# Imaris
# Reference Manual

BITPLANE
SCIENTIFIC SOLUTIONS

# 1 Preface

This Reference Manual provides a description of all menu entries, display modes, functions and parameters.

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For further questions or suggestions please visit our web site at:
www.bitplane.com or contact support@bitplane.com.

**Bitplane AG**
Badenerstrasse 682
8048 Zürich
Switzerland


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## 1.1 Getting Familiar

Today, all optical microscopes commercially available can record several channels simultaneously to produce multi-channel images. Imaris is an application designed to visualize such microscopic data. Imaris uses a special file format to store 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.

It provides 8 different viewing functions for the visualization and production of high quality images for presentation and storage:

- A Slice viewer.
- A Section viewer for simultaneous viewing along three coordinate axes.
- A Gallery viewer for slice image overview and selection.
- The Easy 3D viewer provides a quick image view.
- The Surpass viewer, which offers numerous tools for data preparation, presentation and manipulation of different types of data display as well as any combination of them and the ability to define, combine and group an arbitrary number of objects out of a set of viewing objects.
- Animations can be created from the Slice and 3D modes, or with the key frame animator in Surpass.
- InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view.
- A viewer for Colocalization computation.

It is easy to navigate within the Imaris modules because the frequently used toolbars, menus, and interactive controls remain the same, and can all be operated with the mouse buttons.

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## 1.2 Getting Started

The software is delivered on a standard CD or downloaded from www.bitplane.com. The CD includes a folder containing the necessary manuals, or the manuals can be downloaded.

Minimum hardware/software requirements are:

- Windows NT 4.0, or a more recent version, Windows 2000 or XP
- CD-ROM
- Graphics card with 3D accelerator
- Network facilities for image import from the microscope
- 512 MB RAM (> 1 GB recommended)

Bitplane also recommends:

- A database for storing images (e.g., Image Access)

### Installation

To install the software, please proceed as follows:

- Insert your Imaris CD-Rom in the computer.
- Follow the instructions on the screen.
- The installation is completed automatically.

### Licensing

To run the Imaris system, the appropriate licenses for the required modules, such as the Imaris base (including Surpass), ImarisTime, ImarisColoc, ImarisMeasurementPro, or Topography. Without licenses, the Imaris can only be run in a restricted mode. In case of any license problems, please refer to the support information on our website www.bitplane.com for detailed instructions.

### 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.
- Imaris can be started directly from the Image Access database.

The software opens with the main screen.

### Supported File Formats

The following file formats are supported by Imaris:

- Imaris 3
- Imaris Classic
- ICS file
- Zeiss: LSM510
- Zeiss: LSM410, LSM310
- Zeiss: Axiovision
- Leica: TCS-NT
- Leica: Series
- Leica: LCS
- Biorad: MRC 1024, 600
- Till: TILLvisiON
- Universal Imaging: Metamorph STK
- Delta Vision

- Olympus: FluoView
- Olympus: Cell R
- Olympus: OIF
- Olympus: OIB
- Open Microscopy Environment XML (OME)
- Scanalytics: IPLab
- Tiff (series)
- Tiff (adjustable series)
- BMP (series)

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# 2 Menu File

| | |
|---|---|
| Open... | Ctrl + O |
| Revert to File | Ctrl + R |
| Save as... | Ctrl + S |
| Batch Convert... | |
| Load Scene... | Ctrl + L |
| Export Scene as... | Ctrl + E |
| Snapshot... | Ctrl + T |
| Exit | Ctrl + Q |

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See also:
Addendum - Mouse & Keyboard Mac

## 2.1 Open

Data sets can be loaded from various file formats.

Image File Series

If the data set consists of a whole series of images, each stored as individual file, select only one file to open and the system will automatically load the rest of the images that belong to the data set.

- In the menu select File – Open.
- Select file type from the Files of Type pull-down menu.
- Select a file name from the list and click Open or double-click on the requested file entry.

The file is loaded.

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See also:
Menu File - Batch Convert ...

### 2.1.1 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 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 Menu File - Open or click on the Open button in the Main toolbar.
- Left-click a file to highlight it and click on 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.

- Left-click on Open and the data set is cropped and resampled while loading.

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

### 2.1.2 Settings

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

Leica LCS Settings

A LeicaVista 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.
- Left-click the *.lei file to highlight it and click Settings.

Select the required image stack on the left side of the dialog box. The individual images that belong to that stack will display on the right side.

- Click OK to open the image.

## Leica LIF Settings

A LeicaLIF 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 LIF.
- Left-click the *.lif file to highlight it and click Settings.

Select the required image stack on the left side of the dialog box. The individual images that belong to that stack will display on the right side.

- Click OK to open the image.

## Adjustable Tiff Series Reader Settings

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 selected sequence is shown in the File Arrangement panel. The reader can handle tiff series with single and multiple running numbers.

- Select the menu File - Open or click Open in the Main toolbar.
- Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.
- Open the folder containing the series.
- Click Settings, which is grayed out if the file type selector is on automatic or if the current directory does not contain a series.
- Use Apply Automatic File Filter, Apply, Dimensions, Dimension Sequence, described as follows, to define the series.

**Apply Automatic File Filter**
This is activated automatically when the dialog is opened. It has the same logics as the classic TIFF series reader of Imaris and will pre-select the first series detected in the directory. Be aware that you may not see all files in the directory.

**Button Apply**
Press this button to use the regular expression to the left and select all files in the current directory that match the criterion, i.e.

- *.tif selects all files with the ending *.tif.
- myfile*.tif selects all files that start with “myfile” and are followed by any letter or digit and by the extension *.tif.
- myfile??.tif selects all files that start with “myfile” and are followed by two letters or digits and by the extension *.tif.

Please note: Depending on the filter, not all files in the directory may be visible. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

**Dimensions**
- Define the dimensions of the image starting with Slices (Z), Channels (Ch), and Time Points (T).

Please note: The total number of files in the series, as defined by your selection criteria, is displayed below the Time Points input box. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

**Dimension Sequence**
- Defines how the individual images, which are sorted alphabetically, are to be assigned to Slices (Z), Channels (Ch), and Time Points (T).
- Click OK to return to the Open dialog window.
- Click Resampling Open to open the Resampling dialog box or click Open to open the image.

## 2.2 Revert to File

Re-opens the actual data set.

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## 2.3 Save as ...

Saving in Imaris format is recommended whenever the data set is cropped or the parameters changed. Saving a data set in Imaris file format provides the advantage of a faster loading process and the possibility of using thumbnails. In addition, most parameters are saved with the images.

- In the menu bar select File – Save as... . The Save As window 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.

Available File Formats in Imaris

Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)
Tiff (series)(*.tif *.tiff)
RGBA-Tiff (series)(*.tif *.tiff)
ICS file (*.ics *.ids)
Olympus: cell^R 1.1/standard (*.tif *.tiff)
Open Microscopy Environment Xml (*.ome)
Open Microscopy Environment Tiff (*.tif *.tiff)
BMP (series)(*.bmp)

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See also:
Menu File - Batch Convert...

### 2.3.1 Advanced Save Options

**Button Format Settings...**
A click on the button Format Settings... opens the Imaris Save Options window.

**Time Series**
**Save as Single File**
The time series are saved in a single file.

**Save as Multiple Files**
For each time point a new file is generated.

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

Add to ImageAccess Database

Check the box to add the file to the ImageAccess database.

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## 2.4 Batch Convert ...

With the 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 Open.

### Thumbnail

Here you can select the appearance of the thumbnail in Imaris.

**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.

### Output

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

**Special 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 special folder.

**Format**
The standard formats are:
Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)

To add additional formats please refer to chapter Menu File - Batch Convert ... - Preferences.

### Input

Here you find the selected input file path(s).

Click on the input path to open the window Series Reading Sequences to adjust additional parameters for the conversion. Please refer to chapter Menu File - Open - Settings for details.

### Output

Here you find the selected output file path(s).

Click on the output path to open the window Imaris Save Options to adjust additional parameters for the conversion. Please refer to chapter Menu File - Save as ... - Advanced Save Options for details.

Clear Row

To clear a row click on the red cross at the end of the row.

**Button Clear**
Click on this button to clear all rows in the table.

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

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See also:
Menu File - Open - Reader Configuration
Menu File - Open - Settings
Menu File - Save as ... - Advanced Save Options
Menu File - Batch Convert ... - Preferences

### 2.4.1 Preferences

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

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.

**Cache File Paths:**
Display of the cache file paths.

**Button Add**
**Button Remove**
You can use the buttons to add or 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 licence 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).

**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.

Standard formats:
Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)

Additional formats:
Tiff (series)(*.tif *.tiff)
RGBA-Tiff (series)(*.tif *.tiff)
ICS file (*.ics *.ids)
Olympus: cell^R 1.1/standard (*.tif *.tiff)
Open Microscopy Environment Xml (*.ome)
Open Microscopy Environment Tiff (*.tif *.tiff)
BMP (series)(*.bmp)

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See also:
Menu File - Save as ...

## 2.5 Load Scene ...

A Scene comprises the Surpass Tree including all existing Items. This Scene can be saved as an Imaris Scene File with the extension *.imx. The Scene can be loaded again to the same data set or to another data set.

Load Scene...

Select the directory and requested file to be loaded, and click OK. The Scene File is loaded.

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See also:
Menu File - Export Scene as ...
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

## 2.6 Export Scene as...

A Scene comprises the Surpass Tree including all existing Items. This Scene can be saved as an Imaris Scene File with the extension *.imx. The Scene can be loaded again to the same data set or to another data set.

Export Scene as...

Select the directory and enter the Scene File name and click OK. The Surpass Tree Items are saved as Imaris Scene File.

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See also:
Menu File - Load Scene ...
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

## 2.7 Snapshot

Saving 2D still images is supported by the Snapshot option. Still images can be stored in TIFF-Format or as database records in ImageAccess in order to manage them more efficiently. The process of creating a snapshot in these formats has been semi-automated in Imaris, there is an automatic window size control adjustment implemented.

Image Size

**Template**
Select Windows, Numerical, Size from Image or an Image Size Template.

There are certain points in the image size to be aware of:

- If you choose as image size a very small or very big sized format an exclamation point appears behind the selected size.
- Use big sizes only if you need the image for a poster, in publications or on covers (print media).

**Width**
If you select as Template Numerical the width is adjustable. If you select any other Template, the width is displayed.

**Height**
If you select as Template Numerical the height is adjustable. If you select any other Template, the width is displayed.

**Button Lock Aspect Ratio**
If you select as Template Numerical you can click on this button to lock the aspect ratio.

**Aspect Ratio**
Display of the Aspect Ratio.

Image Output

**Save to File**
Click on the button Save as and select the requested directory. Accept the automatic naming suggestion or enter a new name for the image. Then click on the button Do Snapshot!

**Add to ImageAccess Database**
Make sure that the Database ImageAccess is started. Select the requested directory and enter a name for the image. Click on the button Do Snapshot! The image is saved on the disk and an entry is added to the database.

**Copy to Clipboard**
Click on the button Do Snapshot! and the image is copied to the clipboard. Open another application and select the paste function.

File Path and **Button Save as...**
Here you select the file path for the image output. The image is not saved yet, you just selected the right path and name for the image.

**Button Do Snapshot!**
Click on the button Do Snapshot! to save the image. Automatically the image number for the next snapshot file is increased in consecutively.

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See also:
Menu Edit - Copy Snapshot Image
Menu Edit - Preferences - Display - Off Screen Rendering
Toolbars - Main Toolbar - Snapshot

## 2.8 Exit

Terminates Imaris and returns to the desktop.

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# 3 Menu Edit

| | |
|---|---|
| Undo | Ctrl + Z |
| Copy Snapshot Image | Ctrl + C |
| Image Properties... | Ctrl + I |
| Show Display Adjustment | Ctrl + D |

| | |
|---|---|
| InPress | Ctrl + Shift + P |
| Add Time Points... | |
| Delete Time Points... | |
| Add Channels... | Ctrl + Shift + A |
| Delete Channels... | Ctrl + Shift + B |
| Add Slices... | |
| Delete Slices... | |
| Crop Time... | |
| Resample Time... | |
| Crop 3D... | Ctrl + W |
| Resample 3D... | |
| Change Data Type... | |
| Preferences... | Ctrl + P |
| File Type Associations... | |

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See also:
Addendum - Mouse & Keyboard Mac

## 3.1 Undo

Use the undo function to undo the most recent image processing function.

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## 3.2 Copy Snapshot Image

When an image is ready to be stored, make sure that it is fully visible on the screen and not obstructed by any other windows or displays. To save the image, Imaris reads from the internal buffer, so other objects on the screen would appear superimposed on the image.

- Select Edit - Copy Snapshot Image and the image is copied to the clipboard.

