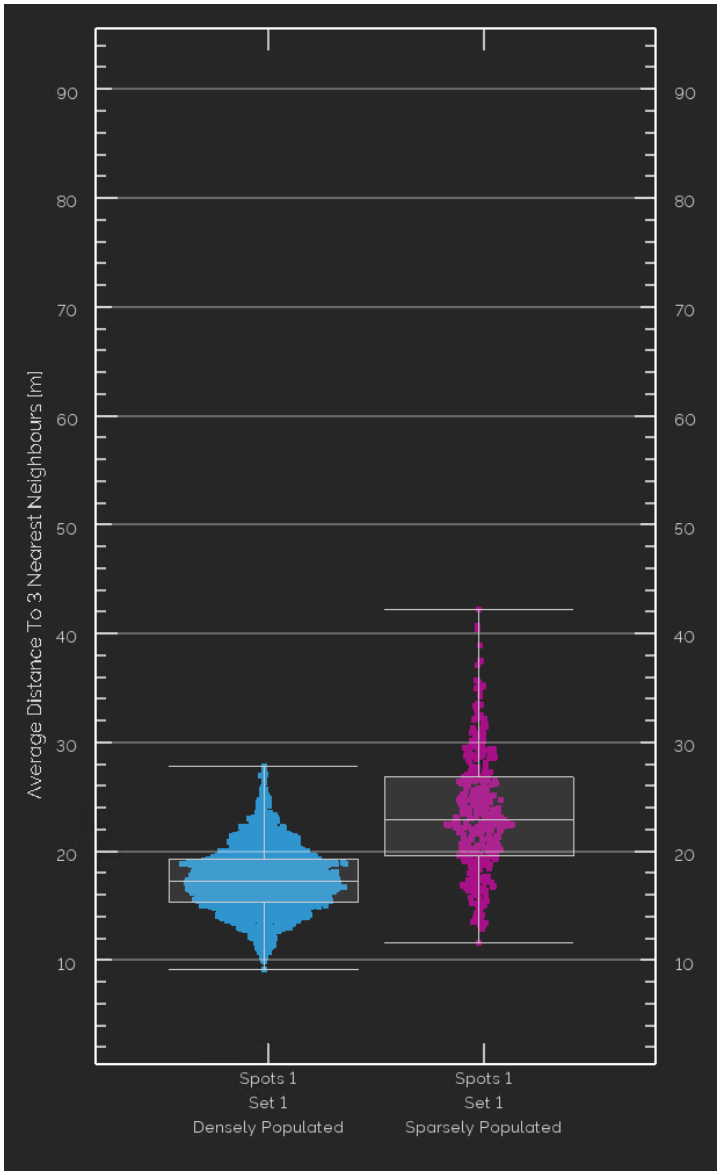
Plot Numbers Area

Check the box Plot Numbers Area to display the tables that contains all statistical values used for the plot creation.

4.3 1D View

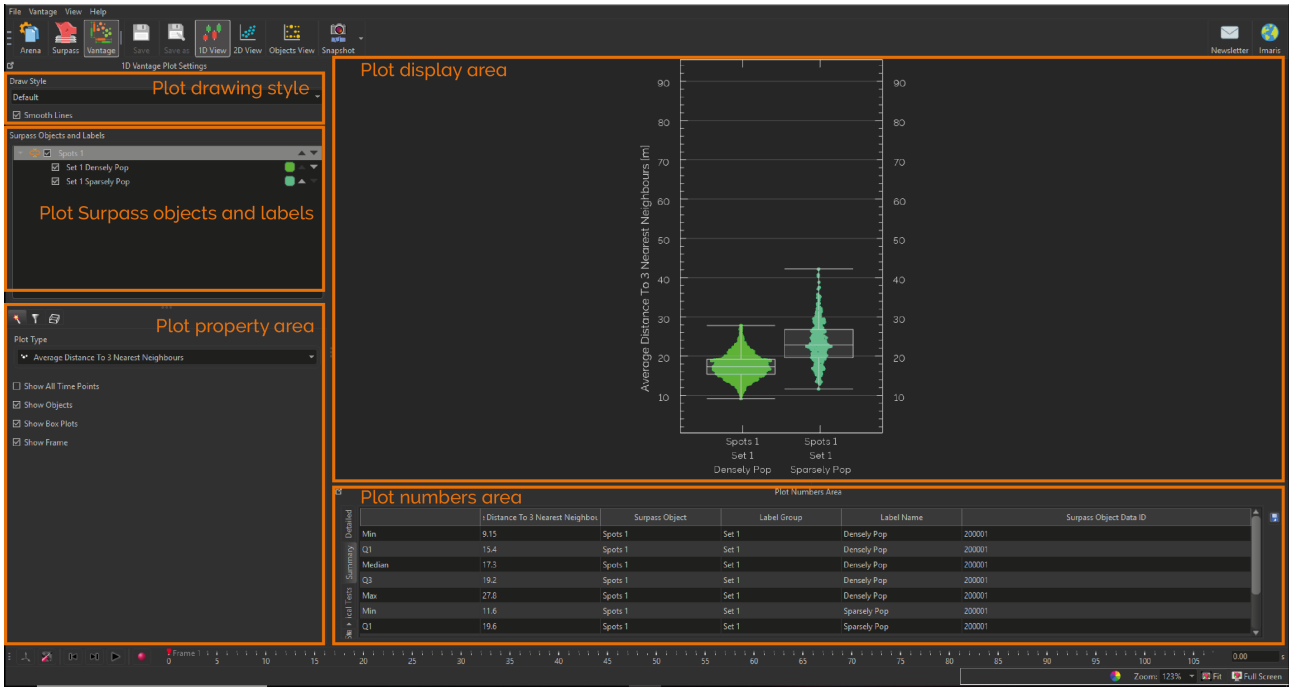
Vantage **1D View** provides an effective way to view patterns and trends in statistical data in the form of one dimensional scatter plots.

1D view allows the user to analyze data, and compare and derive relationships from one or more data sets. 1D View provides a simpler means to analyze and view data sets (for example spots) than Vantage Objects View, however many of the functions found in Objects View are the same and are described previously in Vantage View. In addition the Statistics Tests tab provides powerful statistical tests for direct analysis of data sets within Imaris 1D View.



4.3.1 1D User Interface

The 1D User Interface has the following working areas: Plot Display Area, Plot Drawing Style Area, Plot Surpass Objects and Labels, Plot Property Area and Plot Numbers Area.

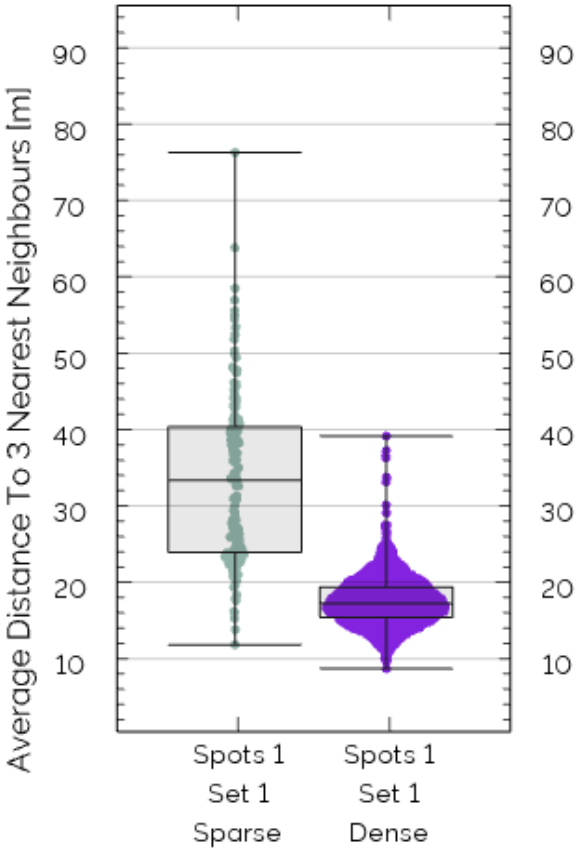


4.3.1.1 1D Plot Drawing Style Area

Change the drawing style of the plot from the options available: Default, Print and Color Print.

Default style displays the plot according to the settings in Preferences Display, for example, for display on the computer monitor.

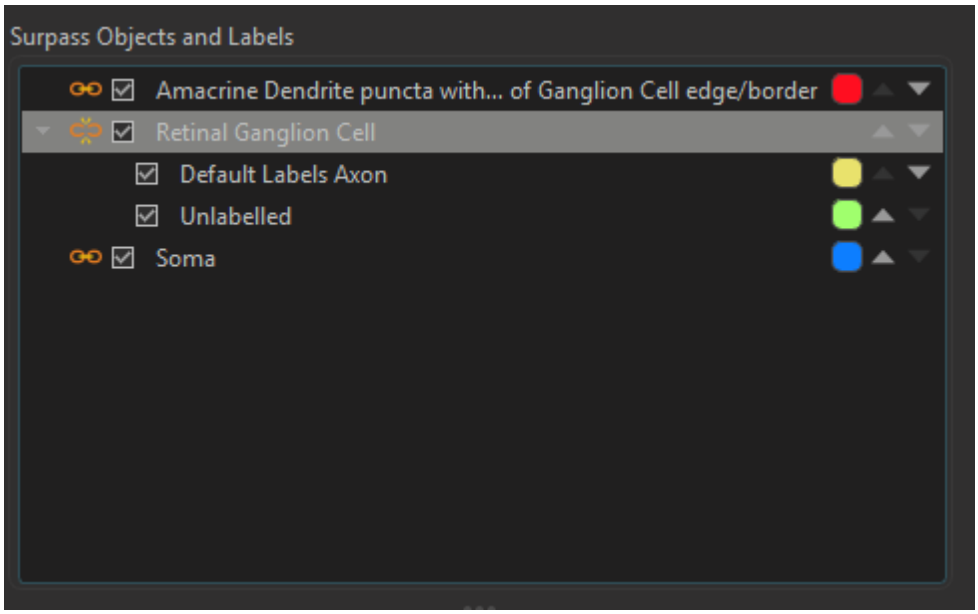
Color Print displays the plot in color with a white background for printing on paper.



Tip: Smooth lines can be used to smooth the appearance of text and lines in the plot.

4.3.1.2 1D Plot Surpass Object and Labels

This section displays all Surpass Objects associated with the image, in addition to their labels.



To **view all labels** associated with an object click on the orange link button, to reveal all labels associated with that object.

To **remove objects** or labels from the plot display uncheck the tick box to the left of their name.

To **change the order of objects** plotted use the up and down arrows to change the position of the object or label in the list and the order in the plot display.

To **change the color** of the object or label within the plot click on the colored square icon to bring up the color slider.

4.3.1.3 1D Plot Property Area

The settings for the data can be modified. These options are grouped together under the following tabs: Settings Tab, Filter Tab and Frame Tab.

Settings Tab

The **Settings Tab** enables selection of required statistical options to analyze data for the 1D Plot. The options available will depend on the input components selected e.g. spots, surfaces, cells or filaments.

Use the Setting tab to select display options: Show all Time Points, Show Objects, Show Box Plots and Show Frame.

4.3.1.3.1 Filter Tab

Filter Tab

The **Filter Tab** allows you to select and apply a number of filters. The filters available are specific to the input components selected e.g. spots, surfaces, cells or filaments. Spots for example, includes the filters: by Area, Diameter, Intensity, Position, Time and Volume. Filters can be added and deleted using the Add and Delete options.

You can use the automatic filter setting, or manually adjust the values as required. Use the slider bar, when this is available, to adjust the parameters for the selected filter. The name of the filter displayed will update if you change the filter settings.

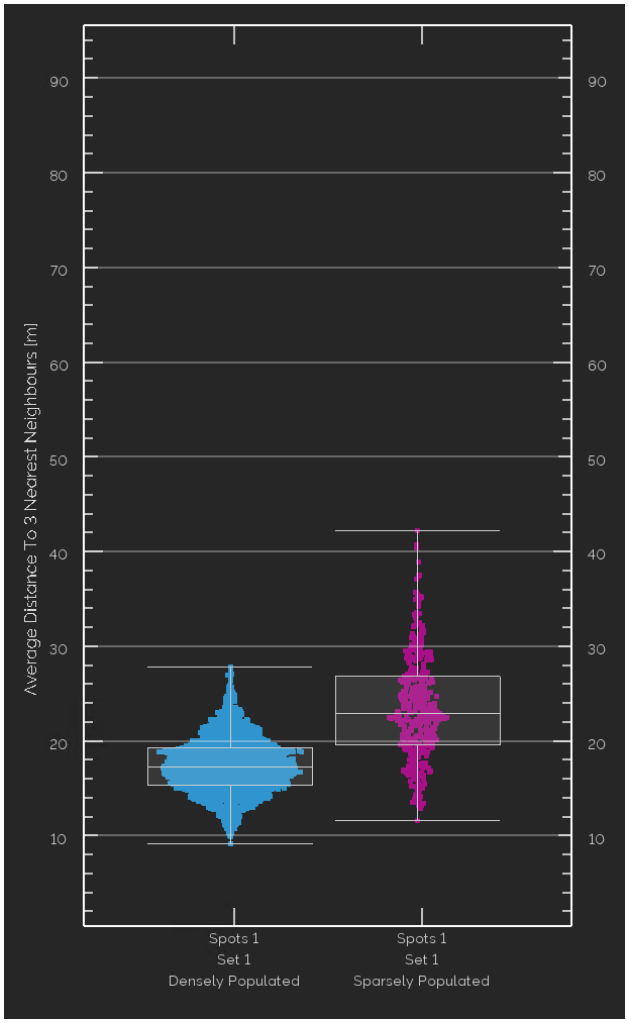
4.3.1.3.2 Frame Tab

Frame Tab

The **Frame Tab** provides you with a range of options to change the appearance and settings used for the displayed plot. This includes the Frame Settings, Grid and Tickmarks, Labels, Axis labels and Font.

4.3.1.4 1D Plot Display Area

The Plot Display Area shows the plots for the selected data set. The plot area is interactive and a specific data point can be selected, the data will be highlighted in the Plot Numbers Area. Unlike Object View, only one data point in a series can be selected at a time.



4.3.1.5 1D Plot Numbers Area

The 1D Plot Numbers Area features the Detailed and Summary tabs, described fully in the Objects View section, follow this link for more information, Plot Numbers Area.

In addition the **Statistical Tests** tab provides automatic statistical data analysis for the surpass objects, meaning that this information can be viewed directly within Imaris. The Statistical Tests functions are outlined below.

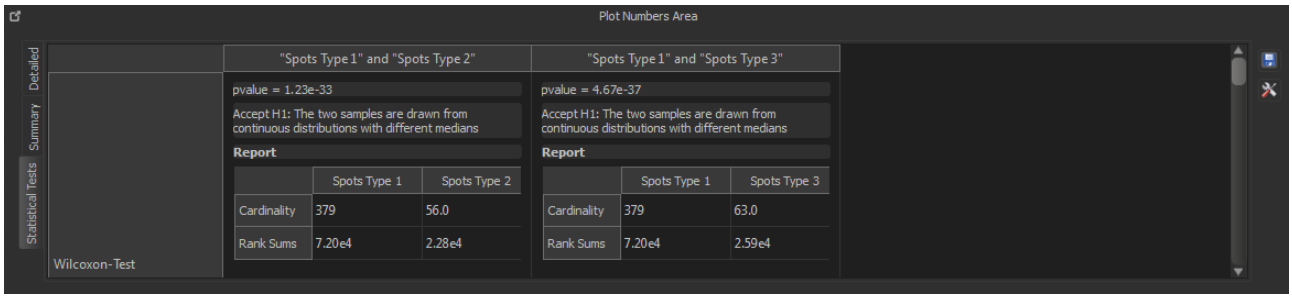
Statistical Tests

The Statistical Tests tab and functions are available through 1D View only- they are not available under 2D or Objects View. The following tests are performed:

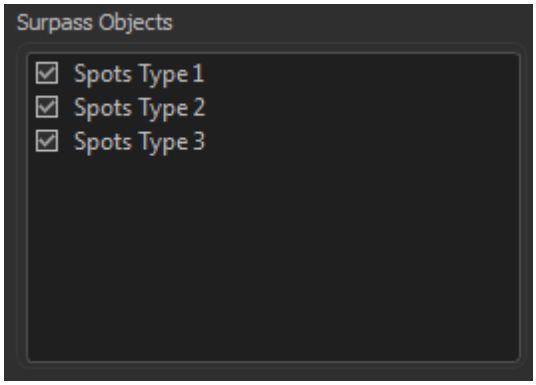
Test	Summary
Wilcoxon-test	The Wilcoxon-test is also referred to as Mann-Whitney test. The Wilcoxin-test enables you to determine whether two sets of surpass data sets with ordinal data, but without normalised distribution i.e. non-parametric, are statistically different from each other.

Test	Summary
t-test	The t-test enables you to test whether the mean of two data sets (for small populations) are statistically different from each other. With this test, there is an option to assume that the variation between the surpass data sets are the same, in this case a *Welch's t-test* is performed. If this is not the case, a *Student's t-test* will be performed.
f-test	The f-test enables you to test (the null hypothesis) that the variance of two data sets are equal. This is done by comparing the ratio of the variance between the two surpass data sets.
Kolmogorov-Smirnov test	The KS-test lets you determine if two data sets differ significantly. The KS-test is distribution free.

The statistics are compared for the first of the surpass objects against the others in the list, and the results are displayed in columns as shown in the example below. If the test does not meet the criteria to run, the reason will be displayed.



As the statistics are compared for the first of the surpass objects against the others in the list, to change the order of the data sets use the arrows to move items to the required order. In the following example, "Spots Type 1" is compared against "Spots Type 2" and "Spots Type 3". "Spots Type 3" could be compared against "Spots Type 1" and "Spots Type 2" by dragging it to the top of the list.



The settings for Statistical Tests can be viewed and edited e.g. confidence level or test type, using the Settings button. This is a shortcut to Statistical Tests preferences.

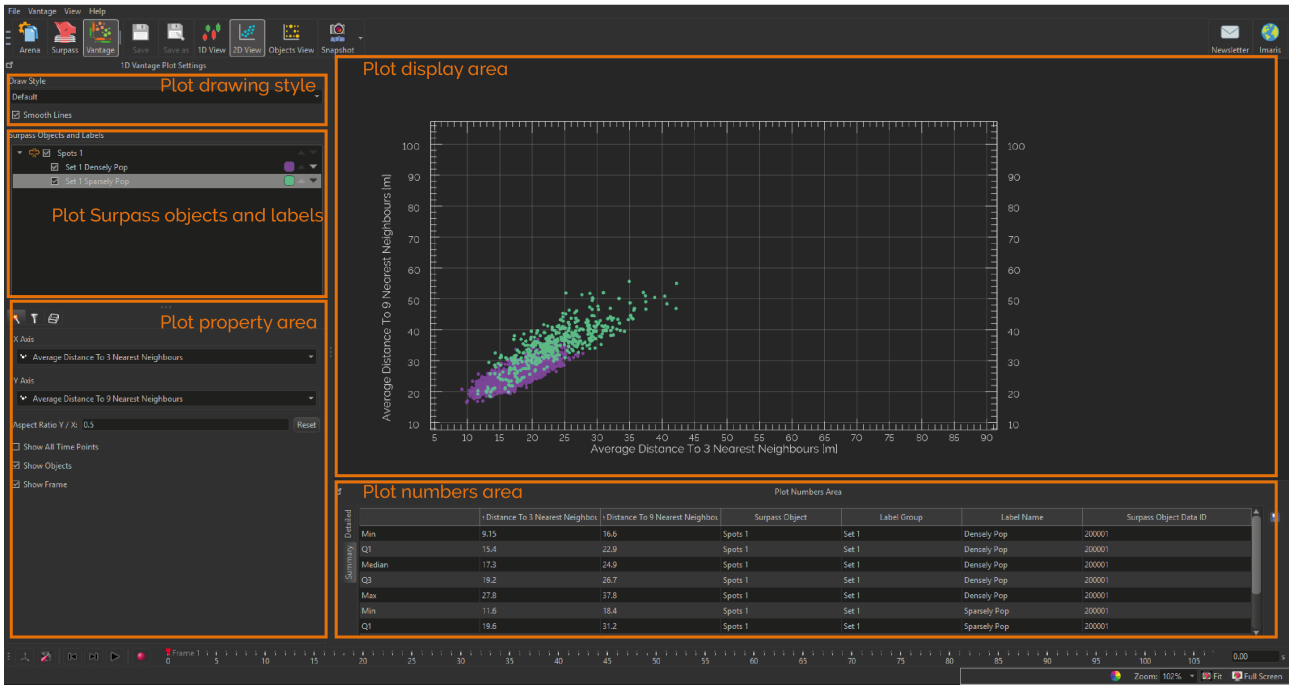
Note that data for each tab can be exported using the button when that tab is highlighted.

4.4 2D View

The **2D view** allows the user to produce scatter plots that quickly display statistical relationships between two variables.

4.4.1 2D User Interface

The 2D User Interface has the following working areas: Plot Display Area, Plot Drawing Style Area, Plot Surpass Objects and Labels, Plot Property Area and Plot Numbers Area.

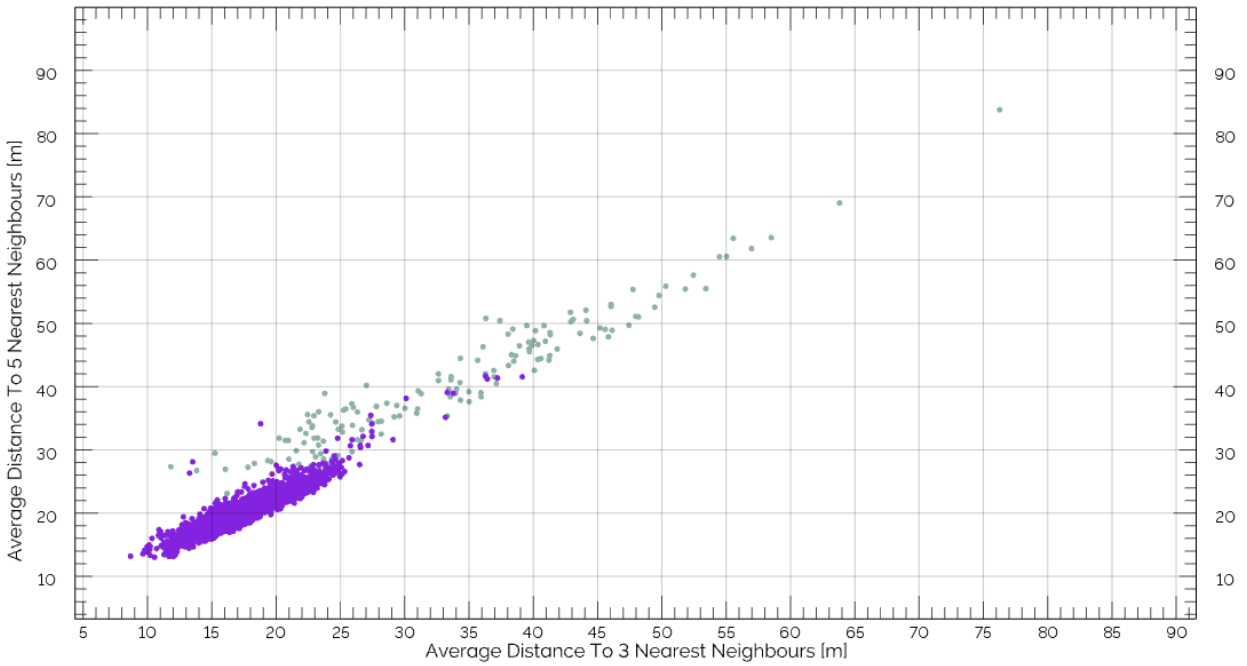


4.4.1.1 2D Plot Drawing Style Area

Change the drawing style of the plot from the options available: Default, Print and Color Print.

Default style displays the plot according to the settings in Preferences Display, for example, for display on the computer monitor.

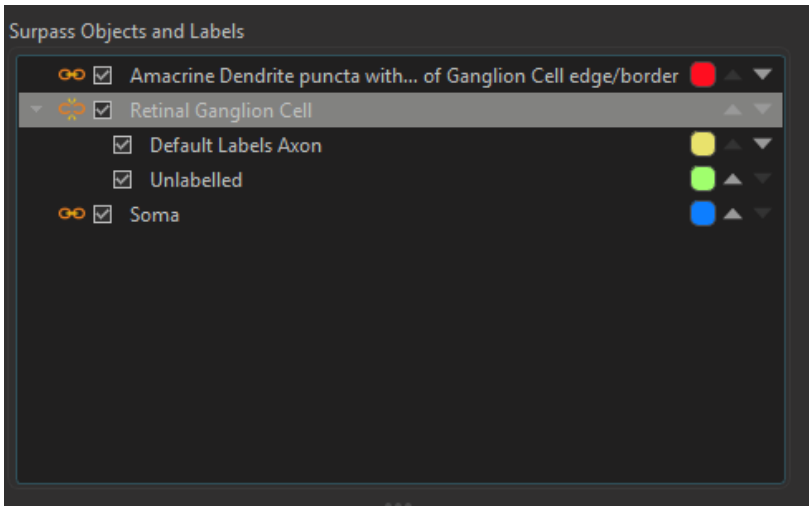
Color Print displays the plot in color with a white background for printing on paper.



Tip: Smooth lines can be used to smooth the appearance of text and lines in the plot.

4.4.1.2 2D Plot Surpass Object and Labels

This section displays all Surpass Objects associated with the image, in addition to their labels.



To **view all labels** associated with an object click on the orange link button, to reveal all labels associated with that object.

To **remove objects** or labels from the plot display uncheck the tick box to the left of their name.

To **change the order of objects** plotted use the up and down arrows to change the position of the object or label in the list and the order in the plot display.

To **change the color** of the object or label within the plot click on the colored square icon to bring up the color slider.

4.4.1.3 2D Plot Property Area

The settings for the data can be modified. These options are grouped together under the following tabs: Settings Tab, Filter Tab and Frame Tab.

Settings Tab

The **Settings Tab** enables the selection of the required statistical options to analyze data by for the 2D Plot. The options available will depend on the input components selected e.g. spots, surfaces, cells or filaments.

Use the Setting tab to select display options: Show all Time Points, Show Objects, Show Box Plots and Show Frame.

4.4.1.3.1 Filter Tab

Filter Tab

The **Filter Tab** allows you to select and apply a number of filters. The filters available are specific to the input components selected e.g. spots, surfaces, cells or filaments. Spots for example, includes the filters: by Area, Diameter, Intensity, Position, Time and Volume. Filters can be added and deleted using the Add and Delete options.

You can use the automatic filter setting, or manually adjust the values as required. Use the slider bar, when this is available, to adjust the parameters for the selected filter. The name of the filter displayed will update if you change the filter settings.

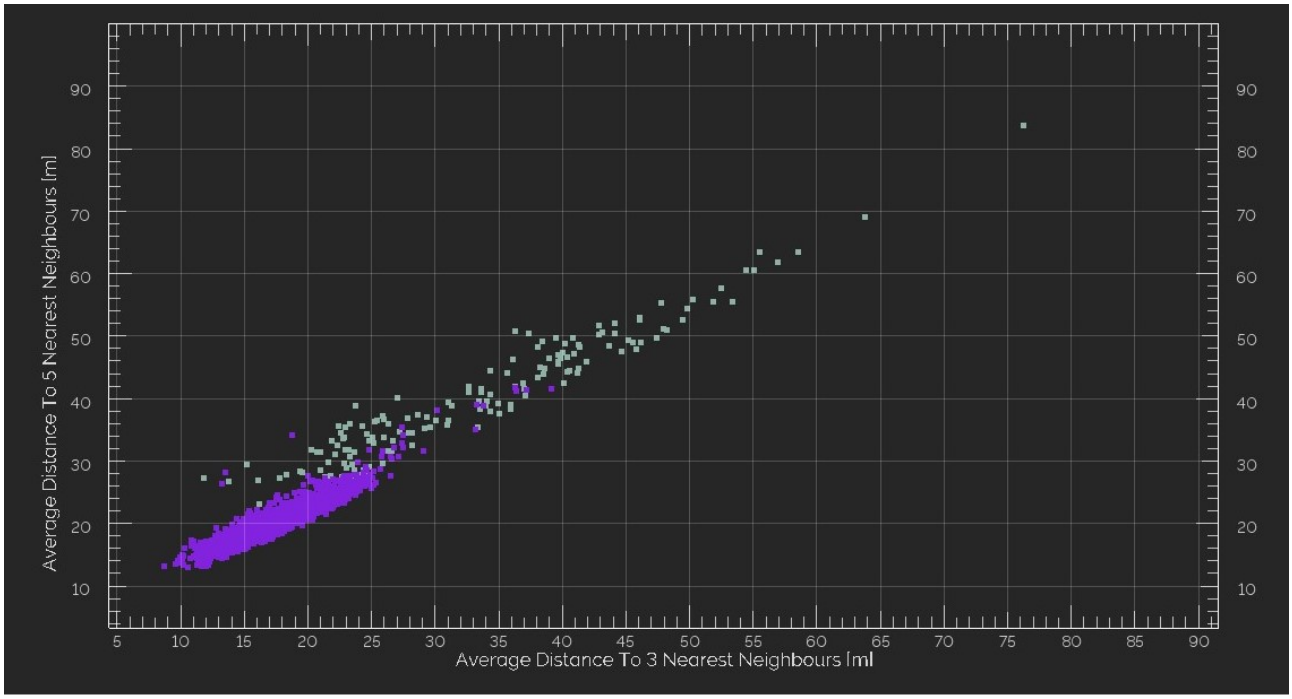
4.4.1.3.2 Frame Tab

Frame Tab

The **Frame Tab** provides you with a range of options to change the appearance and settings used for the displayed plot. This includes the Frame Settings, Grid and Tickmarks, Labels, Axis labels and Font.

4.4.1.4 2D Plot Display Area

The Plot Display Area shows the plots for the selected data sets. The plot area is interactive and a specific data point can be selected, the data will be highlighted in the Plot Numbers Area. Unlike Object View, only one data point in a series can be selected at a time.



4.4.1.5 2D Plot Numbers Area

The 2D Plot Numbers Area features the Detailed and Summary tabs, described fully in the Objects View section, follow this link for more information, Plot Numbers Area.

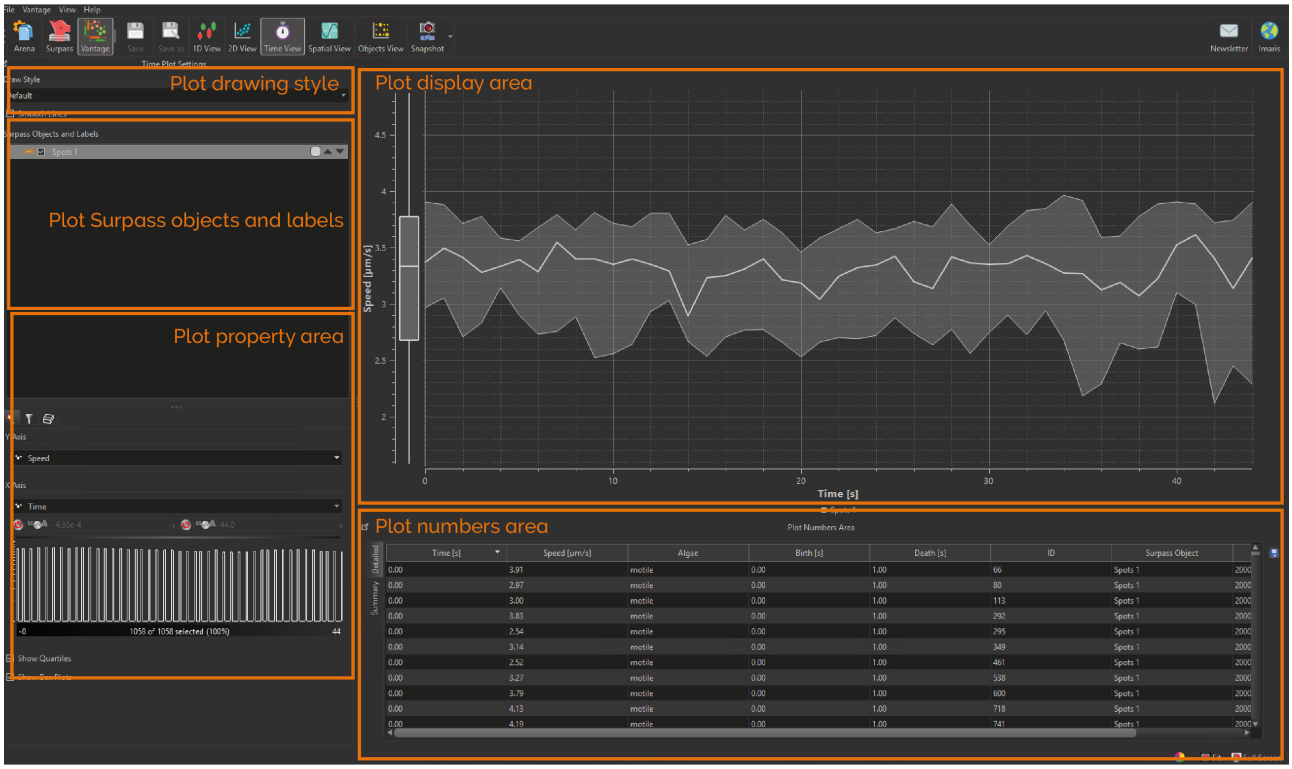
Note that data for each tab can be exported using the button when that tab is highlighted.

4.5 Time View

The **Time View** allows the user to examine time series data within a plot.

4.5.1 Time View User Interface

The Time View Interface has the following working areas: Plot Display Area, Plot Drawing Style Area, Plot Surpass Objects and Labels, Plot Property Area and Plot Numbers Area.

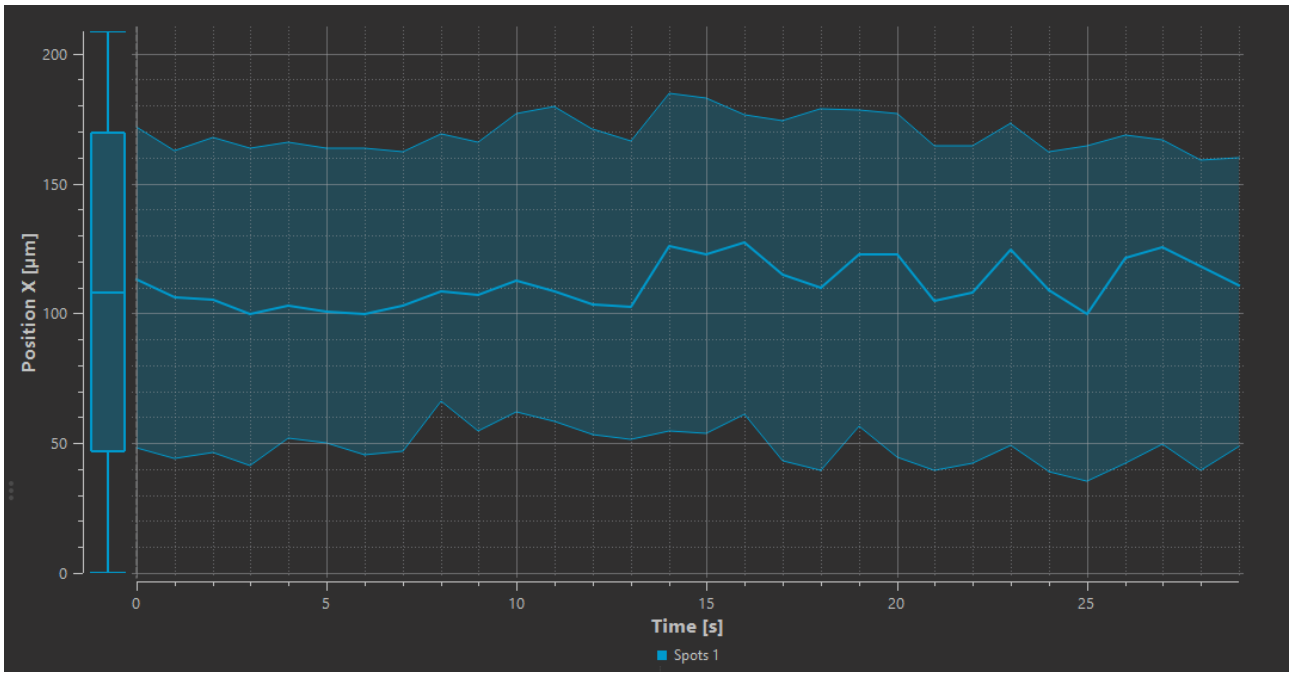


4.5.1.1 Time View Plot Drawing Style

Change the drawing style of the plot from the options available: Default, Print and Color Print.

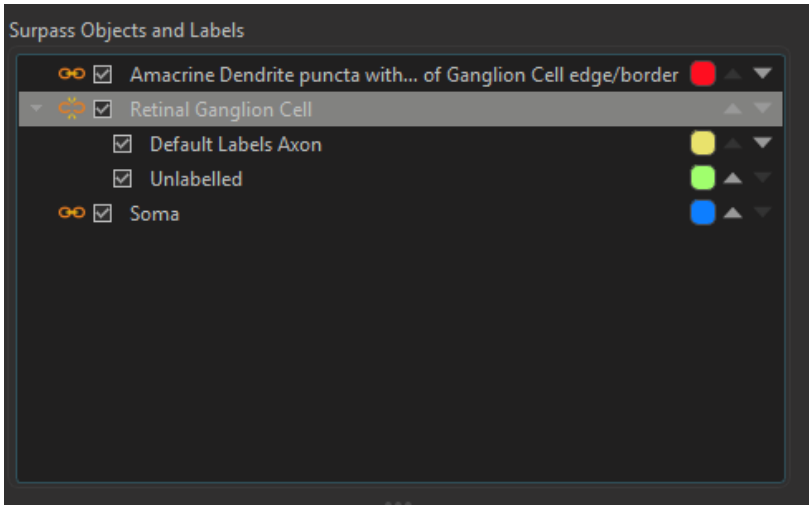
Default style displays the plot according to the settings in Preferences Display, for example, for display on the computer monitor.

Color Print displays the plot in color with a white background for printing on paper.



4.5.1.2 Time View Plot Surpass Objects and Labels

This section displays all Surpass Objects associated with the image, in addition to their labels.



To **view all labels** associated with an object click on the orange link button, to reveal all labels associated with that object.

To **remove objects** or labels from the plot display uncheck the tick box to the left of their name.

To **change the order of objects** plotted use the up and down arrows to change the position of the object or label in the list and the order in the plot display.

To **change the color** of the object or label within the plot click on the colored square icon to bring up the color slider.

4.5.1.3 Time View Plot Property Area

The settings for the data can be modified. These options are grouped together under the following tabs: Settings Tab, Filter Tab and Frame Tab.

Settings Tab

The **Settings Tab** enables you to select the required statistical values from the drop down on the y axis with a time unit on the x axis for analysis. The statistics available for the y axis will depend on the input components e.g. spots, surfaces, cells or filaments.

Use the histogram to select the portion of data shown in the plot display area, drag the yellow vertical line across the histogram to alter the selection.

Use the Setting tab to select display options: Show Quartiles, Show Box Plots and Show "Unassigned" Box Plots.

4.5.1.3.1 Filter Tab

Filter Tab

The **Filter Tab** allows you to select and apply a number of filters. The filters available are specific to the input components selected e.g. spots, surfaces, cells or filaments. Spots for example, includes the filters: by Area, Diameter, Intensity, Position, Time and Volume. Filters can be added and deleted using the Add and Delete options.

You can use the automatic filter setting, or manually adjust the values as required. Use the slider bar, when this is available, to adjust the parameters for the selected filter. The name of the filter displayed will update if you change the filter settings.

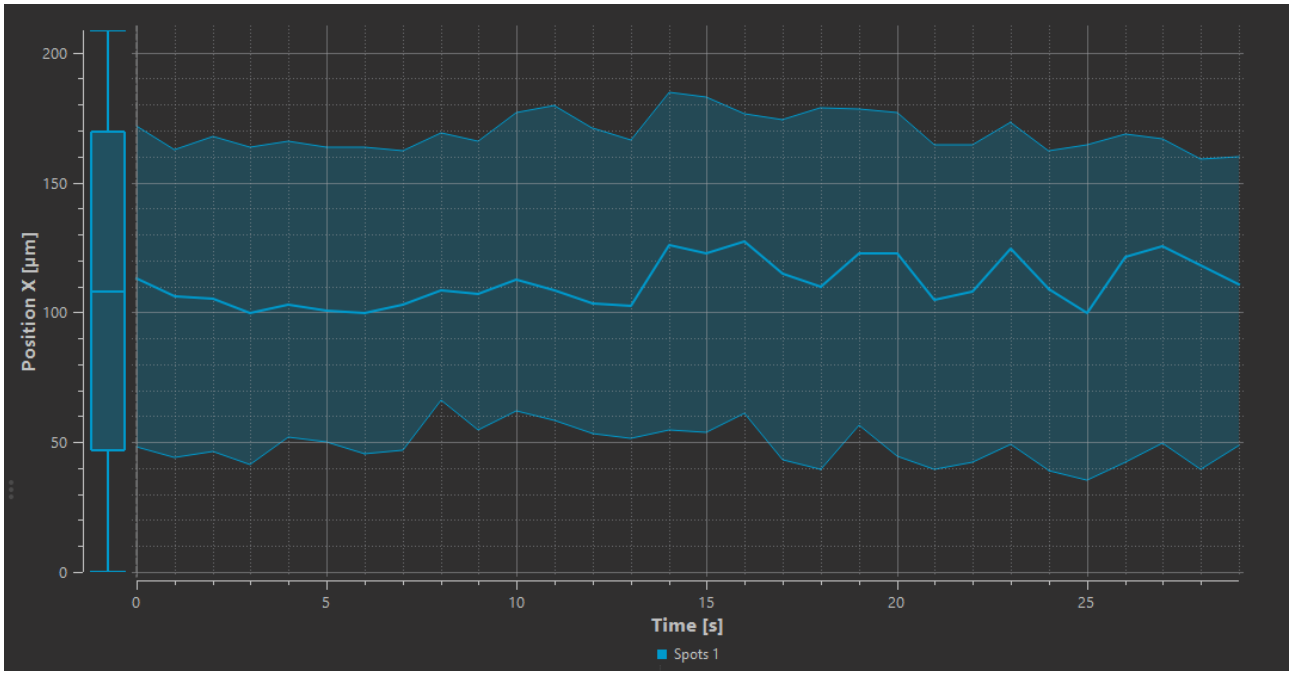
4.5.1.3.2 Frame Tab

Frame Tab

The **Frame Tab** provides you with a range of options to change the appearance and settings used for the displayed plot. This includes the Zoom Direction, Frame Settings, Legend Position, Chart and Box Plot.

4.5.1.4 Time View Plot Display Area

The Plot Display Area shows the plots for your selected data sets. The plot shows median values as well as 25 and 75 percent quartiles over time, these can be removed using the check box in the settings section of the Plot Property Area.



4.5.1.5 Time View Plot Numbers Area

The data displayed in the Plot Display Area are interactively linked with the numerical data shown in the Plot Numbers Area. The numbers in the tables are based on calculated statistical values in the Surpass View.

The Time View Plot Numbers Area features the Detailed and Summary tabs. They are described fully in the Objects View section. Follow this link for more information, Plot Numbers Area.

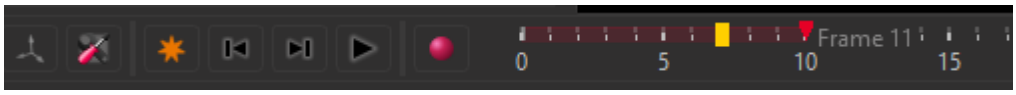
Note that data for each tab can be exported using the button when that tab is highlighted.

4.5.2 Events Concept

Here the concept of implementing events in Imaris is explained. There are three types of event: Image Events, Object Events and Tracking Algorithm Events.

Image Events

An image event can be defined for an entire image (including all objects) at a single time point. To set up image events the time bar of Imaris has an event toggle button  that adds an image event at the current time point. If the current time point has an event the event toggle removes the event from the current time point. Existing image events are rendered on the time axis as yellow rectangles.



Implementation Examples

Use Imaris image events to identify a key moment in an experiment, for example, when a reagent is added or when a photo-stimulation event occurred.

Statistics

Image events calculate event time statistics for all objects in an image so that these event time statistics can be used as the time axis in Vantage Time. Image event statistics are available within the vantage plot view. Statistics for image events have = image event appended at the end of the statistic name. Please read more about Event Statistics on the Event Time Statistics chapter.

Object Events

An Object Event can be defined for an object using classifiers for the entire series of images. There are two ways to create classification based Object Events, via the creation wizard (spots and surfaces only) or events can be manually created via the Edit Events Tab (available for all objects).

Implementation Examples

Use Imaris object events to track events in the sample, for example, cell division.

Statistics

Object events compute statistics for Event time, please read more about Event Statistics on the Event Time Statistics chapter.

Tracking Algorithm Events

Tracking Algorithm Events are computed for object tracks for the entire series of images. Automatically tracked events are created within the creation wizard depending on the algorithm chosen. The Lineage and Connected components and Maximum Overlap (for cells) algorithms will compute the Track Split and Track Merge Event Statistics.

Implementation Examples

Use Imaris Tracking Algorithm Events to understand track merge and track split events in your data series.

Statistics

Object events compute statistics for Event time relating to Track Split and Track Merge Event Statistics.

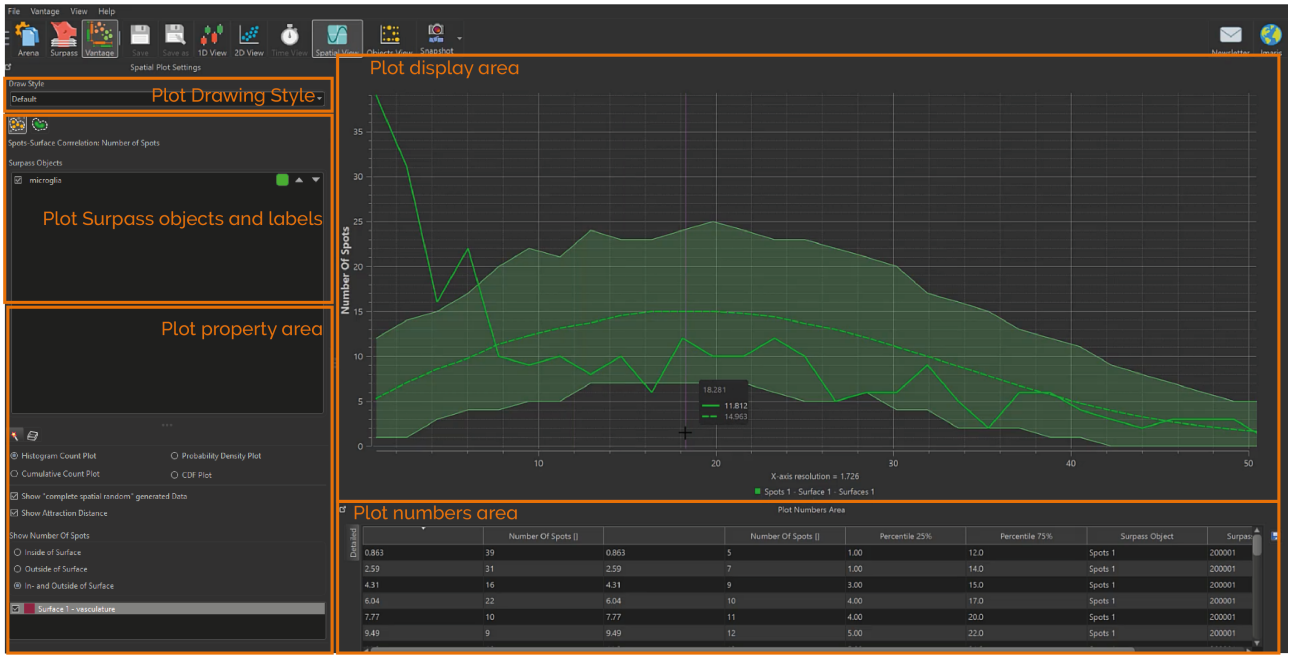
Tracking Algorithm Event statistics have track split and track merge appended to the end of the statistics name. Please read more about Event Statistics on the Event Time Statistics chapter.

4.6 Spatial View

The **Spatial View** allows the user to examine the relationship between spots and surfaces to determine whether the distribution of spots relative to the surface are randomly distributed. Additionally spatial statistics can be used to examine the image intensity with respect to the surface. The following chapters describe the spatial view interface and functionality.

4.6.1 Spatial View User Interface

The Spatial View Interface has the following working areas: Plot Display Area, Plot Drawing Style, Plot Surpass Objects and Labels, Plot Property Area and Plot Numbers Area.



4.6.1.1 Spatial View Plot Drawing Style

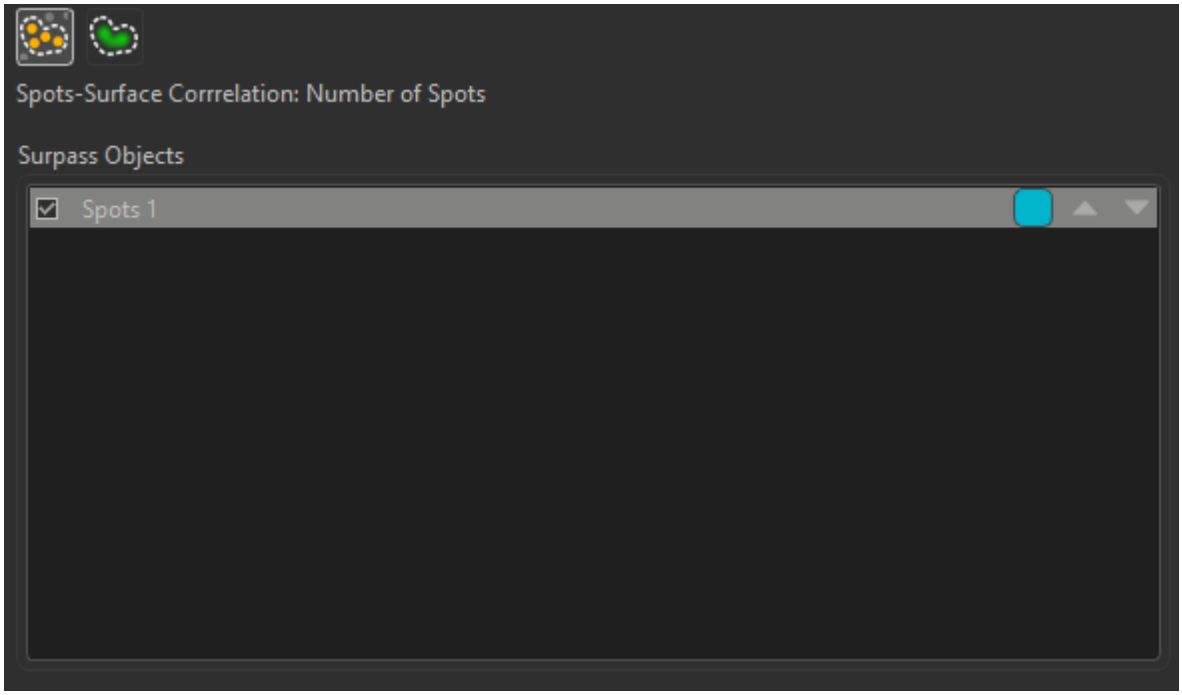
Change the drawing style of the plot from the options available: Default, Print and Color Print.

Default style displays the plot according to the settings in Preferences Display, for example, for display on the computer monitor.

Color Print displays the plot in color with a white background for printing on paper.

4.6.1.2 Spatial View Plot Surpass Object and Labels

This section displays all Surpass Objects associated with the image, in addition to their labels.



In addition the user can choose between **Spots-surface correlation: Number of spots**  and **Surface-Image Correlation: Image Intensity**  .

The **Spots-surface correlation: Number of spots,** allows for the comparison of the distribution of spots with respect to the surface. The **Surface-Image Correlation: Image Intensity,** allows for the comparison of the image intensity with respect to the surface. Please see the Spatial View Concept section for more information on these options.

To **view all labels** associated with an object click on the orange link button, to reveal all labels associated with that object.

To **remove objects** or labels from the plot display uncheck the tick box to the left of their name.

To **change the order of objects** plotted use the up and down arrows to change the position of the object or label in the list and the order in the plot display.

To **change the color** of the object or label within the plot click on the colored square icon to bring up the color slider.

4.6.1.3 Spatial View Plot Property Area

The settings for the plot can be modified. These options are grouped together under the following tabs: Settings Tab and Frame Tab. The **Settings Tab** enables you to select options to analyze spatial statistics data. Different options are available in the settings tab depending on whether **Spots-surface correlation: Number of spots**  or **Surface-Image Correlation: Image Intensity**  is selected. Please see the Spatial View Concept section for more information.

Spots-surface correlation: Number of spots - Settings Tab

Select a plot type:

Histogram Count Plot

The Histogram Count Plot displays the number of spots within a specified distance from the surface. The solid line represents the measured statistical values. The dashed line represents the mean of the simulation of randomly positioned spots relative to the surface, the shaded area represents a 99% confidence interval on the simulated values. The user can observe the distance from the surface that spots occur in greater or lesser numbers than is expected from random positioning.

Probability Density Plot

The probability density plot applies a kernel smoothing width to the data displayed in the histogram plot. The kernel width is displayed beneath the chart in the plot display area.

Cumulative Count Plot

The solid line represents the measured statistical values. The dashed line represents the mean of the simulation of randomly positioned spots relative to the surface, the shaded area represents a 98% confidence interval on the simulated values. Where the observed cumulative function exceeds the simulation it indicates that the observed number of spots within the respective distance to the surface is greater than what is expected from random simulations. Where the observed cumulative function is lower than the simulations it indicates that the observed number of spots within the respective distance to the surface is lower than what is expected from random simulations.

Cumulative Density Function (CDF) Plot

The CDF plot applies a kernel smoothing width to the data displayed in the cumulative count plot. The kernel width is displayed beneath the chart in the plot display area.

Select options using the check boxes below.

Show All Time Points

Show data from all time points on plot.

Show "complete spatial random" generated data

Selecting this option displays the simulated data, the complete spatial random (CSR) values. In the plot display area the dashed line represents the mean of the simulation of randomly positioned spots relative to the surface, the shaded area represents a 98% confidence interval on the simulated values.

Show Attraction Distance

The attraction distance is represented by a red line on the selected plot. It is the distance where the number of spots is equal to the complete spatial random (CSR) value, after being significantly different. There may be multiple attraction distances when the number of spots strongly deviates from the CSR and there are several distances with many more or fewer spots than expected by the CSR.

Select between the following options:

Show number of Spots

Inside of Surface

Show spots that are inside the surface in the plot.

Outside of Surface

Show spots that are outside the surface in the plot.

In- and Outside of Surface

Show spots that are both inside and outside the surface in the plot.

Surface-Image Correlation: Image Intensity - Settings Tab

Show Standard Deviation
Selecting this check box shows the standard deviation on the plot in the Plot Display Area.

Select between the following options:
Show number of Spots
Inside of Surface
Shows in the plot spot distances to the surface for spots that are inside the surface.

Outside of Surface
Shows in the plot spot distances to the surface for spots that are outside the surface.

In- and Outside of Surface
Shows in the plot spot distances to the surface for spots that are both inside and outside the surface.

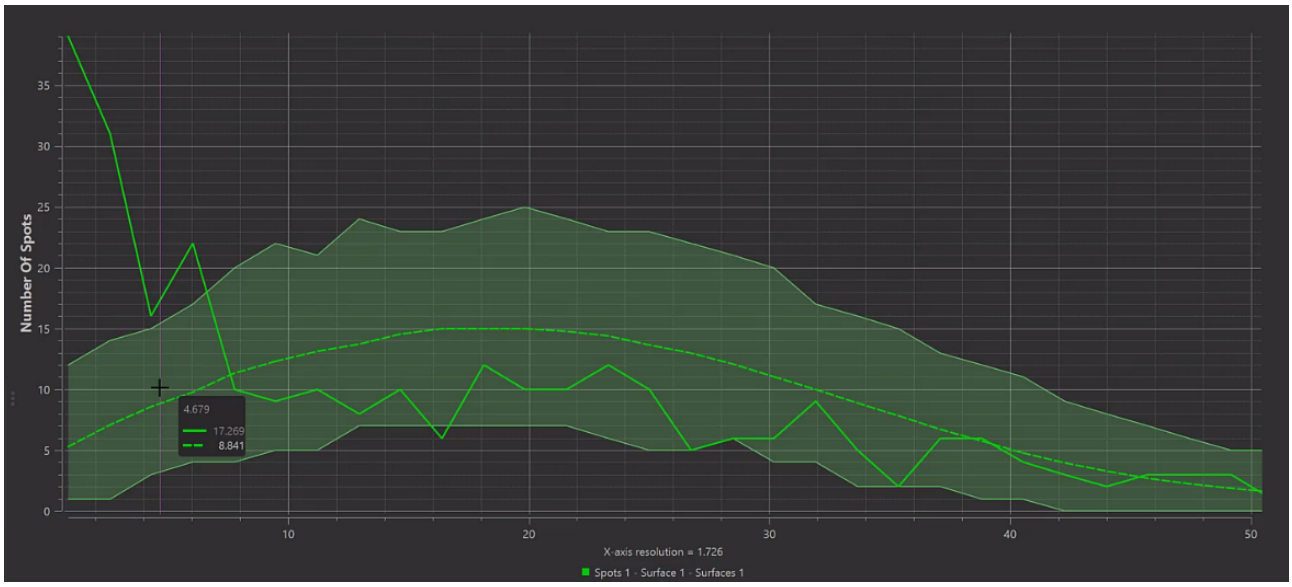
4.6.1.3.1 Frame Tab

Spots-surface correlation: Number of spots and Surface-Image Correlation: Image Intensity - Frame Tab

The **Frame Tab** provides you with a range of options to change the appearance and settings used for the displayed plot. This includes the Zoom Direction, Frame Settings, Legend Position, Chart and Box Plot.

4.6.1.4 Spatial View Plot Display Area

The Plot Display Area shows the plots for selected data sets.



4.6.1.5 Spatial View Plot Numbers Area

The data displayed in the Plot Display Area are interactively linked with the numerical data shown in the Plot Numbers Area. The numbers in the tables are based on calculated statistical values in the Surpass View.

The Spatial View Plot Numbers Area features the Detailed and Summary tabs. They are described fully in the Objects View section. Follow this link for more information, Plot Numbers Area.

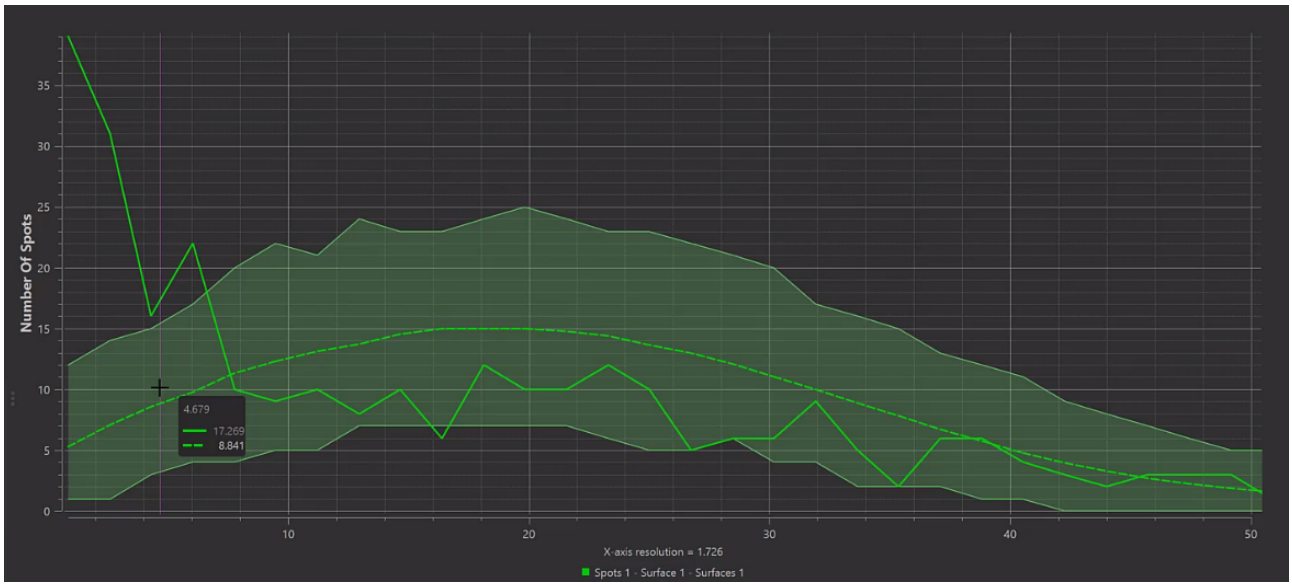
Note that data for each tab can be exported using the button when that tab is highlighted.

4.6.2 Spatial View Concept

In Imaris the spatial view concept allows the user to examine Surface-Spots object relationships and Surface-Image Correlation Intensities.

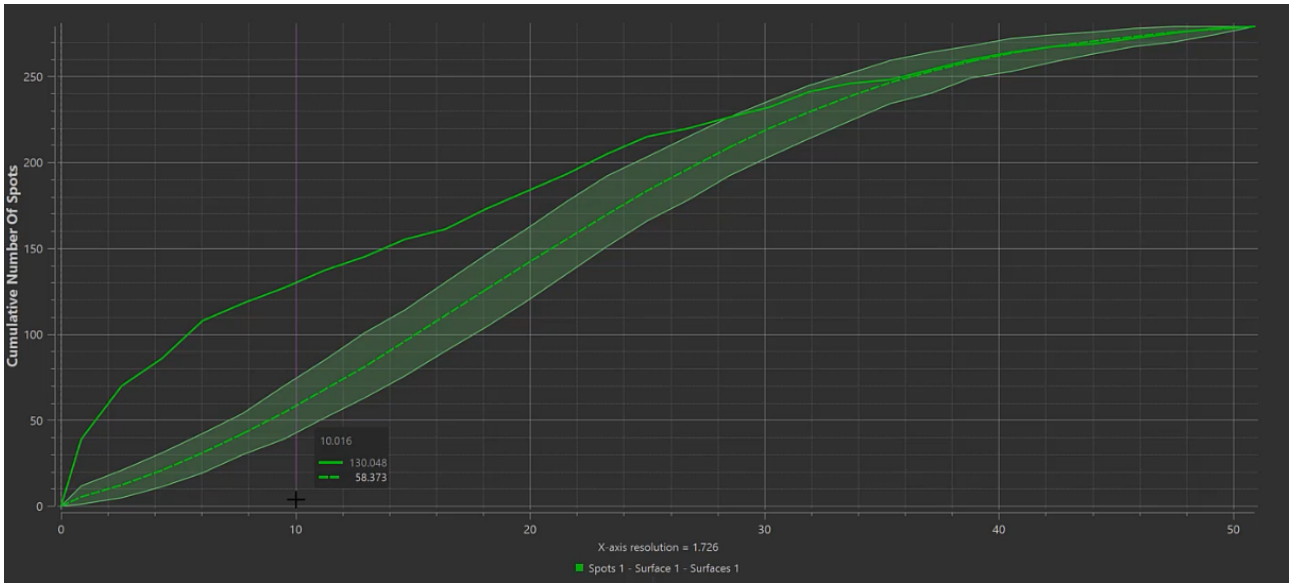
Spots-Surface Correlation: Number of Spots

Spatial statistics are calculated during surfaces and spots object creation. In this analysis the cumulative number of spots within a distance (d) from the surface are measured, in addition to, the number of spots at a distance (d) from the surface. Imaris also simulates randomly positioned spots (the same number as in the image) and generates a confidence band around the expected value of the simulations, facilitating a useful comparison with the measured object statistics. Within Vantage Spatial View, Spatial Statistics are plotted within the Plot Display Area.



Histogram Count Plot (above)

The solid line represents the measured statistical values. The dashed line represents the mean of the simulation of randomly positioned spots relative to the surface, the shaded area represents a 98% confidence interval on the simulated values. In the Histogram Count Plot (above) the user can observe the distance from the surface that spots occur in greater or lesser numbers than is expected from random positioning.



Cumulative Count Plot (above)

The solid line represents the measured statistical values. The dashed line represents the mean of the simulation of randomly positioned spots relative to the surface, the shaded area represents a 98% confidence interval on the simulated values. Where the observed cumulative function exceeds the simulation it indicates that the observed number of spots within the respective distance to the surface is greater than what is expected from random simulations. Where the observed cumulative function is lower than the simulations it indicates that the observed number of spots within the respective distance to the surface is lower than what is expected from random simulations.

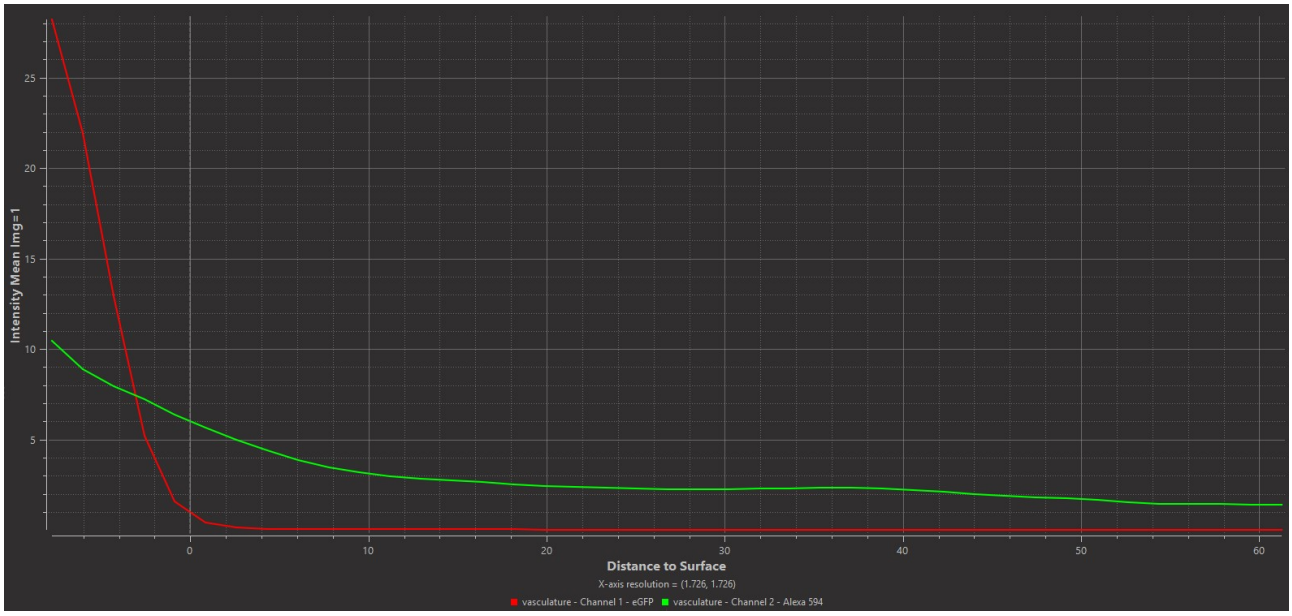
Confidence Band - Simulation Data

The confidence band of the random spots is computed by taking the number of spots in the image and running a thousand simulations to generate the simulated envelope such that it encloses 98% of the simulated values. With 1% of the simulations falling outside of the simulated envelope i.e. 1% of the simulations are above and below the envelope respectively.

The methods employed by Imaris 9.7 are based on well established methods from the field of spatial statistics. In statistical terminology Imaris 9.7 plots the “intensity” of the observed spots as a function of the shortest distance to the surface, Baddeley 2015 chapter 6.6. Randomization envelopes and hypothesis tests are described in Baddeley 2015, chapter 10. The methods for Imaris 9.7 are based on the work and analysis of Gomariz 2018.

Surface-Image Correlation: Image Intensity

Imaris provides a plot showing the average image intensity of each image channel versus distance d from the surface. This plot lets you inspect whether the intensities in some channel change as a function of distance from the surface. Clearly this is expected for the channel from which the surface was computed. Other channels may also exhibit a change of intensity with distance. Spatial statistics are calculated during surface object creation.



Further Reading

[Baddeley 2012] Baddeley, Adrian, Ya-Mei Chang, Yong Song, and Rolf Turner. "Nonparametric estimation of the dependence of a spatial point process on spatial covariates." Statistics and its interface 5, no. 2 (2012): 221-236.

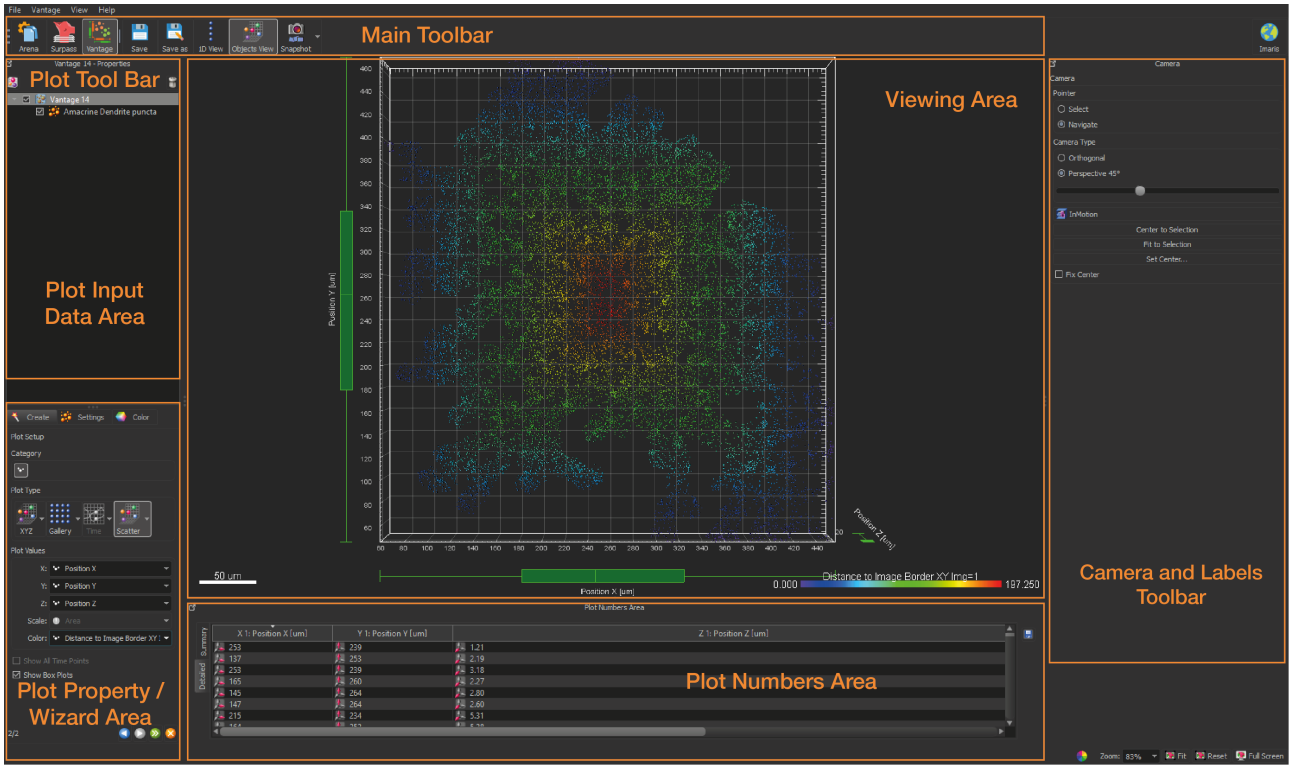
[Baddeley 2015] Baddeley, Adrian, Ege Rubak, and Rolf Turner. Spatial point patterns: methodology and applications with R. CRC press, 2015.

[Gomariz 2018] Gomariz, Alvaro, Patrick M. Helbling, Stephan Isringhausen, Ute Suessbier, Anton Becker, Andreas Boss, Takashi Nagasawa et al. "Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy." Nature communications 9, no. 1 (2018): 1-15.

[Duranton 2005] Duranton, Gilles, and Henry G. Overman. "Testing for localization using micro-geographic data." The Review of Economic Studies 72, no. 4 (2005): 1077-1106.

4.7 Objects View

This Section describes the Vantage user interface for **Objects View**. Objects View consists of five working areas: Plot Tool Bar, Plot Input Data Area, Plot Property Area, Plot Display Area and Plot Numbers Area.



Follow these steps to start Imaris Vantage:

1. Create object(s) in the Surpass tree.
2. In the Toolbars, click on the Imaris Vantage icon.
3. Imaris Vantage is instantly started.
4. Imaris automatically creates a Vantage plot at the beginning of a Vantage session.

4.7.1 Plot Tool Bar

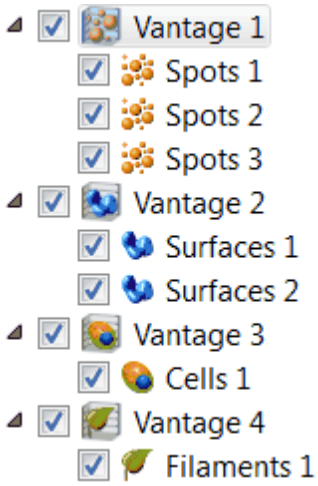
Use the Plot Tool Bar to add or remove plots from Vantage Object View.
The Plot Tool Bar contains two icons:

Imaris Vantage  **Delete** 

- To create a new plot click on the Imaris Vantage icon and follow the Plot Creation Wizard.
- Click an Imaris Vantage icon to instantly generate a new entry in the Plot Input Data Area.
- To remove plots from Imaris Vantage click on the delete icon.

4.7.2 Plot Input Data Area

The Plot Input Data Area in Objects View displays a tree-like list of all created Vantage plots.
Each Vantage plot is organized in a tree-structured hierarchy. At the top of the hierarchy is the Vantage plot and all source objects are listed below.



If in the first step of Plot Creation Wizard the selection of a source object changes, the tree is interactively updated.

Vantage plot symbol reflects the type of the selected source object type. Each element of a Vantage Plot tree has a check-box. Selecting or de-selecting the box next to the plot/object name controls its visibility in the Plot Main Display Area.
The currently active plot/object is selected and highlighted. A set of its adjustable parameters is displayed in Plot Property Area. The selection of the adjustable properties differs depending on the selected tree item; Vantage plot or source object.

For a source object, only tabs that control how objects are individually displayed are available. These are: Settings and Color.
For a Vantage Plot the following tabs are available: Settings, Creation, View, Edit, Frame and Color.
Changing properties for a Vantage Plot, changes also the corresponding properties for all objects in the tree.
To add a new Imaris Vantage plot click on the Imaris Vantage icon within the Plot Tool Bar. Then in the first step of the creation wizard, select an object category and source data. A new Vantage plot is updated and a name and symbol are automatically generated.

Please note: Imaris automatically creates a Vantage plot at the beginning of a Vantage session.
Please note: In the Imaris Vantage, only one object Category can be chosen.

4.7.3 Plot Property Area

In the Properties Area you can create and modify Imaris Vantage plots.
In the Properties Area you can find the Imaris Vantage Plot Creation Wizard and all available parameters for the selected Imaris Vantage object.
The parameters are grouped under different Parameter Tabs:
Setting, Rebuild, Edit, Frame and Color tab.

4.7.4 Plot Display Area

The Plot Display Area shows a variety of Imaris Vantage interactive plots that can be used to provide the best view of the data.
Plots are highly interactive, allowing you to interact with the data in a variety of ways. As in the Surpass View, in the Plot Display Area you can select the object or group of objects by setting the Pointer mode to the Select option and clicking on the object.
Selecting the object in the Plot Display Area highlights the corresponding row in the Plot Numbers Area table. For multiple selections press Ctrl+click (Mac:Command-click).

Zoom and Panning features are available in all created Imaris Vantage plots.
In addition, the Plot Main Display Area can accurately arrange multiple plots allowing different types of visualizations simultaneously.

4.7.5 Plot Numbers Area

The data displayed in the Plot Display Area are interactively linked with the numerical data shown in the Plot Numbers Area. The numbers in the tables are based on calculated statistical values in the Surpass View.

Please Note: If the required statistic variable is not listed as an option, you will need to enable it. To do so go under the Edit menu/Preferences click on the Statistics option.
There you will find all available statistics arranged by category. Only if the box next to a statistical variable is checked, the variable is enabled, it will be calculated. The statistical variable values will appear both under the Statistics tab in the Surpass view and within the Vantage.

In the Plot Numbers' Area two table types are presented: **Detailed** and **Summary**

Detailed

This table contains all statistical variables values used for the plot creation.
The first row contains displays the plot dimension icons, variable name and unit, followed by the relevant object information such as ID, Parent number, Labels and Surpass Object name.
In the corresponding columns are the values of the statistical variables.

A click on any individual row of the table automatically highlights the corresponding object in the Imaris Vantage plot and in the Surpass view. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Summary

This table is available only if show the Show Box Plots option is selected.
The Summary table contains values determined on the basis of all values within a set of the selected variable. The table displays a five-number statistical summary of variables assigned to the plot dimensions.
The first column contains the most important percentiles:

1. Min - the smallest value within a set of values of the selected variable
2. the lower quartile or first quartile-Q1
3. the median - the middle value of the sorted set of values of the selected variable
4. the upper quartile or third quartile-Q3
5. Max - the largest value within a set of values of the selected variable

In the first row the plot dimension icons, variable name unit and Surpass Object name are listed.
The numerical data displayed in the Summary table are represented graphically within the Plot Display Area using a box plot.

In the case that multiple variables assigned to the same plot dimension (see Edit tab) the table columns sorting is bases on the following order: X dimensions (X1 primary, X2 secondary, X3 tertiary), all Y (Y1, Y2, Y3), Z dimension (Z1, Z2, Z3), Scale and Color dimension.

To sort the columns click on the arrow in the column heading. Click again to reverse the sorting.

Exporting Data

Export Button- Export All Statistics to File.
By selecting the Export icon , all of the data that appears in the selected table will be exported.
A click on the Export icon opens the Export Statistics window.
To export data select the file name and the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm).
If you select MS Excel as file type, the program starts automatically.

4.8 Creation Wizard

A Plot Creation Wizard is a component of the **Plot Property Area** that guides you through a sequence of plot creating dialogs. It leads you step by step through the process of selecting object categories, source data, plot type and the various dimension options.
To create a plot, you must first create a Surpass object. The object statistical data will be used for the plot creation.

Please Note: If the required statistic variable is not listed as an option, you will need to enable it. To do so go under the Edit menu/Preferences click on the Statistics option.
There you will find all available statistics arranged by category. Only if the box next to a statistical variable is checked, the variable is enabled, it will be calculated. The statistical variable values will appear both under the Statistics tab in the Surpass view and within the Vantage.

The statistical values of the object Spots, Surfaces, Cells, and Filaments could be used to create the Vantage plots.

To start Imaris Vantage creation wizard follow these steps:

1. Create and select the object in the Surpass tree

2. In the Toolbars, click on the Imaris Vantage icon.

3. Imaris Vantage is instantly started.

The wizard shows a list of all available input components for Imaris Vantage plots.
Select one of the input components from the list (Spots , Surfaces , Cells , or Filaments).

In the next step of the Creation Wizard, specify the plot type that you want to use.
Then assign the selected statistical variables to the plot dimensions.
Click on the Finish button and the Vantage plot is displayed in the viewing area.

To insert a new Imaris Vantage object, click the Imaris Vantage icon to instantly generate a new Plot Creation Wizard.
Please note that only the currently selected component is shown in the plot Main Display Area.

4.8.1 Source Data

All statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Vantage there are the following categories: Spots, Surfaces, Cells and Filaments. The category buttons allow you to choose the object type for the Vantage plot creation. The category icon is active as long as surpass component is created.
Clicking on the Category button selects the corresponding category. According to the selection, all available source objects data are listed.
Based on your selection, the Vantage tree in the Plot Input Data area displays a list of all source data you added.

To remove an object data from the plot creation wizard, deselect the checkbox next to the object name or use the delete button.

Please note: If multiple objects are selected in the first step of the creation wizard, the Gallery plot type is not active in the next step of Creation wizard.
To insert a new Imaris Vantage plot, click the Imaris Vantage icon.

Load Plots Settings

This option lets you use identical creation parameters for multiple plots.
The option Load Plots Settings is only available if the set of the plot's creation parameters has already been saved using the Remember Parameters button under the Rebuild Tab.
When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all the settings, options, and parameters required for plot creation.
After selecting the Load Plots Settings option, the Load Parameters drop-down list appears, Expand the list by clicking on it. The list shows all the creation parameters sets that have been saved previously, according to the chosen object. Simply load your chosen parameters set, by clicking on it, and run the wizard as you have preset, without again defining all its possible options.
Another option is to customize each creation step to meet your specific needs by modifying some parts of the wizard independently - e.g. the same plot type, with a different plot value selection.

4.8.2 Plot Setup

A Category identifies, gathers and summarizes the set of statistical values' calculations of the particular Object Type. Statistical values associated with one particular object type are grouped together in one category. Each Category has its own button, which graphically indicates the Category type.
In Imaris there are following Filter Categories: **Surface, Surface Tracks, Spots, Spots Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Segment, Soma, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks**.
These buttons allow you to choose the Category and determine the list of displayed statistical variables. By clicking one of these buttons the corresponding category is selected and the list of statistical variables will be displayed accordingly.

Please note: In Imaris Vantage, only **one** Object Type Category can be chosen.

Plot Type

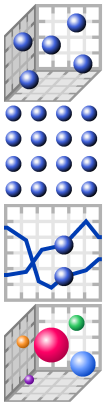
Select the plot type you want to create from the plot type's icons.

XYZ

Gallery

Time Plots

Scatter Plots



To view all options of the selected plot type click on the plot type icon and view the available plot subtypes.
To select a plot type, click the plot type, and then click on the plot subtype that you want.

By XYZ, Time and Scatter Plots each plot dimension represents one selected statistical variable. By default, these plot types could show up to five statistical variables.
The choices of the available statistical variables for the selected object appear in a drop-down list.
To assign it to the selected Plot Value, select one statistical variable from the list.

Imaris Vantage plots allow you to select the same component for multiple plots.

Plot Values

Display the statistical variables' name currently being assigned to the chosen plot dimension.
XYZ, Time and Scatter Plots show five options for the plot dimensions: **X**, **Y**, **Z**, **Scale** and **Color**.
Gallery plots, a selected statistical value is used to sort the objects. The values specified in the column or row fields, define the number of panels to be displayed. In addition, Scale and/or Color could be chosen as a plot dimensions.
The statistical variables for the Vantage can be chosen from the drop-down list. Click on the list and it offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and then the selected statistical variables will be assigned to the chosen plot dimension.
The exclamation mark sign indicates that the statistics value is the same for all objects.

Please note: When creating a plot in the creation wizard, only one variable can be assigned to the chosen plot dimension. However, when a plot creation wizard is completed, under the Edit tab, multiple variables could be assigned to X, Y and Z plot dimensions.

X, Y and Z

Selecting the statistical variable for the X, Y and Z Plot Values define the three-dimensional space of the Vantage plots. The position of the objects within the plot depends on the values of the variables assigned to the X, Y and Z.
The Z-dimension initially points towards the viewer, outward from the screen.
For each Plot type, you can optionally provide an additional statistical variable to individually specify the objects size, color or a combination of these features.

Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale

dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.
Initially all the object dimensions are normalized and then the values of the Scale statistical variable is assigned to the object. The lowest value of the selected statistical variable correspond to 0.25 of the normalized object size, while the highest value of the selected variable is mapped with the 1.25 of the normalized object size. The new values are mapped, providing a new dimension for each object, without altering object shape parameters.

Please Note: Assigning the statistical variable for the Scale plot dimension is only appropriate when the size of the created object doesn't play a biological role.

Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot.
This scalar information of the selected variable is converted into colors using the colormap. The first color of the selected color map is set to the lowest value of the selected statistical variable, while the highest value of the selected variable is mapped with the last color.

Show All Time Points

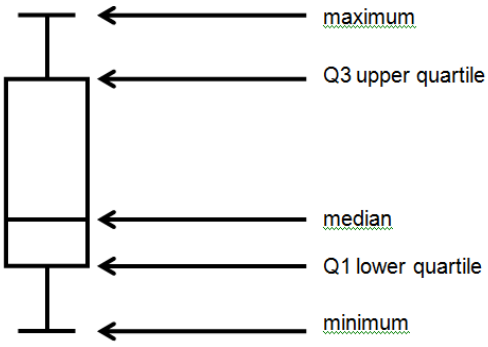
Select this box and the created objects are shown for all time points.

Show Box Plots

Select this box and in the viewing area, the box plots are displayed for the selected plot dimensions.
In addition, in the Summary table in the Plot Numbers' Area, all statistical summary numbers are show for all plot dimensions.
Under the Edit tab, you can modify the display of the summary statistical values for individual plot dimension.

4.8.2.1 Box Plot

A box plot summarizes the data of the selected statistical variable to only five numbers. It is a convenient way to illustrate graphically large group of statistical data through their summaries: the minimum, lower quartile (Q1), median, upper quartile (Q3), and maximum.



The central box represents the values from the lower Q1 to upper quartiles Q3 quartile (25 to 75 percentile respectively).
The line within the box is indicates the median value. A straight lines extending from the ends of the box represent the maximum and minimum values.
In the viewing area box plots can be drawn in all 3 plot dimensions for multiple variables.
If Vantage plot is composed of multiple objects, in the viewing area a separate box for each data set is created.

Box Plot is a useful tool offering several benefits to help you improve understanding of the data:

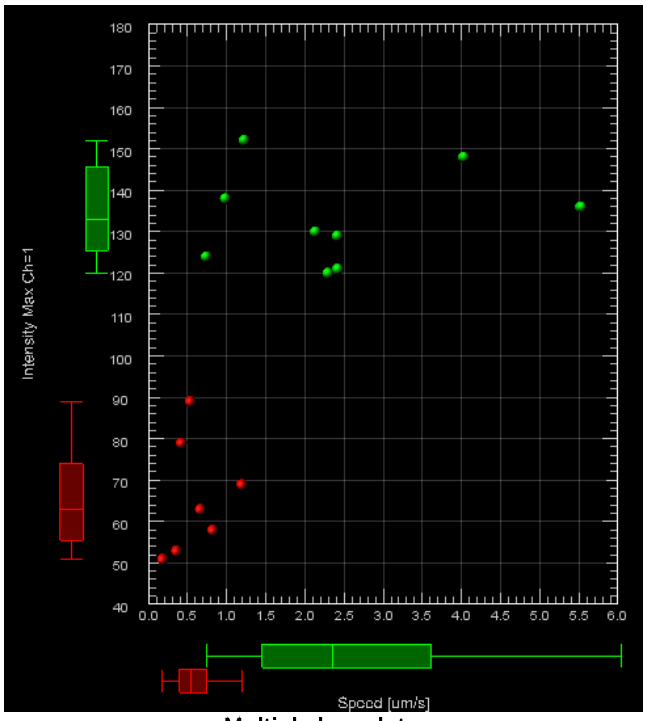
1. A quick visual summary that easily handles extremely large amount of data and shows range, spread and median value of the selected variable values.

2. The distance of the whiskers provides the distribution of the values

3. The space between the different parts of the box indicates the degree of distribution and skewness in the data

4. The position of the median inside the box indicates whether there are more values towards the upper or lower quartile

One of the most important benefits of plot boxes is the possibility to compare more than one data set.
Multiple box plots are drawn side by side providing an efficient method to examine value distributions and highlight differences in variability between several data sets.
The Color of the box plot refers to the color of the Surpass object used as a source data.

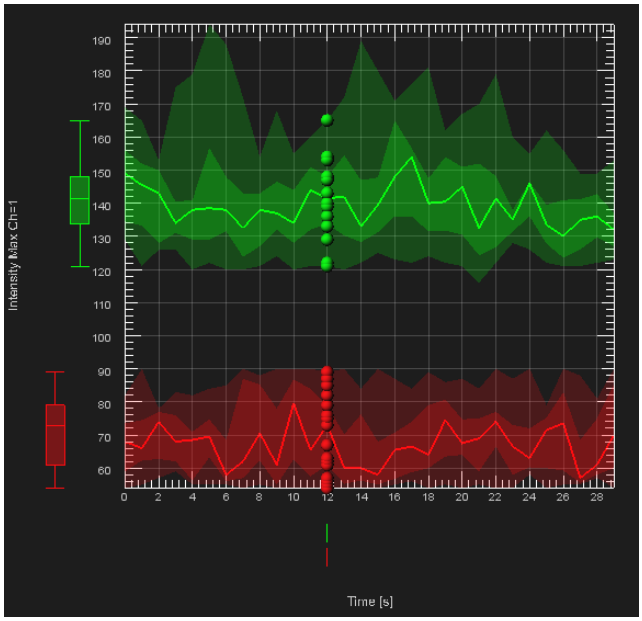


Multiple box plots

Time and box plots

In the case that the Time plot type is selected, the box plots represent a moving statistical summary. The box plot position and shape changes at each time point.
In addition, in the viewing area, 2D summary projection XY, and/or XZ plots show the values of the statistical five-number summary for all time points. The two dimensional plane has the same characteristics as the corresponding box plots and the color of the projections plots correspond to the plot box color.
The summary values of the selected variable are plotted as colored regions and line.
On the projection plot, a tick line indicates where the median value lies. The range between the Q1 and Q3 values is less intensive shade, while the lightly colored area labels the minimum respective the maximum range.

The summary projection plots are especially useful for multiple data sets. You can easily compare how two or more variables change across time based on the line position and size of the regions.



Time box plots

4.8.2.2 XYZ Plot Type

A XYZ plot is used to show the spatial relationship between the selected statistical variables.In XYZ plot type the X, Y and Z Plot Values are predetermined variables, fixed and assigned to the object X, Y and Z position. To uncover other structural connections, an additional statistical variable could be assigned as a Plot value.
To add another dimension to the plot, a fourth or fifth variable can be correlated to the color or size of the plotted object, or a combination of these features.

Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

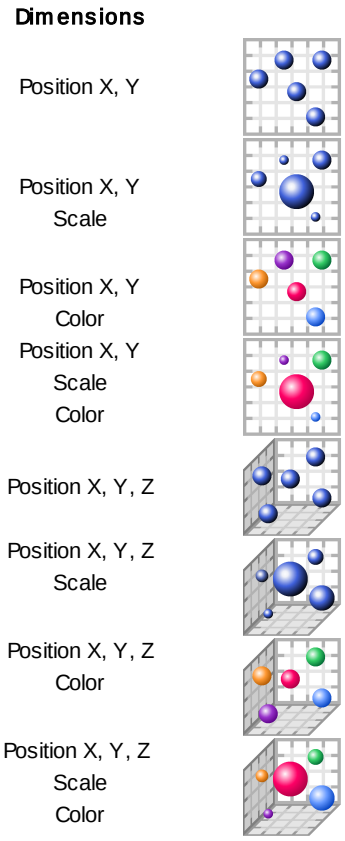
Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

Plot Dimension

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. This list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.
The exclamation mark sign indicates that the statistics value is the same for all objects.
The following XYZ plot types are available:

Assigned Plot	Icons



Please Note: The statistic variable must be enabled under the Edit menu/ Preferences/Statistics options, otherwise it is not listed in the Plot Dimensions.

4.8.2.3 Gallery Plot Type

Gallery plot creates a systematic panel of row and columns. Each individual pane in a Gallery plot displays an object.
In each panel, an object is placed according to the sorted values of the selected statistical variable.

Plot Dimension

Sort by
To select the statistical variable for the Gallery dimensions go under the Plot Values and then click on the Sort by list. The list offers a set of all available statistical variables for the selected object. To select a statistical variable, that can be used to order objects in the Gallery plot, click on its name.
The exclamation mark sign indicates that the statistics value is the same for all objects.
The selected variable value is used to arrange the objects into an ascending/descending order.

The position of the object in the Gallery panel, corresponds directly to the ordered value of the selected variable.
If sorting is done in ascending order , the object with the largest value of the selected variable is positioned in the bottom left corner of the Gallery panel.
The object with the lowest value is positioned in the top right corner of the Gallery panel.
If sorting is done in descending order , the position of the objects within the Gallery plot is reversed.

Columns and Rows
The Gallery plot type assigns vertical and horizontal dimensions to the predetermined variables, which are then

fixed and assigned to the number of Gallery columns and rows. The number of rows and columns defines the Gallery panel size.
The values entered in the column or row fields specify the number of panels to be displayed.

Please note: If the number of objects is higher than the total number of Gallery panes, a subset of the original objects is displayed and some of the objects are automatically skipped.

To add another dimension to the plot, a variable can be correlated to the color or size of the plotted object, or a combination of these features.

Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.
The following Gallery plot types are available:

Assigned Plot Dimensions	Icon
X	
X Scale	
X Color	
X Scale Color	

Please Note: The statistic variable must be enabled under the Edit menu/ Preferences/Statistics options, otherwise it will not be listed in the Plot Dimensions.

4.8.2.4 Time Plots Type

A Time Plot is used to show the time dependency between the selected statistical variables. In Time plot, the X Plot dimension is a predetermined variable, which is fixed and assigned to the time. The successive time intervals are uniformly spaced. Y and Z Plot dimensions could be assigned to any available statistical variable.
The position of the object within the plot depends on the values of the variables selected for the Y and Z dimensions.
To add another dimension to the plot, a variable can be correlated to the color or size of the plotted object, or a combination of these features.

Scale

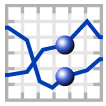
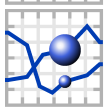
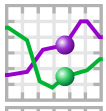
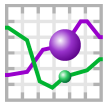
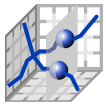
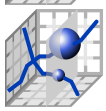
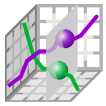
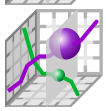
If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

Plot Dimension

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. This list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.
The exclamation mark sign indicates that the statistics value is the same for all objects.
The following Time plot types are available:

Assigned Plot Dimensions	Icon
Time Y	
Time Y Scale	
Time Y Color	
Time Y Scale Color	
Time Y, Z	
Time Y, Z Scale	
Time Y, Z Color	
Time Y, Z Scale Color	

Tip: The statistic variable must be enabled under the Edit menu/ Preferences/Statistics options, otherwise it is not listed in the Plot Dimensions.

To interact with the plot and to go through all the time points click on the play/pause button. Each successive plot shows the data for the next time point.

4.8.2.5 Scatter Plots Type

Scatter plots can show five statistical variables: X, Y, Z, Color, Scale.
Within the Scatter Plot, an object's position depends on the values assigned to the X, Y and Z dimensions.
To add another dimension to the plot, a fourth or fifth variable can be correlated to the color or size of the plotted object, or a combination of these features.

Scale

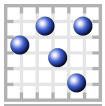
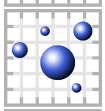
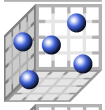
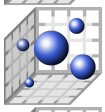
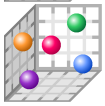
If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

Plot Dimension

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. The list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.
The exclamation mark sign indicates that the statistics value is the same for all objects.
The following Scatter plot types are available:

Assigned Plot Dimensions	Icons
X,Y	
X, Y Scale	
X, Y Color	
X, Y Scale Color	
X, Y, Z	
X, Y, Z Scale	
X, Y, Z Color	

X, Y, Z
Scale
Color



Tip: The statistic variable must be enabled under the Edit menu/ Preferences/Statistics options, otherwise it is not listed in the Plot Dimensions.

4.9 Settings Tab

Depending on the object selection the content under the Settings tab differs.
Please click the appropriate link given below:
Cells
Filaments
Spots
Surfaces

4.10 Rebuild Tab

The rebuild option is useful to store your own customized plots' parameters and re-use them. It also enables you to share the plot creation parameters with other people, allowing them to use an identical set of parameters to perform their own analysis and have them perform their own analysis by using identical set of parameters.

Imaris stores the list of plot creation parameters. Select from the list, which step of the Plot Creation Wizard you want to re-enter.
Source Data
Plot Setup

Rebuild Button
Clicking on this button re-starts the wizard.

Creation Parameters

The Creation Wizard saves parameters so that the Imaris Vantage plot creation can be repeated, and the process of re-creating complex yet standardized plots may be avoided.
The Creation Parameters are listed in the table.

Refer to Store Creation parameters

Preference

When you save the Creation Parameter file, it is automatically added to the list that is displayed under the Edit/ Preference/Vantage Plots

4.11 Store Creation Parameters

Store Parameters Button
The Store Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list. Storing the parameters allows them to be easily reused, modified, or exchanged between users. Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the appropriate plot creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the

Edit/Preference/Creation Parameters.

Clicking on the Remember Parameters Button opens the Store Creation Parameter window.

Store Creation Parameter Window

Enter a name for the Creation Parameter set. This name then identifies the Creation Parameter item, in the Creation Parameter list, used in the Edit/Preference/Creation Parameters and would be used as default name in the subsequent creation wizard algorithms.
Save all of the attributes of your favorite graph style under the Rebuild tab. Add greater speed and efficiency to your analysis by quickly recalling an existing graph type, and then apply this style to your current data set

4.12 View Tab

Under the View Tab you can determine the relative size of the objects, their orientation and their position within the Plots.

-/ + Button
Selecting this button changes the objects size within the View Area by simultaneously changing the Scale values for all X,Y and Z plot dimensions.
Scaling is measured in percent, starting from 100% as the initial object size.

Plus Button

The size of the object is **increased** by 10% every time the **Plus** button is pressed.

Also, every time the **Plus** button is clicked, under the Edit tab, the number in the **Scaling** field for the X, Y and Z plot dimensions is **reduced** by 10%.

Minus Button

The size of the object is **reduced** by 10% every time the **Minus** button is pressed.

Also, every time the **Minus** button is clicked, under the Edit tab, the number in the **Scaling** field for the X, Y and Z plot dimensions is **increased** by 10%.

Scale Objects to Same Size

Select this button to map all objects with equal size distribution.
All objects within the plot are scaled to the largest object

Orientation Horizontal /Vertical slider

To rotate the objects horizontally/vertically and change the object orientation use the sliders.

Align Objects

Select this box to align objects relative to each other.
The objects are aligned along a horizontal or vertical line which represents their original positions.

Reset Orientation button

Select this button to set an original orientation of the objects.

4.13 Edit Tab

The Edit Tab offers the flexibility to customize every detail of your plot appearance by interactively modifying the various plot elements. It gives you independent control of plot dimensions.
Under the Edit Tab, you can adjust the following characteristics of a plot: modify the number and type of the assigned statistical variable to the plot dimension, change the position and scale of the objects, define the objects' binning, alter limits and appearance of the plot dimension scale, and add the annotations' labels and text.

Categories

A Category identifies, gathers and summarizes the set of statistical values' calculations for that particular Object Type. Statistical values associated with one particular object type are grouped together in one category. Each Category has its own button, which graphically indicates the Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determine the list of displayed statistical variables. By clicking one of these buttons the corresponding category is selected and the list of statistical variables will be displayed accordingly.

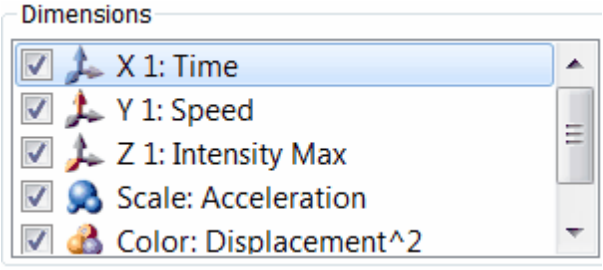
Please note: In Imaris Vantage, only one Object Type Category can be chosen.

Depending on the selected Plot type the content under the Edit tab differs.
XYZ
Gallery
Time Plot
Scatter Plot

4.13.1 XYZ, Time and Scatter Plots

Dimensions

The Edit tab gives you independent control of plot dimensions.
Within the Dimensions window, a list displays the checkbox, icon and assigned variable name for all plot dimensions.



In the list dimensions appear in the following order: X1, X2, X3, Y1, Y2, Y3, Z1, Z2, Z3, Scale and Color.

Selecting or de-selecting the box next to the plot dimension controls its visibility in the viewing area.

The following table gives an overview of the plot dimensions icons:

Plot Dimension	Icon
X	
Y	
Z	
Scale	
Color	

- To adjust the appearance of the plot you can change the various aspects of the plot dimension.
- To modify variables assigned to plot dimensions you first have to select the plot dimension you want to change. Then from the variable list, select a new statistical variable that you want to be mapped.
- To modify the numbers of plot dimensions within the plot, click on the **Add** and/or **Delete** button. By using multiple variables assigned to the plot dimension, more than one criterion can be specified for the data analyses.



Add Button

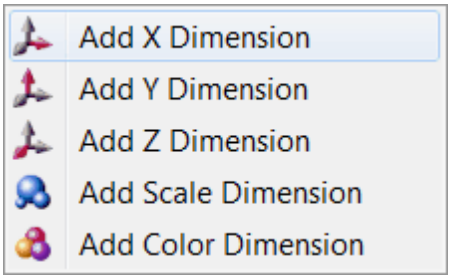


Delete Button

- The statistical variable can be chosen from the drop-down list. The list offers a set of all enabled statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected statistical variable is assigned to the chosen plot dimension.
- The Plot Numerical Table interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new set of values are displayed.

Add Button

To add a plot dimension, click on the Add button.
From the drop-down list, choose a plot dimension.



Then from the variable list, select a new statistical variable that you want to be assigned to this newly created dimension.
The new dimension appears in the Dimensions list.
When the additional plot dimension is added, the plot is re-drawn and a different set of objects is displayed.
Please note: Vantage plot can have only one Scale and Color plot dimension. If a statistical variable is already assigned to Scale or Color plot dimension, the Scale or Color option under the Add button are disabled.
In order to allow a comparison of trends between several variables placed in one plot dimension all axes variables defining the same plot dimension are mutually parallel.
Please note: From a practical concern, it is most likely very difficult to interpret a graph with more than 3 X, Y, and Z axis and therefore Imaris restricts the number of variables assigned to one plot dimension to three.

Delete Button
To remove an unwanted plot dimension, select it and click the Delete button. The selected plot dimension is deleted from the plot.

Plot

The statistical variable can be chosen from the drop-down list. The list offers a set of all enabled statistical variables for the selected object type. To select a particular statistical variable, click on its name in the list and then this selected statistical variable is assigned to the chosen plot dimension.
To remove the assigned plot dimension from the viewing area, deselect the checkbox next to the plot dimension name.
The Plot Number Area interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new sets of values are displayed.

Scale

A scale function specifies how the scale of each plot dimension should be determined from the value of the selected statistical variable.
As the values of the selected variable may be of different magnitudes, during the plot creation, the offset and scaling values are automatically calculated based on each of the selected variables values.
After automatic scaling, the ranges appear to be the same for all of the objects currently being visualized.
The Scale value provides you with options to adapt how the plot dimensions are scaled.

To change the scaling of the selected plot dimension and enter a number in the Scale field.
The entered Scale value multiplies the initially calculated values of the selected plot dimension by the value entered in the Scale field.

Offset

This parameter is used to reposition the objects from their original position within the plots.
Offsetting objects is useful when you have more than one object in the same position and you want to evenly distribute the objects in a particular direction. For example, if you have many objects that you want to shift horizontally in a straight line, you can specify the value, in increments, in the Offset field for the selected X dimension.
The offset values change the object's originally assigned value. This new value is calculated by adding the entered offset value to the value of the selected statistical variable.
The offset value can be positive or negative.
For the X plot dimension, positive offset numbers move the objects to the right, and negative numbers move

them to the left. For the Y plot dimension, positive offset numbers move the objects up , and negative numbers move them down.
The offset value is given in the same units as the selected statistical variable assigned to the selected plot dimension.


Clicking this arrow sets the Scale and Offset back to the original values.

Limit Min Limit Max

During plot creation, the range is automatically adjusted to the actual lowest and highest value of the selected variable.
Min: This is the smallest value of the selected variable to be displayed.
Max: This is the largest value of the selected variable to be displayed.

It is possible to change the Max limit and/or Min limit of the selected plot dimension by entering the values in the Limit Min /Max fields.
Enter a value to the Limit Min /Max to specify the new minimum/maximum values for the selected plot dimension.


Clicking this arrow sets the Limit Min /Max back to the original values.

Bin Size

Binning allows you to sort your data into different groups or bins. The Binning size value divides a plot into a specific number of groups and specifies the new locations of the objects by distributing them into the different bins according to the value of the selected variable.

Gamma

The Gamma function allows you to individually manipulate the appearance of the range of the selected variable. The default value of the Gamma is 1, indicating that the range between the lowest and highest value of the selected variable is extrapolated in a linear mode to the full range of the data set.
If the entered value is different than 1, the linear mode is transferred to a nonlinear mode.
Entering the Gamma values higher than 1, will result in a plot in which the range of the object with the lower values will appear to increase relative to the range of the object with higher values.
Alternatively, entering the Gamma values smaller than 1, will result in a plot in which the range of the object with higher values will appear to increase relative to the range of the object with lower values.
The change is directly visible in the viewing area.

Clamp Limits

If this box is selected, the object outside the plots limits (determined by the frame and range settings) will be repositioned within the limits.
The object with the higher value will be assigned the max value of the selected variable, while the object with the lower value will be assigned the minimum value.

Show Box Plots

Select this box and in the viewing area, the box plots are displayed for the selected plot dimension.

Auto Adjust

Click on this button and the parameters under the Edit tab are returned to the original values.

Load Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

Show All Time Points

Select this box and the created objects are shown for all time points.

4.13.2 Gallery

Sort by:

To modify the statistical variable for the Gallery dimensions go to Plot Values and then click on the Sort by list.


The icons indicates the sort order in the Gallery, ascending or descending order

Cell Columns /Row

To readjust the numbers of panels to be displayed in the Gallery enter the values in the column or row fields.

Skip

The entered value defines the number of displayed objects by defining the skip interval. To display every second object set the value to 1, or to display every third object set the value to 2.

Offset

Adjusting the Offset value is useful when the number of objects is higher than the total number of Gallery panes.
The offset value specifies the first object to be placed in the Gallery. To display first object, set the offset value to 0. To start with the 10^{th} ordered object, set the value to 10.

The Skip and Offset value regulate and limit the Gallery display to a certain number of the displayed objects. In combination, these two values define the subset of the originally ordered objects.

Offset	Skip	Displayed Objects in the Gallery
0	0	all objects
10	0	10^{th} 11^{th} 12^{th} 13^{th} ...

20 15 20^{th} 35^{th} 50^{th} 65^{th} ...

Cell With/Height

The Gallery panel size is equal to the settings in the width/height numerical fields.

Plot Dimensions

The Edit tab gives you independent control of plot dimensions.
Select a plot dimension from the list of icons:

Plot Dimension	Icon
Scale	
Color	

Selecting or de-selecting the box next to the plot dimension name controls its visibility in the viewing area.

- To adjust the appearance of the plot you can change the various aspects of the plot dimension.
- To modify the existing condition, you have to first select the plot dimension you want to change.
- To assign a different statistical variable to the current dimension, select a new statistical variable that you want to be mapped.
- To incorporate a new plot dimension into the plot, select the preferred dimension and assign a new statistical variable.
- The statistical variable can be chosen from the drop-down list. The list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and then this selected statistical variable is assigned to the chosen plot dimension.
- To remove the assigned plot dimension from the viewing area, deselect the checkbox next to the plot dimension name.
- The Plot Numerical Table interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new sets of values are displayed.

Offset

This parameter is used to reposition the objects from their original position within the plots.
Offsetting objects is useful when you have more than one object in the same position and you want to evenly distribute the objects in a particular direction. For example, if you have many objects that you want to shift horizontally in a straight line, you can specify the value, in increments, in the Offset field for the selected X dimension.
The offset values change the object's originally assigned value. This new value is calculated by adding the entered offset value to the value of the selected statistical variable.
The offset value can be positive or negative.
For the X plot dimension, positive offset numbers move the objects to the right, and negative numbers move them to the left. For the Y plot dimension, positive offset numbers move the objects down, and negative

numbers move them up.
The offset value is given in the same units as the selected statistical variable assigned to the selected plot dimension.

Scale

A scale function specifies how the scale of each plot dimension should be determined from the value of the selected statistical variable.
As the values of the selected variables may be of different magnitudes, during the plot creation, the offset and scaling values are automatically calculated based on each of the selected variable values.
After the automatic scaling, the ranges appear to be the same for all of the objects currently being visualized.
The Scale value provides you with options to adapt how the plot dimensions are scaled.
To scale the selected plot dimension enter a number in the Scale field.
The entered Scale value multiplies the initially calculated values of the selected plot dimension by the value entered in the Scale field.


Clicking this arrow sets the Scale and Offset back to the original values.

Limit Min Limit Max

During the plot creation, the range is automatically adjusted to the actual lowest and highest value of the selected variable.
It is possible to change the max limit and/or min limit of the selected plot dimension by entering the values in the Limit Min /Max fields.
Enter a value to the Limit Min /Max to specify the new minimum/maximum values for the selected plot dimension.


Clicking this arrow sets the Limit Min /Max back to the original values.

Bin Size

Binning allows you to sort your data into different groups or bins. The Binning size value divides a plot into a specific number of groups and specifies the new locations of the objects by distributing them into the different bins according to the value of the selected variable.

Gamma

The Gamma function allows you to individually manipulate the appearance of the range of the selected variable.
The default Gamma value is 1, indicating that the range between the lowest and highest value of the selected variable is extrapolated in a linear mode to the full range of the data set.
If the entered value is different than 1, the linear mode is transferred to a nonlinear mode.
Entering Gamma values higher than 1, will result in a plot in which the range of the object with the lower values will appear to increase relative to the range of the object with higher values.
Alternatively, entering Gamma values smaller than 1, will result in a plot in which the range of the object with higher values will appear to increase relative to the range of the object with lower values.
The change is directly visible in the viewing area.

Clam Limits

If this box is selected the object outside the plots limits (determined by the frame and range settings) will be repositioned within the limits.
The object with the higher value will be assigned the max value of the selected variable, while the object with the lower value will be assigned the minimum value.

Auto Adjust

Click on this button and the parameters under the Edit tab are returned to the original values.

Show All Time Points

Select this box and the created objects are shown for all time points.

4.14 Frame Tab

Common aspects of all frames, such as the thickness and color of frame, grid tickmarks and labels setting can be controlled using this tab.

Box and Time Settings

Box
Check the parameter to display a box around the Frame.

Line Width
Adjust the box line width with the respective slider. Moving the slider to the left reduces the line width, moving it to the right increase line width.

Shading
Check this parameter to display shading. Use the slider to adjust the shade contrast.

Shadow
The entire Scene can produce shadows on the three planes of the Frame object. Check this box to display the shadows. Use the Shading slider (see above) to adjust the contrast. The rendering is done in real-time, and it requires a high performance graphic board.

Time Colorbar
Only available if you work with a time data set. Check this box and the Time Colorbar appears in the lower left corner of the viewing area.

Grid and Tick marks Settings

Grid
Check the box Grid to display a grid. Use the slider to adjust the grid width.
The Scale is shown/by a grid behind the object. The frame moves to stay behind the object as it is rotated. The distance represented by each square in the grid, the scale bar, is in the bottom right corner of the screen.

Tickmarks
Check the box Tickmarks to display tickmarks on the edges of the frame.

Spacing
Specify the grid and tickmark spacing for the X, Y and Z plot dimensions.
The frequency of tick marks can be controlled by selecting the appropriate interval values from the drop-down list.

Labels Settings

Axis Labels
Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked, the parameter Label Color and Label Font is available (see below).

Label Color
Only available if the parameter Axis Labels (see above) is checked. Click on the Color Editor window to change the color of the label on the coordinate axes.

Label Font
Only available if the parameter Axis Labels (see above) is checked. Click on the Font Editor window to change the font to label the coordinate axes.

Fix Decoration to Data Set
Check Fix Decoration to Data Set to fix the Frame to the same side when navigating the object.

4.15 Color Tab

Please note: The color coding option is not available under the Vantage Color Tab.

Color

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color slider to select a color or by clicking on the drop down arrow and entering direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Material

Objects are rendered with a Material that can be chosen from a list. Many of the Material choices have bright edges highlighting regions where the surface normal is perpendicular to the line of sight.

Labels Overriding Base Color check box, if selected, the labeled objects will be color coded according to their label. The labels associated with the data set are displayed as a list.

Click **Only show labelled objects** to show only data which have labels associated with them.

Note that if there is more than one label associated with an object, the object will be displayed as a combined color. Non-labelled objects are displayed in the base color.

Transparency

Transparency defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Section 5 File

The options available from the file menu will depend on the current view selected:

Arena File Menu Options

The following options can be selected from the **File** menu under Arena:

Batch Convert
List of recently opened
Surpass files

Surpass File Menu Options

In other modes e.g. Surpass additional options are available and these will change according to what applies to the current image.

Open...	Ctrl + O	
Open Image Only		
Open in a new Window	Ctrl + Shift + O	
Revert to File	Ctrl + R	Use Revert to File to revert to the previously saved version of an image file.
Save	Ctrl + S	Use the Save and Save as options to save the current image file as required.
Save as		
Import Segmentation/ Label		Open the label image from Ilastik or Labkit in Imaris and convert to a surface in Imaris.
Import Scene		The Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called a Scene and can be stored in a Scene file. The Scene can be loaded again to the same data set or to another data set.
Export Scene		The Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called a Scene and can be exported as a Scene file with the extension *.ims.
Import Spots or Tracks		Import tool for data from MaMut and TrackMate.
Add Image/Volume	Ctrl + Shift + I	Add an image or volume into an Imaris Scene for multi-image data sets.

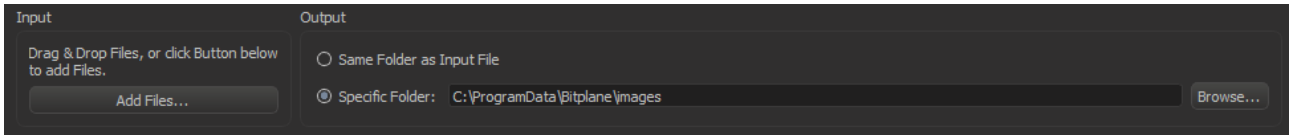
File Menu Options (all views)

Preferences... Ctrl + P
Exit Ctrl + Q
See also:

Addendum - Mouse & Keyboard Mac

5.1 Batch Convert ...

With Batch Convert... (Imaris File Converter) you can convert various image file formats to the Imaris file format *.ims. Select the menu entry Batch Convert... and the Imaris File Converter window displays. From the Windows Start menu select "ImarisFileConverter".



Input

Drag & Drop Files, or click the Button below to add Files.

Button **Add Files ...**
The window Select Files for Conversion displays. Choose the respective file and click on the **Add Files..**..button.

Output

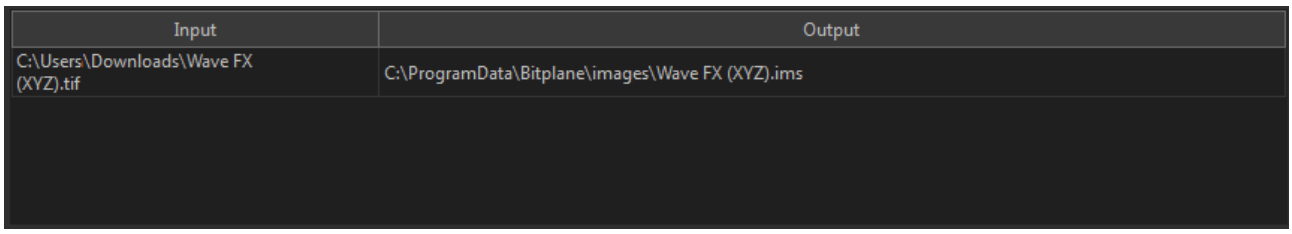
Same Folder as Input File
You find the converted image(s) in the same folder as the input files.

Specific Folder
Here you can select another folder for the converted image(s). Either type in the respective path or use the button **Browse**.

Button **Browse**
Click on this button to browse for the specific folder.

File Format
The format is Bitplane: Imaris 5.5 (*.ims)

Note: Bitplane: Imaris 3.0 (*.ims) and Bitplane: Imaris 2.7 (Classic)(*.ims) options have been removed as of Imaris 8.4.



Input

Here you can see the selected input file path(s).

Output

Here you can see the selected output file path(s).

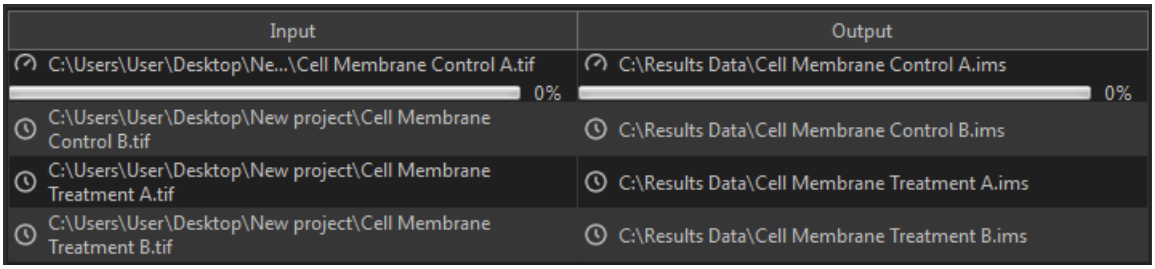
Settings

Provides additional settings for the selected file e.g. to specify voxel size

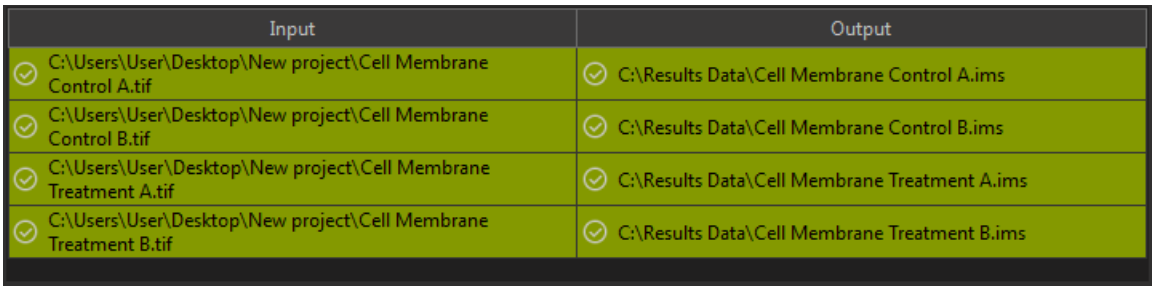
Remove Selected and **Remove All** buttons
Click on these buttons to clear either a selected row or all rows in the table respectively.

Button **Start All**
Click on this button to start the conversion.

When the conversion starts, files are moved to the bottom table. This displays the progress of the conversion for both read progress and write progress. It is possible to add new files and configure settings while a conversion is running.



This then updates on completion of the process:



See also:
Imaris Menu - Open - Reader Configuration
Imaris Menu - Open - Settings
Imaris Menu - Export ... - Advanced Save Options

5.1.1 Preferences

Button **Preferences**
Click on this button to open the Preferences window.

Window: Preferences

Button **Add**
You can use the buttons to add file paths in the list.

Button **Remove**
You can use the buttons to remove file paths in the list.

Output Formats

If you want to extend the list of output formats you have to check the following parameter and enter a valid

license in the next step. Find your license number in Imaris as follows: Click on the menu Help, select the menu entry Licenses and copy the license number (in the License Path at the bottom of the window, next to last enter field).

Include Image Filenames
Select this option and the output file names are assigned by using a default-naming rule.

Suffix Alphabetic
The output file inherits the input file name followed by the successive alphabetic letter.

Suffix Numeric
The output file inherits the input file name followed by the number of the output files being created (consecutive integer numbers).

All Imaris Output File Formats
Check this box to extend the output file formats.

License Location
Type in your license number or use the button **Browse**.

Button **Browse**
Click on this button and select the license path.

Button **OK**
Click on this button and all available Imaris output formats are available.

See also - Save as

5.2 Open

Data sets can be loaded from various file formats.

Image File Series

If the data set consists of a series of images each is stored as an individual file. To open a series of images select the folder and click on Open to open it.
This brings up the Image selection window, which allows you to explore your file system.
The window contains a preview of all the images as well as the following info: Name, Description, Recording date, Size, Time Points, Channels, Total size and Voxel size.
The image series is ordered according to the number, which appears after the file name suffix.

To open the image select a file name from the list and click **Open**

Image Preview
Check the box Thumbnail Preview to activate an image preview.

Search Image Name
Enter the image name for instant image selection.

Note if you want to reopen one of the last six images, you can use the Recent File List at the bottom of the File menu to select the desired image.

Demo Images

A range of demo images is provided so that you may practice with the features and functions of Imaris.

The default location of these files is as follows:

Windows: C:\ProgramData\Bitplane\images
MAC: /Library/Application Support/Bitplane/images/

iQ files in Imaris

To open iQ files in Imaris:

1. Locate and select the **DiskInfo5.kinetic file**.
2. Clicking on the '**Settings**' button opens a new window with a list of images and image information.
3. Check the box Thumbnail Preview to activate an image preview.
4. Select the required image and click on the **OK** button.
5. Then click again on the **Open** button. This will instantly create 3D volume rendered image of the selected data set.

For supported file formats please see Supported File Formats

5.2.1 Reader Configuration

Read only one Time Point

Loads a single time point of a time series.

Resampling Open...

The Resampling Open dialog box can be used as a preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This can be particularly important when reading large data sets over a network.

The revision applies to all views in Imaris and in Surpass.

- Select Imaris Menu - Open or click on the button **Open** in the Main toolbar.
- Click on a file to highlight it and click on the button **Resampling File Open ...** .

Image Preview
The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

Original Size
Display of the original file size.

Subsampling Factor

X,Y,Z, Ch, T
You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

Crop Limits (Min/Max)

X, Y, Z, Ch, T, From, To
The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

Resampled Size
Display of the resampled file size.

- Click on **OK** and the data set is cropped and resampled while loading.

See also:
Menu Edit - Image Properties... - Geometry
Menu Edit - Crop Time...
Menu Edit - Resample Time...
Menu Edit - Crop 3D...
Menu Edit - Resample 3D...

5.2.2 Settings

In the Settings you specify options for reading certain file formats.

Leica LCS Settings

A Leica LCS data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click **Open** in the Main toolbar.
- Select Files of Type: Leica LCS.
- Click on the *.lei file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
By checking the box **Thumbnail Preview** possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.

Select the required image stack on the left side of the dialog box.
- Click **OK** to open the image.

Leica LIF Settings

A Leica LIF data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

1. Select File - Open or click **Open** in the Main toolbar.
2. Select Files of Type: Leica LIF.

3. Click on the *.lif file to highlight it and click Settings. A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
4. By checking the box **Thumbnail Preview** the possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.
5. Select the required image stack on the left side of the dialog box.
6. Click **OK** to open the image.

Series Reading Sequence

If the data set consists of a series of images, individual images can be sorted according to various dimensions (i.e. slices, channels, time points, dimension sequence). The reader can handle Tiff series with single and multiple running numbers.

1. Select the Imaris Menu - the Open option
2. Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.
3. Select the source folder containing the series.
4. The Settings button only becomes enabled when a TIFF file is selected.
5. In the Series Reading Sequence window you can define the options to combine multiple files into one multi-dimensional image.

Reference File

Display the full path of the selected file.

Depending on the structure of the file system and the file name extension there are two options you can use:

1. File Names with Delimiter
2. File Names without Delimiter

File names with Delimiter

You can use this option when your files are numbered in sequential order and contain fields of information.
The dialog allows you to specify the order of the dimension within the image series and defines how they should be arranged in the final sequence. These are Slices: Z, channels: C, time points: T and to split into multiple datasets/images: F (Split).
You may apply as many or as few dimensions as you wish, depending on your image file requirements.
Assign the appropriate dimension by clicking on the arrow and selecting a dimension from the drop-down menu.
The selected dimensions are applied to the series of images.

Summary
Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.
The summary number is updated dynamically.

File names without Delimiter

You can use this option when the files are not numbered and contain no separator or delimiter.
The dialog allows you to specify the order of the dimension within the image series and defines how they should

be arranged in the final sequence. If the file is being loaded, the number of dimensions must be specified by the user so that the algorithm will know when to finish reading the first dimension and continue with the second.
You may apply as many or as few dimensions as you wish, depending on your image file requirements.
Assign the appropriate dimension by clicking on the arrow and select a dimension from the drop-down menu.

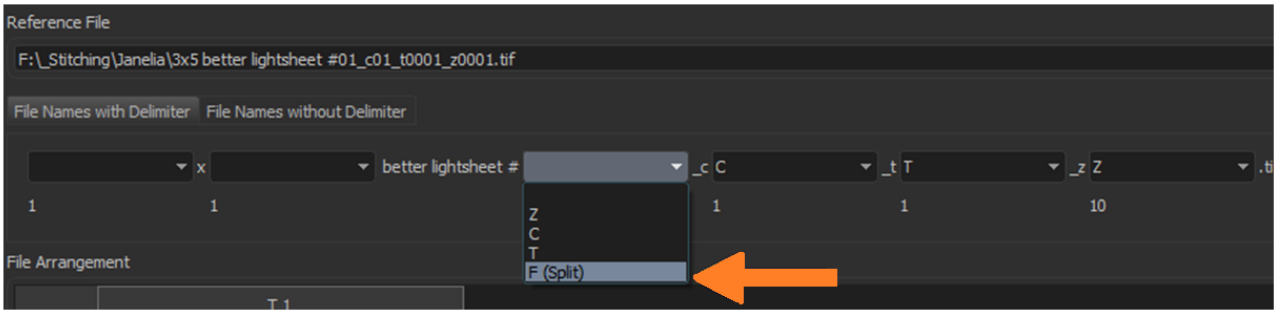
Summary
Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.
The summary number is updated dynamically.

File Arrangement

In the File Arrangement window, the selected sequence and dimension order is shown in tabular form. This display can be used to validate the sequence parameters.
Validate this information before you apply and open the TIFF series.

F(Split)

The split option F (Split) lets you split a collection of images files into multiple DataSets / Images.



This Settings Dialog is present both in Imaris and in ImarisFileConverter, this option is available in the two applications:

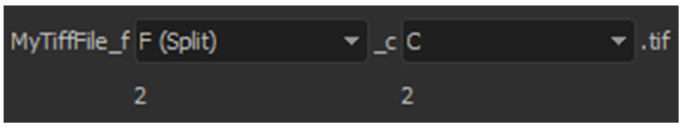
- In ImarisFileConverter, it allows to convert file series into multiple output IMS Files (with the benefit of configuring the layout and applying the X, Y, Z size)
- In Imaris, it allows, to read a single file (or a subset of files) from file series, or load multiple images from subsets of the series for batch run.

The procedure for how to split a number of files into two ims images, with two channels each is as follows:

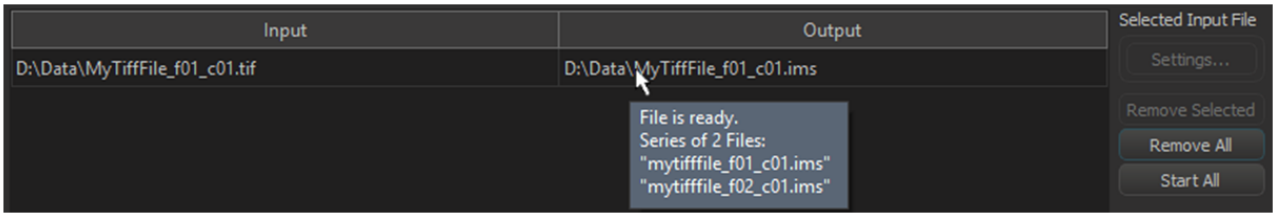
In this example there are 4 TIFF files:

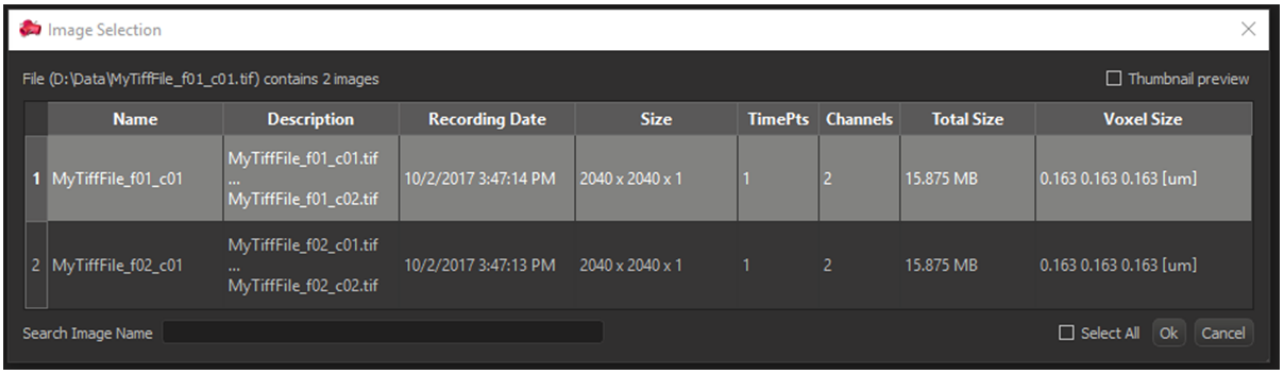
- MyTiffFile_f01_c01.tif
- MyTiffFile_f01_c02.tif
- MyTiffFile_f02_c01.tif
- MyTiffFile_f02_c02.tif

1. Add one of one of the TIFF files to ImarisFileConverter.
2. Click Settings the settings button.
3. Select "F (Split)" for the File Split delimiter. In this case it is “_f".
4. Select “C” for the channel delimiter. In this case it is “_c”.



5. Click OK.
6. By letting the mouse pointer hover over the output, you can see that two IMS Files will be generated (see below).





- The Name and Description categories show which file corresponds to which DataSet.
- The behavior is then the same when loading a multi-image file.

When adding image files as part of a file series, Imaris and ImarisFileConverter will try to automatically detect the layout of image files in the file series. If the detected layout is not as desired, it is possible from to change it from the Series Settings option. It would be cumbersome if you were working with a number of similar image data sets with an incorrect structure to do this each time a new image was opened.

The File Series Delimiters configuration lets you configure the delimiters that are used for the automatic detection. This lets you adjust the process so it matches the image files. (The File Series Delimiters configuration is also accessible in Imaris from Preferences/Loading and in the ImarisFileConverter application under Preferences).

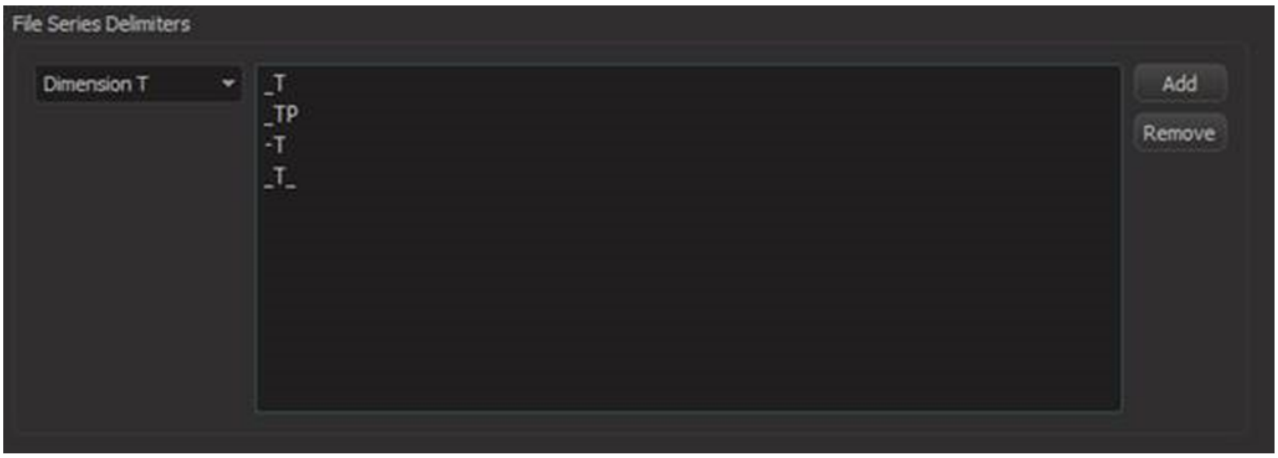
On the left-hand side, you can select the dimension that you want to configure from the drop-down list. The corresponding delimiters will appear in the central area.
For the example above, with “Dimension T” the following images would be correctly detected as time series, because the _T delimiter is in the Dimension T delimiters’ list:

- MyFile_t1.tif
- MyFile_t2.tif
- MyFile_t3.tif
- MyFile_t4.tif

But the following images would not:
- MyFile_t_1.tif
- MyFile_t_2.tif
- MyFile_t_3.tif

- MyFile_t_4.tif

To have these files detected automatically as a time series, the _T_ delimiter must be added:



Dimension F (Split) is by default not detected automatically (it does not contain any default delimiter). Therefore to have File Series Split detected automatically, some delimiters must be added (e.g. _F, _F_, _FLD).

Notes

- **The delimiters are case insensitive.**

5.3 Revert to File

Use Revert to File to revert to the previously saved version of an image file.

5.4 Save

To save an existing data set click **Save** in the File menu or Save icon on the Main toolbar.

Selecting this option stores the image data and (any) created objects within the selected Arena Group. The icon overlaying the image is updated to reflect the newly created surpass objects. The newly created object is added under Properties in the Surpass tab.

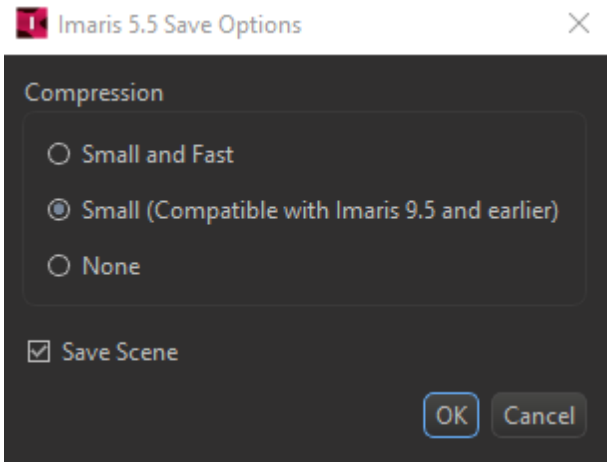
Please note: Save functionality is implemented to be performed at maximum speed. This functionality overwrites the existing file without warning and only writes changed or new data without deleting obsolete ones. As a result, the resulting Save file could actually be larger than the original.

5.5 Save as

To save the changes made to the image in a different file whilst maintaining the original file:

1. Click on the Save as option in the File menu or Save as icon. This option enables you to modify the image name and creates a new image item within the Arena and on the file system. The selected group now contains one original image file which remains unchanged and the new image file with the additionally segmented object. This newly created image item has an overlaid icon indicating the image type and segmented surpass objects. Under the Objects tab the newly created object is added.
2. Enter the name for the file to be saved or confirm the suggestion.
3. Advanced Save Options - Format Settings (apply for the image being saved)

The “Small and Fast” option uses LZ4 compression.
The “Small ” option uses GZIP compression.
The “None” option uses no compression, resulting in the largest file size.



4. Select the required file format and click **OK**.

Available File Formats in Imaris

Bitplane: Imaris 5.5 (*.ims)
BMP (adjustable file series)(*.bmp)
TIFF (adjustable file series)(*.tif *.tiff)
RGBA-Tiff (adjustable file series)(*.tif *.tiff)
Nikon: Image Cytometry Standard ICS (*.ics, *.ids)
Olympus: cellR (*.tif *.tiff)
Open Microscopy Environment TIFF (*.tif *.tiff)
Open Microscopy Environment XML (*.ome)
Move file (slice animation) (*.mp4,*.avi, *.tif, *.tiff)

5.5.1 Advanced Save Options

Button **Format Settings...**

A click on the button **Format Settings...** opens the Imaris Save Options window. See also Open Microscopy Environment: TIFF and Export as Movie File (Slice Animation).

Window: Save Options

Bitplane: Imaris 5.5 (*.ims)

Enable Compression
Check the box to use compression.

Export Scene
Check the box to save the Imaris scene.

Bitplane: Imaris 3.0 (*.ims)

Time Series
Export Single File
The time series are saved in a single file.

Export Multiple Files
For each time point a new file is generated.

Compression
LZW Compression
Check the box to use an LZW compression.

5.5.2 Open Microscopy Environment TIFF Advanced Options

Set the advanced save options when exporting as a **Open Microscopy Environment TIFF** format by clicking the **Format Settings** button. It is possible to set the options for this format from the below:

Dimension order
Select from the following options:
- XY ZCT (default)
- XT TZC
- XT CZT
- XY ZTC
- XY TCZ
- XY CTZ

Number of dimensions on one file
Set the number of dimensions between 2 and 5.

Tiff options
Enable/disable **LZW Compression** (lossless data compression, default: Off) and set image to be composed of either **Tiles** or **Strips** (default).

5.5.3 Movie File (Slice Animation) Advanced Settings

Set the save options when exporting as a **Movie file (Slice Animation)** by clicking the **Format Settings** button. It is possible to set the options outlined below:

Format
Select from the following file formats:
- H264 Movie (default - common high definition format, H.264 compression)
- Raw Movie
- Tiff (series).tiff

Movie Settings
Adjust the Compression factor between 0 (no compression: best quality/largest file size) and 100 (highest compression: lowest quality/smallest file size).

Frame rate
Set the frame rate for the movie file in frames per second.

5.6 Import Segmentation/Label

Open the label image from Ilastik or Labkit in Imaris and convert to a surface in Imaris.

1. First open the raw microscopy image in Imaris Surpass view.
2. Then click the Import Segmentation/Label button within the main toolbar or the File dropdown menu of Surpass View. Select your tiff or h5 file representing your label image.
3. Imaris will then create a Surfaces component, import the label image, and create surface objects from it.
4. Continue to import label images as Surfaces components until all segmentation results from ilastik have been added to your Imaris image file.

See our introductory article on our Learning Center for more information: How To Import Label Images from ilastik

5.7 Import Scene

The Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called a Scene and can be stored in a Scene file. The Scene can be loaded again to the same data set or to another data set using the Import Scene function. Additionally Object components can be imported as a scene and this functionality is compatible with the third party software and associated file types listed below.

Supported File Types

Bitplane	Imaris 5.5	*.ims	Imports all components in scene file to surpass tree
Bitplane	Imaris Scene File	*.imx	Imports all components in scene file to surpass tree
	Imod Binary File	*.imod, *.mod	Generates a surfaces object
	Picasso Binary File	*.hdf5	Generates a spots component
	Mamut/TrackMate XML File	*.xml	Generates a spots component
	CSV Spots File	*.csv	Generates a spots component

5.8 Export Scene

The Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called a Scene and can be exported as a Scene file with the extension *.ims.

5.9 Import Spots or Tracks

Import tool for spots or tracks data from MaMut or TrackMate.

CSV Spots File - Import Guidance

Below details the information that must be contained in the .csv file for import.
The first row should contain any of the following column labels, in any order: **x, y, z, t, r, cell_id, parent_id**. Other labels (e.g. statistics, labels or other features) can be provided, but they (and their corresponding values)

will be ignored by the import reader. The rows below the label rows described should contain the values for the specified labels (one object/spot per row).

x, y, z, are the geometrical coordinates of the object in microns, **r** is the radius in microns, **t** the time point in seconds (rounded to the next lower integer). **X** and **y** labels and values are required, all other labels are optional. **z** and **t** are set to 0 if missing and **r** is set to 1.
Cell_id and **parent_id** are integer values and used to build tracks by connecting cells to their parent, no tracks are generated if cell_id or parent_id are missing.

Import guidance notes and troubleshooting:
- The separator for values is ,
- The decimal separator is .
- Blanks and tabs are ignored
- Negative positions do work
- Negative values for radius or time does not work
- Positions or time are not clipped at the datasets limits
- cell_id from the import file is not kept in Imaris. i.e. if you gave gaps in the numbering in the import file the Spots ID in Imaris will not
- Using the parent_id twice will create branching
- If a entry for a time point is missing within a track the track will bridge the gap without a Spot at the gap
- File coding has to be Unicode UTF-8

Please also see the introductory article on our Learning Center: How To Import Spots and Tracks from MaMuT or TrackMate into Imaris

5.10 Add Image/Volume

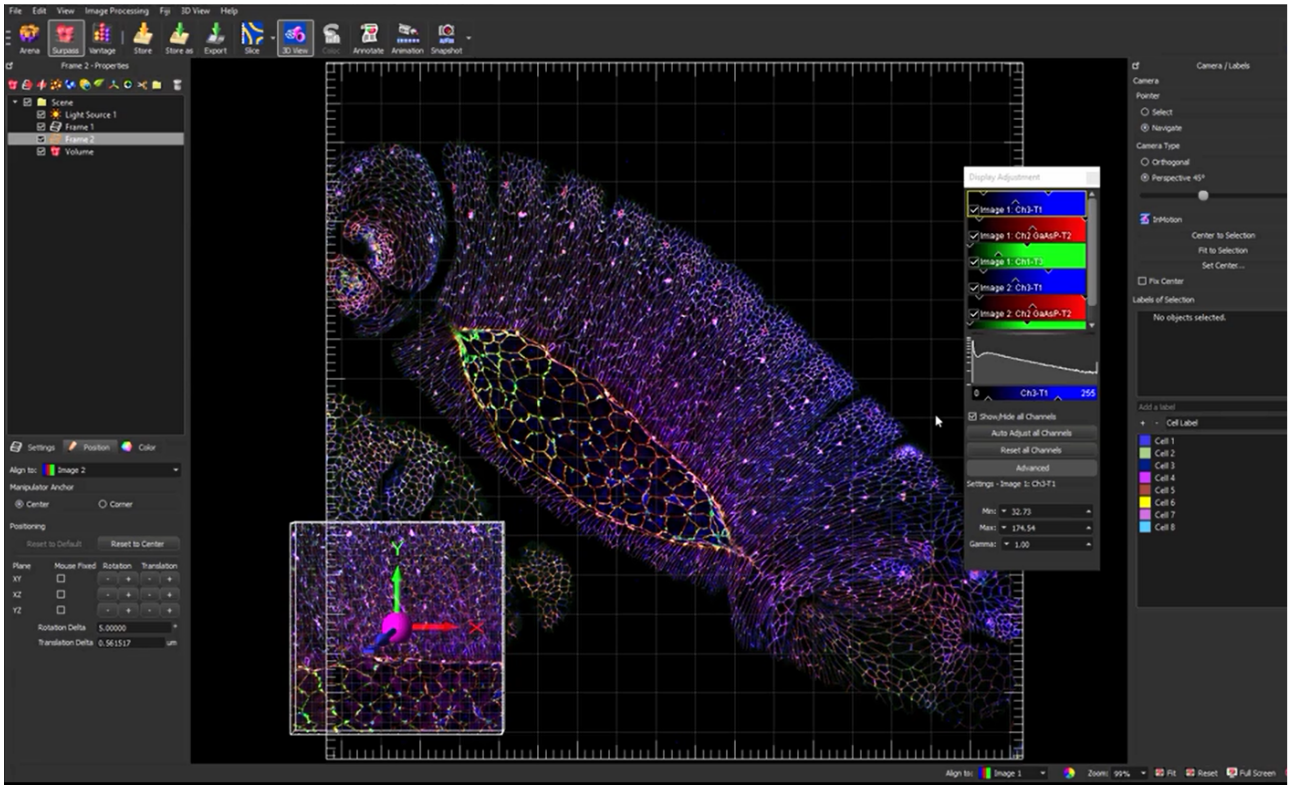
New "multi-image" functionality for working with multiple images in a single Imaris scene was introduced in Imaris 9.1. This feature set may be useful for a range of applications, for example: combining different imaging modalities on the same sample such as electron microscopy and confocal microscopy, or for images of the same modality captured at different magnification levels. It is also possible to perform alignment of 3D objects that were imaged separately.

The features available are described in the following sub-sections along with an overview of how to use these functions on your images.

The first step is how to add additional images:

How to add your images

1. Open the first image in Surpass view.
2. Load additional images using the **Add images...** from the File menu.
3. Each additional image can then be seen to have been loaded in the viewing window.
   a. When the position tab is selected an XYZ tripod shows the orientation of the selected image.
   b. The **Align to:** box displays an indicator color to help identify the appropriate image.
   c. Use the mouse Pointer **Navigate** mode to navigate through the image area, use the **Select** mode to select the required image.



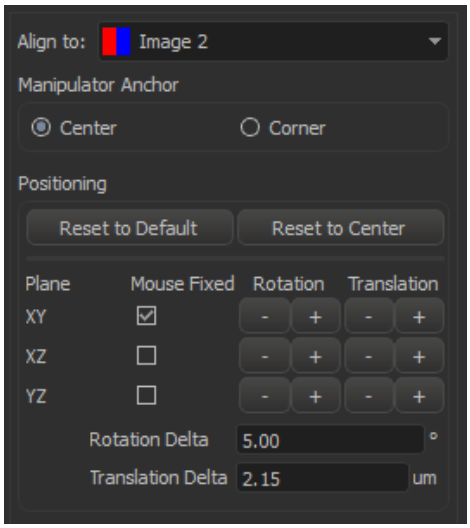
- The Settings section describes how the images are displayed and labelled.
- If there is more than one image loaded, Imaris will let the user chose which image to perform the required action on.
- Image position adjustment tools are described in section Position.

Note: To remove images, use the Remove images... from the Edit menu, see Remove Images.

Settings Tab Menu Options

Refer to the section tab settings for further information on frame component settings.

Position Tab Menu Options



Align to:

Select the image you want to align the selected image to. The color indicator displayed is representative of the appropriate image.

Manipulator Anchor
Select the adjustment/rotation point to be either at the **Center** or the **Corner** of the image.

Positioning
Use the positioning options to adjust the positioning of image in **XY**, **XZ** and **YZ** planes. The **+** and **-** buttons can be used to adjust in increments. The increment size is set in the **Rotation Delta** and **Translation Delta** fields. The positioning can be fixed about the planes by selecting the **Mouse Fixed** checkbox for the appropriate plane. This is useful for locking the movement in the plane/axis as the image is moved.

After making adjustments you can return positioning to the default position or reset to Center using the the **Reset to Default** or **Reset to Center** buttons respectively.

Color Tab Menu Options

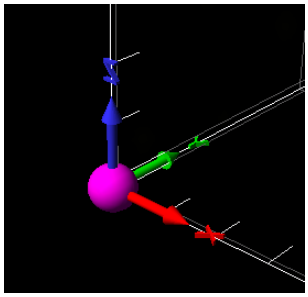
Adjust the color settings.

How to Align Images

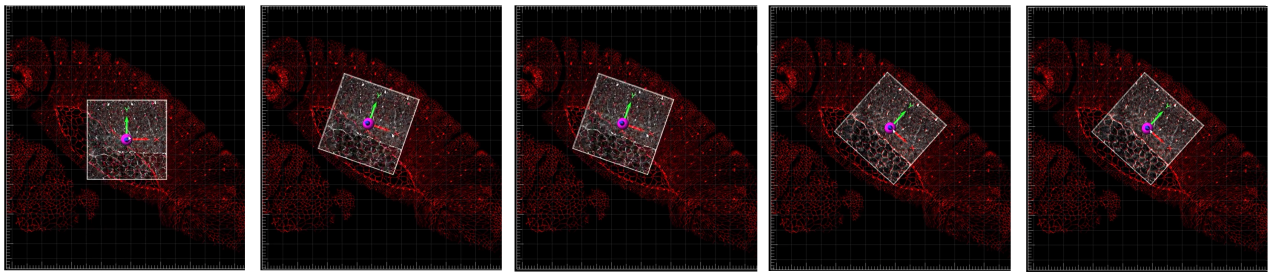
Alignment can be split into two steps:

Performing the Initial Alignment (Approximate)

1. Add the images as described in Add Image... (multi-image)
2. Images can be repositioned in the **Select** Pointer mode by clicking and holding the left mouse button on the pink central section and moving to the required location.
3. To change the orientation about the axes, click on the required X, Y or Z axis arms of the tripod and it will rotate about the selected axis. The plane can be locked in position if required using the **Mouse Fixed** check box for the required plane (axis).



4. When aligning images it is useful to turn off unnecessary channels so you may focus on useful image information to help the alignment. It may also be useful to change the color of the channel you are using for alignment to one that gives good contrast, making alignment easier.

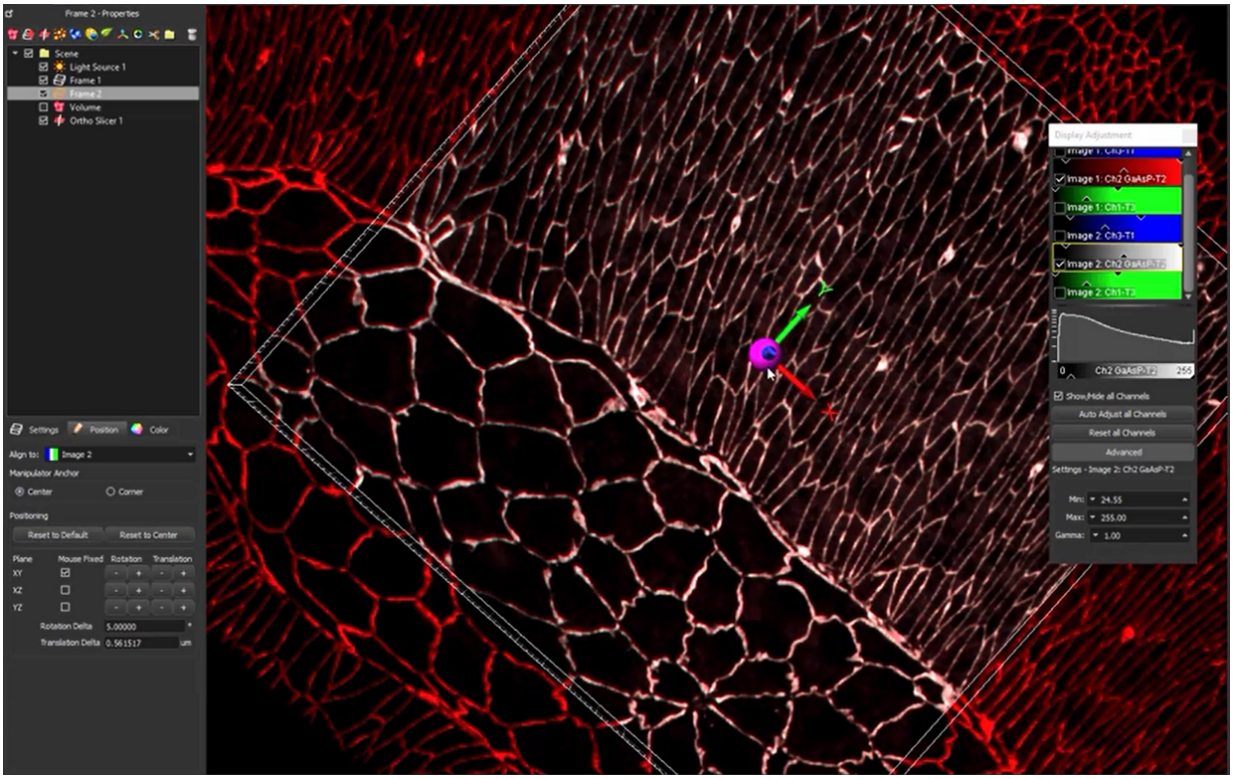


Above: Image is aligned against the underlying image using the tripod to adjust the relative rotation and translation about the XY, XZ and YZ planes. Note that channels for both images have been disabled to help visualize the

alignment of the structures during the alignment process.

Performing Precise Alignment

1. Once the images are aligned approximately you may find it useful to switch to a 2D or Slicer view, zooming in for precise alignment:
2. The plane can be locked in position using the **Mouse fixed** check box for the required plane (axis).
3. The **Rotation** and **Translation -** and **+** buttons can be used to adjust the position in small increments with a specific plane until the desired alignment is achieved. The size of these increments can be set in the fields below. Note that for rotation you can change between having the adjustment in the **Center**, or the **Corner** of the image.
4. This process can be repeated for the other planes and checked until you are happy with the alignment.



Above: Image has been zoomed in and image plane locked in the XY plane. The rotation and Translation + and - buttons are then used to perform the precise alignment.

- Image position adjustment tools are described in section Position.
- To adjust how the images are displayed and labelled refer to the section Settings.

Note: To remove images, use the Remove images... from the Edit menu, see Remove Images.

Performing Image Analysis

- Once the image has been aligned you can perform analysis on the image scene as required. The source image can be selected (from the Align to drop-down box) at step 1 of the creation wizard.

5.11 Preferences...

The adjustable parameters in the Preferences are application specific and Imaris stores these preferences for an individual user.

System
Display
Loading
Calculation
Time
3D View
Creation Parameters
Vantage Plots
Statistics
Statistics Test
Snapshot
Custom Tools
Batch
Licenses
Update
Usage Data
3D Cursor
Annotate
Arena
Advanced

Imaris Configurator can be accessed directly from Preferences so that licenses, data storage locations and other Imaris settings can be configured by a user with administrative privileges.

5.11.1 System

Displays the basic system parameters of your computer.

Graphics

OpenGL Renderer
OpenGL Version
Pixel Shader
GPU Deconvolution
Information about the OpenGL Renderer, OpenGL Version, Pixel Shader, and GPU Deconvolution. For more information on deconvolution please see the Deconvolution section.

Operating System

OS
Version (Build)
Service Pack
Information about the operating system, the installed Version and the service pack.

Memory Status

Physical Memory installed
Physical Memory available
Information about the available amount of internal memory.

Network

Network Interfaces
Information about the available network interfaces

Proxy Settings

Http Proxy Server Name
Enter a proxy server's name. If this field is empty, no proxy server is used.

Http Proxy Server Port
Enter the port to the proxy server. If this field is empty, no proxy server is used.

Test Connection Button
Click on this button to test your proxy settings. If the fields are empty, the connection is tested without using a proxy server.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.2 Display

Select the viewing properties and the basic colors for the backgrounds and selection in the gallery.

Display

Interpolate
If checked, the images are automatically interpolated for a smoother display.

Enable High-DPI Volume Rendering
Enables fast volume rendering on 4k monitor.

Texture Cache Limit (MB)
Before displaying any image data, Imaris converts the data into a configuration (called textures) that is optimized by the graphics hardware. The value of the Texture Cache Limit determines how many textures can be stored in RAM. The Texture Cache Limit is automatically determined from the available memory of the graphics board when Imaris is used for the first time. You can also manually set the value to the memory of your graphics card.

Colors

Background Color
The background color appears when there is no image to be displayed or when the displayed image does not fill the whole View Area.

Please note: The background color setting has not effect on the MIP mode. The default color for MIP(max) is black and MIP(min) is white.

Click on the color selection button to open the color selection window to change the respective color.

Background Color 2
Background marking the original position if an image is moved.

Click on the color selection button to open the color selection window to change the respective color.

Checkered Background for Blending
When using blending mode, a checkered background is displayed in Full 3D blend and in Surpass view.

Tile Size
Allows definition of the tile size for a checkered background.

Linear Color Progress for Blending
The background displays a color gradient in blend progress projections and in Surpass.

Selection Color
Color of selection frame and drawing lines in contour surfaces.

Click on the color selection button to open the color selection window to change the respective color.

Measurement Color
Color of measurements points and lines visible in the image (in the Slice and the 3D view).

Click on the color selection button to open the color selection window to change the respective color.

User Interface Theme
This option allows you to customize the user interface theme.
You can either choose the Native theme (from OS) or select the Dark theme. Click **OK**. The display updates immediately.

Coordinate Axis/Scale TimeColor Bar

Show Coordinates Axis
Show Scale Bar
Show Scale Bar Label
Scale Bar Font Size
Scale Bar Lower Left Corner x
Scale Bar Lower Left Corner y
Show Time Colorbar
Show Date
Show Time

These options change the display of the scale bar, labels and time information in the Slice, Selection, Gallery and 3D View.

Adjusting the Scale Bar

The scale bar position and size can be set in preferences by adjusting the values in the **Scale Bar Font Size**, **Scale Bar Lower Left Corner x**: and **Scale Bar Lower Left Corner y**:

You can also alter the length, width, location and font size of the interactive scale bar when the Pointer is in Select mode. All changes are instantly displayed in the viewing area.
Extend or shorten the scale bar by clicking and dragging one end of it.
Move the scale bar to any location in the viewing by dragging it
Zooming in or out of the image the scale bars are automatically updated.

To choose the style of timestamp to be displayed select either **Show Time** or **Show relative Time.**
Show Time option display the acquisition time.

Show relative Time
For Show relative time the first time point is designated as time zero and all other times are displayed relative to that.

The timestamp presentation format can be chosen and adjusted.
h (hours), min (minutes), s(seconds), f (milliseconds)

Select options to display coordinate axis, date, scale bar, scale bar label or time on screen in the Viewing Area.

Please note: To display the Coordinates Axis in the Surpass view the option Axis Labels in the Frame-Tab Setting must be also selected.

Numbers Representation

You can adjust how numbers are represented for the available options:
- Unit of Length
- Unit of Time
- Significant Digits

Off Screen Rendering (for saving Snapshots and Movies)
If deactivated Imaris will take the images similar like a screenshot directly from the display. Uncheck this option only if you have problems with the Snapshot or Movie export e.g. if the image is distorted. You will not be able to create an output larger than your physical screen size if this option is disabled.

Show System Monitors
Check this box to display the system monitors in the Status Bar at the bottom of the screen. The first window displays the "reads per sec", the second the "writes per sec", the third the "write requests in queue" and the last the "percentage read cache hits". These are useful information especially if you work with huge data sets.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.3 Loading

Allows you to select the color assignment method used when loading data sets and to define the default colors. Images in Imaris format will display in the colors defined in the image file.

Take Colors from:

Default Colors
Use the default color selection to display the loaded data set. The parameter set Default Colors (see below) is available.

File Colors (color table or base color if available, otherwise default colors)
Use the original color definition of the loaded data set (usually stored in a lookup table). The parameter set Default Colors (see below) is not available.

Emission Wavelength (from file if available, otherwise default colors)
Use the color according to the emitted wavelength from the file (corresponds to the appearance under the microscope). The parameter set Default Colors (see below) is not available.

Please note that not all file formats support lookup tables and emission wavelength.

Default Colors

The parameter set Default Colors is available, if you select Default Colors in the parameter set Take Colors from (see above).

First Channel
Second Channel
Third Channel
Other Channels
Display of the defined color.

iQ ImageDisk File

1. Either type in the location of the **DiskInfo5.kinetic** file or use the browse button to find it.
2. Use the Browse button to find and select the DiskInfo5.kinetic file.
3. Open iQ files
4. Clicking on the '**Open iQ files**' opens the Image Selection window with a list of images and image information.
5. Check the box Thumbnail Preview to activate an image preview.
6. Select the required image and click on the **OK** button. This will instantly create a 3D volume rendered image of the selected data set.

Browse Button
Use the Browse button to find and select the DiskInfo5.kinetic file.

Select... Button
Click on this button to open the color selection window to change the respective color.

OK Button

To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.4 Calculation

Calculation

Number of Parallel Jobs
Specify the number of threads (processor cores) used in calculations. See maximum number of available threads in the System box.

History

Maximum Number of Commands in History
Defines the maximum number of operations that can be undone/redone.

Please note: Each level of image processing requires an additional copy of the full image in memory. If your machine runs out of memory, set Maximum Number of Commands in History to 1.

Data Cache

Imaris uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

Memory Limit (MB)
The value of data cache limit controls the amount of data blocks Imaris will keep in memory at any time. Enter a value based on the following table.

PC & MAC	
64 bit	Physical memory installed x 0,5

Cache File Paths:
Imaris utilize advanced caching when working with large images. You can add a Cache File Path by defining a directory where the temporary files should be stored.
Cache File Paths display of the paths to the temporary files (Scratch-disks).

Please note: Setting the Cache File location to a spare or separate hard drive may improve the performance when working on larger images.

Button **Add**
Button **Remove**
You can use the buttons to add or remove file paths in the list.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.5 Time

These are the default parameter settings for the Time Bar.

Play Back

Specify the play back mode for the Time Bar.

Play One Time
All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever
Once the play back has reached the last time point, it starts at the first time point again (continuous loop).

Swing Back and Forth Forever
When the last time point is reached, the time sequence is shown in reverse until the first time point is reached.

Frame Rate: ... Frames per Second
You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

See also:
Toolbars - Time Bar

5.11.6 3D View

Object Creation Buttons

Check the icons to be displayed on the Objects toolbar in the Surpass View.

Button **Move Up**
Button **Move Down**
Highlight an icon and click Move Up or Move Down to define the order of the icons in the Objects toolbar.

Key Frame Animation

Specify the play back mode for the Key Frame Animation.

Play One Time
All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever
Once the play back has reached the last time point, it starts at the first time point again (never ending).

Frame Rate ... Frames per Second
You can further specify the frame rate, i.e. the number of frames per second.

Manual tracking auto-connect (Spots and Surfaces)

Delay before auto advancing (s)
You can specify the value between 0.05 and 5 seconds.
Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

Camera

Enable antialiasing (Smooth Lines)
Check the box to display smooth lines.

Enable Draw Style Settings
Check the box to enable the draw options in the Camera Toolbar.

Enable Stereo Camera Settings
Check the box to enable the stereo options in the Camera Toolbar.

Refer to the Camera Toolbar section for further information.

Zoom Point Under Cursor
This option enables zoom to be centered on the point under the Cursor automatically. This may be useful when you want to zoom to a specific location, for example when using Filament
Autopath and Filament autodepth tracing. This may be disabled for a standard zoom style in which the zoom will zoom in on the center of the viewing area. Default (On).

Auto Adjust Center Of Rotation
This option enables the rotation point to be centered on the center of the visible part of the image/scene automatically. When not enabled the image will automatically rotate around the center of the viewing area window. Default (On).

Zoom Through Data Set
This option provides an auto focus (based on the distance from the camera to the center of the image for zoom and pan speed and center of rotation) so you may zoom smoothly through an image to the other side. This may be useful when the point that you want to zoom to is close to the rear of the image. Default (On).

Torch View

Adjust the options for Torch View. You can select between the three following options:

- Draw entire dataset (default option)
- Draw dataset only around the torch region (full depth)
- Draw dataset only around the torch region (local depth)

5.11.7 Creation Parameters

When you save the Creation Parameter file, it is automatically added to the list that is displayed under the Edit/ Preference/Creation Parameters.

The preference window shows a list of all the parameters sets that have been created. To assist you in finding and organizing the creation parameters, each file is labeled with an object icon, which indicates the type of creation parameters set.

Click on the name of the creation parameter's set, the extended window provides an option to view the entire list of operations, processing instructions and the parameter's value of the selected set.

Remove Button

This function will remove the selected set of creation parameters from the list.
The Export and Import functions provide an easy way to exchange the creation parameters between different users or groups.

Export Button

The creation parameters files can be created and exported.
This can be achieved by selecting the creation parameters set from the list and pressing the Export button.
Specify the directory, where you want to save the creation parameter's file, select a name and save it.

Import Button

The creation parameters files, that can be imported, are generated by the Export command.
Specify the directory of the files you want to import and select the file from the chosen location.

5.11.8 Vantage Plots

When you save the Vantage Plot file, it is automatically added to the list that is displayed under the Edit/ Preference/Vantage Plots.

The preference window shows a list of all the creation parameters' sets. To assist you in finding and organizing the creation parameters, each file is labeled with an object icon, which indicates the type of creation parameters set.

Click on the name of the Vantage Plot, the extended window provides an option to view the entire list of the plot type, dimensions and the selected statistical variables.

Remove Button

This function will remove the selected Vantage Plot parameters from the list.
The Export and Import functions provide an easy way to exchange the Vantage Plot parameters between different users or groups.

Export Button

The Vantage Plot parameters files can be created and exported.
This can be achieved by selecting the Vantage Plot parameters set from the list and pressing the Export button.
Specify the directory, where you want to save the Vantage Plot parameter's file, select a name and save it.

Import Button

The Vantage Plot parameters files, that can be imported, are generated by the Export command.
Specify the directory of the files you want to import and select the file from the chosen location.

5.11.9 Statistics

Imaris features many statistical functions that can be utilised to analyse surpass objects. Statistics are accessed through the Statistics tab in the surpass view, the options available are specific to each of the surpass item types e.g. cells or spots. This section provides an introduction to some of the statistical notations and concepts used in the following statistics topics.

- The time indices where the track objects are present are denoted with **t** ranging from **1** to **n**.
- Descriptions for statistic functions are grouped together for vectors "X, Y, Z" or dimensions "A, B, C". For example, for **Cells - Cell Displacement X, Y, Z** there are three individual functions for each component i.e. **Cells - Cell Displacement X, Cells - Cell Displacement Y** and **Cells - Cell Displacement Z**. Note that for 2D view **X** and **Y** components for statistics are available whereas for objects in 3D view **X, Y** and **Z** components are available. In addition, a Volume statistics value is calculated in 3D view whereas Area is calculated in 2D view.
- Three-dimensional vectors are denoted with an arrow:

$$\vec{r} = [r_x, r_y, r_z]$$

- Length of a vector:

$$|\vec{r}| = \sqrt{r_x^2 + r_y^2 + r_y^2}$$

- Position of an object at time index:

$$\vec{p}(t)$$

- Time of an object at time index:

Show Statistic Values

Statistics values can be selected (for display, export to MS Excel, or sorting). Check the values to be displayed when you open the tab Statistics in the Surpass View.

Show All
Check this box and all statistical values are selected. Uncheck the box and all statistical values are unselected.

For details of the available options please refer to the respective Section:


Menu Edit - Preferences ... Cell
- Statistics


Menu Edit - Preferences ... Filament
- Statistics


Menu Edit - Preferences ... Measurement Points
- Statistics


Menu Edit - Preferences ... Spots
- Statistics


Menu Edit - Preferences ... Surfaces
- Statistics


Menu Edit - Preferences ... Volume
- Statistics

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

See also:
Surpass View - Overview - Properties Area (Tab Statistics)
Coloc - Volume Statistics
Common Statistics - Common Statistics

5.11.9.1 Cells

ImarisCell is an Imaris module that performs automated 2D and 3D time-resolved cell image analysis. It extracts a full spectrum of relevant information, present in the data set, which provides a wide range of options to analyze 3D and 4D image data. ImarisCell detects some features that are not easily detected by a human observer, which can lead to the correlation of potential important and otherwise missed variations in the cell structure and function. Spots statistics can be located in the statistics tab.

Please see the Common Statistics Chapter for statistics common to all objects.

Show Statistic Values - Cell

Cells - Cell Acceleration
Acceleration, a(t), is the change in the Cell velocity over time (t).

Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

For

$$1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|$$

$$\vec{a}(t)$$

as defined in Acceleration X, Y, Z

Acceleration at the first and last time-point is 0.

Cells - Cell Acceleration X, Y, Z

Acceleration is the change in the Cell velocity over time along the selected-axis.

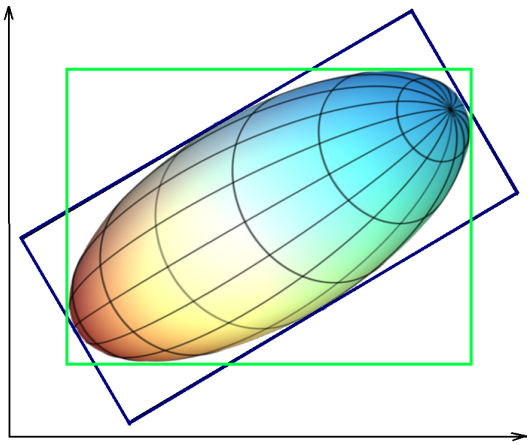
Cells - Cell Area

The area is the sum of the outer voxel faces.

Cells - BoundingBoxAA Length X, Y, Z

A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.
If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X = max(X)-min(X)
BoundingBoxAA Length Y = max(Y)-min(Y)
BoundingBoxAA Length Z = max(Z)-min(Z)



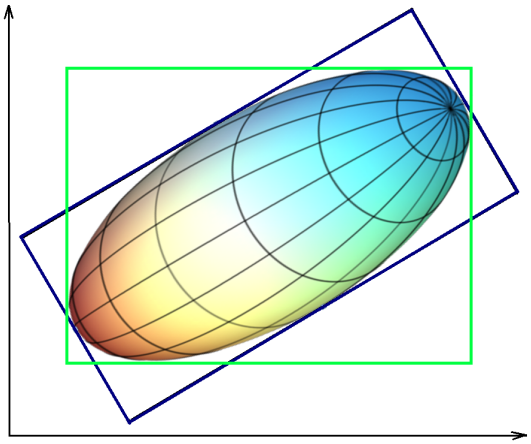
axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

Cells - BoundingBoxOO Length A, B, C

The local axes of the box can be arbitrarily oriented with respect to the coordinate system.
BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis
BoundingBoxOO Length B = Length of the second longest principal axis
BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue
Please note the difference between the ellipsoid axis length and bounding box length.

Cells - Cell Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.
The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

R - center of homogeneous mass (x, y, z)
$\mathbf{r}_i$ - center of a voxel (x_i y_i z_i)
n - number of $i \in$ Cell

Cells - Cell Center of Image Mass X, Y, Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R - center of image mass (x, y, z)
$\mathbf{m}_i$ - voxel intensity
$\mathbf{r}_i$ - center of a voxel (x_i y_i z_i)
M - sum of voxel intensities

Cells - Cell Cytoplasm Area
Measure the area of the cytoplasm within the Cell. It is calculated as sum of Cell area and Nucleus area.

Cells - Cell Cytoplasm Vesicle Diameter Max
The maximum diameter of all Vesicles within the Cell cytoplasm.The value is separately calculated for each vesicle type.

Cells - Cell Cytoplasm Vesicle Diameter Mean
The mean diameter of all Vesicles within the Cell cytoplasm.The value is separately calculated for each vesicle type.

Cells - Cell Cytoplasm Vesicle Diameter Min
The minimum diameter of all Vesicles within the Cell cytoplasm. The value is separately calculated for each vesicle type.

Cells - Cell Cytoplasm Intensity Max
Cells - Cell Cytoplasm Intensity Mean
Cells - Cell Cytoplasm Intensity Min
Cells - Cell Cytoplasm Intensity StdDev
Cells - Cell Cytoplasm Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Cytoplasm (Center, Max, Mean, Min, StdDev and Sum).

Cells - Cell Cytoplasm Number of Vesicles
The total number of Vesicles objects within the cytoplasm. The value is separately calculated for each vesicle type.

Cells - Cell Cytoplasm Number of Voxels

The total number of voxels within the cytoplasm.

Cells - Cell Cytoplasm Volume
Measure the volume of the cytoplasm within the Cell. It is is calculated by subtracting the nucleus volume from the total cell volume.

Cells - Cell Cytoplasm Volume of Vesicles
Volume is quantification of how much space the Vesicles object occupies within the cytoplasm. The value is separately calculated for each vesicle type.

Cells - Cell Vesicle Diameter Min
The minimum diameter of all Vesicles within the Cell objects.

Cells - Cell Vesicle Diameter Mean
The mean value of diameter of all Vesicles within the Cell objects.

Cells - Cell Vesicle Diameter Max
The maximum value of diameter of all Vesicles within the Cell objects.

Cells - Cell Displacement X, Y, Z
The Displacement, d(t), is a measure the distance that an object moves from the first time point position to its current position, p(t). Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

Cells - Cell Displacement ^2
The square of the displacement, d, of the object from the starting point of the track. (Plotting the squared displacement against the value 'Time since track start' is useful for MSD analysis).

$$d^2(t) = |\vec{d}(t)|^2$$

$$\vec{d}(t)$$

as defined in Displacement X Y Z

Cells- Cell Distance from Vesicle to Cell Membrane Mean
The mean distance, calculated by the distance map method, between the Vesicle's centers and the Cell surface border.

Cells- Cell Distance from Vesicle to Cell Membrane Std.Dev.
This value describes the Standard Deviation of the distance between the Vesicle's centers and the Cell surface border.

Cells - Cell Distance from Vesicle to Closest Nucleus Mean
The mean distance, calculated by the distance map method, between the Vesicle's centers and the Nucleus surface border within the Cell object.

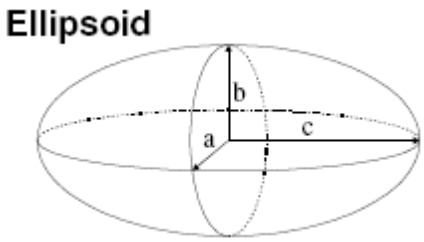
Cells - Cell Distance from Vesicle to Closest Nucleus Std.Dev.
This value describes the Standard Deviation of the distance between the Vesicle's centers and the Nucleus

surface border within the Cell object.

Cells - Distance from Origin
The statistical value measures the distance from each 'Cell Position' to the origin of the dataset. The Cell position is equal to the center of homogeneous mass of the Cell. The origin is the (0,0,0) of the coordinate system of the image. The origin is at the bottom left corner by default (see 'Surpass menu - View - Origin...'). Press 'Surpass - Status Bar - Reset' to see the image in a rotation where the origin is at the position set at 'Surpass menu - View - Origin...'.

Cells - Cell Distance to Image Border X, Y
Cells - Cell Distance to Image Border X, Y, Z
The value specifies the inward distances from the image border to be removed.

Ellipsoid Axis



Cells - Cell Ellipsoid Axis A X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis a.

Cells - Cell Ellipsoid Axis B X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis b.

Cells - Cell Ellipsoid Axis C X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis c.

Cells - Cell Ellipsoid Axis Length A, B, C
Cells - Cell Ellipsoid (oblate)
Cells - Cell Ellipsoid (prolate)

If:

$a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)

- $a \neq b \neq c$ it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \times \left(1 - \frac{a^2 + b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \times \left(1 - \frac{2a^2}{b^2 + c^2}\right)$$

e_{oblate} = oblate Ellipsoid

Cells - Cell Intensity Center
Cells - Cell Intensity Max
Cells - Cell Intensity Mean
Cells - Cell Intensity Median
The Cells - intensity median is estimated from the intensity histogram.
Cells - Cell Intensity Min
Cells - Cell Intensity StdDev
Cells - Cell Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Cell (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells - Cell Intensity Min of Vesicles
This value describes the minimum voxel intensity of the Vesicles within the Cell object.

Cells - Cell Intensity Max of Vesicles
This value describes the maximum voxel intensity of the Vesicles within the Cell object.

Cells - Cell Intensity mean of Vesicles
This value describes the mean voxel intensity of the Vesicles within the Cell object.

Cells - Cell Nucleus to Cytoplasm Volume Ratio
The value is defined as the ratio between Nucleus volume and Cell cytoplasm volume. The Cell cytoplasm volume is calculated by subtracting the volume of the Nuclei from the whole cell object volume.

Cells - Cell Number of Nucleus
The number of the Nucleus objects within the selected Cell object.

Cells - Cell Number of Vesicles
The number of the Vesicle objects within the selected Cell object.

Cells- Cell Number of Tracks
This value describes the total number of the Cell's object tracks.

Cells - Cell Number of Voxels
Number of voxels within the selected Cell.

Cells - Cell Position X, Y, Z
Defines the value of X, Y, Z Cell position (x y z).It is equal to Center of Homogeneous Mass of the object

Cells - Cell Relative Vesicle Position Min
Cells - Cell Relative Vesicle Position Mean
Cells - Cell Relative Vesicle Position Max
This parameter provides Min Max and Mean values of all Vesicle Relative Positions within the cell object. This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.
Vesicle Relative positions is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.
If the Vesicle is located within the Nucleus the value is set to 0. If the value is 1 then the Vesicle is located at the border of Cell object.

Cells - Cell Speed
The Cell speed, s(t), is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Cells - Cell Sphericity
Sphericity, ψ, is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, of a particle is the ratio of the Cell surface area of a sphere (with the same volume, V, as the given particle) to the Cell surface area, A, of the particle:

$$\psi = \frac{\pi^{\frac{1}{3}}(6V_p)^{\frac{2}{3}}}{A_p}$$

Vp - volume of the particle
Ap - surface area of the particle

Cells - Cell Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Cells - Cell Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Cells - Cell Total Number of Voxels
Total number of voxels within the data set for the cell.

Cells - Cell Track AR1Mean

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.

Cells - Cell Track AR1 X, Y, Z

TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$AR1X = \frac{R_x^1}{R_x^0}$$

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

D - Track Displacement
t_L - last time index of track
t_F - first time index of track

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

$P_X(t)$ - x-position of object at time index t

$$\bar{D}_x = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

t_L - last time index of track
t_F - first time index of track

Cells - Cell Track Area Mean

The mean of the triangle Cell object surfaces along the track.

Cells - Cell Track Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the ‘mass’ of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{ie\ object} r_i$$

R - center of homogeneous mass (x, y, z)
$\mathbf{r_i}$ - center of a voxel (x_i y_i z_i)
n - number of i ∈Surfaces

Cells - Cell Track Center of Image Mass X, Y, Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R - center of image mass (x, y, z)
$\mathbf{m_i}$ - voxel intensity
$\mathbf{r_i}$ - center of a voxel (x_i y_i z_i)
M - sum of voxel intensities

Cells - Cell Track Cytoplasm Intensity Max
This value describes the maximum voxel intensity of the Cytoplasm along the tracks.

Cells - Cell Track Cytoplasm Intensity Mean
This value describes the mean voxel intensity of the Cytoplasm along the tracks.

Cells - Cell Track Cytoplasm Intensity Min
This is the minimum value of all minimum (voxel) intensities of the cells along the tracks

Cells - Cell Track Cytoplasm Intensity Sum
This value describes the sum of voxel intensity of the Cytoplasm along the tracks.

Cells - Cell Track Cytoplasm Number of Vesicles Mean
The mean number of Vesicle objects along the track within the Cytoplasm.

Cells - Cell Track Displacement Length
The Track Displacement Length, tdl, is the distance between the first and last Cell's position.

$$tdl = |\vec{tdl}|$$

$$\vec{tdl}$$

defined in Track Displacement X, Y, Z

Cells - Cell Track Displacement X, Y, Z
The object's Track Displacement, tdl, is the distance between the first and last object's position, p, along the selected-axis.

$$\vec{tdl} = \vec{p}(n) - \vec{p}(1)$$

Cells - Cell Displacement Delta XYZ
The difference in position of the current object and the previous object of the same track.

Cells - Cell Displacement Delta Length (a scalar value)
The length of the position difference of the current object and the previous object of the same track.