Open another application and select the paste function. The image is pasted into the new application.

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See also:
Menu File - Snapshot

## 3.3 Image Properties...

Geometry
Data Set
Channels
(Channel 1...n)
Thumbnail
Parameters

When opening a data set, the following parameters should be checked or modified:

- Name and Description (in Data Set).
- Voxel Sizes (in Geometry).
- Channel Colors (in Channel 1...n).

---

### 3.3.1 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.

Coordinates

**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.

**Unit, nm, um, mm, m, unknown**
Here you can select the unit.

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.

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### 3.3.2 Data Set

Name

Data field to type in a data set name.

Description

Data field to type in 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.

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### 3.3.3 Channels

There is no parameter on this card.

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### 3.3.4 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.

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#### 3.3.4.1 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.

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#### 3.3.4.2 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.

To apply the changes click on OK.

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#### 3.3.4.3 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 chapter 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.

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See also:
Coloc - Volume Statistics

### 3.3.5 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.

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### 3.3.6 Parameters

A set of informational parameters is appended to the image file.

**Button Add Group...**
Opens a dialog to add a new group.

**Button Delete Group...**
Deletes a group.

**Button Add Parameter...**
Opens a dialog to add a new parameter to the group.

**Button Delete Parameter...**
Deletes a parameter.

---

## 3.4 Show Display Adjustments

The Display Adjustment function lets 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 is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.

The Channel Visibility adjustment allows you to switch the individual channels on or off and to change channel parameters such as name, color and description. Check or un-check the required channel check-box to switch the channel visibility.

**Button Advanced**
Click on the button to open the Advanced settings.

**Select all Channels**
Click the requested channel to select it, or check Select all Channels to apply the settings to all channels.

Histogram

The histogram shows a linear voxel representation of the selected channel.

Range

**Min**
**Max**
Drag the upper or lower handle of the adjustment line to adjust the Min and Max limits or enter direct values in the Min and Max fields. The effect of the change can be seen on the channels (channels appear brighter or darker).

**Button Reset**
Click the Reset button to set the image back to the original values.

**Button Auto**
When clicking the Auto button the system detects the real high and low values (e.g. 10 - 150) and sets the Max. and Min. limits automatically to these values.
If you check the parameter Select all Channels (see above) all channels have the same Min. and Max. limits.

**Button Auto Blend**
This button is useful if you display your data in the Blend mode (item Volume - tab Settings - Mode Blend). Click on this button and Imaris automatically calculates the optimized Min. and Max. limits for the Blend mode. A good portion of the selected image channel becomes transparent.
If you check the parameter Select all Channels (see above), all channels are calculated consecutively.

Blend Opacity

The Blend Opacity adjustment allows you to change the opacity in real-time in Blend projections in Section, Full 3D, and Surpass Volume views. Drag the Blend Opacity slider bar to adjust the blend opacity. The effect of the change can be seen on the channels (channels appear more or less transparent).

Change Channel Color

Left-click on the channel name to switch directly to the channel properties.

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See also:
Menu Edit - Image Properties... - Channel 1...n

### 3.4.1 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Display Adjustments Window

| | |
|---|---|
| Ctrl + D | Shows the Display Adjustment window |
| Left-click | Select channel |
| Ctrl + left-click | Add channel to selection, or remove channel from selection |
| Left-click & drag | Move left: make image channel brighter<br>Move right: make image channel darker<br>Move up: increase image channel contrast<br>Move down: decrease image channel contrast |
| Right-click & drag | Move left: make image channel transparent<br>Move right: make image channel opaque |
| Ctrl + right-click | Automatic range for Min and Max |

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See also:
Addendum - Mouse & Keyboard PC

### 3.4.2 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Display Adjustments Window

| | |
|---|---|
| Command + D | Shows the Display Adjustment window |
| Click | Select channel |
| Command + click | Add channel to selection, or remove channel from selection |
| Click & drag | Move left: make image channel brighter<br>Move right: make image channel darker<br>Move up: increase image channel contrast<br>Move down: decrease image channel contrast |
| Ctrl + click & drag | Move left: make image channel transparent<br>Move right: make image channel opaque |
| Command + Ctrl + click | Automatic range for Min and Max |

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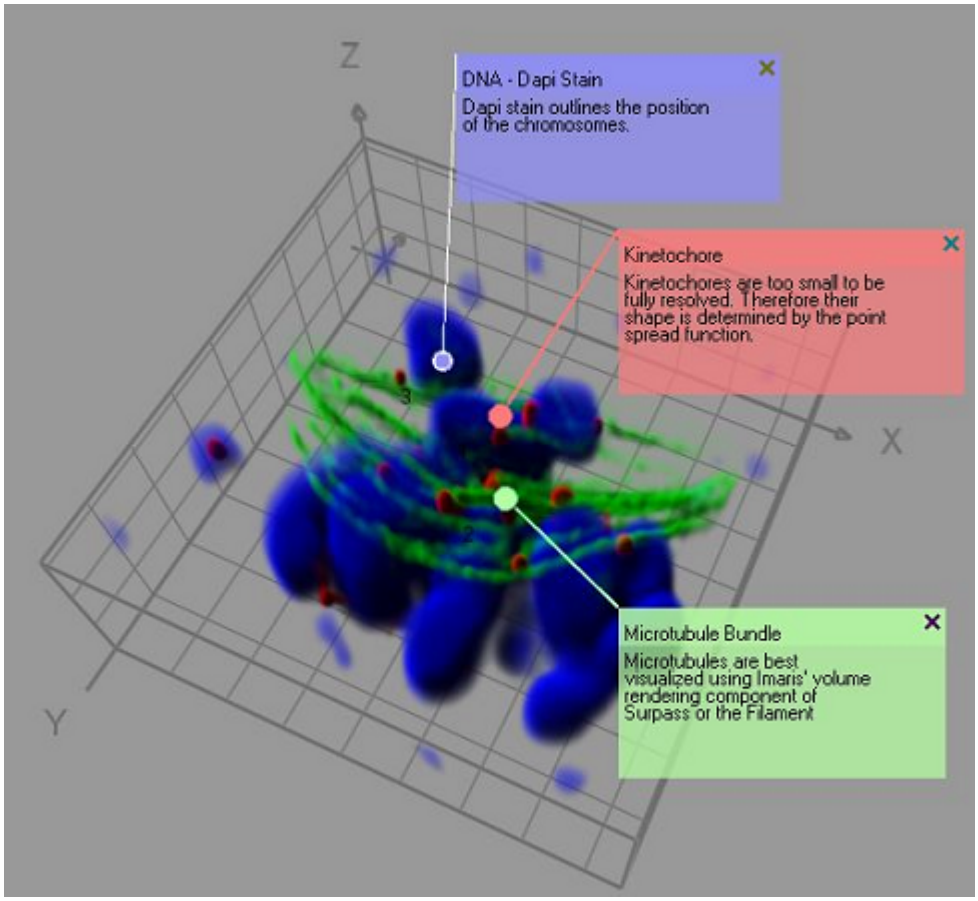
See also:
Addendum - Mouse & Keyboard Mac

## 3.5 InPress

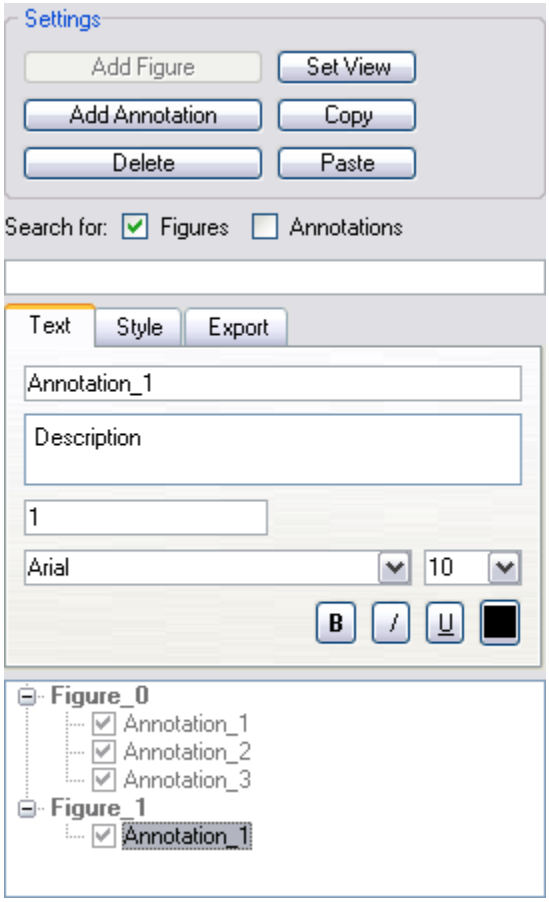



- Figures are composed of annotations or symbols, a 3D attachment site, and text.
- Annotations and symbols are 2D elements which assures optimal reading.
- The attachment point and the line are dynamic and update as you change the view or the camera angle.
- Figures are exported to Html.

---

### 3.5.1 Insert Figures and Annotations


- To activate this module press the InPress button, press Ctrl + Shift + P, or in the menu bar select Menu File – InPress. To close Imaris InPress press the InPress button.
- The Figures window appears on the right side of the screen.
- The Figures window is open in all Imaris views and applies to all views.
- For better handling it can also be un-docked by dragging it off its integrated position or clicking on the docking icon (top right, next to x).




- Labeling that occurs in Imaris InPress has two main parts. A Figure and an Annotation.
- Annotations are the actual labels that are displayed in an image.
- A Figure is a container for the annotations.
- A Figure can contain zero, one, or more than one annotations.
- For a Figure, Imaris remembers the view that you were in and the camera position.

## Create a Figure

- Click on Add Figure. In the first row of the tab Text the standard text header Figure_0 is displayed. To name a figure type the name in the text box. In the second row you can add a figure description. A figure label appears in the Imaris InPress Tree. To jump to the view and camera position associated with a figure double-click the figure name in the Figure Tree. To change the view associated with a particular figure, highlight the name of the figure in the list, move to the Imaris view of interest, and press Set View button from the Settings of Imaris InPress

## Add Annotations

Annotations can be added to any figure you want to create.

- First highlight the figure name in the list that you want the annotation added to.
- Click on Add Annotation. In the first row of the tab Text the standard text header Annotation_0 is displayed. You can edit the text.
- In the second row you can add an annotation text.

## InPress Figure Tree

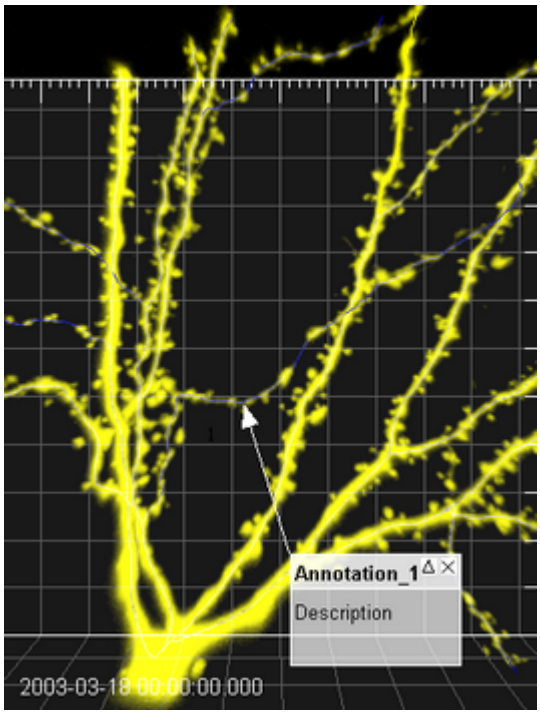
In the Figure Tree all existing figures and annotations are displayed.

---

See also:

Menu File - Export Scene as ...

### 3.5.2 Position Box




- Left-click in the image to set the 3D anchor point.
- Move the mouse to elongate the line. With the second left-click you fix the top left corner of the text box
- Move the mouse to adjust the text box size. With the third left-click you fix the text box size.