Cells - Cell Track Duration
The Track Duration, td, is the duration between the first and last time point, T, within the track.

$$td = T(n) - T(1)$$

Cells - Cell Track Ellipsoid Axis A X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis

Cells - Cell Track Ellipsoid Axis B X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Cells - Cell Track Ellipsoid Axis C X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Cells - Cell Track Ellipsoid Axis Length A, B, C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Cells - Cell Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Cells - Cell Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Cells - Cell Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Cell along the tracks.

Cells - Cell Track Intensity Max
This is the maximum value of all maximum (voxel) intensities of the Cell along the tracks

Cells - Cell Track Intensity Mean
This is the weighted mean of the objects means, equal to the simple mean of all the intensities of all the

objects in a track. Larger objects have a larger impact on the track mean.

Cells - Cell Track Intensity Median
The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

Cells - Cell Track Intensity Min
This is the minimum value of all minimum (voxel) intensities of the Cell along the tracks.

Cells - Cell Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity Sum
This value is the sum of all sum (voxel) intensities of the Cell along the tracks.

Cells - Cell Track Length
The Track Length, tl, is the total length of displacements within the track.

$$tl = \sum_{t=2}^{n} |\vec{p}(t) - \vec{p}(t-1)|$$

Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected tack is continuous in nature.

Cells- Cell Track Number of Nuclei Mean
The mean number of Nuclei objects along the track.

Cells-Cell Track Number of Vesicle Mean
The mean number of Vesicle objects along the track.

Cells - VesicleTrack Number of Branches
The number of branches along the track.

Cells - Vesicle Track Number of Fusions
The number of fusions along the track.

Cells - Cell Tracks Position Mean X, Y, Z
Defines the mean value of X, Y, Z track positions.

Cells - Cell Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Cells - Cell Track Speed Max
The maximum value of the object's speed on the track.

Cells - Cell Track Speed Mean
The Average value of the object's speed on the track. If the Track has no Fuse (merge) or Fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Cell Track Speed Min
The minimum value of the object's speed on the track.

Cells - Cell Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Cells - Cell Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation

μ - Mean value

Cells - Cell Track Sphericity Mean
Describes the mean Cell object Sphericity along the tracks.

Cells - Cell Track Straightness

$$S = \frac{D}{L}$$

S - Track Straightness
D - Track Displacement
L - Track Length

Cells - Cell Track Volume Mean
The mean volume of all Cell objects along the tracks.

Note for all track statistics splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.

Cells - Cell Velocity X, Y, Z
The formulas for calculating the velocity, v, at time points (t) and (1) are calculated as follows:

For

And

$$\vec{v}(n) = \frac{\vec{p}\,(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

Cells - Cell Volume
Volume is quantification of how much space a Cell object occupies.

Cells - Cell Volume of Vesicles
Volume is quantification of how much space the Vesicles object occupies within the Cell.

Cells - Nucleus Acceleration
Acceleration, a(t), is the change in the Nucleus velocity over time.
Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \frac{\left(\frac{\vec{p}\,(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}\,(t) - \vec{p}(t-1)}{T(t) - T(t-1)}\right)}{\left(\frac{T(t+1) - T(t)}{2}\right)}$$

For

$$1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|$$

$$\vec{a}(t)$$

as defined in Acceleration X, Y, Z

Acceleration at the first and last time-point is 0.

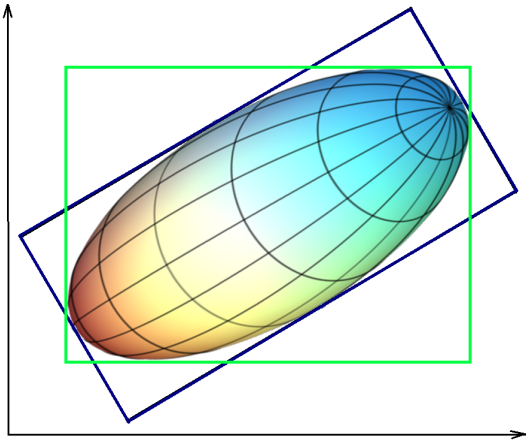
Cells - Nucleus Acceleration X, Y, Z
Acceleration is the change in the Nucleus velocity over time along the selected-axis.

Cells - Nucleus Area
The area is the sum of the outer Nucleus voxel faces.

Cells - Nucleus BoundingBoxAA Length X, Y, Z
A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.
If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X = max(X)-min(X)

BoundingBoxAA Length Y = max(Y)-min(Y)
BoundingBoxAA Length Z = max(Z)-min(Z)



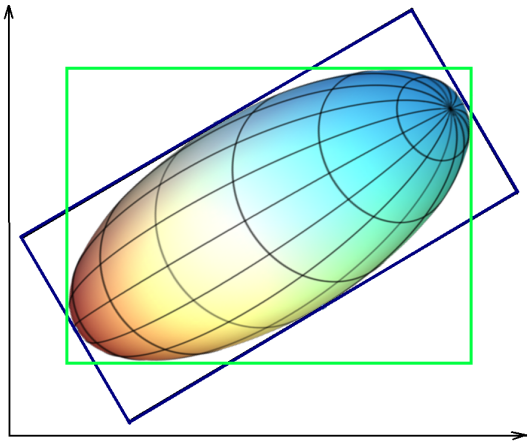
axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

Cells - Nucleus BoundingBoxOO Length A, B, C

The local axes of the box can be arbitrarily oriented with respect to the coordinate system.
BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis
BoundingBoxOO Length B = Length of the second longest principal axis
BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

Cells - Nucleus Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the ‘mass’ of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.
The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{ie\ object} r_i$$

R : center of homogeneous mass (x, y, z)
$\mathbf{r_i}$: center of a voxel (x_i y_i z_i)
n : number of i ∈ Nucleus

Cells - Nucleus Center of Image Mass X, Y, Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R - center of image mass (x y z)
$\mathbf{m_i}$ - voxel intensity
$\mathbf{r_i}$ - center of a voxel (x_i y_i z_i)
M - sum of voxel intensities

Cells - Nucleus Displacement X, Y, Z
The Displacement, d, is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

Cells - Nucleus Displacement²

$$d^2(t) = |\vec{d}(t)|^2$$

$$\vec{d}(t)$$

as defined in Displacement X Y Z

Cells - Nucleus Displacement Delta XYZ
The difference in position of the current object and the previous object of the same track.

Cells - Nucleus Displacement Delta Length (a scalar value)
The length of the position difference of the current object and the previous object of the same track.

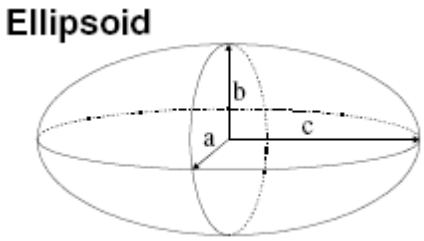
Cells - Nucleus Distance from Center of Mass to Cell Membrane
The minimum distance between the Nucleus centers of mass and the Cell surface border.

Cells - Nucleus Distance from Nucleus Membrane to Cell Membrane
The minimum distance between the Nucleus membrane surface and the Cell surface border.

Cells - Nucleus Distance from Position to Cell Membrane
The minimum distance between the Nucleus centers of homogeneous mass and the Cell surface border.

Cells - Nucleus Distance from Origin
The minimum distance between the Nucleus center of homogeneous mass and the origin.

Ellipsoid Axis



Cells - Nucleus Ellipsoid Axis A X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis a.

Cells - Nucleus Ellipsoid Axis B X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis b.

Cells - Nucleus Ellipsoid Axis C X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis c.

Cells - Nucleus Ellipsoid Axis Length A, B, C
Cells - Nucleus Ellipsoid (oblate)
Cells - Nucleus Ellipsoid (prolate)

If we assume $a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)

- a ≠ b ≠ c it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \times \left(1 - \frac{a^2 + b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \times \left(1 - \frac{2a^2}{b^2 + c^2}\right)$$

e_{oblate} = oblate Ellipsoid

Cells - Nucleus Intensity Center
Cells - Nucleus Intensity Max
Cells - Nucleus Intensity Mean
Cells - Nucleus Intensity Median
The Cells - Nucleus intensity median is estimated from the intensity histogram.
Cells - Nucleus Intensity Min
Cells - Nucleus Intensity StdDev
Cells - Nucleus Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Nucleus (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells- Nucleus Number of Tracks
This value describes the total number of the Nucleus object tracks.

Cells- Nucleus Number of Vesicles
The number of the Vesicle objects within the selected Nucleus object.

Cells-Nucleus Number of Voxels
Number of voxels within the selected Nucleus object.

Cells- Nucleus Position X, Y, Z
Defines the value of X, Y, Z Nucleus position (x y z). It is equal to Center of Homogeneous Mass of the object.

Cells - Nucleus Relative To Cell Acceleration
Acceleration is the change in the Nucleus velocity over time taking into account the relative Nucleus position.

Cells- Nucleus Relative To Cell Acceleration X, Y, Z
Acceleration is the change in the Nucleus velocity over time along the selected-axis, taking into account the relative Nucleus position.

Cells - Nucleus Relative To Cell Displacement X, Y, Z
The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position. The calculation is based on the relative Nucleus position.

Cells - Nucleus Relative To Cell Displacement^2
The Nucleus squared displacement, D, is calculated taking into account the relative Nucleus position.

$$\overline{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

t_L - last time index of track
t_F - first time index of track
$P_X(t)$ - x-position of object at time index t

Cells - Nucleus Relative To Cell Position X, Y, Z
The relative Nucleus position is calculated by subtracting the absolute position of the selected Nucleus from the absolute center position of the parent cell object.

Cells - Nucleus Relative To Cell Speed
The speed, s, is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Cells - Nucleus Relative To Cell Track Displacement Length

The Nucleus Relative To Cell Track Displacement Length is the distance between first and last Nucleus's relative position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

D - Track Displacement

$\mathbf{t_L}$ - last time index of track

$\mathbf{t_F}$ - first time index of track

$\mathbf{P_X(t)}$ - x-position of object at time index t

Cells - Nucleus Relative To Cell Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative Nucleus position.

Cells - Nucleus Relative To Cell Velocity X, Y, Z

The instant velocity vector, v, of an object that has positions x(t) at time t and x(t + Dt) at time t + Dt, can be computed as the derivative of the relative Nucleus position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Nucleus Speed

The Nucleus speed, s(t), is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Cells - Nucleus Sphericity
Sphericity, ψ, is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, of a particle is the ratio of the Nucleus surface area, a, of a sphere (with the same volume, v, as the given particle) to the Cell surface area of the particle:

$$\psi = \frac{\pi^{\frac{1}{3}}(6V_p)^{\frac{2}{3}}}{A_P}$$

Vp = volume of the particle
Ap = surface area of the particle

Cells - Nucleus Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Cells - Nucleus Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Cells - Nucleus Total Number of Voxels
Total number of voxels of the Nucleus voxels within the data set.

Cells - Nucleus Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Cells - Nucleus Track AR1 X, Y, Z
TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$AR1X = \begin{matrix} R_x^1 \\ R_x^0 \end{matrix}$$

AR1X - TrackAR1X

D - Track Displacement
t_L - last time index of track
t_F - first time index of track

$$D_x(t_2, t_1) = P_X(t_2) - P_X(t_1)$$

$P_X(t)$ - x-position of object at time index t

$$\overline{D}_x = \frac{1}{t_L - t_F} \sum_{t = t_f + 1}^{t_L} D_x(t, t-1)$$

t_L - last time index of track

t_F - first time index of track

Cells - Nucleus Track Area Mean

The mean of the triangle Nucleus object surfaces along the track.

Cells - Nucleus Track Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.
The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{ie\ object} r_i$$

R - center of homogeneous mass (x y z)

r_i - center of a voxel (x_i y_i z_i)

n - number of i $\in$ Nucleus

Cells - Nucleus Track Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each

channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R - center of image mass (x, y, z)
m_i - voxel intensity
r_i - center of a voxel (x_i y_i z_i)
M - sum of voxel intensities

Cells - Nucleus Track Displacement Length
The Track Displacement Length is the distance between first and last Nucleus position.

$$tdl = |\vec{tdl}|$$

$$\vec{tdl}$$

defined in Track Displacement X, Y, Z

Cells - Nucleus Track Displacement X, Y, Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Cells - Nucleus Track Duration
The Track Duration is the duration between the first and last time point within the track.

$$td = T(n) - T(1)$$

Cells - Nucleus Track Ellipsoid Axis A X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis a.

Cells - Nucleus Track Ellipsoid Axis B X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Cells - Nucleus Track Ellipsoid Axis C X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Cells - Nucleus Track Ellipsoid Axis Length A, B, C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Cells - Nucleus Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Cells - Nucleus Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Cells- Nucleus Track Intensity Center Mean

This value describes the mean voxel intensity in the center of the Nucleus along the tracks.

Cells- Nucleus Track Intensity Max
This value describes the maximum voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Mean
This value describes the mean voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Median
This value describes the Average of all Intensity Medians of all objects at all time points in the track.

Cells - Nucleus Track Intensity Min
This value describes the minimum voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Sum
This value describes the sum of voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Length
The Track Length, tl, is the total length of displacements within the track.

$$tl = \sum_{t=2}^{n} |\vec{p}(t) - \vec{p}(t-1)|$$

Cells - Nucleus Track Number of Branches
The number of track branches.

Cells - Nucleus Track Number of Fusions
The number of track fusions.

Cells - Track Number of Nucleus
The number of the all Nucleus objects along the tracks.

Cells - Nucleus Tracks Position Mean X, Y, Z
Defines the mean value of X, Y, Z Nucleus track positions.

Cells - Nucleus Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Cells - Nucleus Track Speed Max
The maximum value of the object's speed on the track.

Cells - Nucleus Track Speed Mean
The Average value of the object's speed on the track. If the Track has no fuses(merges) or fragments(splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Nucleus Track Speed Min
The minimum value of the object's speed on the track.

Cells - Nucleus Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Cells - Nucleus Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation

μ - Mean value

Cells - Nucleus Track Sphericity Mean
Describes the mean Nucleus object Sphericity along the tracks.

Cells - Nucleus Track Straightness

$$S = \frac{D}{L}$$

S - Track Straightness
D - Track Displacement
L - Track Length

Cells- Nucleus Track Volume Mean
The mean volume of all Nucleus objects along the tracks.

Cells - Nucleus Velocity X, Y, Z
The formulas for calculating the velocity at time points (t) and (1) are calculated as follows:

$$\vec{v}(t) = \frac{\vec{p}(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

And

Cells - Nucleus Volume
Volume is quantification of how much space a Nucleus objects occupies.

Cells - Number of Cell per Time Point
This value describes the number of Cell object in the selected time point.

Cells - Number of Nuclei per Time Point
This value describes the number of Nuclei object in the selected time point.

Cells - Number of Vesicles per Time Point
This value describes the number of Vesicles object in the selected time point.

Cells - Total Number of Cell
This value describes the total number of Cell objects.

Cells - Total Number of Nucleus
This value describes the total number of Nucleus objects.

Cells - Total Number of Vesicles
This value describes the total number of Vesicles objects.

Cells - Cell Track Number of Cells
The number of the all Cell objects along the tracks.

Cells - Track Number of Nuclei
The number of the all Nucleus objects along the tracks.

Cells - Track Number of Vesicles
The number of the all Vesicles objects along the tracks.

Cells - Vesicle Acceleration
Acceleration is the change in the Vesicle velocity over time. See

$$\vec{a}(t) = \frac{\left(\frac{\vec{p}(t+1)-\vec{p}(t)}{T(t+1)-T(t)} - \frac{\vec{p}(t)-\vec{p}(t-1)}{T(t)-T(t-1)}\right)}{\left(\frac{T(t+1)-T(t-1)}{2}\right)}$$

For

Acceleration at time-point t:

$$\vec{a}(t)$$

as defined in Acceleration X, Y, Z

Acceleration at the first and last time-point is 0.

Cells - Vesicle Acceleration X, Y, Z
Acceleration is the change in the Vesicle velocity over time along the selected-axis.

Cells - Vesicle Area
The area is the sum of the outer Vesicles voxel faces.

Cells - Vesicle Diameter X, Y, Z
This value describes the Vesicle diameter along the selected-axis.

Cells - Vesicles Displacement X, Y, Z
The Displacement, d(t), is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

Cells - Vesicles Displacement^2

$$d^2(t) = |\vec{d}(t)|^2$$

$$\vec{d}(t)$$

as defined in Displacement X Y Z

Cells - Vesicles Displacement Delta XYZ
The difference in position of the current object and the previous object of the same track.

Cells - Vesicles Displacement Delta Length (a scalar value)
The length of the position difference of the current object and the previous object of the same track.

Cells - Vesicle Distance to Cell Membrane
The distance, calculated by the distance map method, between the selected Vesicle centers and the Cell surface border.

Cells - Vesicle Distance to Closest Nucleus
The distance, calculated by the distance map method, between the selected Vesicle center and the closest Nucleus surface border. For Vesicles located inside a Nucleus, this distance is a negative number.

Cells - Vesicle Distance to Closest Nucleus Center
The distance between the selected Vesicle center and the center of the closest Nucleus. For Vesicles located inside a Nucleus, this distance is a negative number.

Cells - Vesicle Intensity Center
Cells - Vesicle Intensity Max
Cells - Vesicle Intensity Mean
Cells - Vesicle Intensity Median

The Cells vesicle intensity median is estimated from the intensity histogram.

Cells - Vesicle Intensity Min

Cells - Vesicle Intensity StdDev

Cells - Vesicle Intensity Sum

The voxel intensity statistics describe the voxels enclosed within the Vesicle (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells - Vesicle Location in Cytoplasm

The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the cytoplasm, while if the value is 0 the vesicle is located within the nucleus (the value of **Vesicle location in Nucleus** is set to 1).

Cells - Vesicle Location in Nucleus

The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the nucleus, while if the value is 0 the vesicle is located outside the nucleus (within the cytoplasm).

Cells - Vesicle Number of Tracks

This value describes the total number of the Vesicle tracks.

Cells - Vesicle Number of Voxels

Number of voxels within the selected Vesicle object.

Cells - Vesicle Position X, Y, Z

Defines the value of X, Y and Z Vesicle position (x y z). It is equal to Center of Homogeneous Mass of the object.

Cells - Vesicle Relative Position

This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.
The value is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.

Cell - Vesicle Relative To Cell Acceleration

Acceleration is the change in the Vesicles velocity over time taking into account the relative vesicle position.

Cell - Vesicle Relative To Cell Acceleration X, Y, Z

Acceleration is the change in the Vesicles velocity over time along the selected-axis, taking into account the relative vesicle position.

Cells - Vesicle Number of Cell per Time Point

This value describes the number of Vesicle object in the selected time point.

Cell - Vesicle Relative To Cell Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position. The calculation takes into account the relative Vesicle position.

Cell - Vesicle Relative To Cell Displacement^2

The vesicles squared displacement is calculated taking into account the relative vesicle position.

$$\overline{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_2, t_1) = P_X(t_2) - P_X(t_1)$$

D - Track Displacement
t_L - last time index of track
t_F - first time index of track
$P_X(t)$ - x-position of object at time index t

Cell - Vesicle Relative To Cell Position X, Y and Z
The relative Vesicles position is calculated by subtracting the absolute position of the selected Vesicles from the absolute center position of the parent cell object.

Cell - Vesicle Relative To Cell Speed
The speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Cells - Vesicle Relative To Cell Track Displacement Length
The Vesicle Relative To Cell Track Displacement Length is the distance between first and last Vesicle's relative position.

D - Track Displacement

t_L - last time index of track

t_F - first time index of track

$P_X(t)$ - x-position of object at time index t

Cell - Vesicle Relative To Cell Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative Vesicle position.

Cell - Vesicle Relative To Cell Velocity X, Y, Z

The instant velocity vector, v, of an object that has positions x(t) at time t and x(t + Dt) at time t + ∆t, can be computed as the derivative of the relative vesicle position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Vesicle Speed

The Vesicle speed, s(t), is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t + 1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t - 1)|}{T(t + 1) - T(t - 1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n - 1)|}{T(n) - T(n - 1)}$$

Cells - Vesicle Time

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Cells - Vesicle Time Index

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Cells - Vesicle Track AR1Mean

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Cells - Vesicle Track AR1 X, Y, Z

TrackAR1X is the AR1 coefficient of the model for the x-positions.

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$a\vec{r}1 = \frac{\sum_{t=2}^{n-1}\left(\vec{d}(t-1,t) - \vec{d}_{avg}\right) \times \left(\vec{d}(t,t+1) - \vec{d}_{avg}\right)}{\sum_{t=2}^{n}\left(\vec{d}(t-1,t) - \vec{d}_{avg}\right)^2}$$

with the displacement between two time points:

$$\vec{d}(t_1,t_2) = \vec{p}(t_2) - \vec{p}(t_1)$$

and the average displacement of the entire track:

$$\vec{d}_{avg} = \sum_{t=2}^{n} \frac{\vec{p}(t) - \vec{p}(t-1)}{n}$$

Cells - Vesicles Track Area Mean
The mean area of all Vesicle objects along the tracks.

Cells - Vesicles Track Diameter Mean
The mean diameter of all Vesicle objects along the tracks.

Cells - Vesicle Track Displacement Length
The Track Displacement Length, tdl, is the distance between first and last Vesicle's position.

$$tdl = |t\vec{d}l|$$

$$t\vec{d}l$$

defined in Track Displacement X, Y, Z

Cells- Vesicle Track Displacement X, Y, Z
The object's Track Displacement, tdl, is the distance between the first and last object's position along the selected-axis.

Cells - Vesicle Track Duration
The Track Duration, td, is the duration between the first and last time point within the track.

Cells - Vesicle Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Max

This value describes the maximum voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Mean
This value describes the mean voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Median
This value describes the Average of all Intensity Medians of all objects at all time points in the track.

Cells - Vesicle Track Intensity Min
This value describes the minimum voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Sum
This value describes the sum of voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Length
The Track Length, tl, is the total length of displacements within the track.

$$tl = \sum_{t=2}^{n} |\vec{p}(t) - \vec{p}(t-1)|$$

Cells - VesicleTrack Number of Branches
The number of track branches.

Cells - Vesicle Track Number of Fusions
The number of track fusions.

Cells- Vesicle Tracks Position Mean X, Y, Z
Defines the mean value of X, Y, Z Vesicles track positions.

Cells- Vesicle Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Cells- Track Number of Vesicle
The number of the all Vesicles objects along the tracks.

Cells - Vesicle Track Speed Max
The maximum value of the object's speed on the track.

Cells - Vesicle Track Speed Mean
The Average value of the object's speed on the track. If the Track has no Fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Vesicle Track Speed Min
The minimum value of the object's speed on the track.

Cells - Vesicle Track Speed Standard Deviation

The Standard Deviation value of the object's speed on the track.

Cells - Vesicle Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation

μ - Mean value

Cells - Vesicle Track Straightness

$$S = \frac{D}{L}$$

S - TrackStraightness
D - TrackDisplacement
L - TrackLength

Cells - Vesicles Track Volume Mean
The mean volume of all Vesicle objects along the tracks.

Cells - Vesicle Velocity X, Y, Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Dt, can be computed as the derivative of the position:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Vesicle Volume
Volume is quantification of how much space a Vesicle objects occupy.

Velocity Angle
The velocity angle is the angle between the velocity vector and the corresponding axis.

Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Event Time Statistics

Please see the Common Statistics chapter for statistics common to all objects for Event Time Statistics.

Track Class Duration Statistics

Please see the Common Statistics chapter for statistics common to all objects for Track Class Duration Statistics.

Lineage Statistics

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

Generations
This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

Track Number of Generations
This statistic shows the number of generations present in the selected track.

Time since first Division
This shows the time elapsed since the first division of the selected object(s).

Time since previous Division
This shows the time between each generation. e.g. the lifespan of an object

Normalized Time since previous Division
Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

Displacement Since Previous Division
This describes the displacement that has occurred since the previous division. The Displacement^2 (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

Time since track start
Displays time since the start of the track. This is only available only for objects that belong to a track. It is different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement^2 over track time start, as in the example at “Displacement Squared Plot” at http://bradleymonk.com/Brownian_Motion.

Cell Cycle Duration
Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

Cell Cycle Displacement
This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division^2 for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

5.11.9.2 Filament

Filament Object Terminology

Filament
The filament created in Imaris is a connected graph-based object. The graph consists of vertices which are

connected by edges.

Vertex
The filament graph consists of vertices and edges. A vertex is a point on the graph and defines the center axis of a segment or spine (see points in Figure 1 and 6).

Edge
An edge is the connection between two vertices. Given vertices, the edges define how to connect the vertices to construct the graph.

Segment
A segment is the part from one end point or a branch point of the graph to the other end point or another branch point of the graph. Segments are classified either as filament segments or spine segments.

Spine
A spine is a sequence of vertices connected by edges, the spine starts at the attachment point and ends at a terminal point. Spines branch off from segments. Spines are comprised of neck, head and ground components.

Neck
The spine neck is defined as all edges that are completely outside the spine attachment and spine terminal point. Note neck is only a component for spines.

Head
The spine head is 25% of the total spine length. Note head is only a component for spines.

Ground
Ground is the remainder of the spine once the neck and head have been accounted for. Note ground is only a component for spines.

Branch
The filament graph is a tree-based structure which has a root point. If traveling along the graph from the root point, the structure can split into several parts. A branch is defined as one of the parts which branches off from a splitting point. After each splitting point on the graph, a branch can be defined, therefore branches can have different levels. Please see Segments Branch Level for a full description.

A simplified representation of the filament tree is shown below in Figure 1 with (•) representing a segment vertex, and (**x**) a spine vertex. Segment and Spine edges are represented with (—) or (····) respectively. Filament segments are noted with D and spine segments with S.

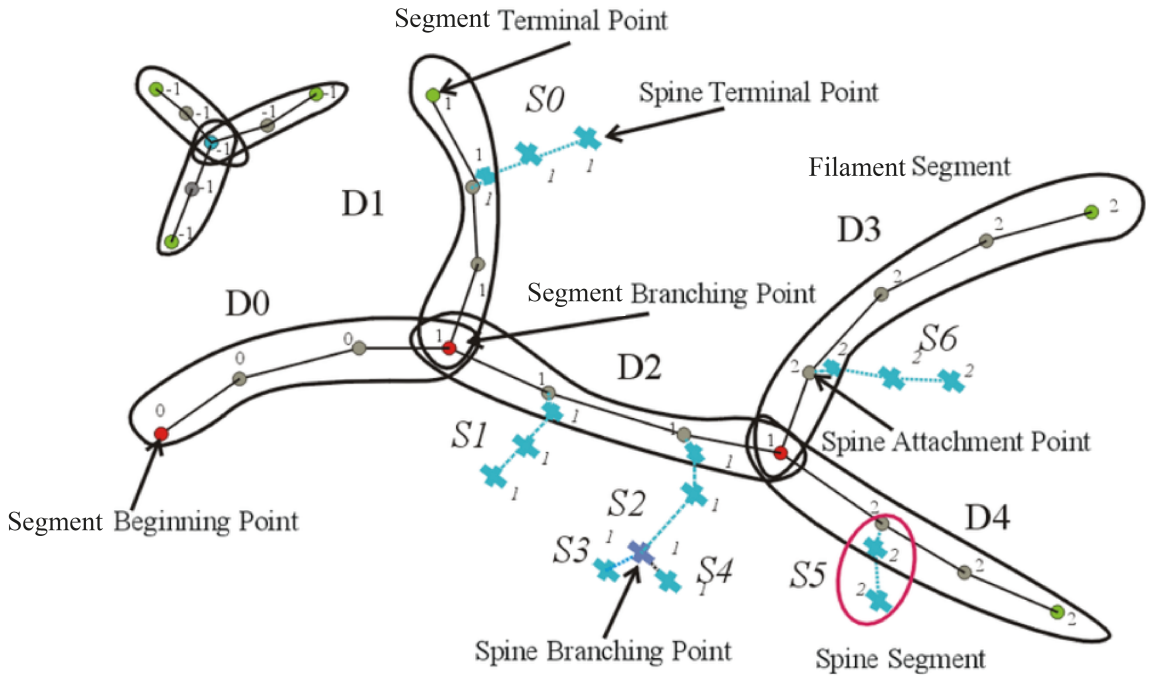


Figure 1

An additional annotated example of the filament structure, terminology and labeling is provided in the Filament section.

Please see the Common Statistics Chapter for statistics common to all objects.

Track Class Duration Statistics

Please see the Common Statistics chapter for statistics common to all objects for Track Class Duration Statistics.

Event Time Statistics

Please see the Common Statistics chapter for statistics common to all objects for Event Time Statistics.

Statistic Values - Filament

Filaments - Filament Acceleration

Acceleration is the change in the filament graph velocity over time.
Object position at time-point *i*:

Acceleration vector x, y, z at time-point *i*

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 \times (t(i+1) - t(i-1))}$$

Acceleration at time-point i:

$$a(i) = |A(i)|$$

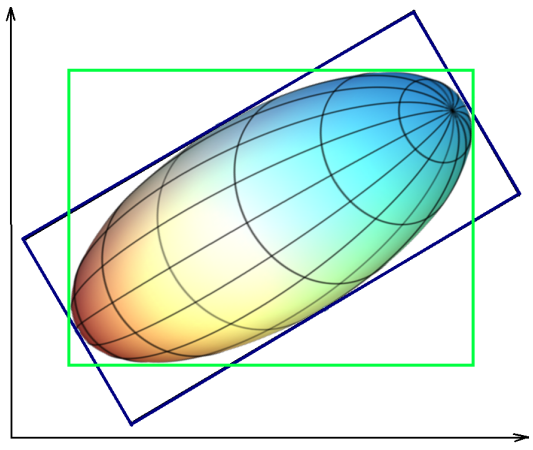
Acceleration at the first and last time-point is 0.

Filaments - Filament Acceleration X, Y, Z
Acceleration is the change in the filament graph velocity over time along the selected-axis.

Filaments - Filament Area (sum)
Defined as the sum of the areas of all filament areas within the graph.

Filaments - BoundingBoxAA Length X, Y, Z
A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.
If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). *BoundingBoxAA* identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X = max(X)-min(X)
BoundingBoxAA Length Y = max(Y)-min(Y)
BoundingBoxAA Length Z = max(Z)-min(Z)



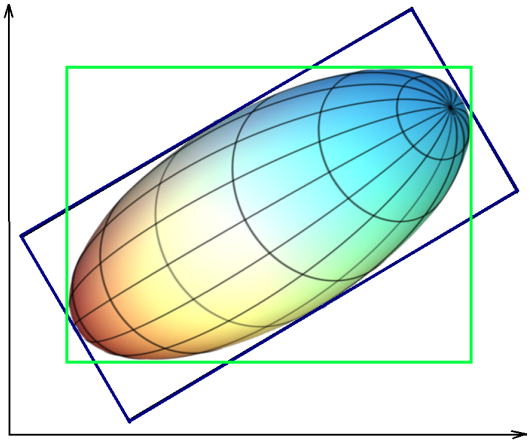
axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

Filaments - BoundingBoxOO Length A, B, C
The local axes of the box can be arbitrarily oriented with respect to the coordinate system.

BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis
BoundingBoxOO Length B = Length of the second longest principal axis
BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

Filaments - Filament Segment Area (sum)

The sum of all segment areas of within the entire filament graph.

Filaments - Filament Segment Length (sum)

The sum of all segment length of within the entire filament graph.

Filaments - Filament Segment Volume (sum)

The sum of the volumes of all segment within the entire filament graph.

Filaments - Filament Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

Filaments - Filament Displacement ^{2}

The square of the displacement of the object from the starting point of the track. (Plotting the squared displacement against the value 'Time since track start' is useful for MSD analysis).

t_F = first time index of track

$P_x(t)$ = x-position of object at time index t

Filaments - Filament Distance from Origin
The distance between the center position of the filament and the origin position (0,0,0). The center position of the filament is the average position of all vertices of the filament object.

Filaments - Filament Full Branch Depth
Branch Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the filament graph. Full Branch Depth is the highest value of Branching Depth for the graph (in Figure 1 value is 2). If no beginning point is defined, the value is set to "-1" (Figure1).

Filaments - Filament Full Branch Level
Full Branch Level is the highest value of Branching Level for the entire filament graph (in Figure 7 value is 4).

Filaments - Filament Length (Sum)
Defined as the sum of the lengths of all edges (both segments and spines) within the entire graph.

Filaments - Filament No. Segment Branch Points
The number of segment branching points in the entire filament graph (in Figure 1 value is 3).

Filaments - Filament No. Segment Branches
The statistics are defined for a filament and a distance value along the segment. This is calculated by following the filament from the segment beginning point along all segments for the defined distance, and then counting how many different branches have been reached. Imaris calculates this as follows:

*Distances 0 to n * DistanceIncrement*

where *DistanceIncrement* has a default value of 1 μm and *n* is dependent on the maximum segment terminal point distance to the beginning point of the filament. Note that the *DistanceIncrement* is equal to the "Sholl Spheres Resolution" and can be adjusted in the Statistics Preferences. The different distance values are visible as Factors "Distance" of the statistics value.

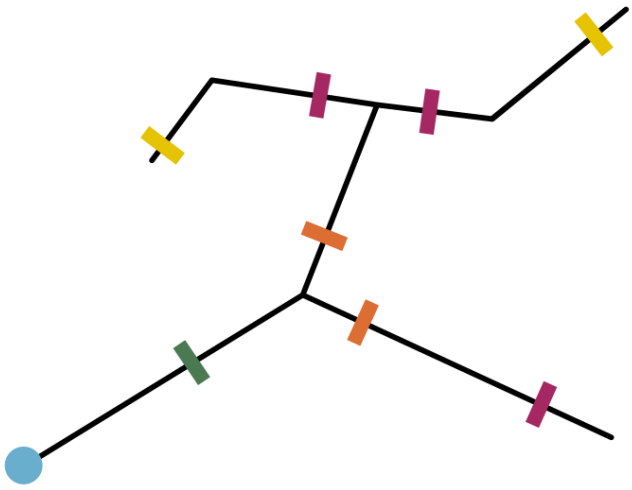


Figure 2 Filament with 7 segments, beginning point at the bottom left and indicators marking the segment branches for different distances.

Distance along segment from segment beginning point	No of Segment Branches
0	0
1	1
2	2
3	3
4	2
5	0

Filaments - Filament No. Segment Segments
The number of segment segments in the entire filament graph (Figure 1 value is 8).

Filaments - Filament No. Segment Terminal Points
The number of segment terminal points (excluding the beginning point) in the entire filament graph (Figure 1 value is 6).

Filaments - Filament No. Edges
The number of connections between vertices in the entire filament graph (Figure 1 value is 34).

Filaments - Filament No. Sholl Intersections
Sholl analysis creates a series of concentric circles (spheres in 3D) around the beginning point of the filament. Filament No. Sholl Intersections is defined as the number of segment intersections (branches) on concentric spheres, defining segment spatial distribution as a function of distance from the beginning point. All spheres have a center at the beginning point (Figure 3). The 1.0 um step resolution for the spheres can be changed under Preferences -> Advanced -> SurfaceAppFilament

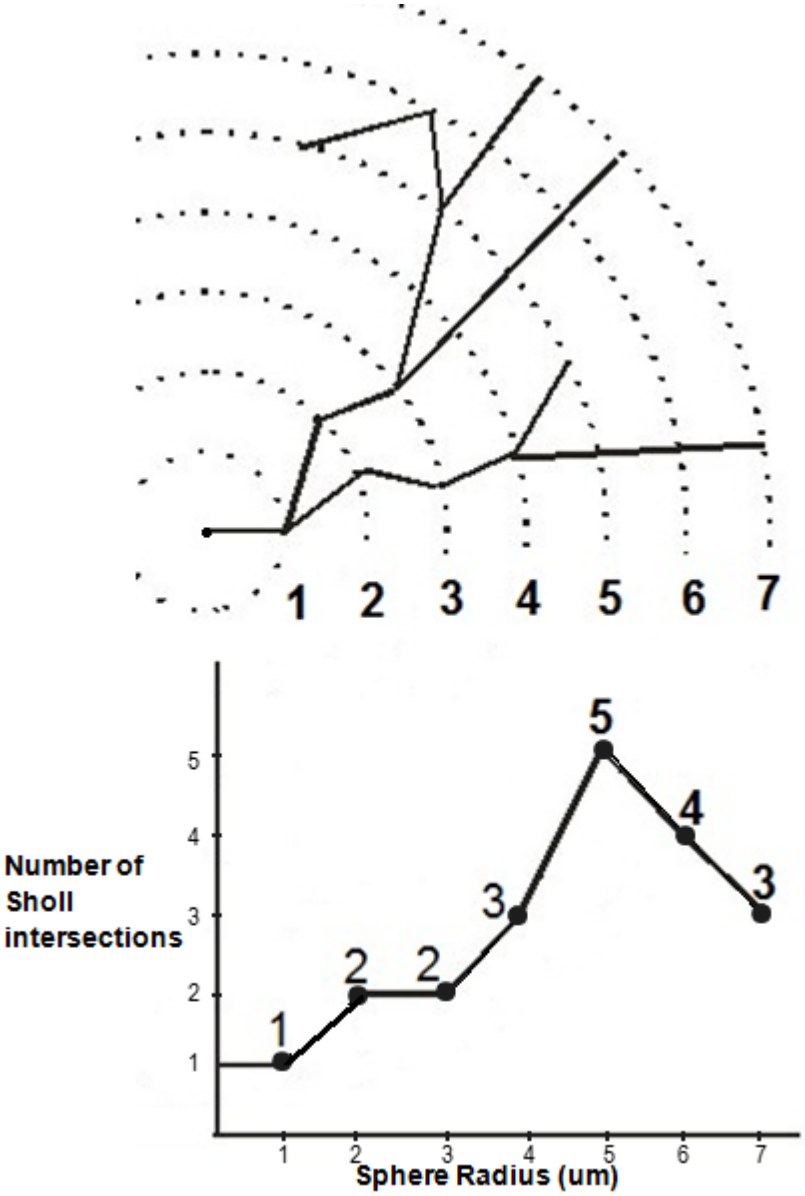


Figure 3

Filaments - Filament No. Spine Branching Points
The number of spine branch points in the entire filament graph (in Figure 1 value is 1). A spine branch point will only exist in a single spine that has more than one terminal point.

Filaments - Filament No. Spine Segments
The spine segment count in entire filament graph (in Figure 1 value is 7).

Filaments - Filament No. Spine Terminal Points
The number of spine terminal points in the entire filament graph (in Figure 1 value is 6).

Filaments - Filament No. Unconnected Parts
The number of unconnected filament graph within the image.

Filaments - Filament No. Vertices

The number of vertices in the filament graph (Figure 1 value is 36).

Filaments – Filament Number of Filament per Time Points
Totals number of filament graph within the image at the specific time point.

Filaments – Filament Number of Tracks
Totals number of tracks for the selected filament graph.

Filaments – Filament Position X, Y, Z
Filament graph X, Y, Z position within the viewing area. The value is equal to Center of Homogeneous Mass of the object.

Filaments – Filament Speed
The filament speed is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Filaments - Filament Spine Area (sum)
Defined as the sum of the areas of all spines segments within the filament graph.

Filaments - Filament Spine Length (sum)
The sum of all spines length of within the entire filament graph.

Filaments - Filament Spine Volume (sum)
The sum of the volumes of all spines within the entire filament graph.

Filaments - Filament Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Filament Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Filaments - Filament Total Number of Filaments

The value indicates the total number of filament graph within the data set.

Filaments - Filament Velocity Angle X, Y, Z
The velocity angle is the angle between the velocity vector and the corresponding axis.
Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Filaments - Filament Velocity X, Y, Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector.
The formula for calculating the velocity $v(i)$ at time point i is calculated as follows:

For objects at time point i at the beginning of a track the velocity $v(i)$ is computed from the position $x(i)$ and time $t^{(i)}$ of the object at time point i and $i+1$:

$$v(i) = \frac{x(i+1) - x(i)}{t(i+1) - t(i)}$$

For objects at time point i in the middle of a track the velocity $v(i)$ is computed from the position $x(i)$ and time $t^{(i)}$ of the object at time point $i-1$ and $i+1$:

$$v(i) = \frac{x(i+1) - x(i-1)}{t(i+1) - t(i-1)}$$

For objects at time point i at the end of a track the velocity $v(i)$ is computed from the position $x(i)$ and time $t^{(i)}$ of the object at time point $i-1$ and i:

$$v(i) = \frac{x(i) - x(i-1)}{t(i) - t(i-1)}$$

Filaments - Filament Volume (sum)

Volume is the quantity of three-dimensional space occupied by an object. The sum of all segment's volumes within the entire filament graph.

Statistic Values - Filament Track

Filaments - Filament Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Note for all track statistics splits or gaps are not taken into account- it is taken that the selected tack is continuous.

Filaments - Filament Track AR1 X, Y, Z
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

t_L = last time index of track

t_F = first time index of track

$$D_x(t_2, t_1) = P_X(t_2) - P_X(t_1)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_x = \frac{1}{t_L - t_F} \sum_{t=t_f+1}^{t_L} D_x(t, t-1)$$

$P_X(t)$ = x-position of object at time index t

t_L = last time index of track

t_F = first time index of track

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

Filaments - Filament Track Displacement Length

The Track Displacement Length is the distance between first and last filament graph position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

D = Track Displacement

t_L = last time index of track

t_F = first time index of track

$P_X(t)$ = x-position of object at time index t

Filaments - Filament Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Filaments - Filament Track Duration
The Track Duration is the duration between the first and last time point within the track.

$$Duration = T(t_L) - T(t_F)$$

Duration = Track Duration
T(t) = time in seconds at time point t
$\mathbf{t_L}$ = last time index of track
$\mathbf{t_F}$ = first time index of track

Filaments - Filament Track Length
The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = Track Length
$\mathbf{t_L}$ = last time index of track
$\mathbf{t_F}$ = first time index of track

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

Filaments - Filament Track No. Branches
The number of parallel outstretched branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

Filaments - Filament Track Number of Filaments
The total number of filament graphs along the track.

Filaments - Filament Track Number of Fusions
A fusion occurs when a filament object is connected to several filament objects of a past time point. The value indicates the number of the related objects.

Filaments - Filament Track Position X, Y, Z Mean
Defines the mean value of X, Y, Z Track positions.

Filaments - Filament Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Filaments - Filament Track Speed Max
The maximum value of the object's speed on the track.

Filaments - Filament Track Speed Mean
The Average value of the object's speed on the track. If the Track has no fuses (merges) or fragments (splits),

then the average speed is given by the Track length divided by the time between first and last object in the Track.

Filaments - Filament Track Speed Min
The minimum value of the object's speed on the track.

Filaments - Filament Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Filaments - Filament Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation
μ - Mean value

Filaments - Filament Track Straightness

$$S = \frac{D}{L}$$

S = Track Straightness
D = Track Displacement
L = Track Length

Statistic Values - Points

Pt
Statistics available for certain types of vertices (points) which are **Segment Terminal, Segment Branch, Spine Terminal, Spine Branch, and Spine Terminal**.

Filaments - Pt Acceleration
Acceleration is the change in the object velocity over time.
Object position *P* at time-point *i*:

Acceleration vector x, y, z at time-point *i*

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 \times (t(i+1) - t(i-1))}$$

Acceleration at time-point i:

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

Filaments - Pt Acceleration X, Y, Z
Acceleration is the change in the filament points velocity over time along the selected-axis.

Filaments - Pt Branching Angle
The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 4 a angles are marked with δ). This value is defined only for filament graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the branching segments (in Figure 4 b angles marked with δ_2). The value is not calculated for branch points with more than three segments.

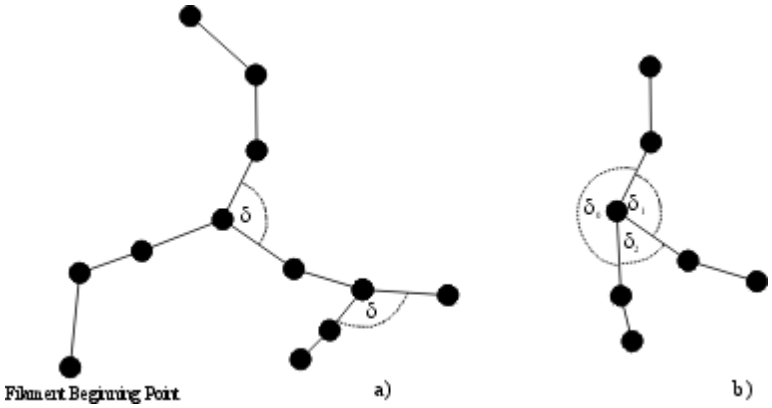


Figure 4

Filaments - Pt Diameter
Defined as the value of the vertices's diameter (Figure 6).

Filaments - Pt Displacement Delta Length (a scalar value)
The length of the position difference of the current object and the previous object of the same track.

Filaments - Pt Displacement Delta XYZ
The difference in position of the current object and the previous object of the same track.

Filaments - Pt Displacement Length
Displacement Length is the length of Displacement (X,Y,Z) (it is a statistics value for Pt).

$$D_l = \sqrt{x^2 + y^2 + z^2}$$

Filaments - Pt Displacement X, Y, Z
The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

Filaments - Pt Displacement ^2

$$\overline{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_L} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

$\mathbf{t_L}$ = last time index of track

$\mathbf{t_F}$ = first time index of track

$\mathbf{P_X(t)}$ = x-position of object at time index t

Filaments - Pt Distance
Length between the vertex and the filament root vertex along the filament structure. If there is no root point, the distance is -1.

Filaments - Pt Distance from Origin
The distance between the vertex (point) and the origin position (0,0,0).

Filaments - Pt Number of Points per Time Point
Number of all beginning, branching, attachment and terminal points in the selected time point.

Filaments - Pt Number of Tracks
It is the total number of tracks of the Pt (vertex). As said before, there are only five special vertex for Pt. Each type vertex is used for building the track.

Filaments - Pt Position X, Y, Z
The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.
These values are only available if the tracking option is selected.

Filaments - Pt Speed
The filaments - Pt speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Filaments - Pt Spine Total Area
This is defined as the sum of the Area of all Spines connected to the Spine Attachment Point.

Filaments - Pt Spine Total Length
This is defined as the sum of the Length of all Spines connected to the Spine Attachment Point.

Filaments - Pt Spine Total Volume
This is defined as the sum of the Volume of all Spines connected to the Spine Attachment Point.

Filaments - Pt Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Pt Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Filaments - Pt Time Since Track Start
Time since track start Displays time since the start of the track. This is only available for objects that belong to a track. It is different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement^2 over track time start, as in the example at “Displacement Squared Plot” at http://bradleymonk.com/Brownian_Motion.

Filaments - Pt Total Number of Points
Number of all beginning, branching, attachment and terminal points.

Filaments - Pt Velocity Angle X, Y, Z
The velocity angle is the angle between the velocity vector and the corresponding axis.
Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Filaments - Pt Velocity X, Y, Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector v of an object that has positions x(t) at time t and x(t + dt) at time t + Dt, can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Statistic Values - Points Track

Filaments - Pt Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Filaments - Pt Track AR1 X, Y, Z
TrackAR1X is the AR1 coefficient of the model for the x positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

$\mathbf{t_L}$ = last time index of track

$\mathbf{t_F}$ = first time index of track

$$D_x(t_2, t_1) = P_X(t_2) - P_X(t_1)$$

$\mathbf{P_X(t)}$ = x-position of object at time index t

$$\bar{D}_x = \frac{1}{t_L - t_F} \sum_{t=t_f+1}^{t_L} D_x(t, t-1)$$

$\mathbf{t_L}$ = last time index of track

$\mathbf{t_F}$ = first time index of track

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

Filaments - Pt Track Displacement Length
The Track Displacement Length is the distance between first and last point position.

D = Track Displacement
$\mathbf{t_L}$ = last time index of track

t_F = first time index of track

$P_X(t)$ = x-position of object at time index t

Filaments - Pt Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Filaments - Pt Track Duration

The Track Duration is the duration between the first and last time point within the track.

$$Duration = T(t_L) - T(t_F)$$

Duration = Track Duration

T(t) = time in seconds at time point t

t_L = last time index of track

t_F = first time index of track

Filaments - Pt Track Length

The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = Track Length

t_L = last time index of track

t_F = first time index of track

$$D_x(t_F,t_L) = P_X(t_L) - P_X(t_F)$$

Note for all track statistics splits or gaps are not taken into account - it is taken that the selected tack is continuous.

Filaments - Pt Track No. of Branches

The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

Filaments - Pt Track Number of Fusions

A fusion occurs when a filament graph beginning, branching, attachment and terminal points are connected to several graph key points of a past time point. The value indicates the number of the related objects along the selected track.

Filaments - Pt Track Number of Points

The total number of points (beginning, branching, attachment and terminal) along the track.

Filaments - Pt Track Position X, Y, Z Mean
Defines the mean value of X, Y, Z Track positions.

Filaments - Pt Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Filaments - Pt Track Speed Max
The maximum value of the object's speed on the track.

Filaments - Pt Track Speed Mean
The Average value of the object's speed on the track. If the Track has no fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

Filaments - Pt Track Speed Min
The minimum value of the object's speed on the track.

Filaments - Pt Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Filaments - Pt Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation

μ - Mean value

Filaments - Pt Track Spine Area Max
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 5).
The value is maximal spine area along the track.

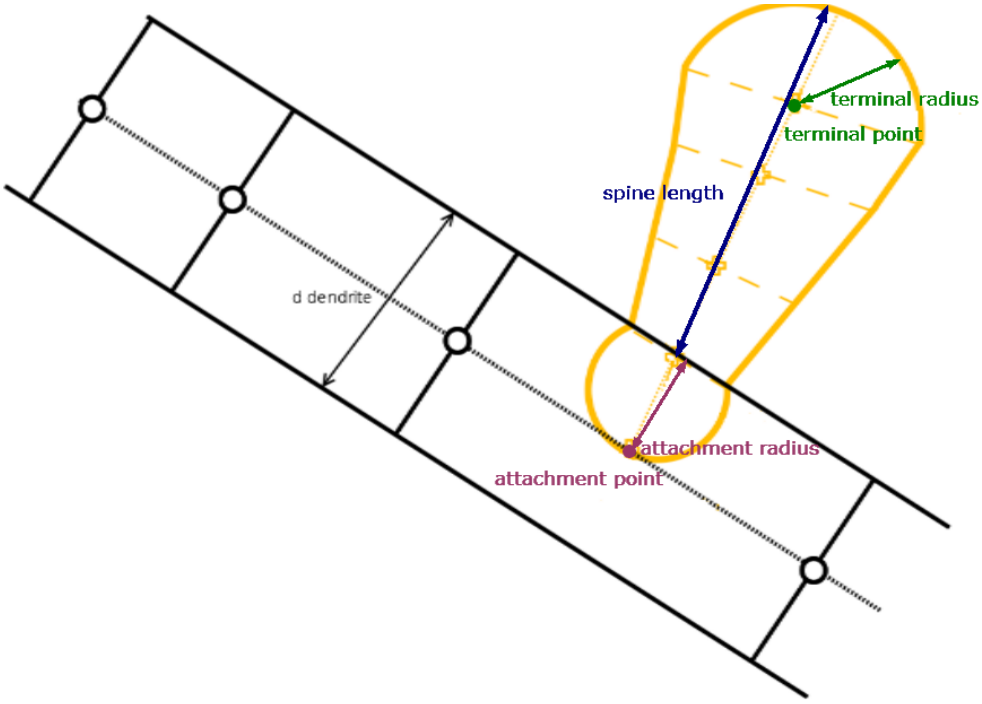


Figure 5

Filaments - Pt Track Spine Area Mean
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 5).
The value is mean spine area along the track.

Filaments - Pt Track Spine Area Min
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 5).
The value is minimal spine area along the track.

Filaments - Pt Track Spine Length Max
The value is the maximal spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

Filaments - Pt Track Spine Length Min
The value is the minimal spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

Filaments - Pt Track Spine Length Mean
The value is the mean spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

Filaments - Pt Track Spine Volume Max
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 5).
The value is maximal spine volume along the track.

Filaments - Pt Track Spine Volume Min

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 5).
The value is minimal spine volume along the track.

Filaments - Pt Track Spine Volume Mean
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 5).
The value is mean spine volume along the track.

Filaments - Pt Track Spine Straightness
Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Segment Straightness, refer to Figure 11).

Statistic Values - Segments

Filaments - Segment Area
This may be defined as the sum of the areas of all the segment edges.
The area of an edge is defined as a surface area of a frustum (truncated cone).

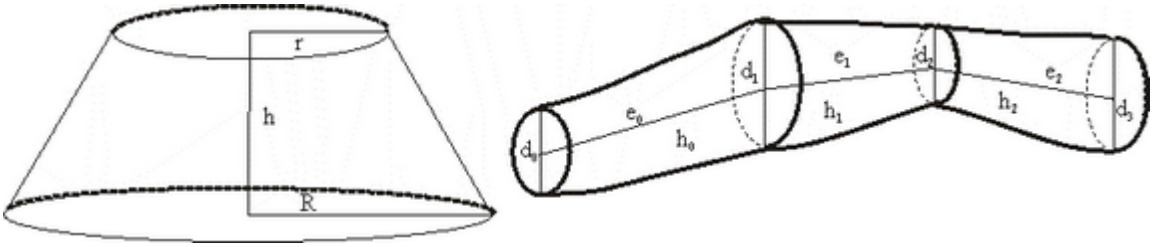


Figure 6

$$A = \sum A(i)_2, i = 0, \ldots n - 1, n$$

n = number of edges of a segment

Filaments - Segment Branch Depth
Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the dendritic graph.

Filaments - Segment Branch Level
The Segment Branch Level is a numerical structure that starts unfolding from the filament beginning toward the terminal points, assigning Branch Level to segments at each branching point.
The Branch Level number is assessed according to the diameter calculations of the individual segments. The initial Branch Level at the beginning point is 1. At each branching point, the filament segment with smaller mean diameter sequentially increases Branch Level, while the filament segment with a greater diameter maintains the same Branch Level.
In the case of two segments with the same diameter, the segment with a smaller Branching Angle (refer to Figure 7) keeps the Branch Level, while the segment with a greater Branching Angle sequentially raises its

Branch Level.
In the Figure 7 diameter d_1 is greater than d_2 ($d_1 > d_2$) and d_3 and d_4 are equal ($d_3 = d_4$). The Segment Branching Angle α_1 is smaller than α_2 ($\alpha_1 < \alpha_2$).

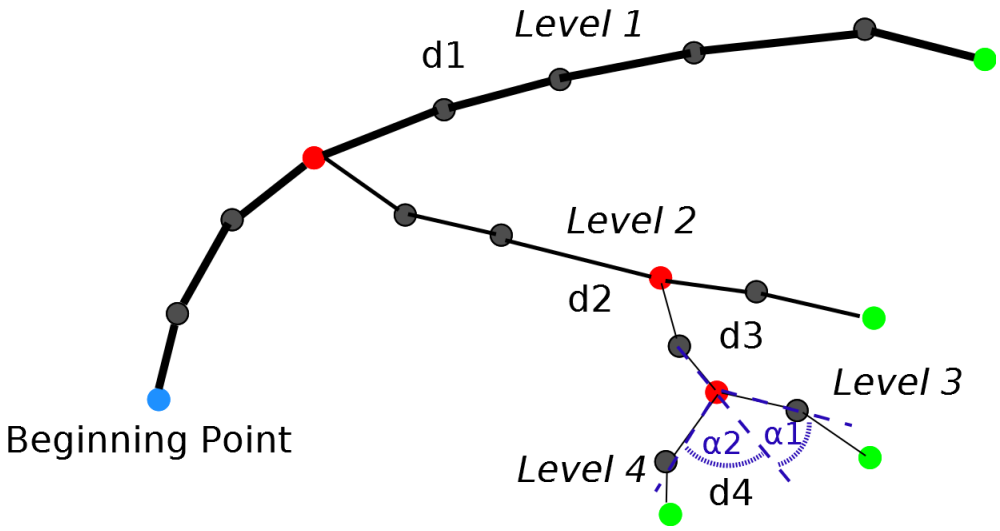


Figure 7

• represents a segment vertex

Filaments - Segment Branching Angle
Defined as the angle between the extending lines from the branch point and its peripheral neighbor vertex (Figure 8 angles marked with dashed blue arcs, α_1 for segment D1 and α_2 for segment D2). Defined only if the beginning point is determined.

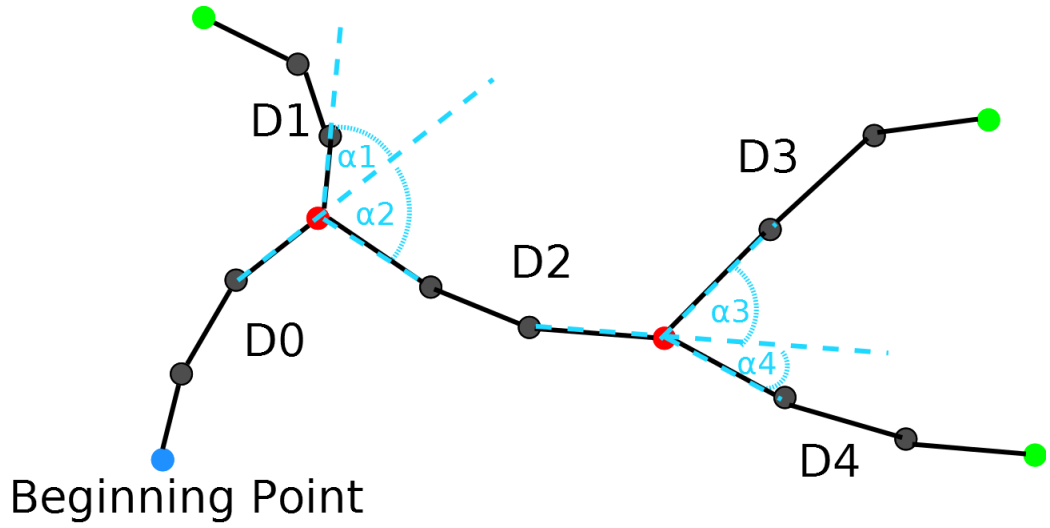


Figure 8

Please note • represents a sgementvertex

Filaments - Segment Branching Angle B
The angle between the extending lines connecting the branch point with the neighboring branch points and the

terminal points, respectively (Figure 9 angles marked with dashed green arcs, β). Defined only if the beginning point is determined.

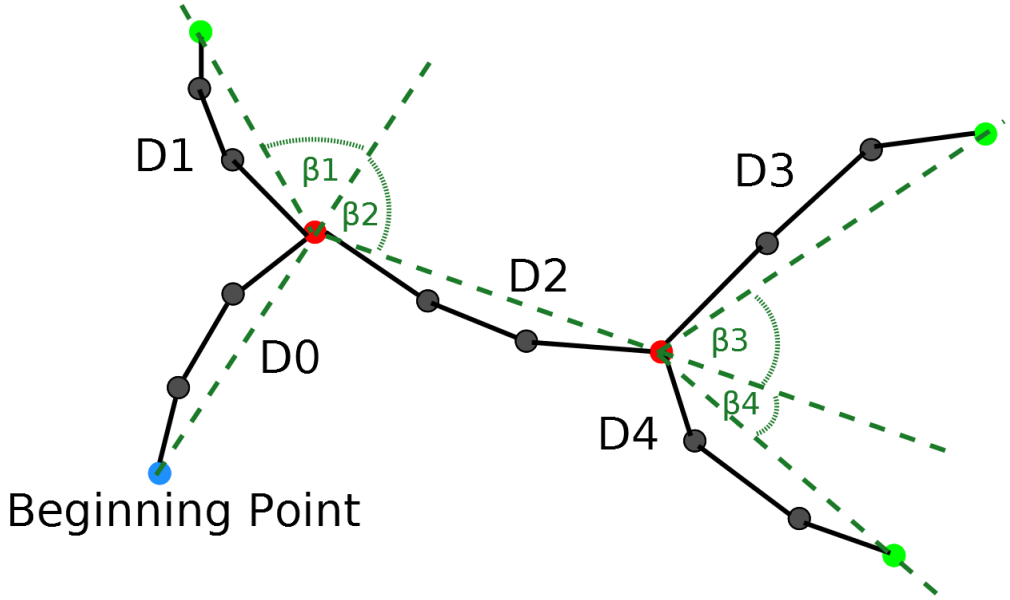


Figure 9

• represents a segment vertex

Filaments - Segment Centre Intensity Max
The maximum intensity of a segment centre voxel

Filaments - Segment Centre Intensity Mean
The mean intensity of a segment centre voxel

Filaments - Segment Centre Intensity Median
The median intensity of a segment centre voxel

Filaments - Segment Centre Intensity Min
The minimum intensity of a segment centre voxel

Filaments - Segment Centre Intensity StdDev
The standard deviation intensity of a segment centre voxel

Filaments - Segment Centre Intensity Sum
The sum intensity of all voxels in a segment centre

Filaments - Segment Centre Number of Voxel
The number of voxels in a segment centre

Filaments - Segment Intensity Max
The maximum intensity of segment voxels

Filaments - Segment Intensity Mean
The mean intensity of segment voxels

Filaments - Segment Intensity Median
The median intensity of segment voxels

Filaments - Segment Intensity Min
The minimum intensity of segment voxels

Filaments - Segment Intensity StdDev
The standard deviation intensity of segment voxels

Filaments - Segment Intensity Sum
The sum intensity of all voxels in a segment

Filaments - Segment Number of Voxel
The number of voxels in a segment

Filaments - Segment Length
Defined as the sum of all edges between two branch points or between a branch point and a terminal point, respectively. (Figure 6 Segment Length e0+e1+e2).

Filaments - Segment Mean Diameter
The mean value of all segment diameters measured from the center to the segment surfaces using the lower threshold (automatic creation) value.

Filaments - Segment No of Spines
The number of spines branching off a filament segment (in Figure 1 D0 0, D11, D2 2, D3 1, D4 1)

Filaments - Segment Orientation Angle
The angle formed between extending line connecting distal vertices of the filament segment and X-axis of image within the XY plane (Figure 10 angles marked with a dashed orange arcs, γ).

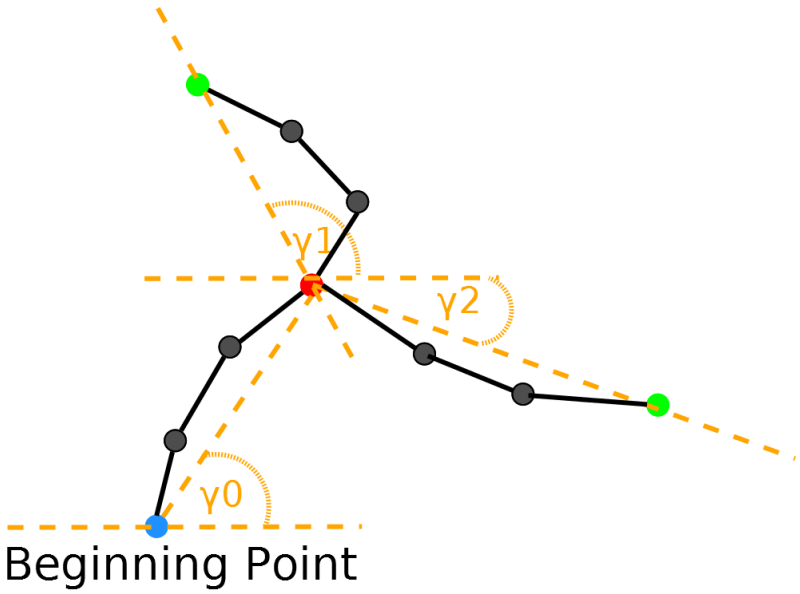


Figure 10

• represents a segment vertex

Filaments – Segment Position X, Y and Z

Segment position within the viewing area on selected axis. The value is equal to Center of Homogeneous Mass of the object.

Filaments - Segment Resistance

Measure of a segment's ability to conduct an electric current. The segment resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account diameter variations. It has units of 1/µm and must be multiplied by specific material resistance in order to obtain a value of a resistor. The resistance is calculated for every pair of consecutive points in the filament.

$$\Omega = \sum \left(\frac{L_{ab}}{\pi \, r_a \, r_b} \right)$$

For all pairs of connected a and b points in the filament

Our model of the filament between two points is a truncated cone with r_a on one side and r_b on the other. The formula is explained here: https://studylib.net/doc/18332217/calculating-resistance-truncated-cone-solution
Note that any point with radius (almost) 0 leads to (almost) infinite resistance, no matter the average segment diameter.

Filaments - Segment Spine Density

Defined as the number of spines per unit length of segment.

Filaments - Segment Straightness

Defined as the ratio between segment length and radial distance between two branch points (h) (Figure 11 value is always smaller than 1. If the segment Straightness is 1 that means that the segment is completely straight.

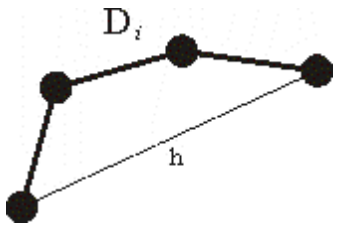


Figure 11

• represents a segment vertex

Filaments - Segment Time

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Segment Time Index

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Filaments - Segment Volume

Defined as the sum of all (frustum) volumes composing a segment (Figure 6).

$$V = \sum V(i)\ i - 0, \ldots n - 1, n$$

n =number of edges of a segment

Soma Statistics

The statistics available for Soma are listed below, their definitions are available in the Surfaces statistics section. For example, the **Soma Area** can be defined in the same way as the **Surfaces - Area.**

Soma Area
Soma BoundingBoxAA Length
Soma BoundingBoxAA Length X
Soma BoundingBoxAA Length Y
Soma BoundingBoxAA Length Z
Soma BoundingBoxOO Length
Soma BoundingBoxOO Length A
Soma BoundingBoxOO Length B
Soma BoundingBoxOO Length C
Soma Center of Homogeneous Mass X
Soma Center of Homogeneous Mass Y
Soma Center of Homogeneous Mass Z
Soma Center of Image Mass X
Soma Center of Image Mass Y
Soma Center of Image Mass Z
Soma Distance from Origin
Soma Distance to Image Border XY
Soma Distance to Image Border XYZ
Soma Ellipsoid Axis A
Soma Ellipsoid Axis A X
Soma Ellipsoid Axis A Y
Soma Ellipsoid Axis A Z
Soma Ellipsoid Axis B
Soma Ellipsoid Axis B X
Soma Ellipsoid Axis B Y
Soma Ellipsoid Axis B Z
Soma Ellipsoid Axis C
Soma Ellipsoid Axis C X
Soma Ellipsoid Axis C Y
Soma Ellipsoid Axis C Z
Soma Ellipsoid Axis Length A
Soma Ellipsoid Axis Length B
Soma Ellipsoid Axis Length C
Soma Ellipticity (oblate)
Soma Ellipticity (prolate)
Soma Generation
Soma Intensity Center
Soma Intensity Max Ch=1 Img=1
Soma Intensity Mean Ch=1 Img=1
Soma Intensity Median
Soma Intensity Min Ch=1 Img=1

Soma Intensity StdDev
Soma Intensity Sum Ch=1 Img=1
Soma Intensity Sum of Square
Soma Number of Disconnected Components
Soma Number of Triangles
Soma Number of Voxels Img=1
Soma Position
Soma Position X
Soma Position Y
Soma Position Z
Soma Sphericity
Soma Time
Soma Time Index
Soma Volume

Statistic Values - Spine

Filaments - Spine Area
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 5).

Filaments - Spine Attachment Pt Acceleration
Acceleration is the change in the object velocity over time.
Object position at time-point *i*:

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector x, y, z at time-point *i*

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 \times (t(i+1) - t(i-1))}$$

Acceleration at time-point i:

Acceleration at the first and last time-point is 0.

Filaments - Spine Attachment Pt Acceleration X, Y, Z
Acceleration is the change in the filament points velocity over time along the selected-axis.

Filaments - Spine Attachment Pt Branching Angle
The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 4 a angles are marked with δ). This value is defined only for filament graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the

branching segments (in Figure 4 b angles marked with δ_2). The value is not calculated for branch points with more than three segments.

Filaments - Spine Attachments Pt Diameter
Diameter of a vertex at the spine attachment point to the segment.

Filaments - Spine Attachments Pt Displacement X, Y, Z
The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

Filaments - Spine Attachments Pt Displacement ^2

$$\overline{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

$\mathbf{t_L}$ = last time index of track

$\mathbf{t_F}$ = first time index of track

$\mathbf{P_X(t)}$ = x-position of object at time index t

Filaments - Spine Attachments Pt Distance
Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 2).

Filaments - Spine Attachments Pt Distance from Origin
The distance between Spine Attachments Pt and the origin (0,0,0).

Filaments - Spine Attachments Pt Position X, Y, Z
The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.
These values are only available if the tracking option is selected.

Filaments - Spine Attachments Pt Speed
The object speed is instantaneous speed of the graph beginning, branching, attachment and terminal points (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

For

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Filaments - Spine Attachments Pt Time
The value is only available for time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Spine Attachments Pt Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Filaments - Spine Attachments Velocity Angle X, Y, Z
The velocity angle is the angle between the velocity vector and the corresponding axis.
Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Filaments - Spine Attachments Velocity X, Y, Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Dt, can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Filaments - Spine Branch Depth
Spine Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the spine attachment point to a given point in the dendritic graph.

Filaments - Spine Branch Level
A Spine inherits the Segemnt Branch Level from the filament segment that it is attached to.

Filaments - Spine Branching
The number of spine branching points in a spine segment. The value is typically 0 or 1 (Figure 12 for S_0 0, S_1 1, S_2 0 and S_3 0).

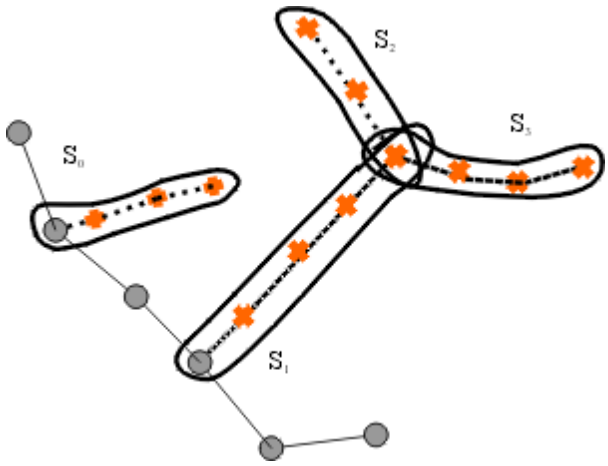


Figure 12

Please note:

- **The spine neck length is set by default to be 50% of the total spine length.**
- **Neck length is measured from the base of the spine head toward the segment.**
- **The spine head is 25% of the total spine length.**
- **The remaining part of the spine, attached to the filament tree, is set to be 25% of the total spine length.**

Filaments - Spine Length

The Spine length is calculated as the sum length of all spine center-line segments including the Spine head radius (the tip of the spine) minus the attachment radius (spine part inside segment, see Figure 5).

Filaments - Spine Max Diameter

The maximal value of spine diameters including attachment point diameter.

Filaments - Spine Min Diameter

The minimal value of spine diameters including attachment point diameter.

Filaments - Spine Mean Diameter

The mean value of spine diameters including attachment point diameter.

Filaments - Spine Neck Length

The Spine Neck length is calculated by subtracting the terminal radius of the spine terminal point from the sum of spine lengths (see Figure 5).

Filaments - Spine Neck Max Diameter
Filaments - Spine Neck Mean Diameter
Filaments - Spine Neck Min Diameter

Maximum, Mean and Minimum value of the spine neck diameter (excluding the spine attachment and terminal pints diameters).

Filaments - Spine Neck Volume

The Spine Neck volume is calculated by subtracting the volume of the spine-ending hemispheres (attachment and terminal point) from the spine volume.

Filaments - Spine Orientation Angle

The angle formed between the extending line connecting distal spine vertices in the spine segment and X-axis of the image within the XY plane (defined in the same way as Segment Orientation Angle, refer to Figure 10).

Filaments - Spine Part Length Ground

The spine ground length is set by default to be 25% of the total spine vertices (points).

Filaments - Spine Part Length Head

The spine head length is set by default to be 25% of the total spine vertices (points).

Filaments - Spine Part Length Neck

The spine neck length is set by default to be 50% of the total spine vertices (points).

Filaments - Spine Part Max Diameter Ground

The maximal value of the spine ground diameter.

Filaments - Spine Part Max Diameter Head
The maximal value of the spine head diameter.

Filaments - Spine Part Max Diameter Neck
The maximal value of the spine neck diameter.

Filaments - Spine Part Mean Diameter Ground
The average value of the spine ground diameter.

Filaments - Spine Part Mean Diameter Head
The average value of the spine head diameter.

Filaments - Spine Part Mean Diameter Neck
The average value of the spine neck diameter.

Filaments - Spine Part Min Diameter Ground
The minimal value of the spine ground diameter.

Filaments - Spine Part Min Diameter Head
The minimal value of the spine head diameter.

Filaments - Spine Part Min Diameter Neck
The minimal value of the spine neck diameter.

Filaments - Spine Part Volume Ground
Measure the volume of the spine ground.

Filaments - Spine Part Volume Head
Measure the volume of the spine head.

Filaments - Spine Part Volume Neck
Measure the volume of the spine neck.

Filaments - Spine Position X, Y, Z
Defines the value of X, Y, Z Cell position (x y z). The value is equal to Center of Homogeneous Mass of the object

Filaments - Spine Resistance
Measure of a spine's ability to conduct an electric current. The spine's resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account the diameter variations. It has units of 1/um and must be multiplied by specific material resistance in order to obtain a value of resistor (defined in the same way as Segment Resistance).

Filaments - Spine Straightness
Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Segment Straightness, refer to Figure 11).

Filaments - Spine Terminal Point Acceleration
Acceleration is the change in the object velocity over time.

Object position at time-point *i*:

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector x, y, z at time-point *i*

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 \times (t(i+1) - t(i-1))}$$

Acceleration at time-point i:

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

Filaments - Spine Terminal Point Acceleration X, Y, Z
Acceleration is the change in the filament points velocity over time along the selected-axis.

Filaments - Spine Terminal Point Diameter
Diameter of a vertex at the spine terminal point.

Filaments - Spine Terminal Point Displacement X, Y, Z
The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

Filaments - Spine Terminal Point Displacement ^2

$$\overline{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t - \Delta t)^2 + D_y(t, t - \Delta t)^2 + D_z(t, t - \Delta t)^2$$

$\mathbf{t_L}$ = last time index of track

$\mathbf{t_F}$ = first time index of track

$\mathbf{P_X(t)}$ = x-position of object at time index t

Filaments - Spine Terminal Point Distance
Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 2).

Filaments - Spine Terminal Point Distance from Origin

The distance between Spine Terminal Point and the origin (0,0,0).

Filaments - Spine Terminal Pt Intensity Center
Defined as the intensity of the spine terminal center voxel.

Filaments - Spine Terminal Point Position X, Y, Z
The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.
These values are only available if the tracking option is selected.

Filaments - Spine Terminal Point Speed
The object speed is instantaneous speed of the graph beginning, branching, attachment and terminal points (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Filaments - Spine Terminal Point Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Spine Terminal Point Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Filaments - Spine Terminal Point Velocity Angle X, Y, Z
The velocity angle is the angle between the velocity vector and the corresponding axis.
Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Filaments - Spine Terminal Point Velocity X, Y, Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Dt, can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Filaments - Spine Terminal Pt. Volume
Spine Terminal Pt. Volume is calculated as a volume of the sphere with the diameter of a spine terminal point vertex (Figure 5).

Filaments - Spine Time
The value is only available for time series data sets. The number indicates the time associated with each time step for the selected object.

Filaments - Spine Time Index
The value is only available for time series data sets. The number indicates the number for the current time step in the series.

Filaments - Spine Volume
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 5).

Lineage Statistics

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

Generations
This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

Track Number of Generations
This statistic shows the number of generations present in the selected track.

Time since first Division
This shows the time elapsed since the first division of the selected object(s).

Time since previous Division
This shows the time between each generation. e.g. the lifespan of an object

Normalized Time since previous Division
Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

Displacement Since Previous Division
This describes the displacement that has occurred since the previous division. The Displacement^2 (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

Time since track start
Displays time since the start of the track. This is only available only for objects that belong to a track. It is

different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement^2 over track time start, as in the example at “Displacement Squared Plot” at http://bradleymonk.com/Brownian_Motion.

The following statistics are available as lineage statistics for Filaments in Imaris:

Filament Cell Cycle Displacement Length
Filament Cell Cycle Displacement X, Y and Z
Filament Cell Cycle Duration
Filament Displacement Delta Length
Filament Displacement Delta X, Y and Z
Filament Displacement Length
Filament Displacement Since Previous Division X, Y and Z
Filament Displacement Since Previous Division^2
Filament Generation
Filament Normalized Time Since Previous Division
Filaments - Filament Time Since First Division
Filaments - Filament Time Since Previous Division
Filaments - Filament Time Since Track Start
Filaments - Filament Track Number of Generations
Filaments - Pt Cell Cycle Displacement Length
Filaments - Pt Cell Cycle Displacement X, Y and Z
Filaments - Pt Cell Cycle Duration
Filaments - Pt Displacement Length
Filaments - Pt Generation
Filaments - Pt Normalized Time Since Previous Division
Filaments - Pt Time Since First Division
Filaments - Pt Time Since Previous Division
Filaments - Pt Track No. of Generations
Filaments - Spine Attachment Pt Cell Cycle Displacement Length
Filaments - Spine Attachment Pt Cell Displacement X, Y and Z
Filaments - Spine Attachment Pt Cell Cycle Duration
Filaments - Spine Attachment Pt Displacement Delta Length
Filaments - Spine Attachment Pt Displacement Delta X, Y and Z
Filaments - Spine Attachment Pt Displacement Length
Filaments - Spine Attachment Pt Displacement Since Previous Division X, Y, Z
Filaments - Spine Attachment Pt Displacement Since Previous Division^2
Filaments - Spine Attachments Pt Generation
Filaments - Spine Attachments Pt Normalized Time Since Previous Division
Filaments - Spine Attachments Pt Time Since First Division
Filaments - Spine Attachments Pt Time Since Previous Division
Filaments - Spine Terminal Point Displacement Delta Length
Filaments - Spine Terminal Point Displacement Delta Length X, Y and Z
Filaments - Spine Terminal Point Displacement Length
Filaments - Spine Terminal Point Displacement Since Previous Division X, Y, Z
Filaments - Spine Terminal Point Displacement Since Previous Division2
Filaments - Spine Terminal Pt Cell Cycle Displacement Length
Filaments - Spine Terminal Pt Cell Displacement X, Y and Z
Filaments - Spine Terminal Pt Cell Cycle Duration
Filaments - Spine Terminal Point Generation
Filaments - Spine Terminal Normalized Time since Previous Division
Filaments - Spine Terminal Time since First Division

Filaments - Spine Terminal Time since Previous Division

5.11.9.3 Measurement Points

Measurement Point statistics are automatically computed for each Measurement Point.

Please see the Common Statistics Chapter for statistics common to all objects.

Show Statistic Values - Measurement Points

Measurement Points - Angle
This is the angle between two adjacent measurement points that share a common point. This statistic is only calculated in Polygon (ABCD…) Line Mode.

Measurement Points - Distance
This is the distance between the measurement points.

Measurement Points - Distance Sum
The Distance Sum is the sum of all measured distances.

Measurement Points - Number of Points
This is defined as the number of measurement points.

Measurement Points - Point Intensity Center
The Point Intensity Center is the value of the point intensity center (average) in the different channels.

Measurement Points - Point Position X
Point position in x-axis.

Measurement Points - Point Position Y
Point position in y-axis.

Measurement Points - Point Position Z
Point position in z-axis.

5.11.9.4 Spots

Spots statistics can be located in the statistics tab. Spots store a diameter and a specific time point. The intensity of the spot is defined by their statistics, intensity therefore changes when the image changes, which means that it is not an inherent part of the Spot itself (and not given a different treatment than the other statistics). Position X Y Z, diameter X Y Z and the time point are the values needed for the full description of a Spot. These values can be edited in the Edit tab options.

Please see the Common Statistics Chapter for statistics common to all objects.

Show Statistic Values - Spots

Spots - Acceleration
Acceleration is the change in the Spot(s) velocity over time.

Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \frac{\left(\frac{\vec{p}\,(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}\,(t) - \vec{p}(t-1)}{T(t) - T(t-1)}\right)}{\left(\frac{T(t+1) - T(t-1)}{2}\right)}$$

For

$$1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|$$

$$\vec{a}(t)$$

as defined in Acceleration X, Y, Z

The acceleration at the first and last time-point is 0.

Spots - Acceleration X, Y, Z
The acceleration is the change in the velocity of a spot over time along the selected-axis.

Spots - Area
The sum of the area of the sphere / ellipsoid surface (surface of the spot).

Spots - Average Distance to 3 Nearest Neighbours
Measures the average distance to the nearest 3 spots within the same spots component.

Spots - Average Distance to 5 Nearest Neighbours
Measures the average distance to the nearest 5 spots within the same spots component.

Spots - Average Distance to 9 Nearest Neighbours
Measures the average distance to the nearest 9 spots within the same spots component.

Spots - Diameter X, Y, Z
The diameter specifies the size of spherical object (i.e. the spot) along the selected-axis.

Spots - Displacement X, Y, Z
The Displacement is a measure of the distance that a spot moves from the first time point position, to its current position. This is calculated for each axis by subtracting the first time point position from a selected time point position:

Spots - Displacement ^2
The square of the displacement of the object from the starting point of the track. (Plotting the squared displacement against the value 'Time since track start' is useful for MSD analysis).

$$d^2(t) = |\vec{d}(t)|^2$$

$$\vec{d}(t)$$

as defined in Displacement X Y Z

Spots - Distance from Origin
The statistical value measures the distance from each 'Spots Position' to the origin of the dataset. The Spot position is equal to the center of homogeneous mass of the Spot. The origin is the (0,0,0) of the coordinate system of the image. The origin is at the bottom left corner by default (see 'Surpass menu - View - Origin...'). Press 'Surpass - Status Bar - Reset' to see the image in a rotation where the origin is at the position set at 'Surpass menu - View - Origin...'.

Spots - Spots Distance to Nearest Neighbour
Measures the distance to the nearest spot within the same spots component.

Spots - Intensity Center
The Intensity Center is the intensity of the voxel at the center of the Spot.

Spots - Intensity Max
Spots - Intensity Mean
Spots - Intensity Median
The Spots - intensity median is estimated from the intensity histogram.

Spots - Intensity Min
Spots - Intensity StdDev
Spots - Intensity Sum
The spots intensity statistics provide a description for the voxels enclosed within a Spot (Max, Mean, Median, Min, StdDev and Sum).

Spots - Intensity Sum of Square
The sum of the squared difference between the measured value x and its mean, where i ≤ n.

$$Total\ sum\ of\ squares = \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Spots - Number of Spots per Time Point
This is the number of spots at each time point.

Spots - Number of Tracks
Number of tracks.

Spots - Number of Voxels
Number of voxels within a Spot. A voxel is considered in a spot object if the centre of the voxel is located within the spot. When spots have a small diameter compared to the voxel size, this statistic value can be 0

Tip: When spots with voxel count 0 are reported it can help to resample the image in the z direction so that the voxel z size is smaller than the spot diameter

Spots - Position X, Y, Z
The position specifies the spot position (X, Y, Z). The position of the center of the spot:

$$\vec{p} = [p_x, p_y, p_z]$$

Spots - Shortest Distance To Spots
Computes the shortest distance from the center of each spot of “this” Spots object to the centers of all spots from the other Spots objects.

Spots - Shortest Distance To Surfaces
Computes the shortest distance from the center of each spot of “this” Spots object to the border of all surfaces from the Surfaces objects. Distances are positive for spots outside of the Surfaces, and negative for spots inside of the Surfaces.

Spots - Speed
The spots speed is the instantaneous speed of the spot s(t) (µm/s).The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Spots - Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Spots - Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series

Spots - Total Number of Spots
The total number of spot objects.

Spots - Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y and TrackAR1Z.

Note for all track statistics, any splits or gaps are not taken into account- it is taken that the selected

track is continuous in nature.

Spots - Track AR1 X, Y, Z

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$a\vec{r}1 = \frac{\sum_{t=2}^{n-1}\left(\vec{d}(t-1,t) - \vec{d}_{avg}\right) \times \left(\vec{d}(t,t+1) - \vec{d}_{avg}\right)}{\sum_{t=2}^{n}\left(\vec{d}(t-1,t) - \vec{d}_{avg}\right)^2}$$

with the displacement between two time points:

$$\vec{d}(t_1,t_2) = \vec{p}(t_2) - \vec{p}(t_1)$$

and the average displacement of the entire track:

$$\vec{d}_{avg} = \sum_{t=2}^{n} \frac{(\vec{p}(t) - \vec{p}(t-1))}{n}$$

Spots - Track Area Mean

The mean of the triangle Spots surfaces along the track.

Spots - Track Diameter Mean

The mean diameter of all spots along the track.

Track Displacement X, Y, Z

$$t\vec{d}l = \vec{p}(n) - \vec{p}(1)$$

Spots - Track Displacement Length

The Track Displacement Length (TDL) is the distance between first and last spot's position.

$$tdl = |t\vec{d}l|$$

$$t\vec{d}l$$

defined in Track Displacement X, Y, Z

Spots - Track Displacement X, Y, Z

The Track Displacement is the distance between the first and last spot's position along the selected-axis.

Spots - Displacement Delta XYZ

The difference in position of the current object and the previous object of the same track.

Spots - Displacement Delta Length (a scalar value)

The length of the position difference of the current object and the previous object of the same track.

Spots - Track Duration

The Track Duration is the duration between the first and last time point within the track.

Spots - Track Intensity Center Mean

This is the mean center intensity of all spots along the track.

Spots - Track Intensity Max

This is the maximum value of all maximum (voxel) intensities of the spot along the tracks

Spots - Track Intensity Mean
This is the weighted mean of the spot mean values, equal to the simple mean of all the intensities of all the spots in a track. Larger spots have a larger impact on the track mean.

Spots - Track Intensity Median
The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

Spots - Track Intensity Min
This is the minimum value of all minimum (voxel) intensities of the spots along the tracks

Spots - Track Intensity StdDev
The standard deviation track intensity of all spots along the track.

Spots - Track Intensity Sum
The sum of the track intensity of all spots along the track.

Spots - Track Length
The Track Length is the total length of displacements within the track.

$$tl = \sum_{t=2}^{n} |\vec{p}(t) - \vec{p}(t-1)|$$

Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.

Spots - Track Number of Branches
The number of track branches.

Spots - Track Number of Fusions
The number of track fusions.

Spots - Track Number of Spots
The number of track Spots.

Spots - Tracks Position Mean X, Y, Z
Define the mean value of X, Y, Z track coordinates positions.

Spots - Tracks Position X, Y, Z Start
Define the value of the X, Y, Z track start positions.

Spots - Track Shortest Distance To Spots
Measures the shortest distance of any spot within a track to any spot of another Spots component. The value corresponds to the minimum of the SpotsShortestDistanceToSpots statistics value of all spots within the given track.

Spots - Track Shortest Distance To Surfaces
Measures the shortest distance of any spot within a track to any surface of another Surfaces component. The value corresponds to the minimum of the SpotsShortestDistanceToSurfaces statistics value of all spots within the given track.

Spots - Track Speed Max
The maximum value of the object's speed on the track.

Spots - Track Speed Mean
The Average value of the spot's speed on the track. If the Track has no fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

Spots - Track Speed Min
The minimum value of the spot's speed on the track.

Spots - Track Speed Standard Deviation
The Standard Deviation value of the spot's speed on the track.

Spots -Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ- Standard Deviation

μ- Mean value

Spots - Track Straightness

$$S = \frac{D}{L}$$

S = Track Straightness
D = Track Displacement
L = Track Length

Spots - Track Volume Mean
The mean volume of all Spots along the tracks.

Spots - Track Velocity X, Y, Z
The formulas for calculating the velocity at time points (t) and (1) are calculated as follows:

For

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)}$$

And

$$\vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

Spots - Volume
Volume is a quantification of how much space an Spots object occupies.

Velocity Angle
The velocity angle is the angle between the velocity vector and the corresponding axis.

Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Spatial Statistics

Please see the Common Statistics chapter for statistics common to all objects for Spatial Statistics.

Event Time Statistics

Please see the Common Statistics chapter for statistics common to all objects for Event Time Statistics.

Track Class Duration Statistics

Please see the Common Statistics chapter for statistics common to all objects for Track Class Duration Statistics.

Lineage Statistics

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

Generations
This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

Track Number of Generations
This statistic shows the number of generations present in the selected track.

Time since first Division
This shows the time elapsed since the first division of the selected object(s).

Time since previous Division
This shows the time between each generation. e.g. the lifespan of an object

Normalized Time since previous Division

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

Displacement Since Previous Division
This describes the displacement that has occurred since the previous division. The Displacement^2 (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

Time since track start
Displays time since the start of the track. This is only available only for objects that belong to a track. It is different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement^2 over track time start, as in the example at “Displacement Squared Plot” at http://bradleymonk.com/Brownian_Motion.

Cell Cycle Duration
Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

Cell Cycle Displacement
This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division^2 for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

5.11.9.5 Surfaces

Surfaces statistics are automatically computed for each Surface. These values can be viewed in the statistics tab.

Please see the Common Statistics Chapter for statistics common to all objects.

Show Statistic Values - Surfaces

Surfaces - Acceleration
Acceleration is the change in the Surface velocity over time.

Acceleration X, Y and Z are the components of the acceleration vector (at time-point t):

For

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|$$

$$\vec{a}(t)$$

as defined in Acceleration X, Y, Z

The acceleration at the first and last time-point is 0.

Surfaces - Acceleration X, Y, Z

Acceleration is the change in the Surfaces velocity over time along the selected-axis.

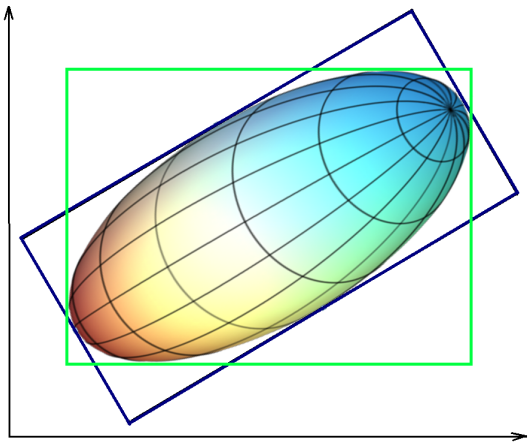
Surfaces - Area

The sum of the triangle surfaces.

Surfaces - BoundingBoxAA Length X, Y, Z

A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.
If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X = max(X)-min(X)
BoundingBoxAA Length Y = max(Y)-min(Y)
BoundingBoxAA Length Z = max(Z)-min(Z)



axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

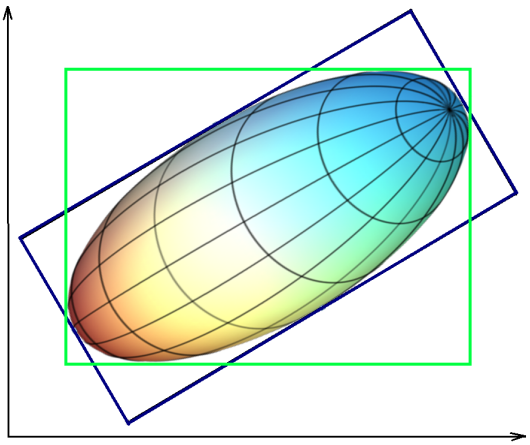
Please note the difference between the ellipsoid axis length and bounding box length.

Surfaces - BoundingBoxOO Length A, B, C

The local axes of the box can be arbitrarily oriented with respect to the coordinate system.
BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis

BoundingBoxOO Length B = Length of the second longest principal axis
BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length

Surfaces - Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (i.e. voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of the homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.
The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{ie\ object} r_i$$

R : center of homogeneous mass (X, Y, Z)
$\mathbf{r_i}$: center of a voxel (x_i y_i z_i)
n : number of i ∈ Surfaces

Surfaces - Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R : center of image mass (x y z)
$\mathbf{m_i}$: voxel intensity
$\mathbf{r_i}$: center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Surfaces - Circularity
Circularity is defined as:

$$Circularity = \frac{4\pi \times Area}{Perimeter^2}$$

Surfaces - Displacement X, Y, Z
The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position:

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

Surfaces - Displacement ^2
The square of the displacement of the object from the starting point of the track. (Plotting the squared displacement against the value 'Time since track start' is useful for MSD analysis).

$$d^2(t) = |\vec{d}(t)|^2$$

$$\vec{d}(t)$$

as defined in Displacement X Y Z

Surfaces - Distance from Origin
The statistical value measures the distance from each 'Surface Position' to the origin of the dataset. The Surface position is equal to the center of homogeneous mass of the Surface. The origin is the (0,0,0) of the coordinate system of the image. The origin is at the bottom left corner by default (see 'Surpass menu - View - Origin...'). Press 'Surpass - Status Bar - Reset' to see the image in a rotation where the origin is at the position set at 'Surpass menu - View - Origin...'.

Surfaces - Distance to Image Border X, Y
Surfaces - Distance to Image Border X, Y, Z
The value specifies the inward distances from the image border to be removed.

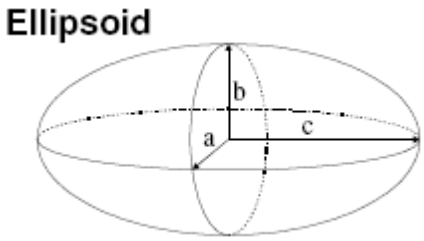
Ellipsoid Axis

In mathematics, an Ellipsoid is a type of quadric that is a higher dimensional analogue of an Ellipse.

The equation of a standard Ellipsoid in an x-y-z Cartesian coordinate system is:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$

where a, b and c (the lengths of the three semi-axes) are fixed positive real numbers determining the shape of the Ellipsoid.



Fitting ellipsoid into the surface

To calculate the dimension of a three principal axes of the best-fitted ellipsoid into the surface, the moments of inertia along the axes of ellipsoid is computed and optimized to match the moment of inertia of the surface. The ellipsoid has homogeneous mass distribution within the surface object. For more information:

http://en.wikipedia.org/wiki/Moment_of_inertia#Principal_moments_of_inertia

Please note: Only if the shape of the surface matches to an ellipsoid, the lengths of the three ellipsoid axes are exactly equal to the surface axis.

Surfaces - Ellipsoid Axis A X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis a.

Surfaces - Ellipsoid Axis B X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis b.

Surfaces - Ellipsoid Axis C X, Y, Z
These three parameters define the Vector of the Ellipsoid Axis c.

Surfaces - Ellipsoid Axis Length A, B, C
Surfaces - Ellipsoid (oblate)
Surfaces - Ellipsoid (prolate)

If we assume $a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)

- a ≠ b ≠ c it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

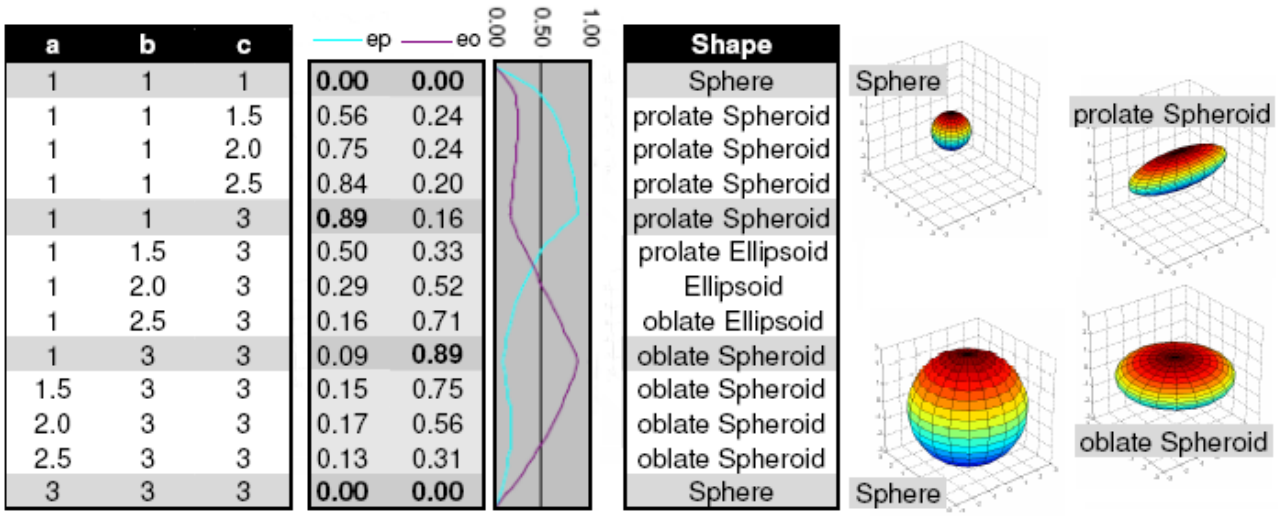
- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \times \left(1 - \frac{a^2 + b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \times \left(1 - \frac{2a^2}{b^2 + c^2}\right)$$

e_{oblate} = oblate Ellipsoid



a	b	c	ep	eo	Shape
1	1	1	**0.00**	**0.00**	Sphere
1	1	1.5	0.56	0.24	prolate Spheroid
1	1	2.0	0.75	0.24	prolate Spheroid
1	1	2.5	0.84	0.20	prolate Spheroid
1	1	3	**0.89**	0.16	prolate Spheroid
1	1.5	3	0.50	0.33	prolate Ellipsoid
1	2.0	3	0.29	0.52	Ellipsoid
1	2.5	3	0.16	0.71	oblate Ellipsoid
1	3	3	0.09	**0.89**	oblate Spheroid
1.5	3	3	0.15	0.75	oblate Spheroid
2.0	3	3	0.17	0.56	oblate Spheroid
2.5	3	3	0.13	0.31	oblate Spheroid
3	3	3	**0.00**	**0.00**	Sphere

Surfaces - Intensity Center
Surfaces - Intensity Max
Surfaces - Intensity Mean
Surfaces - Intensity Median
The Surfaces - intensity median is estimated from the intensity histogram.
Surfaces - Intensity Min
Surfaces - Intensity StdDev
Surfaces - Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Surfaces (Center, Max, Mean, Median, Min, StdDev and Sum).

Surfaces - Intensity Sum of Square
The sum of the squared difference between the measured value x and its mean, where i ≤ n.

Surface- Number of Disconnected Components
The number of disconnected surfaces in the selected Surface object.

Surface- Number of Disconnected Components per Time Point
The number of created disconnected surfaces in the selected time point.

Surfaces - Number of Surfaces per Time Point
Number of Surfaces per actual Time Point.

Surfaces - Number of Tracks
Number of tracks.

Surfaces - Number of Triangles
Number of the Surface object triangles.

Surfaces - Number of Vertices
Number of the Surface object vertices.

Surfaces - Number of Voxels
Number of voxels within the selected Surface object.

Surfaces - Position X, Y, Z
The X, Y and Z coordinates of the surface object. The value is equal to the value of the Center of Homogeneous Mass of the object.

Surfaces - Shortest Distance To Spots
Computes the shortest distance from the border of each surface of “this” Surface object to the center of all spots from the Spots object.

Surfaces - Shortest Distance To Surfaces
Computes the shortest distance from the border of each surface of “this” Surface object to the border of all surfaces from the other Surfaces. Distances are positive for surfaces that are entirely outside the other Surfaces. Distances are negative for surfaces that are entirely inside the other Surfaces. Distances are zero for surfaces that intersect the other Surfaces.

Surfaces - Speed
The surfaces speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

For

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

Surface - Sphericity
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, Ψ, of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle:

$$\psi = \frac{\pi^{\frac{1}{3}}(6V_p)^{\frac{2}{3}}}{A_p}$$

Vp = volume of the particle
Ap = surface area of the particle

Surface - Time
The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

Surfaces - Time Index
The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

Surface- Total Number of Disconnected Components
Total number of created disconnected surfaces.

Surfaces - Total Number of Surfaces
Total number of Surfaces.

Surfaces - Total Number of Triangles
Total number of Triangles.

Surfaces - Total Number of Voxels
Total number of voxels of the Surface objects within the data set.

Surfaces - Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Note for all track statistics splits or gaps are not taken into account- it is taken that the selected tack is continuous.

Surfaces - Track AR1 X, Y, Z
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

t_L = last time index of track

t_F = first time index of track

$$D_x(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_x = \frac{1}{t_L - t_F} \sum_{t=t_f+1}^{t_L} D_x(t, t-1)$$

t_L = last time index of track

t_F = first time index of track

Surfaces - Track Area Mean
The mean of the triangle surfaces along the track.

Surfaces - Track Center of Homogeneous Mass X, Y, Z
The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.
The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{ie\ object} r_i$$

R : center of homogeneous mass (x, y, z)
$\mathbf{r_i}$: center of a voxel (x_i y_i z_i)
n : number of i ∈Surfaces

Surfaces - Track Center of Image Mass X, Y, Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.
The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

R : center of image mass (x, y, z)
$\mathbf{m_i}$: voxel intensity
$\mathbf{r_i}$: center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Surfaces - Track Circularity Mean
The mean value of all objects' circularity from one track.

Surfaces -Track Displacement X, Y and Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis:

Surfaces - Track Displacement Length

defined in Track Displacement X, Y, Z

Surfaces - Displacement Delta XYZ
The difference in position of the current object and the previous object of the same track.

Surfaces - Displacement Delta Length (a scalar value)
The length of the position difference of the current object and the previous object of the same track.

Surfaces - Track Duration
The Track Duration is the duration between the first and last time point within the Track.

$$td = T(n) - T(1)$$

Surfaces - Track Ellipsoid Axis A X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis a.

Surfaces - Track Ellipsoid Axis B X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Surfaces - Track Ellipsoid Axis C X, Y, Z Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Surfaces - Track Ellipsoid Axis Length A, B, C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Surfaces - Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Surfaces - Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Surfaces - Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Surfaces.

Surfaces - Track Intensity Max
This is the maximum value of all maximum (voxel) intensities of the surface along the tracks

Surfaces - Track Intensity Mean
This is the weighted mean of the objects means, equal to the simple mean of all the intensities of all the objects in a track. Larger objects have a larger impact on the track mean.

Surfaces - Track Intensity Median
The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

Surfaces - Track Intensity Min
This is the minimum value of all minimum (voxel) intensities of the surface along the tracks

Surfaces - Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity Sum
This value describes the sum of voxel intensity of the Surfaces along the tracks.

Surfaces - Track Length
The Track Length is the total length of displacements within the Track.

$$tl = \sum_{t=2}^{n} |\vec{p}(t) - \vec{p}(t-1)|$$

Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.

Surfaces - Track Number of Branches
A branch occurs when a Surface object is connected to several other Surface objects of a future time point.

Surfaces - Track Number of Fusions
A fusion occurs when a Surface object is connected to several Surface objects of a past time point.

Surfaces - Track Number of Surfaces
The total number of Surfaces along the track.

Surfaces - Track Number of Triangles
Number of Triangles along the track.

Surfaces - Track Number of Voxels
Number of Voxels along the track.

Surfaces - Track Position X, Y, Z Mean
Defines the mean value of X, Y, Z track coordinates positions.

Surfaces - Tracks Position X, Y, Z Start
Defines the value of the X, Y, Z track start positions.

Surfaces - Track Shortest Distance To Spots
Measures the shortest distance of any surface within a track to any spot of another Spots component. The value corresponds to the minimum of the SurfacesShortestDistanceToSpots statistics value of all surfaces within the given track.

Surfaces - Track Shortest Distance To Surfaces
Measures the shortest distance of any surface within a track to any surface of another Surfaces component. The value corresponds to the minimum of the SurfacesShortestDistanceToSurfaces statistics value of all surfaces within the given track.

Surfaces - Track Speed Max, Mean, Min, StdDev
Maximum, mean, minimum and standard deviation of the value of the object's speed on the track.

Surfaces - Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$v = \frac{\vartheta}{\mu}$$

σ - Standard Deviation

μ - Mean value

Surfaces - Track Sphericity Mean
Describes the mean Surfaces Sphericity along the tracks.

Surfaces - Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Surfaces - Velocity X, Y, Z
The formula for calculating the velocity t at time point 1 is as follows:

$$\vec{v}(t) = \frac{\vec{p}\,(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}$$

For

$$1 < t < n$$

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)}$$

And

$$\vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

Surfaces - Track Volume Mean
The mean volume of all Surfaces along the tracks.

Surfaces - Volume
Volume is a quantification of how much space a Surface object occupies.

Velocity Angle
The velocity angle is the angle between the velocity vector and the corresponding axis.

Velocity Angle X: The angle between the velocity vector and the x-Axis (1,0,0).
Velocity Angle Y: The angle between the velocity vector and the y-Axis (0,1,0).
Velocity Angle Z: The angle between the velocity vector and the z-Axis (0,0,1).

Surfaces Overlap Statistics

To help study the relation between two sets of surfaces Imaris calculates statistics values measuring surface-surface overlap (if you have overlapping surfaces).

Overlapped Volume to Surfaces Surfaces

The volume of the overlap region between this specific surface and all surfaces from the other Surface.

Overlapped Volume Ratio to Surfaces

The ratio of the Overlapped Volume to Surfaces and the Volume of the specific surface.

Spatial Statistics

Please see the Common Statistics chapter for statistics common to all objects for Spatial Statistics.

Event Time Statistics

Please see the Common Statistics chapter for statistics common to all objects for Event Time Statistics.

Track Class Duration Statistics

Please see the Common Statistics chapter for statistics common to all objects for Track Class Duration Statistics.

Lineage Statistics

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

Generations

This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

Track Number of Generations

This statistic shows the number of generations present in the selected track.

Time since first Division

This shows the time elapsed since the first division of the selected object(s).

Time since previous Division

This shows the time between each generation. e.g. the lifespan of an object

Normalized Time since previous Division

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

Displacement Since Previous Division

This describes the displacement that has occurred since the previous division. The Displacement^2 (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

Time since track start

Displays time since the start of the track. This is only available only for objects that belong to a track. It is

different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement^2 over track time start, as in the example at “Displacement Squared Plot” at http://bradleymonk.com/Brownian_Motion.

Cell Cycle Duration
Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

Cell Cycle Displacement
This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division^2 for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

5.11.9.6 Volume

Volume statistics are automatically computed for each Volume object. These can be viewed from the statistics tab.

Please see the Common Statistics Chapter for statistics common to all objects.

Show Statistic Values - Volume

Volume - Data Intensity Mean
Volume - Data Intensity StdDev
Volume - Data Intensity Sum
The volume intensity statistics describe the voxels of the whole data set (Mean, StdDev and Sum).

Volume - Data Volume
The Data Volume is a quantification of how much space an object occupies.

Volume - Number of Voxels
Number of the Volume voxels.

5.11.9.7 Common Statistics

The following sections discuss statistics common to all objects.

Event Time Statistics
Track Class Duration Statistics
Spatial Statistics

5.11.9.7.1 Event Time Statistics

Event Time statistics are computed for all events with Imaris including Image Events, Object Events and Tracking Algorithm Events.

Image Events - Event Time Statistics

Time Since Event = ImageEvent
Time since nearest previous Event. Before the first Event it has negative values.

Time to Event = ImageEvent
Time to nearest next Event. After the last Event it has negative values.

Event Time = ImageEvent
Statistic returns Event Time equal to Time Since Event if Time Since Event is less than Time To Event. Or if Time Since Event is greater or equal to Time To Event the statistic returns Event Time equal to negative Time To Event. i.e.
if(TimeSinceEvent < TimeToEvent) {EventTime = TimeSinceEvent; } else { EventTime = -TimeToEvent;}

Object Events Event - Event Time Statistics

Time Since Event
Time since nearest previous Event. Before the first Event it has negative values.

Time To Event
Time to nearest next Event. After the last Event it has negative values.

Event Time
Statistic returns Event Time equal to Time Since Event if Time Since Event is less than Time To Event. Or if Time Since Event is greater or equal to Time To Event the statistic returns Event Time equal to negative Time To Event. i.e.
if(TimeSinceEvent < TimeToEvent) {EventTime = TimeSinceEvent; } else { EventTime = -TimeToEvent;}

Tracking Algorithm Events - Event Time Statistics

When objects are tracked using the lineage tracking or connected components tracking algorithm or maximum overlapping cells or manual tracking, then the objects immediately before a division or immediately after a merge will get a TrackSplit or TrackMerge Event and for all objects within a track that has a division or a merge an event time “Event Time Event= TrackSplit” will be computed.

Time Since Event = TrackSplit or Event Time = TrackMerge
Time since nearest previous Event. Before the first Event it has negative values.

Time to Event = TrackSplit or Event Time = TrackMerge
Time to nearest next Event. After the last Event it has negative values.

Event Time = TrackSplit or Event Time = TrackMerge
Statistic returns Event Time equal to Time Since Event if Time Since Event is less than Time To Event. Or if Time Since Event is greater or equal to Time To Event the statistic returns Event Time equal to negative Time To Event. i.e.
if(TimeSinceEvent < TimeToEvent) {EventTime = TimeSinceEvent; } else { EventTime = -TimeToEvent;}

5.11.9.7.2 Track Class Duration Statistics

Track class duration statistics are calculated for tracks that are split into classes during object creation for time series data. The statistics indicate how long the objects of a track have belonged to a class. Track Class duration statistics can be implemented for all objects in time series data with assigned classes. Here we discuss an example between a surface and a single spot object at 11 different points in time as shown in Figure 1 below.

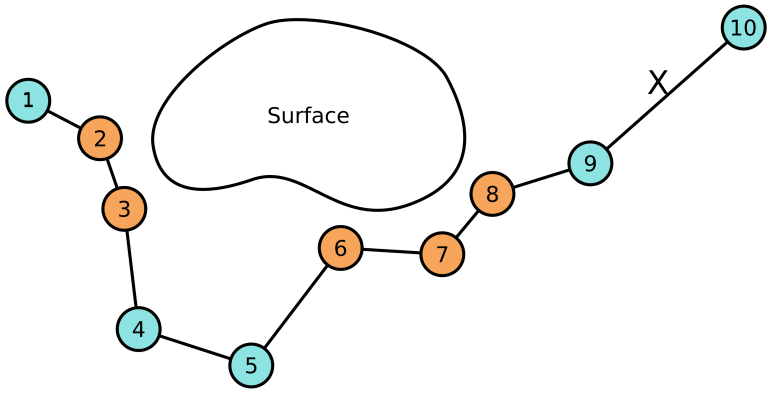


Figure 1: A surface object and a single spot object at 11 different time points. Numbers within spots indicate the spot ID. Orange spots indicate that at this time point the spot is in close proximity to the surface and has been assigned the class Set 1::Close. Blue spots indicate a time point where the spot is far from the surface Set 1::Far.

When objects are tracked and assigned classes, several Class Duration Statistics are calculated. Figure 1 shows a surface and a single spot object tracked over 11 time points. In this example, the spot has been assigned a class dependent on its distance from the surface over time. The spot at positions 1, 4, 5, 9 and 10 were manually labeled as **Set 1::Far (blue)** and the spot at positions 2, 3, 6, 7 and 8 were labeled as **Set 1::Close (orange)**. Note that Class Duration Statistics are not dependent on the surface, it is only in this example for illustrative purposes. Note also that at time point "9 seconds", no spot object is present (denoted by X in the figure). The following table shows the time in seconds, the spot ID and the labels of the spots:

Time [s]	Spot ID	Class
0	1	Set 1::Far
1	2	Set 1::Close
2	3	Set 1::Close
3	4	Set 1::Far
4	5	Set 1::Far
5	6	Set 1::Close
6	7	Set 1::Close
7	8	Set 1::Close
8	9	Set 1::Far
9	no object	-
10	10	Set 1::Far

Class Duration statistics are calculated for tracked objects that are labeled. Labeled but untracked

objects, as well as tracked but unlabeled objects do not have these statistics. For a track and a label, a Contact Span is defined as the sequence of objects that are connected and have the same label. Each labeled object in the track has one associated Contact Span. In the example shown in Figure 1 there are 3 Contact Spans for label Set 1::Far: (1), (4,5) and (9,10), and 2 Contact Spans for label Set 1::Close: (2,3) and (6,7,8).

Class Duration

Class Duration is a statistic that is calculated for objects that are tracked and labeled. It is the time difference of the last object and the first object of the associated Contact Span. All objects in the same Contact Span have the same Class Duration statistics. In the example shown in Figure 1, these are the Class Duration statistics:

Time [s]	Spot ID	Class Duration Class Set 1::Far	Class Duration Class Set 1::Close
0	1	0	-
1	2	-	1
2	3	-	1
3	4	1	-
4	5	1	-
5	6	-	2
6	7	-	2
7	8	-	2
8	9	2	-
9	no object	-	-
10	10	2	-

Track Class Duration Longest

Track Class Duration Longest is a Track Statistic and defined as the maximum value of Class Duration of all objects belonging to a track. This statistical value is reported for each class of the objects that are part of the track.

Track Class Duration Mean

Track Class Duration Mean is a Track Statistic and defined as the sum of Class Duration values of the objects, divided by the number of Contact Spans in that track. Note that for each Contact Span, the value is added only once, not for every object. This statistical value is reported for each class of the objects that are part of the track.

Track Class Duration Shortest

Track Class Duration Shortest is a Track Statistic and defined as the minimum value of Class Duration of all objects belonging to a track. This statistical value is reported for each class of the objects that are part of the track.

Track Class Duration Sum

Track Class Duration Sum is a Track Statistic and defined as the sum of Class Duration values of the objects. Note that for each Contact Span, the value is added only once, not for every object. This statistics value is reported for each class of the objects that are part of the track.

Track Number of Contacts
Track Number of Contacts is a Track Statistic and defined as the number of Contact Spans in a track. This statistical value is reported for each class of the objects that are part of the track.

For the example shown in Figure 1 the track statistics values are shown below:

Track Class Duration Longest Class Set 1::Far	2
Track Class Duration Mean Class Set 1::Far	1
Track Class Duration Shortest Class Set 1::Far	0
Track Class Duration Sum Class Set 1::Far	3
Track Number of Contacts Class Set 1::Far	3

Track Class Duration Longest Class Set 1::Close	2
Track Class Duration Mean Class Set 1::Close	1.5
Track Class Duration Shortest Class Set 1::Close	1
Track Class Duration Sum Class Set 1::Close	3
Track Number of Contacts Class Set 1::Close	2

5.11.9.7.3 Spatial Statistics

Surfaces

The surfaces spatial statistics measurements are performed in equidistant shells around a surface that are also restricted to the inside of the image. This enables plotting statistics values as a function of “distance to surface”. Imaris reports the measurements in a table with the following layout:

Distance to Surface	StatisticsValue

For each statistics value Imaris reports three variants.
1. **StatisticsValue**: Measurement on the entire image.
2. **StatisticsValue Inside**: Measurement only inside the surface.
3. **StatisticsValue Outside**: Measurement only outside the surface.

The statistics values reported by Imaris are the following:
Intensity (per channel) - measures the total intensity of all pixels inside an equidistant shell around the surface that does not extend outside of the image.
Volume - measures the volume of an equidistant shell around the surface that does not extend outside of the

image.
Cumulative Volume - measures the volume inside a thick shell extending from the surface to the respective distance but not outside of the image.

Spots

Spatial statistics are computed for spots relative to a surface with the aim of studying the question: “Is there an interaction between spots and surfaces?” The spatial statistics approach to answer this question is the following: Within equidistant shells around the surface, count the observed number of spots inside each shell. Then run simulations that generate spots with “complete spatial randomness” (CSR) and determines the number of spots inside each shell from the simulations. Imaris runs 1000 simulations of CSR spots. From these it computes for each measurement value the 1st and the 99th percentile, Imaris also computes the expectation value which falls between the 1st and 99th percentile. These values appear in columns with the titles “CSR”, ”CSR min”, and “CSR max”. To summarize for every type of measurement that Imaris reports for spots spatial statistics it reports a table with the following layout:

Distance to Surface	StatisticsValue	StatisticsValue CSR	StatisticsValue CSR min	StatisticsValue CSR max

For each statistics value Imaris reports three variants.
1. **Statistics Value**: Observed spots are counted anywhere in the entire image and CSR simulation randomly “throws” spots anywhere in the image.
2. **Statistics Value Inside**: Observed spots are counted only on the inside of the surface and CSR simulation “throws” spots only inside the surface.
3. **Statistics Value Outside**: Observed spots are counted only outside of the surface and CSR simulation “throws” spots only outside the surface.

The different statistics values reported by Imaris are the following:
Number of Spots - measures the number of spots inside an equidistant shell around the surface that does not extend outside of the image.
Number of Spots Density - same as Number of Spots divided by total number of spots for all distances times 100.
Cumulative Number of Spots - measures the number of spots inside a thick shell extending from the surface to the respective distance but not outside of the image.
Cumulative Number of Spots Percentage - same as Cumulative Number of Spots divided by the total number of spots at all distances times 100.
Spots Density Kernel - same as Number of Spots Density smoothed with a Gaussian filter kernel averaging values at different distances.

5.11.10 Statistics Tests

The settings for the Statistics Tests calculated and displayed in the Statistics Tests tab of 1D view can be viewed or edited as required.

Common Preferences

Set the Confidence level for all tests. The default is 0.05.

Wilcoxon Test

Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

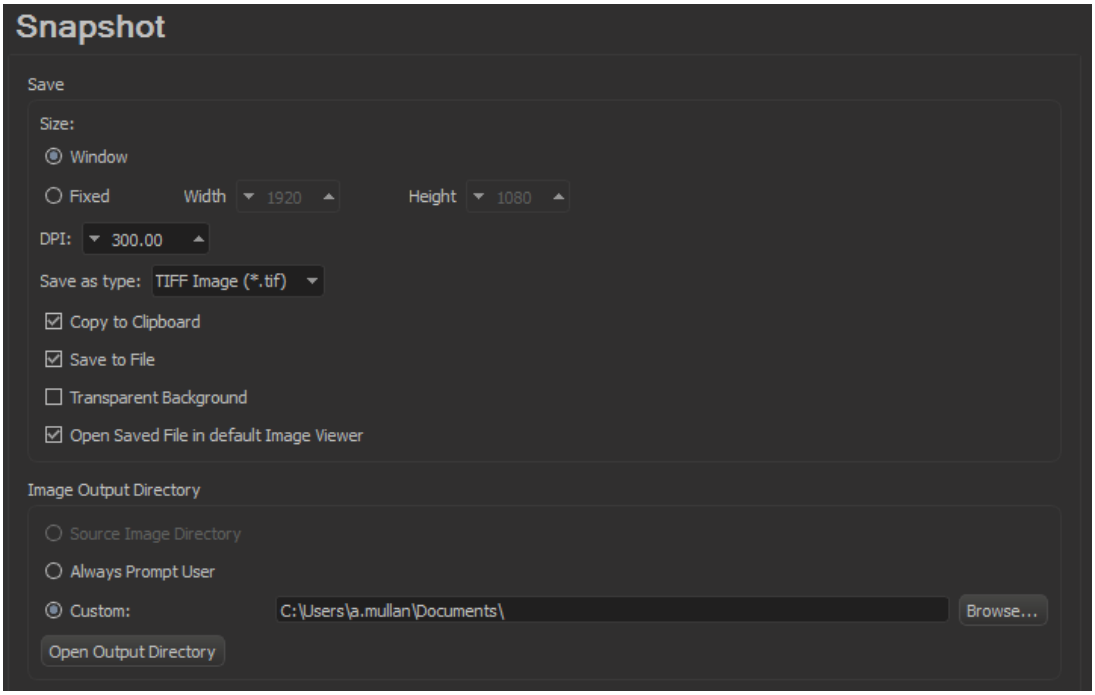
F test
Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

T Test
Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.
Select whether the assumption will be made that the original populations have the same variances. The default is unselected- in this case a *Student's t-test* is performed. If this option is selected, a *Welch's t-test* will be performed.

Kolmogorov-Smironov Test
Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

5.11.11 Snapshot

Configure the preferences for Snapshot. Note that the default file format is tiff.



Save

Size
Adjust the size of the snapshot image. Select the image size either as that of the **Window** size or a **Fixed** size. Set the Fixed size to a required resolution using the Width and Height fields.

DPI
Adjust the print resolution of the snapshot image. The default value is 300dpi. This can be increased for higher resolution, or decreased if required.

Save as type
Select the file format: TIFF (default), PNG and JPEG).

Copy to Clipboard
Set the snapshot image to save to clipboard e.g. so the image may be pasted into a document. Note this mode of copying will not support transparent backgrounds.

Save to File
Set Save to file to save the snapshot image directly to file- this will save the image directly to the set image output directory so that multiple snapshot image may be taken quickly and easily.

Transparent Background
Save images so that the background of the image is transparent.

Open Saved File in Default Image Viewer
Selecting this option will result in the snapshot image opening automatically in your default image browser.

Image Output Directory

Set your preferred save location options for Snapshot images.

Source Image Directory
Not available when Imaris has been configured for Arena (Arena is the default, recommended option)- this option enables snapshot images to be saved to the source image directory location.

Always Prompt User
Select this option to always prompt the save location each time you take a snapshot image.

Custom
Imaris will use a logical location by default to save snapshot files to, but you can change this to a more suitable location if required. The current location is shown and the Browse button can be used to set a new location

Open Output Directory Button
Open the set output directory to new the folder contents.

5.11.12 Custom Tools

ImageJ / Fiji Application

The **Browse button** allows you to browse to a desired location within the file system.
Here you can specify the Fiji folder in which the Fiji.exe file resides. Either type in the respective path or use the button **Browse**.
Please note: Fiji can be downloaded from the Customer Portal.
portal.imaris.com

Default Paths:
Windows: C:\Program Files\Fiji.app\ImageJ-win64.exe
OS X:/Applications/Fiji.app/Contents/MacOS/ImageJ-macosx

Checkboxes
Show Labkit Pixel Classifier option for Surfaces segmentation
Enable GPU for Labkit Pixel Classification when reusing a trained classifer

MATLAB

MATLAB Compiler Runtime enables the execution of MATLAB files on systems without a fully installed MATLAB version. Imaris will automatically detect if the required MATLAB versions are present, if not, a download link is shown.

Version Installation Guidance
For Windows and Mac use 2023a.
For older Imaris releases, these versions should be used:
R2014b, Imaris 8.3 - 9.5
R2020a, Imaris 9.6 Windows and Imaris 9.6 or above OSX
R2020b, Imaris 9.7 or above Windows only

The **Add** button allows you to add additional compilers to those shown as default e.g. if running different Xtensions.
The **Browse...** button allows you to specify the MATLAB application if it is not in the default location.
The **Remove** button enable you remove an additional compiler - you can not remove the default versions.

Python Application

The **Browse** button allows you to specify the Python application.
Either type in the respective path or use the button **Browse**.

Default Paths:
Windows: C:/Users/username/AppData/Local/Programs/Python/Python37/python.exe
Mac: /Library/Frameworks/Python.framework/Versions/3.7/bin/python3.7

Note: Python 3 needs to be installed from python.org for Windows and Mac platforms.

XTension Folders

The **Browse** button allows you to specify the XTensions directories.
Either type in the respective path or use the **Browse** button.

Default Paths (examples shown below for Imaris 9.9)

Windows:
Matlab XTensions
\Bitplane\Imaris x64 IMARIS 9.9.0 \XT\matlab\
Compiled MATLAB XTensions
Bitplane\Imaris x64 9.9.0\XT\rtmatlab
Python XTensions
\Bitplane\Imaris x64 IMARIS 9.9.0 \XT\python

OS X
Matlab XTensions
/Applications/Imaris IMARIS 9.9.app/Contents/SharedSupport/XT/matlab
Compiled MATLAB XTensions
/Applications/Imaris IMARIS 9.9.app/Contents/SharedSupport/XT/rtmatlab
Python XTensions
/Applications/Imaris IMARIS 9.9.app/Contents/SharedSupport/XT/python

Please note: Fiji and MATLAB Compiler Runtime can be downloaded from the Bitplane Customer Portal. MATLAB Compiler Runtime enables the execution of Matlab files on systems without a fully installed Matlab version.
portal.imaris.com

Add Button
To add a XTensions directory, click on the Add button.

Remove Button
Click on the Remove button to remove the selected XTensions directory from the list.

OK Button
To apply the changes click on **OK**.

Cancel Button
If you do not want to save the changes click on **Cancel**.

5.11.13 Batch

Batch

Batch Memory Limit
Data Cache RAM memory limit shared by number of running batches, equally divided by number of concurrently running batches.

Max Number of Concurrent Batches
Number of Batch processes. If more batches are started they are put in a queue. Whenever a batch on an image has finished, a new one is processed from the queue until the queue is empty.

Enable GPU Batch Deconvolution
If true and supported, uses GPU for calculation the deconvolution. Otherwise, deconvolution is done on the CPU. To see if GPU deconvolution is supported, view Preferences/System/Graphics/GPU Deconvolution. GPU deconvolution in batches running in the background may affect the frame rate of graphics applications.

Batch Logging

Log Severity Level
By default, batches are executed without any logging.
To deactivate logging, chose "NONE" as Log Severity Level By specifying a folder and setting a Log Severity Level, batches create log files in the specified directory with increasing details:
NONE No logging
ERROR Log only errors
WARNING Log errors and warnings
INFO Log info messages
DEBUG Log Debug messages
TRACE Log everything

Log Files written to Directory

Select the directory to which you would like log files to be saved to.

Batch with Subfolders

When running Batches in Arena with the "Run Batch Including Subfolders" option, this setting controls how many subfolder levels should be included.

Batch Recursivity Limit (-1 no limit)
Select the batch recursivity limit, a value of 1 means run in current folder and direct subfolders.

5.11.14 Licenses

Overview

Node-locked License
Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use the Imaris Configurator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine. Please refer to the offline license method for further instructions.

Floating License
Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.
The Imaris Configurator application easily let you enable a floating license.

Demo License
A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open the Imaris Configurator application and open the Demo License you received from your local support representative.

Evaluation License
Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.
To get an Evaluation License you first have to register at:

https://imaris.oxinst.com/downloads

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

Imaris Open Developer License
To get an Imaris Open Developer License you first have to register at:
http://open.bitplane.com/

If you can install multiple license locations, (Floating license and a Node-Locked license), Imaris will search all the locations until it finds the license for the requested features.

This Section contains information related to licensing management, options and procedures. It includes the following sections:
Preferences Dialog
Installation of Licenses
Imaris Configurator

5.11.14.1 Preferences Dialog

Imaris Configurator enables the licenses of Imaris applications (and Imaris data and system settings) to be managed by the Administrator. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this system folder we strongly advise you not to do so. It is recommended to go through the Imaris Configurator application instead.

Imaris Configurator

This starts the Imaris Configurator. Unless you want to use an Evaluation license you need to use the Imaris Configurator to set up your licenses.

Please note that you'll need administrative privileges in order to start the Imaris Configurator.

Any change of licenses will require Imaris to be restarted.

Except the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need administrative privileges.

If you have access to an administrative system account, Imaris Configurator will start normally.

Administrate
This button starts Imaris Configurator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use Imaris Configurator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Configurator.
Any change of licenses will require a new start of Imaris.

Evaluation License

After you entered your RegistrationID and Passcode you can click on the Light Bulb button  to activate the Evaluation License.
This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on  .
You can also disable your Evaluation License by clicking again on Light Bulb button and the light bulb will turn off. This indicates that you aren't using any Evaluation Licenses.
Any change of licenses will require a new start of Imaris.

RegistrationID
Please enter your RegistrationID you received by mail previously.

Passcode
Please enter your Passcode you received by mail previously.

Imaris Open Developer License

After you entered your OrderID click on the Imaris Open button  to activate the Developer License.
This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the button will turn on  .

Any change of licenses will require a new start of Imaris.

RegistrationID
Please enter your RegistrationID you received by mail first.

Available Licenses

The table displays all existing Imaris licenses on the system and additional information for each feature:
Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given in the Licenses section.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.

License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
- Green: License is valid.
- Yellow: License is valid, but all licenses are currently in use.
- Red: License has expired.
- No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

Check/Uncheck All
Check this box to enable or disable all available Imaris licenses on the system.
Please note that the Feature “ImarisBase” can't be disabled because this would render Imaris unusable.

5.11.14.2 Installation of Licenses

First-time Installation

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

• If you have a Node-locked or a Floating License you should use Imaris Configurator application for the further license setup. This is done by simply clicking on the “Start Imaris Configurator” button. Please note that Imaris Configurator application needs administrative privileges.

• If you want to evaluate Imaris first, please register at:
https://imaris.oxinst.com/downloads

The automatic mail response contains your Registration ID and Passcode.
To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation License” button.
Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

Installation of Additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start Imaris Configurator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

5.11.15 Update

Here you can adjust the default update settings.

How often should you search for updates?

Daily
Weekly
Never
Select either daily, weekly, or never.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.16 Usage Data

Here you can enable the capturing of data usage.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

5.11.17 3D Cursor

Here you can adjust the default 3D Cursor settings.

3D Cursor

Box
Sphere
Cross
Torus
Here you can select the shape of the 3D Cursor.

Size
Color
Here you can select the size and color of the 3D Cursor.

Animation

Frequency
Adjust the animation frequency.

Amplitude X
Amplitude Y
Adjust the amplitude in x-, and y-direction.

Shear
Rotate
Select shear or rotate as animation mode.

Continuous
Discrete
Select continuous or discrete as animation sequence.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.18 Annotate

Here you can adjust the default Annotation settings.

Format

Title Format
Text Format

Legend Format
Choose title format, text format or legend format and adjust the following parameter set.

Title, Text or Legend

Font
Font Size
Color
Format
Bold
Italic
Underlined
The font, font size, color, and format can be selected (from any installed system font).

Color Selection Button
Click on this button to open the color selection window to change the respective color.

Style

Arrow Color
Arrow Width
Arrow Style
Text Box Color
Text Box Transparency
Text Box Style
You can adjust the arrow color, width and style (Sphere, Arrow, Magnifier), text box color, transparency and style (Unicolor, Black Text, Black Title).

Color Selection Button
Click on this button to open the color selection window to change the respective color.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

5.11.19 Arena

Folder Lists
Add or remove folder locations for your images.

Prior to Imaris 9.3, Arena used a different system for image file management. Data from prior Imaris versions can be imported into the new system using Imaris Arena Migrator. The Imaris Arena Migrator can be downloaded from our customer portal if required.

5.11.20 Advanced

Displays a window similar to the parameters window. It shows internal settings and parameters in groups and parameter lists.

Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations.

Reset Preferences Button
Click on this button reset preference values to their default settings.

OK Button
To apply the changes click on **OK**.

Cancel Button
If you do not want to save the changes click on **Cancel**.

5.11.21 Imaris Configurator

This Section contains information related to licensing management, and the data storage and system options that can be configured through Imaris Configurator.



This Section includes the following sections:

License Management:
Administrative Privileges
License Information
Add License
Data Management
System Preferences

Advanced Preferences

You will need to restart Imaris for any changes to take effect.

5.11.21.1 Administrative Privileges

There are some cases where the proper privileges can not be provided automatically and requires you to start the application manually.

Windows 7:
If the User Account Control (UAC) is disabled and you are logged in with a standard user no elevation prompt is shown because the UAC setting omits these prompts. Therefore you can't get the right administrative permissions and you will have to start Imaris Configurator manually. This is done by opening the executable by a right click while holding down the “Shift” key. Then you can select “Run as different User” which prompts you for another account.

Mac OS X:
No cases known where problems occur.

Default Imaris Configurator Paths

Windows 8 and 10
64-bit: C:\Program Files\Bitplane\Imaris 9.0\ImarisConfigurator.exe

Mac OS X
/Applications/Imaris 9.0.app/Contents/MacOS/ImarisConfigurator

5.11.21.2 License Information

Types of license

A description of the license types available is given in the Licenses Section.

License Location

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use the **Add License Location** button to add a new license location.

Add License Location Button
Use this button to add new licenses. A description is given in the Add License Section.

Delete License Location
If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you have selected the right table entry. In the case of a node-locked license, you are asked if you want to keep the license. It is recommended that you keep all license files.

License Location Table
This table lists all available license locations. Normally it would be just enough to have one license location defined. But there are also situations in which it is useful to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations

and the licensed features are merged on your system automatically.

The table shows also some additional Information:
- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers show you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

Licenses Table
In this table you have a detailed overview of all licensed features of a license location. You can switch among different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :
Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given in the Licenses section.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.
License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
- Green: License is valid.
- Yellow: License is valid, but all licenses are currently in use.
- Red: License has expired.
- No color: License is not available (e.g. this additional license feature hasn't been purchased).

Finish Button
This button is only enabled if you have added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes Imaris Configurator and you can proceed using Imaris.

5.11.21.3 Add License

First, you have to select your licensing scheme:

Download Node-locked License

If you decide to add a Node-locked License, the software will verify that your computer is connected to the internet. If the connection could be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

Online-Licensing Wizard
This wizard has three steps:

1. Select the Network Interface

You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

2. Check your Order-ID for newly ordered Products:
If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the “Check Order-ID” button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.
If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

3. Select the Licenses for Installation
In this step you just have to mark all products you want to install by checking them in the left table.
The right table is for your information only and shows all currently installed products.
When you click on the “OK” button, the license file is downloaded and the corresponding entry is added to the License Location table.

Offline-Licensing Wizard
If you are not connected to the Internet, this could be due to either of the following:
1. your computer is behind a web proxy server which does not permit a direct connection or
2. the PC is unplugged from the internet.

Therefore you have the following choices:

A) Set up a Proxy Server
Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.
If not, either the credentials you entered are not correct or the connection is broken at another place in the network. In that case please contact your network administrator or Support teams eusupport_imaris@andor.com or ussupport_imaris@andor.com

If you want to delete a previously set up proxy server, just delete both fields and click on the button again.
The proxy server settings can also be altered in Imaris' system preferences dialog.

B) Download the Node-locked License from another Computer
You can download the license from another computer connected to the internet.
On that machine log on to http://www.bitplane.com/activation/acti_offline_ident.html
and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and afterwards you could transfer the file to the offline computer (e.g. by using a USB stick).
The instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.
When you click **OK** the license file is copied to the proper location.

Define Floating License Server

In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

Install License from Existing File

If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

Show floating license selection dialog upon start of Imaris

This option enables you to enforce active individual selection of all Imaris add-on modules licenses and secure the optimal use of the licenses available on the server. It prevents unintentionally overuse of the purchased add-on modules licenses and helps you with your short- and long-term plans for Imaris licenses spending and allows you to better control your software cost.
Select the Show floating license selection dialog upon start of Imaris and you are configuring the License Selection table to starts automatically. So every time Imaris is started Imaris, the License Selection table is displayed for each user.

5.11.21.4 License Selection

It is important to understand that the License Selection option is not designed to minimize your Imaris usage. It lets you manage and optimize use of all Imaris add-on modules licenses that are available within your license purchased pool.
When you launch Imaris, the License Selection table will be shown. With the License Selection table the user has the flexibility to mix and match add-on modules and to suit changing scientific requirements in a flexible form. The License Selection table allows selection of all add-on Imaris modules to be simultaneous used by any number of users up to the maximum number of licenses agreed upon at the time of purchase.

The table displays only the Imaris add-on module license that does not match the overall number of purchased Imaris basic licenses.
This table displays: Imaris product, feature, version, license type and status and expiration date.

From the modules lists, you can select the required license.
Choose from the table the modules that are needed and available and select only the add-on modules that will be needed for the current Imaris session. Activate your license by clicking the appropriate check-box.
After activating the license, for the selected module the total license number available in the pool is reduced.
Once all available licenses are checked out (the activated license number has been exceeded the maximal number of license have been purchased), the corresponding Imaris product will not be shown within the table. The product will not be available for your future selection until one of the current users is finished and the license is checked back into the licensing pool.

Do not show this dialog

If you select this option, you won't have a license table opened on your system, and the opt-in licenses file will not be available for your future selection.

5.11.21.5 Data Management

The administrator can set the default location that the data set and batch data files are saved to using **Set Data Storage Path**. This can be either a local, or a remote network server location. This is described in the Arena Section. The user can change the default location if they require.

5.11.21.6 System Preferences

System Preferences allows an administrator to set the key system parameters for Imaris users. Default values are detected for the computer by the software and automatically set in the Number of Threads, Data Cache Memory Limit and Texture Cache Limits fields. These values can be set manually if required by free typing or using the up and down arrows in each field.

System Preferences are set by default so they are editable by the user. This can be disabled by deselecting the **Is editable by User** check-box.

Select **Reset to Factory Defaults** to change any modified values back to the original default values. Click the **Finish** button when you are finished making changes. You will need to restart Imaris for any changes to take effect.

Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations. Refer also to the Calculation Section.

Calculation

The number of processing threads can be adjusted from the default value depending on the system resource requirements of the computer running Imaris.

Data Cache

Adjust the size of the **Data Cache Memory Limit** and set and select the **Data Cache File Path** to be used.

Display

Set the maximum memory limit allocated to the texture cache used for display. Adjusting the Texture Cache Limit can be used to improve image quality or performance or according to the size of the images and the computer resources available.

Settings

Set the frequency for maintenance and and software updates. The default setting is Weekly.

Usage Data

Enable or disable the capturing of usage data. Refer also to the Usage Data Section.

5.11.21.7 Advanced Preferences

In Advanced Preferences it is possible to edit parameters in Imaris and set whether they may be editable by the user under the Advanced option of preferences. Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations. To return values to the original default values select **Reset to Factory Defaults**. Click the **Finish** button when you are finished making changes. You will need to restart Imaris for any changes to take effect.

5.12 Exit

Terminates Imaris and returns to the desktop.

Section 6 Edit

Undo	Ctrl + Z
Snapshot	Ctrl + C
Image Properties...	Ctrl + I
Show Display Adjustment	Ctrl + D
Add Time Points...	
Delete Time Points...	
Add Channels...	Ctrl + Shift + A
Delete Channels...	Ctrl + Shift + B
Add Slices...	
Delete Slices...	
Remove images	
Crop Time...	
Resample Time...	
Crop 3D...	Ctrl + W
Resample 3D...	
Change Data Type...	

See also:
Addendum - Mouse & Keyboard Mac

6.1 Undo

The Undo command reverses the last action, when necessary. It supports multiple undo actions, starting with the most recent one and working backwards in sequence.
All actions in the Draw, Edit and Edit Tracks tabs as well as the image processing functions can be undone by selecting the Undo command or by pressing **Ctrl+Z**.
The number of any previously applied command that can be Undone/Redone is defined in the Edit menu-Preferences-Calculations-**Maximum Number of Commands in History** field. By default, the number of steps is 4.

6.2 Redo

The Redo command repeats the last action, if appropriate. The command supports multiple redo actions, starting with the most recent one and working backwards in sequence.

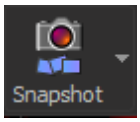
All action in the Edit, Edit tracks, and Draw tabs as well as image processing functions can be redone by selecting the Redo command or by pressing **Ctrl+Y**.

The number of any previously applied command that can be Undo/Redo is defined in the Edit menu-

Preferences-Calculations-**Maximum Number of Commands in History** field.

6.3 Snapshot

The snapshot button in the main toolbar immediately produces a snapshot of the current view. This may be either the image is Surpass, or a 2D, 3D or 4D Vantage plot as it currently appears in the viewing area. It creates a new file containing your Vantage plot as well any scale information, measurements data, and box plots that are displayed.



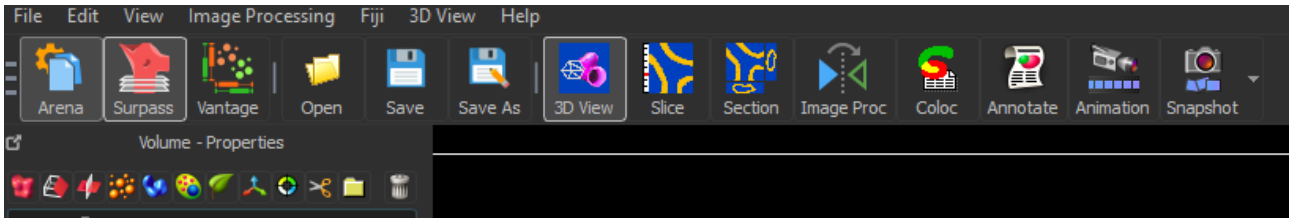
By default the snapshot is saved to a file in the source image directory and after creating the snapshot it is opened in the default image viewer to show the user what the snapshot looks like. The snapshot is by default also copied to the operating system clipboard for use in other applications.

To create the snapshot, simply click the Snapshot button.

To view and adjust the settings of the snapshot, click Preferences from the Snapshot button drop-down box. Refer to Snapshot Preferences for further information.

Notes:

- **To help the user see the size of the screen render area, Imaris displays this size in the top left hand corner of the render-area whenever the size is changed (shown below).**



- **The view-menu has "Save Window State" and "Load Window State" options which function on a per viewer basis, to save and load the size and visibility of all windows.**
- **Snapshots always have the same aspect ratio as the render-area of the current view. (In previous versions of Imaris it was possible to create snapshots with aspect ratios differing from those of the current view).**

6.4 Image Properties...

Geometry
Data Set
Channels
(Channel 1...n)
Thumbnail
Parameters

When opening a data set, the following parameters should be checked or modified as required:

- Name and Description (in Data Set).
- Voxel Sizes (in Geometry).
- Channel Colors (in Channel 1...n).

6.4.1 Geometry

The geometrical settings of the actual data set are displayed.

Type

Data Type
Display of the image type.

Size

Size X, Y, Z, T
Display of the image size.

Image size:
Shows the uncompressed image size.

Coordinates (um)

Voxel Size, X, Y, Z
The voxel sizes directly influence the views because they control the height of the image relative to its width. Check the parameters and adjust the **Voxel Size** and/or other settings if necessary.

Min, X, Y, Z
The minimum value of the coordinate axes.

Max, X, Y, Z
The maximum value of the coordinate axes.

Time Point

First Box
Select the time point.

Date
Enter the collection data.

Time
Enter the collection time.

Button **All Equidistant...**
If the data set is a time series, enter the date/time for each time point or click **All Equidistant** to open the Set Equidistant Time Points dialog box.

- Enter the **Start Date** and **Start Time** and the **Time Interval**.
- Click **OK** when finished. Imaris will calculate the time for each time point in the series.

The data set must be saved to retain the changes. Click **OK** when finished or select another heading for further adjustments.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

6.4.2 Data Set

Name

Data field to enter a data set name.

Description

Data field to enter a data set description.

Numerical Aperture (N.A.)

Reads out the numerical aperture (as defined in the menu Edit - Image Properties... - Parameters).

Log

Display of processing steps.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

6.4.3 Channels

There are no parameters available on this menu.

6.4.4 Channel 1...n

In the Index of the Image Properties box click the **Channel** entry (Channel 1, Channel 2 etc.) to select the required channel.

Name

Data field to type in the channel name.

Description

Data field to type in a channel description.

Emission Wavelength

Reads out the emission wavelength.

Excitation Wavelength

Reads out the excitation wavelength.

Pinhole Radius

Reads out the pinhole radius.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

6.4.4.1 Tab Base Color

Red, Green, Blue
Reads out the assigned color. To change the color either adjust the values or move the square in the color circle.

To apply the changes click on **OK**.

6.4.4.2 Tab Mapped Color

Selected Color

Button **Edit...**
Click on a square in the grid and click on the button **Edit...** . Select a color and click on **OK**. The selected color is displayed in the square.

Button **Copy**
Click on a square in the grid and then on the button **Copy** to copy the color.

Button **Paste**
Click on a square in the grid and then on the button **Paste** to paste the color.

Interpolation

Colorspace

RGB
The RGB color model is an additive model in which red, green and blue are combined in various ways to reproduce other colors.

HSV
The HSV (Hue, Saturation, Value) model, defines a color space in terms of three constituent components.

- Hue, the color type.
- Saturation, the vibrancy of the color.
- Value, the brightness of the color.

Button **Interpolate**
Select two squares in the grid and click on the button **Interpolate**. The colors between the two selected squares are interpolated.

Color Table File

Button **Import ...**
Imaris comes with a set of pre-defined color tables. Click on **Import...** to open the Import Color Table File window. Select a color table file and click on **OK**.

Button **Export...**
Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

A large number of predefined colormaps are available. If the default colormap (Spectrum) is not appropriate for your application, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

To apply the changes click on **OK**.

6.4.4.3 Tab Coloc Statistics

The tab Coloc Statistics is available when a data set contains a Coloc channel and the Coloc channel is selected in the channel selection of the Image Properties.

All statistics about the resulting colocalized volume are displayed. Definitions of the displayed values and further information are given in Section Coloc - Volume Statistics.

Button **Export...**
Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

See also:
Coloc - Volume Statistics

6.4.5 Thumbnail

Type

None
Select None if no thumbnail should be displayed.

Middle Slice
Thumbnail is the middle slice.

MIP
Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend
Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Preview

Displays a preview of the thumbnail image.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

6.4.6 Parameters

A set of informational parameters is appended to the image file.

Button **Add Surpass Group...**
Opens a dialog to add a new group.

Button **Delete Surpass Group...**
Deletes a Surpass group.

Button **Add Parameter...**
Opens a dialog to add a new parameter to the group.

Button **Delete Parameter...**
Deletes a parameter.

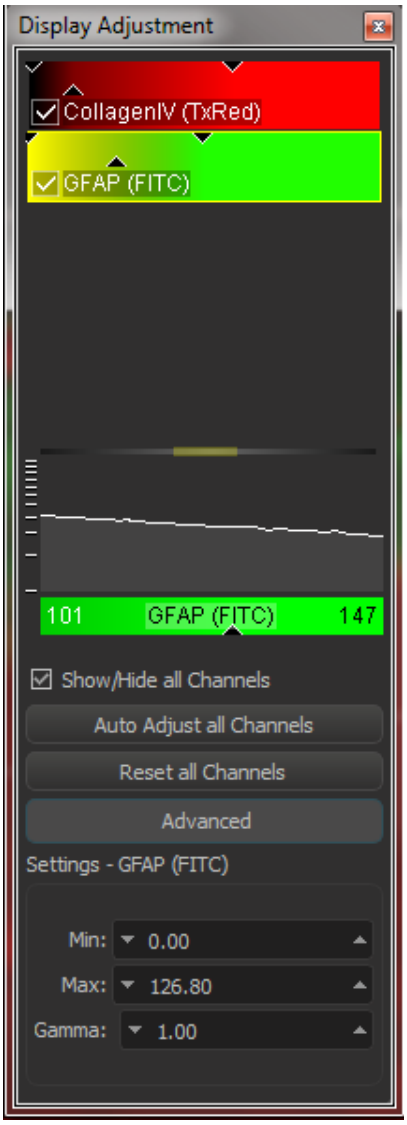
Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

6.5 Show Display Adjustments

The Display Adjustment options let you choose the channel visibility as well as improve the image display by concentrating on a limited color contrast range of voxels. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0 - 255).

The Display Adjustment function lets you set an upper limit for maximum color and a lower limit for minimum color (i.e. black). The range between these two limits - represented by triangle symbols, is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.



Display Adjustment Dialog (one for each channel)

Switch the individual channels on or off.

- Check or uncheck the required channel check-box to switch the channel visibility.

Change the channel parameters such as name, color and description.

- Click on the channel name to open the Image Properties. For a detailed description please refer to Section

Menu Edit - Image Properties - Channels 1...n.

Show/Hide all Channels
Check or uncheck to show or hide all channels as required.

Button **Auto Adjust all Channels**
The auto function works as follows: the minimum value is set to the first mode (maximum) of the histogram. The maximum value is set to a value that is around the 99.8 percentile of the histogram which effectively disregards high intensity outliers.

Button **Reset all Channels**
Click on the button to set the image back to the original values.

Button **Advanced**
Click on the button to open the Advanced settings (see below).

Advanced Settings

Min
Max
- Enter direct values in the Min (lower limit for minimum color) and Max (upper limit for maximum color) fields.
- Alternatively drag in the display adjustment dialog the upper or lower handle of the adjustment line to adjust the Min and Max limits.

The effect of the change can be seen on the channels (channels appear brighter or darker).

Gamma
The default value of the gamma correction is 1 (the range between lower and upper limit is extrapolated in a linear mode to the full data set range). Enter a value below 1 and the linear mode is transferred to a nonlinear mode, the lower intensities appear brighter. The effect of the change is directly visible in the viewing area.
- Enter the value in the respective field.
- Alternatively click onto the middle triangle in the display adjustment dialog and drag it to the left to increase brightness/to the right to decrease brightness.

List of Shortcuts in the Display Adjustment Dialog

Please refer to Section Menu Edit - Show Display Adjustments - Mouse & Keyboard PC.
Please refer to Section Menu Edit - Show Display Adjustments - Mouse & Keyboard Mac.

Histogram

A histogram is a statistical data analysis, representing linear voxel within an image of the selected channel.

Change Channel Color

Click on the channel name to switch directly to the channel properties (Menu Edit - Image Properties - Channels 1...n).

See also:
Menu Edit - Image Properties... - Channel 1...n

Menu Edit - Show Display Adjustments - Mouse & Keyboard PC
Menu Edit - Show Display Adjustments - Mouse & Keyboard Mac

6.5.1 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Display Adjustments Window

Ctrl + D	Shows the Display Adjustment window
Left-click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Left-click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
Ctrl + right-click	Automatic range for Min and Max

See also:
Addendum - Mouse & Keyboard PC

6.5.2 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Display Adjustments Window

Command + D	Shows the Display Adjustment window
Click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Command + click	Add channel to selection, or remove channel from selection
Click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
ctrl + click & drag	Move left: make image channel transparent Move right: make image channel opaque
Command + ctrl + click	Automatic range for Min and Max

See also:
Addendum - Mouse & Keyboard Mac

6.6 Add Time Points...

Additional time points can be added to the data set.

- Select the required image and click **Open**.

The time points to be added must contain the same number of channels and be of the same format as the current data set. A warning message is displayed if the formatting does not match.

Append with Small Time Gap
By default, the check box **Append with Small Time Gap** is activated. This triggers the same behaviour as in Imaris versions before Imaris 9.0: The original time information of newly added time points is altered by a

translation on the time line to the end of existing time data. Relative time differences in the added data set stay the same. The offset between last existing time point and first time point of the added data is set to 1 second.

If the check box is not activated, new time points are added without modifications. This could result in big time gaps on the time line.
If there is an overlap on the time line between existing time points and newly added ones, the same translation is applied as described above.

6.7 Delete Time Points...

Any time points that are not needed can be deleted.

Parameters

Delete From, To
Specify the time points to be deleted and click **OK**. The selected time points are automatically deleted from the data set.

6.8 Add Channels...

Adding channels is used to extend or complete the data set information if a microscope can only export one channel as a file at one time. All channels of a selected data set are added. The size of the data sets must be identical.

Window: Add Channels

- Select the required file and click **Open**.

All channels from this data set are added automatically. If the size of the data set does not match a warning message is displayed.

6.9 Delete Channels...

Deleting channels is used to erase unwanted information in a data set.

Select Channels to Delete

Channel 1...n
Select the channel to be deleted in the Delete Channels box and click **OK**. The effect is immediately visible on the image.

See also:
Imaris Menu - Open - Reader Configuration

6.10 Remove Images

Use Remove images to remove images from the current set of open images.

To remove images from a multi-image scene:
1. Check the checkbox beside the required images

2. Click OK to remove the image(s) or Cancel to cancel.

For more about multi-image functionality refer to Add Image... (multi-image)

6.11 Add Slices...

Adding slices may become necessary, if through manipulation, or during file export or formatting the data set consists of less than the originally acquired number of slices.

Window: Add Slices

- Select the requested file and click **Open**.

All slices from this data set are added automatically. The x-, and y-values of the two data sets must be identical and the same number of channels is required.

6.12 Delete Slice...

Parameters

Slice number [1...n]
Specify the slice to be deleted and click **OK**. The slice is automatically deleted from the data set.

6.13 Crop Time...

The total number of time points can be reduced at the beginning or end of the series of images.

Parameters

From, To
Enter the time points to be included in the data set and click **OK**. All other time points are deleted from the data set.

See also:
Imaris Menu - Open - Reader Configuration

6.14 Resample Time...

The total number of time points can be reduced to display the images at a faster speed.

Parameters

New Number of Time Points
Specify the number of time points needed and click **OK**.

The process of resampling the time points takes a while. When the process is finished, the time bar displays the new number of time points.

See also:
Imaris Menu - Open - Reader Configuration

6.15 Crop 3D...

Cropping the data set allows you to crop the images down to the region of interest. Cropping reduces the size of the data set and makes it easier and faster to handle the viewing and storing of the images.

Preview

Displays a sectional view of the actual image (current time point). A rectangle, representing the region of interest (ROI), is overlaid on all three views.

Select Crop Dimensions

X, Y, Z, From, To, Size

To modify the size and the position of the region of interest you can either enter the ranges of x, y, and z coordinates into the corresponding fields or adjust the region of interest box using the handles directly in the viewer window.

- To move the ROI, click inside the rectangle, hold the mouse button down & drag the entire ROI around.
- To shape the ROI, click on a handle, hold the mouse button it down and reshape the ROI. Side handles affect one direction, corner handles two directions.

The modifications apply to all slices and all time points of the image.
The displayed values correspond to the changeable voxel value.

Click **OK** when finished. The data set is cut down to the marked ROI. The rest is erased.

See also:
Imaris Menu - Open - Reader Configuration

6.16 Resample 3D...

Resampling reduces the voxel density in a data set to increase the processing speed. Reducing the data size also reduce the resolution. Resampling reduces the number of voxels in a grid but keeps the original relationship between the voxels.

New Size

X, Y, Z
The fields display the current x-, y-, and z-values. The requested values can be directly entered in the fields.

Aspect Ratio

Fixed Ratio X/Y
Fixed Ratio X/Y/Z
The Aspect Ratio of the data set's dimensions can be kept by checking the respective Fixed Ratio options. Clicking the **OK** button resamples the data set to the entered values.

Use a Gaussian filter as low-pass before sampling down an image.

See also:
Imaris Menu - Open - Reader Configuration
Menu Image Processing - Smoothing - Gaussian Filter

6.17 Change Data Type...

Type

From
Displays the current data set type.

To
Drop-down list to select the requested data set type from the supported types:

- unsigned 8 bit for the range 0...255.
- unsigned 16 bit (0...65535).
- 32 bit float.

Range Adjustment

Check field to determine how the data values are translated during the change.

None
Data values are imported in the new type.

Source Range to Target Range
Maximum data values are scaled to the new range (e.g., 0...255 to 0...65535).

Data Range to Target Range
Actual data range values are interpolated to the new range (e.g., 0...150 to 0...65535).

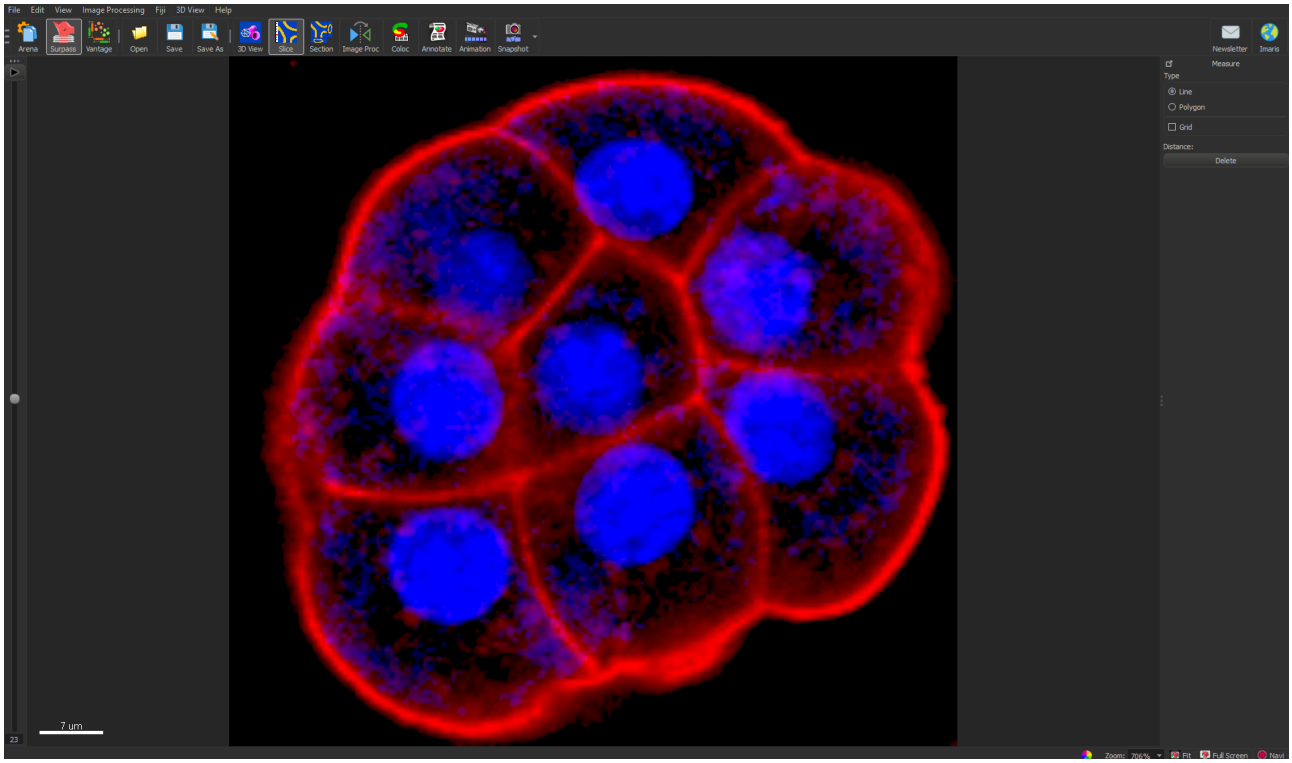
Section 7 View

		Please refer to Section:
3D View	Ctrl + 1	3D View
Slice	Ctrl + 2	Slice
Section	Ctrl + 3	Section
Animation	Ctrl + 4	Animation
Image Proc	Ctrl + 5	Image Proc
Coloc	Ctrl + 6	Coloc
Fit	Ctrl + B	
Reset	Ctrl + A	
Full Screen	F11	
Navi	Ctrl + N	
Load Window State		
Save Window State		
Origin Bottom Left		
Origin Top Left		
Origin Bottom Right		
Origin Top Right		
Status Bar		Toolbars - Status Bar
Refresh	Ctrl + F	
Event log		

See also:
Addendum - Mouse & Keyboard Mac

7.1 Slice

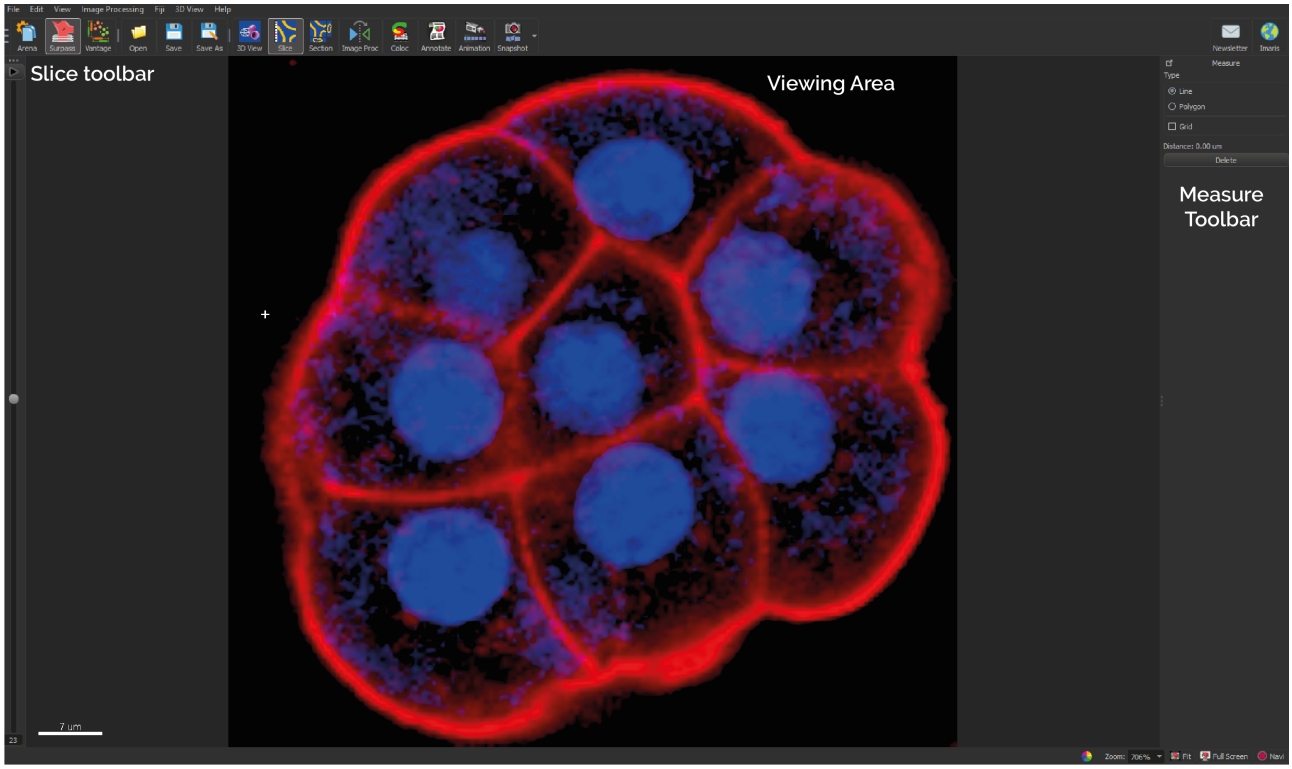
The Slice mode is the view in which an image is displayed when loading a data set into Imaris. The Slice representation shows a 2D view from the top in the z-axis. It shows all images in the data set along the z-axis, so you can inspect any slice along the z-axis for a general overview of the data set.



See also:
Overview
Slice Toolbar
Measure Toolbar
Mouse & Keyboard PC
Mouse & Keyboard Mac

7.1.1 Overview

The illustration below shows the slice view screen, with different areas of the view highlighted.



When moving the cursor over the image, the current voxel intensity and the 3D position in the data set (in brackets) is displayed in the lower left corner of the view.

See also:
Slice Toolbar
Measure Toolbar

Additional Information in the Status Bar

The Slice also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)
The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:
Toolbars - Status Bar

7.1.2 Slice Toolbar

Slider

To inspect any slice in the data set along the z-axis you use the Slider. Use the slider as follows:

- Drag the slider handle in the left control bar up and down.
- Select the slider handle with a left mouse click and then use the Arrow-keys up/down on the keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first and last slice respectively.

- Select the slider handle with a left mouse click and then press the PageDown-key or the PageUp-key to scroll through the first and last slice respectively in steps.
- Enter the desired slice number in the number field at the bottom of the slider.

Button **>**

Click on the **Play/Pause** button to see a continuous display through all the slices in the stack. To pause re-click on the button.

Slice Number Field

The respective slice number is displayed on the bottom of the slider in the number field.

7.1.3 Measure Toolbar

Type

Line

Set the two Measurement Points with two consecutive clicks. The result is displayed in the status field Distance.

Polygon

All Measurement Points are consecutively connected by lines and the displayed distance is the sum of the distances between the points.

Grid

Check this box to display a grid in the image.

Distance

The result of the measurement is displayed in the status field distance.

Button **Delete**

Click on **Delete** to clear the status field and start a new measurement.

7.1.4 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Slice

Ctrl + 2	Activates the Slice
Left-click	Set a measurement point
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

7.1.5 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Slice

Command + 2	Activates the Slice
Click	Set a measurement point

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

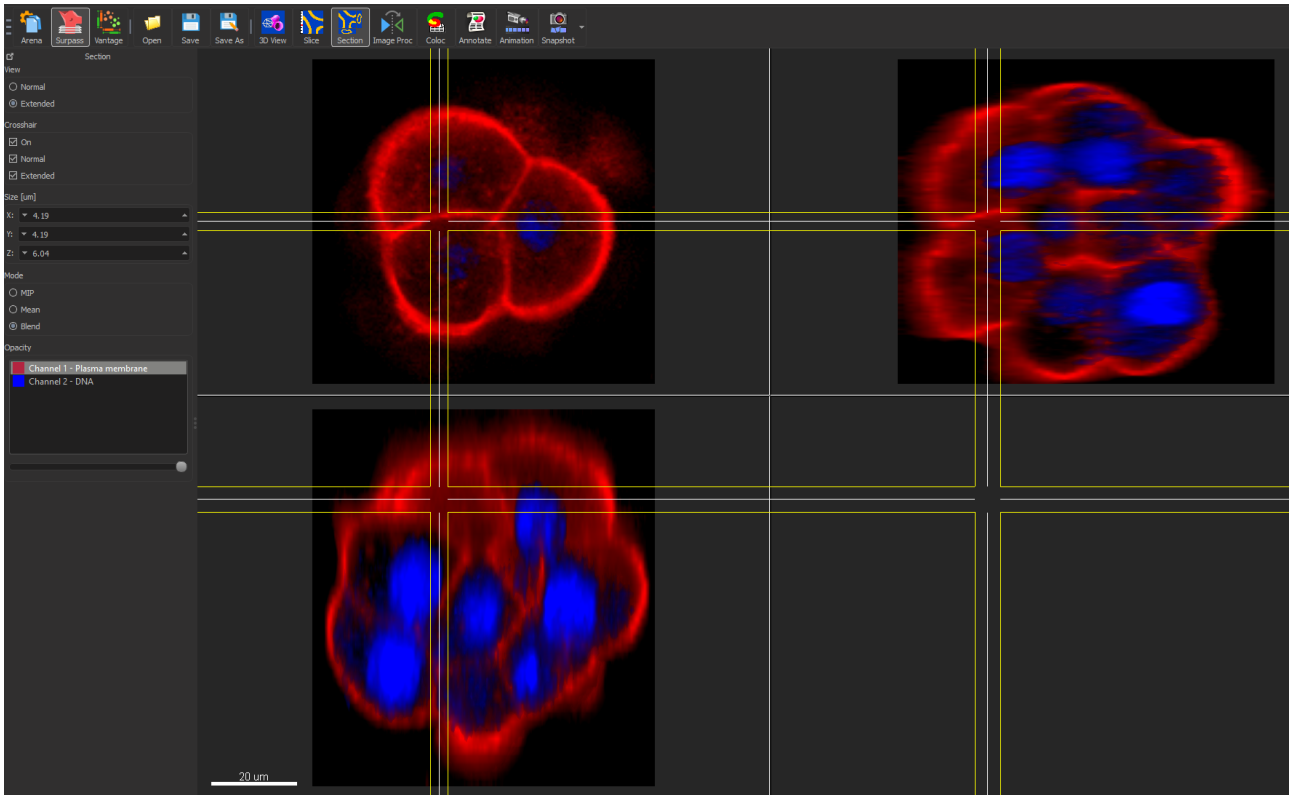
Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

7.2 Section

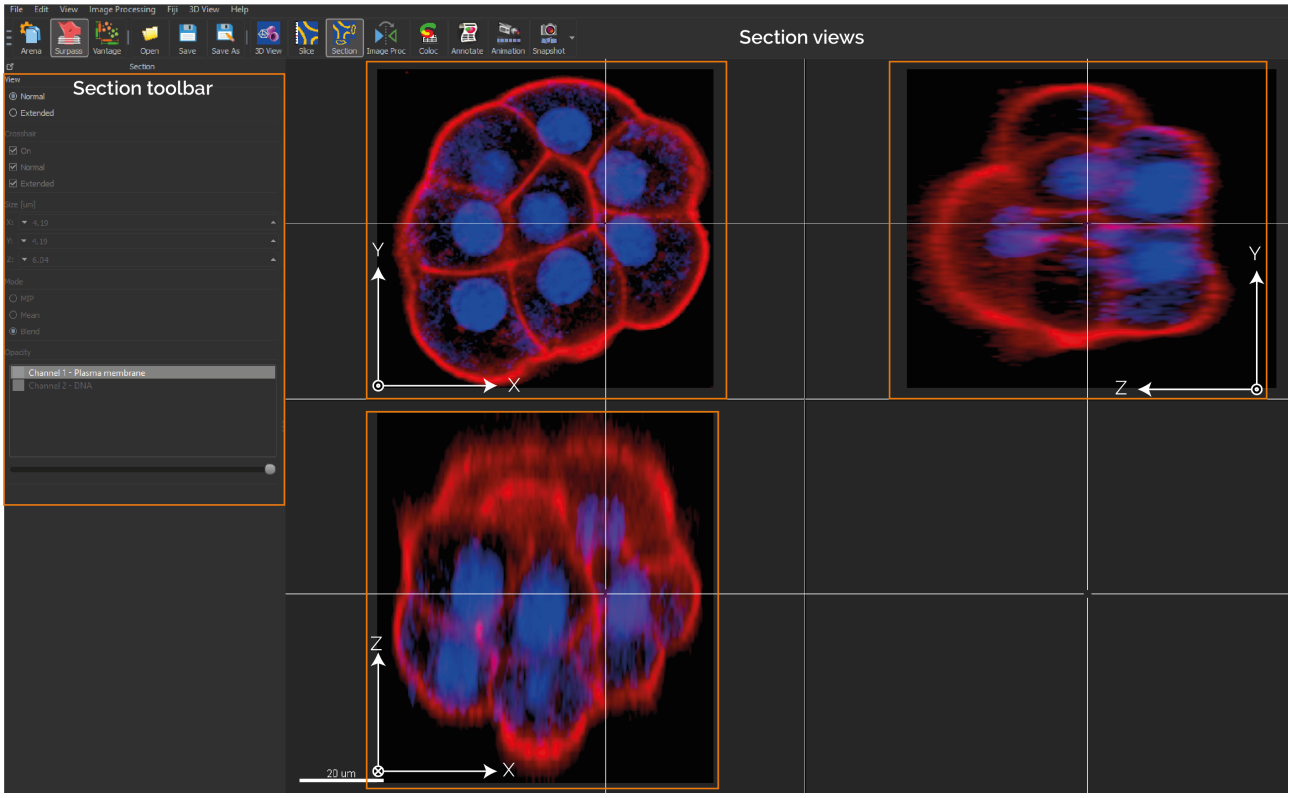
The Section lets you inspect the environment of any given point within the data set by showing the cut through the point in the x-, y-, and z-axes or a defined portion of the data set (several slices together).



See also:
Overview
Section Toolbar
Mouse & Keyboard PC
Mouse & Keyboard Mac

7.2.1 Overview

The illustration below shows the coordinates in the three different display areas of the section.



To select a point, click in any of the three views, hold the button down & drag the crosshairs to the requested point. The crosshairs immediately snap to the cursor when clicking anywhere in the image. The other views, connected by one coordinate, automatically follow the movements.

See also:
Section Toolbar

Additional Information in the Status Bar

The Section also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)
The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:
Toolbars - Status Bar

7.2.2 Section Toolbar

View

Normal
Selects a single cut through the data set.

Extended

Additional colored lines are displayed in the viewing area. If Extended is checked the parameter sets Crosshair, Size and Mode are available.

Crosshair

The parameter set Crosshair is only available, if Extended is checked in the parameter set View (see above).

Normal
You may eliminate the Crosshair display on the image by unchecking the box. It is then only visible at the edges.

Extended
Select a portion of the data set in the x-, y-, and z-direction to be displayed. In the main screen, separate the two crosshairs. The planes between the crosshairs are rendered in the views showing the other axis. This allows you to see only certain interior structures without both the top and the bottom.

- The image shows the white crosshair line together with colored focus range lines.
- The focus range can be set in any of the three directions as follows: Move the cursor on a colored line. The cursor changes its form to a straight arrow with a bar.
- Drag the line in the respective direction. The range is extended or reduced. Its depth is reflected in the x-, y-, and z-fields in the Size display. The Size values are indicated in the data set's dimensions, e.g., in µm. It can also be adjusted directly by entering values in the Size fields (see below) or by means of their up and down arrows.

Uncheck the Crosshair – Normal and/or Crosshair – Extended boxes to eliminate the crosshair and/or the focus range lines display on the image.

Size fields

The parameter set Size is only available, if Extended is checked in the parameter set View (see above). Display of the actual x-, y-, and z-position of the extended Crosshair. The positions can also be adjusted directly by entering values in the fields or by means of their up and down arrows.

Mode

The parameter set Mode is only available, if Extended is checked in the parameter set View (see above). Here you may choose the best display mode for the slice portion.

MIP
Calculated maximum over all slices in the range.

Mean
Calculated mean value over all slices in the range.

Blend
Calculated upon the transparency of the slices.

7.2.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Section

Ctrl + 3	Activates the Section
Left-click	Set the section center
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

7.2.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Section

Command + 3	Activates the Section
Click	Set the section center

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

7.3 Coloc

In fluorescence microscopy, colocalization describes the presence of two fluorochromes at the same physical location. ImarisColoc provides extended functionality for the analysis and visualization of colocalization in multi-

channel data sets. It provides an automated selection of colocalization based on a method introduced by Costes & Lockett at the NIH, NCI/SAIC.

Coloc processes 2D, 3D, and 4D data sets. It operates on two channels simultaneously and measures the degree of overlap of the two channels.

With Coloc you can determine and display locations within the data set with common information from all channels, e.g., to illustrate where all types of proteins can be found simultaneously or to detect low-density tissues in all channels.

The desired range for signal analysis can be limited by defining intensity thresholds or sets of intensity values for each of the involved channels. All voxels falling inside everyone of these channel-specific intensity limits represent a colocalization. They are emphasized in the view and statistics are calculated and displayed. When the selection is changed, the numeric and visual feedback happens in real-time.

The result of the colocalization as a whole can be saved as a separate channel, which can then be processed and viewed as any originally acquired channel in Imaris. Multiple colocalization images are easily combined into a single view in Imaris Surpass.

Coloc is an optional module within the Imaris program and is not available as a standalone program.

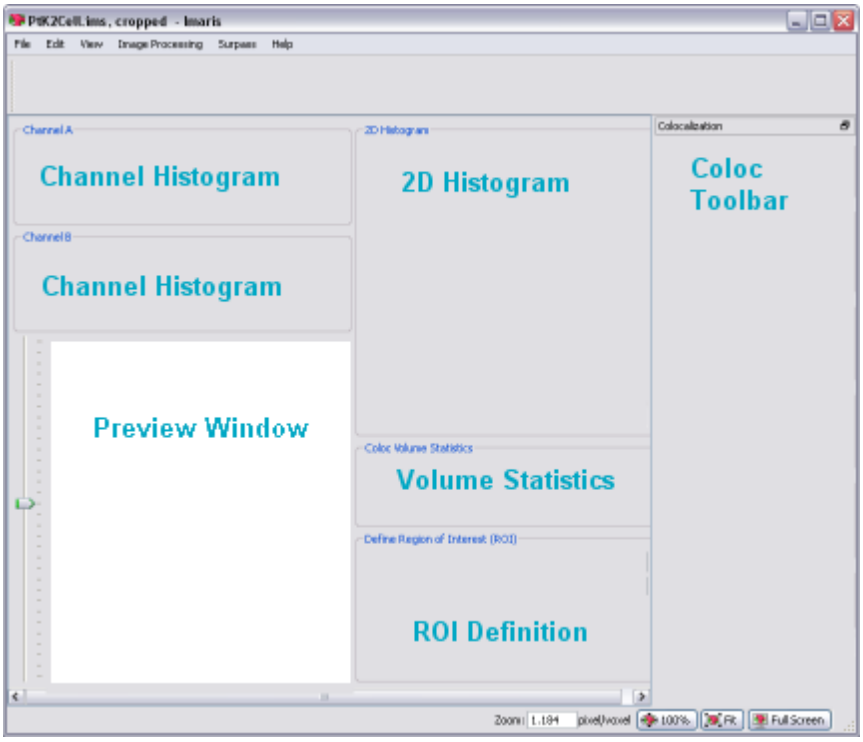
How to Display the Coloc Main Screen?

- Click on the Coloc icon in the Main toolbar,
- Select View - Coloc,
- Press the key combination Ctrl + 7.

See also:
Coloc - Mouse & Keyboard Mac

7.3.1 Overview

The Coloc main screen consists of different areas:



- Channel Histogram (channel A, channel B). This area allows the selection of the two channels for the colocalization analysis.
- Preview Window. This area visualizes the data set together with a preview of the colocalized region.
- 2D Histogram. This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.
- Volume Statistics. This area displays statistics about the resulting colocalized volume. The display adapts instantly to all modifications in the selection.
- ROI Definition. This feature allows you to define a region of interest (ROI) for the entire analysis.
- Coloc Toolbar. The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

7.3.2 Channel Histogram

This area allows the selection of the two channels for the colocalization analysis. The intensity histograms are displayed for the currently selected channels.

Select Channel A and Channel B

The two channels for colocalization detection must be selected first.

- Select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Threshold

- The threshold for each channel can then be defined by clicking in the histogram, dragging the colored line directly, or by entering the value in the threshold field.

Adjust Histogram Settings

If necessary, you can adjust in the Coloc toolbar the histogram mode and histogram options.

See also:
Coloc - Coloc Toolbar
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

7.3.3 Preview Window

This area visualizes the data set together with a preview of the colocalized region. It displays a Slice representation of the data set, i.e. a 2D view from the top in the z-axis direction. On the left hand side, a sliding bar with a handle allows inspecting and displaying the single slices in the data set along the z-axis. The Preview window updates in real-time the display of the colocalized region based on the actual settings of the colocalization parameters. The original channels can be switched on or off in the Display Adjustments window.

Time Settings

There are no time-dependent parameters for the colocalization analysis. The Preview window always displays the currently selected time point of the data set. The values in the Statistics window always refer to the volume of the current time point. However, when the **Build Coloc Channel** button is pressed, the Coloc channel and statistical values for all time points are calculated.

See also:
Menu Edit - Show Display Adjustment
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

7.3.4 2D Histogram

This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.

Adjust Channel Thresholds

Effects of the modification are instantly visible in the channel histogram A and B, and in the Preview window.

Selection Mode Threshold
The channel thresholds are visible in form of a rectangle.

- Point with the cursor on the 2D histogram.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

Selection Mode Polygon
The channel thresholds are visible as an intensity area in form of a free configurable polygon.

You draw the polygon on the 2D histogram. This method is especially useful for eliminating channel bleed through regions along the border of the lower intensity bins. After checking the Polygon mode, the overlaid

threshold rectangle in the 2D histogram is converted into a polygon with four corner points. You can now start to modify this polygon or to draw a completely new one. The initial point of the polygon is visible as white filled.

Set Point
Press and hold the Shift-key. The cursor turns into a cross with a little sign. Click on the 2D histogram with the left mouse button to place a point (vertex). Any additional point connects with lines to the initial point and the last point inserted.

Insert Point in a Line
Press and hold the Shift-key. The cursor turns into a cross with a little " + " sign. Point on the line between the two vertices and click on the line with the left mouse button to add a point.