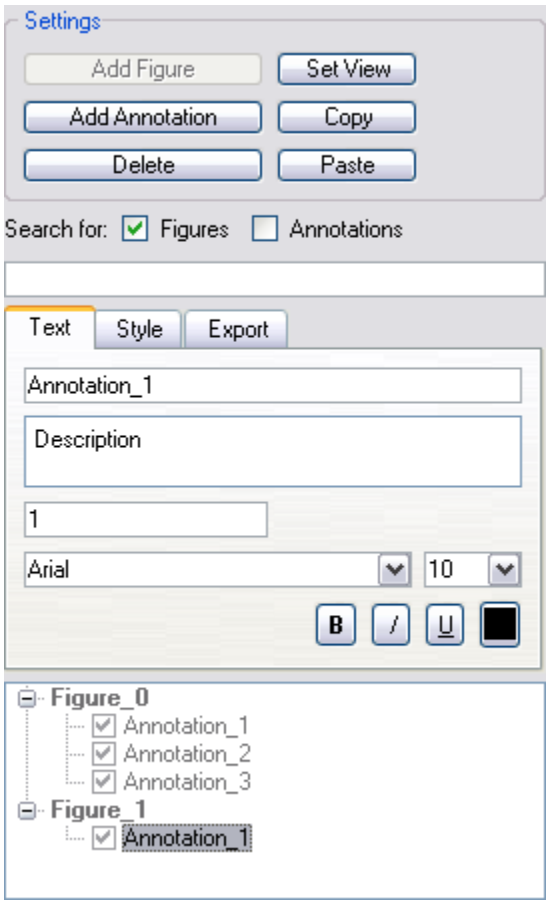
#### Move, Resize, and Hide the Annotation Box

- The 3D anchor point can be moved by putting the mouse over the anchor point (until it changes to a cross) then left-click and drag the anchor point to a new location.
- The annotation box can be moved by putting the mouse on the upper region of the box (until it changes to a cross) then left-click and drag the annotation box to a new location.
- The annotation box can be resized by moving the mouse to an edge of the box (until the mouse changes to a resize icon) and then left-click and drag the edge.
- The description area of the annotation can be closed (hidden) or expanded (shown) by clicking the triangle on the top right of any annotation box.
- The annotation can be hidden by clicking the “X” on the top right corner of the box or hidden / show by (un) checking the box in front of the annotation in the InPress list view
- The annotation number can be moved by putting the mouse over it (until it changes to a cross) then left-click and drag the annotation number to a new location.

#### Save Figures and Annotations in Scene File

The figure legends are saved in the scene file of a data set.

### 3.5.3 Settings




The first buttons in the left row are to Add Figure, Add Annotation and Delete selected figures/annotations. With a left-click on the button Set View you can save the actual image view for the selected figure. Left-click on the button Copy to copy the selected figure/annotation. Left-click on Paste to paste the copied figure/annotation.

Search for Figures and Annotations

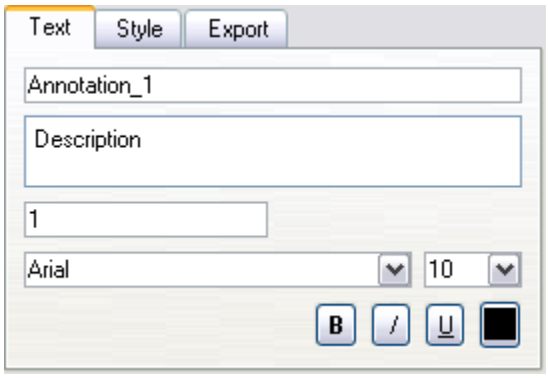
You can search for the initials in the title field (first row in the tab Text) of a Figure or Annotation.

---

### 3.5.4 Tab Text - Style - Export

Tab Text

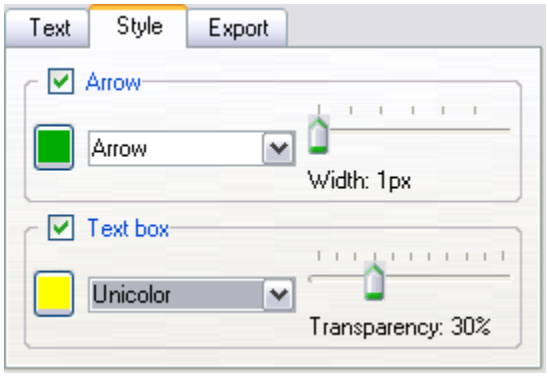
The tab Text allows for the entry of all the text fields for an annotation.




- The top line controls the annotation title field.
- The second line controls the description field (text).
- The third box by default contains the annotation running number (legend). To remove the number from the image, delete it from the text field
- The font, font size, and color can be selected (from any installed system fonts). The settings for the different rows (title filed, text field, and legend field) can be adjusted separately. To display the default settings click on the Menu Edit - Preferences... and select InPress).

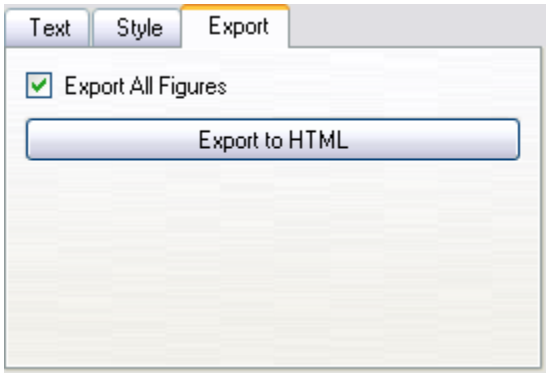
## Tab Style

The tab Style changes the style and color of the text box and pointer for an annotation.




- The first check-box turns on or off the Arrow.
- With a left-click on the color selection you can change the color of the graphical element. Click it to change the pointer to any desired color.
- In the drop-down list you select the shape of the pointer. The pointer can have three possibilities; a sphere, an arrow, or a magnifier.
- The slider controls the size of the line and the pointer.
- The second check-box turns on or off the Text box.
- With a left-click on the color selection you can change the color of the text box.
- The pointer can be colored in three ways; uni-color, black text area, or a black title area.
- The slider controls transparency of the text box.

## Tab Export




- Check the check-box if you want to export all figures.
- Highlight the figure in the InPress Figure Tree that you want to Export.
- With a click on the button Export to HTML you open the Export Figure as HTML window.
- Choose the desired file path, enter a file name to save the document as.
- A web page is created that contains the image name and location, each channel name, the number of time points, the image size, the number of slices, the voxel sizes, a snapshot of the view that is associated with the figure, and the figure name.

## 3.6 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 appears if the formatting does not match.

## 3.7 Delete Time Points...

Superfluous time points 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.

## 3.8 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 requested 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 issued.

## 3.9 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:
Menu File - Open - Reader Configuration

## 3.10 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.

---

## 3.11 Delete Slices...

Parameters

**Slice number [1...n]**
Specify the slice to be deleted and click OK. The slice is automatically deleted from the data set.

---

## 3.12 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:
Menu File - Open - Reader Configuration

## 3.13 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:
Menu File - Open - Reader Configuration

## 3.14 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

Display of 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**
Modify the size and the position of the region of interest by entering the direct values in the corresponding x-, y-, and z-fields or as follows:

- To move the ROI, click inside the rectangle with the left mouse button, hold it down and drag the entire ROI around.
- To shape the ROI, click on a handle with the left mouse button, hold 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.

Click OK when finished. The data set is cut down to the marked ROI. The rest is erased.

---

See also:
Menu File - Open - Reader Configuration

## 3.15 Resample 3D...

Resampling reduces the voxel density in a data set to fasten its processing. Reducing the data size also deteriorates 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:
Menu File - Open - Reader Configuration
Menu Image Processing - Smoothing - Gaussian Filter

## 3.16 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).

---

## 3.17 Preferences...

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

System
Display
Loading
Calculation
Time
Surpass
Statistics
Licences
Tools
Update
3D Cursor
InPress
Advanced

---

### 3.17.1 System

Displays the basic system parameters of your Windows computer.

Processor

**Number of Processor**
**Processor Architecture**
**Processor Speed**
Information about the number of Processors, Processor Type and Processor Speed.

Graphics

**OpenGL Renderer**
**OpenGL Version**
**Pixel Shader**
**OpenGL Extensions**
Information about the OpenGL Renderer, OpenGL Version, Pixel Shader, and OpenGL Extensions.

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.

**Button Hardware Settings**
Click on this button to open the Hardware Settings window (see below).

Data Cache

**Memory Limit**
The memory limit defines how much RAM memory Imaris can use before caching on the disk starts. The value must stay below the total amount of installed RAM on the system to work properly. 32-bit systems can not handle more than 2-3 GB per application.

Display

**Texture Cache Limit**
The texture cache limit defines how much VideoRAM, RAM Imaris can use for textures. This should be set to the same value as the amount of VideoRAM on your graphics board.

**Open GL Test**
The result of the open GL test displays on the right hand side.
- Congratulations, your graphics board is able to display huge data.
- Your graphics board is not capable of displaying huge data. Some features will be unavailable.

## 3.17.2 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.

**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. Set the value to the memory of your graphics card.

Colors

**Background Color**

Normal background if an image does not completely fill a view. Background color for Surpass.

**Button Select...**
Click on this 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.

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

**Checkered Background for Blending**
When using the blending mode, a checkered background displays in Full 3D blend and in Surpass.

**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.

**Button Select...**
Click on this 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 view and the Surpass view).

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

Coordinate Axis/Scale Bar

**Show Coordinates Axis**
**Show Date**
**Show Scale Bar**
**Show Time**
**Show relative Time**
Select to display coordinate axis, date, scale bar or time on screen in Slider, Section, or Gallery view.

**Off Screen Rendering (for saving Snapshots and Movies)**
Check this box to save only the viewing area. For the Snapshot and Movie Imaris hides additional control elements. If you un-check this box the actual screen display is saved, e.g. if the window Display Adjustments is in front of the viewing area this window is saved as well.

**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.

---

See also:
Menu File - Snapshot

### 3.17.3 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 Cannel**
**Second Channel**
**Third Channel**
**Other Channels**
Display of the defined color.

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

Automatic File Format Detection Sequence

Allows you to specify the order in which the file format readers are called when loading images. Move the most frequently used file format readers to the top of the list to hasten file loading.

**Button Move Up**
**Button Move Down**
- Highlight a file reader.
- Click Move Up or Move Down to arrange the format readers in the desired sequence.

## 3.17.4 Calculation

Calculation

**Number of Processors**
Specify the number of processors used in calculations. See maximum number of available processors in the System box.

Image Processing History

**Maximum Number of Commands in History**
Defines the maximum number of image processing operations that can be undone. 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
32 bit Physical memory installed x 0,5; but not higher than 1.2 GB
64 bit Physical memory installed x 0,5

Mac
32 bit Physical memory installed x 0,5; but not higher than 2 GB

**Button Apply**
Press this button to apply the changes.

**Cache File Paths:**
Display of the cache file paths.

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

---

## 3.17.5 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 (never ending).

**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.

---

See also:
Toolbars - Time Bar

## 3.17.6 Surpass

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 Interpolation

**Object Rotation Center (optimizing default user interaction)**
This is the default parameter, select Object Rotation Center to create a rotated animation. The distance from

the camera to the object rotation center is always the same.

**Camera Rotation Center (optimizing fly through animation)**
Select Camera Rotation Center to create a fly through animation. The distance from camera to object is always the same, the rotation is not around a fixed rotation center.

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.

---

See also:
Surpass View - Overview - Surpass Tree (Objects toolbar)

## 3.17.7 Statistics

Show Statistic Values

The most desired set of statistics values can be specified (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. Un-check the box and all statistical values are un-selected.

For details please refer to the respective chapter:

Menu Edit - Preferences ... - Statistics - Contour Surface
Menu Edit - Preferences ... - Statistics - Filament
Menu Edit - Preferences ... - Statistics - IsoSurface
Menu Edit - Preferences ... - Statistics - Measurement Points
Menu Edit - Preferences ... - Statistics - Spot
Menu Edit - Preferences ... - Statistics - Spots
Menu Edit - Preferences ... - Statistics - Surface Object
Menu Edit - Preferences ... - Statistics - Track
Menu Edit - Preferences ... - Statistics - Volume

---

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

### 3.17.7.1 Contour Surface

Contour Surface statistics are automatically computed for each Contour Surface.

Show Statistic Values - Contour Surface

**Contour Surface - Area**
The sum of the triangle surfaces.

**Contour Surface - Center of Image Mass X**
**Contour Surface - Center of Image Mass Y**

**Contour Surface - Center of Image Mass Z**
Center of Image Mass (the voxel intensities are not equal):

$$R = \frac{1}{M} \sum_{i \in IsoSurface} m_i r_i$$

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

**Contour Surface - Center of homogeneous Mass X**
**Contour Surface - Center of homogeneous Mass Y**
**Contour Surface - Center of homogeneous Mass Z**
Center of homogeneous Mass (voxel intensities are equal):

$$R = \frac{1}{n} \sum_{i \in IsoSurface} r_i$$

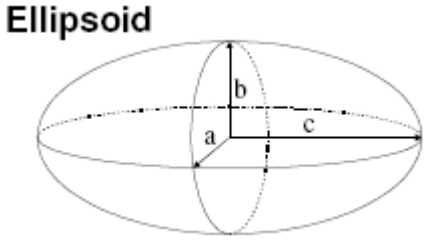
R : center of homogeneous mass (x y z)
$r_i$ : center of a voxel ($x_i$ $y_i$ $z_i$)
n : number of i € IsoSurface

## 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.




**Contour Surface - Ellipsoid Axis A X**
**Contour Surface - Ellipsoid Axis A Y**
**Contour Surface - Ellipsoid Axis A Z**
These three parameters define the Vector of the Ellipsoid Axis a.

**Contour Surface - Ellipsoid Axis B X**
**Contour Surface - Ellipsoid Axis B Y**
**Contour Surface - Ellipsoid Axis B Z**
These three parameters define the Vector of the Ellipsoid Axis b.

**Contour Surface - Ellipsoid Axis C X**
**Contour Surface - Ellipsoid Axis C Y**
**Contour Surface - Ellipsoid Axis C Z**
These three parameters define the Vector of the Ellipsoid Axis c.

**Contour Surface - Ellipsoid Axis Length A**
**Contour Surface - Ellipsoid Axis Length B**
**Contour Surface - Ellipsoid Axis Length C**

**Contour Surface - Ellipsoid (oblate)**
**Contour Surface - 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)
- $a \neq b \neq c$ 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)

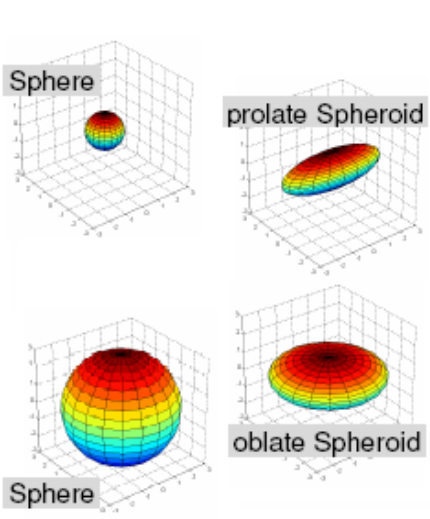
$$e_{prolate} = \frac{2a^2}{a^2+b^2} * \left(1 - \frac{a^2+b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2+c^2} * \left(1 - \frac{2a^2}{b^2+c^2}\right)$$

$e_{oblate}$ = 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 |



**Contour Surface - Intensity Max**
**Contour Surface - Intensity Mean**
**Contour Surface - Intensity Median**
**Contour Surface - Intensity Min**
**Contour Surface - Intensity StdDev**
**Contour Surface - Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Contour Surface (Max, Mean, Median, Min, StdDev and Sum).

**Contour Surface - Number of Triangles**
Number of the Contour Surface triangles.

**Contour Surface - Number of Voxels**
Number of the Contour Surface voxels.

**Contour Surface - Sphericity**
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, $\Psi$, 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

**Contour Surface - Volume**
Volume is a quantification of how much space an object occupies.

---

See also:
Surpass View - Contour Surface - Tab Statistics
Addendum - Terminology (Standard Deviation)

### 3.17.7.2 Filament

Filament statistics are automatically computed for each Filament.

Each point of a filament line has its individual measured diameter. The diameter is measured as shortest distance from the center line to the IsoSurface defined by the lower threshold (automatic creation).

Show Statistic Values - Filament

**Filament - Branch Point Diameter**
The diameter at a branch point.

**Filament - Full Depth**
Each point of the graph has a depth depending on the root point. The depth of a point is defined by the number of branch point of the (shortest) path to the root point. Full depth is the depth of the point with the largest number of branchings.

**Filament - Full Length**
The sum of the lengths of all lines within the filament.

**Filament - No. Branch Points**
The number of branch points.

**Filament - No. Edges**
The filament graph is composed of a lot of small linear components called edges. The size depends on the voxel size of the original data set.

**Filament - No. End Segments**
Equal to the number of end points.

**Filament - No. Segments**
A segment is the path between branch points or end points. This value counts also the number of end segments.

**Filament - No. Unconnected Filaments**
Parts of the filament graph without any connection. When splitting the filament the same number of filament objects will be created. Each new filament will consist of one connected piece.

**Filament - No. Vertices**
A vertex is a point within the filament graph which is connected through edges. Some special vertices are end points, branch points or the root point.

**Filament - Segment Avg. Diameter**
Average diameter within a segment. If a root point is defined there is also a corresponding depth available.

**Filament - Segment Length**
The sum of length of all edges which compose a segment. If a root point is defined there is also a corresponding depth available.

**Filament - Segment Resistance**
This value is proportional to the length and indirect proportional to the cross-section area. A variable diameter is considered. Multiply this value with an electrical material constant to obtain an electrical resistor (composed from small cones).

**Filament - Segment Volume**
The sum of volume of all edges (cones) which compose a segment. If a root point is defined there is also a corresponding depth available.

**Filament - Terminal Point Diameter**
The diameter of an end point.

---

See also:
Surpass View - Filament - Tab Statistics
Surpass View - Filament - Tab Edit (Button Split)

#### 3.17.7.3 IsoSurface

IsoSurface statistics are automatically computed for each IsoSurface.

The IsoSurface 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 real volume object.

Show Statistic Values - IsoSurface

The Surface is made up of connected triangles. The Surface statistics describe the Surface mesh (number of triangles, surface area, enclosed volume).

**IsoSurface - Area**
The sum of the triangle surfaces.

**IsoSurface - Center of Image Mass X**
**IsoSurface - Center of Image Mass Y**
**IsoSurface - Center of Image Mass Z**
Center of Image Mass (the voxel intensities are not equal):

$$R = \frac{1}{M} \sum_{i \in IsoSurface} m_i r_i$$

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

**IsoSurface - Center of homogeneous Mass X**
**IsoSurface - Center of homogeneous Mass Y**
**IsoSurface - Center of homogeneous Mass Z**
Center of homogeneous Mass (voxel intensities are equal):

$$R = \frac{1}{n} \sum_{i \in IsoSurface} r_i$$

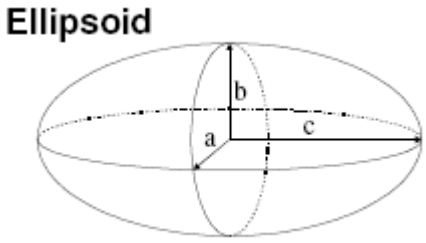
R : center of homogeneous mass (x y z)
$r_i$ : center of a voxel ($x_i$ $y_i$ $z_i$)
n : number of i € IsoSurface

## 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.




**IsoSurface - Ellipsoid Axis A X**
**IsoSurface - Ellipsoid Axis A Y**
**IsoSurface - Ellipsoid Axis A Z**
These three parameters define the Vector of the Ellipsoid Axis a.

**IsoSurface - Ellipsoid Axis B X**
**IsoSurface - Ellipsoid Axis B Y**
**IsoSurface - Ellipsoid Axis B Z**
These three parameters define the Vector of the Ellipsoid Axis b.

**IsoSurface - Ellipsoid Axis C X**

**IsoSurface - Ellipsoid Axis C Y**
**IsoSurface - Ellipsoid Axis C Z**
These three parameters define the Vector of the Ellipsoid Axis c.

**IsoSurface - Ellipsoid Axis Length A**
**IsoSurface - Ellipsoid Axis Length B**
**IsoSurface - Ellipsoid Axis Length C**

**IsoSurface - Ellipsoid (oblate)**
**IsoSurface - 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)
- $a \neq b \neq c$ 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)

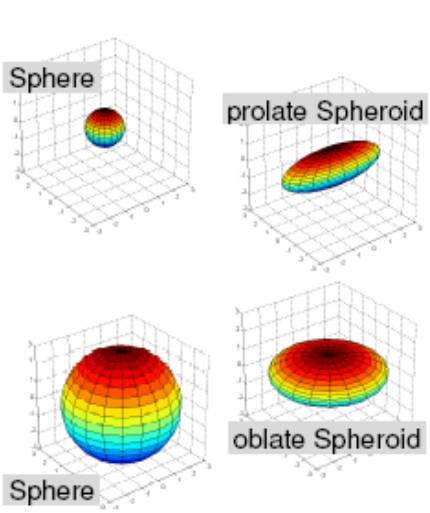
$$e_{prolate} = \frac{2a^2}{a^2+b^2} * \left(1 - \frac{a^2+b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2+c^2} * \left(1 - \frac{2a^2}{b^2+c^2}\right)$$

$e_{oblate}$ = 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 |




**IsoSurface - Intensity Center**

**IsoSurface - Intensity Max**
**IsoSurface - Intensity Mean**
**IsoSurface - Intensity Median**
**IsoSurface - Intensity Min**
**IsoSurface - Intensity StdDev**
**IsoSurface - Intensity Sum**
The voxel intensity statistics describe the voxels enclosed within the IsoSurface (Center, Max, Mean, Median, Min, StdDev and Sum).

**IsoSurface - Number of Triangles**
Number of the IsoSurface triangles.

**IsoSurface - Number of Voxels**
Number of the IsoSurface voxels.

**IsoSurface - Sphericity**
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, $\Psi$, 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

**IsoSurface - Volume**
Volume is a quantification of how much space an object occupies.

---

See also:
Surpass View - IsoSurface - Tab Statistics

#### 3.17.7.4 Measurement Points

Measurement Point statistics are automatically computed for each Measurement Point.

Show Statistic Values - Measurement Points

**Measurement Points - Distance**
Distance between the measurement points.

**Measurement Points - Distance Sum**
Sum of all measured distances.

**Measurement Points - Number of Points**
Number of measurement points.

**Measurement Points - Point Intensity Center**
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.

See also:
Surpass View - Measurement Points - Tab Statistics

### 3.17.7.5 Spot

Spot statistics are automatically computed for each Spot object. To each Spot belongs a spatial position along the x-, y-, and z-axis, as well as the intensity of the point it represents.

Show Statistic Values - Spot

**Spot - Area**
The surface area of the Spot..

**Spot - Intensity Center**
Intensity of the voxel in the Spot center.

**Spot - Intensity Max**
**Spot - Intensity Mean**
**Spot - Intensity Median**
**Spot - Intensity Min**
**Spot - Intensity StdDev**
**Spot - Intensity Sum**
The voxel intensity statistics describe the voxels enclosed within the Spot (Max, Mean, Median, Min, StdDev and Sum).

**Spot - Number of Voxels**
Number of the Spot voxels.

**Spot - Position X**
**Spot - Position Y**
**Spot - Position Z**
Spot position (x y z).

**Spot - Volume**
Volume is a quantification of how much space a Spot object occupies.

See also:
Surpass View - Spots - Tab Statistics
Addendum - Terminology (Standard Deviation)

### 3.17.7.6 Spots

Spots statistics are automatically computed for each Spots object. To each Spot belongs a spatial position along the x-, y-, and z-axis, as well as the intensity of the point it represents.

Show Statistic Values - Spots

**Spots - Area**
The sum of the triangle surfaces.

**Spots - Intensity Center**
Intensity of the voxel in the Spot(s) center.

**Spots - Intensity Max**
**Spots - Intensity Mean**
**Spots - Intensity Median**
**Spots - Intensity Min**
**Spots - Intensity StdDev**
**Spots - Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Spots (Max, Mean, Median, Min, StdDev and Sum).

**Spots - Number of Voxels**
Number of the Spot(s) voxels.

**Spots - Position X**
**Spots - Position Y**
**Spots - Position Z**
Spots position (x y z).

**Spots - Volume**
Volume is a quantification of how much space an Spots object occupies.

---

See also:
Surpass View - Spots - Tab Statistics
Addendum - Terminology (Standard Deviation)

### 3.17.7.7 Surface Object

Surface object statistics are automatically computed for each Surface object.

Show Statistic Values - Surface Object

**Surface Object - Area**
The sum of the triangle surfaces.

**Surface Object - Center of Image Mass X**
**Surface Object - Center of Image Mass Y**
**Surface Object - Center of Image Mass Z**
Center of Image Mass (the voxel intensities are not equal):

$$R = \frac{1}{M} \sum_{i \in IsoSurface} m_i r_i$$

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

**Surface Object - Center of homogeneous Mass X**
**Surface Object - Center of homogeneous Mass Y**
**Surface Object - Center of homogeneous Mass Z**
Center of homogeneous Mass (voxel intensities are equal):

$$R = \frac{1}{n} \sum_{i \in IsoSurface} r_i$$

R : center of homogeneous mass (x y z)
$r_i$ : center of a voxel ($x_i$ $y_i$ $z_i$)
n : number of i € IsoSurface

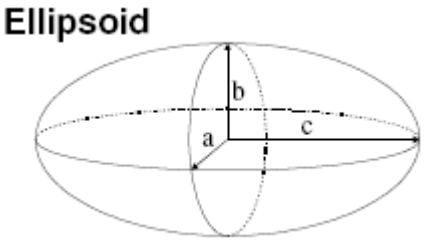
Ellipsoid Axis

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.