Automatic Point Setting
Press and hold the Ctrl-key. The cursor turns into a cross with a little " + " sign and a stack symbol. Drag the cursor around. New vertices are added automatically at each change of cursor position.

Move Point
Point on one of the vertices, click & drag the vertex around to the desired location. The polygon changes its shape.

Move Line
Point on a line between two vertices, click & drag the line around to the desired location. The polygon changes its shape.

Move Polygon
Point somewhere beside of the polygon edge, click & drag the polygon around to the desired location.

Delete Point
Shift + click on a point to delete the point.

See also:
Coloc - Coloc Toolbar (selection Mode Threshold or Polygon)

7.3.5 Volume Statistics

The values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. The values are calculated by the following formulas.

Please note: The calculated Coloc Volume Statistics are not automatically saved in the data set. To save or export the statistics you have to build a coloc channel (Coloc - Coloc Toolbar - Build Coloc Channel). For publishing Coloc statistical values please use only the statistics reported in the Channel Properties.

Due to a real-time response, some of the statistical values shown in the main Coloc window are generated from a sub-sampled data set. The accuracy of these values depends on the variability introduced by the sub-sampling procedure.
Depending on the data set, the difference between the values reported in the Channel Properties (a high accuracy values, produced from the entire data set) and these values (calculated from sub-sampled data set) may differ significantly.

Definitions

Regions:

Data set	All data set voxels
Coloc	Colocalized voxels
Object A	Voxels with channel A intensity above threshold A
Object B	Voxels with channel B intensity above threshold B
ROI	Region of interest

Indicators:

N	Number of voxels
SAi	Channel A intensity of voxel
SBi	Channel B intensity of voxel
SAavg, Coloc	Average channel A intensity in the Coloc region

Volume A	Number of voxel channel A x voxel volume
Volume B	Number of voxel channel B x voxel volume
Material A	Channel A voxel signal intensity
Material B	Channel B voxel signal intensity

Number of colocalized voxels

Total count of colocalized voxels N_{coloc} .

Please note:

- **Ncoloc is the number of colocalized voxels for the defined region of interest (ROI).**
- **Colocalization statistics are all calculated using the ROI.**

% of data set colocalized

Percentage of total data set voxels colocalized.

$$\frac{N_{coloc}}{N_{dataset}} . 100$$

% of volume A above threshold colocalized

Percentage of channel A voxels above threshold A colocalized.

% of volume B above threshold colocalized

Percentage of channel B voxels above threshold B colocalized.

% of material A above threshold colocalized

Percentage of channel A material above threshold A that is colocalized.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in objectA} SA_i} . 100$$

% of material B above threshold colocalized
Percentage of channel B material above threshold B that is colocalized.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in objectA} SB_i} . 100$$

% of ROI colocalized
Percentage colocalization of channel A and channel B volume inside the region of interest.

$$\frac{N_{coloc}}{N_{mask}} . 100$$

% of ROI material A colocalized
Percentage colocalization of ROI channel A material.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in mask} SA_i} . 100$$

% of ROI material B colocalized
Percentage colocalization of ROI channel B material.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in mask} SB_i} . 100$$

Pearson`s coefficient in colocalized volume
Pearson correlation of channel A and channel B inside the colocalized region.

Please note: The correlation value ranges between 1 and -1. A value of 1 represents perfect correlation, 0 no correlation, and -1 perfect inverse correlation.

Pearson`s coefficient in data set volume
Pearson correlation of channel A and channel B inside the entire data set volume.

$$R_{AB,dataset} = \frac{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})(SB_i - SB_{avg,dataset})}{\sqrt{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})^2 \sum_{i \in coloc} (SB_i - SB_{avg,dataset})^2}}$$

Pearson`s coefficient in ROI volume
Pearson correlation of channel A and channel B inside the region of interest.

$$R_{AB,\ mask} = \frac{\sum_{i \in mask} (SA_i - SA_{avg,\ mask})(SB_i - SB_{avg,\ mask})}{\sqrt{\sum_{i \in mask} (SA_i - SA_{avg,\ mask})^2 \sum_{i \in mask} (SB_i - SB_{avg,\ mask})^2}}$$

Original Mander`s coefficient A
Original Mander's coefficient A = Sum(SAiSBcoloc) / Sum(SAi)
SBcoloc=1 if SBi>0, otherwise SBcoloc=0

Original Mander`s coefficient B
Original Mander's coefficient B = Sum(SBiSAcoloc) / Sum(SBi)
SAcoloc=1 if SAi>0, otherwise SAcoloc=0

Mander`s coefficient A (thresholded)
Thresholded Mander's coefficient A = Sum(SAiSBcoloc) / Sum(SAi)
SBcoloc=1 if SBi>thresholdB, otherwise SBcoloc=0

Mander`s coefficient B (thresholded)
Thresholded Mander's coefficient B = Sum(SBiSAcoloc) / Sum(SBi)
SAcoloc=1 if SAi>thresholdA, otherwise SAcoloc=0

See also:
Menu Edit - Image Properties... - Channel 1...n - Tab Coloc Statistics (to export the statistics)
Coloc - Coloc Toolbar - Build Coloc Channel (to build the coloc channel)

7.3.6 ROI Definition

Define Region of Interest (ROI)

This feature allows you to define a region of interest (ROI) for the entire analysis. A third channel can be selected as a masking area for the entire analysis. A masking channel is e.g. a third microscope channel acquired with settings that allows defining a region of interest by simple intensity thresholding. All voxels outside of the region of interest defined by the mask channel are ignored for the colocalization analysis. The volume excluded by the mask appears hatched in the Preview window. The mask channel is used in conjunction with the Automatic Threshold function. For this function, the selection of an appropriate region of interest is essential.

Mask data set
Check this box to activate the function.

X% of Data selected
Displays the percentage of selected data.

Channel Selection
Select the channel to be used from the drop-down list.

Channel Histogram
Point with the cursor into the histogram and click to set the threshold. A bolded line marks the threshold in the histogram and the corresponding value is indicated in the threshold field. The masked volume of the data set appears hatched in the Preview window. Drag the cursor in the histogram to modify the threshold. Effects of the modification are instantly visible in the threshold field as well as in the Preview window.

Threshold Value
Editable threshold value field.

Mask Channel and Automatic Threshold

When the mask is set as required, click the button **Automatic Thresholding** to calculate the thresholds. Build the colocalization channel by clicking the button **Build Coloc Channel**.

See also:
Coloc - Coloc Toolbar - Automatic Thresholding
Coloc - Coloc Toolbar - Build Coloc Channel
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

7.3.7 Coloc Toolbar

The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

Selection Mode

You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bold line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bold lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bold lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

Threshold
To adjust the threshold value using the mouse (see above 1. and 3.), select this mode.

Polygon
To draw a polygon line in the 2D histogram (see above 4.) select this selection mode.

Automatic Thresholding

Please refer to Section Coloc - Coloc Toolbar - Automatic Thresholding.

Histogram Mode

The histogram mode options define the voxels selection for the colocalization calculation within the data set.

Single Slice
The 2D histogram is calculated and displayed for the current slice only.

Single Time Point
The 2D histogram is calculated and shown for the entire 3D stack of the current time point. This mode allows for faster browsing through the data set along the z-axis.

All Time Points
The 2D histogram is calculated and shown for an entire time data set, i.e. for all time points within the 4D data set.

Histogram Options

The histogram mode options are used to define the display of the 1D and 2D histograms.

Ignore Border Bins
A border bin is always the first and last value in a histogram. Often a lot of voxels fall into the bin with the lowest intensity. In a frequency plot this causes a massive and unnecessary downscale of all other intensity bins which are of more interest. The border bins can therefore be ignored when scaling the display of the histogram. Ignore is selected as default. With most data sets, checking the Ignore Border Bin and Logarithmic options allows a more representative histogram display.

Logarithmic
The frequency plot is shown in a logarithmic scaling instead of a linear scaling.

Color Coded
Applies a false color look-up table to the histogram to improve the visibility of intensity differences. The high frequencies (bins in the 2D histogram into which a lot of voxels accumulate) are shown in yellow-to-white, bins with little voxels are shown in blue-to-black.

Coloc Intensities

The Coloc intensities option defines the brightness of the calculated colocalization displayed in the Preview window.

Source Channels
Uses the intensities of the two channels for displaying the intensity of a colocalized voxel as the square root of the product of the intensities of the original channels.

Constant Value
Displays the intensity of a colocalized voxel defined by the specified value. No distinction is made whether high-intensity or low-intensity voxels colocalize as long as they are within the selection.

Coloc Color

Coloc color allows you to specify personal color settings for the colocalization display in the Preview window.

Button **Edit**
Clicking on the button **Edit** opens the Color window. You can either select one of the predefined colors to apply on the colocalization areas or define, add, and apply your own color definitions.

Button **Build Coloc Channel**
Please refer to Section Coloc - Coloc Toolbar - Build Coloc Channel.

Button **Build Time Dep. Coloc**
Please refer to Section Coloc - Coloc Toolbar - Build Coloc Channel.

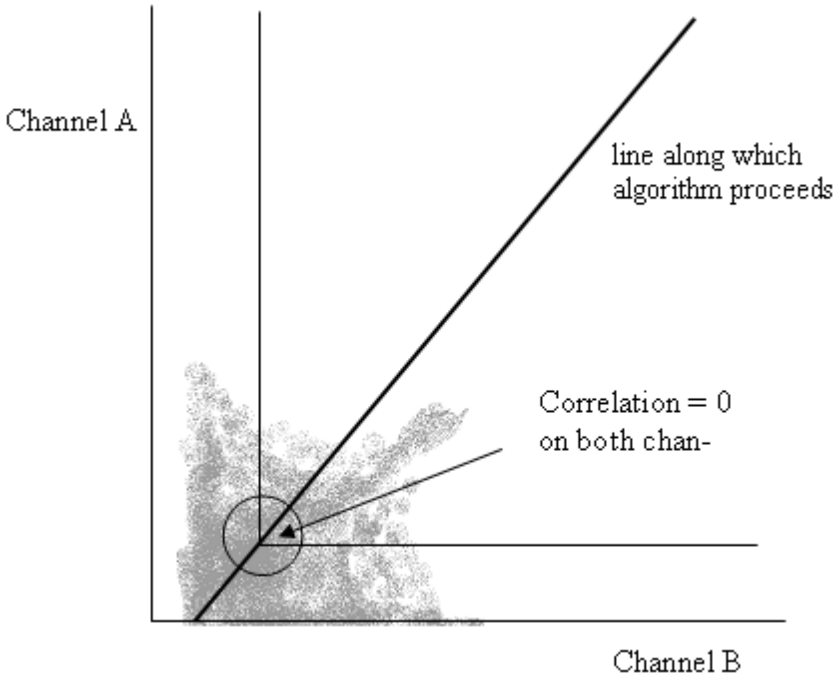
Button **Channel Statistics**
Please refer to Section Menu Edit - Image Properties... - Channel 1...n - Tab Coloc Statistics.

See also:
Coloc - Channel Histogram
Coloc - Preview Window
Coloc - 2D Histogram

7.3.7.1 Automatic Thresholding

Button **Calculate Thresholds**
This button performs an automatic threshold run on both channels based on an algorithm developed by Costes and Lockett at the National Institute of Health, NCI/SAIC, which is based on the exclusion of intensity pairs that exhibit no correlation (Pearson's correlation below zero).

The automatic threshold search is done using the 2D histogram. Starting with the highest intensity value, the algorithm reduces the threshold value step by step along a line (see figure below) and computes the correlation coefficient of the image using only voxels with intensities below the threshold. The algorithm continues reducing the thresholds until the correlation reaches 0, thus defining the automatic threshold.



P-Value
PSF Width
As the size of the Point Spread Function please enter the length along the Z Axis. This can be a calculated theoretical length.
You can find links to calculators on the web.
The default in Imaris is not actually related to the PSF but only to the diagonal of the voxel size.

For more information about the P-Value and PSF Width please refer to the following publication:

Automatic and Quantitative Measurement of Protein-Protein Colocalization in Live Cells

Sylvain V. Costes,*y Dirk Daelemans,z Edward H. Cho,* Zachary Dobbin,* George Pavlakis,z
and Stephen Lockett*

* Image Analysis Laboratory, National Cancer Institute, Frederick, Maryland;
y National Cancer Institute/Science Applications International Corporation, Frederick, Maryland; and
z Human Retrovirus Section, National Cancer Institute, Frederick, Maryland

Biophysical Journal Volume 86 June 2004 3993-4003

7.3.7.2 Build Coloc Channel

Button **Build Coloc Channel**
Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set. The channel can then be saved and is part of the data set.

Button **Build Time Dep. Coloc**
Clicking this button calculates the colocalization channel according to the selected settings (calculating an automatic threshold individually for each time point) and adds it to the data set. The channel can then be saved and is part of the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File – Export or click the **Export** button in the toolbar. The Export box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

Button **Channel Statistics** (for the new Coloc channel)
After building the Coloc channel, its statistics become available in the Image Properties. The button **Channel Statistics** displays directly the Image Properties window with the statistics for the calculated channel. The name of the channel, the description of the source channels and the selection method are indicated and displayed in the Image Properties on the Tab Coloc Statistics.

Export Statistics

The statistics can be exported.

Button **Export**
In the Image Properties window click the **Export** button. The Export Coloc Statistics window is displayed. Select the directory and enter the name for the file to be saved. The statistics are saved as .csv Excel file and can be opened directly with Microsoft Excel.

See also:
Coloc - Volume Statistics

7.3.8 Basic Operation

The basic use of Coloc in the daily work flow comprises the following steps:

- Select the channels.
- Adjust selection mode and histogram mode.
- Set thresholds.
- Check the statistics.
- Build and save the Coloc channel.

Select Channel A and Channel B

In the 1D histograms select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Selection Mode and Histogram Mode

Before you set the thresholds, please check the appropriate parameters in the Coloc toolbar on the right hand side of the screen:

- Check as Selection Mode Threshold.
- Decide, which Histogram Mode you need. Choose Single Slice for calculating the histogram for the current slice only. Or choose Single Time Point to calculate the histogram for the entire 3D time point. Or choose All Time Points for the entire 4D image.

Set Thresholds

Colocalization occurs where the voxels are above threshold in channel A and channel B. You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bold line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bold lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bold lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

A. On the 1D Channel Histogram

- Point with the cursor on the histogram. The cursor turns into a cross.
- Click on the histogram to determine a threshold. The bold left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the line and drag it to another position in the histogram. Effects of the modification are instantly visible in the other indications as well as in the Preview window.
- Perform for both channels until the settings are as desired.

B. By Entering a Value

- Click in the Threshold field of one of the channels and enter a value, then press Enter. The bold left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot. Effects of the modification are instantly visible in the other indications as well as in the Preview display.
- Perform for both channels until the settings are as desired.

C. On the 2D Histogram

- Point with the cursor on the 2D histogram. The cursor turns into a cross.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

D. On the Preview Window

- Click with the cursor on the image in the Preview window. An active contour is displayed, outlining image elements brighter than the location clicked.
- Drag the line on the image to define the threshold of channel A. The selection works best when starting with brighter spots and dragging towards darker areas.
- Press and hold the Shift-key, click with the cursor on the image, then drag the line to determine the threshold of channel B.

Check the Statistics

The statistical values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. For details please refer to Section Coloc - Volume Statistics.

Build Coloc Channel

Button **Build Coloc Channel**
Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File - Export or click the **Export** button in the toolbar. The Export box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

7.3.8.1 Advanced Examples

The colocalization channel can be visualized like any other channel in Imaris. In the following examples, a Coloc channel was built on the retina data set.

Visualize the Coloc Channel in Easy 3D

Example 1: Visualize and Adjust the Coloc Channel in Imaris Easy 3D

- After building the Coloc channel, click on the **Easy 3D** button in the Imaris menu bar.
- Check the desired Rendering mode parameters and adjust the Settings if necessary, then click the **Calculate** button. The 3D image of channel is calculated and displayed.

As the colocalized region always lays inside the initial channels, making the initial channels invisible or more transparent allows a better view of the colocalized region.

- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustments window uncheck one of the two original channels, then click the button **Calculate** again. The 3D image of the original and the Coloc channel is calculated and displayed.
- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustment window select the Coloc channel and adjust its settings.
- In the Display Adjustment window select the remaining original channel and adjust its settings. In this example the channel is set as partly opaque.
- After each modification, click the **Calculate** button again to calculate the result.

Visualize the Coloc Channel in Surpass

The colocalization channel can be visualized like any other channel in Surpass.

Example 2: Create Surfaces of the Coloc Channel and Add the Two Initial Channels as Volume Rendering

- After building the Coloc channel, click on the **Surpass** button in the Imaris menu bar.
- In the Objects toolbar click the **Add New Surfaces** button.
- In the Surfaces Properties window check and select the Coloc channel.
- Select the desired parameters. In this example, we set the threshold just below the lowest intensity values in

the histogram.

- In the menu Surpass select Volume.
- In the Imaris menu bar select Edit – Show Display Adjustment and uncheck the Coloc channel. The Surpass Volume object displays only the two initial channels. Then select the two initial channels and adjust their settings, e.g. decrease the blend opacity.

7.3.9 Mouse & Keyboard PC

Coloc Mouse & Keyboard Functions

Ctrl + 6	Activates the Coloc view

Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Left-click on histogram	Set new threshold
Left-click on line & drag	Move threshold

2D Histogram

Left-click	Set channel A and channel B thresholds
Left-click on line & drag	Move channel A and channel B thresholds

Preview Window

Left-click & drag	Set threshold channel A
Shift + left-click & drag	Set threshold channel B
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + left-click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Left-click & drag (on point or line)	Move point or line
Left-click & drag (outside or inside polygon)	Move polygon

7.3.10 Mouse & Keyboard Mac

Coloc Mouse & Keyboard Functions

Command + 6	Activates the Coloc view

Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Click on histogram	Set new threshold
Click on line & drag	Move threshold

2D Histogram

Click	Set channel A and channel B thresholds
Click on line & drag	Move channel A and channel B thresholds

Preview Window

Click & drag	Set threshold channel A
Shift + click & drag	Set threshold channel B

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Command + click	Add multiple new points
Click & drag (on point or line)	Move point or line
Click & drag (outside polygon)	Move polygon
Delete	Delete single point

7.4 Animation

The Key Frame Animation function defines several subsequent views (key frames) of your image, which will be used to create an animation. Imaris generates smooth transitions between the selected views by interpolation.

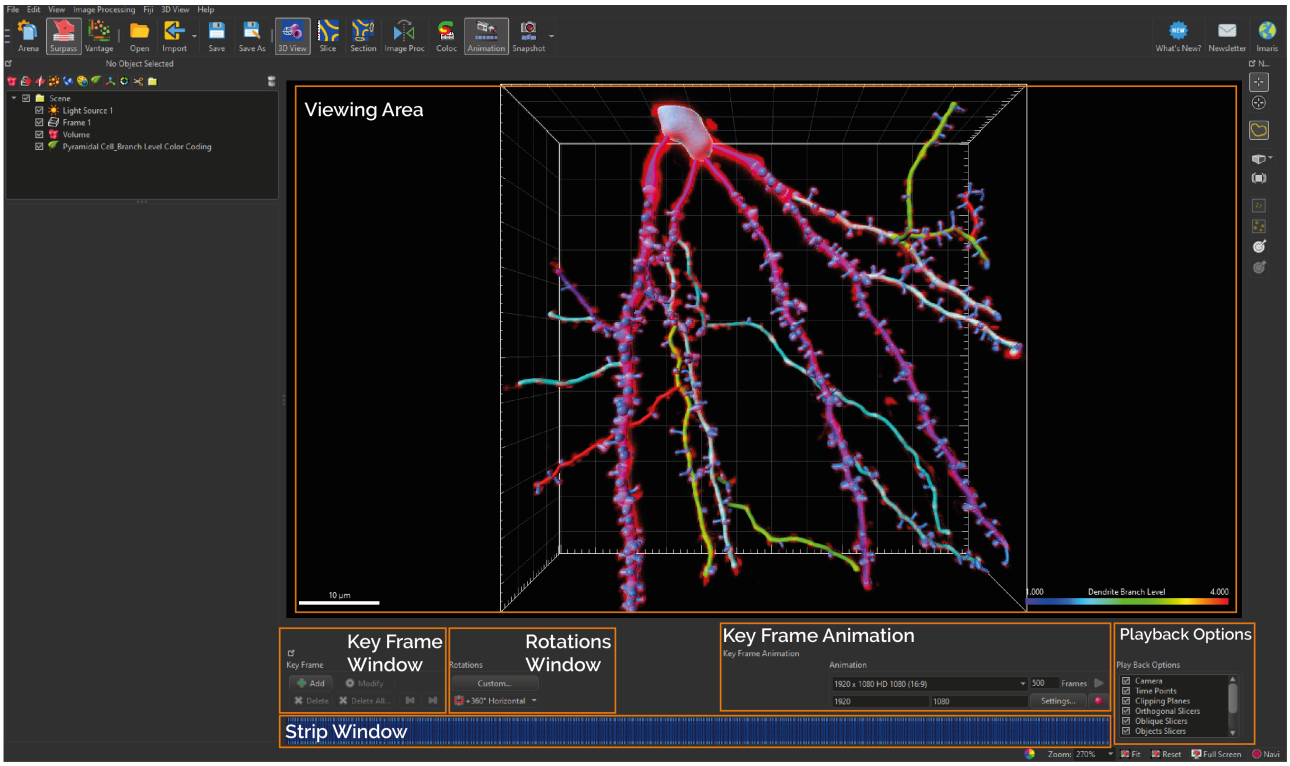
How to Open the Key Frame Animation Window?

1. Click on the icon in the Main toolbar.
2. Select View - Animation.
3. Press the key combination Ctrl + 6.

See also:
Menu Edit - Preferences... - 3D View
Animation - Mouse & Keyboard Mac

7.4.1 Overview

The Key Frame Animation consists of different areas:



At the bottom of the screen the following windows are displayed: the Key Frame Window, the Rotations Window, the Key Frame Animation Window, the Playback Options selection, and the Strip Window.

For a video tutorial on how to make Animations in Imaris please see our Imaris Homeschool Session Create Eye Catching Animations and Snapshots using Imaris or for a shorter demonstration please see Create an Amazing Video from Microscopy Data.

7.4.2 Key Frame Window

In the Key Frame window you define and manage the key frames of your movie.

Button **Add**
Use this button to insert user-defined key frames in the strip sequence.

Button **Modify**
Use this button to modify user-defined key frames in the strip sequence.

Button **Delete**
Use this button to delete user-defined key frames in the strip sequence.

Button **Delete All**
Click on this button to delete all user-defined key frames in the Strip window.

Large spaces between the user-defined key frames (thick blue lines) will cause that section of the movie to play more slowly. Placing the lines close together will cause that section of the movie to play more quickly. Use this button to set a constant speed throughout the entire movie.

Button **II<**
Button **II>**
With the arrow buttons you step to the previous (arrow to the left) or next (arrow to the right) user-defined key frame.

Buttons Overview

Add	**Add** new user-defined key frame
Mod.	**Modify** active, user-defined key frame
Del	**Delete** active, user-defined key frame
Del. All	**Delete** all key frames
II<	Go to **Previous** user-defined key frame
II>	Go to **Next** user-defined key frame

For the complex use of these buttons please refer to Section Animation - Strip Window.

See also:
Animation - Create Animation

7.4.3 Rotations Window

Use the templates to quickly define standard camera motions for the key frame animation system.

Templates
Select one of the predefined templates.
You can customize your animation rotation by choosing one of the following options: +360 horizontal, + 180 horizontal, - 360 horizontal, - 180 horizontal, +360 vertical, + 180 vertical, - 360 vertical, - 180 vertical

The key frames are inserted automatically. Click on the button **Play** to see the animation.

Button **Custom**
Click on this button to display the Custom Rotation window to freely define the rotation (see below).

Space

Screen
The rotation axis is associated to the x-, y-, and z-axis of the screen.

World
The rotation axis is associated to the x-, y-, and z-axis of the data set.

Axis

X
Y
Z
Turn along the x-, y-, or z-axis.

Direction

+
Turn to the left.

-
Turn to the right.

Angle

Select or type in the desired angle.

Button **OK**
Click on this button to accept the changes.

Button **Cancel**
Click on this button to cancel the changes.

7.4.4 Animation Window

Frames
The number in the field **Frames** indicates the number of thin blue lines within the Strip Panel. This number

defines the duration of animations.

Button **Settings ...**
To open and adjust the Key Frame settings click on this button. For details please refer to Section Menu Edit - Preferences... - 3D View.

Button **>**
Click on this button (arrow to the right - **Play/Pause**) to start the movie preview. To pause the movie preview re-click on this button.

Button **O**
To record the movie click on this button (red dot - **Record**). Selecting this button the Export Movie window is displayed (see below).

Window: Export Movie Window

File name
Enter a file name.

Export type
Select the desired file type (TIFF series, or AVI).

Movie Settings

Compression Factor
Select a compression factor between 0 (high quality) and 100 (low quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate
Define how many frames are displayed per second.

Play Movie with default Media Player when finished
Check the box to play the movie with the default media player when finished.

Menu Edit - Preferences... - Surpass (default Key Frame settings)

7.4.5 Playback Options

In the State selection you can adjust the play back settings.

Camera
- Check: Play back the previously recorded Camera positions for each key frame.
- Uncheck: Do not play back the recorded Camera positions. The Camera stays in the same position. You can change the Camera position manually while playing the animation.

Time Points
- Check: Play back the previously recorded Time Points for each key frame.
- Uncheck: Do not play back the recorded Time Points. The Time Points do not automatically change during the animation. You can change the Time Points manually while playing the animation.

Colors
- Check: Play back the previously recorded Colors for each key frame.
- Uncheck: Do not play back the recorded Colors. The Colors do not automatically change during the animation. You can change the Colors manually while playing the animation.

Display Adjustments
- Check: Play back the previously recorded Display Adjustments for each key frame.
- Un-check: Do not play back the recorded Display Adjustments. The Display Adjustments do not automatically change during the animation. You can change the Display Adjustments manually while playing the animation.

Clipping Plane (Position)
- Check: Play back the previously recorded Clipping Plane positions for each key frame.
- Uncheck: Do not play back the recorded Clipping Plane positions. The Clipping Plane positions do not automatically change during the animation. You can change the Clipping Plane positions manually while playing the animation.

Orthogonal Slicer (Position)
- Check: Play back the previously recorded Orthogonal Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Orthogonal Slicer positions. The Orthogonal Slicer positions do not automatically change during the animation. You can change the Orthogonal Slicer positions manually while playing the animation.

Oblique Slicer (Position)
- Check: Play back the previously recorded Oblique Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Oblique Slicer positions. The Oblique Slicer positions do not automatically change during the animation. You can change the Oblique Slicer positions manually while playing the animation.

Objects Show/Hide (Object Visibility)
- Check: Play back the previously recorded objects for each key frame.
- Uncheck: The visibility of the objects in the viewing area can be changed manually during the animation. Check or un-check Surpass Tree Items during the animation and the effect is directly visible in the viewing area.

See also:
Animation - Create Animation

7.4.6 Strip Window

The Strip window provides the working area. To display the views of the different key frames left-click on a line to display the corresponding image in the viewing area.

Lines in the Strip Window

Thick Blue Line
A thick blue line indicates a user-defined key frame.

White Line
A white line represents the active key frame.

Thin Blue Line
A thin blue line stands for an interpolated frame.

Actions in the Strip Window

Add user-defined Key Frame

- Click on a thin blue line (interpolated key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add** to insert a user-defined key frame in the strip sequence.
- Click on a thick blue line (user-defined key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add**. Each time you click on the button Add you insert a new user-defined key frame right to the last active key frame.

Please remember: If you select a thin blue line, you define a new user-defined key frame exactly on this location. If you select an already user-defined key frame the new key frame is halfway between the selected and the next user-defined key frame on the right hand side.

Delete user-defined Key Frame
Click on a key frame and then on the button **Delete**.

Move user-defined Key Frame
To move a key frame in the Strip window drag the key frame line while holding down the mouse button. The first and last key frames can not be moved.

Modify user-defined Key Frame
Select the key frame in the Strip window. Turn the image to a new position and click on the button **Modify**. The new position for the selected key frame is saved.

Copy user-defined Key Frame
To copy a key frame in the Strip window select the initial key frame with a click. The scene in the viewing area changes accordingly. Right-click on the key frame in the Strip window at the desired copy position. The scene in the viewing area does not change. Click on the button **Add** to insert the user-defined key frame.

Please remember: Click on a key frame to change the camera position in the viewing area accordingly. Right-click (if you use a three button mouse) on a key frame and the view does not change.

Strip Window Overview

Click on key frame	Select key frame and change view accordingly
Right-click on key frame	Select key frame, view does not change
Button **Add**	**Add** new user-defined key frame
Button **Mod.**	**Modify** selected, user-defined key frame
Button **Del**	**Delete** selected, user-defined key frame

Menu Edit - Preferences... - 3D View (default interpolated frame settings)

7.4.7 Create Animation

The Key Frame Animation feature in Surpass allows you to create an animation from views (key frames). It interpolates frames between the user-defined views to create a smooth movie.

1. Adjust Pre-Settings

- Create an object (Surfaces, Volume, etc) from which to create the animation.
- In the Main toolbar click on the icon **Animation**. The Key Frame Animation window displays on the bottom of the screen and is divided in the Key Frame window, Rotations window, Animation window, State window, and Strip window.
- In the Animation window you find the data field Frames. Enter the number of frames to be included in the movie.
- Click the **Settings** button and enter the number of frames per second.

2. Add Key Frames

- Turn the image view to the first position.
- Capture the first view with a click on the button **Add** in the Key Frame window (records position). The key frame appears in the Strip window.
- Move the image to the next viewing position.
- Click on the button **Add** in the Key Frame window to set the next key frame position (records new position). The new key frame appears in the Strip window.
- Repeat as desired, until all user-defined key frames are added.

3. Select Play Back Settings

- In the State selection you can adjust the play back settings. Please refer to Section Animation - Play Back State for details.

4. Play the Animation

- In the Animation window click on the button **Play** to start the animation.

Example: Handle Camera

Use 360° Template

Use the templates to quickly define standard camera motions for the key frame animation system.

- In the Rotations window select +360° Horizontal. Five key frames are inserted automatically. The first and the last key frames are identical.
- In the State window check Camera.
- In the Animation window click on the button **Play** to see the animation. The camera rotates 360° horizontally.

Capture Individual Camera Positions

- With the pointer in Navigate mode, position the image to create the view for the first key frame.
- In the Key Frame window click the button **Add**. With the first click on **Add** you insert the first and last key frames, they are identical. The key frames appears in the Strip window.
- Position the image to the desired view for the next key frame and click the **Add** button to place the next key

frame.
- Repeat as desired, until all user-defined key frames are added.
- In the State window check Camera.
- To preview the movie, click in the Animation window on the button **Play**. The camera position changes automatically.

Move the Camera Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Camera.
- Play the animation and change the Camera position during the play back manually.

Example: Handle the Orthogonal Slicer

Individual Orthogonal Slicer Positions

- Add the Surpass Tree Item Orthogonal Slicer and check the box in the Surpass Tree.
- Adjust the Orthogonal Slicer for the first key frame and click on the button **Add**.
- Move the Orthogonal Slicer before you add the next key frame and so on.
- In the State window check Orthogonal Slicer.
- Play the animation. The Orthogonal Slicer position changes automatically.

Change Orthogonal Slicer Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Orthogonal Slicer.
- Play the animation and change the Orthogonal Slicer position during the play back manually.

See also:
Animation - Overview
Animation - Key Frame Window
Animation - Rotations Window
Animation - Animation Window
Animation - Play Back State
Animation - Strip Window
Animation - Save Animation

7.4.8 Save Animation

When you are satisfied with the movie, there are two possibilities to save the animation; either as an Imaris Scene File or as a movie-file.

In Imaris as Scene File

Whenever you save the Scene File in Imaris, the actual animation is saved automatically as part of the Scene File.

Save Different Animations in Different Scene Files

Saving different animations of the same data set requires saving of different Scene Files. After generating a new movie by the Key Frame Animation, rename each time the Scene File, e.g. add "Movie" at the end of the Scene File name.

Record the Animation as Movie

To save the animation as movie click in the Animation window on the button **Record** (red dot). The Export Movie dialog window is displayed on screen. As File name type in the corresponding Scene File name, select the file type and choose the Movie Settings. Click on **Save**.

The **Export type** option allows you to select the movie output file format and save the settings as a preset for future use.

Imaris lets you **save** and **create movie** files on **any platform**. The movie file type specifies the video format and predetermines the codes required to view saved animation.
To playback the movie you must use the appropriate Media player for the selected movie file type.

Available Movie File Formats

- H264 Movie (*.mp4)
- Raw Movie (*.avi)
- Tiff (series) (*.tif, *.tiff)

Please note: The number and type of media player codes available depends on your operating system version, video card, and other software installed on your system. If you have experienced problems with viewing the created animation files we recommend installing the free and platform independent, VLC player.

Multi-threaded Movie Writers

Multi-threading offers a significant (up to 30%) increase in speed during movie creation and are therefore recommended.

The following movie writer is multi-threaded:

- MPEG-4 Movie (*.mp4)

See also:
Surpass View - Overview - Scene File Concept
Animation - Animation Window (Button Record)

7.4.9 Mouse & Keyboard PC

Animation Mouse & Keyboard Functions

Ctrl + 4	Activates the Key Frame Animation

In the Strip Window

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

7.4.10 Mouse & Keyboard Mac

Animation Mouse & Keyboard Functions

Command + 4	Activates the Key Frame Animation

In the Strip Window

With a one-button mouse:

Click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Ctrl + click on key frame	Select key frame, view does not change

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

7.5 Fit

Fits the entire scene into the view.

In the Status Bar you find the respective button **Fit**.

See also:
Toolbars - Status Bar

7.6 Reset

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

7.7 Full Screen

Displays the viewing area to full size of the monitor. To return to the standard window, click on the button **Full Screen** in the lower left corner.

In the Status Bar you find the respective button **Full Screen**.

See also:
Toolbars - Status Bar

7.8 Navi

The Navigation window displays an overview of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. You can activate the Navigation window individually for each Imaris view.

In the Status Bar you find the respective button **Navi**.

Change Position

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Double-click the Navigation window again to switch the automatic "snap-to-corner-mode" back on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section the position of the Navigation window is fixed (lower right corner) and can not be moved.

7.9 Origin

The location of the coordinate system origin can be set to any corner of all the views. It can be set to: Top Left (default) or Bottom Left. A selection is stored and used each time you start Imaris.

7.10 Status Bar

At the bottom of the screen you find the Status Bar.

7.11 Event log

Event Log is a window that displays detailed information about Imaris Arena activity. This can be helpful when troubleshooting problems and errors.

7.12 Refresh

Refresh the screen.

Section 8 Image Processing

The Image Processing menu provides a range of functions to change the image data. Frequently used functions are Baseline Subtraction, and Threshold Cutoff from the Image Thresholding submenu or the Gaussian Filter from the Image Smoothing submenu. Image processing is performed on a per channel basis. The copy and paste buttons for each command can be copied and pasted so that settings can be applied across multiple channels easily.

Button **OK**
Clicking **OK** applies the image processing function to all time points.

Flip ...
Rotate...
Free Rotate
Channel Shift...
Swap Time and Z
Swap Time and Channels
Image Processing Dialog
AutoQuant...
XTensions

8.1 Flip...

The channels can be flipped together or separately.

On the left hand side of the window Flip Channel(s) the actual channel(s) are displayed. Select the channels to be flipped by marking the check-box on the left.

All Channel(s)

Direction X, Y, Z
Check the requested Direction box.

Button **Apply**
Button **OK**
Button **Cancel**
Click the **Apply** button. The effect of the change can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

8.2 Rotate...

Rotate direction applies to all channels together. Single channel application is not possible.

All Channel(s)

Axis X, Y, Z
Enter the requested axis.

Orientation
Counter Clockwise, Clockwise
Select the respective orientation.

Button **Apply**
Button **OK**
Button **Cancel**
Click the **Apply** button. The effect result can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

8.3 Free Rotate

The Free Rotation function rotates an image by any degree and offers additional image resizing options. The camera location determines the rotation angles and directions. The center of the image is the center point of the rotation. The operation is preformed for every time point and channel.
Please note selecting just one channel for free rotation will create two data sets.

X, Y, Z

These fields display the current x-, y-, and z axis vector values.
To alter the image position you can write the values directly into the fields or you can as well adjust the image position in the Surpass View.

- The x, y and z axis values are the components of a vector from the origin, to the point with the specified x,y, and z value.
- The rotation will rotate the image around this axis by a specified amount (angle).
- The amount of rotation is specified as an angle. The angle can have any value. 0 means no rotation. Rotations will repeat themselves after 360 degrees. For example, a rotation of 45 degrees is the same as one of 405 degrees, the same as one of -315 degrees and the same as one of 765 degrees etc.

Angle

This field displays the current value of the angle of rotation.

Coordinate resize factor

The image and voxel values define the size of the new data set after free rotation.
These values are used to build a map of all the co-ordinates that will be used to display the newly rotated image.

Free rotation can be an expensive memory operation. Adjusting the image dimensions can reduce the size of the data set, decreasing the time needed to read the file and speeding up calculation. This can be particularly important when reading large data sets.

Voxel

Changing the x, y, and z values creates a new image with different voxel spacing.

These values determine the resampling points. Resampling reduces or increases the voxel density in a data set.
Reducing the resampling points deteriorates the image but speed up any operations on the data.

X, Y, Z
In the Voxel X, Y and Z fields, enter the factor by which to multiply the current resampling interval.
For decimation (fewer samples), enter a factor greater than 1 to increase the sampling interval by this factor.
For interpolation (more samples), enter a factor less than 1 to decrease the sampling interval by this factor.

Image

If the border of the new image needs to be adjusted, change the x, y, and z values and create a new image with the different image size. This option helps you to crop (minimize the size) the data set without loosing significant data.

X, Y, Z
The parameters X, Y and Z allow you to specify the size of a rotated image by defining the range of an original image.
In the Image X, Y and Z fields, enter the crop factor of the lower and upper image range.
The values of X, Y and Z should be in the range between 0 and 1.

Padding Intensity

When you create a new image with free rotation some of the new voxel surroundings are included with the data set. The padding voxels define an area between the previous outside boundary of the original image and newly rotated image content.
This padding intensity field lets you edit the newly created pad voxel intensity.

Linear interpolation

After rotation and data size adjustment, a new image with different voxel spacing is created and the new voxel coordinates need to be calculated.
The difference between the voxel spacing on the new and old image means that an interpolation scheme is required as the new image's voxels are unlikely to coincide with those in the old image.
The Linear interpolation box allows you to select the interpolation method.
If the box is selected for new voxel coordinates then the tri-linear interpolation method is used (all surrounding values are used for calculations).
If the box is not selected the voxel spacing will be calculated based on the nearest neighbor interpolation
Depending on the data set and parameter values the interpolation algorithm could involve some rather time intensive internal calculations. When speed is favored, please consider selecting the less-time costly interpolation method (nearest-neighbor).

8.4 Channel Shift...

The Channel Shift function allows you to move channels relative to one another.

On the left hand side of the window Channel Shift the actual channel(s) are displayed. Select the channels that should be moved relative to the remaining channels.

Pixel Shift for All Selected Channel(s)

X, Y, Z
The translation is specified in terms of integer numbers of voxels to move along each of the x-, y-, and z-directions.

8.5 Swap Time and Z

Exchange the t-axis and the z-axis and re-format the image in such a way that the time dimension is mapped onto the z-axis and the z-dimension is mapped onto the t-axis. The section now shows x-t-, and y-t-views.

Start Function

- Select in the menu Image Processing - Swap Time and Z.

The z-, and t-axes are exchanged. This allows you to play the images over z and t.

Play the Series

- Click on Start (arrow to the right) in the Time Bar to play the series of images along the z-axis.

The Time Bar is visible in all display modes (Slice, Section, Gallery 3D Preview, and Surpass). It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Example:
Your data set has four Time Points, each Time Point has one Slice in z-dimension. Then you swap time and z. The result is a new data set with one Time Point and four Slices in z-dimension.

See also:
Toolbars - Time Bar

8.6 Swap Time and Channels

Exchange the t-axis and the channels and re-format the image.

Start Function

- Select in the menu Image Processing - Swap Time and Channels.

The t-axes and the channels are exchanged.

Example:
Your data set has four Time Points, each Time Point has one channel. Then you swap time and channel. The result is a new data set with one Time Point and four channels.

See also:
Menu Edit - Image Properties - Channel 1 ... n

Menu Edit - Show Display Adjustment

8.7 Extract Channel

The Extract Channels compare the color content of an image to reference color information. This function extracts and displays color data from the image referent point and use it as a representative color, for a new color channel creation. The new channel is generated based on the difference in the reference color point and the whole image color values.
The difference relates how closely the color information in the region matches the information represented by the color reference point, assigning the higher intensity for smaller difference.The result of the Extract Channel is a separate channel, which can be processed and viewed as any originally acquired channel in Imaris and used for easier segmentation of similarly colored multi-channel data sets.

1. Click on the **Slice** icon in the Main toolbar.
2. Change pointer mode from Navigate to **Select**.
3. Select the reference color and click on it.
4. Click on the **Get value from the last measurement point** button displays associated RGB values in the numerical fields.
5. Specify Color Difference by selecting the color space (either RGB or Perceptual).
6. Select the Apply button. The effect of the change can be seen on the screen.
7. When the results are satisfactory, click the **OK** button.
8. The new channel is created and it's a part of the data set.
9. To restore the initial image click Cancel.

The new extracted channel can be visualized like any other channel in Imaris by selecting the Edit menu and choosing the option Display Adjustment Window. In the Display Adjustment Window uncheck original channels, select the newly created channel and adjust its setting.

Please note:

Channel selection in the Extract channel window switches to match Display Adjustment channel selection when you click Get value from the last measurement point button

Extracted channel values

1. The number of channels switched on in Extract Channel window influence extracted channel values.
2. Display Adjustment Window color parameters (min/max/gamma) influence the extracted channel values.
3. However, the number of channels switched on in Display Adjustment Window does not influence the extracted channel values

Channels

The channels can be selected by clicking on the check-box.

Color of Interest

The Color of Interest option allows the reference color point to be present either as separate value for red green and blue or as the intensity value for a selected channel.
The reference color point is shown within the color wheel.

RGB

The RGB triple value is displayed for the color reference point.

Intensity

The Intensity of the selected channel is displayed for the color reference point.

Get value from the last measurement point Button
Clicking this button calculates the RGB or intensity values according to the selected color reference point.

Color difference
This option defines the color space for a newly created channel.

RGB
The RGB color space is an additive color model in which values for Red, Green and Blue color information are combined together to reproduce any given color.

Perceptual
Implements the color extraction using Lab color space. The Lab color model provides a perceptually uniform color space. A perceptually uniform color space indicates that a change in a color value produces a change of about the same visual importance. Lab color is designed to approximate human vision.
For more specific information about Lab color space: http://en.wikipedia.org/wiki/Lab_color_space

8.8 Image Alignment

Two options are available:

Set Reference Frames
This option analyses the image and automatically corrects the drift. You can select if only **Translational Drift** or **Translational and Rotational Drift** should be corrected. The result of this option is the creation of a Reference Frame surpass object. The image and other objects are not affected.

Align Image
This option analyses the image and automatically corrects the translational and rotational drift. As a result, the image will be changed. In the Objects options section, you can choose if none, all or the selected surpass objects should be adjusted along with the image.

The **Result dataset size** section lets you control the resulting image size.

Crop Largest common region creates a dataset that contains the overlapping region between the original and the corrected dataset.
New size equal to current size creates a dataset that has the same size as the original dataset and contains the overlapping region.
Include entire result creates a dataset that fully contains the original and corrected dataset.

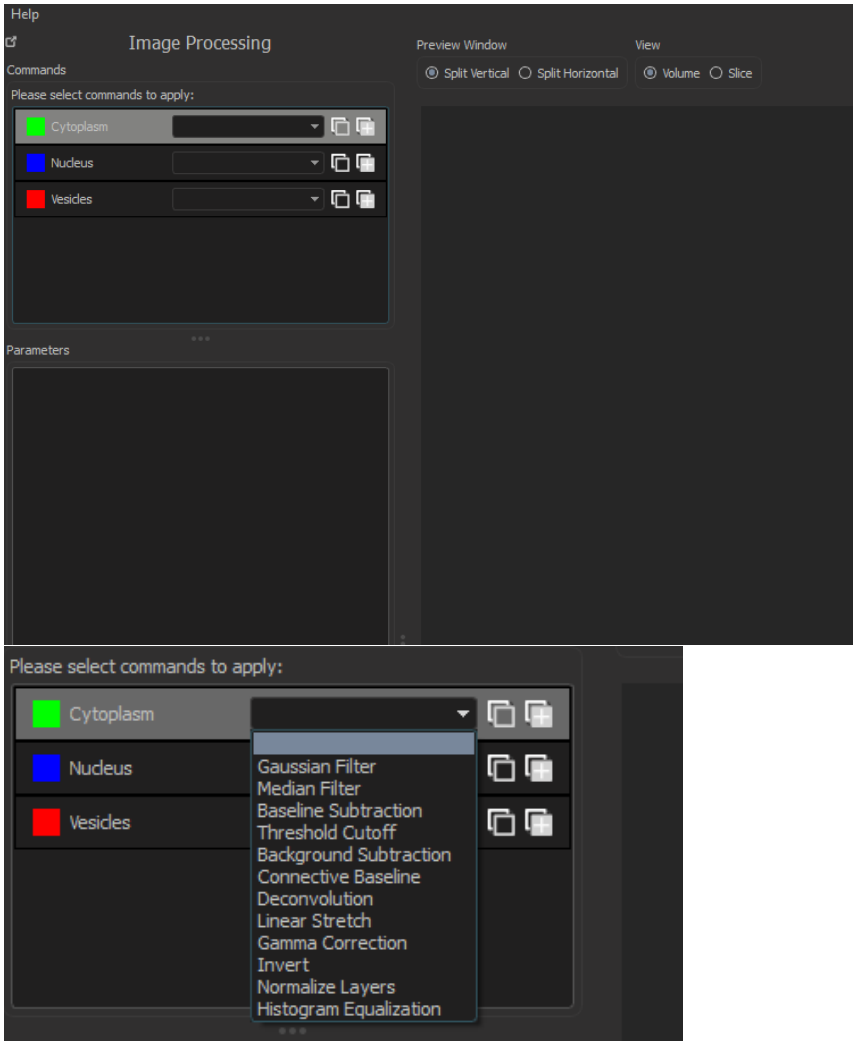
The **Preview** section illustrates the resulting dataset in pink, compared to the original and corrected image in black.

Drift Correction is also possible from Tracked Objects and from the Reference Frame.

8.9 Image Processing Dialog

The Image Processing Dialog can be opened by selecting 

Once open options for image processing are available to select from the drop down menus.

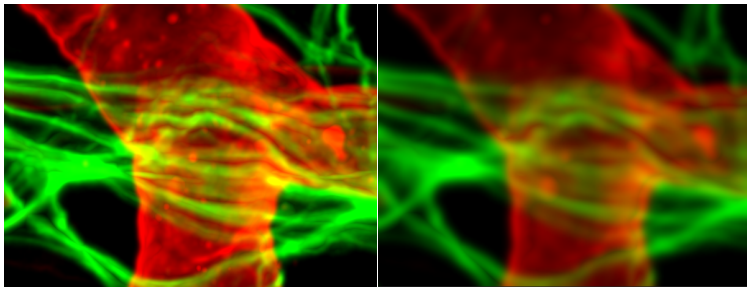


More information on each of the image processing options is available in the following sections of the reference manual:

Gaussian Filter...
Median Filter...
Baseline Subtraction...
Threshold Cutoff...
Background Subtraction...
Connective Baseline...
Deconvolution
Linear Stretch...
Gamma Correction...
Invert...
Normalize Layers...
Histogram Equalization

8.9.1 Gaussian Filter

The Gaussian filter smooths the image.



Retina demo image. Original on left and Gaussian filter applied on red and green channel on the right.

Applying a Gaussian Filter to an image means that:

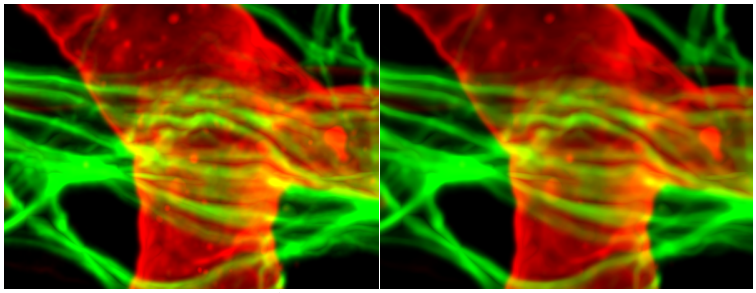
- Total image intensity is preserved.
- Noise is suppressed evenly across the image.
- No artefacts are introduced.

Parameters

Filter Width
Higher values result in a greater level of smoothing, whereas, lower values result in less smoothing. The filter width parameter field can be adjusted for each channel individually and structures smaller than the filter width will be significantly attenuated, while structures larger than the filter width will be less impacted. This can help removing noise.

8.9.2 Median Filter

The Median filter smooths the image.



Retina demo image. Original on left and Median Filter applied on red and green channel on the right.

The Median filter replaces the intensity of each voxel with the statistical median of the intensities of the neighboring voxels. The median filter is useful when the images are corrupted by 'salt and pepper' noise.

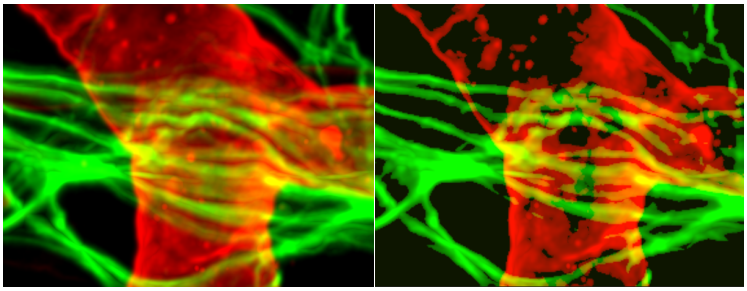
Parameters

Filter size
Select from one of the following options:
3 x 3 x 1
3 x 3 x 3
5 x 5 x 5

The filter size parameter field can be selected for each channel individually.

8.9.3 Threshold Cutoff

The Threshold Cutoff allows voxels in the image below a user selected threshold to be replaced with an alternative value.



Retina demo image. Original on left and Threshold Cutoff applied on red and green channel on the right.

Threshold Cutoff compares the intensity of every voxel in the image to the threshold value. If the intensity of the voxel is greater than the threshold, it remains unchanged. If a voxel has an intensity value less than the threshold value it is assigned a new intensity value which is 0 by default. The default value of 0 can can be changed by setting the **Set values below to** parameter field.

Parameters

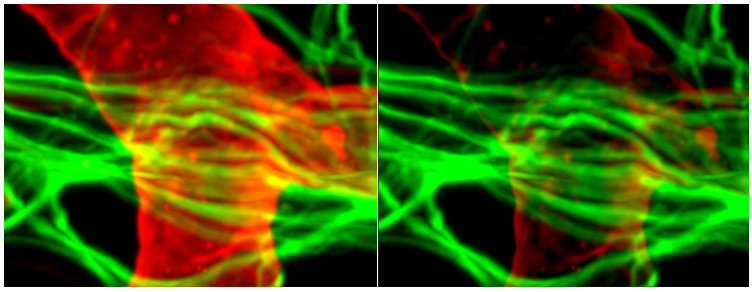
Threshold
Specify a threshold value, which all other voxels are compared against.

Set values below to
Allows the user to set the intensity of all voxels below the threshold parameter to this value.

The parameter values of **Threshold** and **Set values below to** may be adjusted on each channel individually.

8.9.4 Baseline Subtraction

Baseline Subtraction allows the user to remove voxels below a selected baseline intensity value.



Retina demo image. Original on left and Baseline Subtraction applied on red and green channel on the right.

Baseline Subtraction subtracts the baseline value from the intensity of every voxel in the image. If the result is positive, this is the new intensity value, if it is negative, the intensity of the voxel is set to zero.

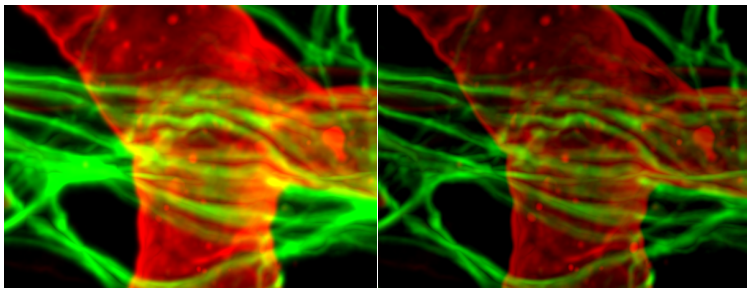
Parameters

Baseline
Specify a baseline value of intensity, which is then subtracted from the intensity value of all other voxels in the image.

The baseline parameter values may be set for each channel individually.

8.9.5 Background Subtraction

Background Subtraction applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background.



Retina demo image. Original on left and Background Subtraction applied on red and green channel on the right.

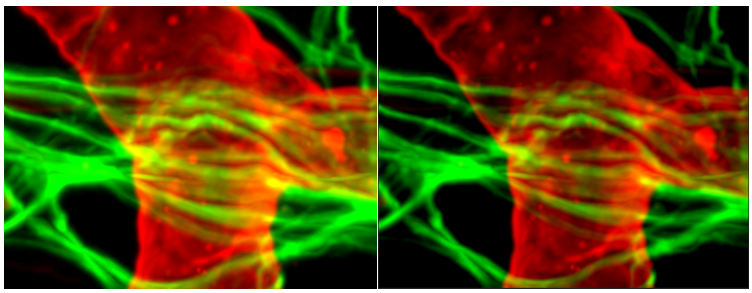
Parameters

Filter Width

Higher values result in a greater level of smoothing, whereas, lower values result in less smoothing. The filter width parameter field can be adjusted for each channel individually and structures smaller than the filter width will be removed.

8.9.6 Connective Baseline

The Connective Baseline smooths the image.



Retina demo image. Original on left and Connective Baseline applied on red and green channel on the right.

The Connective Baseline works with two threshold values, a lower baseline and an upper threshold. It applies these thresholds to divide the image into regions and then processes each region with a Baseline Subtraction using either the lower or the upper threshold. The three types of regions the method creates are the following:

- Connected region of voxels where the intensity of every voxel within the region is below the lower threshold (baseline) (Type A).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold (baseline) and no voxel within the region has an intensity greater than the upper threshold (Type B).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold (baseline) and at least one voxel within the region has an intensity greater than the upper threshold (Type C).

The intensities of voxels from regions of type A are set to zero, as are the intensities of voxels from regions of type B. Only voxels from regions of type C retain a nonzero intensity, namely the original intensity less the lower threshold value.

Parameters

Baseline
Lower threshold value for the intensity of the voxel.

Threshold
Upper threshold value for the intensity of the voxel.

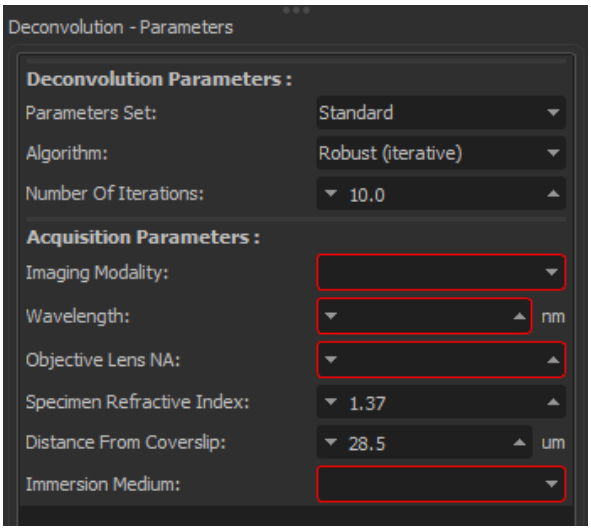
Both the baseline and threshold parameter fields can be adjusted for each channel individually.

8.9.7 Deconvolution

Deconvolution is available where supported and enables deconvolution to be applied to the image. Deconvolution is supported on NVIDIA graphics cards with CUDA Compute Capability 3.0 or above and drivers supporting CUDA 10, or other Graphics Cards supporting OpenCL 1.2 or later. At least 4GB of on-board GPU RAM is recommended. Deconvolution can be used to apply a useful improvement to image quality, the level of improvement will depend on the image mode, settings and performance of the optical and imaging setup. Due to the nature of this process more information on the imaging modality and parameters are required:

Deconvolution Parameters

Select between standard and advanced parameters. Both the standard and advanced parameters allow the user to select the algorithm and number of iterations.



Algorithm
Select between Robust (Iterative) and Fastest (Non-Iterative).

Robust (iterative)

Maximum Likelihood Estimator, slowest but most resistant to noise.

Fastest (non-iterative)

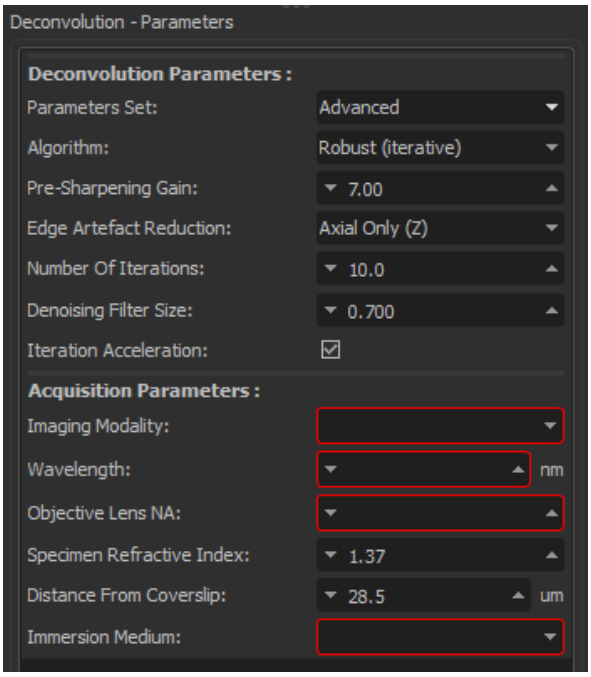
Inverse Filter, also known as Wiener filter, fastest.

The robust algorithm is recommended in all cases (especially for images with a low signal to noise ratio), unless speed is a higher priority to the user.

Number of Iterations

When Robust (Iterative) algorithm is selected the number of image iterations performed can be selected. Increasing iterations may improve image quality at the expense of time.

Advanced Parameters also provide the following additional settings.



Pre-Sharpening Gain

Degree of pre-sharpening to apply to the input image. Higher values provide a larger degree of pre-sharpening, but may result in noisier outputs and the possibility of artefacts.

Edge Artefact Reduction

Reduces the artefacts sometimes seen at the edge of the volume. Most commonly seen as bright areas, especially at the top and bottom of the stack. The greater the degree of artefact reduction, the longer the processing time.

Denoising Filter Size

The strength of de-noising filter applied during processing. This value relates to the standard deviation of the Gaussian Filter applied during the denoising process. Larger values relate to a larger standard deviation and are recommended for noisier images. Valid values range from 0.0 to 1.0.

Iteration Acceleration

Apply iteration acceleration during deconvolution. Fewer iterations are required with iteration acceleration on, but processing overhead is increased slightly.

These additional parameters may be used to fine tune the effectiveness of the algorithm.

Acquisition Parameters

It is important to match the settings with those of the imaging acquisition setup as the values and imaging modalities are used by the algorithm.

Imaging Modality
Select your experimental imaging system, select from the following options Spinning Disk Confocal, Widefield Fluorescence, Brightfield, Laser Scanning Confocal, TIRF.

Wavelength
Input the wavelength of light used to illuminate the sample during the experiment.

Objective Lens NA
Input the numerical aperture of your objective lens.

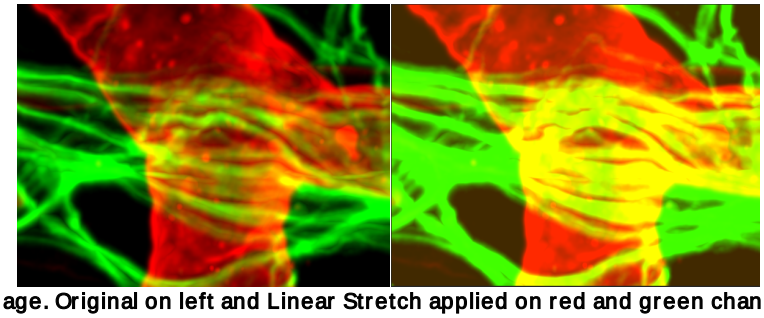
Specimen Refractive Index
Input the refractive index of your specimen.

Distance from Coverslip
Input the distance from the coverslip in microns.

Immersion Medium
Select your immersion medium from the following options Air, Oil, Water, Glycerol, Silicon Oil or set up a custom value.

8.9.8 Linear Stretch...

Linear stretching results in a picture displaying dense data in a wider contrast range.



Retina demo image. Original on left and Linear Stretch applied on red and green channel on the right.

Linear stretching may be necessary whenever 12 bit data is read in 16 bit mode, the contrast can be extended to the new limits. Image data is changed after linear stretching.

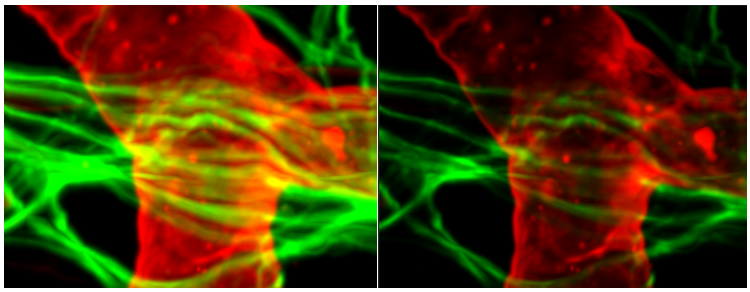
Parameters

Current minimum/maximum
These values define the limits of data set intensities. Intensities below the Current minimum will be set to the new minimum and intensities above the Current maximum will be set to the new maximum.

New minimum/maximum
These values define the intensity range of the output data set. By default, they are set to the minimum/ maximum achievable intensities of the data set.

8.9.9 Gamma Correction

Applies the gamma correction function to every voxel intensity of a channel.



Retina demo image. Original on left and Gamma Correction applied on red and green channel on the right.

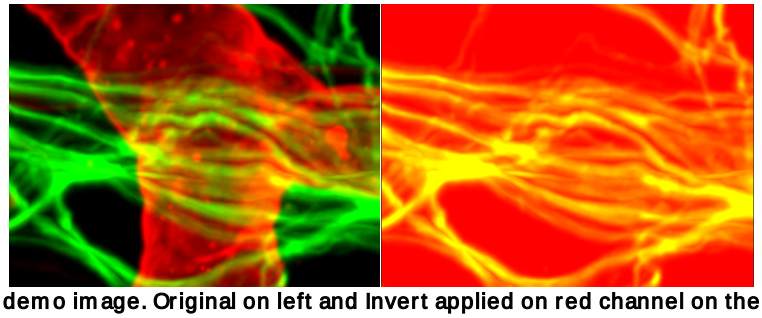
The Gamma correction function allows to improve the image display by adjusting the intensity value of a specific range of voxels. This can be used to provide more contrast to the interesting region or homogenize the intensities along the entire range when they are concentrated in a smaller range.

Parameters

Gamma
The Gamma value can be adjusted on each available channel.

8.9.10 Invert...

Invert modifies the intensity of each voxel by mirroring around the central value.
The central value is dependent on the data type (8bit, 16bit, Float). See Edit/Image Properties/ Data Type.



Retina demo image. Original on left and Invert applied on red channel on the right.

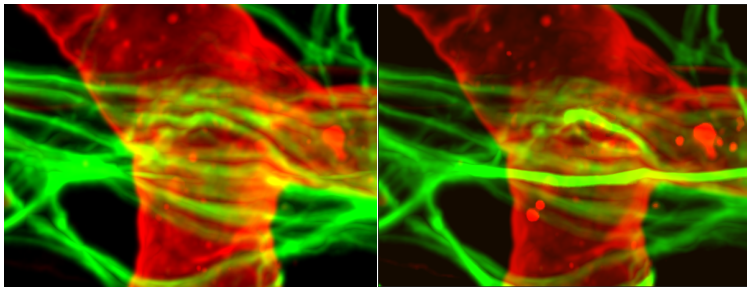
For 8 bit, the voxel intensities range from 0 to 255, the central value is 127.5.
Example: A voxel with intensity 0 has afterwards intensity 255. A voxel with intensity 200 has afterwards intensity 55.

For float and 16 bit, inverts the intensity maintaining the current range. The central value is computed from all intensities of a channel as follows:
Central Value = (Intensity Min + Intensity Max) / 2.0.

The channels can be inverted individually.

8.9.11 Normalize Layer(s)

The normalization of layers adjusts brightness and contrast of each image.



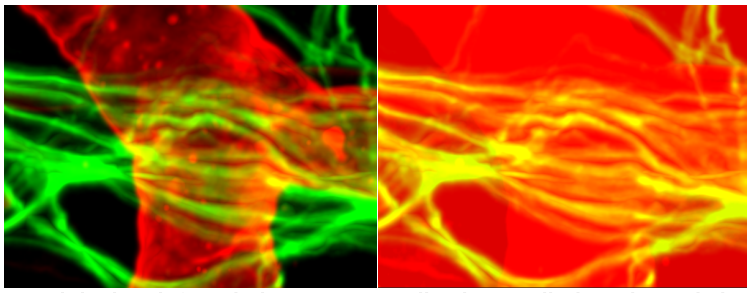
Retina demo image. Original on left and Normalize Layer applied on red and green channel on the right.

The normalization adjusts brightness and contrast of individual z-slices to a uniform level. It is most useful for correcting illumination variations of scans from physical sections and in some cases it can also reduce attenuation effects from z-stacks.

The image processing function computes the mean and standard deviation values of the intensity for all individual z-slices, then it adjusts the intensities of each z-slice with a linear transformation to match their average mean and standard deviation value. This procedure is applied for all z-slices of the data set, for each time point and for each channel independently.

8.9.12 Histogram Equalization

Histogram equalization is a method of image processing via contrast adjustment using the image's histogram.



Retina demo image. Original on left and Histogram Equalization applied on the red channel on the right.

The histogram equalization image processing method is useful in images with close contrast values; in particular, this method can lead to better views of electron microscopy images. Through this adjustment, the dynamic range and contrast of an image is altered. The resulting image features the highest possible contrast, uniformly distributed over the whole contrast range. This method increases the global contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. In histogram equalization, each pixel is assigned a new intensity value based on its previous intensity level so that the output image contains a uniform distribution of intensities. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

Parameters

Current minimum/maximum

These values define the limits of data set intensities. Intensities below the Current minimum will be set to the new minimum and intensities above the Current maximum will be set to the new maximum.

New minimum/maximum
These values define the intensity range of the output data set. By default, they are set to the minimum/ maximum achievable intensities of the data set.

8.10 AutoQuant...

The AutoQuant deconvolution software is integrated into Imaris. It requires a separate license. The pathway to AutoQuant must be set in the menu File - Preferences... - Advanced - Files - AutoQuantExeFileName.

When opening AutoQuant, the software will prompt for a destination for the file that is transferred to AutoQuant and will automatically create an image in the ids/ics format and open it in AutoQuant.

- Select the menu Image Processing - AutoQuant ....

An error message appears if the program is not installed or the pathway is not set.

Section 9 XTensions

This Section gives an overview of all Imaris XTensions.
Imaris XTensions is a multi-functional two-way interface from Imaris to classic programming languages, Matlab and other imaging software programs.

Please note:

- **Windows 8 and 10 do not come with the .net 3.5 and prior framework required for the Matlab runtime which Imaris XTensions use.**
- **In order to enable this 'Windows Feature' you will need an administrator account. You can set this under Control Panel -> Programs and Features, left hand side panel contains 'Turn Windows features on or off'.**

In order to use the supplied Matlab runtime extensions Matlab runtime also needs to be installed. This can be found on the Customer Portal: portal.imaris.com

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9.1 Project to 2D

This XTension compresses the data set into two selected dimensions. The user first selects the projection plane (XY, XZ or YZ) and then selects the projection mode (MIP or Mean).
In the projection plane window, select the projection plane XY, XZ or YZ.

Projection mode MIP or Mean
MIP- max intensities over the projection line
Mean- over the projection line divided by the number of voxels

9.2 Attenuation Correction

When working with 3D images you may find that optical sections from deeper within the sample may appear to have lower intensity when compared to the top layers. This XTension corrects this effect, by amplifying the intensity of pixels from slices at the bottom of the z stack.

To apply the AttenuationCorrection XTension to an image:

1. Select the relevant channel.
2. Find a position in the first and last slice of the image stack that should have the same intensities.
3. Determine the intensities at these positions
4. Select the AttenuationCorrection XTension
5. In the Attenuation Correction window, enter the channel number and corresponding intensity of the top and bottom optical slices.
6. Press **OK** and the XTension increases the intensities of the bottom layers while leaving the intensities of the top layers unchanged

Each channel is handled separately with the relevant correction.

It is based on the following calculation:

α=log (value2)-log(value1)/ pixel size (z)

$I_{layer\ new} = I_{layer\ old} * e^{-\alpha(\text{slice number}-1)}$

9.3 Crop Borders

This XTension computes the bounding box of voxels with intensity higher than the user specified value. Starting from the borders, it searches the image for the largest possible border containing an intensity value above the entered threshold. All voxels with intensity values below the threshold are cut away.
This XTension minimizes the size of the data set without losing meaningful data by removing peripheral pixels along any of the specified three axes.
The cropping direction X,Y,or Z should be specified and the threshold voxel intensity should be defined.

9.4 Camera Functions

This Section outlines the Camera XTensions:

Camera Manager
Set the Angle of the Camera

9.4.1 Camera Manager

This XTension saves, loads or clears camera settings in .mat files. With this Xtension you can save the camera position and you can load the same camera positions for other data sets as well.
Available settings are: position, orientation, perspective, orthographic, focus and height. This XTension lets you store and load an arbitrary number of viewpoints. This XTension can be also used for different images with a similar size.
To change the default location (Matlab), select the Browse button. Select the directory and enter the name for the file to be saved.

To save a setting:

1. Adjust the data set to the position you need
2. Start Camera Manager
3. Browse to a location where you want to save the file
4. Save the file

To reload a saved setting:

1. Execute the Camera Manager
2. Browse to the location where you saved the file
3. Confirm the dialogue for the overwrite file
4. Select the Load button to apply the same

If you open in the Camera.mat file (Matlab directly) 10 values can be found.

1-3: Camera Position
4-6: Camera Orientation
7: Angle
8: Perspective or Orthogonal
9: Focus
10: Height (width of the perspective)

9.4.2 Set the Angle of the Camera

The angle of the camera is defined by using the Azimuth, Elevation, Angle coordinates. The camera is first rotated around axis Y/the Y axis by Angle, then around axis X/the X axis by Elevation, then around the object axis by Azimuth.
Coordinates can be ambiguous: A different Azimuth, Elevation, and Angle can determine the same camera orientation.
Moving the sliders or entering a numeric value in the selected fields changes the camera position.

9.5 Channel Arithmetics

This XTension supports the common arithmetical operators on the channels. Matlab expressions are executed by entering a mathematical expression in the Matlab syntax field. The expression is executed in Matlab itself. As a result, the intensity values are converted into double floating-point precision, calculated and converted back to the original data type and shown in Imaris as an additional channel.

Channels "ch1, ch2, ch3, etc." and numbers can be used.

Note: avoid using NaNs (Not a Number values) in mathematical expressions.

The following examples are shown in the following table for your reference:

Example	Expression
New channel is the sum of two existing channel intensities	ch1 + ch2
New channel is the first channel with an intensity of 20 added	ch1 + 20
New channel is the second channel intensity, divided by two (note that you must use "./" and not just "/")	ch2 ./ 2
New channel is the first channel multiplied by 3.5 plus the second channel divided by 2 (note that you must use ".*" and not just "*")	ch1 .* 3.5 + ch2 ./ 2
New channel is 255 minus the first channel intensity, i.e. the inverse of the first channel in case of an 8bit image	255 - ch1
New channel is the square root of the multiplication of the first and second channel intensity	sqrt(ch1 .* ch2)
Masking is also possible. The new channel has intensity 1 for those voxels that have channel1 higher than 200 ch1 > 200	ch1 > 200
Visualization is easier when the intensity is higher than one. In this case the new channel has intensity 150 for those voxels that have channel1 higher than 200	(ch1 > 200) .* 150
New channel has intensity 150 where the first channel has intensity higher than 50 and the second channel has intensity higher than 30	(ch1 > 50 & ch2 > 30) .* 150
The new channel has intensity 150 where the first and second channel has an intensity of 0	(ch1 == 0 & ch2 == 0) .* 150
New channel has intensity 150 where the first or second channel has an intensity higher than 200	(ch1 > 200 | ch2 > 200) .* 150

9.6 Add Similarity Statistics Value

The Add Similarity Statistic Value is an XTension that allows quantification of the degree of the similarity of a set of objects with the pre-selected reference object(s).

This function creates a new statistical value, which can be used under the Filter, Statistical and Color tab.

To create a Similarity Statistic Value:

1. Select the reference objects according to your requirements
2. Enter an appropriate name for the new statistical value.
3. In the Similarity-Selection Box select one statistical value or in combination them from the list.
4. To select a statistical value, click on the name of the variable in the list. For multiple selection use Crtl + click. The newly created statistical value is based upon the aggregate weighted value of the statistical characteristics of the selected object(s).
5. The Matlab window confirms the creation of the new statistical value.

The benefits of the Similarity Statistic Values function are the precise identification of the objects with similar complex statistical properties or on the other hand, it offers the benefit of determining the objects which are the least similar to the reference object.

9.7 Distance Transformation

For Surface objects

This XTension computes and creates a new channel. The created channel intensity indicates the shortest distance to a surface object border. The border of the surface objects defines the origin of the distance transformation. The intensity value of the voxels at the border of the surface is always set to 0.

There are two options for the distance transformation calculation:
- Inside Surfaces object
- Outside Surfaces object

Depending on the choice for this option, either distances inside or outside of the surfaces object will be calculated. The distances are always positive values.

Each voxel is mapped with the intensity representing the measured minimum distance to the nearest surface border. The intensity value correlates directly to the distance to the border. The higher the intensity value corresponds to the larger the distance.

The XTension converts all the channels of the image to 32 bit float.

To modify the channel visibility contrast and range open the Display Adjustment window under the Edit tab.

See also the Distance Transform functionality in the Mask Channel Window that performs a distance transform where inside and outside distance are calculated at the same time.

For Spots objects

This XTension computes and creates a new channel. The created channel intensity values are based on the distance to a spot object. Each voxel is mapped with an intensity representing the minimum distance measured to the nearest spots centre. A higher intensity value corresponds to a larger distance to an object. The center of the spot object defines the origin of the distance transformation. Voxels in the centre of the spot object are always set to have an intensity value of 0.

The XTension converts all the channels of the image to 32 bit float.

To modify the channel visibility contrast and range open the Display Adjustment window under the Edit tab.

See also the Distance Transform functionality in the Mask Channel Window that performs a distance transform where the distance to the sphere border (instead of the center) is calculated.

9.8 Surfaces Functions

This Section covers the XTensions relevant to surfaces:

Distance Transformation- refer to Distance Transformation
Spots And Surfaces Distance
Surfaces Split
Surface Merge

9.8.1 Spots And Surfaces Distance

This XTension computes the distance between all Surface and Spots objects that are in the same Surpass group.

This XTension first computes the centroid of each object component. The centroid of the Spots objects is equal to the mean of the coordinates of all the single spots. The centroid of a surface object is calculated based on the mass of the object's center.

Then, the XTension calculates and displays the distance between the centroids.
The results of this XTension are presented in the Matlab window as a table.

9.8.2 Surfaces Split

This XTension splits one Surfaces object in multiple Surfaces of disconnected components.
As a result, in the 3D View tree new Surfaces entries are created, one for each surface. The number in brackets is just a running number without special meaning.

9.8.3 Surface Merge

This XTension merges all the Surfaces objects that are in the same folder into a single Surfaces object.
As a result, in the Surpass tree a new Surfaces object is created. The number in brackets indicates the number of merged Surfaces objects.

9.9 Spots Functions

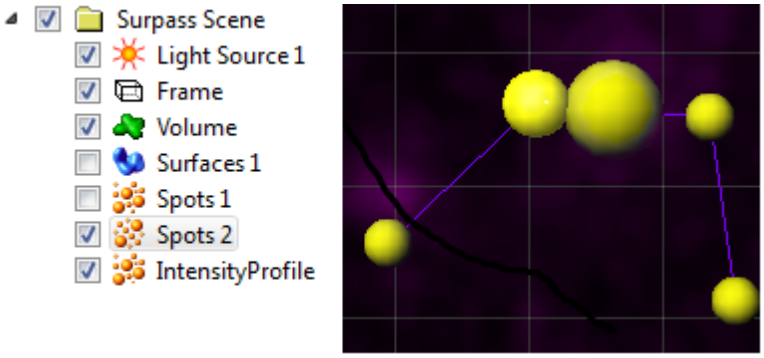
This Section covers the XTensions relevant to spots.

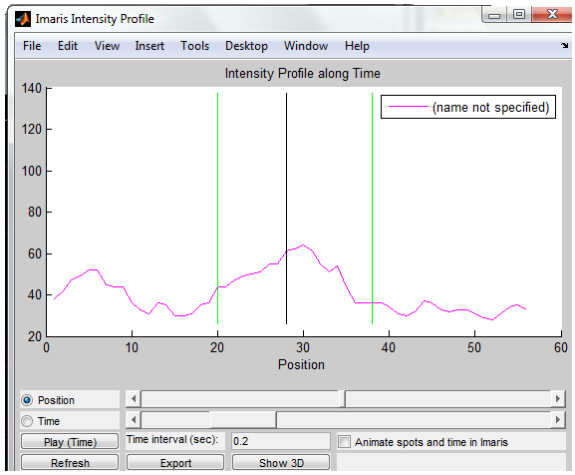
Distance Transformation- refer to Distance Transformation
Intensity Profile
Spots And Surfaces Distance
Spots to Spots Closest Distance
Find Spots Close To Filaments
Colocalize Spots
Split Spots
Split Spots Into Surface Objects
Super Resolution Localization Data To Image
Super Resolution Localization Data To Spots

9.9.1 Intensity Profile

This XTension creates and plots the intensity profile of the created spots. The Matlab window displays the intensity profile as a function of the position or time. The x-axis represents the position /time and the y-axis represents the intensity range.

The black vertical line indicates the current position/time point. The green lines indicate the intermediate positions. To change the position/time use the slider.





The channel colors are displayed in the plot. The intensities of the channels are shown using different colors respective to the channels. Several options allow the user to have control over the parameters of the calculation and how the results are displayed.
The length/time selection is located below the plot.

In the animation line you can find the following options:

- Play/Stop button
- Time interval field
- Animate spots and time in Imaris

Click on the **Play** button to play the time series. To stop the continuous display through all the time points click on the **Stop** Button.

In the **Time intervals** field the current value is displayed. By entering a new value, you can adjust the time interval duration.

Selecting or de-selecting the box next to the **Animate spots and time in Imaris** controls the animation.

Refresh

Clicking on the Refresh button imports any changes that were made to the data in Imaris and displays the most recent version of the data.

Export

For some further statistical analysis you can export the data. Click on the button Export to open the Export window and then select the file type (Comma Separated Values *.csv, Excel file *.xls, or Text file *.txt).

Show 3D

3D plots are used to represent data on three axes and to show the relationship between the three variables: time, position and intensities.

Please note: The best way to analyze the Intensity Profile information is by using a limited amount of data.

9.9.2 Spots Close to Surface

This XTension finds the subset of Spots that are closer to the surface than the user specified threshold (in μm). The coordinates of the Spot and Surface objects are used for the distance calculation. The distance is measured for the centre of the spot, not the surface of the spot. As a result, in the Surpass tree two new Spots objects are created; a subset of spots, whose location lies within the specified distance to the surface and the other subset of spots, whose location exceed the specified threshold value. In the viewing area, the subsets of the spots are represented by different colors.
The user can specify which Surfaces and Spots objects should be used. If multiple Spots or Surfaces are selected, each pair of Spots/ Surfaces are analyzed. The number of the Spots objects created is 2 times the 'number of selected Spots items' multiplied by 'number of the selected Surfaces'.

9.9.3 Spots to Spots Closest Distance

This XTension calculates and displays the distance to the closest neighbour of the Spots objects.
1. Choose between the 3 possibilities of how the results can be displayed.

The results can be displayed as:

a. New statistical values under the Statistical tab (distance max, distance mean, distance min)
b. New spot radius, where the new the spot size corresponds to the calculated minimum distance
c. New channel, where the channel intensity values depend on the minimum distance.

A possibility of multiple selections is available by pressing the **Select All** button.

2. Select the distance calculation mode.

The distance can be calculated either from the centre of the spot object or from the border.

9.9.4 Find Spots Close To Filaments

To run this XTension the user has to create a Spots and a Filaments component.
This XTension splits one Spots object into two Spots objects. One Spots object will include all the spots that are close to the filamentous structure (colored red). The other Spots object will include the spots that are far from the reference filaments (colored blue).
To account for significantly different filament diameters a ratio instead of an absolute distance between spots and filaments is used.

The ratio is given by r=a/b

a- Distance between the spot location and the centre of the filament
b- Filament radius

Multiple Spots/Filaments objects:
This XTension produces result for pair of the selected Spots Filaments objects.
The selected spots objects are determined by the fact that you run the XTension from it's 'Tool' tab.
If multiple Filaments objects are created, by default, the selected Filament object used in calculation is the one which appears first in the top down order of the Surpass tree
To change the order of Filaments objects in the Surpass tree please use the drag and drop functionality.

9.9.5 Colocalize Spots

This XTension creates new Spots objects including spots that exist within a user-defined distance of each other (using the currently set Imaris unit).
If the distance between two spots is less or equal to the user defined value then the two spots are considered to “colocalize” and will be placed within a new Spots object, (the distance is measured between the centre of each spot).

In the Surpass tree a new Spots object group is created with an automatically generated name. The name indicates the threshold value and the selected spot’s object.

9.9.6 Split Spots

This XTension splits one Spots object in multiple Spots objects.
Two Spots objects are created if the minimal distance between their spot elements is greater than the user-defined threshold.
All spots elements are assigned to the same Spots object if the shortest distance between two neighbouring spots is less or equal to the user-defined threshold.

Each new Spots object is individually colored.

A spot element can belong to only one Spots object.
In the Surpass tree a new Spots object is created (the number in brackets indicates the threshold value). The names of the newly created Spots objects are automatically generated indicating the number of spots in the Spots object (and the Spots object ID number).

9.9.7 Split Spots Into Surface Objects

To run this XTension the user has to create a Spots and a Surface component.

For each Surface object located in the same Surpass group as the Spots object, this XTension creates a new subset of Spots that contains only the spots that lie inside the Surface.

9.9.8 Super Resolution Localization Data To Image

The Super Resolution Localization Data To Image XTension reads localization data files produced by super-resolution microscopy. This XTension is very similar to the Super Resolution Localization Data To Spots XTension, but creates an ***Imaris image*** as the output.

Super Resolution Microscopy

Super-resolution techniques allow you to capture images with a higher resolution than is possible with conventional confocal microscopy techniques.
A super-resolution data set from PALM and STORM methods (also known as PALMIRA, dSTORM, sptPALM, GSDIM, SMLM, 'blink' microscopy) is composed of:
a) a sequence of a large number of images where individual sub-diffraction particles need to be detected (typically 10.000-100.000 images)
b) a particle list (typically 1.000.000 - 10.000.000 particles) that is generated after the particle detection analysis.
The particle information that is the result of this technique is composed of the intensity, x-position, y-position, z-position and additional metadata.

For more information please refer to:

http://en.wikipedia.org/wiki/Super_resolution_microscopy

This XTension allows to load super resolution localization data into Imaris as an image. The supported format of the localization data is the QuickPalm particles tables tif file, as well as CSV (https://en.wikipedia.org/wiki/Comma-separated_values) and TXT files.

You can find further QuickPALM information at these locations:

http://code.google.com/p/quickpalm/

http://www.nature.com/nmeth/journal/v7/n5/full/nmeth0510-339.html

To perform this XTension:

1. Press **Choose file** in the top left of the Dialog and choose the super-resolution file you want to process The file name and the first few lines of the file are displayed in the filename and preview area of the dialog. Depending on the format, the column settings in the bottom left area of the dialog will be enabled. For quickpalm files, these settings are not available and step 2 can be skipped.
2. For csv and txt files, the bottom left area of the dialog allows you to specify which columns should be used to generate the spots. The dropdown boxes are filled with the text of the first line of the file.

a. **Position**
Use the dropdown boxes to choose which columns should be used for Position X and Position Y values. Position Z is optional and contains "None" as last element in the dropdown. By choosing "None" for Position Z, all spots will have Position Z set to 0.

b. **Unit**
With the "Unit" dropdown, choose which unit the spot positions should have. The available options are "m", "mm", "µm", and "nm", default is "µm".

c. **Channel**
With the "Channel" dropdown you can specify a column for the channel. The XTension will generate an separate channel in the image for each distinct "Channel" value. It is possible to set "Channel" to None, then the created image will only contain one channel.

3. Press **OK**. A second dialog will appear. Enter the voxel x y z sizes for the each dimension in the input dialog. Default values are 10 µm.
4. Press **OK** to start reading the whole file. Depending on the size of the file, this can take a while. A progress is shown and at the end, the image that Imaris displays is changed. A scene is created if Imaris does not yet contain a scene.

The entered voxel size is used to create a 2D/3D image covering a volume equal to the volume occupied by the particles. Each voxel has an intensity value corresponding to the number of particles found within. Voxel

intensities are initialized to zero and then incremented by one every time a particle is found inside them. The result is voxels with high intensities where many particles were found and lower intensities where fewer particles were found.

Please note: Smaller voxel size create a higher resolved image, but requires more time and memory for processing. If the value of the voxel size is set to be too large, the benefits of the super resolution technique are lost. Finding the optimal voxel size is a difficult task that might need several tries.

9.9.9 Super Resolution Localization Data To Spots

The Super Resolution Localization Data To Spots XTension reads localization data files produced by super-resolution microscopy.
The XTension is very similar to the Super Resolution Localization Data To Image XTension, but produces ***Spots Components in Imaris*** as the output.

Super Resolution Microscopy

Super-resolution techniques allow you to capture images with a higher resolution than is possible with conventional confocal microscopy techniques.
A super-resolution data set from PALM and STORM methods (also known as PALMIRA, dSTORM, sptPALM, GSDIM, SMLM, 'blink' microscopy) is composed of:
a) a sequence of a large number of images where individual sub-diffraction particles need to be detected (typically 10.000-100.000 images)
b) a particle list (typically 1.000.000 - 10.000.000 particles) that is generated after the particle detection analysis.
The particle information that is the result of this technique is composed of the intensity, x-position, y-position, z-position and additional metadata.

For more information please refer to:

http://en.wikipedia.org/wiki/Super_resolution_microscopy

This XTension allows to load super resolution localization data into Imaris as an image. The supported format of the localization data is the QuickPalm particles tables tif file, as well as CSV (https://en.wikipedia.org/wiki/Comma-separated_values) and TXT files.

You can find further QuickPALM information at these locations:

http://code.google.com/p/quickpalm/

http://www.nature.com/nmeth/journal/v7/n5/full/nmeth0510-339.html

To perform this XTension:

1. Press **Choose file** in the top left of the Dialog and choose the super-resolution file you want to process The file name and the first few lines of the file are displayed in the filename and preview area of the dialog.

Depending on the format, the column settings in the bottom left area of the dialog will be enabled. For quickpalm files, these settings are not available and step 2 can be skipped.

2. For csv and txt files, the bottom left area of the dialog allows you to specify which columns should be used

to generate the spots. The dropdown boxes are filled with the text of the first line of the file.

a. **Position**

Use the dropdown boxes to choose which columns should be used for Position X and Position Y values. Position Z is optional and contains "None" as last element in the dropdown. By choosing "None" for Position Z, all spots will have Position Z set to 0.

b. **Load Spot Sizes From File**

Check the "Load Spot Sizes From File" checkbox if the spot sizes should be read from the file. If checked, the dropdown box below will be activated to choose a column. If "Load Spot Sizes From File" is checked, the "PSF & Camera Gain" area is disabled. For csv and txt files, if "Load Spot Sizes From File" is not checked, the intensities are set to 1, for quickpalm files, the intensities are read from the file.

c. **Unit**

With the "Unit" dropdown, choose which unit the spot positions should have. The available options are "m", "mm", "µm", and "nm", default is "µm".

d. **Channel**

With the "Channel" dropdown you can specify a column for the channel. The XTension will generate a separate Spots component for each distinct "Channel" value. It is possible to set "Channel" to None, then only one Spots component will be generated.