**Surface Object - Ellipsoid Axis A X**
**Surface Object - Ellipsoid Axis A Y**
**Surface Object - Ellipsoid Axis A Z**
These three parameters define the Vector of the Ellipsoid Axis a.

**Surface Object - Ellipsoid Axis B X**
**Surface Object - Ellipsoid Axis B Y**
**Surface Object - Ellipsoid Axis B Z**
These three parameters define the Vector of the Ellipsoid Axis b.

**Surface Object - Ellipsoid Axis C X**
**Surface Object - Ellipsoid Axis C Y**
**Surface Object - Ellipsoid Axis C Z**
These three parameters define the Vector of the Ellipsoid Axis c.

**Surface Object - Ellipsoid Axis Length A**
**Surface Object - Ellipsoid Axis Length B**
**Surface Object - Ellipsoid Axis Length C**
**Surface Object - Ellipsoid (oblate)**
**Surface Object - Ellipsoid (prolate)**

If we assume $a \le b \le c$ , then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)
- $a \ne b \ne c$ 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)

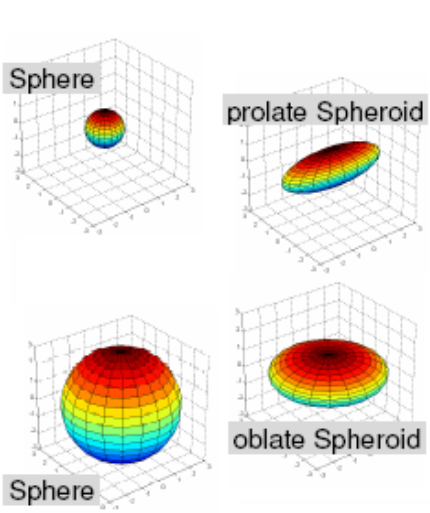
$$e_{prolate}=\frac{2a^2}{a^2+b^2}*\left(1-\frac{a^2+b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} * \left(1 - \frac{2a^2}{b^2 + c^2}\right)$$

$e_{oblate}$ = 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 |




**Surface Object - Intensity Max**
**Surface Object - Intensity Mean**
**Surface Object - Intensity Median**
**Surface Object - Intensity Min**
**Surface Object - Intensity StdDev**
**Surface Object - Intensity Sum**
The voxel intensity statistics describe the voxels enclosed within the Surface object (Max, Mean, Median, Min, StdDev and Sum).

**Surface Object - Number of Triangles**
Number of the Surface object triangles.

**Surface Object - Number of Voxels**
Number of the Surface object voxels.

**Surface Object - Sphericity**
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, $\Psi$, 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 Object - Volume**
Volume is a quantification of how much space an object occupies.

See also:
Surpass View - Surface Object - Tab Statistics

### 3.17.7.8 Track

When a tracking is done statistics such as Track Length, Track Speed, Number of Track Branches and Number of Track Fusions, are made available.

Show Statistic Values - Track

**Track - Number Of Branches**
A branch occurs when a Surface object is connected to several other Surface objects of a future time point.

**Track - Number of Fusions**
A fusion occurs when a Surface object is connected to several Surface objects of a past time point.

**Track - AR1**
An autoregressive time series model of order 1 is fit to the first difference of track positions in X, Y, Z, respectively.

**Track - AR1Mean**
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

**Track - AR1X**
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F+1}^{t_L-n} (D_X(t,t-1) - \overline{D}_x)(D_x(t+n,t+(n-1) - \overline{D}_x)$$

$t_L$ = last time index of track
$t_F$ = first time index of track

$$D_X(t_1,t_2) = P_X(t_1) - P_X(t_2)$$

$P_x(t)$ = 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

**Track - AR1Y**
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

**Track - AR1Z**
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

**Track - Track Displacement**
The Track Displacement is the distance between first and last position.

$$D = \sqrt{D_x(t_L, t_F)^2 + D_Y(t_L, t_F)^2 + D_Z(t_L, t_F)^2}$$

$$D_X(t_1, t_2) = P_X(t_1) - P_X(t_2)$$

D = TrackDisplacement
$t_L$ = last time index of track
$t_F$ = first time index of track
$P_x(t)$ = x-position of object at time index t

**Track - Track Displacement^2**

$$\overline{D}^2(\Delta t) = \sum_{t=t_F+\Delta t}^{t_L} D_X(t, t-\Delta t)^2 + D_Y(t, t-\Delta t)^2 + D_Z(t, t-\Delta t)^2$$

$$D_X(t_1, t_2) = P_X(t_1) - P_X(t_2)$$

$t_L$ = last time index of track
$t_F$ = first time index of track
$P_x(t)$ = x-position of object at time index t

**Track - Track Duration**
The Track Duration is the duration between the first and last time point within the Track.

$$\text{Duration} = T(t_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
$t_L$ = last time index of track
$t_F$ = first time index of track

**Track - Track Length**
The Track Length is the total length of displacements within 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 = TrackLength
$t_L$ = last time index of track
$t_F$ = first time index of track

$$D_X(t_1,t_2) = P_X(t_1) - P_X(t_2)$$

$P_x(t)$ = x-position of object at time index t

**Track - Track Speed**
The Track Speed is the instantaneous speed of the object.

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_Y(t,t-1)^2 + D_Z(t,t-1)^2}}{T(t) - T(t\text{-}1)}$$

S(t) = TrackSpeed

$$D_X(t_1,t_2) = P_X(t_1) - P_X(t_2)$$

$P_x(t)$ = x-position of object at time index t
T(t) = time in seconds at time point t

**Track - Track Speed Avg**
Average of Track Speed. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

**Track - Track Speed Variability**
Standard Deviation of TrackSpeed.

**Track - Track Straightness**

$$\text{Straightness} = \frac{\text{Displacement}}{\text{Length}}$$

Straightness = TrackStraightness
Displacement = TrackDisplacement
Length = TrackLength

---

See also:
Surpass View - Track - Tab Statistics

### 3.17.7.9 Volume

Volume statistics are automatically computed for each Volume object.

Show Statistic Values - Volume

**Volume - Data Intensity Mean**
**Volume - Data Intensity StdDev**
**Volume - Data Intensity Sum**
The voxel intensity statistics describe the voxels pf the whole dataset (Mean, StdDev and Sum).

**Volume - Data Volume**
Volume is a quantification of how much space an object occupies.

**Volume - Number of Voxels**

Number of the Volume voxels.

---

See also:
Surpass View - Volume - Tab Statistics
Addendum - Terminology (Standard Deviation)

### 3.17.8 Licenses

Here you can adjust the default license settings.

Available Licenses/Licenses Found

**Feature Licenses**
Display of all existing Imaris licenses on the system. If a particular license is currently not available (another computer is probably using it), it will be displayed grayed. 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 and all available Imaris licenses on the system are selected.

**File Reader Licenses**
Display of the actual available file reader licenses on the system.

**Check/Uncheck All**
Check this box and all available file reader licenses on the system are selected.

**Information**
Information text field how to configure the settings.

---

See also:
Menu Help - License...

### 3.17.9 Tools

Custom Tools/Objects

You can custom tools and objects using the following buttons.

**Button Import (Overwrite)...**
A click on this button opens the window Import Custom Tools File.

**Button Import (Merge)...**
A click on this button opens the window Import Custom Tools File.

**Button Export...**
A click on this button opens the window Export Custom Tools File.

---

### 3.17.10 Update

Here you can adjust the default update settings.

How often should be searched for updates?

**Daily**
**Weekly**
**Never**
Select either daily, weekly, or never.

---

## 3.17.11 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

This are the default animation parameters for the function InMotion.

**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.

---

See also:
InMotion

## 3.17.12 InPress

Here you can adjust the default InPress 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).

**Button Select...**
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).

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

---

### 3.17.13 Advanced

Displays a window similar to the parameters window. It shows internal settings and parameters in groups and parameter lists.

Please keep in mind: Changing the advanced parameter settings may endanger your system and lead to erroneous shutdowns!

---

## 3.18 File Type Associations...

The file association dialog for the thumbnail creator (window Imaris Icon and Thumbnail Settings) is displayed.

File Extensions

Thumbnails will be created and MetaData will be extracted from all checked file types.

**.ims**
Imaris Series, Imaris Classic

**.imx**
Imaris Scene

**.ics .ids**
Nikon ICS

**.lsm**
Zeiss LSM510

**.zvi**
Zeiss Axiovision

**.lei**
Leica LCS

**.lif**
Leica LIF

**.pic**
Biorad MRC 1024/600

**.rbinf**
TILLvisION

**.stk**
MetaMorph STK

**.r3d .dv**
Delta Vision

**.oib .oif**
Olympus OIB/OIF

**.ipl**
Scanalytics IPLab

**.ome**
Open Microscopy Environment XML

**.inf .info**
Leica Series

**.tif .tiff**
Zeiss LSM 410, Zeiss LSM 310, Leica TCS-NT, Leica Series, Leica LCS, Olympus FluoView, Olympus cellR, PerkinElmer, TIFF series

**More**
Separated by spaces: e.g. ".ims .ics .lsm .zvi".

2D/3D Indicator

The generated thumbnails are created with or without a 2D/3D indicator.

**Show**
Check this box to show the indicator at the left upper corner of the thumbnail. There are four different icons to indicate the image type:

- 2D Image (colored square).
- 2D Time Image (pink square and bars).
- 3D Image (green cube).
- 3D Time Image (blue cube and bars).

# 4 Menu View

| | | Please refer to chapter: |
|---|---|---|
| Slice | Ctrl + 1 | Slice View |
| Section | Ctrl + 2 | Section View |
| Gallery | Ctrl + 3 | Gallery View |
| Easy 3D | Ctrl + 4 | Easy 3D View |
| Surpass | Ctrl + 5 | Surpass View |
| Animation | Ctrl + 6 | Animation |
| InMotion | Ctrl + 7 | InMotion |
| Coloc | Ctrl + 8 | Coloc |
| Fit | Ctrl + B | |

| | |
|---|---|
| 100% | Ctrl + A |
| Full Screen | F11 |
| Navi | Ctrl + N |

Origin Bottom Left
Origin Top Left
Origin Bottom Right
Origin Top Right

| | |
|---|---|
| Status Bar | Toolbars - Status Bar |

| | |
|---|---|
| Refresh | Ctrl + F |

---

See also:
Addendum - Mouse & Keyboard Mac

## 4.1 Fit

Fits the entire scene into the view.

---

See also:
Toolbars - Status Bar (Button Fit)

## 4.2 100%

Select 100% to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

---

See also:
Toolbars - Status Bar (Button 100%)

## 4.3 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.

---

See also:
Toolbars - Status Bar (Button Full Screen)

## 4.4 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.

### Change Position

- In the pointer mode Select you can click and 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 view the position of the Navigation window is fixed (lower right corner) and can not be moved.

### Button Overview

In the Status Bar you find the respective button Navi.

---

See also:
Section View
Toolbars - Status Bar (Button Navi)
Surpass View - Overview - Camera Toolbar (Pointer Select)

## 4.5 Origin

The origin of the coordinate system can be set to any corner of all the views: Bottom Left, Top Left, Bottom Right, or Top Right.

---

## 4.6 Refresh

Refresh the screen.

---

# 5 Menu 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.

**Button Apply**
**Button OK**
If a time series is loaded, clicking Apply applies the image processing function to the current time point displayed on the screen. Clicking OK applies the image processing function to all time points.

Flip ...
Rotate...
Channel Shift...
Swap Time and Z
Swap Time and Channels

Smoothing
- Gaussian Filter...
- Median Filter...

Thresholding
- Baseline Subtraction...
- Threshold Cutoff...
- Background Subtraction...
- Connective Baseline...

Contrast Range
- Linear Stretch...
- Gamma Correction...
- Invert...
- Local Contrast...

AutoDeblur...

---

## 5.1 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.

---

## 5.2 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.

---

## 5.3 Channel Shift...

The Channel Shift function allows 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.

---

## 5.4 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 viewer 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

## 5.5 Swap Time and Channel

Exchange the t-axis and the channels and re-format the image.

Start Function

- Select in the Menu Image Processing - Swap Time and Channel.

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

## 5.6 Smoothing

Whether or not images need to be smoothed depends on their quality. Inspect the image to see if any smoothing is required. For image smoothing several different filters are available:

- Gaussian Filter
- Median Filter

---

### 5.6.1 Gaussian Filter

User Interface

The Gaussian filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures smaller than the filter width will be removed.