3. **PSF & Camera Gain**

Quickpalm files contain intensities for every spot. With PSF and Camera Gain and the intensities the spot diameters are calculated according to the formula below. Adjust PSF and Camera Gain accordingly:

$$R = \frac{S}{\sqrt{\frac{I}{G}}}$$

R - The diameter of the created spots
S - The full-width-half-maximum of the microscope point-spread-function
I - Intensity
G - The gain factor of the camera to convert particle intensities into photons

4. Press **OK** to start reading the whole file. Depending on the size of the file, this can take a while. A progress is shown and at the end, a group with one or more Spots components is created in Imaris.

9.10 Filaments Functions

This Section covers the XTensions relevant to Filaments:

Filaments Angles Statistics
Filaments Branch Hierarchy
Filaments Convex Hull
Create Channel from Filaments
Filaments Points Track
Classify Filaments Spines
Split Filaments Into Branches

9.10.1 Filaments Angles Statistics

This XTension measures and displays the angles statistics of the filament, the angle between segments and branches. The calculated data is plotted. Two plots are created: Angles between the segments and Angles between the branches.

The x-axis represents the measurement of the angle and the y-axis represents the number of segments or

number of branches.

Please note: All the angles calculations are based on segment vertex positions.

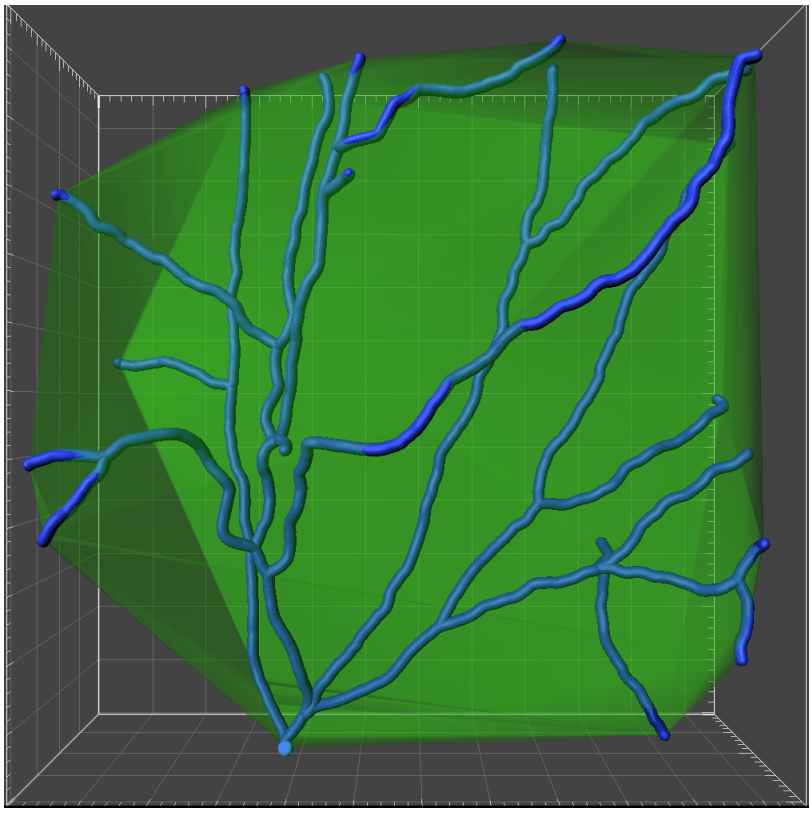
9.10.2 Filaments Branch Hierarchy

This XTension calculates and displays segment branch levels. All segments with the same branch level are assigned to a new Filaments object. Each new Filaments object is individually colored. Each segment branch can belong only to one Filaments object.
In the Surpass tree multiple Filaments objects are created (the number in brackets indicates the branch level).

9.10.3 Filaments Convex Hull

This XTension calculates the convex hull for the filament object.
The convex hull is the minimal convex polyhedron that contains all the points in the selected filament object. A convex hull is defined by the minimal convex set of points that envelops a terminal points of the arbor. Based on the convex hull calculation, a new Surface is created that completely overlaps the filament object. The region of the filament object is obtained from the statistical values of the newly created surface.

Convex hull measurements are more robust than tortuosity (branch length divided by linearity) to artifacts of optical microscopy, and allow a better measurement of segment spread - 'spanned area' preserving the spatial component of the neuron.



9.10.4 Create Channel from Filaments

This XTension creates and computes a new channel. The channel intensity values depend on the diameter of the selected filament object. The user can choose how the new channel is created by selecting how the intensity is calculated along the filament diameter.
If the channel is created by interpolating along the segment/spine diameter, the maximum intensity will be assigned to the voxel at the centre of the filament. If not, the uniform intensity is mapped at the location of the

filament.

Tip: To modify the channel visibility contrast and range, open the Display Adjustment window under the Edit tab.

9.10.5 Filaments Points Track

This XTension tracks Filament significant points (beginning, segment branching and terminal points, spine attachments and spine branching and terminal points) and plots the track statistics.
It provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Filament significant points (beginning, segment branching and terminal points, spine attachments and spine branching and terminal points). Based on these predicted positions, the best matches to candidate Filament significant points can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while the Autoregressive Expert mode combines the changes in position and the intensity to compute the total cost.

Brownian Motion
This algorithm models the motion of each of the Filament significant points as a Brownian motion. This model is appropriate if the Filament significant points perform similar motions to a Brownian motion (the random movement with very frequent changes in direction and no discernible pattern).
The only prediction applied is that the Filament significant points do not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each of the Filament significant points as an autoregressive AR1 process. This model is appropriate if the Filament significant points perform any kind of continuous motion. The AR1 model looks back to one time-point and predicts that the Filament significant points will move the same distance and in the same direction again. The user-specified maximum distance value is the distance in which the Filament significant points are allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each of the Filament significant points as an autoregressive AR1 process (see above). It is suitable for the tracing of multiple objects with adjacent or crossing paths, especially if the neighbouring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components
For this algorithm, Filament significant points in adjacent time-points are considered connected if the Filament significant points' spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All objects with an overlap will be assigned the same track ID.
This is the only algorithm that automatically handles lineage (Filament significant points that split or merge). It will only work with images that have excellent time resolution because the Filament significant points can't jump a distance larger than their own size, Max Distance.

Max Distance

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the object's connection is automatically established is based on the value defined in the Max Gap Size file.

The Max Gap Size defines the maximum number of the consecutive time points, which are allowed to be missing in order for the track to be continuous.

Intensity Weight

Based on the information obtained from the measured Filament significant point's intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

TotalCost = (all connections) DistanceCost + IntensityWeight * IntensityCost

Track Filters

The Track filtering is incorporated into the process. Enter the filtering type to be used in order to classify the tracks and specify the measurement of the criteria.

As a result of this XTension, a Matlab plot can be created. The plot displays the number of Filament significant points as a time plot. Each Filament significant point is represented using a special symbol.

In Imaris,a new Spot's object is created in the Surpass tree.

9.10.6 Classify Filaments Spines

This XTension determines the differences in spine morphology. It classifies spines into the following default categories: stubby, mushroom, long/thin, and filopodial based on their length, head and neck morphology.

Classification Rule

All detected spines are classified according to the current classification rules. All classification rules are written as Matlab syntaxes and are fully editable. These are logic statements returning a value of 1 if true and 0 if false. These rules are applied in order of precedence (i.e. top to bottom). All spines that comply with the first rule (the first classification rule is true-total spine length is less than 1μm) are set as stubby. For all remaining spines, the second rule is tested and the process of classification continues.

Any spine that was categorized by the highest rule in the list, is excluded from further classification. Therefore, a spine can be part of one class only.

Setting the New Rule

You can classify spines according to the default classification rules, or you may create new classification rules by defining output classes based on the new criteria.

To adjust a classification of the spines morphology into more relevant classes, it is possible to change a set of classification criteria. In the data set, any detected spine can be selected and measured and then the different 3D features obtained from the spine, can be used for classification. The suitable morphological feature could be used for setting the new rule.

The list of variables used for setting rules are displayed.

To create or adjust the classification rules combine the spine morphology categories (**Spine**, **Head**, **Neck**,

Ground) with the following variables:
Volume, length, mean/ minimum/ maximum/ width.

Insert variable button
Select the Insert variable button to insert the selected combination of spine morphology and statistical variables.

Any new rule must be based on Matlab syntaxes. The default classification rules show you a pattern of the syntax needed to complete these logic statements.

- To adjust the rule that controls classification, click on the rule line and enter a new set of classification criteria.
- To change the name of the class, click on the name line and enter a new name.
- To remove the selected class, click on the appropriate **Remove class** button.

In this XTension, the total spine length is divided in 3 parts: ground, neck and head. The spine neck length is set by default to be 50% of the total spine length. Neck length is measured from the base of the spine head toward the segment. The spine head is 25% of the total spine length. The remaining part of the spine, attached to the filament tree, is set to be 25% of the total spine length. To change the default length ratio enter the new values in the head/ ground length fields.

Create one Filament
Under the Create One Filament option you can find different outputs.

Create one Filament object for each class
As a result, in the Surpass tree a new Surpass group is created which is composed of multiple Filament objects. All spines (and segments of the source) categorised in the same class are assigned to the same Filaments object. Each Filaments object is individually colored.

Create one Filament with custom statistics
As a result, in the Surpass tree a new Filaments object is created. Under the Statistical tab a new variable - Spine class is added. The spine class is indicated by a numerical value (this value corresponds to the rule number).
Please note: In both output options every branched spine is replaced with an unbranched one, starting from the attachment point wherever a branching point is encountered, a new spine is created.

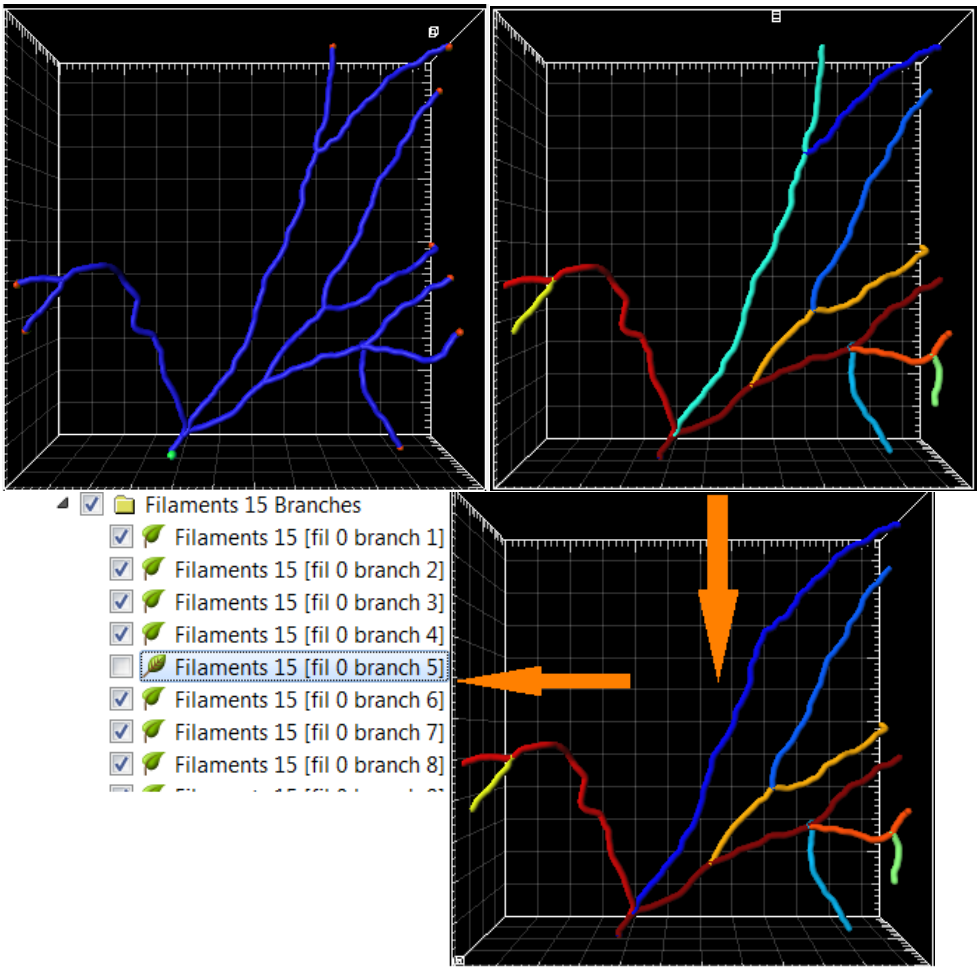
Plot class statistics
A new window plots the selected spine statistics. For each spine class the following statics can be plotted: count, density, average volume, average length, average mean/ min/max with for total spine, head, neck and ground.
Variables can be selected interactively.
Density is defined as the total number of selected spines divided by the total length of the segment

Add new class button
Select this button and fields for the new classification rule and name are added.

Classify spines
Select this button and the Classify spine XTension is executed.

9.10.7 Split Filaments Into Branches

This XTension splits one Filaments object into different Filaments objects. Starting from a common beginning point and going towards a terminal point, whenever a branching point is encountered a new Filament object is created. As a result, the newly created Filaments do not have branches and for each branch point, there is only one beginning and one terminal point. The number of Filaments created is equal to the number of terminals and branch points of the original Filaments object.
In the Surpass tree a new filament object is created.



9.11 Histogram Equalize Layers

This XTension adjusts the contrast layer by layer by using the image's histogram. Histograms are created for each layer separately and for the whole image. The XTension enhances the contrast by transforming the intensity values in each of the layer's histograms, so that the resulting histogram approximately matches the whole image histogram.

For more information, please refer to:

http://www.mathworks.ch/help/toolbox/images/ref/histeq.html

The resulting image features the highest possible contrast, uniformly distributed over all the layers.
This method increases the contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

Define the value of the lower limits of intensities. Voxels with an intensity below the entered value are excluded from this calculation - they are interpreted as background.

9.12 Normalize Time Points

This XTension adjusts the brightness and contrast of the individual time points in a 4D data set to a uniform level.
It first computes the mean and standard deviation values of the intensity for all of the time points. Then, it adjusts the intensities of each time point to match the overall mean and Standard Deviation value.

It adjusts the brightness of all the time points in the data set and each channel independently.

Voxels with intensity below the entered value are excluded from this calculation - they are interpreted as background.

9.13 Open Object Manager

This XTension saves, loads or clears single Imaris objects in .mat files.
It can be applied to Spots, Surfaces, Tracks, and Filaments objects and Groups. Groups are saved with all their content. Only first-level objects (mScene direct children) can be saved.
To change the default location (Matlab), select the Browse button. Select the directory and enter the name for the file to be saved.

9.14 RGB to Gray

This XTension creates and computes a new gray channel. The intensity values of the gray channel are calculated as a total weighted sum of the selected channels' intensities.
The user defines the coefficients of the sum for each channel.

9.15 Smooth Time

This XTension smoothes the intensity of each voxel along the time dimension. The amount of smoothing is defined by the Gaussian filter width.
The user specifies the channel and the filter width value.

9.16 Time Projection

This XTension projects all channels of a time data set as a single time point. For each voxel the intensity is computed using a MIP or a mean projection of all time points. The projection mode is defined by the user.

9.17 Time Subtract Average

This XTension calculates for each voxel a new intensity value, by subtracting the time-averaged intensity value from the initial intensity. It eliminates all motionless objects since the difference in intensities for motionless objects are negative values.

There are two options:
1. The user can set all negative values to zero
2. To avoid these negative values and loss of data, any intensity which is zero or negative can be equal to absolute values

To speed up the processing of **2D time series** you can use Image processing - **Swap Time and Z** before and after starting the XTension.

1. After exchanging the Time and Z dimensions start the XTension.
2. Define the number of slices that should be processed.
3. Select to set Negative values either to zero or to absolute values
4. Run the XTension
5. Finally, under the Image processing menu, select again the Swap Time and Z option

9.18 Tracks Functions

This Section covers the XTensions relevant to tracking functions:

Connect Tracks
Plot Angles of selected Tracks
Plot Distance Between Tracks
Plot Length of selected Track
Split Tracks
Translate Tracks

9.18.1 Connect Tracks

For each initial object within the track, this XTension assesses the position at the previous time point.
Then at the previous time, it measures the distance between the calculated position and the positions of all the other objects. It creates a track connection if the distance between the anticipated object position and any other object at the previous time point is less than the user specified maximal distance value.

The new connections can be seen under the Edit track tab.

Please note: Two tracks, which track an object through the same time point, cannot be connected.

9.18.2 MSD Plot

The MSD XTension creates a plot of the Mean Square Displacement of a tracked Spots or Surfaces object. The MSD is the mean (average) distance of objects from their starting point, plotted over time since the beginning of the tracks. The self-diffusion coefficient for the plot is computed according to http://www.cs.unc.edu/Research/nbody/pubs/external/Keffer/selfD.pdf.

The plot also shows the best linear and square fit for the MSD curve. A precise linear fit would suggest that the objects move randomly (Brownian motion), while a good square fit would indicate a constant/linear trajectory (autoregressive motion).

Clicking “Export Data…” allows the user to save all information and plot values to a csv file.

9.18.3 Plot Angles of selected Tracks

This XTension creates four plots that display the angles statistics of the selected track. The first plot shows the spatial orientation of the selected track and the following plots are illustrating the XY, XZ and YZ planes.

9.18.4 Plot Distance Between Tracks

This XTension creates four plots that display the distance between the selected track objects and all other tracks objects that are in the same group. The first plot shows the absolute spatial distance of the selected track and all other tracks. The following are illustrating planes X, Y and Z distance. The following are illustrating the distance of the selected track and all other tracks in X, Y and Z planes.

9.18.5 Plot Length of selected Track

This XTension calculates the difference in the object position within the selected track between two consecutive time points. If the distance between particle (e.g. a vesicle or a cell) positions in two connective time points is smaller than the entered threshold value, then it is considered as a pause.
As a result, this XTension creates four plots that display the length of the track steps. The first plot shows the spatial step size of the object within the selected track. The other three plots illustrate the XY, XZ and YZ planes.

9.18.6 Split Tracks

This XTension splits tracked objects into individual Spots objects.

- All objects within one track are assigned to the same Spots object.
- Each Spots is individually colored.
- Each track can belong to only one Spots object.
- As a result, in the Surpass tree a new objects group is created. The subgroup names are automatically generated indicating the object type and track number.

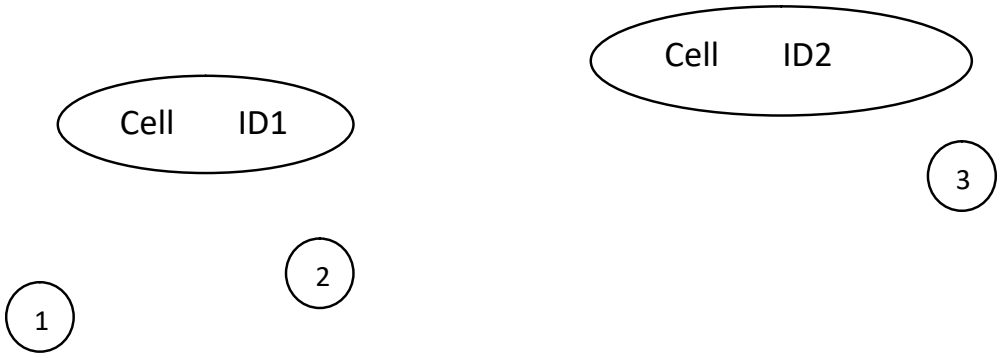
9.18.7 Translate Tracks

This XTension makes a copy of all tracks that are in the same group of the selected track. The copies are then translated and as a result they all have the same starting point.
In the Surpass tree a new object is created.

9.19 Vesicle Outside Cell

The XTensions VesicleOutsideCell function calculates for each Spot object, the closest Cell, and the distance to it.

XTensions VesicleOutsideCell illustration



The figure illustrates two Cells (ID 1, ID 2) and three Spots (1, 2, 3).
The results of the XTensions VesicleOutsideCell function are two statistic values per Spot object:
Nearest Cell ID and Nearest Cell Distance.

Spot 1
Nearest Cell ID: 1
Nearest Cell Distance: 10 mm

Spot 2
Nearest Cell ID: 1
Nearest Cell Distance: 7 mm

Spot 3
Nearest Cell ID: 2
Nearest Cell Distance: 3 mm

Section 10 Fiji

Imaris Fiji compatible plugins are aimed at performing additional image processing and offering a solution for specific problems with image analysis.
The Imaris Fiji menu allows you to use your current Fiji routines and still continue with your work within Imaris. The Fiji plugins are integrated into Imaris and can be executed from within the application, enhancing and extending the functionality of Imaris.
In the Fiji menu you can find a list of plugins organized into a menu structure. Each plugin collection is displayed as a sub menu on this menu. The plugins and macros are stored and run from the Fiji folder.

Image From Fiji
Image To Fiji
Labkit Segmentation
File
Image
Process
Analyze
Plugins
Options...
Help..

Please note: This is not an Fiji tutorial. These Sections describe only the usage of Fiji as an integrated part of Imaris.

10.1 Fiji-Imaris

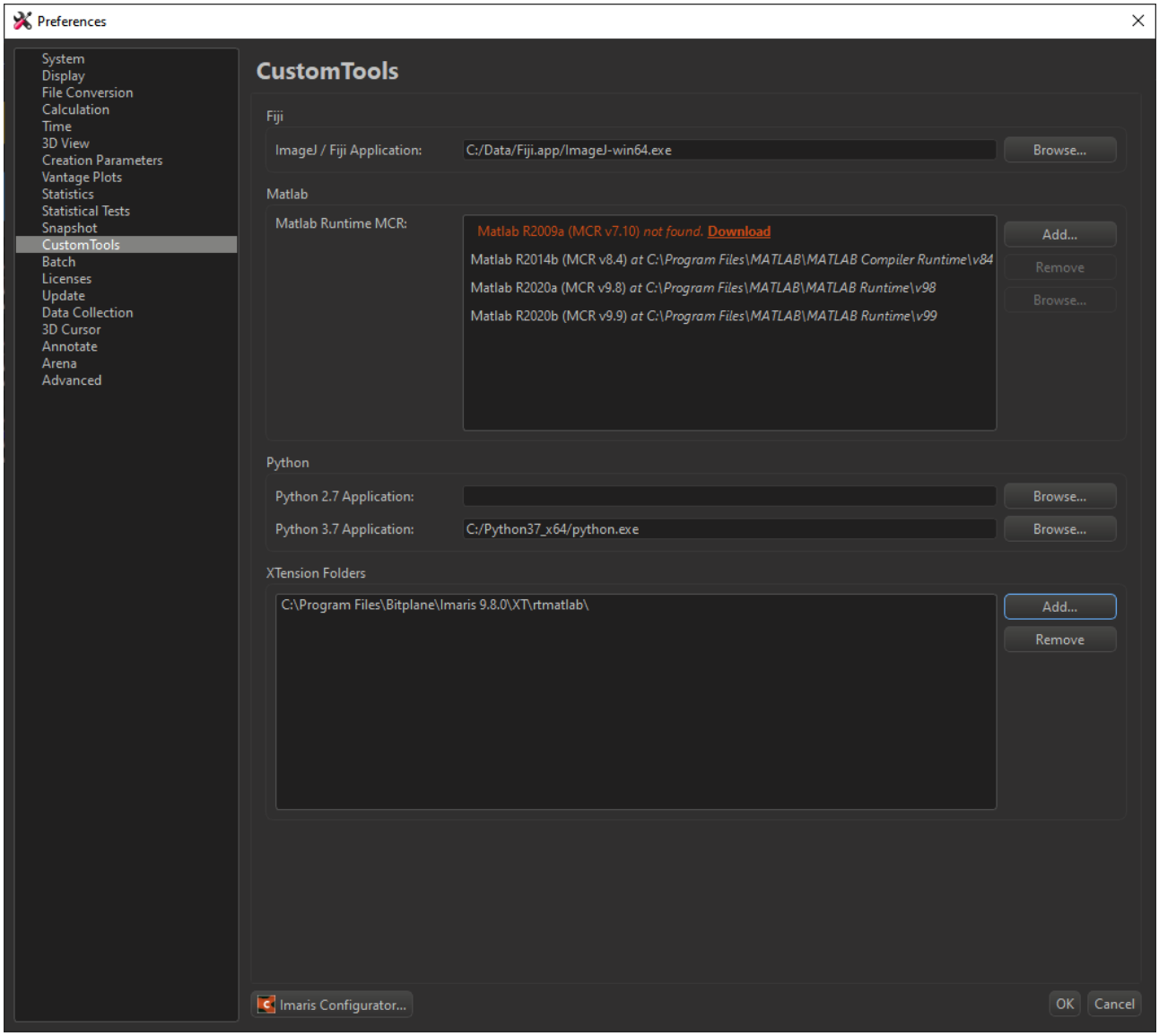
In this Section you will learn how to integrate Fuji plugins in Imaris by means of the Fiji menu and how to establish connections between the Fiji and Imaris.

If you already have Fiji installed, skip down directly to the to the **Options...** section. If you don't have Fiji installed, you must install it first. The latest version can be downloaded from the following locations:
Fiji: https://imagej.net/software/fiji/

Depending upon your operating system select the appropriate link.
To complete download follow the specific download instructions.

Options...

The first step of integrating the Fiji plugins within Imaris is to specify the folder with the Fiji.exe file. To do that open the Fiji menu and choose the last item in the list **Options...**
The Custom Tools window opens. In the field Fiji application either type in the respective path or use the button Browse.
Specify the Fiji folder and select the .exe file. Click on the **OK** button.



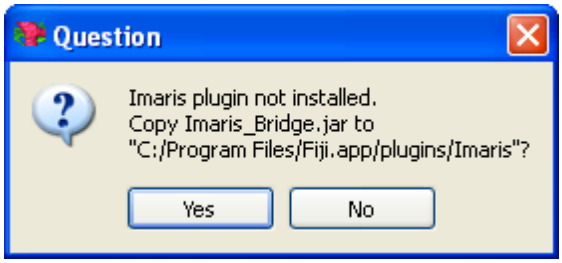
For Mac users:

/Applications/Fiji.app/Contents/MacOS/fiji-macosx

In the Fiji menu all options are now active.

10.2 Imaris Bridge.jar File

The downloaded plugins do not include the file needed to run your Fiji plugins in Imaris.
In order to run your Fiji plugins in Imaris you need to install the file, **Imaris_Bridge.jar file**; which provides the link between the two applications.
The installation starts automatically as soon as you select a plugin from the list.
The new window appears asking you to install Imaris_Bridge.jar file



You can confirm installation by selecting **Yes.**
The Imaris_Bridge.jar file is placed in a new subfolder, Imaris, within the Fiji application.

Security Alert window: Depending upon how your computer security is set, you may see a Security Warning. Select the option Unblock.



After installing the Imaris bridge file all plugins are ready to be used and no additional steps are required.

10.3 Configuration File

This section will show you how to expand the pre-existing collection of plugins and embed initially unlisted plugins into the Imaris Fiji menu.

You can customize the Fiji menu by the integration of new plugins.

Download Plugin

To incorporate the new Fiji plugin into Imaris, you are not required to modify, or access the Imaris code. Simply select a plugin from the Fiji list, download it and place it in the plugin menu or one of the submenus.

Configuration File

To properly run the plugin in Imaris it is important to create a **configuration file**.
The configuration file is needed to fully read/execute/and write permissions to the Fiji directory and

subdirectories. The configuration file handles all the details of incorporating, executing and presenting the results of this new plugin within Imaris.

The configuration file has two main elements. The first is executed with Imaris. It has several lines that connect the plugin to Imaris. By adding a special header to the file you define the Menu or the Submenu and the name of the plugin in Imaris.
The second part is written as any plugin and executed by Fiji. This part of the file runs the Imaris-Bridge file, which provides a connection between the Fiji and Imaris, loads data set information, performs some actions, sends images back to Imaris, closes Fiji and terminates the connection.
Once a file is created, copy the file to the Imaris Fiji plugins folder in the current Imaris Program Files path and you will have access to new plugins within Imaris.
The following is the example configuration file.

```
// <CustomTools>
//   <Menu name="Fiji">
//     <Submenu name="submenu name (e.g. Process)"> -This line is optional (embedding the plugin in the submenu)
//       <Item name="plugin name" icon="Fiji">
//         <Command>Fiji::submenu name_plugin name </Command> - Please make sure that the plugin name is the same as the configuration file name
//       </Item>
//     </Submenu>
//   </Menu>
// </CustomTools>

call("Imaris_Bridge.In", getArgument());
run("plugin name");
call("Imaris_Bridge.Out", getArgument());
call("Imaris_Bridge.Terminate", getArgument());
```

Save the file, and copy it to the folder:"

Windows: C:\Program Files\Bitplane\Imaris x64 X.X.X\XT\fiji
MAC: /Applications/Imaris\ 9.2.0.app/Contents/SharedSupport/XT/fiji/

Please note: The Imaris version cannot be set differently than the currently running version

Restart Imaris and the newly inserted plugin will appear in the Fiji menu.

Please note: Imaris Fiji compatible plugins are plugins that provide image data as a result.

10.4 Image From Fiji

Transfer the image to Imaris by selecting the Image From Fiji option.

10.5 Image To Fiji

Transfer the image to Fiji by selecting the Image to Fiji option.

10.6 Labkit Segmentation

Labkit is an open source machine learning pixel classification software and is available in Imaris. With the Labkit pixel classifier an additional channel can be created on the volume or surface objects can be added, for subsequent analysis in Imaris. See more on the options below for using Labkit with Imaris.

Note that this feature is not an extension and cannot be edited.

Labkit Segmentation (Fiji Menu)

Labkit segmentation is available from the Fiji menu, by selecting this option, an additional channel can be created on the volume.

Select Labkit Segmentation from the dropdown, use Labkit to train a pixel classifier, to identify items of interest in the image. Once finished press “SendResultToImaris” in the bottom left hand corner of Labkit to send the result back to Imaris. On completion this adds a channel to the volume, available for further analysis in Imaris.

Surfaces Creation With Labkit

In Imaris, the surfaces creation with Labkit can be used to detect surfaces in an image. It can be used with all or one channel(s) and for batch analysis.
During the surface creation wizard the Labkit checkbox is available in the Source Channel step. Please see Surfaces - Creation Wizard - Source Channel for more information on this step.

When this option is checked Labkit will be started automatically on clicking “next” in the creation wizard and the image from Imaris is automatically rendered in Labkit. Use Labkit to train a pixel classifier and when finished press “SendResultToImaris” in the bottom left hand corner of Labkit to send the result back to Imaris and continue in the surface creation wizard.

Labkit is a Fiji plugin that needs to be activated after the installation of Fiji. If the options in the drop down or creation wizard are greyed out please ensure you have installed Labkit correctly please see Labkit Installation.

10.7 Labkit Installation

Labkit is a Fiji plugin that needs to be activated after the installation of Fiji.
1. Download Fiji from https://imagej.net/software/fiji/ and unpack it anywhere (to a location without whitespaces in the path). Ensure to configure the path to Fiji in Imaris’ Preferences, Custom Tools. See Fiji-Imaris for full instructions.
2. Run Fiji.app/ImageJ-win64.exe and the update manager should popup automatically:
   a) Choose the menu Help > Update…
   b) Press the “Manage update sites” button to open the “Manage update sites” dialog.
   c) Check the “clij” and “clij2” items. This will add support for GPU accelerated Labkit segmentation. Labkit segmentation also works without clij and clij2, but it is then much slower and it is recommended to use clij and clij2.
   d) In the same dialog, press the “Add update site” at the bottom
   e) Enter a new site with name imarislabkit and url https://sites.imagej.net/imarislabkit/ and check it
   f) Click Close, then Apply changes
   g) Close Fiji

Once configured correctly, Labkit Segmentation available from the Fiji Dropdown and during the surface creation wizard, the Labkit for Pixel Classification check box will be present in the second step (Source Channel).

For further instructions please see our Learning Center article: Configuring Imaris with Labkit.

10.8 Initially Installed Plugins

File
Open
Save

Process

Find Edges
Morphology
Gray Morphology

Sharpen

If you would like to find out more about these plugins, additional information is available in the form the Fiji website.

Analyze
Color inspector 3D

Plugins

LOCI
Bio-Formats Exporter
Bio-Formats Importer

Plugins are not only used to implement new processing functions, they are also used to support reading and writing file formats, which are not normally supported by Imaris.

Process

Dilate (3D)
Distance Transform (3D)
Erode 3D
Exact Euclidean Distance Transform (3D)
Exact Signed Euclidean Distance Transform (3D)
Gradient (3D)
IFT (3D)
Laplace (3D)
Maximum (3D)
Median (3D)
Minimum (3D)
Particle Analyzer (3D)
Smooth (3D)

For the detail description it is recommend to visit the Fiji website.
There are a lot of examples and tutorials, which could help you to understand the features of these plugins.
http://pacific.mpi-cbg.de/wiki/index.php/Fiji's_Menu

Registration

Correct 3D Drift
Linear Stack Alignment with SIFT

Skeleton

Analyze Skeleton

http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:analyzeskeleton:start

Skeletonize (2D/3D)

http://imagejdocu.tudor.lu/doku.php?id=plugin:morphology:skeletonize3d:start

10.9 Options...

Custom Tools
In Custom Tools you can specify the Fiji folder. Either type in the respective path or use the button **Browse.**

10.10 Fiji Help

You can find further Fiji information by clicking on the following link:

http://fiji.sc/wiki/index.php/Fiji

Section 11 2D View

2D images open automatically in 2D view. In surpass view all available options under the 2D View menu item are fully described in the 3D View section.

Section 12 3D View

	Please refer to Section:
Cells	Cells
Clipping Plane	Clipping Plane
External Object	External Object
Filament	Filament
Frame	Frame
Surpass Group	Surpass Group
Light Source	Light Source
Measurement Point	Measurement Point
Oblique Slicer	Oblique Slicer
Ortho Slicer	Ortho Slicer
Spots	Spots
Surfaces	Surfaces
Volume	Volume

Export Selected Objects...	
Delete Selected Objects...	Del
Switch View Mode	Esc
InMotion	InMotion

12.1 Cells

ImarisCell is an Imaris module designed specifically to identify, segment, track, measure and analyze Cell, Nucleus and Vesicles in 2D and 3D time-resolved images. ImarisCell provides simultaneous detection, measurement and analysis of cell objects combined with the interactive identification in the viewing area.
ImarisCell calculates and displays detailed statistics values for all the cell's components and their tracks. Interactive image exploration, simultaneous measurement and analysis of the many features in the image provide quick, accurate, quantitative and reproducible results.

How to Add new Cells?

- Select menu 3D - Cells or 2D View - Cells.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Cells - Properties.

For information on cells statistics calculations used refer to Cells.

12.1.1 Creation Wizard

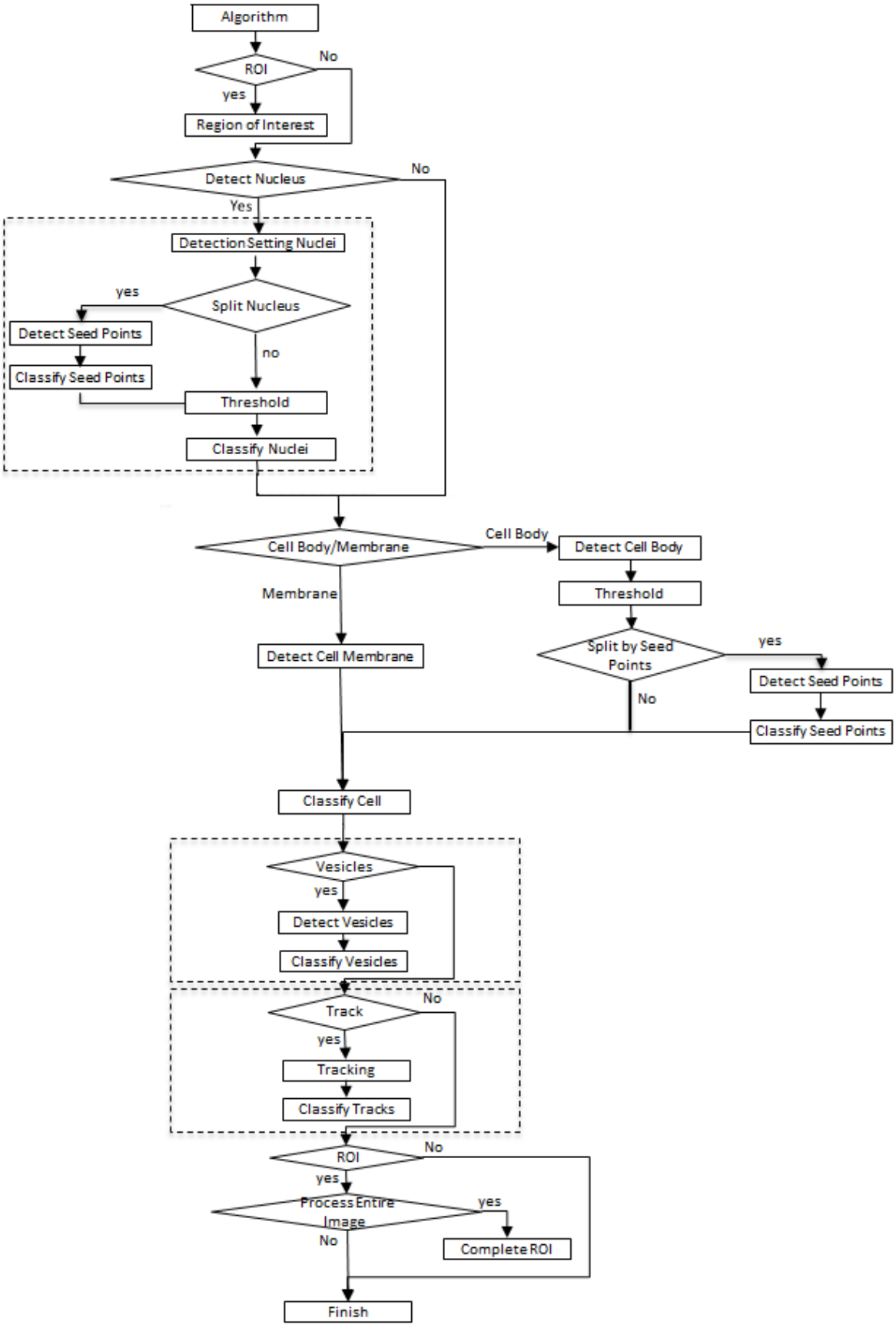
In the first window of the Cell Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters, or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual Cells creation.

If the image data is for a multi-image scene an option to select which source image to be analyzed is available

from the Align to: drop down box.

Depending on the chosen algorithm setting the Creation Wizard could consists of the following major steps:


Algorithm
ROI
No
yes
Region of Interest
Detect Nucleus
No
Yes
Detection Setting Nuclei
yes
Split Nucleus
Detect Seed Points
no
Classify Seed Points
Threshold
Classify Nuclei
Cell Body/Membrane
Cell Body
Detect Cell Body
Threshold
Membrane
Detect Cell Membrane
Split by Seed Points
yes
Detect Seed Points
No
Classify Seed Points
Classify Cell
Vesicles
yes
Detect Vesicles
Classify Vesicles
Track
No
yes
Tracking
Classify Tracks
ROI
No
yes
Process Entire Image
yes
No
Complete ROI
Finish

12.1.1.1 Algorithm

Note: If you do not want to use the automatic cells creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual edit options.

Favourite Creation Parameters

In the creation wizard for all objects the algorithm step allows for the creation of favourite creation parameters and are described in the Algorithms common concepts section.

Algorithm Settings

Depending on the used cell staining techniques and sample preparation Imaris Cell lets you choose from four different detection types.

You can select to detect **Nucleus, Cell and Vesicles**  , detect **Nucleus and Cell**  , detect **Cell and Vesicles**  or just to detect **Cell**  .

Segment only a Region of Interest
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
This option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Un-check this box and only the region of interest is processed.

Track Cells (over Time)
Check this box if you want to track the resulting Cells over time, for details please refer to the Tracking Section.

Creation Preferences
This check box **Start Creation with Slicer view** can be used to activate Slicer view in the creation wizard.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.2 Region of Interest

Please see our common concepts page on the Region of Interest.

12.1.1.3 Nuclei Detection

Source Channel
Select the respective source channel, from which the Nuclei are to be computed.

Nucleus Diameter
The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value.

Advanced Options

Smooth
Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

Filter Width
The value of the Filter Width determines the Gaussian filter width.
The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value is given by image units and not by the amount of voxels.

Background Subtraction
Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.
If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

Sphere Diameter
The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the data set.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Split Nuclei by Seed Points
Select this option to apply filters to the seed points of the nuclei. Refer to the topic Classify Nuclei Seed Points for more information on this step.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.4 Filter Nuclei Seed Points

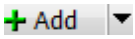
The Seed Points filtering is incorporated into the creation process. This tab only appears, if the Split Nuclei by Seed Points option has been selected.

Filters may allow objects that would interfere with interpretation of results to be removed.

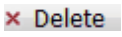
The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.5 Nuclei Threshold

Nucleus Threshold (Absolute Intensity)

In this creation step the Nucleus objects are detected and segmented.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.6 Classify Nuclei

The Nuclei classification is incorporated into the creation process under Nuclei Detection Advanced options.

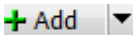
This step allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

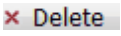
Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.7 Cells Detection

Depending on the cell staining techniques and sample preparation used, you can select either to detect:

1.Cell Boundary from Cytoplasm 

2.Cell Boundary from Cell Membrane 

Cell Body Detection

Source Channel
Select the respective source channel, from which the Cells are to be computed.

Smooth
Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

Filter Width
The value of the Filter Width determines the Gaussian filter width.
The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value is given by image units - not by the amount of voxels.

Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the largest sphere that fits into the Object from the intensity of the original channel.
If the option Background Subtraction is selected then the Sphere Diameter should be determined.

Sphere Diameter
The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of Sphere Diameter should be slightly larger than the diameter of the largest object expected to be found in the data set.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.8 Cells Threshold

Cell Body Detection Threshold (Absolute Intensity)

When Cell Body Detection has been selected the following options are available in this creation step in which the Cell objects are detected and segmented.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Split Touching Cells

Don't Split
When this option is selected the Split Cells' algorithms are **not** activated.

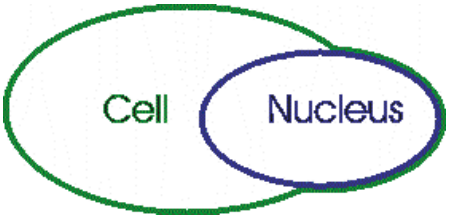
One Nucleus per Cell
When the automatic algorithm does not properly segment the cells, creating cell objects containing two or more nuclei, this option can be used. When activated, this option performs a separation of cells, resulting in every single cell containing only one Nucleus. The algorithm used for this separation is the **Marker Controlled Watershed**.

Split by Seed Points
This option supports the separation of two or more of the Cells objects,that have been identified as one, in the Threshold step.
The Split Cells method for segmentation in Imaris starts with defined seed points. You use the Cells as seed points for the Cells segmentation that will be performed in the next steps. During the process, the region around each seed point grows until it reaches the border.

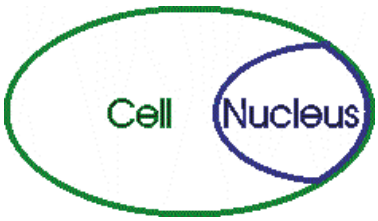
Seed Points Diameter
Type in the estimated diameter of a seed point.

Nuclei Options

Expand Cell on Nucleus
This option defines the voxel set which is assigned, in the segmentation step, as a boundary of the Cell object. Areas of the image that have been initially segmented as Cells are expanded outside the original borders, to include a set of voxels which are defined in the segmentation step as Nuclei.
Resulting in a union between the set of voxels defining the Cells and the set of voxels initially defined as Nuclei.



Erase Nucleus outside Cell
This option defines the voxel set which is assigned as a boundary for the segmented Nucleus.
Any parts of Nuclei that are segmented outside the Cell object is removed from the preview area, leaving only set of voxel assigned as Nucleus within the Cell object. This means that the segmented Nuclei boundary will be reduced to the voxel set defined as the Cell object.



Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.9 Cell Membrane Threshold

Cell Membrane Threshold

When the Cell Membrane Detection type has been selected the following options are available for Cell Membrane Threshold Detection:

In the previous Cells Detection step, there are two filter options "Local Contrast" and "Smooth" that set the available cell membrane threshold settings.

For the "Local Contrast" filter, two threshold values can be adjusted to fuse the basic regions. The first value is Intensity which is the average intensity value of the border segment of the regions on the smooth image. The histogram of the Intensity represents the values of all border segments. By adjusting the Intensity value, the border segment whose value is below the threshold is removed so that its connected two basic regions are fused together. By adjusting the Intensity value threshold, the small sub-regions which are located on the background region can be easily fused together to generate a better background region. The second value is Quality which is the value of the border segment of the fused regions which are generated by adjusting the first

value Intensity on the filtered image. So the Quality value is based on the fused regions generated by adjusting the Intensity.

For the "Smooth" filter, one threshold value can be adjusted to fuse sub-regions. The Quality is the value of the border segment of the basic regions on the smooth image. By adjusting the Quality value threshold, the basic regions can be fused together to generate the better results.

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

12.1.1.10 Filter Cells

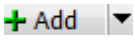
The Cell object classification is incorporated into the creation process.

Filters may allow objects that would interfere with interpretation of results to be removed.

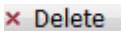
The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.11 Vesicles Detection

Select this option to start the automatic creation process for Vesicles.

The drop-down list shows the types of Vesicles that will be detected in the creation process.

To detect the additional vesicles types
To add a new Vesicles type, click on the button and choose the name. The new Vesicles type name appears in the list.

1. Click on the **Add New Type** Button
2. Enter a Vesicle name in the **Vesicles name** field
3. The new Vesicles type name appears in the list
4. Select the Source Channel
5. Define the Estimated Diameter
6. Decide on the Background Subtraction

Vesicle Name
This field allows you:

1. To enter the name for a newly added vesicle type or
2. To edit a name already defined

Source Channel
Select the respective source channel, from which the Vesicles are to be computed.

Estimated Diameter
Objects with size smaller than the specified **Estimated Diameter** are not detected.

Background Subtraction
If checked, the background will be removed prior to Vesicle detection. Technically the background subtraction is used to smooth the image. The intensity equals the Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

Different Vesicles Sizes (Region Growing)
The following options only appears, when the **Different Vesicles Sizes (Region Growing)** box is checked.

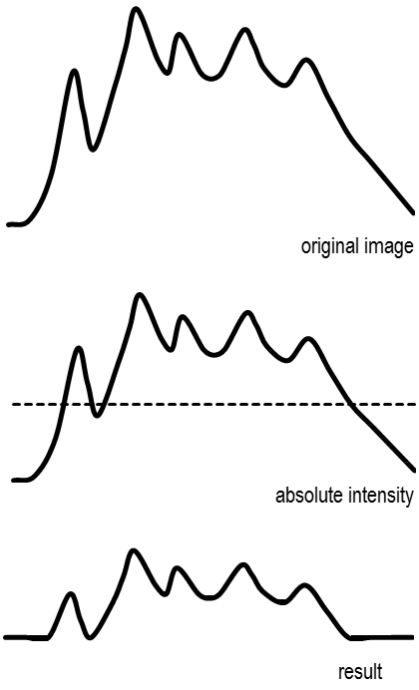
The region method for segmentation in Imaris starts with defined seed points. You use Vesicles as seed points for the region growing that is performed. During the region growing process around each seed point the region grows until a border stops the growing.

Vesicles Regions from

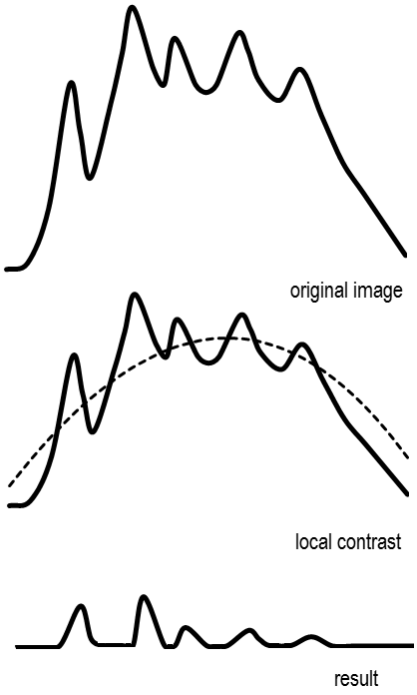
Absolute Intensity
Local Contrast
You can set the border using the **Absolute Intensity** or the **Local Contrast** as criteria. In both cases as next step the selected channel is displayed in the viewing area.

Local Contrast
This option should only be used when the **Absolute Intensity** method does not properly segment all objects.
Please note: Local Contrast applies a Gaussian filter in order to estimate the intensity value of each voxel. Baseline Subtraction is then performed by subtracting the variable background from every voxel in the image. As a result, it uses more memory than the Absolute Intensity method.

Gaussian Smoothing replaces the voxel intensity value with a newly calculated value. The new value is a weighted sum, defined using a Gaussian function, of the voxel and it neighbouring voxels.
The degree of the smoothing is directly dependent on the shape of this kernel, which in turn is a function of the standard deviation. A Gaussian kernel is a symmetric bell shaped curve.



Absolute Intensity



Local Contrast

Add New Type Button
To add a new vesicles types, click on the button Add and choose the appropriate settings.

Delete Button
Select a Vesicles type in the list and click on this button to delete it.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.12 Classify Vesicles

Vesicle filtering is incorporated into the creation process.

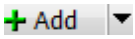
Click on the drop-down list and select the required Vesicle type to be classified.

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria.
First select the Vesicle type. Then select the filter type by selecting a variable from the drop-down list, and finally define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analysis.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign indicates that the statistics value is the same for all objects.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.13 Vesicle Regions

This tab only appears, if **Different Vesicle Sizes (Region Growing)** is checked.

Vesicle Regions

The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local Contrast.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Diameter from

Region Volume
The Vesicles radius is calculated from the region volume (the region volume and the Vesicles volume are equal).

Create Color Coded Region Channel
Additionally the Vesicles the Region Channel is displayed. Each region has a different color.

Region Border
The radius of the calculated Vesicles is the smallest distance from each Vesicles center to the region border.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.14 Tracking Cells

Tracking allows objects to be traced throughout sequential frames of a time data set, so each object maintains a unique identity in the output measurements.
Tracking the Nucleus and Vesicle objects with respect to the inertial image coordinates is less significant than tracking their relative motion within the cell.
In order to cope with the spatial position of Nuclei and Vesicles within the Cell, the concepts of relative tracking are required.

Tracking of Nuclei and Vesicles objects is presented in relative terms of their parent Cell objects, rather than their real coordinates on the screen.
Since the Cell object itself can be moving (relative to the inertial image reference frame), the tracking of Nuclei and Vesicle is **relative** to the parent Cell of a specified reference point.
The position of the Nuclei and Vesicle objects is specified as a relative distance from a reference point. This reference point is the Cell object center of mass.
The position of the individual Nuclei and Vesicles objects is calculated using two types of information. One is the absolute position of the selected object in terms of the inertial image reference coordinates and the other is the absolute position of the parent Cell object center. The **relative** Nuclei and Vesicles object's **position** is calculated by subtracting these two positional differences and is then tracked.
This concept of relative tracking allows direct measurements and statistical data comparison between moving cells to be calculated.

Algorithm

Brownian Motion

This algorithm models the motion of each object (Nuclei or Vesicles) as a Brownian motion. This model is appropriate if the object performs similar to a Brownian motion (the random movement of objects with very frequent changes in direction and no discernible pattern).
The only prediction applied is that the object does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process. This model is appropriate if the object performs any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components

For this algorithm, objects in adjacent time-points are considered connected if the object spheres occupy some of the same space (the spheres would overlap if two time-points were fused into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.
Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that fuse or fragment). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object (Nuclei or Vesicles) as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that

undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

Maximum Overlapping
For a cell at time point t the overlap with all cells at the previous time point t-1 is computed and the cell is linked to that cell from the previous time point with which it has the largest overlap. The link is only created if the ratio of the volume of the smaller of the two linked cells to the larger is greater than the "maximum overlapping ratio".

Parameters

Max Distance Cell
The value of the Max Distance specifies the maximum distance that a track Cell object could possibly move between two consecutive time points. This parameter disallows the connection between a Cell and a candidate match if the distance between the predicted future position of the Cell and the candidate position exceeds the maximum distance.

Max Relative Distance Nucleus
The value of the Max Distance specifies the maximum distance that a track Nucleus could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Max Relative Distance Vesicles
The value of the Max Distance specifies the maximum distance that a track Vesicles could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Options

It can happen that the object segmentation fails for some time points. This can break a Track apart into two Tracks. A gap-closing algorithm links track segment ends to track segment starts in order to recover tracks that were interrupted by the temporary disappearance of particles.

Close Gap
Select this option and the gap-closing algorithm is implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two

consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Track Nuclei and Vesicles

Select this option to track nuclei and vesicles. Refer to Tracking Nuclei and Vesicles for information (This option is only available if the relevant detection algorithm has been selected).

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

Track
Select track to select a complete track. Refer to Tab Edit Tracks for editing information.

12.1.1.15 Classify Cells Tracks

Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Cells (over Time)** is checked (Algorithm).

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.
Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type
Select the filter you want to apply from the drop-down list.

Filter category
A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object

Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.16 Tracking Nuclei and Vesicles

Tracking allows objects to be traced throughout sequential frames of a time data set, so each object maintains a unique identity in the output measurements.
Tracking the Nucleus and Vesicle objects with respect to the inertial image coordinates is less significant than tracking their relative motion within the cell.
In order to cope with the spatial position of Nuclei and Vesicles within the Cell, the concepts of relative tracking are required.
Tracking of Nuclei and Vesicles objects is presented in relative terms of their parent Cell objects, rather than their real coordinates on the screen.
Since the Cell object itself can be moving (relative to the inertial image reference frame), the tracking of Nuclei and Vesicle is **relative** to the parent Cell of a specified reference point.
The position of the Nuclei and Vesicle objects is specified as a relative distance from a reference point. This reference point is the Cell object center of mass.
The position of the individual Nuclei and Vesicles objects is calculated using two types of information. One is the absolute position of the selected object in terms of the inertial image reference coordinates and the other is the absolute position of the parent Cell object center. The **relative** Nuclei and Vesicles object's **position** is calculated by subtracting these two positional differences and is then tracked.
This concept of relative tracking allows direct measurements and statistical data comparison between moving cells to be calculated.

Track

Track Nuclei
Select this option to apply the required algorithm and parameters to Nuclei.

Track Vesicles
Select this option to apply the required algorithm and parameters to Vesicles.

Algorithm

Brownian Motion
This algorithm models the motion of each object (Nuclei or Vesicles) as a Brownian motion. This model is appropriate if the object performs similar to a Brownian motion (the random movement of objects with very frequent changes in direction and no discernible pattern).
The only prediction applied is that the object does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process. This model is appropriate if the object performs any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components
For this algorithm, objects in adjacent time-points are considered connected if the object spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.
Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that diverge). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

Lineage
The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object (Nuclei or Vesicles) as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

Parameters

Max Distance Cell
The value of the Max Distance specifies the maximum distance that a track Cell object could possibly move between two consecutive time points. This parameter disallows the connection between a Cell and a candidate

match if the distance between the predicted future position of the Cell and the candidate position exceeds the maximum distance.

Max Relative Distance Nucleus
The value of the Max Distance specifies the maximum distance that a track Nucleus could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Max Relative Distance Vesicles
The value of the Max Distance specifies the maximum distance that a track Vesicles could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Options

It can happen that the object segmentation fails for some time points. This can break a Track apart into two Tracks. A gap-closing algorithm links track segment ends to track segment starts in order to recover tracks that were interrupted by the temporary disappearance of particles.

Close Gap
Select this option and the gap-closing algorithm is implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.17 Classify Nuclei and Vesicles Tracks

Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Cells (over Time)** is checked (Algorithm).

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common

Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.1.18 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.1.2 Tab Settings

On Tab Settings you can choose the rendering methods and adjust the rendering quality to produce faster performance of the display.

Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris. The render on slicer options are available on every tab of the surpass property area, and are explained in the Surpass Property Area section.

Object Type

A choice of Object types is presented. For time series images the choice of the Object Type is in the drop-down list. After choosing the Object type, a selection of the Setting Tab is listed.

Cell/Nuclei
Cell Tracks
Nuclei Tracks
Vesicle Type and Tracks

Volume rendering type

The option **Volume Display** is used to identify the different objects and distinguish them from the surrounding by visualizing the volume around a created object. It extracts a subset of the volume data set by using the specified axis-aligned limits.
The border around each object is defined independently and corresponds to the regions equal to bounding box of the created object.

Shaded Volume
This option displays the subvolume around a certain object in the shaded volume mode. In this mode, all values

along the viewing direction including their transparency are used for the calculation.

MIP
In the Maximum Intensity Projection mode only the point with maximum intensity of all layers along the viewing direction displayed. If your data set has more then one channel, the combination color is displayed.

Slicer display options are described in the Cell/Nuclei section.

12.1.2.1 Cell/Nuclei

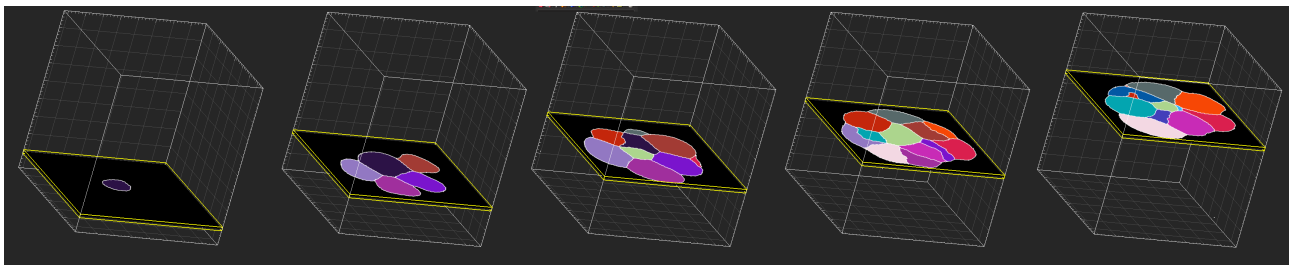
Cell and Nuclei Style

Cell visible
This option shows or hides the Cell objects in the viewing area.

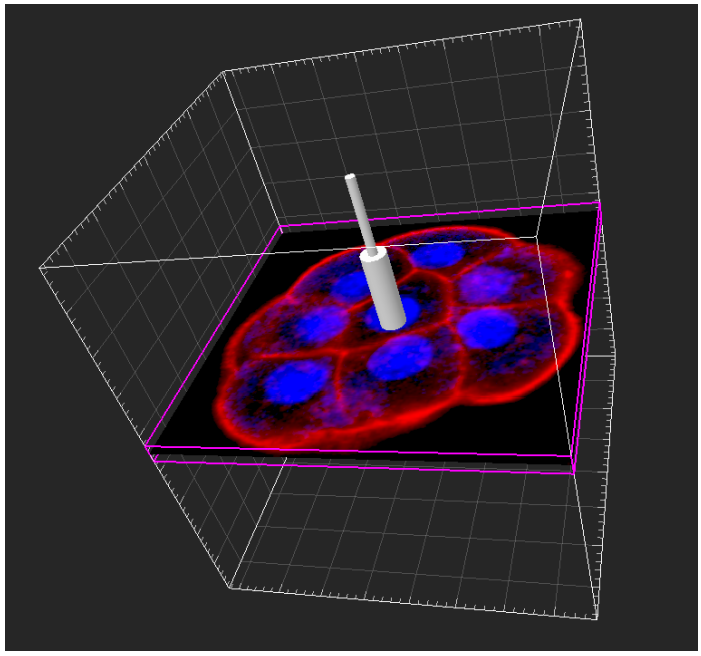
Nucleus visible
This option shows or hides the Nucleus objects in the viewing area.

Slicer Display

Slicer Direction
The Slicer display represents an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. Three orthoslicer planes show their intersection with a plane perpendicular to two of the coordinate axes
Change the slice direction if required by selecting the appropriate XYZ plane option.
The position of slices can be changed in the viewing area. Switch the pointer to the Select mode and then click on the plane and drag it to a new position. A series of slices is shown below for XY Slicer direction, Border rendering and Solid color region rendering.



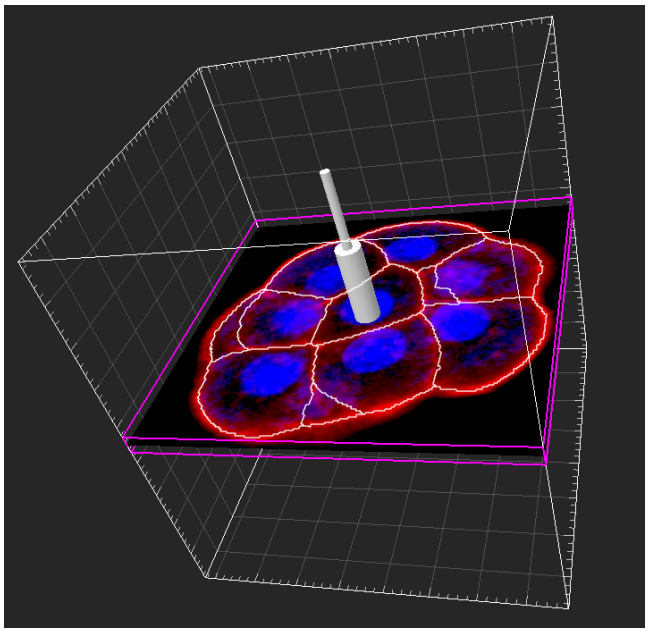
The check buttons labeled as **XY**, **XZ** and **YZ** direction, are used to select the visibility of the corresponding slice. The **Oblique** option represents a plane that can be freely moved and rotated within the data set and allows the user to look inside any object. The Oblique Slicer (shown below) consists of a colored frame with a white double rod in the middle.



The position of the Oblique Slicer can be changed. Choose the pointer mode Select, highlight the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will appear highlighted as it is selected, allowing you to move it and to set the Oblique Slicer to the required position.
The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In the Select mode click the smaller diameter rod and it will be highlighted as it is selected. To rotate the Oblique Slicer, move the smaller rod.

Border Rendering

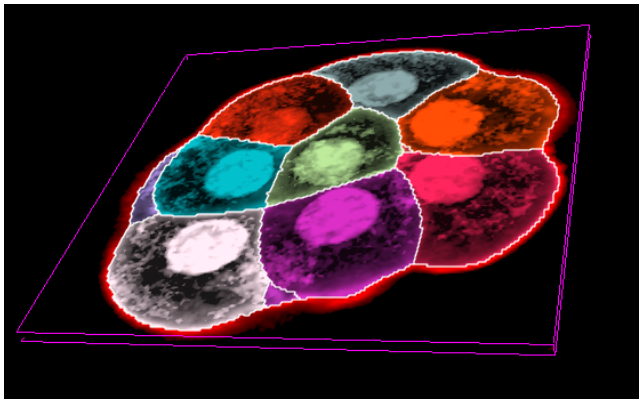
Border rendering can be a useful visualization tool- it displays the cell borders that have been calculated during the cell creation process. This can be seen in the following image compared to the previous image without Border rendering. The width of the border may be changed using the Border width slider bar.



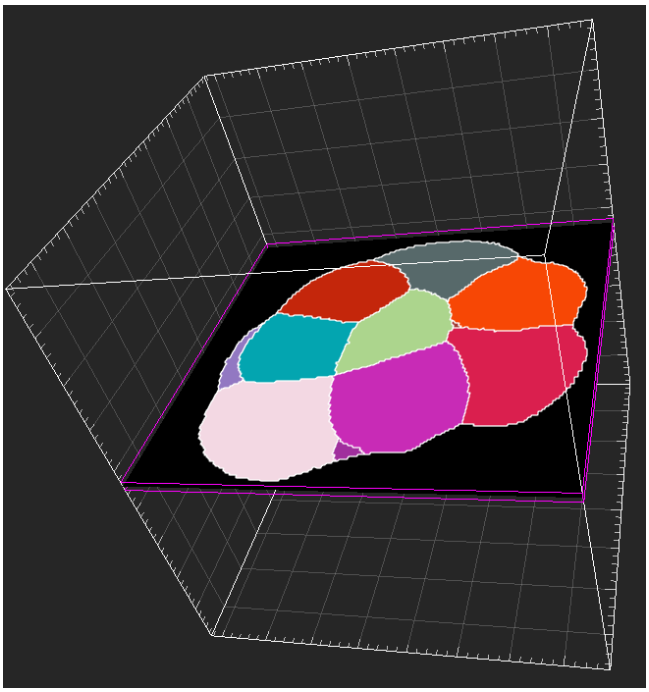
Region rendering

The region rendering tool enables the individual regions of the cell to be visualized as separate colors. There are two options available: Colored data set and Solid colors.

Colored data set- showing the regions of the cell in transparent colors so you may see features of the image.



Solid colors- showing each region as a solid color, without cell image detail, so you may view the arrangement and relationship of each region clearly.



12.1.2.2 Cell/Nucleus Tracks

Cell/Nucleus/Vesicles Tracks

Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

Cylinder
Select this option to display the tracks connections as cylinders.

Line
Select this option to display the tracks connections as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter/Render quality
Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

Radius Scale
An Edit field for the displacement vector radius.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider position.

Slider
The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

12.1.2.3 Vesicle Type and Tracks

Vesicles Style/Quality

On the tab Settings you can adjust the rendering style or quality.

Center Points
This option displays the Vesicles as center points.

Off
Select this option to disable the visualization of the Vesicles.

Sphere
Select this option to display the Vesicles as spheres.

Radius Scale box
The size of the Vesicles can be changed via the Radius Scale box.

The Render Quality
The Render Quality is adjustable using the slider. When the number of Vesicles is very large, adjust the Render Quality, to produce a faster display performance.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

Cylinder
Select this option to display the tracks connections as cylinders.

Line
Select this option to display the tracks connections as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter/Render quality
Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

Radius Scale
An edit field for the displacement vector radius.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider position.

Slider
The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

12.1.3 Tab Rebuild

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list, which step of the Cell Creation Wizard you want to re-enter.

For details please refer to Section:
Menu 3D View - Cells - Algorithm
Menu 3D View - Cells – Tracking

Rebuild Objects Button
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so that the Cell computation can be repeated for the same region, a different region, or the entire image. The Creation Parameters are listed as the table.

Store Parameters for the Batch
The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters:
- Arena
- Favorite Creation Parameters

Arena
When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, Creation Parameters, is created and displayed.
Once stored, Imaris Batch applies the creation parameters set to all images within the selected Group.

Favorite Creation Parameters
To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.
When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/ Favorite Creation Parameters. This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the 3D View view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

Store Creation Parameter

Refer to Store Creation Parameters

12.1.4 Tab Edit

Once the Cell creation wizard is completed, use these buttons for manual editing of the Cell objects. It allows you:

1. To import Surfaces or Spots and incorporate them in the Cells analysis.
2. To export objects from the Cells.
3. To clear the selection or delete all selected component of the cell object.

Import

Import Surface to Cells
To import the Surface as the Cell or the Nuclei Objects, click on this button. The window, **Import to Cells Objects**, will appear.

Import Spots to Vesicles
To import the Spots as the Vesicles, click on this button. The window, **Import to Cell Object**, will appear.

Import to Cell Object window

Choose one of the objects from the drop-down list. Choose how the selected object type is to be imported and specify the selection and the time.
The Surface can be imported either as the Cell or the Nuclei.
The Spots can be imported only as the Vesicles.

Import as
Choose to import the object either as a new Vesicle type or as a previously detected Vesicle type.

To import the Spots object as an already segmented Vesicle type, please select the correct name from the drop-down list.

Selection
The user can decide either to import the **Selection** or the **All** objects of the specified component.

Time
The **time point** of the object selected can be imported either as the **Current time point** or the **All time points**.

Export

Selection
The **Selection** checkbox is used to export only the currently selected object.

All Timepoints
The **All Timepoints** checkbox is used to export the object for all time points.

Export Cells to Surface
With a click on this button you export the Cell objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Cell Export**. The exported objects can be then manually edited.

Export Nuclei to Surface
With a click on this button you export the Nuclei objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Nucleus Export**. The exported objects can be then manually edited.

Export Vesicles to Spots
With a click on this button you export the Vesicles to a Spots object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Vesicles Export**. The exported

objects can be then manually edited.

Full Export
With a click on this button you export all the Cell objects. The **Cell** and **Nuclei** objects are exported **to Surface**, while the **Vesicles** objects are exported **to Spots**.
The Surpass Tree list is updated and three new surpass objects are generated (two Surface items and one Spots item) with the automatically generated names **Cells n object-name Export**. Pressing this button has the same effect as pressing the three buttons above.

Selection

Clear Button
Click this button to clear the current object selection.

Delete Button
Click on this button to delete all the currently selected components.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

Selection Visible Time Point

Fuse and Fragment are available only when cells were detected using **Detect Cell Boundary from Cell Membrane** option.

Fuse Button
Select this to fuse two or more selected cell objects within the visible time point.

Fragment Button
Select this to split selected cell objects within the visible time point. When fragment is selected it will return all previously fused objects back to their original dimensions.

12.1.4.1 Import

Import Surface to Cell
To import the Surface as the Cell or the Nuclei Objects, click on this button. The window, **Import to Cell Object**, will appear.

Import Spots to Vesicles
To import the Spots as the Vesicles, click on this button. The window, **Import to Cell Object**, will appear.

Import to Cell Object window

Choose one of the objects from the drop-down list. Choose how the selected object type is to be imported and specify the selection and the time.
The Surface can be imported either as the Cell or the Nuclei.
The Spots can be imported only as the Vesicles.

Import as
Choose to import the object either as a new Vesicle type or as a previously detected Vesicle type.

To import the Spots object as an already segmented Vesicle type, please select the correct name from the drop-down list.

Selection
The user can decide either to import the **Selection** or the **All** objects of the specified component.

Time
The **time point** of the object selected can be imported either as the **Current time point** or the **All time points**.

12.1.5 Tab Edit Tracks

This tab is common concept for all objects, please read more here Tab Edit Tracks.

12.1.6 Tab Edit Labels

This is a common concept for all Objects. Please find more information at the following link Tab Edit Labels.

12.1.7 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determine the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects the Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Compact Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Duplicate Selection to New Surfaces Button
Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

12.1.8 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.1.9 Tab Color

The Color tab is a common concept for all objects in Imaris, see 3D View - Common Concepts - Surpass Objects - Tab Color.

12.2 Clipping Plane

The Clipping Plane cuts away objects on one side of the plane, the data is only visually hidden and using the clipping pane does not have an impact on statistics or object creation. It can be freely moved and rotated in the scene and allows you to look inside any object. The Clipping Plane consists of a colored frame with a sphere and arrow manipulator.
To change the position and orientation of the Clipping Plane in the Viewing Area the Pointer must be in Select mode.

How to Add a new Clipping Plane?

- Select menu **3D View - Clipping Plane** or **2D View - Clipping Pane**.

The Surpass Tree list is automatically updated and in the Clipping Plane - Properties the Settings parameters

are displayed. The Clipping Plane is displayed in the viewing area.

To clip another object, this object must be placed beneath the clipping plane within the Surpass Tree; any object beneath the clipping pane will be clipped. It must also be in the same group as the Clipping Plane.

Change Position of Clipping Plane

The position of the Clipping Plane can be changed. In Selection pointer mode, Left mouse click on the circle of the manipulator and drag to move the clipping pane back and forth along the current plane. Right click and drag on the circle to move the position of the manipulator in its current plane.

Rotate Clipping Plane around Axes

The orientation of the Clipping Plane can be rotated around the x-, y-, and the z-axes. In Selection pointer mode left mouse click and hold down on the arrow pointer of the manipulator and drag to rotate the Clipping Plane. To reset to a YZ, XZ or XY plane use the orientation buttons on the settings tab.

See also:
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

12.2.1 Tab Settings

Position

Button **Recenter**
The Clipping Plane center is moved to the data set center.

Orientation

Button **YZ Plane**
Button **XZ Plane**
Button **XY Plane**
The Clipping Plane is oriented parallel to either the yz-plane, the xz-plane or the xy-plane.

Button **Flip**
Click on the **Flip** button to flip the orientation of the clipping plane.

Configuration

Button **Store**
Button **Recall**
After changing the position and/or orientation of the Clipping Plane, the new configuration can be stored by clicking on the **Store** button. The position previously stored can be recalled by selecting the **Recall** button.

Camera

Button **Orthogonal View**
The camera is set to a position which is perpendicular to the Clipping Plane.

Show Frame
To make the frame visible, check this box.

Show Manipulator
To make the manipulator visible, check this box.

Perpendicular Movement

The pointer must be in the pointer mode Select. Place the mouse within the image. To change the orientation of the rod press the Ctrl button. The Clipping Plane is moved in the directions parallel to the plane.

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

12.2.2 Mouse & Keyboard PC

Clipping Plane Mouse & Keyboard Functions

Left-click on the sphere	Move plane to desired position
Left-click on the pointer arrow	Rotate plane to desired position
Right-click on sphere	Move manipulator to different position in plane

See also:
Addendum - Mouse & Keyboard PC

12.2.3 Mouse & Keyboard Mac

Clipping Plane Mouse & Keyboard Functions

Click on the sphere	Move plane to desired position
Click on the pointer	Rotate plane to desired position
Right-click on sphere	Move manipulator to different position in plane

See also:
Addendum - Mouse & Keyboard Mac

12.3 External Object

Surpass provides the ability to import External objects (both, non-Imaris and Imaris objects) and display them concurrently with a Surpass object for comparison.

Only the External objects with the extensions ***.iv** (Inventor file) or with extensions *.**vrml** (VRML2 files) or .**wrl** (WRL files) can be imported.

The imported External objects can be rotated, scaled and translated.

For example, previously calculated Surfaces, which have been exported as Inventor files, can be imported to another data set.

How to Add a new External Object?

- Select menu 3D View - External Object.

The Surpass Tree list is automatically updated and in the External object - Properties the Settings parameter are displayed.

See also:
Menu 3D View - Export Selected Objects ...

12.3.1 Tab Settings

The object properties area displays a number of parameters for the positioning and display of the External object. The following display/handling options can be checked:

Box Handle
The object shows a surrounding box with handles. These handles allow you to transform/distort the object in the x-, y-, and z-direction.

Trackball
The object shows surrounding circular lines. These lines allow rotation of the External object in any direction.

File Path Field
Display of the file path (to load the object, see below).

Button **Load...**
To select and load a data set as a External object click on the button **Load...** and the Load External Object File window is displayed. Select the file to be loaded as External object. The External object will be only loaded if the selected files have the extension ***.iv** (Inventor file), ***.vrml** (VRML2 files) or **.wrl** (WRL files). Click on the button Open.

Button **Reset Size and Location**
Clicking this button sets the External object back to its original size and position before any translational, scaling or rotational operations have been performed.

Fix Ratio
If this option is checked and if scaling is performed on the External object by entering a value, the same scaling factor is applied to all three dimensions.

Position/Scaling

The following positional/scaling options are available:

Translation
Use these fields to move the External object in the x-, y-, or z-direction.

Rotation
Use this field to rotate the External object around the x-, y-, or z-axis.

Scaling
Use this field to scale the External object in one of the dimensions (or all together if Fix Ratio, see above, is checked). Fix Ratio is only valid with this method of scaling.

12.3.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

The quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting is for no specular intensity.
- Emission- light emitted from the material defines the self-illumination. Default setting is for no emission of light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

12.3.3 Mouse & Keyboard PC

External Object Mouse & Keyboard Functions

Left-click white box & drag	Scale object from center
Left-click white box + Ctrl & drag	Scale object from opposite corner
Left-click trackball & drag	Rotate object
Shift + left-click trackball & drag	Set up a rotation axis

See also:
Addendum - Mouse & Keyboard PC

12.3.4 Mouse & Keyboard Mac

External Object Mouse & Keyboard Functions

Click white box & drag	Scale object from center
Click white box + Ctrl & drag	Scale object from opposite corner
Click trackball & drag	Rotate object
Shift + click trackball & drag	Set up a rotation axis

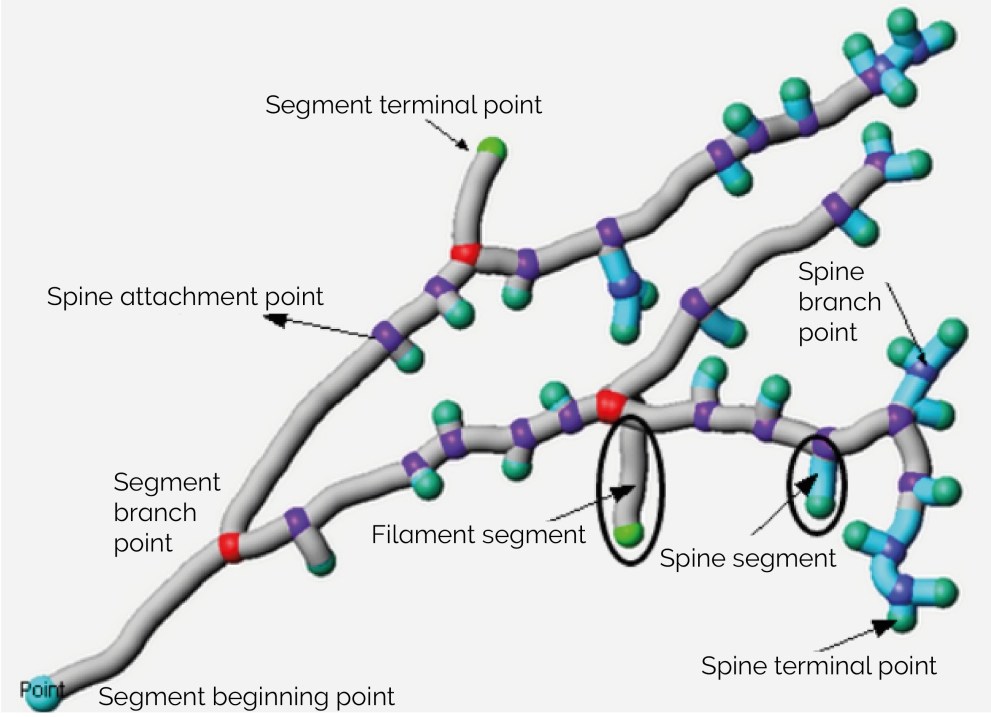
See also:
Addendum - Mouse & Keyboard Mac

12.4 Filament

In Imaris filament trees are represented by a set of **vertices** and **edges**. A **Vertex** is drawn as a node or point and defines the center axis of a segment. An **edge** is drawn as a line connecting two vertices. Thus, a filament graph can be understood as a combination of vertices and edges. A filament may also have a Soma at the beginning point, which is represented by a surface. For a comprehensive list of definitions for filament terminology please see the filaments statistics section.

The vertices and edges of a filament can receive different names depending on their position in the graph. As shown in the color-coded image below **starting, ending and branching vertices** are classified into **segment beginning point (light blue), segment branch point (red), segment terminal point (light green), spine attachment point (purple), spine branch point (blue), and spine terminal point (teal green)** depending on their position in the graph. Please note that the colors shown in the image below are the default colors for the different sections; these settings can be changed using Tab color.

Segments are defined as the collection of edges between two starting, ending, or branching vertices. Segments are classified either as **filament segments (grey)** or **spine segments (light blue)**. Please note the default color for spine segments is white; this has been amended using Tab Color in this example for clarity. In a segment the edge is called a **segment edge**, whereas in a spine it is called a **spine edge**.



The Imaris FilamentTracer offers features to segment, track, edit, draw, display, measure and annotate Filaments. The FilamentTracer processes one channel (color) at a time. After automatic Filament creation (see Creation Wizard) you can use the Edit tab to delete Filaments, connect Filaments, or draw new Filaments. You may also skip the automatic Filament creation and process the data with semi-automatic or manual segmentation (see the Tab Draw).

Generated Filament objects will be visualized in the context of Surpass view. This will allow multiple viewing combinations in which the Filaments are displayed individually or together with other cellular components that may have been imaged in other channels. A wide selection of measurements referring to the whole filament graph or its individual sections (Points, Edges, Segments and Spines) are automatically generated and can be checked within the Statistics Tab. They can also be exported to different formats.

How to Add a new Filament?

- Select menu 3D View - Filament or 2D View - Filament.

The Surpass Tree list is automatically updated and you start in the first window of the Creation Wizard (see below).

See also:
Surpass View - Overview - Surpass Tree
3D View - Filament - Creation Wizard (for automatic Filament creation)
3D View - Filament - Tab Draw (for interactive Filament tracing method)
For information on statistics calculations used refer to the statistics section for Filament

12.4.1 Data Input

The FilamentTracer is designed to operate on 3D time-resolved images. These images can be multi-channel (multiple colors) but only one channel will be traced at a time. The following input images are acceptable:

- The ideal input is a fluorescently labeled confocal image. Such images generally have good contrast (due to selective labeling) and good resolution in all three spatial dimensions.
- 3D images can also be acquired using a standard microscope (non-confocal) equipped with a CCD camera and a stepper motor. Non-confocal optical arrangements do not provide resolution along the optical axis which is why every single plane consists of in-focus components and out-of-focus components. These images require deconvolution prior to tracing. The technique of deconvolution uses information about the imaging process (the point spread function) to remove the out-of-focus components from every section.
- Finally there are 3D images taken in transmission light microscopy. Staining is usually less specific than for fluorescence techniques and it will therefore be more difficult to automatically segment the Filament. Deconvolution can also be applied to transmission images although its performance is theoretically weaker than for fluorescence methods.

12.4.2 Creation Wizard

The automatic filament graph creation is guided by a wizard-like user interface. Selection of the appropriate algorithm can be carried out in Step 1 of the wizard (or quit the automatic filament creation and draw the filaments interactively). Specific steps in the creation wizard will be determined by the algorithm chosen, (please see Algorithm for more information). Optimal parameters can be found by moving forwards and backwards through the wizard.

If the image data is for a multi-image scene an option to select which source image to be analyzed is available from the Align to: drop down box.

Stored creation parameters can be loaded in Step 1 of the Creation Wizard. Decide whether to load a set of previously stored creation parameters or to create a new one. The most convenient way to apply the same creation parameters to different data sets is to use the Load Parameters option.

12.4.2.1 Algorithm

Different pre-defined algorithms are available for automated filament creation.

Note: If you do not want to use the automatic filament creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the interactive filament tracing method.

Favourite Creation Parameters

In the creation wizard for all objects the algorithm step allows for the creation of favourite creation parameters and are described in the Algorithms common concepts section.

Algorithm Settings

The automatic filament creation provides two main algorithms to choose from:
The Tree Autopath Algorithm

The automated AutoPath Algorithm

The following options are available on the first step of the Filaments creation wizard.



Icon	Algorithm	Steps in Creation Wizard
	**The Tree Autopath Algorithm** **Autopath (no loops) and no spines** • Based on local intensity contrast • Identifies large start points or somas • Identifies segments from seed points • Connects points to form tree-like Filaments	1. Algorithm 2. Region of Interest (optional) 3. Soma Starting Points 4. Filter Starting Points 5. Check Soma Segmentation 6. Seeds Points for Segments 7. Filter Seed Points for Segments 8. Seed Points Classification (optional) 9. Segment Classification (optional) 10. Segment Postfilter 11. Tracking (time series data only)
	**The Tree Autopath Algorithm (with spines)** **Autopath (no loops) with spines** • Based on local intensity contrast • Identifies large start points or somas • Identifies segments from seed points • Connects points to form tree-like Filaments • Detects spines	1-10 same as **The Tree Autopath Algorithm, Autopath (no loops) and no spines** 12. Spine Detection 13. Filter Spine Points 14. Spine Points Classification 15. Tracking (time series data only)
	**Automated AutoPath Algorithm: Autopath loops with soma no spines** • Produces a filament with loops • Is based on local intensity contrast • Connects seed points • Connects to a soma	Same as **The Tree Autopath Algorithm, Autopath (no loops) and no spines**
	**Automated AutoPath Algorithm: Autopath loops with soma and spines** • Produces a filament with loops • Is based on local intensity contrast • Connects seed points • Connects to a soma • Detects spines	Same as **The Tree Autopath Algorithm (with spines), Autopath (no loops) with spines**
	**Automated AutoPath Algorithm: Autopath loops no soma and no spines** • Produces a filament with loops	1. Algorithm 2. Region of Interest (optional) 3. Seeds Points for Segments 4. Filter Seed Points for Segments

Icon	Algorithm	Steps in Creation Wizard
	• Is based on local intensity contrast • Connects seed points	5. Seed Points Classification (optional) 6. Segment Classification (optional) 7. Segment Postfilter 8. Tracking (time series data only)
	**Automated AutoPath Algorithm: Autopath loops no soma and with spines** • Produces a filament with loops • Is based on local intensity contrast • Connects seed points • Detects spines	1-7 same as **Automated AutoPath Algorithm: Autopath loops no soma and no spines** 9. Spine Detection 10. Filter Spine Points 11. Spine Points Classification 12. Tracking (time series data only)

Segment a Region of Interest-ROI
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analysis. Defining and processing ROI allows other parts of an image to remain unchanged.

Process entire Image finally
Use the Region of Interest to determine parameters, but on the final step process the entire image.

Track (over time)
Check this box if you want to track Filament over time, for details please refer to the tab Tracking.

Object-Object Statistics
Select to calculate statistics between objects in the image file.

Creation Preferences
This check box **Start Creation with Slicer view** can be used to activate Slicer view in the creation wizard.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.2 Region of Interest

Please see our common concepts page on the Region of Interest.

12.4.2.3 Soma Starting Points

Select Source Channel

Select the respective source channel, from which the Soma structure is to be created.

Starting Points

A new Soma for the filament will be determined based on the value given for the largest diameter.

Estimated Largest Diameter
Enter a value of the maximum (largest) diameter of Soma to specify the maximum Soma diameter.

12.4.2.4 Filter Starting Points

Starting Points Threshold

Thresholds are used to classify the starting points. The detected starting points are visualized in the image. The respective histogram is displayed. The threshold can be set manually or automatically.

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Calculate Soma Model
Check this box if you want to calculate a Soma model during Filament object creation.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.5 Check Soma Segmentation

Choose between Soma models and starting points

The Soma model computes a shape representative of the actual structure around each soma position. The computation of the soma shape requires a good staining of the soma itself. If you discover that the detected soma shape is not a good representation of the biological shape you can disable the soma model and use beginning points instead.

Select **Use Soma Models** or **Use Starting Points**, the viewing area is updated with your selection.

Choose which model to use and press next to move to the next step. For example, use a starting point if the soma structure detected is not appropriate for the dataset.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.6 Seeds Points for Segments

Seed Points

Thinnest Diameter
Enter a value of the minimum (smallest) diameter of segment to specify the Seed points diameter.

Multiscale Points
Select to enter **Largest Diameter** Value

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.7 Filter Seed Points for Segments

A threshold is used to classify the seed points for segments. The detected seed points are visualized in the viewing area and the respective histogram is displayed. The threshold can be set manually or automatically.

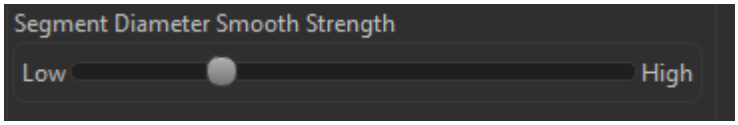
Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

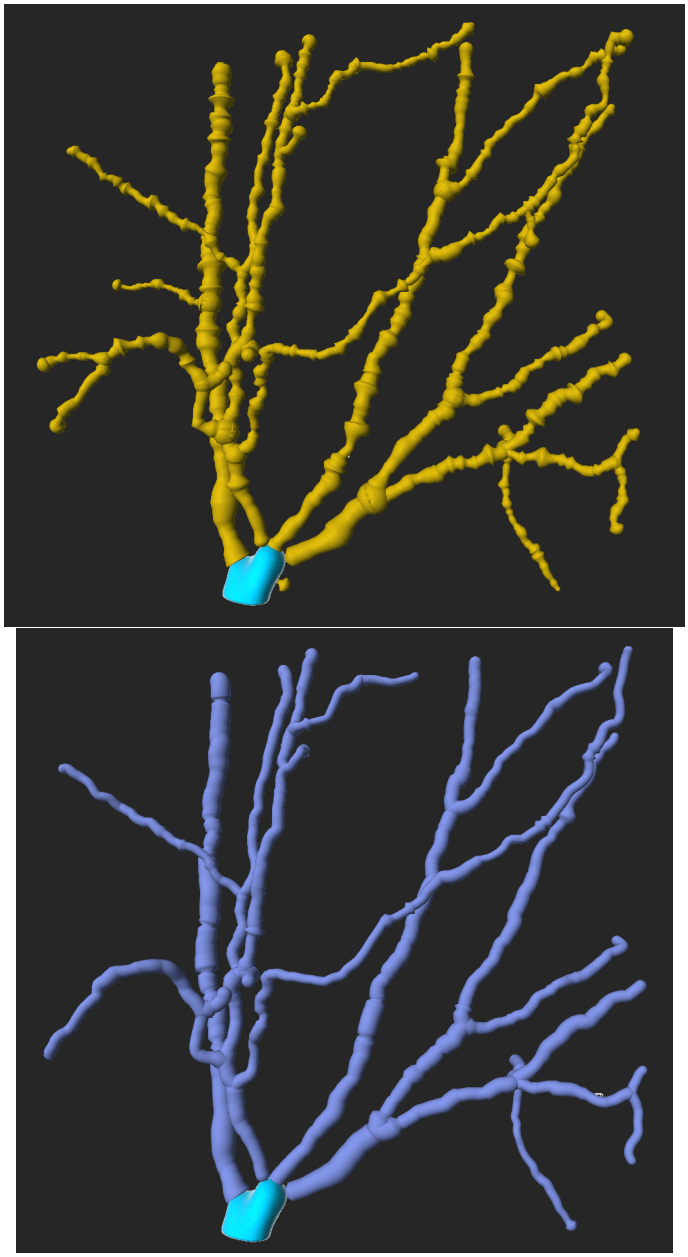
Remove Seed Points Around Starting Point(s)
When selected, any seed points below a distance defined by the **Diameter of Sphere Region(s)** from the starting point will be excluded from further computation.