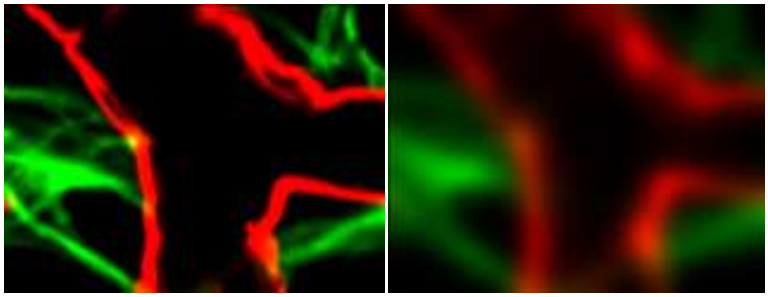
- Select the Menu Image Processing - Image Smoothing - Gaussian Filter. The Gaussian Filter box is displayed.
- Select the channels to be filtered by marking the check-box on the left of the channel.
- Enter the requested filter width in the parameter field and click Apply. The effect of the change can be seen

on the screen.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. To restore the initial image click Cancel.

Function

The Gaussian filter smoothes the image.



It has some nice properties:

- Total image intensity is preserved.
- Noise is suppressed homogeneously.
- No artefacts are introduced.

---

See also:
Surpass View - Filament - Data Input and Filtering

### 5.6.2 Median Filter

User Interface

The Median filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures that are smaller than the filter width will be removed.

- Select the Menu Image Processing - Image Smoothing - Median Filter. The Median Filter box is displayed.
- Select the channels to be filtered by marking the check-box.
- Check the requested Filter Size and click the Apply button. The effect of the change can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original image.

Function

The Median Filter replaces the intensity of each voxel with the statistical median of the intensities of neighboring voxels. There are several choices for the neighboring voxels to be included in the median calculation. The median filter is useful when the image data are corrupted by salt and pepper noise.

---

## 5.7 Thresholding

Whether or not it is necessary to apply a thresholding function to an image depends on its quality. Inspect the image to see if any action is required.

For image thresholding a number of functions are available. The following paragraphs describe the user

interface to the following filters:

- Threshold Cutoff
- Baseline Subtraction
- Background Subtraction
- Connective Baseline

---

### 5.7.1 Threshold Cutoff

Threshold Cutoff compares the intensity of every voxel in the image to the threshold value. If the intensity is greater than the threshold, it remains unchanged. If a voxels has an intensity less than the threshold value it is assigned a new intensity value. This value is 0 by default. The Threshold Cutoff values for the channels may differ.

- Select the Menu Image Processing - Image Thresholding - Threshold Cutoff. The Threshold Cutoff box is displayed, showing a histogram of the voxels in the data set.
- Select the channels to be cut by marking the check-box to the left of the channel bar.
- Highlight a channel.
- A colored bar in the histogram shows which channel has been selected.
- Enter the requested values for that channel in the Threshold field or drag the bar in the histogram.
- Repeat for each channel (if applicable).
- Click Apply. The effect of the changes can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original image.

---

### 5.7.2 Baseline Subtraction

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 voxels intensity is set to zero. The Baseline Subtraction values for the channels may differ.

- Select the Menu Image Processing - Image Thresholding - Baseline Subtraction. The Baseline Subtraction box is displayed, showing a histogram of the voxels in the data set.
- Select the channels to be cut by marking the check-box to the left of the channel bar.
- Highlight a channel.
- A colored bar in the histogram shows which channel has been selected.
- Enter the requested value for that channel in the Baseline field or drag the bar in the histogram.
- Repeat for each channel (if applicable).
- Click Apply. The effect of the changes can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original image.

---

### 5.7.3 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. The filter width is the only parameter of the background subtraction method.

- Select the Menu Image Processing - Thresholding - Background Subtraction. The Background Subtraction box is displayed.

- Select the channels by marking the check-box on the left of the channel.
- Enter the requested filter width in the parameter field and click Apply. The effect of the change can be seen on the screen.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

---

### 5.7.4 Connective Baseline

The Connective Baseline works with two threshold values, a lower 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 (Type A).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold 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 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.

---

See also:
Surpass View - Filament - Data Input and Filtering

## 5.8 Contrast Change

Linear Stretch...

Linear stretching may be necessary whenever 12 bit data is read in the 16 bit mode. Then the contrast can be extended to the new limits. Picture data is changed when stretching. Linear stretching results in a picture displaying dense data in a wider contrast range.

- Select the Menu Image Processing - Contrast Change - Linear Stretch. The Linear Stretch box is displayed.
- Select the channels to be stretched by marking the check-box.
- Enter the requested values in the parameter fields New Maximum and New Minimum and click the Apply button. The effect of the change can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original image.

Gamma Correction...

The Gamma correction function lets you improve the image display by intensifying the gray value of a specific range of voxels. Usually the color contrast values of the voxels stretch linear over a wide range (e.g. 0 - 255). The Gamma correction function lets you set a new gamma value according to which the gamma curve for the voxels is calculated automatically. Thus the brightness of the voxels can be intensified or weakened in certain areas.

- Select the Menu Image Processing - Contrast Change - Gamma Correction. The Gamma Correction box is displayed with the default Gamma Value set to 1, therefore showing a straight line as a linear voxel representation.
- Select the requested channels by clicking marking the check-box.
- Enter the requested value in the Gamma value field or click directly in the gray Gamma Curve area to set

the new gamma value point. The curve and the field value are set accordingly and the effect of the change can be seen on the channels (channels appear differently).
- Press Apply.
- If necessary, readjust the value.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original image

Invert...

The channels can be inverted together or separately.

- Select the Menu Image Processing - Invert. The Invert box is displayed.
- Select the channels to be inverted by marking the check-box on the left. The effect of the change can be seen in the image.
- When the results are satisfactory, click the OK button.

The data set is modified. Click Cancel to return to the original data set.

---

## 5.9 Auto Deblur ...

The AutoDeblur deconvolution software is integrated into Imaris. It requires a separate license. The pathway to AutoDeblur must be set in the Menu Edit - Preferences... - Advanced - Files - AutoDeblurExeFileName.

When opening AutoDeblur, the software will prompt for a destination for the file that is transferred to AutoDeblur and will automatically create an image in the ids/ics format and open it in AutoDeblur.

- Select the Menu Image Processing - AutoDeblur ....

An error message appears if the program is not installed or the pathway is not set.

---

# 6 Menu Surpass

| | Please refer to chapter: |
|---|---|
| Clipping Plane | Surpass View - Clipping Plane |
| Contour Surface | Surpass View - Contour Surface |
| External Object | Surpass View - External Object |
| Filament | Surpass View - Filament |
| Frame | Surpass View - Frame |
| Group | Surpass View - Group |
| IsoSurface | Surpass View - IsoSurface |
| Light Source | Surpass View - Light Source |
| Measurement Point | Surpass View - Measurement Point |
| Oblique Slicer | Surpass View - Oblique Slicer |
| Ortho Slicer | Surpass View - Ortho Slicer |
| Spots | Surpass View - Spots |
| Topography | Surpass View - Topography |
| Track | Surpass View - Track |
| Volume | Surpass View - Volume |

| | |
|---|---|
| Export Selected Objects... | |
| Delete Selected Objects... | Del |
| Switch View Mode | Esc |

## 6.1 Export Selected Objects...

In the Surpass Tree highlight all Items to be exported. To export all IsoSurfaces, Contours, and External objects, select the entire Surpass Scene folder.

- 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 an Inventor file with the extension *.iv and can then be loaded as External objects to another data set.

---

See also:
Surpass View - Overview - Scene File Concept
Surpass View - External Object

## 6.2 Delete Selected Objects ...

In the Surpass Tree highlight all Items to be deleted. To delete all IsoSurfaces, Contours, and External objects, select the entire Surpass Scene folder.

- Select Menu Surpass - Delete Selected Objects… .

The Delete selection window with a confirmation question is displayed.

---

See also:
Surpass View - Overview - Surpass Tree - Objects toolbar - Button Delete ...
Surpass View - Overview - Scene File Concept

## 6.3 Switch View Mode

In the Surpass View there are two viewing modes: Select and Navigate.

**Select**
The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set some Measurement Points on the object surface.

**Navigate**
The cursor becomes two turning arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

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.

---

See also:
Surpass View - Overview - Camera Toolbar (Pointer Select or Navigate)

# 7 Menu Help

| | |
|---|---|
| Quick Start Tutorials | Ctrl + F1 |
| Keyboard and Mouse | F1 |
| Reference Manual | |
| Programming Interface | |

License... F2
Check for Updates now... F3

Bitplane
About Imaris...

---

See also:
Addendum - Mouse & Keyboard Mac

## 7.1 Quick Start Tutorials

The Quick Start Tutorials provide you with the basic information 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 ten 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 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.

---

## 7.2 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 User:

Addendum - Mouse & Keyboard PC

For Mac User:

Addendum - Mouse & Keyboard Mac

---

## 7.3 Reference Manual

The Reference Manual provides you with the basic information about Imaris: The Menu(s), the different Imaris Views and all 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 massage 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 massage the Reference Manual opens in the actual browser and displays the corresponding page.

---

## 7.4 Programming Interface

Select Programming Interface to open the Imaris COM Interface Documentation for ImarisXT.

---

## 7.5 License...

Two different licensing schemes are available for the software, node-locked and floating. Node-locked licenses grant permission to run the licensed software on one computer (node) only. 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.

Node-locked licenses are the easiest way to license the software. The installation of node-locked licenses is guided by the Imaris software itself.

Floating licenses require the installation of a separate license server that can run either on a Windows or on an Irix computer. The server installation requires more technical know how.

### Install and Upgrade Product Licenses for this Computer

**If this computer is connected to the Internet, Imaris can directly download the license information from "www.bitplane.com". Press the "Online" button and follow the instructions.**
**Button Online...**
Press this button and follow the instructions.

**If this computer has no Internet access you can download the license information via another computer with Internet access. Press the "Offline" button and follow the instructions.**
**Button Offline...**
Press this button and follow the instructions.

**If this computer should apply license information from a floating license server, or if you would like to configure a special license file, press this button and follow the instructions.**
**Button Config...**
Press this button and follow the instructions.

**Button Next**
Press this button and the window for the Hardware Settings displays (see below). The button Next is only available after the initial installation of a new Imaris version.

### Hardware Settings

The window for the hardware settings is only available after the initial installation of a new Imaris version (see above) when you click on the button Next. Imaris will try to figure out the optimal settings for your system and displays the values in the fields. You can edit the values. To insert the calculated values again press the button Set Default. The hardware settings are adjustable for each user individually. If you want to change the limits afterwards please go for the Memory Limit to Menu Edit - Preferences... - Calculation and for the Texture Cache Limit to Menu Edit - Preferences... - Display.

### Data Cache

**Memory Limit**
The memory limit defines how much RAM memory Imaris can use before caching on the disk starts. The value must stay below the total amount of installed RAM on the system to work properly. 32-bit systems can not handle more than 2-3 GB per application.

### Display

**Texture Cache Limit**
The texture cache limit defines how much VideoRAM, RAM Imaris can use for textures. This should be set to the same value as the amount of VideoRAM on your graphics board.

**Open GL Test**
The result of the open GL test displays on the right hand side.
- Congratulations, your graphics board is able to display huge data.
- Your graphics board is not capable of displaying huge data. Some features will be unavailable.

Automatic Updates

**Enable**
Check this box and Imaris will look for available updates once a week.

**Button Set Default**
Click on this button to insert the calculated values (see above).

Licenses Running on this Computer

**Name**
Display of all existing Imaris products and their names.

**Type**
Display of the status of the corresponding license on the computer.

**License Path**
Display of the license path. Editable text field.

**Button Clear**
Click on this button to clear the license path.

**Host ID**
Display of the host ID. Editable text field.

---

See also:
Menu Edit - Preferences... - Calculation
Menu Edit - Preferences... - Licenses
Menu Edit - Preferences... - Display

## 7.6 Check for Updates now ...

If your computer is connected to the Internet, Imaris can directly check, if a software Update is available.

---

## 7.7 Bitplane AG

If your computer is connected to the Internet, Imaris can directly open the Bitplane AG homepage (www.bitplane.com).

---

## 7.8 About Imaris...

The Imaris start screen is displayed.

**Imaris**
Version number and release date.



European Patent Registry No. 05009677.7

Patent Pending.

Using XML parser expat: Copyright
1998, 1999, 200. Thai Open Source
Software Center Ltd and Clark Cooper

www.bitplane.com
welcome@bitplane.com

---

# 8 Toolbars

In the standard Imaris View there are three different toolbars available. The Main Toolbar, the Status Bar and the Time Bar.

---

## 8.1 Main Toolbar

| | Please refer to chapter: |
|---|---|
| Open | Menu File - Open |
| Save as | Menu File - Save as |
| Snapshot | Menu File - Snapshot |
| InPress | Menu File - InPress |

The display modes Slice, Section, Gallery and Easy 3D are in one icon group. Click on the arrow to select the respective mode.

| | Please refer to chapter: |
|---|---|
| Slice | Slice View |
| Section | Section View |
| Gallery | Gallery View |
| Easy 3D | Easy 3D View |
| Surpass | Surpass View |
| Animation | Animation |
| InMotion | InMotion |
| Coloc | Coloc |
| Bitplane AG | Menu Help - Bitplane AG |

---

## 8.2 Status Bar

At the bottom of the screen you find the Status Bar.

**Zoom ... pixel/voxel**
Enter the zoom factor.

**Button 100%**
Click on this button to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

**Button Fit**
Click on this button to pan the position to best fit in the window and adjust the 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 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 and drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner. In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

Mouse Clicks to Zoom in Slice View, Section View, Gallery View, Easy 3D View, and Surpass View

- 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.

Please note: In the Surpass view be sure that the mouse is in the pointer Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

---

See also:
Menu View - Fit
Menu View - 100%
Menu View - Full Screen
Menu View - Navi
Section View
Surpass View - Overview - Camera Toolbar (Pointer Navigate/Select)

## 8.3 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, a Time Settings button, Play and Pause button (arrow to the right), and a Record button (red dot).

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. The views change when selecting a new Time Point.

Play and Record Time Series

**Button Time Settings...**
Click on this button and you open directly the Time default parameter settings.

**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.

**Button O**
To record the time series as movie click on the button Record (red dot). The Save As 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.
- 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.

Save as Movie Window

**File name**
Enter a file name.

**Save as type**
Select the desired file type (QuickTimeMovie, 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.

**Add to ImageAccess database**
Check the box to add the file to the ImageAccess database.

**Play Movie with default Media Player when finished**
Check the box to play the movie with the default media player when finished.

QTVR Settings

The QuickTimeVR Settings are not available.

---

See also:
Menu Edit - Preferences ... - Time (default parameter settings)
Surpass View - Overview - Camera Toolbar (Pointer Select)
Surpass View - Overview - Camera Toolbar - QuickTimeVR

# 9 Slice View

How to Open the Slice View?

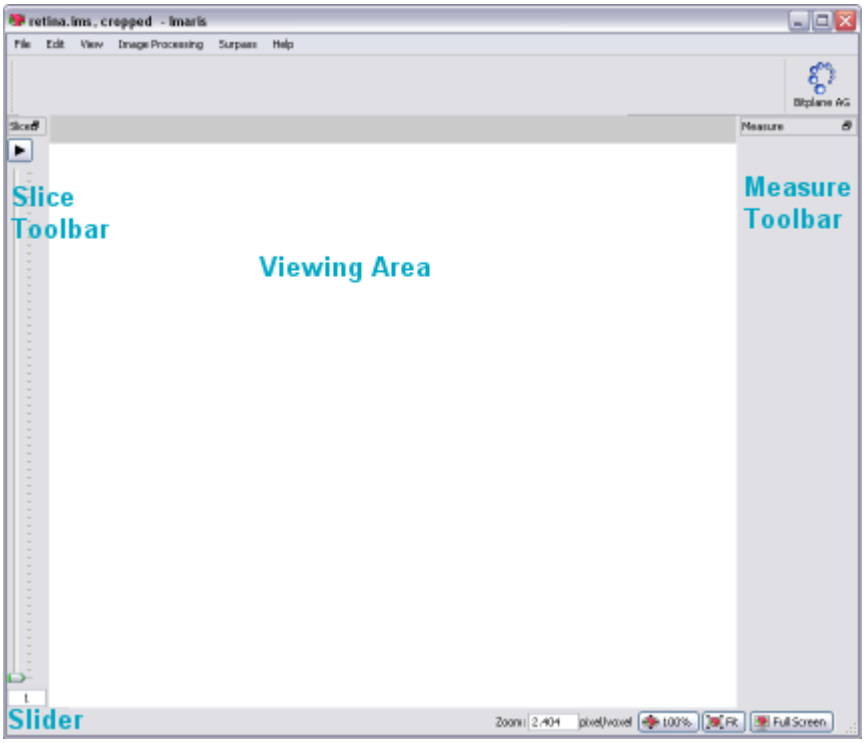
- Click on the icon in the Main toolbar. If the icon Slice is not displayed click on the arrow in the group: Section, Gallery, Easy 3D, and select Slice.
- Select Menu View - Slice.
- Press the key combination Ctrl + 1.

---

See also:
Slice View - Mouse & Keyboard Mac

## 9.1 Overview

The Slice view 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.




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.

Additional Information in the Status Bar

The Slice view 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

## 9.2 Slice Toolbar

Slider

To inspect any slice in the data set along the z-axis you use the Slider. Navigate 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 respectively last slice.
- Select the slider handle with a left mouse click and then press the PageDown-key or the PageUp-key to display the first respectively last slice.
- 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 Filed**

The respective slice number is displayed on the bottom of the slider in the number field.

## 9.3 Measure Toolbar

Type

**Line**
Set the two Measurement Points with two consecutive left-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.

## 9.4 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Slice View

| | |
|---|---|
| Ctrl + 1 | Activates the Slice View |
| Left-click | Set a measurement point |
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>Move down: zoom in |
| Right-click & drag | Pan image |

See also:
Addendum - Mouse & Keyboard PC

## 9.5 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Slice View

| | |
|---|---|
| Command + 1 | Activates the Slice View |
| Click | Set a measurement point |

With a one-button mouse:

| | |
|---|---|
| Shift + Ctrl + click & drag | Move up: zoom out<br>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<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard Mac

# 10 Section View

How to Open the Section View?

- Click on the icon in the Main toolbar. If the icon Section is not displayed click on the arrow in the group: Slice, Gallery, Easy 3D, and select Section.
- Select Menu View - Section.
- Press the key combination Ctrl + 2.

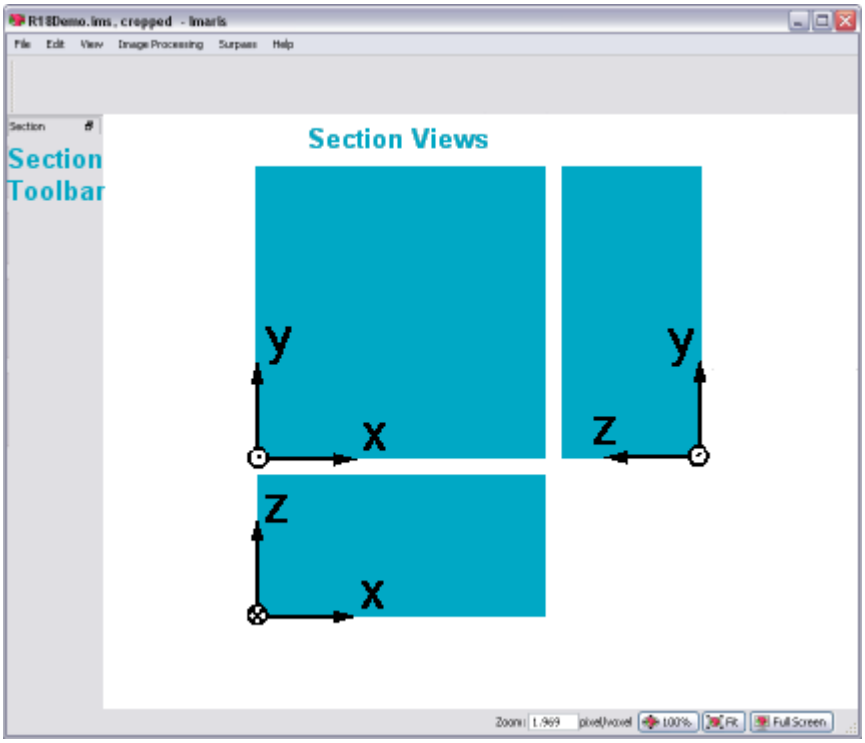
---

See also:
Section View - Mouse & Keyboard Mac

## 10.1 Overview

The Section view 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).

The illustration below shows the coordinates in the three different display areas of the section view.




To select a point, click with the left mouse in any of the three views, hold the button down and 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.

### Additional Information in the Status Bar

The Section view 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

## 10.2 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 un-checking 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 cross hairs. The planes between the cross hairs 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.

Un-check 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.

---

## 10.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Section View

| | |
|---|---|
| Ctrl + 2 | Activates the Section View |
| Left-click | Set the section center |
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard PC

## 10.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Section View

| | |
|---|---|
| Command + 2 | Activates the Section View |
| Click | Set the section center |

With a one-button mouse:

| | |
|---|---|
| Shift + Ctrl + click & drag | Move up: zoom out<br>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<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard Mac

# 11 Gallery View

How to Open the Gallery View?

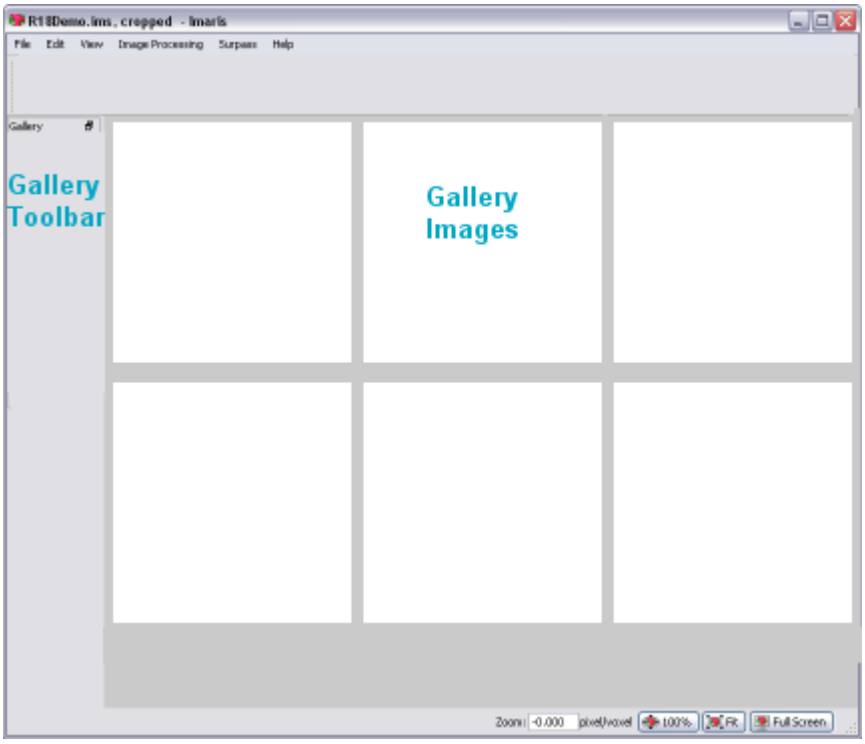
- Click on the icon in the Main toolbar. If the icon Gallery is not displayed click on the arrow in the group: Section, Slice, Easy 3D, and select Gallery.
- Select Menu View - Gallery.
- Press the key combination Ctrl + 3.

---

See also:
Gallery View - Mouse & Keyboard Mac

## 11.1 Overview

The Gallery view displays all available slice images of the data set. In the Gallery view you can adapt the display to your needs to gain more information from the slice series. When the Gallery view is selected, all slices are displayed. Click on images to select or deselect them. Selected images are marked with a colored frame. Holding the Shift-key lets you select a continuous series of images. Hold the Ctrl-key to select various images spread over the gallery.




Additional Information in the Status Bar

The status bar indicates which of how many available pages is currently being displayed, and how many slices have been selected. Changes in the gallery view only affect the current display and do not crop the data set.

---

See also:
Toolbars - Status Bar

## 11.2 Gallery Toolbar

In the Gallery view the following display and selection controls are available:

Slice

By clicking on the arrows, you move up and down the images in the gallery one by one. This is especially useful whenever the display on the screen does not include all the slices.

Page

If there are more images than displayed on screen, move up and down the pages by clicking on the arrows or go directly to the first or last page by using the respective buttons.

Columns

Specify the number of columns to be currently displayed on the screen by clicking on the up/down arrows or by entering the requested number.

Skip

Select every second image from the data set by selecting 1 image to skip, or every third image by selecting 2 images to skip (or any other skip interval analogously, by clicking on the up/down arrows or entering the appropriate number directly).

Display

**Button Select...**
Highlight image(s) and click on the button Select... . Only the selected image(s) is(are) displayed in the gallery. Re-click on the button Select... and all images are displayed in the Gallery.

Slice Number Display

The slice number is displayed beneath each slice.