Classify Seed Points
Check this box if you want to classify Seed Points in the following step.
Classify Segments
Check this box if you want to classify Segments in the following step.

Segment Diameter Smooth Strength
Use the slider to adjust the final smoothing of the filament segments. On moving the slider nothing will change in the viewing area, only the smoothing of the final filament object will be impacted. At the lowest setting (Low) the minimum amount of smoothing is applied to the final render of the segments in the filament object. Whereas using the highest setting (High) on the slider will give the most smoothing of the segments in the final filament object. See Figure below.

The value used by the slider is determined using the seed points radius chosen in Seed Points for Segments. The minimum value is a 1x modifier while the maximum value is a 5x modifier. The default value on the slider is a 2x modifier. The smoothing effect applied using the slider takes into account neighbouring segment vertices and uses them to Gaussian smooth the final filament segment shape.
If using only the thinnest diameter seed point then this is the value that is used. If using multiscale points the thinnest and the average of thinnest and largest diameter are used for two group segments.
Gaussian filter width = seed points radius x segment diameter smooth strength





Left: Final filament object for lowest (Low) slider setting on Segment Diameter Smooth Strength. Right: Final filament object for highest (High) slider setting on Segment Diameter Smooth Strength.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

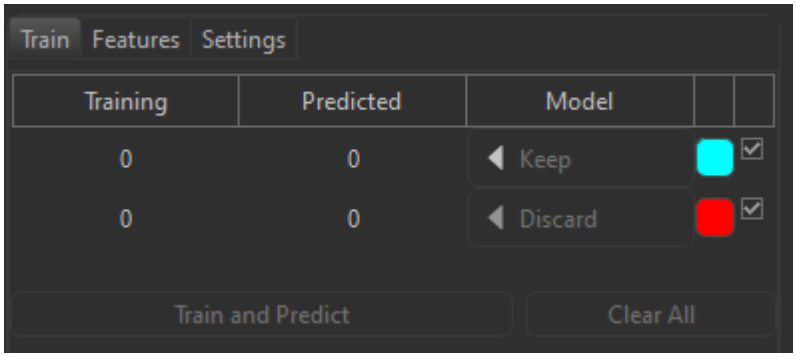
12.4.2.8 Seed Points, Segments or Spines Classification

The Filaments creation wizard Classification step has a similar interface for Seed Points, Segments or Spines. The classification steps are only available in the creation wizard if **Classify Seed Points, Segments or Spines** have been selected in the previous step.

The train dialog allows the user to add specific selected Seed Points, Segments or Spines to the keep or discard classes to develop a training set for the machine learning algorithm. First, some example Seed Points, Segments or Spines which are representative of the keep and discard classes are added to the training set. The first class displayed in the train dialog is the keep class and the second the discard class. To add a Seed

Point, Segment or Spine to a class, select the object(s) using the selection tool in the viewing area and then press the Add button of the class you want to add them to. Actual training and prediction of the machine learning algorithm occurs when the Train and Predict button is selected. As a result of this, the class of each object not in the training set is predicted by the machine learning algorithm.

To get accurate predictions you will need to add at least 50 Seed Points, Segments or Spines to the respective keep and discard classes.



Train Tab

Training - lists the number of objects manually assigned to that class, which are used by the machine learning algorithm to assign classes to all other objects in the image which are unassigned. Note: at least 50 Seed Points, Segments or Spines must have been assigned to a class before the Train and Predict button can be selected.

Predicted - lists the number of objects that are predicted to be part of the class using the machine learning algorithm. Note: values only populated after the machine learning algorithm has been run using the Train and Predict button.

Keep/Discard buttons - allow selected objects from the image to be added to that class.

Colour block icon - Change the colour of the keep and discard classes by clicking on this icon.

Check box - Once objects are assigned to the keep and discard classes, use these check boxes to show (checked) or hide (unchecked) the classified objects in the viewing area.

Train and Predict - Run the train and predict algorithm.

Clear all - Clear all assigned classes and restart classification.

If some objects have not been classified correctly during the first Train and Predict run, retraining can be carried out by manually selecting and adding the wrong predictions to the correct class and then selecting Train and Predict again. These steps can be repeated as many times as is required to classify Seed Points, Segments or Spines in the image appropriately.

If you decide not to use the classification step, leave both classes empty and press next to move to the next step in the creation wizard.

12.4.2.9 Terminal Segment Postfilter

Filter items to be included or excluded from the final filament object. Setting thresholds and using histograms are a common concept used throughout the Imaris creation wizard. However, the key difference in the Segment Postfilter step is that the selected section (in red) represents segments that will be removed from the final filaments object.

This filtering can be achieved by selecting the filter type from the drop-down list and defining the filter values using automatic or manual thresholds, which will then limit the selection in the histogram to those objects that meet these criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between, shown in red. **In the Segment Postfilter step the objects with the relevant properties between the lower filter value and higher filter value will be excluded in the final filament object, and the objects outside the defined interval will be included.**

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Setting thresholds and using histograms are a common concept used throughout the Imaris creation wizard and are described in Thresholds. However, the key difference in the Segment Postfilter step the selected section (in red) represents segments that will be removed from the final filaments object.

12.4.2.10 Spine Detection

Select Source Channel

Select the respective source channel, from which the spine structures are to be created.

Seed Points

Spines will be determined based on the value given for the **Thinnest Diameter**.

Maximum Length
Enter a value for the maximum (largest) length of a spine.

Allow Branch Spines
Select to allow branch spines in the computation.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.11 Filter Spine Points

Spine

Seed Points Threshold

Thresholds are used to classify the spines seed points. The detected Spine seed points are visualized in the image. The respective histogram is displayed.

The threshold can be set manually or automatically.

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Classify Seed Points
Check this box if you want to classify Seed Points in the following step.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.12 Tracking

The filament object consists of a set of segments, spine points and the connections between them. Tracking allows objects to be traced throughout sequential frames of a time data set. Each object maintains an unique identity in the output measurements.

This tab only appears if the Track (over Time) is checked (Algorithm Settings).

The Filament tracking tab provides Autoregressive Motion algorithms, which models the filament movement.
The algorithm utilizes the object's direction and speed information from one single time point to predict the best object position at the next time point.
Based on the tracking of the entire Filament graph, the algorithm predicts the future positions of the Soma, Branching and Terminal Points as well as the Spine Attachments and Spine Terminal Points. Based on these predicted positions, the best matches to the candidate points can be determined.

Autoregressive Motion

This algorithm models the motion of the filament graph's center of gravity as an autoregressive AR1 process. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified Maximum Distance value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the value of the maximum distance parameter, and because the AR1 algorithm performs correlations only to one previous time-point.

Track Filaments Center of Gravity (Autoregressive Motion)

Max Distance
The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.
It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

Close Gap with Max Gap Size
Select this option and the gap-closing algorithm will be implemented.

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and

the creation of track fragments. Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points which are allowed to be missing in order to a track to be continued.

Track Segments (Autoregressive Motion)

Select this option and the Segment points will be tracked.
When the Track Segment option is not selected the drop-down list remains inactive.

From the drop-down list you can select the type of Segment points to be tracked. There are two options:
- Segment Branch Points
- Segment Terminal Points

Please note: The tracking of the Soma is automatically computed based on the tracking of the entire Filament structure.

Enable
Check the box to enable the selected Segment points to be traced

Max Distance
The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.
It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

Close Gap with Max Gap Size
Select this option and the gap-closing algorithm will be implemented.

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions. Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

Track Spines

Please note: The spine tracking options are only available if the spines have been previously created.

Select this option and the Spine points will be tracked.

From the drop-down list select the type of Spine points to be tracked. There are three options:

- Spine Attachment Points
- Spine Branch Points
- Spine Terminal Points

Enable track
Check the box to enable the selected Spine points to be traced.

Close Gap
Select this option and the gap-closing algorithm will be implemented.

Close Gap with Max Gap Size

Select this option and the gap-closing algorithm will be implemented.

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions. Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.13 Finish ROI Processing

The selected parameters will be used to complete the computation of a Filament structure in an image. By selecting one of six finishing options a Filament structure will be created in the following ways:

1. **Additional Regions** - re-enters creation process (in step two-ROI) and uses the same set of parameters for a new ROI to create the filament structure.This option is only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for all time points with the same set of parameters.

2. **Entire Image (in All Time Points)-** the entire image is processed with the same parameters used for the ROI processing and the filament structure is created for the entire image and all time points. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.

3. **All Done**- creates a filament structure only within an ROI.

Note: The Use Existing Filament option requires that the new ROI overlaps with some portion of the existing filament.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.2.14 Finish

Finish

To validate the automatically generated graph and the prior segmentation, you can inspect the result together with numerous Surpass modules: Ortho Slicer, Volume, Surfaces etc. You can optionally superimpose the segments with the original slices of the image stack using Imaris OrthoSlicers. OrthoSlicers are planes, which are perpendicular to any of the three spatial axes and extend throughout the entire image volume.

Build Filaments for all Time Points
Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters. They will be visible within the Surpass Tree. However the 3D Filament graph is only visible according to its time point.

Delete Working Channel
The Filament Working channel can be kept for another use. Principally it is not needed any more when finishing the Filament graph.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.4.3 Filament Properties

When the creation process is finished, the Tab Rebuild disappears while some other property Tabs appear. They allow the manipulation of the filament graph:

Tab Rebuild
Only appears when you Add a new Filament.

Tab Settings
Change Filament appearance and inspect selection statistics.

Tab Rebuild
Re-enter the creation process.

Tab Draw
Manually draw filaments and connections.

Tab Edit
Select automatically filament parts and delete or smooth them.

Tab Statistics
Inspect all statistic values.

Tab Color
Change the color appearance (material) of the filament.

See also:
3D View - Filament (Add a new Filament)

3D View - Filament - Tab Settings
3D View - Filament - Tab Rebuild
3D View - Filament - Tab Draw
3D View - Filament - Tab Edit
3D View - Filament - Tab Statistics
3D View - Filament - Tab Color

12.4.4 Tab Settings

On the tab Settings you can edit and adjust the display options.
Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris. The render on slicer options are available on every tab of the surpass property area, and are explained in the Surpass Property Area section.
Select the Filament option to adjust quality and style of the filament graph.
Select the Filament Tracks option and you can edit and adjust settings that determine the display of connections between the Track filament graphs.
Select the Point Tracks option and you can edit and adjust settings that determine the display of connections between the **beginning, branch and terminal points, as well as spine attachment, branch and terminal points** of the filament graphs.

Select Show Segments and/or Show Spines to render shapes on the volume representative of the shape of the filament components. Under Show Segments select Soma/Beginning Point, Branch Points, Terminal Points or under Show Spines select Attachment Points, Branch Points to render spherical points on the volume not representative of the actual shape of the filament. These can then be color coded on Tab Color.

12.4.4.1 Filament

Style

Line
Cylinder
Cone
The visualization of the graph is done as lines with a specified thickness (pixel), as cylinders (with a defined diameter), or cones (using the measured graph diameter).

Show Segments

Check the box in the heading to highlight segment points in the filament. The key points are highlighted with color-coded spheres.

Soma/Beginning Point
Check this box to highlight the segment beginning points.

Branch Points
Check this box to highlight the segment branch points.

Terminal Points
Check this box to highlight the segment terminal points.

Show Spines

Check the box in the heading to highlight spine points in the filament. The key points are highlighted with color-coded spheres.

Attachment Points
Check this box to highlight the spine attachment points.

Branch Points
Check this box to highlight the spine branch points.

Terminal Points
Check this box to highlight the spine terminal points.

Render Quality

With the slider you can adjust the render quality of the filament. Moving the slider to the left reduces the render quality, moving it to the right increases render quality.

12.4.4.2 Filament Track

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Cylinder
This option displays the tracks connections drawn as cylinders.

Line
This option displays the tracks connections drawn as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Filament Tracks

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale
Edit field for the displacement vector radius.

Color Editor
Select a desired color for the displacement arrow.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

12.4.4.3 Point Tracks

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Cylinder
This option displays the tracks connections drawn as cylinders.

Line
This option displays the tracks connections drawn as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Point Tracks

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale
Edit field for the displacement vector radius.

Color Editor
Select a desired color for the displacement arrow.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

12.4.5 Tab Rebuild

Recompute

Select from recompute Filaments, Somata, Segments or Spines and the creation wizard opens at the links below:

- Filaments
- Somata
- Segments
- Spines
- Track (time series data only)

Keep Data

The option **Keep Data** should be selected depending on whether the existing Filament graph should be **discarded**, or **re-used** in the Filament rebuild process.
If this option is selected, the previously created filament structure is kept. The Wizard creates a newly added filament that is merged with the existing one.
If this option is not selected, the previously created filament structure is discarded and the Wizard creates a new filament structure.

All Time Points

The option **All Time Points (time series data only)** should be selected depending on whether the parameters used for creating the Filament structure should be applied to the selected time point only or to all time points.
If this option is selected, the same set of creation parameters used for selected time point is reused to the create filament structure for all time points.
If the option is not selected, the creation parameters are used only for the selected time point.

In the rebuild process the following structure of the filament creation is applied:
1. According to the setting of the **Keep Data** option the existing Filament structure is discarded or re-used
2. The selected Rebuild algorithm is completed
3. In the case of the time resolved data sets, according to the setting of **All Time Points** options, the parameters are either used to create the filament structure for the selected time point only or to create the filament structure for all time points.

Store Parameters for Batch

Click on the Store Parameters for the Batch button to open the Store Creation Parameter window. The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters
Arena
Favorite Creation Parameters

Arena
When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, Creation Parameters, is created and displayed.
Once stored, Imaris Batch applies the creation parameters set to all images within the selected Group.

Favorite Creation Parameters
To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.

When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/Creation Parameters. This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

12.4.6 Tab Draw

Method

When it is not possible to use Imaris automatic filament creation algorithms, either semi-automatic or manual creation will allow you to create filament objects by manually marking the object location in using method AutoPath, AutoDepth or Soma.

AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available. If you select this method please refer to Section AutoPath (3D View - Filament - Tab Draw - AutoPath).

AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed). If you select this method please refer to Section AutoDepth (3D View - Filament - Tab Draw - AutoDepth).

Soma

The Soma function allows for the manual addition of a Soma surface. If you select this method please refer to Section Soma (3D View - Filament - Tab Draw - Soma).

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

See also:
Menu Edit - Show Display Adjustment
3D View - Overview - Camera Toolbar - Pointer
3D View - Filament - Tab Draw - AutoPath
3D View - Filament - Tab Draw - AutoDepth
3D View - Filament - Tab Draw - Soma

12.4.6.1 AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available.

- Use the Channel drop-down options to specify the channel on which the calculation runs.
- Placing a new start point cancels the previous calculation and starts again from the new start point.
- When a start point is placed close to an end point the path will automatically connect.
- The most recently drawn filament is automatically highlighted.
- Press Shift+T to switch between Torch View and Full View modes.
- Pressing Ctrl+8 is useful immediately after adding a path to turn on InMotion for validation of the tracing.
- Autopath mode respects clipping planes: It chooses the start and end points only within the visible part of the volume. (Note that the clipping plane frames and handles may be turned off so they do not selected by mistake when making other adjustments to the image).

Setting

Diameter

Set the Diameter for the cursor. The size of the cursor determines the resolution at which the tracing is calculated (larger cursor provides lower resolution but is a faster calculation and less precision- but often these low resolution paths are effective).

Type

Select between segment and spine options

Select Source Channel

Select the respective source channel, from which the structure is to be created.

Correction

AutoCenter

This option runs an automatic centering when the path is added (after pressing shift+left-click).

AutoDiameter

Select this option to run an automatic diameter calculation when the path is added (after pressing shift+left-click). The calculation takes the mouse cursor size on shift+left-click as an input parameter (initial guess of diameter). The mouse cursor size should roughly correspond to the diameter of the filament for the calculation to succeed.

View

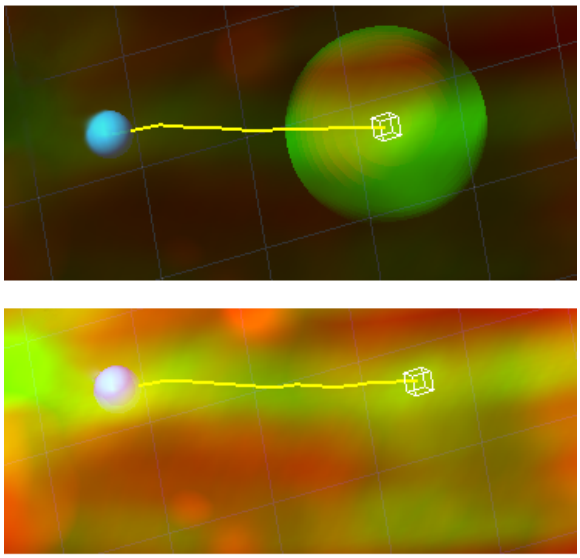
Select between the two viewing options:

Torch View

Provides a highlighted area to help allow you to focus on an area of interest (top image). The size of the area illuminated by the torch can be adjusted using the mouse wheel.

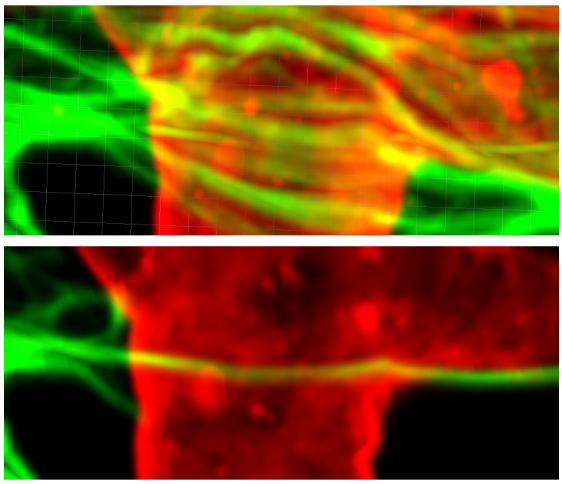
Full View

Provides a normal view through the full image area (bottom image).



Depth Visibility

When working through an image with depth, it may have features that can obscure an area or structures of interest. You may enable Depth Visibility and then use the sliders to adjust the area of visibility between Near and Far and between a Narrow and Wide field to help obtain a suitable view as shown in the following image. Unchecking the Depth Visibility will return the image to the normal view.



Drawing in AutoPath Mode on a PC:

1. Select AutoPath and Shift + right-click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.
2. Place the mouse cursor at the ends of the structure. The computed path is displayed.
3. Shift + click and the displayed path is drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

PC Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point

Shift + Ctrl + right-click	Connect and restart.
Shift + left-click	Add the path
Shift + Ctrl + left-click	Add path and restart
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

Drawing in AutoPath Mode on a Mac:

1. Select AutoPath and Shift + right click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.

2. Place the mouse cursor at the ends of the structure. The computed path is displayed.

3. Shift + click and the displayed path is drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

Mac Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point
Shift + Command + right-click	Connect and restart.
Shift + left-click	Add the path
Shift + Command + left-click	Add path and restart
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

Notes

There is no visual indication of the progress of the autopath calculation. One way to find this out iindirectly is by moving the mouse pointer to different positions in the image. For those positions where the calculation has already arrived a tracing will be shown. For those positions where the calculation has not yet arrived no tracing is shown.
Since it is very easy to trace a small piece and then continue the tracing from there users can work without interrupt.

12.4.6.2 AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed).

- An autosnap feature enables easy drawing along the filament structure as you do not need to click in the exact centre. When ambiguities arise, autodepth keeps track of multiple paths until the ambiguities resolve. (Only the best path is displayed to the user).
- Press Shift+T to switch between Torch View and Full View modes.
- Pressing Ctrl+8 is useful immediately after adding a path to turn on InMotion for validation of the tracing.
- Autodepth mode respects clipping planes: It chooses start and end points only within the visible part of the

volume. (Note that the clipping plane frames and handles may be turned off so they do not selected by mistake when making other adjustments to the image).

Setting

Diameter

Set the Diameter for the cursor. The size of the cursor determines the resolution at which the tracing is calculated (larger cursor provides lower resolution but is a faster calculation and less precision- but often these low resolution paths are effective).

Type

Select between segment and Spine options

Select Source Channel

Select the respective source channel, from which the structure is to be created.

Correction

AutoCenter

This option runs an automatic centering when the path is added (after pressing shift+left-click).

AutoDiameter

Select this option to run an automatic diameter calculation when the path is added (after pressing shift+left-click). The calculation takes the mouse cursor size on shift+left-click as an input parameter (initial guess of diameter). The mouse cursor size should roughly correspond to the diameter of the filament for the calculation to succeed.

View

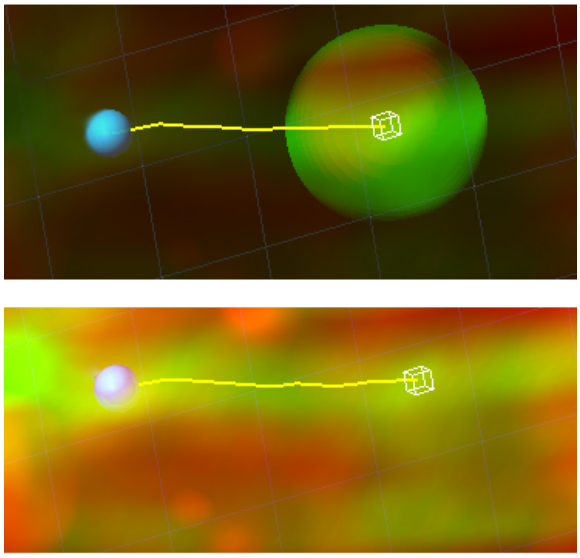
Select between the two viewing options:

Torch View

Provides a highlighted area to help allow you to focus on an area of interest (top image). The size of the area illuminated by the torch can be adjusted using the mouse wheel.

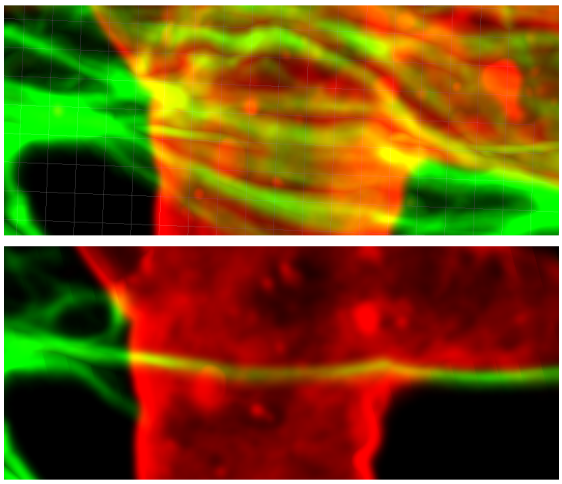
Full View

Provides a normal view through the full image area (bottom image).



Depth Visibility

When working through an image with depth, it may have features that can obscure an area or structures of interest. You may enable Depth Visibility and then use the sliders to adjust the area of visibility between Near and Far and between a Narrow and Wide field to help obtain a suitable view as shown in the following image. Unchecking the Depth Visibility will return the image to the normal view.



Drawing in AutoDepth Mode on a PC:

Select AutoDepth, press the Shift-key and hold down the left mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. If this is the case, pre-filtering would be then necessary.

PC Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

Drawing in AutoDepth Mode on a Mac:

Select AutoDepth, press the Shift-key and hold down the mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. If this is the case, pre-filtering would then be necessary.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

Mac Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

Notes
Pressing the d-key on the keybord while drawing enables an eraser so you can work backwards and redraw from an earlier point. This works only on those parts of a filament that were drawn right before pressing the d-key.

12.4.6.3 Soma

The soma model computes a shape representative of the actual structure around each soma position. The computation of the soma shape requires a good staining of the soma itself. If you discover that the detected soma shape is not a good representation of the biological shape you can disable the Soma model and use beginning points instead.

Setting

Diameter
Set the Diameter for the selection box volume. The size of the selection box determines the the size limit of the Soma.

Select Source Channel

Select the respective source channel, from which the structure is to be created.

PC Mouse & Keyboard Function Overview in the Soma Mode

Shift + left-click & drag	Draw a Soma in the volume
Shift + middle scroller	Change size of selection box

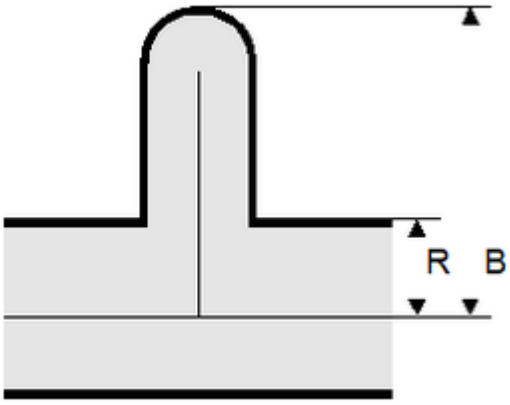
12.4.7 Tab Edit

Up to this point in the analysis process, most steps have been carried out automatically. You have influenced the tracking process by specifying a set of parameters. The Tab Edit allows you to make changes to the graph topology and the number of nodes and segments in the filament graph. You make these changes directly by processing part or all of the existing filament graph, or by operating on the 3D view of the graph.

Selection of components within the filament object can be made using the camera and selection toolbar.

Select Parts from Filament

There are various methods to make automatic selections on the whole Filament graph.



Button **Rel. Branch...**
Rel. Branch selects short parts of the Filament defined by the ratio of the branch length to trunk radius B/R.

Button **Loops...**
Loops finds circular closed Filaments limited by a maximum length.

Button **Invert**
Invert selects all unselected parts of the graph and de-selects all selected parts.

Button **Abs. Branch**
Abs Branch selects all branches shorter than an absolute length B.

Button **Path**
Path requires at least two selected points from the Filament graph. The shortest path between these two points will be selected.

Button **Clear**
Clear removes the selection.

Selection

The following buttons apply only to the previously selected parts (see above) of the Filament.

Button **Assign as Segment**
Click on this button to assign the selection as segment.

Button **Assign as Spine**
Click on this button to assign the selection as spine.

Button **Assign as Segment Beginning Point**
Click on this button to assign the selection as segment beginning point.

Button **Smooth**
Removes the roughness of the current selection. It affects the position and the diameter of all segments except the branch points and end points.

Button **Duplicate**
Click on this button to create a duplicate of the current selection. The new Filament item appears in the Surpass Tree.

Button **Delete Soma**
Click to delete Soma, and replace with beginning point.

Button **Delete**
Click on this button to delete the current selection from the filament object.

Process Filaments

The following buttons apply to the entire Filament.

Clear Segment Beginning Point
Click on this button to remove the segment beginning point.

Button **Smooth**
Smooth removes roughness from the Filament path. It affects the position and the diameter of all segments except the branch points and end points.

Button **Split**
Unconnected parts of the Filament can be split into separate Filament objects. The new objects will appear as new Items in the Surpass Tree. This allows individual coloring and individual statistics.

Button **Merge...**
In Imaris it is possible to merge Filament objects into one Filament objects. This button is only available if you have two or more Filament items in the Surpass Tree. Click on this button and the window **Merge selected Objects to "Filament X"** displays. In the Merge selected Objects to "Filament X" window there are the following options:

Check the boxes in front of the desired Filaments or use the button **Select All**.

Button **Clear All**
Use this button to clear the Filaments selection.

Button **OK**
If you want to merge the Filaments click on **OK**.

Button **Cancel**
If you do not want to merge the Filaments click on **Cancel**.

Connect & Translate selected Edges (Vertices)
Check this box to connect and translate selected edges.

Button **Import Soma...**
Click Import Soma to import a surface object to the filament object as a Soma. If a surfaces object is available in the surpass tree, the **Import Surfaces to Filament as Soma** window will open. Select the surface object from the drop down. In Selection choose Selection or All. For a time series data set select whether this applies

to the current time point or all timepoints. Click OK to proceed or cancel to return to Tab Edit.

Button **Export...**
The whole Filament graph can be stored in the Neuron file format (*.hoc). The Neuron application is free and is able to do electrical simulations.

Process Intensity-based

Source Channel
Select the respective source channel, from which the structure is to be created.

Diameter Smooth Strength
This is the same as the Diameter Smooth Strength slider in Filter Seed Points for Segments. Note that the viewing area will not automatically update, next select whether you want the **Diameter Smooth Width** to apply to a selected segment or to the entire filament, using the buttons below.

Selection (only available if a selection has been made)

Button **Center**
Click on this button to re-center the selection after adjusting the **Diameter Smooth Width**.

Button **Diameter**
Click on this button to recalculate the diameter of the selection to the value set in the **Diameter Smooth Width**.

Button **Join**
Select the objects to be joined in the image by Ctrl + left-click (Mac: Command+ left-click). The selected point or segment will be highlighted, then click Join. A new filament structure will be created structurally joining previously unconnected points or segments.

Filaments

Button **Center**
Click on this button to re-center the entire filament object after adjusting the **Diameter Smooth Width**.

Button **Diameter**
Click on this button to recalculate the entire filament object to the value set in the **Diameter Smooth Width**.

Check box **Object-Object Statistics**
Select to calculate statistics between objects in the image file.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

12.4.8 Tab Edit Tracks

This tab is common concept for all objects, please read more here Tab Edit Tracks.

12.4.9 Tab Edit Labels

This is a common concept for all Objects. Please find more information at the following link Tab Edit Labels.

12.4.10 Tab Filter

Please note: Before using, you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select the filter you want to apply from the drop-down list.

Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Duplicate Selection to New Filament Button
Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

12.4.11 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.4.12 Tab Color

The Tab Color is a common concept for all objects in Imaris, see 3D View - Common Concepts - Surpass Objects - Tab Color.

However, please note that to color code the following in the filaments drop down:

- Segment Beginning Point
- Segment Branch Point
- Segment Special Branch Point
- Segment Terminal Point
- Spine Attachment Point
- Spine Branch Point
- Spine Terminal Point

their corresponding check boxes must be selected in the Tab Settings.

12.4.13 Mouse & Keyboard PC

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

Shift + right-click	Set a starting point into volume. Multiple paths/branches can be drawn from this start point.
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	Draw the path visible on screen to the cursor. Does not change the start point or calculation thread from the start point.
Shift + Control + Left Click	Draw the path visible on screen to the cursor. Starts a new calculation from the end of a drawn path. Continues on same filament path.
Shift + Control + Right Click	Start a new calculation from an existing point on a drawn segment. This will create a new branch point, if it is in the vicinity of a drawn segment.
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:
Addendum - Mouse & Keyboard PC

12.4.14 Mouse & Keyboard Mac

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	Draw the path visible on screen to the cursor. Does not change the start point or calculation thread from the start point.
Shift + Command + Left Click	Add the path.
Shift + Command + Right Click	Connect and restart.
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

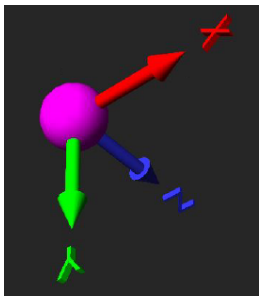
Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Command + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:
Addendum - Mouse & Keyboard Mac

12.5 Reference Frame

Reference frames can be used for image alignment. When time series need to be rotated or shifted for better alignment this transformation can be achieved using reference frames. Reference frames are orthogonal coordinate systems that can be positioned manually. To facilitate reporting of Object Statistics in the reference frame coordinate systems all positional and vectorial statistics values are automatically reported both in image coordinates and in reference frame coordinates.



A time play mode lets you play through a movie so that reference frames stay still.

The Reference Frame Coordinate System

A reference coordinate system consisting of three orthogonal axes can be positioned anywhere within the image and oriented in any direction by the user. The reference coordinate system can be positioned independently for each time point. A user can for example, place a reference coordinate system so that one axis aligns with the anterior-posterior axis of a Zebrafish.

Reference frames are positioned manually by clicking into the 3D View.

To adjust orientation of the axes
- Click on arrow of an axis and hold the left mouse button to adjust the orientation of the axes.

To move position of the axes
- Click on the central sphere to translate/move the position of the axes within the image.

For a time series it is not necessary to place a reference frame at every time point. It is possible to position a few frames at a few time points and all frames in between will be interpolated.

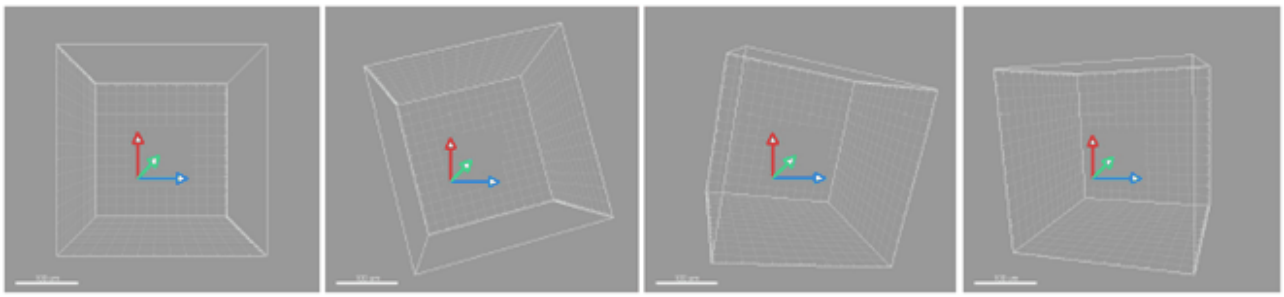
It is possible to create multiple reference frames within one image.

Statistics Values reported in Reference Frame Coordinates

All statistical values of surpass objects (spots, surfaces, cells, filaments) are computed both in world coordinates and in reference coordinates. In the Zebrafish example Imaris would report track-velocity vectors in the reference coordinate system such that it is easy to filter for objects that move toward the head of the fish for example.

Time Series with Fixed Reference Frame

When reference frames have been set for different time points of a time series it is possible to play the movie in such a way that the reference frames stay still. This is depicted in the following sequence:



ImarisXT Interface

Reference Frames can also be used and set through the ImarisXT interface.

Statistics Values

Reference frame specific statistics values for each surpass object are:

- Distance from origin of reference frame.
- Velocity angles with axes of reference frame.

These statistics values allow you to color objects by their distance to a reference frame, or by their direction of motion relative to the reference frame.

12.5.1 Settings Tab

Key Frame
Use the **Previous** and **Next** buttons to move between the key frames. The default option is interpolated (displayed in the Key Frame Type field. To set a translational key frame enable the **T** option, or to set a rotational key frame enable **R** option. Activating these options will update the description in the Key Frame Type field.

Planes
Set the frame plane options between **XY**, **XZ** and **YZ** planes.

Tick the **Visible** checkbox to toggle the visibility of the Reference frame on or off.

Use the **fix** checkbox to fix the plane in position.

Set the **extended section** for each plane by using the up and down arrows to increase or decrease the value

incrementally through each plane, or enter the required value directly.

Interpolation mode
Select between **No Interpolation** and **Linear Interpolation**.

No Interpolation: the translational and rotational positions will remain fixed between each reference frame. They may therefore appear to "jump" between reference frames.
Linear Interpolation the translation and rotation positions between reference frames will be calculated in linearly interpolated increments. This will provide a smooth transition between reference frames.

Resample Image to align Reference Frames
Press this button to resample the image in the reference frame coordinates.

For information on drift correction please refer to the section Drift Correction.

12.6 Correct Drift

If there is object drift (e.g. a cell drift) during the image acquisition this can be corrected in Imaris using the Correct Drift function.

- Drift correction can be applied to images with multiple time points, or to tracked objects.
- The result of the drift correction is either a translational and rotational correction of image time points or a reference frame object.

Drift correction on an image

To apply drift correction to an image:
1. Load an image with multiple time points into Imaris.
2. Choose Image Processing / Image Alignment / Align Image, or Image Processing / Image Alignment / Set Reference Frame.

Drift correction on an image is a parameter free method. It performs an automatic spots detection and tracking on the image and then applies drift correction on these points. The spots are never visible in Imaris and there is no way to adjust the spots detection. If this procedure does not produce adequate results, detect and track objects using e.g. the Spots wizard and use the Correct Drift functionality on the Track Editor Tab of the tracked objects.

Drift correction based on tracked objects

To perform drift correction based on tracked objects, load an image with multiple time points in Imaris, create and track a surpass object, e.g. Spots. Switch to the Edit Tracks Tab and press the Correct Drift button. Then configure the Drift Correction Options dialog to produce a new dataset or reference frames.

Drift Correction Options Dialog

Depending on the context, the Drift Correction Options dialog displays a subset of the following options.

Objects options

This section controls which objects should be affected by the drift correction.

Correct image only
Only the loaded image will be corrected, surpass objects that have already been detected remain unchanged.

Correct image and selected object
The image and those surpass objects that are selected in the surpass tree will be corrected.

Correct image and all objects
The image and all surpass objects will be corrected.

Reference Frame correction
The image and selected objects don't change, but a new Reference Frame will be added to the surpass scene.

Algorithm

This section controls how the drift correction should be calculated.

Translational drift
The image/objects will be corrected by only shifting the objects.

Translational and rotational drift
The image/objects will be corrected by shifting and rotating the objects.

Result dataset size

This section controls the size of the corrected image. After drift correction, a resampled image is created.

Crop largest common region
Only those parts of the image that overlap after drift correction will be present in the new image.
This produces the smallest result image.

New size equal to current size
The image after drift correction will have the same size as the original image.

Include entire result
The image after drift correction will contain all data from the original image.
This option produces the biggest image.

A Preview image at the bottom of the dialog illustrates how the result will look like.

12.7 Frame

The Frame serves to outline the object in the viewing area. In the Surpass Tree check the check-box Frame to display the frame in the viewing area. Uncheck the box to make the frame invisible.

How to Add a new Frame?

- Select menu 3D View - Frame or 2D View - Frame.

The Surpass Tree list is automatically updated and in the Frame - Properties the Settings parameter are displayed.

Surpass View - Overview - Surpass Tree

12.7.1 Tab Settings

Highlight Frame in the Surpass Tree and click on the tab Settings. The Frame Settings display in the properties area.

Box and Time Settings

Box
Check the parameter to display a box around the Frame.

Line Width
Adjust the box line width with the respective slider.Moving the slider to the left reduces the line width, moving it to the right will increase line width.

Shading
Check this parameter to display a shading. Use the slider to adjust the shade contrast.

Time Colorbar
Only available if you work with a time data set. Check this box and the Time Colorbar displays in the lower left corner of the viewing area.

Grid and Tickmarks Settings

Grid
Check the box Grid to display a grid. Use the slider to adjust the grid width.
Scale is shown using a grid behind the object. The frame moves to stay behind the object as it is rotated. The distance represented by each square in the grid, the scale bar, is shown in the bottom right corner of the screen.

Tickmarks
Check the box Tickmarks to display tickmarks on the edges of the frame.

Spacing
Specify the grid and tickmark spacing.

Labels Settings

Axis Labels
Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked the parameter Label Color and Label Font is available (see below).

Label Color
Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Color Editor window to change the color of the label on the coordinate axes.

Label Font
Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Font Editor window to change the font to label the coordinate axes.

Fix Decoration to Data Set
Check Fix Decoration to Data Set to fix the Frame to the same side when navigating the object.

12.7.2 Tab Color

Tab Color edit, manage and assign colors material to the Frame.

For Frame the Color Type available is Base.

Color

RGB

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color slider to select a color or by clicking on the drop down arrow and entering direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

12.8 Surpass Group

Surpass Groups are folders in the Surpass Tree that contain other Surpass objects. They are visible in the objects area and allow you to create a treelike hierarchy of Surpass objects. When the first Surpass object is created, it is automatically placed in a Group called Scene. Additional Groups can be created in order to sort objects of the scene.

How to Add a new Group?

- Select menu Surpass - Surpass Group.

The Surpass Tree list is automatically updated and in the Group - Properties the Settings parameter are displayed.

How to Add Items in a new Group?

- Click on an Item in the Surpass Tree list and drag it onto the Group object. Once released, the Item is added to the Group.

Like all other objects, Groups have a visibility check-box. This box affects the visibility of all objects in the Group.

See also:
3D View - Overview - Surpass Tree

12.8.1 Tab Settings

Number of Objects
Displays the number of objects in the Group.

Button **Show All**
If you click this button the check-box(es) of the Item(s) in the Group are checked.

Button **Hide All**
If you click this button the check-box(es) of the Item(s) in the Group are unchecked.

12.8.2 Tab Color- Base

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
- Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

12.8.3 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.9 Light Source

A Light Source serves to illuminate objects in the viewing area. A light source node serves as the location and orientation of a light source.

How to Add a new Light Source?

- Select menu 3D View - Light Source.

The Surpass Tree list is automatically updated and in the Light Source - Properties the Settings parameter are displayed. Multiple light sources can be added in the Surpass Tree.

Move Light Source

To change the Light Source position and direction, choose the pointer mode Select and drag the cone or line.

Light Intensity

Click on the cylinder above the cone and move it closer or further away from the object to change the light intensity.

12.9.1 Tab Settings

Fix to Data Set
Check the box to fix the Light Source to the same side when navigating the object. When the box is unchecked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

12.9.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

For light source the Color Type available is Base.

Color

RGB

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color slider to select a color or by clicking on the drop down arrow and entering direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

12.9.3 Mouse & Keyboard PC

Light Source Mouse & Keyboard Functions

Left-click cone (or line) & drag	Change direction
Left-click cylinder & drag	Change intensity

See also:
Addendum - Mouse & Keyboard PC

12.9.4 Mouse & Keyboard Mac

Light Source Mouse & Keyboard Functions

Click cone (or line) & drag	Change direction
Click cylinder & drag	Change intensity

See also:
Addendum - Mouse & Keyboard Mac

12.10 Measurement Point

How to Add a new Measurement Point?

- Select menu 3D View - Measurement Point or 2D View - Measurement Point.

The Surpass Tree list is automatically updated and in the Measurement Point - Properties the Edit parameters are displayed.

12.10.1 Tab Settings

Point Shape

Tetrahedron
Cube
Sphere
Here you can choose from three different shapes for the Measurement Points (tetrahedron, cube, or sphere).

Filled
Check the box and the point shape is solid.

Point Diameter

Here you can define the Measurement Point diameter.

Point Labels

Name
Check the check-box to display the name of a Measurement Point in the viewing area.

Intensity
Check the check-box to display the intensity of a Measurement Point in the viewing area. The intensity of the channel is displayed in brackets.

Angle-Polygon

This option is only available when Line Mode Polygon is selected in Settings. In the Pairs mode this option becomes inactive.
For each pair of adjacent measurement points, the angle between them at their common point is computed and displayed next to the angle arc.

Line Mode

Pairs (AB, CD, ...)
Any two consecutive points are connected by a line.

Polygon (ABCD ...)

All points in the Group are connected consecutively (P1-P2-P3-P4, etc.).

Line Width

Here you define the line width.

Line Labels

Distance
The measured distance between two points is displayed next to the line connecting the two Measurement Points. Check the check-box to display the distances in the viewing area.

Velocity
Only accessible if you have a time data set. Check the box to display the speediness between two Measurement Points.

Tip: If you do not have the module ImarisTrack you can easily use the velocity function to track single objects over several time points and measure the velocity.

Button **Color**
Button **Font**
Label Color and Font
To change the color or font of the label (display of the line length) click on the respective icon.

Tip: You can find the parameters to change the color of the Measurement Points and connecting lines on the tab Colors.

Visible for all Time Points
Only accessible if you have a time data set. Check the box to display the inserted Measurement Points, lines, and annotations at all time points.

See also:
3D View - Measurement Point - Tab Color

12.10.2 Tab Edit

AutoDepth Position

In the mouse pointer mode Select:
Shift-click with the left mouse button to add a new point. Left-click to select an existing point. Shift-left-click to reposition a selected point.

Intersect with

The first two parameters are relevant if you want to insert Measurement Points in a volume rendered data set.

all visible Channel
Select this parameter and all visible channel are relevant for the placement of the Measurement Points.
Please note: In the Display Adjustments you can decide, whether a channel is visible or not in the viewing area.

specific Channels
Select this option for the placement of the Measurement Points and choose the relevant channel directly from the drop-down list.
Please note: In the Display Adjustments you decide, whether a channel is visible or not in the viewing area.

Surface of Object
Select this option and the Measurement Points are placed on the surface of an object. Uncheck the box and you can also place Measurement Points in an object.

Center of Object
Select this option and the Measurement Points are placed in the center of the object.

Please note the difference with the different objects types.

For Spots objects (also Vesicles and segment or spines beginning, branching, attaching, terminal points): Select this option and a new measurement point is placed at the center of the sphere.
For Surfaces objects: Select this option and a new measurement point is placed at the center of the object homogeneous mass
For Cells objects (Cell and Nucleus): Select this option and a new measurement point is placed at the center of the object homogeneous mass.
For Filament objects (Segment and Spines): Select this option and a new measurement point is placed at the segment/spine center axis. (For more information please refer to the Filament description).

Button **Delete All Points**
Click on this button to delete all Measurement Points. Alternatively click on the Delete-key on the keyboard.

Selected Point

Click to select an existing point. If no point is selected the following parameters are grayed. Re-click on a selected point to un-select the point.

Button **Delete**
Click on this button to delete the selected Measurement Point.

Name
Text field to edit the Measurement Point name.

Position
X
Y
Z
T
Data field of the x-, y-, z- and t-coordinates.

12.10.3 Tab Intensity

Imaris reads out the intensity profile of each voxel against the distance. Here you select the channels that should be displayed in the histogram and you choose the desired connecting lines between the Measurement Points.

Histogram

The histogram displays the intensity profile along the lines. The labeled lines represent the Measurement Points. The x-axis represents the length of the profile. On the left hand side of the histogram you find the display of the channel(s) intensity range. The channel selection is located below the histogram. Here you can switch channels in the intensity profile on or off. Below the channel selection you find the profile selection. In the drop-down list select the desired line and the histogram updates accordingly.

Button **Snapshot**
Click on this button to do a Snapshot of the histogram. The window Save Snapshot as... opens.

See also:
Imaris Menu - Export... Snapshot

12.10.4 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.10.5 Tab Color

Color Tab edit, manage and assign material colors of an Object.

Color

RGB

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color slider to select a color or by clicking on the drop down arrow and entering direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

12.10.6 Mouse & Keyboard PC

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + left-click	Add a new measurement point
Shift + left-click	Reposition selected measurement point
Left-click on existing point	Select measurement point

Mouse wheel in Add mode	Turn wheel up: Increase size of next measurement point Turn wheel down: Decrease size of next measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of the shape around the mouse pointer changes accordingly. This helps when handling 3D data sets.

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard PC

12.10.7 Mouse & Keyboard Mac

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + click	Add a new measurement point
Shift + click	Reposition selected measurement point
Click on existing point	Select measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of shape around the mouse pointer changes accordingly. This helps when handling 3D data sets.

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard Mac

12.11 Oblique Slicer

The Oblique Slicer represents a plane on which original data are projected. It can be moved and rotated freely within the data set. The Oblique Slicer consists of a colored frame and yellow pointer.
The Oblique Slicer, and related tools, allows you to re-slice your data sets in any chosen rotation and position. Please see the Slicer Interaction section for a full explanation of slicer interactivity in Imaris.

How to Add a new Oblique Slicer?

- Select menu 3D View - Oblique Slicer.

The Surpass Tree list is automatically updated and in the Oblique Slicer - Properties the Settings parameter are displayed. The Oblique Slicer is displayed in the viewing area (depending on the default parameter on the tab Settings).

12.11.1 Tab Settings

Under the Setting tab you can adjust the position and orientation of the Oblique Slicer, adjust the slice thickness and control the visibility of the Frame.
Please see the Slicer Interaction section for a full explanation of slicer interactivity in Imaris.

Orientation

Button **YZ Plane**
Button **XZ Plane**

Button **XY Plane**
Change the Slicer direction, if required, by selecting the adequate plane.

Position

Button **Recenter**
Click on this button to re-center the Slicer.

Extended Section

The section is a single cut through the data set, the highlighted lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame
Check the box to display a colored frame around the Slicer.

Camera

Button **Orthogonal View**
The camera is set to a position which is perpendicular to the Slicer.

12.11.2 Tab Measure

Shift + Left Mouse Click to add the first measurement point, then Shift + Left Mouse click to add the second measurement point. The distance will be displayed in the measure tab.

To delete the drawing press the **Delete Drawing** button. The delete drawing button is enabled as soon as an initial measurement point is drawn on the image.

12.12 Ortho Slicer

Ortho Slicer represent an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. When adding slices, within the Surpass view per default suggested are the directions z, x, and y as sequence for the first three and all subsequent slices. Please see the Slicer Interaction section for a full explanation of slicer interactivity in Imaris.

How to Add a new Ortho Slicer?

- Select menu 3D View - Ortho Slicer or 2D View - Ortho Slicer.

The Surpass Tree list is automatically updated and in the Ortho Slicer - Properties the Settings parameter are displayed. The first Slice is displayed in the viewing area (depending on the default parameter on the tab Settings).

12.12.1 Tab Settings

Please see the Slicer Interaction section for a full explanation of slicer interactivity in Imaris.

3D View

Slice Orientation

YZ Plane
XZ Plane
XY Plane
The check XY, XZ and YZ plane, to select the visibility of the corresponding slice.

Extended Section

The section is a single cut through the data set, the colored lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame
Check the box to display a colored frame around the active slice.

Slice Position

Slider
Choose the relative position of the Slice in the data set by means of the slider or a direct entry of the slice position given as the offset distance from the data set origin (equals the absolute coordinates).

2D View

Show Frame
Check the box to display a colored frame around the image.

12.12.2 Tab Measure

Shift + Left Mouse Click to add the first measurement point, then Shift + Left Mouse click to add the second measurement point. The distance will be displayed in the measure tab.

To delete the drawing press the **Delete Drawing** button. The delete drawing button is enabled as soon as an initial measurement point is drawn on the image.

12.13 Spots

The Spots object is available to model point-like structures in the data. It provides a procedure to automatically detect point-like structures, an editor to manually correct detection errors, a viewer to visualize the point-like structures as spheres, and statistics output.

How to Add new Spots?

- Select menu 3D View - Spots or 2D View - Spots

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Spots - Properties.

See also:
Surpass View - Overview - Surpass Tree
For information on statistics calculations used refer to Spots

12.13.1 Creation Wizard

The Creation Wizard allows you to move forward or backwards until the optimal parameters are found.

If the image data is for a multi-image scene an option to select which source image to be analyzed is available from the Align to: drop down box.

In the first window of the Spots Creation Wizard, you will find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

In the first window of the Creation Wizard you find the algorithm settings. Here you have to decide, if you want to segment only a **Region of Interest (ROI)**, if you want to use the **Region Growing** for the Spots detection, and if you want to create **Tracks** after the **Spots** detection.

For further details please refer to Section Algorithm, Region of Interest, Source Channel, Classify Spots, Spots Region Type, Spots Regions, Edit Spots, Tracking, and Classify Tracks.

12.13.1.1 Algorithm

Note: If you do not want to use the automatic spots creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates manual spots creation.

Favourite Creation Parameters

In the creation wizard for all objects the algorithm step allows for the creation of favourite creation parameters and are described in the Algorithms common concepts section.

Algorithm Settings

Segment only a Region of Interest
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Uncheck this box and only the region of interest is processed.

Different Spots Sizes (Region Growing)

Check this box if you want to apply the region growing, starting on the tab Spots Region Type.

Track Spots (over Time)
Check this box if you want to track Spots over time, for details please refer to tab Tracking.

Classify spots
This check box can be used to classify spots using conditions set by the user. Read more about this in the step Classify Spots.

Object-Object statistics
This check box can be used to activate or deactivate the shortest distance statistics. For information on **Shortest Distance To Spots** and **Shortest Distance To Surfaces** statistics please see Statistics Surfaces

Creation Preferences
This check box **Start Creation with Slicer view** can be used to activate Slicer view in the creation wizard.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.2 Region of Interest

Please see our common concepts page on the Region of Interest.

12.13.1.3 Source Channel

Source Channel

Select the respective source channel, from which the Spots are to be computed.

Spots Detection

Estimated XY Diameter

This parameter specifies the dimension of the Spots object in the X and Y direction. Objects with size smaller than the specified **Estimated Diameter** are not detected. The values are reported under the statistics tab as separate variables.
By default the radius X and Y are equal.

Detect Ellipsoids

To change the detection and creation of the spherical Spots objects to ellipsoid shape objects select the **Detect Ellipsoids** box.
Check the Detect Ellipsoids box to activate the Estimate Z Diameter field.

If both the **Detect Ellipsoids** and and **Different Spots Sizes (Region Growing)** options are selected, the value entered in the estimated diameter field is used only to determine the position of the spots.
The spot sizes are finally determined by the region growing parameters (Region Growing type and Regions threshold).

Estimate Z Diameter

This parameter specifies the dimension of the Spots object in the Z direction. Objects with size smaller than specified **Estimated Diameter** are not detected. This value is reported under the Statistics tab as a separate variable.
The value of the specified Z diameter in comparison to the XY diameter determines the overall object shape.
If Z < X,Y the created shape is an oblate spheroid
If Z > X,Y then the r the created shape is a prolate spheroid
If Z = X,Y then the diameter the created shape is a perfect sphere

Background Object Subtraction
If checked, the background will be removed prior to Spots detection. Technically background subtraction smoothes the image. The intensity is the one of a Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.4 Classify Spots

Spots filtering is incorporated into the creation process.

Filters allow the removal of objects that would interfere with interpretation of results.

Quality: The 'Quality' is the intensity at the center of the spot in the channel the Spots was detected.

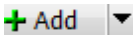
If 'Background Subtraction' was not checked this is the intensity of the channel Gaussian filtered by 3/4 of spot radius.
If 'Background Subtraction' was active the intensity is the one of a Gaussian filtered channel from above minus the intensity of the original channel Gaussian filtered by 8/9 of spot radius.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Note: The "Quality" statistics value of spots during the spots creation wizard is the intensity value of the detected spot's center on the working image which is created by applying Gaussian smooth or Background subtraction on the original image (based on the creation parameters) for detecting the spots. The Quality statistics value is only present during the creation wizard steps, but not in the statistics or filter tab.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.5 Edit Spots

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Click and drag to enter navigate mode or press 3.

AutoDepth Draw

Intersect with
The auto depth function will automatically calculate the Spots depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spots.

all visible Channel
specific Channel - selected channel will be used for Spots depth computation
solid Objects
Select as reference for the Spots depth the first visible channel, all visible channels, or other solid objects.

Process Selection

	PC:	Mac:
Select spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new spot	Shift + left-click	Shift + click
Delete spot	Left-click to select, then click on the button Delete or Shift + left-click on a selected spot	Click to select, then click on the button Delete or Shift + click on a selected spot
Change spot diameter	Mouse wheel	Mouse wheel

Button **Duplicate**
Click this button and all selected Spots appear in a new Surpass Group in the Surpass Tree (Spots Selection).

Button **Recenter**
Click on the Recenter button results in change of the selected Spots position. The new center is placed in position of the local intensity maximum in the vicinity of the initial spot center.

Button **Delete**
Click on this button to delete the selected Spot(s).

Button **Clear**
Click this button to clear the Spots selection.

No Spots Selected/Selected Spots

Position X: Y: Z: [um]
Display of the Spots position.

Diameter [um]
The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index
The number indicates the current time point in the series for the selected object.

Spots

In Imaris it its possible to merge two or several Spots items into one Spots item.

Button **Merge ...**
This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

Window: Merge selected Objects to "Spots X"

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **Clear All**
Use this button to clear the Spots selection.

Button **OK**
If you want to merge the Spots click on **OK**.

Button **Cancel**
If you do not want to merge the Spots click on **Cancel**.

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Spots.

Mask All... Button
Mask Selection ... Button
Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask
When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map
Constant Inside/Outside
Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:
The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:
If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points
Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.6 Tracking

Tracking allows objects to be traced throughout sequential frames of a time data sets, so each object maintains a unique identity in the output measurements.
This tab only appears, if **Track Spots (over Time)** is checked (Algorithm Settings).

Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions, the best matches to candidate Spots can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

Brownian Motion
This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernible pattern).
The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each Spot as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components
For this algorithm, Spots in adjacent time-points are considered connected if the Spot spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.
Connected Components and Lineage are the only algorithms that automatically handle lineage (spots that diverge). It will only work with images that have excellent time resolution, because the Spot cannot ever jump a distance larger than its own size.

Lineage
The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

Parameters

Max Distance
A maximum distance disallows the connection between a spot and a candidate match if the distance between the predicted future position of the spot and the candidate position exceeds the defined maximum distance.
The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order for a track to continue.

This parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Intensity Weight
Based on the information obtained from the measured Spots intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is

determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

TotalCost = Σ(all connections) DistanceCost + IntensityWeight * IntensityCost

The parameter is only available if you select Autoregressive Motion Expert (see above).

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

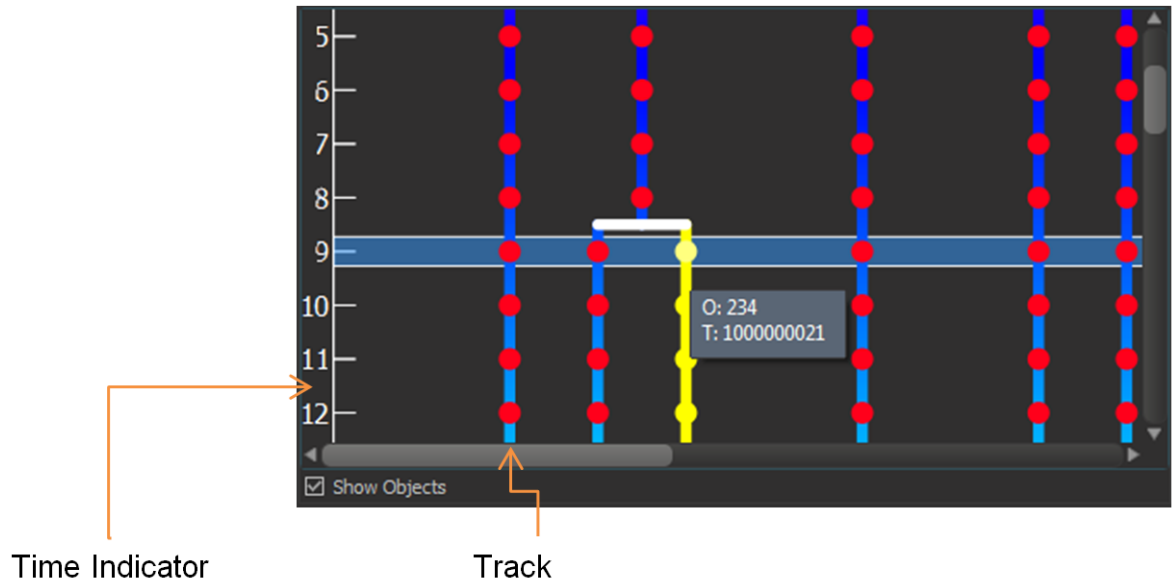
Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Tracks automatically click on **Cancel**.

Lineage

Table Layout

The track editor table:



Time indicator
The axis on the left displays the frame number.

Track
Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects diverge, e.g. cells undergoing division.

Object
Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.
If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

Interactive Selection

When you click on an individual column, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (column in the table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table. Using the mouse wheel will have the same effect.

Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

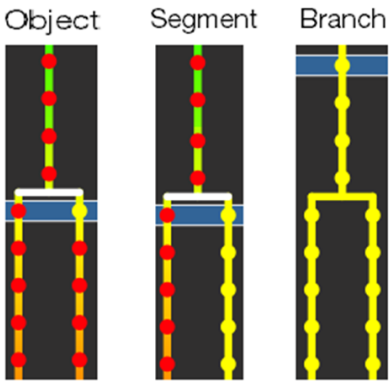
Mouse Selects

This option allows you to select objects or tracks as follows:

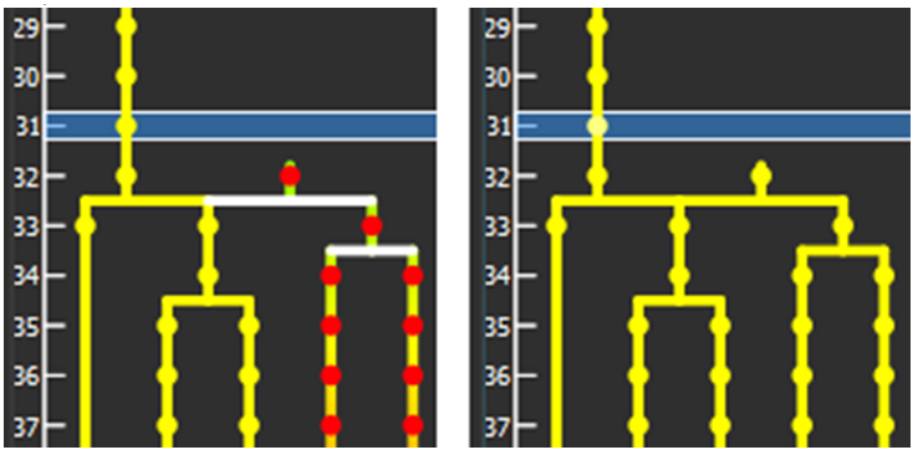
Object
Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.

Segment
Click Segment to select a specific segment (between branch points).

Branch
Select Branch to select the segment and the branched segments and objects that appear later in time only.



Pivot
Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.
For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



Track
Select track to select a complete track.

Editing

Track Editor enables you to modify your tracks.

Connect Button
Click this button to connect two existing objects. Select two objects in two different tracks at the subsequent time points (just one object per time point) and click on the button Connect. The connection is automatically created between all selected objects.

Disconnect Button
Select an object and click on the button Disconnect. The connection between the two selected objects in the time sequence is broken.

< Button
Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

> Button
Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves

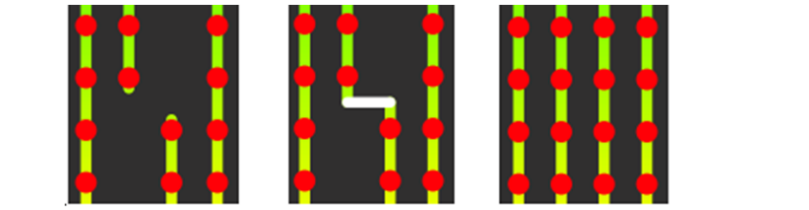
down.

<< Button
Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

>> Button
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

Flip
The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

Reorganize
The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.


Editing tracks. Connection added. Tracks reorganized.

Invert Axis
Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

12.13.1.7 Classify Tracks

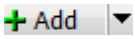
Track filtering is incorporated into the creation process. This tab only appears, if **Track Spots (over Time)** is checked (Algorithm Settings).

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.8 Classification

Please see the section on common concepts, Creation Wizards - Classification for more information.

12.13.1.9 Event Setup

Please see the section on common concepts, Creation Wizards - Event Setup for more information.

12.13.1.10 Spot Region Type

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked (Algorithm Settings).

The region method for segmentation in Imaris starts with defined seed points. You use Spots as seed points for

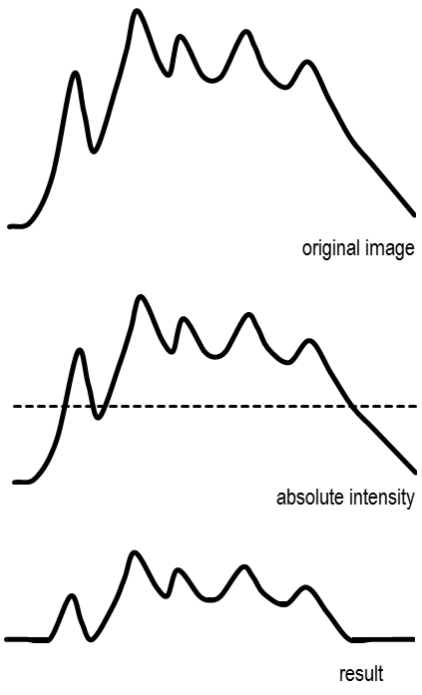
the region growing that is performed in the next steps. During the region growing process around each seed point the region grows until a border stops the growing.

Spots Regions from

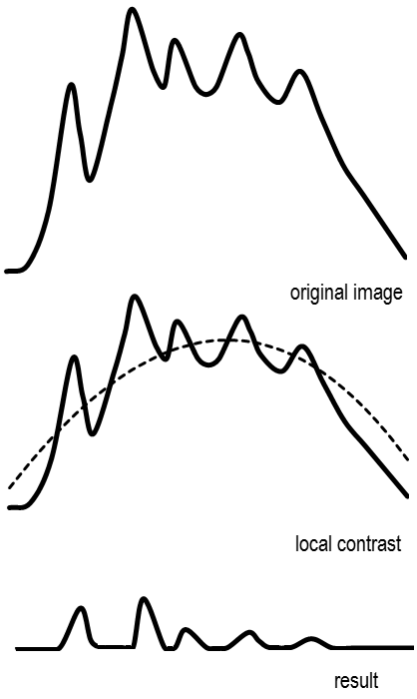
Absolute Intensity
Local Contrast
You can set the border using the Absolute Intensity or the Local Contrast as criteria. In both cases as next step the selected channel is displayed in the viewing area.

Local Contrast
This option should only be used when the **Absolute Intensity** method does not properly segment all objects.
Please note: Local Contrast applies a Gaussian filter in order to estimate the intensity value of each voxel. Baseline Subtraction is then performed by subtracting the variable background from every voxel in the image. As a result, it uses more memory than the Absolute Intensity method.

Gaussian Smoothing replaces the voxel intensity value with a newly calculated value. The new value is a weighted sum, defined using a Gaussian function, of the voxel and it neighbouring voxels.
The degree of the smoothing is directly dependent on the shape of this kernel, which in turn is a function of the standard deviation. A Gaussian kernel is a symmetric bell shaped curve.



Absolute Intensity



Local Contrast

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.11 Regions Threshold

This tab only appears, if **Different Spots Sizes (Region Growing)** is checked (Algorithm Settings).

Spots Regions

The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local Contrast.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Diameter from

Region Volume
The Spot(s) radius is calculated from the region volume (the region volume and the Spots volume are equal).

Region Border
The radius of the calculated Spot(s) is the smallest distance from each Spot center to the region border.

Create Color Coded Region Channel
Additionally the Spots the Region Channel is displayed. Each region has a different color.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.1.12 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm Settings).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.13.2 Tab Settings

Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris. The render on slicer options are available on every tab of the surpass property area, and are explained in the Surpass Property Area section.

On the tab Settings you can adjust the rendering quality to produce faster performance of the display when the number of Spots is very large. The size of the Spots can be changed via the Radius Scale box.

Spots Style/Quality

Sphere
This option displays the Spots as spheres.

Center Point
This option displays the Spots as center points.

Off
Select this option to disable the visualization of the Spots.

Please note: The computation of Spheres is time consuming. If you have many Spots in your data set Imaris automatically switches to the display mode Center Points. If you would like to switch to the Spheres mode again select Sphere and confirm the changes.

Tracks Display

Full Tracks
Show full tracks.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Off
Select this option to disable visualization of the Tracks.

Tracks Style

The box Style/Quality has settings that determine the display of connections between the Track objects.

Off
Select this option to disable the visualization of the Tracks.

Line
This option displays the tracks connections dawn as lines.

Cylinder
This option displays the tracks connections dawn as cylinders.

Width/Diameter

Depending on the previous selection adjust the width of the line or diameter of the cylinder.

Track Displacement Display
Check the box Track Displacement Display to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Color Editor
Select a desired color for the displacement arrow.

12.13.3 Tab Rebuild

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the Spots Creation Wizard you want to re-enter the creation process.

For details please refer to Section:
Menu 3D View - Spots - Creation Wizard - Algorithm
Menu 3D View - Spots - Creation Wizard - Region Growing
Menu 3D View - Spots - Creation Wizard - Tracking

Button **Rebuild Objects**
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Spots computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters
- **Arena**
- **Favorite Creation Parameters**

Arena
When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, Creation Parameters, is created and displayed.
Once stored, Imaris Batch applies the creation parameters set to all images within the selected Group.

Favorite Creation Parameters
To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.
When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/Creation Parameters. This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should

you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

Store Creation Parameter

Refer to Store Creation Parameters

12.13.4 Tab Edit

Once the Spots creation wizard is completed, use these buttons for manual editing of the Spot objects. It allows you:

1. To add Spots and incorporate them into analysis.
2. To delete selected Spots.
3. Create tracks from Selection
4. Merge selected spots.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Add/Delete (Cursor intersects with):

All visible Channels
The All-visible channel option places a spot at the cursor position while the depth through the volume is determined by the most intense voxel of all the channels

Specific Channel
Select the required channel from the drop-down list and selected channel will be used for Spots computation. A new spot will be placed at the most intense voxel of the selected channel, even if that channel is overlaid with other channels of higher intensity.

Surface of Object
Select this option and a new spot will be placed on the surface of an object.

Center of Object
Select this option and a spot will be placed in the center of the object.

For Spots object (also Vesicles and segment or spine beginning, branching, attaching, terminal points): Select

this option and a new measurement point is placed at the center of the sphere.

Selection

Select between a pointer, circle or box shaped selection.

The pointer mode provides the normal pointer function making it easy to select specific individual spots,

In circle mode a 2D circle appears around the mouse pointer on the screen. Press left-click to select all the spots that have center points within the circle. Press Ctrl+left-click will add spots to the existing selection, if some unselected spots are within the circle, otherwise it will deselect all spots within the circle. Use the mouse wheel to adjust the size of the on-screen selection circle.

In box mode a 3D box appears on the screen around the mouse pointer. Use left-click or Ctrl+left-click the spots that have center points within the 3D box to add or remove them from the selection.

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click
Delete Spot	Left-click to select, then click on the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

Clear Button
Click this button to clear the Spot selection.

Delete Button
Click on this button to delete the selected Spot(s).

Duplicate Button
Click this button and all selected Spots appear in a new Surpass Group in the Surpass Tree (Spots Selection).

Recenter Button
Clicking on the Recenter button changes the position of the selected Spots. The new center is placed in the position of the local intensity maximum, which is in the vicinity of the initial spot center.

Mask button
Click this button to define the mask functions for the current selection- see Mask Channel Window section for more details.

Create Track from Selection Button
Clicking this button creates a track for the selected Spot(s) - see the Create Track from Selection section for more details

No Spot Selected/Selected Spot

Position X: Y: Z: [um]
Display of the Spot position.

Diameter [um]
The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index
The number indicates the current time point in the series for the selected object.

Edit All

Merge selected Objects to "Spots X" Button

Click this button to combine multiple spot objects into a single spot item. See the **Merge selected Objects to "Spots X"** section for more details.

Mask... button
Click this button to define the mask functions for the image- see Mask Channel Window section for more details.

Manual Tracking

The Manual Tracking option can either be used when automatic tracking algorithms require modifications and adjustments, or to create an entire track from scratch.

The manual tracking option allows you to track objects within a time-resolved data set by manually specifying the object location in each time point. Essentially, the manual tracking is done by clicking on the structure in the image, which serves as a visual reference (template).

This option can be activated by clicking in the box **Auto-connect to selected spot**.

To start manual tracking, move the time slider to the first time point of the feature you want to track. Then use the pointer to Shift and then left click on the feature in the data set.

After each mouse click, the time slider advances one frame, allowing you to see the subsequent image of the temporal stack. To continue manual tracking, keep clicking on the structure on the image. As you add more manual tracking points by clicking within the viewing area, a track is created allowing you to follow the moving features, frame by frame and from point to point, until you've built a complete motion path.

Please note: Manual tracking behaviour is governed by the Selection mode under Track Edit tab.

Auto-connect to the selected spot box

Select the option to add all selected or added objects to one track and advance to next time point when a new object is added or an existing is selected.

Please note: Objects are always added at the current time point (t).

The result of Manual tracking can be very different depending on which selection is chosen in the Track Edit tab.

If the option **Track** is selected, a connection is established between added object at time point t and the last added object of the selected track at time point t-1.
If the option **Spots** is selected, a connection is established between the selected object at the immediately preceding time point (t-1) and the newly added object at time point t.

Please note: If there is no the immediately preceding time point, a new track will be constructed.

The box on the drawing plane represents the created object diameter. The diameter of the object can be changed, during the manual tracking, by using the mouse wheel. Use the mouse wheel to increase/decrease the radius of the created object.

Enable Delay before auto-advancing

The Enable Delay before auto advancing option sets a delay before proceeding to the next time point. When this option is selected, the created object will be displayed for the duration of the delay in the viewing area. The visual feedback of the created object gives you an opportunity to adjust it.
The delay duration is adjustable under the Edit menu/Preference/Surpass. You can specify the value between 0.05 and 5 seconds.

Object-Object Statistics

This check box can be used to activate or deactivate the shortest distance statistics. For information on **Shortest Distance To Spots** and **Shortest Distance To Surfaces** statistics please see Statistics Spots

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

12.13.4.1 Merge selected Objects to "Spots X" Function

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **OK**
If you want to merge the selected spot objects click on **OK**.

Button **Cancel**
If you do not want to merge the selected spot objects click on **Cancel**.

12.13.4.2 Create Track from Selection

1. Select the Source channel

2. Spot Detection
Estimated Diameter
This is the diameter of the inserted Spots. Spots with a diameter smaller than this are not detected.
There are two options for a Surface creation Threshold:
Background Subtraction
If checked, the background will be removed prior to spot detection.

3.Tracking algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions the best matches to candidate Spots can be determined.

There are two algorithms to choose from:

Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction . The user-specified **Maximum Distance** value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Lineage

4. Max Distance

The maximum search distance that a tracked object are looked for is specified with the Max Distance value. The parameter Maximum Distance disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.

5. Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

6. End Time Point

The parameter specifies the last time point for the tracking algorithm implementation.

OK Button

Cancel Button

12.13.5 Tab Edit Tracks

This tab is common concept for all objects, please read more here Tab Edit Tracks.

12.13.6 Tab Edit labels

This is a common concept for all Objects. Please find more information at the following link Tab Edit Labels.

12.13.7 Tab Edit Events

Please see the section on common concepts, Surpass Objects - Tab Edit Events for more information.

12.13.8 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values

is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign  indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Button **Duplicate Selection to New Spots**
Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

12.13.9 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.13.10 Tab Color

The Color tab is a common concept for all objects in Imaris, see 3D View - Common Concepts - Surpass Objects - Tab Color.

12.13.11 Mouse & Keyboard PC

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Left-click
Select multiple Spots	Ctrl + left-click
Add new Spot	Shift + left-click
Delete Spot	Left-click to select, then click on the button Delete
Change Spot diameter	Mouse wheel

See also:
Addendum - Mouse & Keyboard PC

12.13.12 Mouse & Keyboard Mac

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Click
Select multiple Spots	Command + click
Add new Spot	Shift + click
Delete Spot	Click to select, then click on the button Delete
Change Spot diameter	Mouse wheel

See also:
Addendum - Mouse & Keyboard Mac

12.14 Surfaces

The Surfaces visualization is a computer-generated representation of a specified gray value range in the data set. It creates an artificial solid object in order to visualize the range of interest of a volume object.

How to Add a new item Surfaces?

- Select menu 3D View - Surfaces or 2D View - Surfaces

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Surfaces - Properties.

For information on statistics calculations used refer to Surfaces

12.14.1 Creation Wizard

The Creation Wizard allows you to move forward or backwards until the optimal parameters are found.

If the image data is for a multi-image scene an option to select which source image to be analyzed is available from the Align to: drop down box.

In the first window of the Surface Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

In the first window of the Creation Wizard you find the algorithm settings. Here you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection, use the **Seeded Region Growing** for the Surfaces detection or to **Skip automatic creation** and start Contour Surface drawing .

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual Surface creation.

For further details please refer to Section Algorithm, Region of Interest, Source Channel, Threshold, Classify Seed Points, Classify Surfaces, Tracking, Edit Surfaces, Classify Tracks and Complete ROI.

12.14.1.1 Algorithm

Note: If you do not want to use the automatic surfaces creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates manual Surface creation.

Favourite Creation Parameters

In the creation wizard for all objects the algorithm step allows for the creation of favourite creation parameters

and are described in the Algorithms common concepts section.

Algorithm Settings

Segment only a Region of Interest
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Uncheck this box and only the region of interest is processed.

Track Surfaces (over Time)
Check this box if you want to track the resulting Surfaces over time, for details please refer to tab Tracking.

Object-Object statistics
This check box can be used to activate or deactivate the shortest distance statistics. For information on **Shortest Distance To Spots** and **Shortest Distance To Surfaces** statistics please see Statistics Surfaces

Creation Preferences
This check box **Start Creation with Slicer view** can be used to activate Slicer view in the creation wizard.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.2 Region of Interest

Please see our common concepts page on the Region of Interest.

12.14.1.3 Source Channel

Source Channel

Select the respective source channel, from which the Surfaces are to be computed.

Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the **Surface Area Detailed Level** value should be determined. Use either the automatically given value or type in a new value in the data field.

Method

Absolute Intensity Thresholding

Background Subtraction (Local Contrast)
Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.
If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

Diameter of the largest Sphere which fits into the Object:
The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the data set.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Machine Learning Segmentation
Use Imaris' integrated machine learning segmentation for surface creation.
All channels checkbox - check for all channels

Following option is only available if selected in Preferences - Custom Tools tab
Labkit for Pixel Classification
Use the open source machine learning pixel classification software Labkit for surface creation. Use Labkit to train a pixel classifier to your liking and when you are done press “SendResultToImaris” in the bottom left hand corner of Labkit to send the result back to Imaris and proceed in the surface creation wizard. Note that Fiji and Labkit must be installed for this option to be available, see **Imaris Labkit Installation** in Labkit Installation for instructions.
All channels checkbox - check for all channels

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.4 Machine Learning Training

In this step of the surfaces object creation wizard train the machine learning segmentation to segment the surfaces in the image; simply paint some foreground and background pixels and use the machine learning system to determine the classification of the data set. Repeat this process until you are happy with the surfaces classification shown in the viewing panel.

In Imaris the machine learning segmentation uses a Gradient Boosted Trees classifier [Friedman 2001] algorithm to segment the surfaces. The classifier receives derivative of Gaussian features up to second order computed at a wide range of scales [Koenderink 1987], specifically 1, 2, 4, 8, 16, and 32 times the sigma specified in the user interface.

Need to provide information on Imaris Machine Learning Segmentation for the Methods section of your publication? Please see Machine Learning Publication Information

Machine Learning Training Usage Tips

The machine learning segmentation attempts to infer rules that fit your painting. The way you paint influences how it learns. Here are a few suggestions:

- **Paint small regions**

If you paint large regions the machine learning segmentation needs to work through large amounts of data to infer its rules and that takes longer. It is often sufficient to paint a few small regions.

- **Don't confuse the machine learning segmentation**

If you make mistakes painting foreground where you want background or painting background where you want foreground, you confuse the machine learning system. This makes learning harder.

- **Clarify borders (without confusion)**

If you stay away from borders the machine learning segmentation cannot learn where exactly you want the boundary. Suppose the machine learning segmentation should learn a simple threshold. If you label some very bright pixels as foreground and some very dark pixels as background and don't label any pixels in between, then you are not telling the machine learning segmentation what to do for intensities in between.

- **Train several rounds**

It is useful to start simple (to avoid confusion), see what the machine learning segmentation picks up, and then refine.

- **Be prepared to stop**

The machine learning segmentation cannot solve every problem. If you find that adding more training data doesn't improve the prediction, then perhaps you are trying to teach the machine learning segmentation something that it cannot learn.

Multiple Images and Batch processing

Once appropriate parameters for surface creation using machine learning segmentation have been created, the same classifier can be applied to multiple images using batch processing.

Alternatively the machine learning segmentation can be trained using several images. When you store Surfaces creation parameters the classifier and the training data are stored within the creation parameters. These stored creation parameters can be opened on a new image to continue training on this new image.

Training Data

Select between background and foreground options and then use Shift + Mouse Left Click to begin drawing with brush strokes on the data set. Adjust the size of the brushstroke using Control + Middle Scroll wheel.



Background and foreground selection options, with foreground currently selected.

Click on  to change the colour option, using the slider. Click on  to change the display of the background/foreground in the viewing panel. When just an outline is selected  just the outline of the foreground/background will be shown in the viewing panel. When the icon displays a semi-transparent fill  the foreground/background will appear with this fill in the viewing panel.

Delete Last - delete the last brush stroke from the viewing panel. This can continue to be pressed until all brush strokes are removed.

Delete All - delete all brush strokes from the viewing panel.

Export Labels - Available from the dropdown, allows the user to export training voxels as a TIF Image (.tiff) file.

Show/Hide - Check or uncheck to display the training voxels used in the machine learning segmentation.

Classifier

Train and Predict - Press this button to train and predict surfaces. Once training is complete foreground and background pixels can be added as required using the brushstrokes as described above and the surfaces retrained using the **Train and Predict** button. This process can be repeated to refine surfaces as required.

Show/Hide - Check or uncheck to display the surfaces determined from the machine learning segmentation in the viewing panel.

Training progress: displays the percentage of training progress
Preview display progress: shows the percentage of the predicted data required for full preview

Settings
Show/Hide Training Voxels Checkbox - Check to see the voxels used during training the data set
Interpolate Data Checkbox - Check to turn on data interpolation to smooth the display of the trained data
Slicer Extended Section - adjust the thickness of the slicer

References
[Friedman 2001] Friedman, Jerome H. "Greedy function approximation: a gradient boosting machine." Annals of statistics (2001): 1189-1232.
[Koenderink 1987] Koenderink, Jan J., and Andrea J. van Doorn. "Representation of local geometry in the visual system." Biological cybernetics 55.6 (1987): 367-375.

12.14.1.4.1 Machine Learning Publication Information

Please use the following text to explain Machine Learning Segmentation in the methods section of your publications.

Machine Learning Segmentation in Imaris is a pixel classification algorithm for automatic segmentation. The algorithm uses a Gradient Boosted Tree classifier [Friedman 2001] to assign each pixel/voxels into one of two classes, foreground and background. The classifier receives as input Derivative of Gaussian features up to second order computed at a wide range of scales [Koenderink 1987], specifically 1,2,4,8,16, and 32 times the sigma specified in the user interface.

A Gradient Boosted Tree is a supervised machine learning method, and as such must be trained. In Imaris, training pixels are selected manually by the user with a “brush” tool that allows the user to paint brush strokes on the image. The training voxels used for machine learning are those voxels within a brush stroke that the user sees while making the brush stroke.

The set of training voxels can be exported as an Ome Tiff image and together with the original image this can be used to reproduce the segmentation in other tools such as LABKIT [Labkit 2022]. Note that this also requires setting Labkit to use all Gaussian Derivative features up to second order at the same scales used by Imaris, i.e. 1,2,4,8,16, and 32 times the sigma specified in the user interface.

The machine learning segmentation in Imaris is part of the Imaris Surface object which provides an entire object segmentation workflow including splitting by seed points, object filtering based on statistics, classification and tracking in the case of time series. The full Surface creation method can be easily repeated inside the Imaris software by storing and re-using the Surface Creation Parameters. Creation Parameters include all parameters necessary to reproduce the results, including the user-defined training data.

[Friedman 2001] Friedman, Jerome H. "Greedy function approximation: a gradient boosting machine." Annals of statistics (2001): 1189-1232.

[Koenderink 1987] Koenderink, Jan J., and Andrea J. van Doorn. "Representation of local geometry in the visual system." Biological cybernetics 55.6 (1987): 367-375.

[Labkit 2022] Arzt M, Deschamps J, Schmied C, Pietzsch T, Schmidt D, Tomancak P, Haase R and Jug F (2022) LABKIT: Labeling and Segmentation Toolkit for Big Image Data. Front. Comput. Sci. 4:777728. doi: 10.3389/fcomp.2022.777728

12.14.1.5 Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Thresholds for more information.

Split touching Objects (Region Growing)

This option supports the separation of two or more objects that are identified as one. The region method for segmentation in Imaris starts with defined seed points. During the region growing process around each seed point the region grows until a border stops the growing.
If you want to apply the seeded region growing for the Surfaces creation check the respective option. The threshold you set above is the border of the region growing.

Seed Points Diameter
Type in the estimated diameter of a seed point.

Select from:
Intensity based, seed points are placed at intensity peaks of the detected channel.
Morphological Split is shape based. A distance transform inside the surface is performed, and the seed points are detected on this distance transformed channel.

Preview Region Info

For large data sets that exceed a certain memory limit, a Surface preview is only created for a centred sub-region for which the volume is highlighted. The size of the sub-region matches a memory limit defined in the advanced preferences, see Preferences -> Advanced, the parameter bpSurfacesModelViewer->3DPreviewMemoryLimitMB for 3D volumes and bpSurfacesModelViewer->4DPreviewMemoryLimitMB for time resolved data. For placing the Surface preview region manually, a ROI needs to be defined.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.6 Filter Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if **Split touching Objects (Region Growing)** is checked (Algorithm Settings).

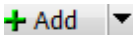
Filtering allows the removal of objects that would otherwise interfere with the interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up

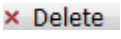
criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note that if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.7 Filter Surfaces

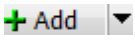
The Surfaces filtering is incorporated into the creation process.

Filters allow the removal of objects that would interfere with interpretation of results.

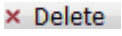
The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting the required variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values

is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign  indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.8 Edit Surfaces

Here you find the manual editing tools for the Surfaces.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Edit Mode

Add/Delete
This activates a semi-automatic surface segmentation (see below). Single objects can be added by one mouse click. If you select **Add/Delete** as edit mode, the Add/Delete Properties are available (see below).

Cut
This activates the Surface Scissors function (see below). You can manually split two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available (see below).

AutoDepth Draw

The auto depth function will automatically calculate the Surface depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:
all visible Channels
specific Channel
solid Objects
Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Add/Delete Properties

The Add/Delete Properties are only available, if you select **Add/Delete** as edit mode (see above).

There are two different techniques available:

Marching Cubes

Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

- Position the autodepth cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the autodepth cursor to the border of the object (the size of the box does not influence the result).
- Shift + click to create the new object.

Process Selection

Button **Clear**

Click this button to clear the Surfaces selection.

Button **Duplicate**

Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

Duplicate to all Timepoints

If you check this box the selection is not only active for the current time point but for all time points in which the object appears.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.

- Change to the pointer mode Select.
- Select as Edit Mode the mode Cut (see above).

- Shift + click on the Surface object.
- A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular).
- Click on the button **Cut Surface** (see below) and the object splits into two individual Surface objects with two different colors.

Button **Cut**
Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear**
Click on this button to clear the selection.

Close Borders
Check this box to add a surface along the cutting line and close the surface.

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**
Button **Mask Selection ...**
Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Surfaces

In Imaris it its possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**
This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask
When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map
Constant Inside/Outside
Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:
The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:
If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points
Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**
If you want to apply the mask click on **OK**.

Button **Cancel**
If you do not want to apply the mask click on **Cancel**.

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button **Select All**.

Button **Clear All**
Use this button to clear the Surfaces selection.

Button **OK**
If you want to merge the Surfaces click on **OK**.

Button **Cancel**
If you do not want to merge the Surfaces click on **Cancel**.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.9 Classification

Please see the section on common concepts, Creation Wizards - Classification for more information.

12.14.1.10 Event Setup

Please see the section on common concepts, Creation Wizards - Event Setup for more information.

12.14.1.11 Tracking

Tracking allows objects to be traced throughout sequential frames of a time data sets, so each object maintains a unique identity in the output measurements.
This tab only appears, if **Track Surfaces (over Time)** is checked (Algorithm Settings).

Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Surfaces. Based on these predicted positions the best matches to candidate Surfaces

can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

Brownian Motion
This algorithm models the motion of each surface as a Brownian motion. This model is appropriate if your Surface performs similar to a Brownian motion (the random movement of Surface with very frequent changes in direction and no discernible pattern).
The only prediction applied is that the Surface does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each Surface as an autoregressive AR1 process. This model is appropriate if your Surface perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Surface will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the Surface is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each Surface as an autoregressive process. This model is appropriate if your Surfaces perform any kind of continuous motion. In the expert mode the tracks are predicted by the additionally established algorithm called Intensity Weight (see below).

Connected Components
For this algorithm, Surface objects in adjacent time-points are considered connected if the Surface spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID. Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that diverge). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

Lineage
The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

Parameters

Max Distance
The maximum search distance that a tracked object are looked for is specified with the Max Distance value. This parameter disallows connections between a Surface and a candidate match if the distance between the predicted future position of the Surface and the candidate position exceeds the maximum distance.
The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected, the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Intensity Weight
Tracks are computed by minimization of a cost function that combines distance based and intensity based costs. "Intensity Weight" is the factor by which IntensityCost is taken into account:
TotalCost = sum_(all connections) DistanceCost + IntensityWeith * IntensityCost
The parameter is only available if you select Autoregressive Motion Expert (see above).

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

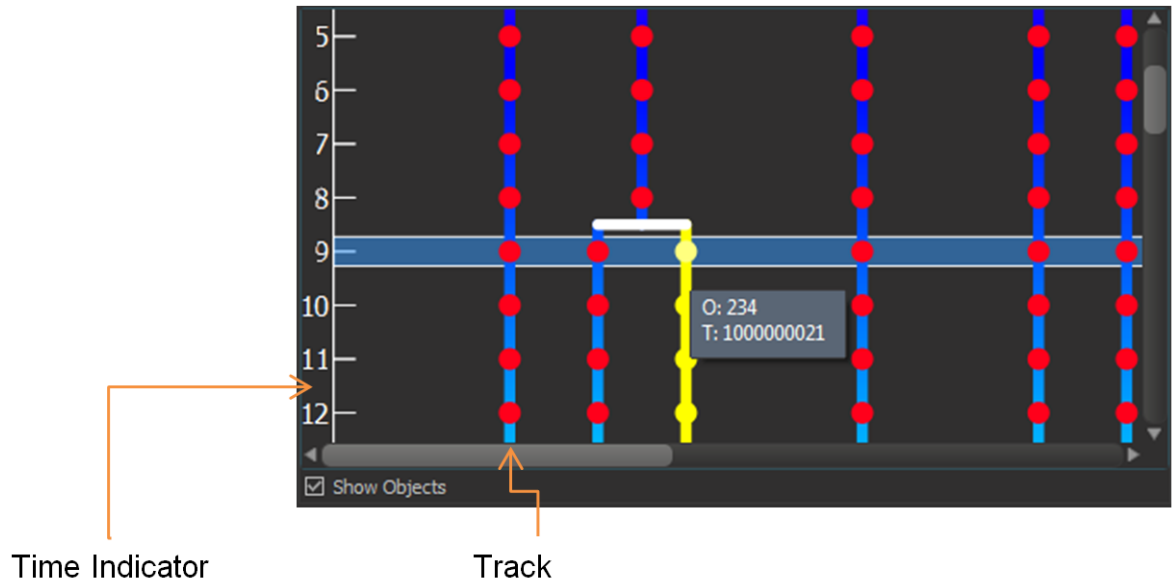
Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

Lineage

Table Layout

The track editor table:



Time indicator
The axis on the left displays the frame number.

Track
Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects diverge, e.g. cells undergoing division.

Object
Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.
If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

Interactive Selection

When you click on an individual column, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (column in the table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table. Using the mouse wheel will have the same effect.

Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

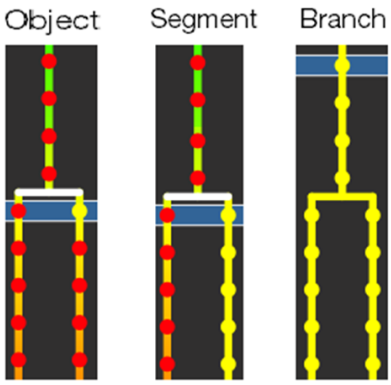
Mouse Selects

This option allows you to select objects or tracks as follows:

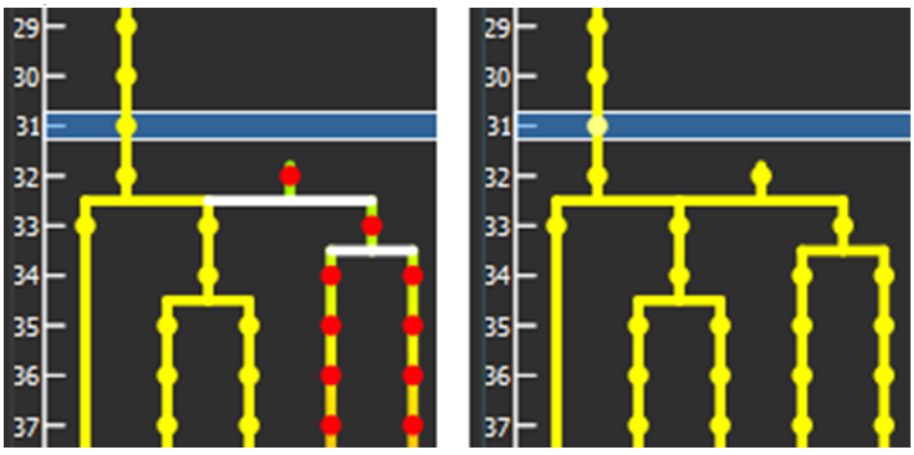
Object
Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.

Segment
Click Segment to select a specific segment (between branch points).

Branch
Select Branch to select the segment and the branched segments and objects that appear later in time only.



Pivot
Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.
For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



Track
Select track to select a complete track.

Editing

Track Editor enables you to modify your tracks.

Connect Button

Click this button to connect two existing objects. Select two objects in two different tracks at the subsequent time points (just one object per time point) and click on the button Connect. The connection is automatically created between all selected objects.

Disconnect Button

Select the two adjacent objects you want to disconnect (using Ctrl) and then click on the button Disconnect. The connection between the two selected objects in the time sequence is broken.

< Button

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

> Button

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

<< Button

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

>> Button

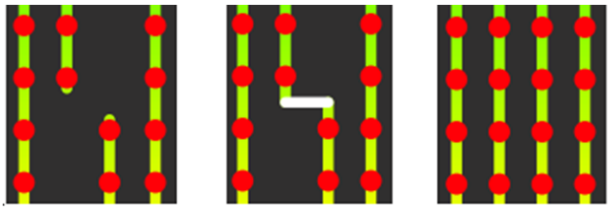
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

Flip

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

Reorganize

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

Invert Axis

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

12.14.1.12 Classify Tracks

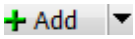
Track filtering is incorporated into the creation process. This tab only appears, if **Track Surfaces (over Time)** is checked (Algorithm Settings).

Filters allow the removal of objects that would otherwise interfere with the interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting the required variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add 

and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button 

to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Lower and Upper Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common

Concepts - Creation Wizard - Thresholds for more information.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.1.13 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm Settings).

Region of Interest

The parameters you have set up will now be used to compute Surfaces (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Creation Wizard Buttons

More information on the Creation Wizard buttons can be found at the following link.

12.14.2 Tab Settings

Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris. The render on slicer options are available on every tab of the surpass property area, and are explained in the Surpass Property Area section.

Surfaces Style/Quality

Surface
Select this option and the created Surfaces is displayed in the viewing area.

Center Point
This option displays the Surfaces as center points.

Off
Select this option to disable the visualization of the Surfaces.

Tracks Display

Select how to visualize the tracks:

Full Tracks
Select this option to show the complete track.

Dragon Tail
Select this option to view a dragons tail for the object in motion. The parts of the track ahead and following the object can be adjusted (in seconds) using the Next and Last fields respectively.

Off
Select this option to disable the visualization of the tracks.

Tracks Style

Set the display of connections between the Track objects.

Line
This option displays the tracks connections drawn as lines.

Cylinder
This option displays the tracks connections drawn as lines.

Diameter
Depending on the previous selection you can adjust the width of the line (in pixels) or diameter of the cylinder (in um) using the slider.

Tracks Displacement Display
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Arrow Scale
Adjust the thickness of the displacement arrows by changing the value in the Arrow Scale field.

Color Editor
Select a desired color for the displacement arrow.

12.14.3 Tab Rebuild

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the Surfaces Creation Wizard you want to re-enter the creation process.

For details please refer to Section:
Menu 3D View - Surfaces - Algorithm
Menu 3D View - Surfaces - Tracking

Rebuild Objects Button
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Surfaces computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters
Arena
Favorite Creation Parameters

Arena
When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, Creation Parameters, is created and displayed.
Once stored, Imaris Batch applies the creation parameters set to all images within the selected Group.

Favorite Creation Parameters
To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.
When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/Creation Parameters. This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

Store Creation Parameter

Refer to Store Creation Parameters

12.14.4 Tab Draw

The Draw tab allows the user to semi-automatically create further Surface objects.
The Draw tab has two sub tabs:
Add/Delete
Contour

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

12.14.4.1 Add/Delete

Add/Delete
This activates a semi-automatic surface segmentation.
Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:
all visible Channels
specific Channel
solid Objects

Select as reference for the Surface depth the first visible channel, all visible channels, or other solid objects.

Add Surface using:

There are two different techniques available for adding the Surface Objects:

Marching Cubes
Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

- Position the cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

Magic Wand
Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the cursor to the border of the object (the size of the box does not influence the result).
- Shift + click to create the new object.

Manual Tracking

The Manual Tracking option can either be used when automatic tracking algorithms require modifications and adjustments, or to create an entire track from scratch.

The manual tracking option allows you to track objects within a time-resolved data set by manually specifying the object location in each time point. Essentially, the manual tracking is done by clicking on the structure in the image, which serves as a visual reference (template).

This option can be activated by clicking in the box **Auto-connect to selected Surface**.

To start manual tracking, move the time slider to the first time point of the feature you want to track. Then use the pointer to Shift and then left click on the feature in the data set.

After each mouse click, the time slider advances one frame, allowing you to see the subsequent image of the temporal stack. To continue manual tracking, keep clicking on the structure on the image. As you add more manual tracking points by clicking within the viewing area, a track is created allowing you to follow the moving features, frame by frame and from point to point, until you've built a complete motion path.

Please note: Manual tracking behaviour is governed by the Selection mode under Track Edit tab.

Auto-connect to the selected Surface box

Select the option to add all selected or added objects to one track and advance to next time point when a new object is added or an existing is selected.

Please note: Objects are always added at the current time point (t).

The result of Manual tracking can be very different depending on which selection is chosen in the Track Edit

tab.

If the option **Track** is selected, a connection is established between added object at time point t and the last added object of the selected track at time point t-1.
If the option **Surface** is selected, a connection is established between the selected object at the immediately preceding time point (t-1) and the newly added object at time point t.

Please note: If there is no the immediately preceding time point, a new track will be constructed.

The box on the drawing plane represents the created object diameter. The diameter of the object can be changed, during the manual tracking, by using the mouse wheel. Use the mouse wheel to increase/decrease the radius of the created object.

Enable Delay before auto-advancing

The Enable Delay before auto advancing option sets a delay before proceeding to the next time point. When this option is selected, the created object will be displayed for the duration of the delay in the viewing area. The visual feedback of the created object gives you an opportunity to adjust it.
The delay duration is adjustable under the Edit menu/Preference/Surpass. You can specify the value between 0.05 and 5 seconds.

12.14.4.2 Contour Surface

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. You can employ this method whenever simple threshold does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

It is recommended to uncheck any other object in the Surpass Tree before drawing the contour, so that only the contour is visible in the viewing area.

The Contour Tab has three sub-tabs:
Board
Mode
Autofit
There are common concepts between the three tabs explained in Common Concepts - Contour Surface Tabs

12.14.4.2.1 Board

A drawing plane is displayed in the viewing area.

Orientation

This option defines the orientation of the drawing plane. Once orientation of the drawing plane is selected, the drawing planes of all contours are set in the same position. By default the orientation is set to be in the XY plane-z-direction.
Choose the **YZ**-plane to draw in the x-direction. Choose the **XZ**-plane to draw in the y-direction. Choose the **XY**-plane to draw in the z-direction.

Note for 2D view only **XY** Orientation is available.

Visibility

This option defines the Visibility of previously drawn contour lines.

None
The contour currently being drawn is made visible.
Next
The contour(s) next to the contour currently drawn is/are shown.
All
All contours are shown.

Resolution

This option controls the smoothness and complexity of the surface created by the contour lines. It can be used to reduce the number of triangles in a surface object according to a user-defined value. There are two options:
Automatic
The Automatic resolution depends on the contour line vertex spacing.
Manual
The manual resolution is controlled with the slider. Moving the slider to the left reduces the resolution, moving it to the right increase resolution.
Setting the Resolution to 100% corresponds to the data set resolution. Setting the resolution to 1% reduces the voxel resolution to 1% of original data set.

Preserve feature

If the box Preserve feature is selected, a Surface calculation is then performed using anisotropic Gaussian filtering. This feature aims to reduce image noise without removing significant parts of the image content, ensuring that thin and sharp features are not absent from the created surface. The filter size is calculated adaptively and separately for each plane. It produces a family of contour lines, and each resulting line is a combination between the original image and a filter that depends on the local content of the original image. As a consequence, in the created Surface noise is removed, and significant parts of the image content (like elongated and narrow features) are preserved.

If the Preserve feature is not selected an isotropic Gaussian filter is used for the Surface creation. The smoothing is made along the structures, resulting in consistently smooth Surface.

Please note: As a result of applying isotropic Gaussian filter, thin and sharp Surface features may fail to be created.

Selection

The following button actions apply to the previously created contour lines.

Button Cut
To cut a contour line in a plane click on the button Copy.

Button Copy
To copy a contour line in a plane click on the button Copy.

Button Paste
To paste a contour line in click on the button Paste.

Button Delete
To delete a contour line click on the button Delete

Button Smooth
To smooth a contour line edge click on the button Smooth.

Please see Common Concepts - Contour Surface Tabs for information on Slice, Slice Position, Drawing modes and Create Surface options.

12.14.4.2.2 Mode

Several segmentation tools are provided allowing you to select areas in the current slice for contour line drawing.

Select one of the shape object tools in the Drawing Mode. Left clicking on a tool icon activates a drawing tool. Once a drawing style tool is selected you may adjust the specific parameters for that drawing mode.
Once a segmentation tool is activated and adjusted, activate the drawing mode by changing the pointer mode to Select (press the ESC-key) and then once press the **Draw button or Ctrl+space key**. The Drawing mode is activated.

By default, contour lines are drawn in a transparent gray color. The color of the contour lines can be modified using a dialog under the Color Tab.

Click

Clicking within the image inserts a point, a so-called vertex. You can now define subsequent vertices by clicking with the left mouse button. Successive points will be connected with straight-line segments.

Time

Click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms), specified in the corresponding field. The interval can be changed by manually specifying the required value. This drawing style allows you to draw a structure that contains fine details.

Insert a vertex after ms
The number specifies the time interval (in ms) between two inserted vertices.

Distance
Click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (um), which is specified in the corresponding field. To change the interval, specify the required value in the object properties area. This drawing style is recommended if the structure is smooth.

Vertex spacing um

The number specifies the distance interval (in um) between inserting two inserted vertices.

Circle

The Circle drawing tool inserts a circular contour line. The Circle tool always draws a contour line from the center; that is, the place you initially click will be the center point of the contour line. To change the circle radius specify the required value in the object properties area. In the parameters you can also specify the number of contour vertices to be inserted. To create the contour line, move the mouse pointer over the orthogonal slicer plane. A preview outline of the circle is then displayed. To create a contour line, click with the left mouse button on the desired position.

Radius um
Defines the radius of the circle in um.

Tip: Scrolling the mouse wheel can also change the radius of circle.

Number of vertices
Defines the number of vertices in the circle line.

Isoline

The Isoline drawing tool generates contours at a fixed iso-value so the geometrical features of the contour line takes into account the image's intensity values. Connecting the points with a constant intensity value forms the contour line.
The Isoline drawing mode selects the maximum intensity iso-value under the mouse pointer and creates a contour line by tracing points of uniform intensity. Clicking with the left mouse button on the orthogonal slicer plane selects the largest connected area that contains the all voxel with the same intensity values. The current iso-value under the mouse pointer is displayed in the parameter field Intensity and is updated automatically as you move the mouse. To create a contour line, click with the left mouse button on the desired position.

Reduce density to numerical field %
Defines the density of inserted vertex in relation to the data set.
A density of 100% inserts a contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Intensity
Displays the value of the voxel intensity under the mouse pointer.

Magic wand

The Magic wand tool creates a contour line by selecting the largest connected area that contains the maximum intensity voxels and all voxels within a user-defined tolerance range. The Tolerance range defines the tool's sensitivity; the higher value you set, than wider image area that is selected. The Tolerance range can be specified in a box in the Parameter panel.
Move the mouse pointer over the orthogonal slicer plane and a preview of the contour line is displayed. The Intensity value in the Parameter panel is updated as the mouse pointer is moved across over the orthogonal slicer plane. To create a contour line, click with the left mouse button on the desired position.
The Intensity with the corresponding Tolerance value is stored in the parameter section of the label field. This makes it possible to easily correct a selection at a later time.
It is possible to modify the range even after the seed voxel has been selected.

Reduces the density to numerical field %
Defines the density of the inserted vertex in relation to the data set.
A density of 100% inserts contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Tolerance
Defines the range of intensity limits in percentages.
The tolerance 0% selects pixels with exactly the same intensity value as the point under the mouse pointer. The tolerance value of 100% selects all intensities within the intensity range of the data set. Values in between are interpolated linearly. The tolerance can also be changed incrementally by scrolling the mouse wheel.

Intensity

Displays the value of the voxel intensity under the mouse pointer and the tolerance range.

Detects holes box
This option enables you to create a contour line that contains holes of selected areas. If the Detects holes box is selected the line is generated around holes, outlining the hole. If the box in unchecked, the outer line of the selected region is drawn and the holes are not visible.

Channel Selection

All Visible Channel
Specific Channel
Select the respective source channel, from which the Contour surface is to be computed.

Please see Common Concepts - Contour Surface Tabs for information on Slice, Slice Position, Drawing modes and Create Surface options.

12.14.4.2.3 Autofit

Autofit is a method that can help the user automate a manual segmentation.
Autofit is a deformable contour model used to create and optimize the contour line segmentation, so that the localization and accuracy of the segmented object is improved.
It is a semi-automatic, user interactive detection method that is relatively insensitive to image noise. The contour line creation procedure consists of two steps: initial user segmentation and refinement by the Autofit algorithm.
In the first step the model requires the user input, an initial curve (rough outline) closed to the boundaries of the object to be segmented.
The next step is to perform an Autofit algorithm. The algorithm then tries to deform this initial shape so that it fits onto the contours of the image. The Autofit algorithm performs an iterative optimization process where the sum of external and internal energies along the contour line approach a local minimum.
The external energy is computed from the original image intensity gradients and it should be minimal when the contour line is located at an object boundary.
The internal energy is based on the shape of the contour line. The Autofit algorithm simulates the contour line as an elastic rubber band and the internal energy is defined by the physical constraints that describe the contour. It should be minimal when it meets some regularity and smoothness constraints regarding the shape of the line (curvature is minimized and contour vertices are encouraged to be equidistant).
The outcome of the Autofit algorithm is an optimized line, a good fit between the external forces, which aims at the object boundaries, and deformable internal forces that targets regularity and smoothness.
The method, which is applied by Autofit, is known under the name of active contour model or snakes. Kass, Witkin and Terzopoulos published the seminal work on the method.

M. Kass and A. P. Witkin and D. Terzopoulos (1988), Snakes: Active Contour Models, International Journal of Computer Vision, 1(4): 321-331

The Autofit algorithm in Imaris is based on the following publication:

Donna J. Williams and Mubarak Shah (1992), A Fast Algorithm for Active Contours and Curvature Estimation, CVGIP: Image. Understanding, 55, (1), 14-26.

Please Note: The Autofit algorithm does not solve the entire problem of finding contours in images, but rather, depends on mechanisms like interaction with a user, interaction with some higher level image understanding process and information from image data.The accuracy and computation time

depends on the convergence criteria used in the Autofit. The model is sensitive to the initial contour line estimate and algorithm parameters.The automatically determined parameters provide the optimal values for the Autofit algorithm.

Perform Autofit button

Clicking on this button performs an Autofit calculation.

Presets

Shape Smooth slider Accurate

This setting affects the shape of the resulting contour line. If the Shape slider is set to be more on one side the resulting contour line can be encouraged either to be **smooth** or more **accurate** with original image intensity gradients.

Impact Weak slider Strong

The impact signifies how strongly the algorithm will affect the contour line.
If the impact is set to be **Weak**, the algorithm performs only a few iterations and does not move the vertex very far from the initial contour line.
If the impact is set to be **Strong**, the number of iterations will increase and the vertexes can be moved to find edges that are more distant from the initial contour line.

Advanced

This option activates only if the **Presets** option is not selected.

Continuity

The Continuity value affects the vertices space regularity and tends to keep the distances between each pair of adjacent vertices equal. Setting a high continuity value enforces the contour vertices to be equally spaced on the line.

Curvature

The Curvature value constrains the shape of the contour line. Setting a high value for Curvature, results in a circularly shaped smooth contour line.

Image Energy

The Image Energy value constrains the intensity gradients on the contour. Setting a high value places the contour line at the intensity high gradients.

Reach Distance um

This parameter controls the maximal distance the contour vertex can move away from the original position during a single iteration.
Setting a high value enables the algorithm to find edges at more distant locations from the initial contour line and therefore decreases the risk of falling into local minima. However, the algorithm then has a tendency of detecting edges different from the desired object.

Max iteration

This parameter defines the Maximum number of iterations to be performed. The Autofit algorithm terminates if the specified maximum iterations have been performed.

SSC iterations

Scale space continuation is a method for preventing the Autofit algorithm to place a contour line at the local

minima created by noise. First a large Gauss filter is used and then the Autofit algorithm iterates while slowly reducing the size of the Gauss filter. SSC iterations define the number of Autofit algorithm iterations
For example, if the maximum number of iterations is set to 30 and the number of SSC iterations is set to 3, the Autofit algorithm is run three times. The first time with a Gauss filter with three times the specified standard deviation, then for the second time with two times the standard deviation and finally, for the third time with the specified standard deviation. 10 iterations are performed maximally each time.

Gauss numerical field um
The standard deviation of the Gauss filter applied prior to the algorithm.

Please see Common Concepts - Contour Surface Tabs for information on Slice, Slice Position, Drawing modes and Create Surface options.

12.14.4.2.4 Common Concepts - Contour Surface Tab

Slice Position

Note that slice position is only available in 3D view.

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slice (data field)

The respective slice number is displayed in the number field.
The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

Lock button
This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.
Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.
The L key allows the switch between locked and unlocked orthogonal slicer.

Drawing Modes

Draw button

Click on this button to alter between the two Drawing Modes: Active and Inactive

In the contour line, creations there are two different drawing modes: **Active** and **Inactive**.
Active
The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.
Inactive
In the inactive drawing mode, the existing contour lines can be modified or deleted.

You can select the respective mode by using the **Draw button** or **Ctrl+space** key.
Press the **Draw button** or **Ctrl+space** key to alter **Drawing mode** between **Active** and **Inactive** mode.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Create Surface button
To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

Please refer to the section 'Shortcuts Overview' for a listing of the respective shortcuts.

12.14.4.2.5 Shortcuts Overview

You can select the drawing mode by pressing once the **Ctrl+space** key. The pointer appearance on the screen depends on the selected drawing mode. The symbol on the screen appears as an **arrow** in the **Inactive** drawing mode and as a **cross** in the **Active** drawing mode.

Contour Line Mouse & Keyboard Functions

up or down arrow keys	Move up / down one slice
left/right arrow keys	Move up / down to next slice which contains contour lines
C key	Copy contour lines from current slice
X key	Cut contour lines on current slice
V key	Paste contour lines to current slice
Del key	Delete all contour lines on current slice
O key	Smooth all contour lines on current slice
A key	Perform Autofit Algorithm on current slice
+ key or .	Increase contour line width
- key or ,	Decrease contour line width
Mouse wheel	Change circle radius or magic wand tolerance
	Drawing mode **Active**
Backspace key	Abort drawing of currently opened contour line
Enter key	Stop drawing currently opened contour line and leave it open
U key	Change to contour line Union mode Union is indicated by a "+" sign next to the mouse cross cursor

Drawing mode **Inactive**	
L key	Toggle lock/unlock orthogonal slicer
T key + Left-click on line (vertex)	Translate contour line or vertex
R key + Left-click on line (vertex)	Rotate contour line
S key + Left-click on line (vertex)	Scale contour line
D key + Left-click on line (vertex)	Delete contour line or vertex

Union mode
The result of Union mode is a single contour line whose area is the minimum superset of the overlapping input contour line areas.

12.14.5 Tab Edit

Here you will find the manual editing tools for Surfaces.

Cut

The options in Cut allow for manually splitting surface objects. The buttons **Cut Surface** and **Clear Scissor** appear greyed out on selecting the edit tab.

Scissors Properties
Activate the cut buttons:
- Change to pointer mode Select.

Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.
- Press Shift + left-click on the Surface object to enable the scissor and set a vertical scissor line.
- The **Cut Surface** and **Clear Scissor** buttons are enabled.
- A blue colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular). The line acts as a preview for the cut which will be performed.
- Inspect it to check for the correct position. Rotate the scene to change the angle of the cut applied to the Surface object. Use the **Clear Scissor** button if you would like to select a different Surface object to cut.
- Click on the button **Cut Surface** to apply the change and cut the surface object. The Surface object splits into two individual Surface objects.

Button **Cut Surface**
Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear Scissor**
Click on this button to clear the selection.

Selection

Button **Clear**
Click this button to clear the Surfaces selection.

Button **Duplicate**
Click this button and all selected Surfaces appear in a new Surpass Group in the Surpass Tree (Surfaces Selection).

Button **Delete**
Select a Surface and click on this button to delete the Surfaces.

Button **Duplicate to all Timepoints**
Click this button and the selection is not only active for the current time point but for all time points in which the object appears.

Button **Unify**
Click this button to unify (group) the selected Surfaces into one Surface object.

Please note: Selecting the Unify button does not connect, or bridge, the two objects by adding any new pixels. The new, unified object will consist of a group of disconnected components.

Button **Split**
Click this button and the selected Surfaces are separated into two Surface objects.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Edit

Sometimes it is useful to apply a certain function, not to the entire image, but only for a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask Selection ...**
Button **Mask All...**
Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see Mask Channel Window).

Button **Merge ...**
In Imaris it its possible to merge two or several Surfaces items into one Surfaces item. This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button **Select All**.

Button **Clear All**
Use this button to clear the Surfaces selection.

Button **OK**
If you want to merge the Surfaces click on **OK**.

Button **Cancel**
If you do not want to merge the Surfaces click on **Cancel**.

Object-Object Statistics

This check box can be used to activate or deactivate the shortest distance statistics. For information on **Shortest Distance To Spots** and **Shortest Distance To Surfaces** statistics please see Statistics Surfaces

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

12.14.6 Tab Edit Tracks

This tab is common concept for all objects, please read more here Tab Edit Tracks.

12.14.7 Tab Edit Labels

This is a common concept for all Objects. Please find more information at the following link Tab Edit Labels.

12.14.8 Tab Edit Events

Please see the section on common concepts, Surpass Objects - Tab Edit Events for more information.

12.14.9 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop-down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Threshold

Setting thresholds are a common concept used throughout the creation wizards in Imaris please see Common Concepts - Creation Wizard - Thresholds for more information.

Duplicate Selection to New Surfaces Button
Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

12.14.10 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.14.11 Tab Color

The Color tab is a common concept for all objects in Imaris, see 3D View - Common Concepts - Surpass

Objects - Tab Color.

12.15 Volume

How to Add a new Volume?

- Select menu 3D View - Volume.

The Surpass Tree list is automatically updated and in the Volume - Properties the Settings parameter are displayed. A data set can have only one volume.

For information on statistics calculations used refer to Volume

12.15.1 Display Adjustments

To set the correct Display Adjustments for the Volume rendering of your data set please refer to Section Menu Edit - Show Display Adjustment.

12.15.2 Tab Settings

Visibility

Select or deselect the option for Auto-Hide in the presence of slicers. Once selected, if Ortho Slicer is chosen from the top ribbon the volume frame will be hidden.

Mode

MIP (max)
In the Maximum Intensity Projection mode only the brightest point (the point with maximum intensity) of all layers along the viewing direction displays. If your data set has more than one channel, the combination color displays. A layer in the data set with high red and high green intensity displays yellow.

Normal Shading
The result of the normal shading is a depth effect on the screen. The surfaces turned away from the light source appear darker.

Blend
The result of the blend mode is a depth effect on the screen. One effect of the blend mode is, that the borders appear darker. In the blend mode all values along the viewing direction including their transparency are used for the calculation.

For further information on **Normal Shading** and **Blend** functionality see Volume Rendering.

Shadow Projection
The result of the shadow projection is a depth effect on the screen. An object between light source and the surface you look at projects a shadow.

Rendering Quality

If the rendering quality is set to 1.000, then 1 pixel represents 1 voxel. If you decrease the rendering quality the image quality is reduced and becomes coarse-grained.

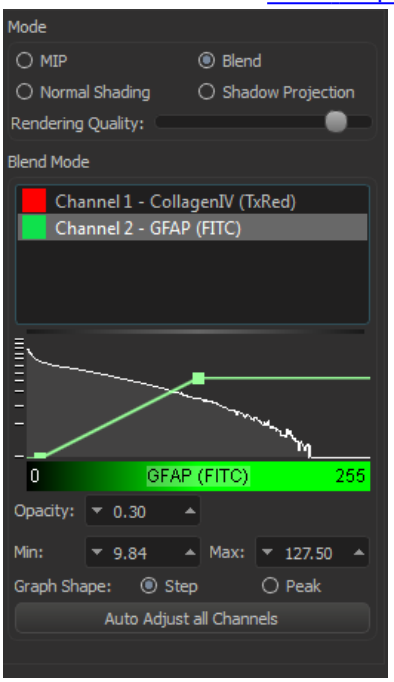
View Aligned Planes
Check this box to see the display planes. Examine the planes while rotating the data set in the viewing area.

See also:
Preface - Terminology

12.15.3 Volume Rendering

Imaris 9.0.0 onwards introduces new **Blend** and **Normal** shading functionality to make it easier to adjust, and control settings, so you can obtain optimal visualization of your rendered image data under these modes.

The Transfer Function Histogram (shown below) enables you to set the Opacity and the min/max thresholds for the selected channel. Changes to these values are restricted to the selected Blend and Normal mode and channel selected. The Display Adjustment settings allow you to adjust how the rendered data set using the transfer Function Histogram is displayed. Refer to the section Show Display Adjustment.

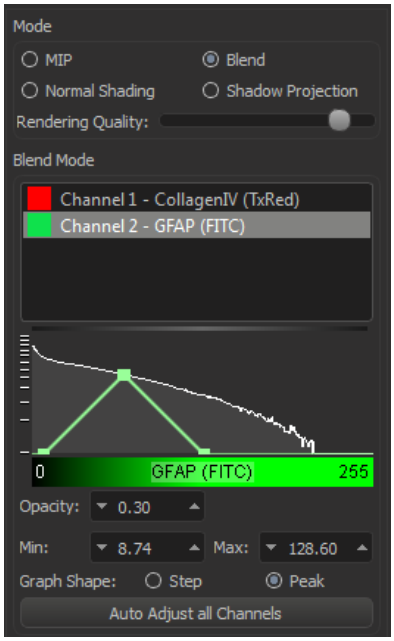


Opacity
Opacity sets how transparent or solid the rendered data will appear. The value can be set between 0.0 (fully transparent) and 1.0 (solid). The level of opacity can be adjusted by entering the value directly in the Opacity field (or adjusting the value using the up down arrows), or alternatively by clicking and dragging the upper adjustment point in the Transfer Function Histogram in the y-axis.

Min/Max
The Min and Max thresholds for the data that is to be rendered using a 0.00 and 255.00 range. Values for Min and Max can be entered directly in the Min and Max fields (or by adjusting the value using the up down arrows). You can also adjust the min and max values from the histogram by clicking and dragging the adjustment points in the x-axis. In the "Step" Graph Shape the two points in the graph may be adjusted independently. For the "Peak" Graph Shape the limits of the peak will increase or decrease as the adjustment points are moved.

Graph Shape

Step is the default graph shape and applies a "step" profile so that opacity and Min/max limit can be adjusted using two points (shown in the previous image). Peak is available for Blend Mode only and applies a "peak" profile so that the values can be adjusted using three points (shown below).



Auto Adjust all Channels Button
Use the AutoAdjust all Channels Button to automatically set all channels to calculated values.

Notes

- **Display Adjustment settings that map intensities to colors apply to all visualizations. Changing them in MIP changes them for blend and normal shading as well (Control + D).**
- **Zoom in/out on histogram using the mouse wheel**
- **Double right click the histogram to reset zoom level to default**

12.15.4 Tab Draw

Volume Painter

On the tab Draw you can find the settings for the Volume Painter. Use this Volume Painter to adjust the voxel intensities before you do a segmentation based on Surfaces, Spots, or Filaments. (If an automatic algorithm is not able to separate two objects, the intensity between these two objects can be "erased" in advance.) You can draw disks, spheres, or hollow spheres into the selected image channel.

1. First select the desired channel and painter shape.
2. Rotate the view to execute a vertical cut.
3. Then customize the painter settings.
4. Change to the pointer mode Select.
5. Move the cursor to the desired position.
6. Hold down the Shift-key and click to add the shape.
7. In the viewing area you see a preview of the painter tool.
8. To re-edit a shape use the point selection.
9. Set intensity to 0 to erase a region, or set the intensity to 255 to fill a region.
10. To fix the shape click on the button **Set Intensity**.

Please note: The button Set Intensity changes the data set.

Selected Channel

Select the respective source channel.

Painter Shape

Here you select the painter shape.

Disk
The painter is disk shaped. In the settings selection adjust the diameter, width and intensity of the disk (see below).

Sphere
The painter is a sphere. In the settings selection adjust the diameter and intensity of the sphere (see below).

Hollow Sphere
The painter is a hollow sphere. In the settings selection adjust the diameter and intensity of the hollow sphere (see below).

Selection

Diameter
Here you select the diameter of a disk, sphere, and hollow sphere. If your mouse has a wheel, you can use the wheel to adjust the diameter.

Width
Here you select the width of a disk.

Intensity
Here you select the painter shape intensity. The shape preview in the viewing area changes accordingly. If you want to cut parts out of the channel select 0 as intensity value.

Button **Set Intensity**
In the last step you click on this button to fix the shapes. The button **Set Intensity** changes the data set and it is not possible to re-edit the shapes again.

Button **Delete All**
Click on this button to delete all inserted shapes in the preview.

Selected Point

You can use the following data fields and the button **Delete** to re-edit or delete a selected point.

Position [um] X, Y, Z
To select a point change to the pointer mode Select and click on a point. The color of the selected point changes. The point position displays in the x, y, and z data field. To move the point click on the new position in the viewing area or edit the x, y, or z data field.

Button **Delete**

Click on this button to delete the selected point.

12.15.5 Tab Statistics

The Statistics tab is available for all objects in Imaris and is explained in the common concepts section 3D View - Common Concepts - Tab Statistics

12.16 Export Selected Objects...

In the Surpass Tree highlight all Items to be exported. To export all Surfaces, Contours, and External objects, select the Export Selected Objects... .

- Select menu Surpass - Export Selected Objects... .

The Save selection as External Objects box is displayed.

- Select the directory and enter the name for the file to be saved and click **OK**. The object is saved as either as an Inventor file with the extension *.iv, VRML2 file with extension *.vrml or WRL files with the extension .wrl. The saved object can be loaded as External objects to another data set.

12.17 Delete Selected Objects ...

In the Surpass Tree highlight all Items to be deleted. To delete all Surfaces, Spots, Contours, and External objects, select the entire Scene folder.

- Select menu Surpass - Delete Selected Objects.

The Delete selection window with a confirmation question is displayed.

12.18 Switch Pointer Mode

In the Surpass view there are two pointer modes: Pointer Select Mode and Circle Selection Mode.

Pointer Select Mode
Use the Pointer Select Mode to select an object in the image, e.g. to set Measurement Points on the object surface. Hold control to select multiple objects.

Circle Selection Mode
Use the Circle Selection Mode to select multiple objects simultaneously. Hold control and use the scroll function on the mouse to adjust the size of the circle, this will allow for the simultaneous selection of a smaller or greater number of objects as desired.

Navigate
Once either the Pointer Select Mode or the Circle Selection Mode have been selected, by dragging the cursor accross the screen, the cursor becomes two turning arrows and you can use either pointer to navigate, move, rotate or zoom the image in the viewing area.

The shortcut keys 1, 2, 3 let you quickly switch between Pointer Select Mode, Circle Selection mode and Navigate respectively.

12.19 InMotion

The function InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression with smooth animation of the image. This helps to get an understanding of the image depth, while manipulating with Surpass objects: Spots, Filaments, Measurement Points.

How to Start the InMotion Mode?

- Click on the icon in the Main toolbar.
- Select View - InMotion.

To switch the mode off, click on the icon Surpass in the Main toolbar.

Adjust 3D Cursor Settings

To adjust the 3D cursor settings click on menu Edit - Preferences... - 3D Cursor.

12.20 Store Creation Parameters

Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Arena view and in the Creation Parameter list used in the Edit/Preference/Creation Parameters and would be used as default name in the subsequent creation wizard algorithms.

12.21 Common Concepts

This section provides an overview for topics that are common concepts within Imaris.

12.21.1 Creation Wizards

The following sections present information on common concepts within creation wizards.

12.21.1.1 Algorithms

In the creation wizard for all objects the algorithm step allows for the creation of favourite creation parameters and are described in this Algorithms common concepts section.

Favourite Creation Parameters

This option lets you use the identical creation parameters for multiple data sets.
The Favourite Creation Parameters option is only available if the set of the creation parameters has already been saved under the Creation tab using the Remember Parameters button.
Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Favourite Creation Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your

chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

12.21.1.2 Region of Interest (ROI)

The region of interest (ROI) is a common concept for all objects in Imaris. The ROI can be used, for example, to detect in a specific region of the image because only a small subsection is interesting. Alternatively the region of interest can be used to quickly set parameters on a small area of the image before detecting a surpass object over the entire image.

Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

X, Y, Z, Time
Min, Max, Size
Minimum, maximum and Size of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields.
Alternatively the location and size of the ROI can be adjusted directly in the viewing area.
Size of the ROI:
1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X ,Y and Z.
Location of the ROI:
1. Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.
The numbers in the value fields are updated automatically.

Memory Usage:
Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**
Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**
Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:
Display of the memory usage for the image size within the ROI.

12.21.1.3 Thresholds

Thresholds are a common concept used throughout the creation wizards in Imaris.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated  . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form  indicating an activated Automatic threshold to  indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the histogram and viewing area.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.

12.21.1.4 Classification

The Classification Step in the creation wizard can be used to set up rules for automated classification based on available statistics values for spots and surfaces. This automated classification enables efficient classification of large numbers of spots objects within a single image or a batch of images.

The Classification Selection Table

Lets the user set up the number of classifiers to be applied. To add another class use the green plus button  . To remove any of the added classes use the trash button  .

Sets

To change the name of the set click on the table cell containing the current name and enter the new name.

Input

The options avaliable as input will depend on the object selected. For spots the input options are Spots or All (Spots) Tracks. For surfaces the input options are Surface or All (Surface) Tracks.

Filter Type

Select the filter type suitable for your data set, Filter 1D, Filter 2D and Machine Learning (ML).

Filter 1D allows the user to split objects into classes by specifying thresholds for a specific single statistics value.

Filter 2D allows the user to split objects into classes using two statistics values.

Machine Learning allows the user to add different spots and/or surfaces objects to specific groups to develop a training set. Once enough objects have been selected to develop an effective training set, this option allows for the automatic classification of further objects within this image or for batch processing purposes.

Group Name Statistics Filter

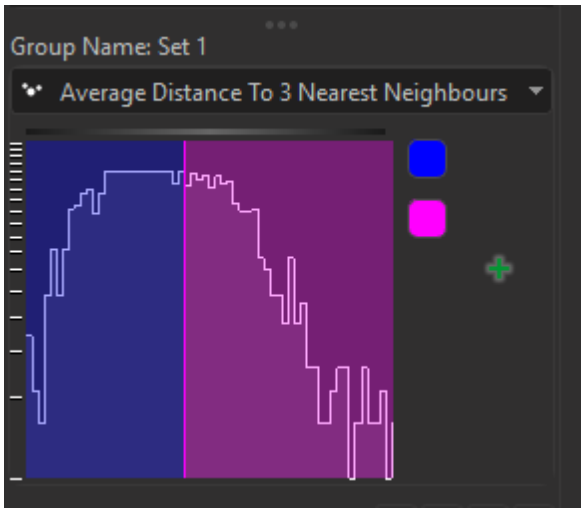
A Category identifies and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
For the classification step there are following Filter Categories: Surface, All (Surface) Tracks, Spots, All (Spots) Tracks the selection available will depend what is selected as the input in The Classification Selection Table.
This dropdown allows you to select the statistics value of interest of Object Types selected in buttons allow you to choose the Category and determines the list of displayed statistical values. The exclamation mark sign indicates that the statistics value is the same for all objects.

Histogram

Histogram using Filter 1D

In the histogram the regions above and below the threshold values are displayed in the default colors below. Change the threshold values by clicking on the colored line separating the two regions and drag to the required threshold position, changes are instantly visible in the viewing area.

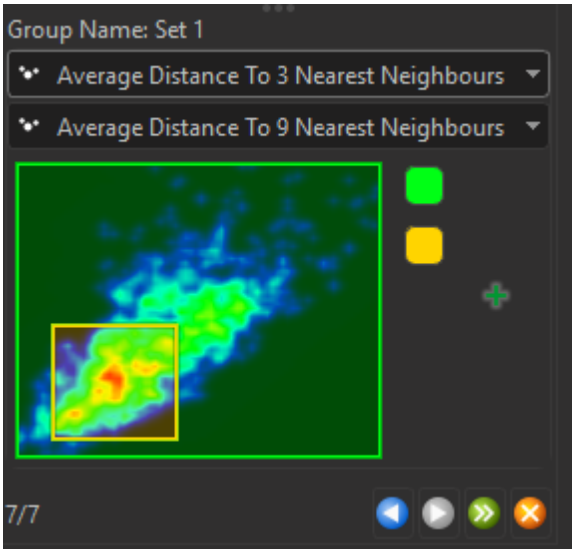
To change names or the color of your classes and regions on your histogram, click on the colored icons to the right of the histogram to show a color slider and labeling options. To add another class and identify another set of threshold values use the green plus button  . To remove any of the classes or regions use the trash button  .



Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display, alternatively, hold control and use the scroll function on the mouse when hovering over the histogram. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.

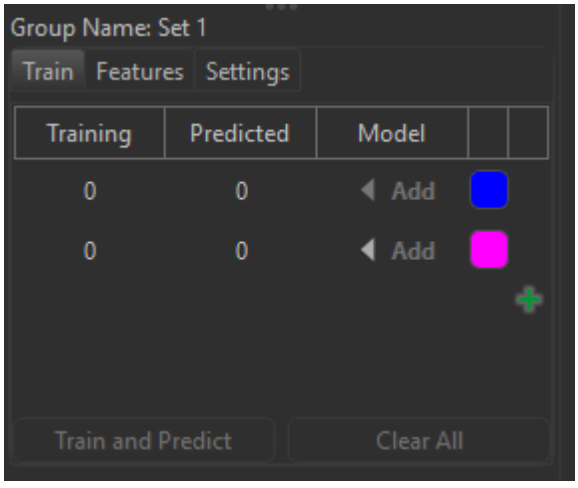
Filter 2D

Objects can be separated into classes in the Filter2D dialog that can be defined using rectangles.



Machine Learning

The Machine Learning (ML) dialog looks like the image shown below prior to assigning classes of objects. This dialog allows the user to add specific selected objects to a class to develop a training set for the machine learning algorithm. First, some example objects which are representative of the different classes the user wants to assign are added to the training set. To add an object to a class, select the object(s) using the selection tool and press the add button of the class you want to add them to. Actual training and prediction of the machine learning algorithm occurs when the Train and Predict button is selected. As a result of this the class of each object that was not in the training set is predicted by the machine learning algorithm.



Train Tab

Training - lists the number of objects assigned to that class, which are used by the machine learning algorithm to assign classes to all other objects in the image which are unassigned. Note: some objects must have been assigned to a class before the Train and Predict button can be selected.

Predicted - lists the number of objects that are predicted to be part of the class using the machine learning algorithm. Note: values only populated after the machine learning algorithm has been run using the Train and Predict button.

Add buttons - allow selected objects from the image to be added to that class.

If some objects have not been classified correctly during the first Train and Predict run, they can be manually selected added to the correct class and the Train and Predict button selected again to rerun the class

allocation process using machine learning. These steps can be repeated as many times as is required to classify objects in the image appropriately.

For the interested reader we recommend the following paper that describes a machine learning approach similar to the approach implemented in Imaris: Ranzato, M., et al. "Automatic recognition of biological particles in microscopic images." Pattern recognition letters 28.1 (2007): 31-39. Ranzato et al. make use of multi-scale features invariant to shift and rotation as described originally by Koenderink and van Doorn, "Representation of local geometry in the visual system." Biological cybernetics 55.6 (1987): 367-375.

To remove all existing class allocations and start assigning classes again select **clear all**.

Features Tab
The Machine Learning algorithm analyzes the statistics values of the training data set and weights them. The features tab shows the rank, statistic and its relative weight.

Settings Tab
The list of machine learning statistics is visible only on the Settings tab of the machine learning user interface. The list is available mainly to allow experts to look “under the hood” and see which values the machine learning classifier gets as input.

12.21.1.5 Event Setup

Events can be defined in the creation wizard of all objects. Please note in the first step of the creation wizard that the check box Track Object (over time), must be selected for the Events Setup option to appear during the creation wizard.

Overview

The event setup dialog lets you add any number of events and for each event you can define the class-change. For example an “inside” and an “outside” class based on the shortest distance between spots and surfaces to label spots as “inside” when their distance to the surface is negative and “outside” otherwise. Then an event can be set up to capture when a spot changes from “outside” to “inside” and another event for when it changes from “inside” to “outside”. Each event you define produces a corresponding event time statistics for all those objects that are in a track which has such an event. This statistics value then becomes available as an x-axis for Vantage Time plots.

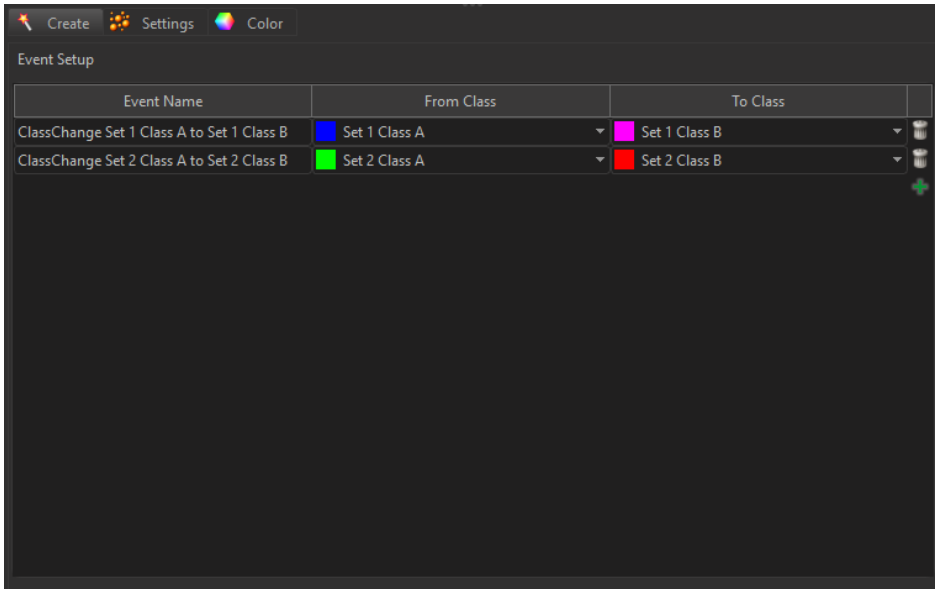
Event Tab in the Creation Wizard

Events can be defined in the creation wizard of all Spots and Surface. The user must classify objects into different classes (in the previous classification step) and then set up “ClassChange” events for objects within a track when they “switch” from one class to another.

The Classification Selection Table

Lets the user set up Events. To add another event use the green plus button  . To remove any of the added events use the trash button  .

The event name is based on the name of the classes set up in the previous step, the name can be changed manually in the Tab Edit Events, once the creation wizard set up is complete. To change the From Class and To Class options use the drop down to make a selection.



12.21.1.6 Buttons

During object creation, there are a set of four buttons at the bottom of every step of the creation wizard. The buttons allow the user to navigate through the creation wizard steps, and are summarized below.

Button **Back** 

With **Back** you return to the previous window of the Creation Wizard.

Button **Next** 

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish** 

Click on **Finish** to calculate the desired Object and display the Object in the viewing area.

Button **Cancel** 

If you do not want to create an Object click on **Cancel**.

12.21.2 Surpass Objects

The following sections present information on common concepts within surpass objects.

12.21.2.1 Slicer Interaction

Slicers can be added to all surpass objects or to visualize slices of the volume using the Ortho or Oblique slicer. Common properties and interactivity exist between all slicers including, Ortho slicer, Oblique slicer and the Render on Slicer option available in the Surpass Property Area, and are described here.

When to use Ortho, Oblique or Object based Slicers.
Ortho slicers can be placed in fixed plane orientations XY, XZ, YZ, for the entire volume.

Oblique slicers can be placed in any plane of orientation, for the entire volume.
Object based slicers can be placed on any surpass object in any plane of orientation.
Please note that Ortho and Oblique slicers allow for multiple slicers to be overlaid on the image. However, object based Renders on Slicer do not allow more than one slicer per object type.

Mouse and Keyboard Functionality

Right click on yellow pointer	Change orientation of plane of slice (fixed plane positions XY, XZ, YZ)
Right click and drag on yellow pointer	Move pointer position
Left-click on the yellow arrow & drag	Rotate plane to desired position (Ortho slicer fixed plane positions XY, XZ, YZ only)
Left-click on the yellow arrow, hold shift & drag	Rotate plane to desired position in XY, XZ, YZ plane orientation (Oblique and object based slicers only)
Up arrow	Move up through render slice in discrete steps
Down arrow	Move down through render slice in discrete steps

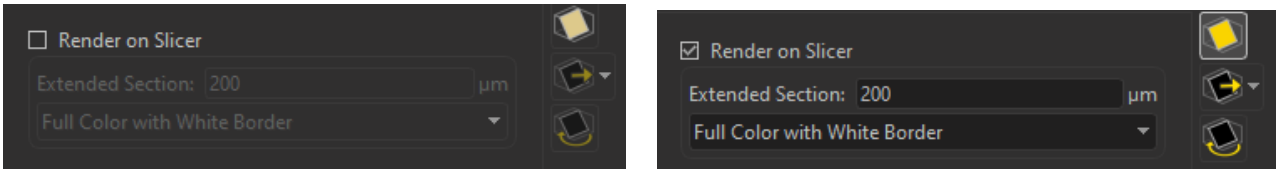
Users with two wheel mouse

Slide with side scroller	Move through render slice continuously
Click side buttons	Move through render slicer in discrete steps

See also:
Addendum - Mouse & Keyboard PC
Addendum - Mouse & Keyboard Mac

12.21.2.2 Surpass Property Area

Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris.



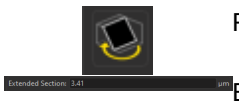
Render on Slicer option turned off (left) and turned on (right).

See below for outline of functionality of each button or section.



Turn on Slicer, note that this can also be done using the check box labeled Render on Slicer.
Translate Slicer to center of selected objects and fit window to them.
From the drop down select from:

- Translate slicer to selection
- Align Slicer to XY Plane
- Align Slicer to XZ Plane
- Align Slicer to YZ Plane
- Store Slicer Configuration



Rotate, zoom and center viewing area to fit orthogonal slicer window

Extended section, use this to set the thickness of the slice



Select one of the following shading options from the drop down:

- Colored border

- Colored border with shading
- Full Color
- Full Color with white border


Help button, opens the online reference manual to the relevant section

Render on slicer is a common concept between Surfaces, Spots, Filaments and Cells objects in Imaris and the following render on slicer button options are available on every tab of the surpass property area.



The render on slicer options that are available on every tab of the surpass property area.

12.21.2.3 Tab Edit Labels

The edit labels tab allows users to manually label objects.

Sets

The add button  can be used to set up sets ready to have labels assigned.

Set

To change the name of the set click on the current name and enter the new name.

Input

In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.

Set Name

Use this section to set up labels within the selected group. To assign a label to an object select the object in the 3D View and then click the “Assign” button for the respective label.

Class - Name of class.
Count - Displays the number of objects assigned to this class.
Assign to class - once an object has been selected within the 3D view, it can assigned to a specific class using the buttons in this column.
Color block - Click on this to bring up the color options slider and change colors and/or label names.

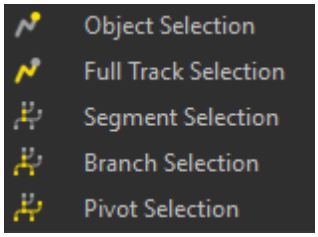
To clear all assignments select the **Clear Classes** button at the bottom of the dialog.

12.21.2.4 Tab Edit Tracks

Please note edit tracks is only available as an option when an object with time series data set is selected.

Camera Toolbar Mouse Selects

The drop down in the camera toolbar allows you to select an object/track within an image according to its object/track type. There are the following options in the camera toolbar:



The differences between these options is described in Tracking

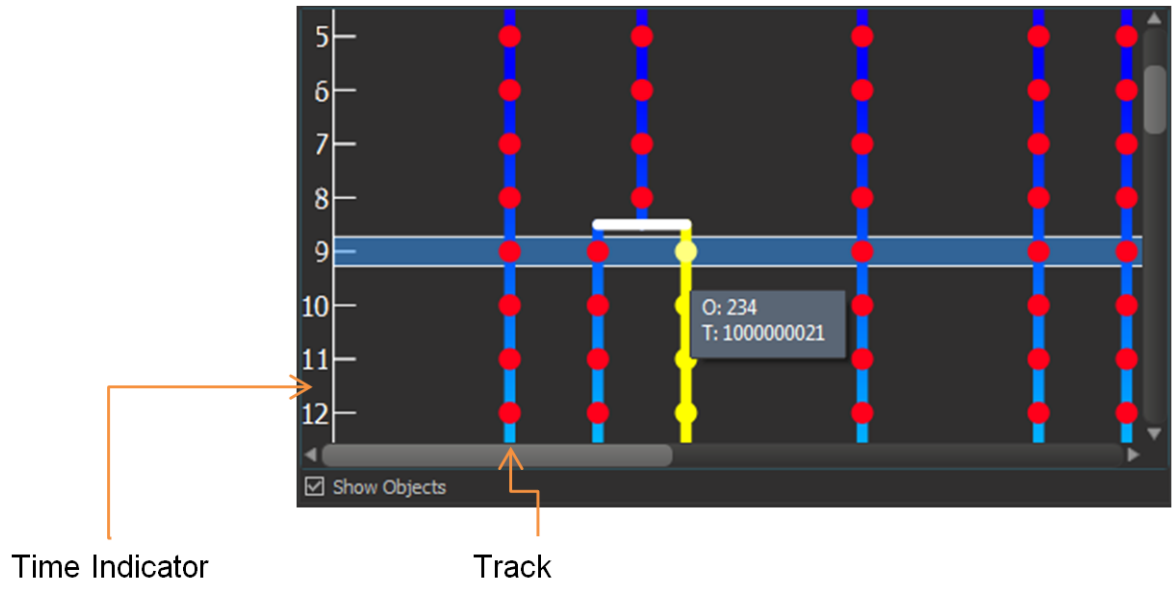
To edit the properties of multiple objects simultaneously, select the required object type from the drop down list in the camera toolbar and hold control when selecting objects.

Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.
The first row of the track-editing table displays the timeline. Each number represents a time point in the series.
The second row of the track-editing table displays the **object name**.
Multiple object types can be tracked easily since every object type appears in different colors.

Using the Track Editor

Table Layout
The track editor table:



Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.
Using the mouse wheel will have the same affect.

Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

Time indicator
The axis on the left displays the frame number.

Track
Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects fuse or fragment, e.g. cells undergoing division.

Object
Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.
If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

Show Objects
Use this option to select to show or hide each object in the tracks from being visible.

Camera icon
Use the camera icon to save an image of the currently displayed tracks (either .tif or .svg formats) to a specified location. The image will automatically open in the default graphics applicatin after saving is complete.

Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.
If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.

Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Editing

Track Editor has several features that help you modify your tracks

Connect Button
Click this button to connect track components such as objects or segments as selected under Mouse Selects. For example, select objects in different tracks at the subsequent time points (just one object per time point). The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Connect (you can also press the "c" key as a shortcut). The connection is automatically created between all selected objects.

Disconnect Button
Use this option to disconnect track components such as objects or segments as selected under Mouse Selects. For example, select object(s) to be disconnected. The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Disconnect (you can also press the "d" key as a shortcut). The button will update to show the number of objects selected. The connection between the selected objects in the time sequence is broken.

< Button
Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

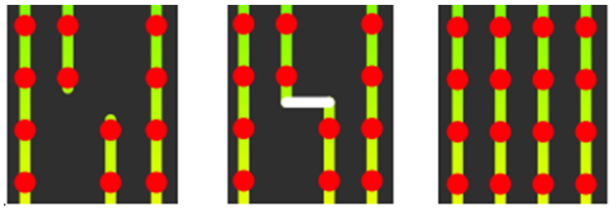
> Button
Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

<< Button
Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

>> Button
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

Flip
The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

Reorganize
The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.


Editing tracks. Connection added. Tracks reorganized.

Invert Axis
Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

Correct Drift

Refer to Drift Correction.

Algorithms

The Drift Correction is calculated based on the two algorithms, translational drift and translational and rotational drift.

Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

Please note: The number of the selected tracks affects the algorithm calculation.

Selection of a single track gives a complete description of a translation.
Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).
A better fit between the time points is achieved when three or more tracks are selected.

Focus Tracks

On selecting Focus Tracks, up to 15 tracks are displayed in the track editor.

Rebuild Around Selection

Rebuild (around Selection) lets the user choose which tracks should be displayed in the editor. Select one or more tracks, then select Rebuild, so that tracks in the region of the selected ones can be inspected and adjusted in the track editor.

Select All

Select All selects all tracks “focused” in the track editor.

Object Type

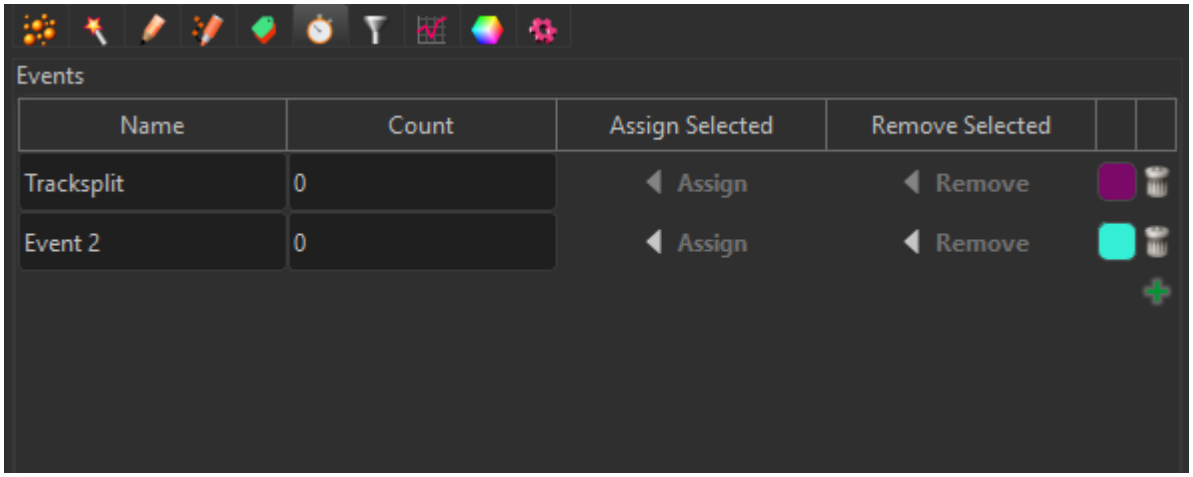
Allows the user to select from a drop down if there are multiple objects which have tracks i.e. for cells and nucleus options within the cells object.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

12.21.2.5 Tab Edit Events

Manual Event Editing

Events can be manually edited after Object creation in the “Edit Events” tab. To assign an event to an object, select the object and press “assign” on the event you want to assign to that object.



Name - Name of Event. To change the name of the event click on the current name and enter the new name.
Count - Displays the number of objects assigned to this event.
Assign to class - once an object has been selected within the 3D view, it can assigned to a specific event using the buttons in this column.
Color block - Click on this to bring up the color options slider and change colors and/or event names.

The add button  can be used to manually add a new event.

12.21.2.6 Tab Statistics

Surfaces statistics are automatically computed. For the statistical values please refer to the Sections:
Menu Edit - Preferences... - Statistics - Surfaces
Menu Edit - Preferences... - Statistics - Spots
Menu Edit - Preferences... - Statistics - Filament
Menu Edit - Preferences... - Statistics - Cells

The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.
Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data.
The Statistics View is configured as follows:
Overall
Spatial (Surfaces and Spots only)
Detailed
Selection
In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics

that are not measured on a per-object basis are displayed here.

Category

A Category identifies and summaries a set of statistical values or calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: **Surface**, **Surface Tracks**, **Spots**, **Spots Tracks**, **Filament**, **Filament Tracks**, **Spines**, **Spine Tracks**, **Point**, **Point Tracks**, **Segment**, **Soma**, **Cell**, **Cell Tracks**, **Nucleus**, **Nucleus Tracks**, **Vesicle** and **Vesicles Tracks**.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the statistics value is the same for all objects.

Spatial Tab (Surfaces and Spots only)

Select the spatial statistic from the drop down, the values are populated in the table. Statistics values are explained in File - Preferences - Statistics - Common Statistics - Spatial Statistics.

The statistics volume, cumulative volume and intensity for current time point or all time points (all time points appended) are available from the drop down, and dependent on the selection, the table will populate with the statistical information.

Detailed Tab

The drop down list offers two types of detailed statistical calculations **Specific Values** or **Average values**:

Specific Values

In the Specific Values, a second drop down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed. Categories are available for selection.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum) - the smallest value within a set of values of the selected variable
Max (Maximum) - the largest value within a set of values of the selected variable
Mean - value of the sum of all values divided by number of values within a set
StdDev (Standard deviation) - a measure of the values distribution around the mean values
Median - the middle value of the sorted set of variables
Percentile 25% - indicates where 25% of results are lower than this value
Percentile 75% - indicates where 75% of results are lower than this value
Sum - the sum of all values within a variable set
Count - number of values within a set
Unit - variable unit

Channel
Class
Image

Once **Specific** or **Average** values are selected from the drop down the statistics data is populated in the table below. For time series data a **Time Plot** is available in the statistics tab, multiple selections of data in different rows is available via the **interactive two-way selection.**

Time Plots 
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+click, will plot a superimposed second selected value over the first one.
Time Plot Average Values: The **orange** line on the time plot indicates where the **median** value of the set lies, the **shaded light gray region** indicates the **percentiles**.
Time Plot Specific Values: The **orange** indicates the actual values for the selected object, **blue** line on the time plot indicates where the **median** value of the set lies, the **shaded light gray region** indicates the **percentiles**.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. The changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

Selection Tab

Statistical tables for the selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

The drop down list offers two types of statistical calculations **Specific Values** or **Average values**:

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This

choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

Once **Specific** or **Average** values are selected from the drop down the statistics data is populated in the table below. For time series data a **Time Plot** is available in the statistics tab, or make multiple selections in the image to view the **interactive two-way selection.**

Time Plots 

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.
Time Plot Average Values: The **orange** line on the time plot indicates where the **median** value of the set lies, the **shaded light gray region** indicates the **percentiles**.
Time Plot Specific Values: The **orange** indicates the actual values for the selected object, **blue** line on the time plot indicates where the **median** value of the set lies, the **shaded light gray region** indicates the **percentiles**.

Interactive Two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections in the image is available by pressing Ctrl+click (Mac: Command +click). See the button overview below.

Buttons Overview

	PC:	Mac:
Select Object	Left-click	Click
Select multiple Objects	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings**  - Configure a List of visible statistics values.
Click on the button Settings, preferences window opens. See below for more information on the specific object statistics.
Menu Edit - Preferences... - Statistics - Surfaces
Menu Edit - Preferences... - Statistics - Spots
Menu Edit - Preferences... - Statistics - Filament
Menu Edit - Preferences... - Statistics - Cells

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button Search.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Surfaces/Spots/Cells/Filaments** - Duplicate Selection to new Surfaces/Spots/Cells/Filaments.
Click on this button and all selected elements are copied to a new object. The new Object appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export**  - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
When the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export**  - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export all the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects. When the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

12.21.2.7 Tab Color

Tab Color edit, manage and assign colors material to Object components or Tracks. Tab Color can be used at any time to assign colors to components when using the creation wizard. Once optimal parameters and color settings have been assigned using the creation wizard they can also be employed for batch processing.

Object Type

The available Object types are presented in a drop-down window. Select either Surfaces/Spots/Filaments/Cells or Tracks and select from the color types available below:

Base
Object ID
Track ID
Statistics Coded
Time Mapped

12.21.2.7.1 Color Type - Base

Tab Color edit, manage and assign material colors of a Object/Track.

Color

RGB

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color slider to select a color or by clicking on the drop down arrow and entering direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Material

Objects are rendered with a Material that can be chosen from a list. Many of the Material choices have bright edges highlighting regions where the object normal is perpendicular to the line of sight.

Labels Overriding Base Color check box, if selected, the labeled objects will be color coded according to their label. The labels associated with the data set are displayed as a list.

Click **Only show labelled objects** to show only data which have labels associated with them.

Note that if there is more than one label associated with an object, the object will be displayed as a combined color. Non-labelled objects are displayed in the base color.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

12.21.2.7.2 Color Type - Object ID

Assigns a random color to every object. This is available for all surpass components. Please note that Object ID is only available for objects, not for tracks.

12.21.2.7.3 Color Type - Track ID

Track ID assigns a fixed, random color to each object. This mode is useful for validating tracks as an object will have the same color through the track duration. Erroneous behavior can easily be detected and corrected using the Edit Tracks  functions. Refer to the Tab Edit Tracks section.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image

12.21.2.7.4 Color Type - Statistics Coded

Statistics Coded

The Statistical variable selection is listed in the drop-down window depending on the object type selected (either Objects or Tracks). For example, if objects is chosen as the Object type, the available Statistical type will be the object linked variable. Similarly, Statistical variables for Tracks will be listed, if the Object type Tracks is selected.

Statistics Colorbar Properties

The Statistics colorbars are displayed in the viewing area. The upper one is the colorbar range for objects, and lower one is the colorbar for the Tracks.

Show Colorbar
Check the box to see the statistics colorbar in the viewing area.

Show Title
Check this box to see the title in the statistics colorbar.

Show Range
Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**
Click the **Reset** button to set the image back to the original values.

Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

Colormap range

Colormap range-specify the limits for colorbar.
Min - lower color limit
Max - upper color limit
Button **Auto**
Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

12.21.2.7.5 Color Type - Time Mapped

Time Mapped
This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

12.21.2.8 Mask Channel Window

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask
When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map
Constant Inside/Outside
Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:
The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:
If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Distance Transform
A new 32 bit float image is created that contains in each voxel the distance to the closest surface/spot. The distance is negative inside and positive outside the surfaces/spots.

See also the XTension XTDistanceTransform that performs a distance transform either inside or outside of the surface with always positive values. For Spots, it performs a distance transform where distances are measured from Spot centers.

Apply to all Time Points
Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**
If you want to apply the mask click on **OK**.

Button **Cancel**
If you do not want to apply the mask click on **Cancel**.

Section 13 Help

Learning Resources	
Quick Start Tutorials	Ctrl + F1
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How to find the pdf based help for PC Users:
To open the pdf help file in Imaris first select **Start**- **All Programs** – **Imaris**.
The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

How to find the pdf based help for Mac Users:
To open the pdf help file in Imaris first locate and select the Imaris application file.
By default, the file is in the Application folder and the file name is Imaris 8.3.app.
With the right-click select the option **Show Package Contents**.
The new window will open.
Select the **Contents** folder and with a double-click select the **Shared Support** folder.
The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

See also:
Addendum - Mouse & Keyboard Mac

13.1 Learning Resources

Opens the Imaris Welcome Page, which offers new and experienced users of Imaris a single location to access all available help materials. For example, easy video tutorials to familiarize yourself with the Imaris user interface, or easy access to our FAQ section and Learning Center where you can browse for specific content.

Available at imaris.oxinst.com/welcome

13.2 Quick Start Tutorials

The Quick Start Tutorials provide you with the basic information on how-to-use Imaris but may also show yet unrecognized new features of the software to the advanced user.

The tutorials are designed to be followed sequentially, but if you are already familiar with Imaris the basic lessons may be skipped. The tutorials are cross-referenced by hyperlinks highlighted in blue underlined font. The Table of Contents and the Index provide further support for navigation in the tutorials.

Each tutorial is a hands-on seminar, therefore it is recommended to move Imaris to the left hand side of the screen and adjust the online tutorial on the right hand side of the screen and practice step-by-step.

13.3 Keyboard and Mouse

Click on this menu entry and the Reference Manual opens in the actual browser. The page for the global Mouse & Keyboard functions displays.

Global Mouse & Keyboard Functions

For PC Users:

Addendum - Mouse & Keyboard PC

For Mac Users:

Addendum - Mouse & Keyboard Mac

13.4 Reference Manual

The Reference Manual provides you with the information about Imaris: the menu(s), the different Imaris views and the adjustable parameters in the program.

To find a specific parameter use the "Search function" in the online version of the Reference Manual.

Context Sensitive Help

For PC User:

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

For Mac User:

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

13.5 Statistics Reference

This link opens the statistics section of the Imaris reference manual, available at File - Preferences - Statistics.

13.6 Programming Interface

Select this to open the Imaris XT programming Interface.

13.7 XTension Documentation

This Section provides a link to descriptions of XTension documentation.

13.8 Imaris Open

If your computer is connected to the Internet, Imaris can directly open the Imaris Open webpage (http://open.bitplane.com/).

13.9 License...

Overview

Node-locked License
Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use Imaris Configurator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine.

Floating License
Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.
The Imaris Configurator application easily let you enable a floating license.

Demo License
A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open Imaris Configurator application and open the Demo License you received from your local support representative.

Evaluation License
Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.
To get an Evaluation License you will have to register yourself first at:

https://imaris.oxinst.com/downloads

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

Imaris Open Developer License
To get an Imaris Open Developer License you first have to register at:
http://open.bitplane.com/

After you entered your OrderID click on the Imaris Open button  to activate the Developer License.
This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the button will turn on  .

RegistrationID
Please enter your RegistrationID you received by mail first.

First-time Installation

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

• If you have a Node-locked or a Floating License you should use the Imaris Configurator application for the further license setup. This is done by simply clicking on the “Start Imaris Configurator” button. Please note that the Imaris Configurator application needs administrative privileges.

• If you want to evaluate Imaris first, please register yourself first at:

https://imaris.oxinst.com/downloads

The automatic mail response contains your RegistrationID and Passcode.
To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation License” button.
Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

Installation of additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start the Imaris Configurator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

Imaris Configurator

Here you can adjust the default license settings.
Imaris Configurator is an application designed to manage the licenses of various Bitplane applications. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this folder we strongly advise you not to do so. It is recommended to use the Imaris Configurator application instead.

Imaris Configurator Button
This button starts the Imaris Configurator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the Imaris Configurator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Configurator.
Any change of licenses will require Imaris to be re-started.

Except for the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need administrative privileges.

If you have access to an administrative system account, Imaris Configurator should just start normally.

Start Imaris Configurator Button
This button starts Imaris Configurator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use Imaris Configurator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Configurator.
Any change of licenses will require a new start of Imaris.

Evaluation License

Use Evaluation License Button
After you entered your RegistrationID and Passcode you can click on this button to enable the Evaluation License. This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on.
You can also disable your Evaluation License by clicking again on this button and the light bulb will turn off. This indicates you aren't using any Evaluation Licenses.
Any change of licenses will require a new start of Imaris.

RegistrationID
Please enter your RegistrationID you received by mail.

Passcode
Please enter your Passcode you received by mail.

Available Licenses

The table displays all existing Imaris licenses on the system and additional information for each feature:
Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given at the very beginning of this page.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.
Expiration
Shows the number of days until this license expires.

License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
– Green: License is valid.
– Yellow: License is valid, but all licenses are currently in use.
– Red: License has expired.
– No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

Check/Uncheck All
Check this box to enable or disable all available Imaris licenses on the system.
Please note that the Feature “ImarisBase” can't be disabled because this would render Imaris unusable.

The default Imaris Configurator executable can be found here:

Windows 7, 8 and 10:
64-bit: C:\Program Files\Bitplane\Imaris 9.0\ImarisConfigurator.exe

Mac OS X:
/Applications/Imaris 9.0.app/Contents/MacOS/ImarisConfigurator

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use the **Add License Location** button to add a new license location.

Add License Location Button
Use this button to add new licenses. A description is given in the Add License Section.

Delete License Location
If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you selected the right table entry. In case of a node-locked license you are asked if you want to keep the license. It is recommended that you keep all license files.

License Location Table
This table lists all available license locations. Normally it would be just enough to have one license location defined. But there exist also good reasons to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations and the licensed features are merged on your system automatically.

The table shows also some additional Information:
- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers shows you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

Licenses Table
In this table you have a detailed overview of all licensed features of a license location. You can switch between different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :
Version, Type, Status, Expiration
The validity of a license is also indicated by different colors.

Finish Button
This button is only enabled if you added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes Imaris

Configurator and you can proceed using Imaris.

Add License Window

First, you have to select your licensing scheme:

Download Node-locked License:
If you decide to add a Node-locked License, it will check that your computer is connected to the internet. If the connection can be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

Online-Licensing Wizard:
This wizard has three steps:

1. Select the Network Interface
You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

2. Check your Order-ID for newly ordered Products:
If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the “Check Order-ID” button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.
If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

3. Select the Licenses for Installation
In this step you just have to mark all products you want to install by checking them in the left table.
The right table is for your information only and shows all currently installed products.
When you click on the “**OK**” button, the license file is downloaded and the corresponding entry is added to the License Location table.

Offline-Licensing Wizard:
If you are not connected to the Internet, this could be due to two reasons:
1. your computer is behind a web proxy server which omits a direct connection or
2. your PC may be unplugged from the internet .

Therefore you have the following choices:

A) Set up a Proxy Server
Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.
If not, either the credentials you entered are not correct or the connection is cut at another place in the network. In that case please contact your network administrator or Support teams eusupport_imaris@andor.com or ussupport_imaris@andor.com

If you want to delete a previously set up proxy server, just delete both fields and click on the button again.
The proxy server settings can also be altered in Imaris' system preferences dialog.

B) Download the Node-locked License from another Computer
You can download the license from another computer connected to the internet.
On that machine log on to http://www.bitplane.com/activation/acti_offline_ident.html

and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and you could transfer the file afterwards to the offline computer (e.g. by using a USB stick).
This instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.
When you click **OK** the license file is copied to the proper location.

Define Floating License Server:
In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

Install License from Existing File:
If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

See also:
Menu Edit - Preferences... - Calculation
Menu Edit - Preferences... - Licenses
Menu Edit - Preferences... - Display

13.10 Maintenance Plan

Imaris Infinity

A message is shown when your Imaris Infinity is about to expire.

If your contract has lapsed, the message indicates when that occurred.

You can renew your software maintenance contract for one or more years at a time. During the validity period of your Imaris Infinity contract, you may install all updates to the program, contact our team for technical support as well as access a wide range of educational and training services/tools.

Click on the Contact Sales button to send an email requesting a renewal of your Imaris Infinity contract.

Click on the **Remind me later** button to be reminded again in seven days.

If you click **No, Thanks** button and later change your mind and do wish to be reminded, change the setting in the the Preferences/Update dialog.

13.11 Check for Updates now ...

If your computer is connected to the Internet, Imaris can directly check, if a software update is available.

If new updates are available, a list of the available software versions is shown.

For a detailed list of new features, improvements and fixed bugs, please refer to the release notes.

13.12 System Information

Opens a new window with system information, copy (Ctrl+C) this information in case you run into issues and wish to send to support.

13.13 What's New

Opens a link to our What's New webpage imaris.oxinst.com/newrelease

13.14 Newsletter

Opens a link to sign up for our Imaris Newsletter, available at imaris.oxinst.com/newsletter-sign-up

13.15 Imaris

If your computer is connected to the Internet, Imaris can directly open the homepage imaris.oxinst.com

13.16 About Imaris

The Imaris start screen displays.

Imaris
Version number and release date.

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All rights reserved

European Patent Registry No. 05009677.7
Patent Pending.

Using XML parser expat: Copyright
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Section 14 Toolbars

In the standard Imaris view there are three different toolbars available. The Main Toolbar, the Status Bar and the Time Bar.

See also:
Surpass View - Overview - Camera Toolbar

14.1 Main Toolbar

In All Views		
	Arena	
	Surpass	
	Vantage	
	What's New	
	Newsletter	
	Imaris	
In Arena View		
	Observe Folder	
	New Folder	
	New Batch	
	New Plot	
In Surpass View		
	Open	
	Import (drop down)	Import Segmentation/Label Import Scene Import Spots or Tracks Add Image/Volume
	Save	


Save As


3D View


Slice


Section


Image Processing


Coloc


Animation


Snapshot

In Vantage View


Save


Save As


1D View


2D View


Time View


Spatial View


Objects View


Snapshot

14.2 Status Bar

At the bottom of the screen you find the Status Bar.

Zoom %

Imaris lets you enlarge or reduce the viewing size of your data set as is required with the Zoom function. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value. At 100% zoom factor one pixel on screen represents one voxel.

Mouse Clicks to Zoom

With a three-button mouse:

- Click with the middle mouse button on the image, hold down the middle mouse button, and drag the mouse up or down.
- Hold the Shift-key and click with the right mouse button on the image, and drag the mouse up or down.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Button Fit

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button Reset

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

Button Full Screen

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window, click on the button Full Screen in the lower left corner.

Button Navi

Click on this button to toggle the display of the Navigation window of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. In the pointer mode Select you can click & drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner.

Change Position of the Navigation Window

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section the position of the Navigation window is fixed (lower right corner) and can not be moved.

14.3 Time Bar

If a time series is loaded, a Time Bar displays at the bottom of the main window. It contains a slider showing all Time Points in the series, and control buttons (described below).

The Time Bar is visible in the display modes Slice, Section, Gallery 3D Preview, and Surpass. It allows you to view a particular Time Point or to automatically play 4D images. With the cursor on the time bar, users with a two wheel mouse can use the side scroller to move through the time series.

Play and Record Time Series

Reference Frame button 
Sets the reference frame playmode. Refer to Reference Frame.

When using reference frames, colored tick marks indicate where the reference frames are located in time as shown below for frames 0, 3, 5, 8 and 10:



The color of the tick mark indicates if the Keyframe is a Translation (green) and/or Rotational (magenta) Reference Frame. In the above example, the reference frame located at frame 3 is both translational and rotational, frame 5 is rotational only.

Time Settings button 

Click on the time settings button to open the time parameter settings.

Jump to previous event and **Jump to next event** buttons 
Use these options to move between event increments in the playback e.g. frames.

Play button 
Click on this button to play the time series. To pause the continuous display through all the Time Points in the time series re-click on the button.

Record button 
To record the time series as movie click on the record button. The Export Movie window is displayed (see below).

Slider
To inspect any the Time Point in the time series manually navigate the slider as follows (be sure that the Pointer is in the Select mode):

- Drag the slider handle to the left or right.
- Moving the slider to the left reduces the time point, moving it to the right increase time point.
- Select the slider handle with a left mouse click and then use the Arrow-keys to the left/to the right on the

keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last Time Point.

The respective Time Point number is displayed on the right hand side of the slider in the number field.

Window: Export Movie

File name
Enter a file name.

Export type
Select the desired file type (TIFF series, or AVI).

Movie Settings

Compression Factor
Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. Moving the slider to the left reduces the compression point, moving it to the right increase compression pint. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate
Define how many frames are displayed per second.

Play Movie with default Media Player when finished
Check the box to play the movie with the default media player when finished.

See also:
Menu Edit - Preferences ... - Time (default parameter settings)
Surpass View - Overview - Camera Toolbar (Pointer Select)

Section 15 Addendum

In this addendum you find the Imaris global Mouse & Keyboard PC and Mouse & Keyboard Mac functions, some tips and tricks on how to customize your Imaris Interface for your daily routine, and a Terminology table with frequently used technical terms and specific image processing terms.

15.1 Mouse & Keyboard PC

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

1	Pointer selection mode
2	Circle selection mode
Ctrl + middle scroll	Changes the size of the circle in circle selection mode
Drag	Navigate
O	for object selection mode
T	for track selection mode
Ctrl + 1	Activates the 3D view
Ctrl + 2	Activates the Slice
Ctrl + 3	Activates the Section
Ctrl + 4	Activates the Key Frame Animation
Ctrl + 5	Activates the Image Processing
Ctrl + 6	Activates the Coloc
Ctrl + A	Centers the image to the window
Ctrl + B	Adjusts zoom factor and pan position to best fit the image into the window
Ctrl + C	Copies current view to the clipboard
Ctrl + D	Shows the Display Adjustment window
Ctrl + E	Saves Scene file
Ctrl + F	Refreshes the textures of all views
Ctrl + I	Allows to inspect and edit the image properties
Ctrl + L	Opens Scene file
Ctrl + N	Toggles display of Navigation window
Ctrl + O	Opens existing file
Ctrl + P	Allows to edit the settings of Imaris
Ctrl + Q	Exits the application
Ctrl + R	Discards all changes and opens the current data set again
Ctrl + S	Saves the current data set
Ctrl + T	Saves the current view as a *.tif file
Ctrl + W	Crops X, Y, or Z
Ctrl + Z	Revokes the last action
Ctrl + Shift + A	Adds channels of another image
Ctrl + Shift + B	Deletes selected channels
Ctrl + Shift + C	Copies the Imaris version, date and build to the clipboard
Ctrl + Shift + I	Open an additional image without closing the existing image.

Ctrl + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F4	Displays or updates the license status
F3	Automatic check for updates
F11	Maximizes the viewing area to the full size of your monitor
Ctrl + F1	Opens Quick Start Tutorials
Alt + F4	Exits the application
Del	Deletes selected objects
Right-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Left-click & drag	Rotate image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective Section in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - Mouse & Keyboard PC
Slice -	Mouse & Keyboard PC
Section -	Mouse & Keyboard PC
3D View -	Overview - Mouse & Keyboard PC
	Clipping Plane - Mouse & Keyboard PC
	Contour Surface -Shortcuts Overview
	External Object - Mouse & Keyboard PC
	Filament - Mouse & Keyboard PC
	Light Source - Mouse & Keyboard PC
	Measurement Point - Mouse & Keyboard PC
	Oblique Slicer - Slicer Interaction
	Ortho Slicer - Slicer Interaction
	Surface-Shortcuts Overview
	Spots - Mouse & Keyboard PC
Animation -	Mouse & Keyboard PC
Coloc -	Mouse & Keyboard PC

Context Sensitive Help

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

15.2 Mouse & Keyboard Mac

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

1	Pointer selection mode
2	Circle selection mode
Ctrl + middle scroll	Changes the size of the circle in circle selection mode
Drag	Navigate
O	for object selection mode
T	for track selection mode
Command + 1	Activates the 3D View
Command + 2	Activates the Slice
Command + 3	Activates the Section
Command + 4	Activates the Key Frame Animation
Command + 5	Activates the Image Processing
Command + 6	Activates the Coloc
Command + A	Centers the image to the window
Command + B	Adjusts zoom factor and pan position to best fit the image into the window
Command + C	Copies current view to the clipboard
Command + D	Shows the Display Adjustment window
Command + E	Saves Scene file
Command + F	Refreshes the textures of all views
Command + I	Allows to inspect and edit the image properties
Command + L	Opens Scene file
Command + N	Toggles display of Navigation window
Command + O	Opens existing file
Command + ,	Allows to edit the settings of Imaris
Command + Q	Exits the application
Command + R	Discards all changes and opens the current data set again
Command + S	Saves the current data set
Command + T	Saves the current view as a *.tif file
Command + W	Crops X, Y, or Z
Command + Z	Revokes the last action
Command + Shift + A	Adds channels of another image
Command + Shift + B	Deletes selected channels
Command + Shift + C	Copies the Imaris version, date and build to the clipboard
Command + Shift + P	Allows to edit figures and annotations
Ctrl + Shift + I	Open an additional image without closing the existing image.
F1	Opens Reference Manual
F4	Displays or updates the license status
F3	Automatic check for updates
Command + F11	Maximizes the viewing area to the full size of your monitor

Command + F1	Opens Quick Start Tutorials
Del	Deletes selected objects
Ctrl-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective Section in this Reference Manual for details.

Edit -	Show Display Adjustments - Mouse & Keyboard Mac
Slice -	Mouse & Keyboard Mac
Section -	Mouse & Keyboard Mac
3D View -	Overview - Mouse & Keyboard Mac
	Clipping Plane - Mouse & Keyboard Mac
	Contour Surface - Shortcuts Overview
	External Object - Mouse & Keyboard Mac
	Filament - Mouse & Keyboard Mac
	Light Source - Mouse & Keyboard Mac
	Measurement Point - Mouse & Keyboard Mac
	Oblique Slicer - Slicer Interaction
	Ortho Slicer - Slicer Interaction
	Surface-Shortcuts Overview
	Spots - Mouse & Keyboard Mac
Animation -	Mouse & Keyboard Mac
Coloc -	Mouse & Keyboard Mac

Context Sensitive Help

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

15.3 Customize Interface

In this Section you find a collection of tips and tricks to customize the Imaris interface for your daily routine.

Histogram

Logarithmic or linear histogram display.

- On the left hand side of a histogram there are horizontal lines representing a linear or logarithmic histogram display.
- Click on these lines to change the display from logarithmic to linear and vice versa.

The default setting is logarithmic.

Windows

You can plug windows in Imaris in or out.

Example: Key Frame Animation window

Plug out:
- Click on the header of the Key Frame Animation window and drag the window to any free space on the screen.
- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged out, you can drag it on any free space on the screen.

Plug in:
- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged in on its original place.

Tabbed Toolbars

Try to undock the toolbars and dock them all at the same side. Especially in combination with Annotation, this saves a lot of screen area.

Example: In the Section you can bring the toolbar Figures (Annotation), Camera, and Properties to the left side at the bottom.



Objects Toolbar

You can adjust the Objects toolbar to your personal preferences.

- Open the menu Edit - Preferences - 3D View - Object Creation Buttons to adjust your personal Objects toolbar.

Display Mode Blend and Bland Opacity

You can select the Display Mode Blend in the Surpass view for a Volume object, in the Section (Extended) and in the Easy 3D . Any change in the Display Adjustment window in the parameter Blend Opacity is only visible, if a Blend Mode is selected.

15.4 Terminology

Term	Description
Channel	A channel is a particular piece of information that has been recorded for all voxels. Most of the time in light microscopy, the different channels are intensities that have been measured at different wavelengths. For instance, a two-channel data set contains two intensities for each voxel, one intensity measured in channel 1 (e.g., green light) and one intensity in channel 2 (e.g., red light). Ideally, the intensity values present in different channels are independent of one another.
Coordinates	Any position in the data set can be described either in the voxel grid (e.g., voxel no. 5 in x-direction) or by a coordinate, which corresponds to a m). The distance relative to a defined origin in the real world (e.g. 2 latter) requires the correct calibration of the data set, i.e. that the voxel size is defined appropriately. Generally, in Imaris the origin of the data set is in the left bottom corner. If the data set was cropped from a larger data set, the bottom left corner may have minimum coordinates that are not equal to (0,0,0).
Image stack	A 3D data set is sometimes called an image stack consisting of 2D images stacked on each other.
MIP	A maximum intensity projection (MIP) is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.
Original Data Set	A 3D data set that cannot be derived from any other data set using Bitplane software. Usually "Original data sets" have been acquired using an image sensor and are loaded into Imaris.
Rendering	A technique that visualizes a multi-dimensional data set as a two-dimensional image that can be displayed on a computer screen or printed on a laser printer.
Resultant Image	An image that has been computed using Imaris.
Standard Deviation	In probability and statistics, the standard deviation of a probability distribution, random variable, or population or multiset of values is a measure of the spread of its values. It is defined as the square root of the variance. The standard deviation is the root mean square (RMS) deviation of values from their arithmetic mean. For example, in the population {4, 8}, the mean is 6 and the standard deviation is 2. This may be written: {4, 8} 6. In this case 100% of the values in the population are at one standard deviation of the mean. The standard deviation is the most common measure of statistical dispersion, measuring how widely spread the values in a data set are. If the data points are close to the mean, then the standard deviation is small. Conversely, if many data points are far from the

	mean, then the standard deviation is large. If all the data values are equal, then the standard deviation is zero. The standard deviation (s) of a population can be estimated by a modified standard deviation (s) of a sample.
Threshold	A threshold in Imaris is a gray value, which acts as a limit. All gray values above (or below) are assigned a particular functionality.
Time Point	A time point is a single 3D image containing one or many channels. The term is used regardless of what data is actually stored in the channels. In particular, the channels could contain data taken at different points in time with a changing object.
Volume Rendering	A rendering technique which operates directly on the voxel data. The input image consisting of a collection of voxels arranged in a regular grid is converted directly into the 2D output image.
Voxels	A volume data set is composed of volume elements called voxels. The voxels are the smallest units within the image about which we have distinct information in the form of a measured intensity. The dimensions of an individual voxel are specified in most image file formats supported by Imaris.

OXFORD INSTRUMENTS **IMARIS**



Image credit: 16-cell sea urchin embryo. DNA and endosomes are marked with Hoechst and Rhodamine. Courtesy Dr. Joseph Campanale