---

## 11.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Gallery View

| | |
|---|---|
| Ctrl + 3 | Activates the Gallery View |
| Left-click | Select slice |
| Ctrl + left-click | Add single slice to selection |
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard PC

## 11.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Gallery View

| | |
|---|---|
| Command + 3 | Activates the Gallery View |
| Click | Select slice |
| Shift + click | Add slices to selection |
| Command-click | Add single slice to selection |

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

# 12 Easy 3D View

How to Open the Easy 3D View?

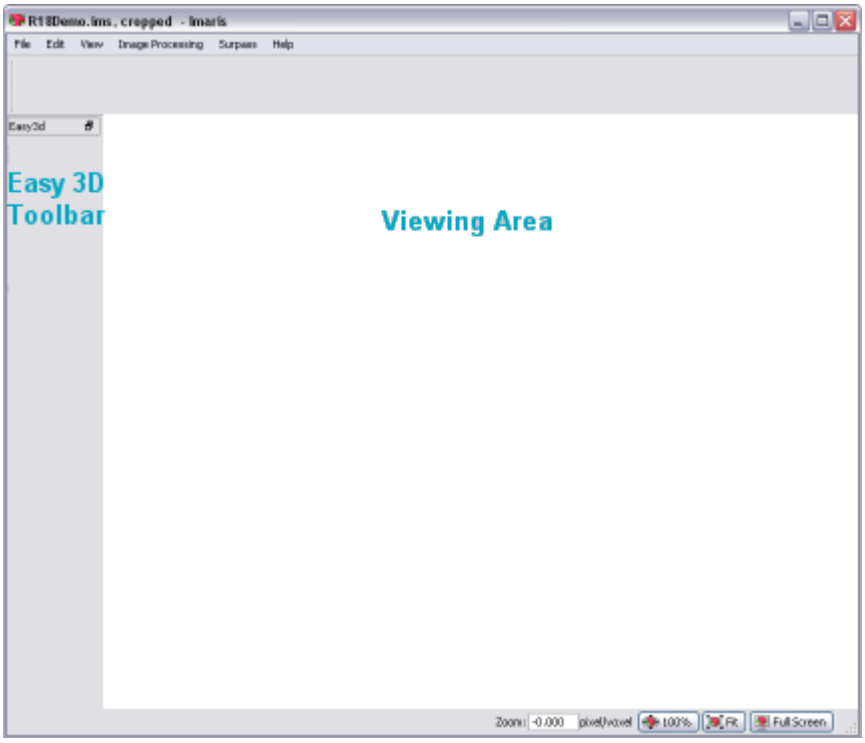
- Click on the icon in the Main toolbar. If the icon Easy 3D is not displayed click on the arrow in the group: Section, Gallery, Slice, and select Easy 3D.
- Select Menu View - Easy 3D.
- Press the key combination Ctrl + 4.

---

See also:
Easy 3D View - Mouse & Keyboard Mac

## 12.1 Overview

The Easy 3D view provides a quick fixed view with a few parameters for rendering and light setting. This lets you explore the data set in the 3D mode before doing any further processing.




## 12.2 Easy 3D Toolbar

### Rendering

**MIP**
(Maximum Intensity Projection) - Shows the maximum intensity of all layers along the viewing direction.

**Blend**
Shows the color obtained by blending all values along the viewing direction and including their transparency. If Blend is selected, the parameter set Light is available (see below).

### Light

If Blend is selected (see above), the parameter set Light is available.

**Light**
Check the box to switch the light on and adjust the following parameters. There are two predefined light setting parameters in Easy 3D Blend to choose from.

**Left**
Light from the left, shadow to the right.

**Right**
Light from the right, shadow to the left.

**Button Settings...**
In Blend mode, with light enabled, click the button Settings.... The Easy 3D Settings dialog box displays (see below).

### Calculate

**Auto**
The image is calculated and displayed automatically.

**Manual**
The image calculation starts after you click on the button Calculate. If you select Manual as calculation mode, the button Calculate is available.

**Button Calculate**
Click on this button to start the calculation for the Easy 3D image view. The button is only available, if you select Manual as calculation mode.

Easy 3D Settings Window

Light

**Directional Intensity**
Controls the brightness of structures that are illuminated from the light source.

**Ambient Intensity**
Controls the brightness of structures that are in the shadow. The default (0) means that structures completely in the shadow are black. The higher the value the brighter the picture and the lower the shadowing contrasts.

Channel Opacity Combination

Allows you to control the opacity of the image.

**Opaque**
**Translucent**
**Custom**
Select Opaque, Transparent, or Custom opacity. It affects only the behavior of interleaving structures of two or more channels.

---

## 12.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Easy 3D View

| | |
|---|---|
| Ctrl + 4 | Activates the Easy 3D View |
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard PC

## 12.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Easy 3D View

| | |
|---|---|
| Command + 4 | Activates the Easy 3D View |

With a one-button mouse:

| | |
|---|---|
| Shift + Ctrl + click & drag | Move up: zoom out<br>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<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard Mac

# 13 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.
- Grouping functions.

How to Open the Surpass View?

- Click on the icon in the Main toolbar.
- Select Menu View - Surpass.
- Press the key combination Ctrl + 5.

The window Please select as initial Scene displays (default setting).

Window Please select as initial Scene

**Ortho Slice**
**Volume**
**IsoSurface**
**Spots**
**Empty**
Select the desired Surpass Scene Item or Empty (the viewing area in the Surpass main screen is empty).

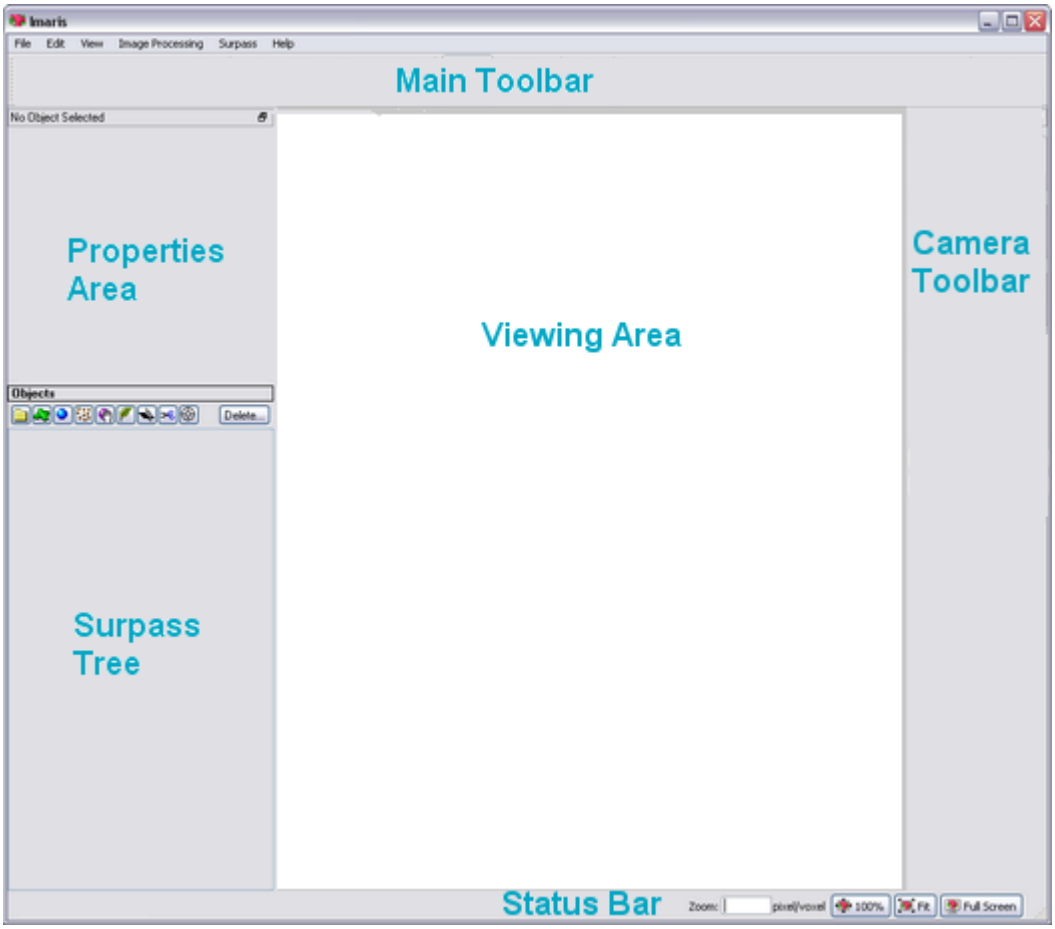
**Show the dialog whenever the Surpass scene is empty**
Check the box and the dialog is displayed whenever the Surpass Scene is empty.

---

See also:
Surpass View - Overview - Mouse & Keyboard Mac
Surpass View - Ortho Slice
Surpass View - Volume
Surpass View - IsoSurface
Surpass View - Spots

## 13.1 Overview

The Surpass main screen consists of six different areas:




- Properties Area, displays 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.
- Surpass Tree to handle the Scene File Concept.
- Viewing Area, this area displays all objects that are added to the Surpass Tree and are checked visible.
- Camera Toolbar includes a number of tools for data preparation, presentation and manipulation, most of which are displayed as buttons.

---

See also:
Toolbars - Main Toolbar
Toolbars - Status Bar

### 13.1.1 Properties Area

The Properties Are 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 |
|---|---|
| Clipping Plane | Tab Settings |

| | |
|---|---|
| Contour Surface | Tab Settings<br>Tab Statistics<br>Tab Color |
| External Object | Tab Settings<br>Tab Color |
| Filament | (Tab Create)<br>Tab Settings<br>Tab Draw<br>Tab Edit<br>Tab Statistics<br>Tab Color |
| Frame | Tab Settings<br>Tab Color |
| Group | Tab Settings<br>Tab Color |
| IsoSurface | (Tab Create)<br>Tab Settings<br>Tab Tracking<br>Tab Statistics<br>Tab Color |
| Light Source | Tab Settings<br>Tab Color |
| Measurement Point | Tab Settings<br>Tab Edit<br>Tab Intensity<br>Tab Statistics<br>Tab Color |
| Ortho Slicer | Tab Settings |
| Oblique Slicer | Tab Settings |
| Spots | (Tab Create)<br>Tab Settings<br>Tab Edit<br>Tab Tracking<br>Tab Statistics<br>Tab Color |
| Spot Track Group | Tab Tracks<br>see Track - Tab Settings |
| Surface Object | Tab Settings<br>Tab Edit<br>Tab Statistics<br>Tab Color |
| Topography | Tab Geometry<br>Tab Coloring<br>Tab Base Color |
| Track | Tab Settings<br>Button Edit Track<br>Tab Statistics<br>Tab Sort |

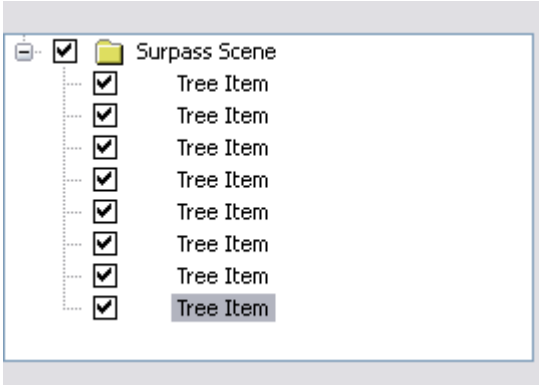
| | |
|---|---|
| | Tab Color<br>Tab Spots<br>see Spots - Tab Settings |
| Volume | Tab Settings<br>Tab Draw<br>Tab Statistics |

---

See also:
Surpass View - Overview - Surpass Tree

### 13.1.2 Surpass Tree

The Surpass 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 adding or deleting a Surpass Tree Item. The first added object generates a group (Surpass Scene). All following new objects are stored in this group. A name is generated automatically for each Surpass Tree Item. To change the name, double-click on the entry and enter a new name. Move objects or groups from one group 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. You find a list of all Items in the chapter 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. Un-check 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.

#### 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 a 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 chapter Menu Edit - Preferences... - Surpass (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:

| | |
|---|---|
| Clipping Plane | Clipping Plane n |
| Contour Surface | Contour n |
| External Object | External Object n |
| Filament | Filament n |
| Frame | Frame |
| Group | Group n |
| IsoSurface | Iso_txxx_cy_n txxx is the current threshold limit, cy is the channel number and n is a consecutive number |
| Light Source | Light Source n |
| Measurement Point | Measurement Points n |
| Ortho Slicer | Ortho Slicer n |
| Spots | Spots n |
| Surface Object | Result after splitting an IsoSurface |
| Topography | Topography n |
| Track | Track 1 |
| 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.

---

See also:
Menu Edit - Preferences... - Surpass (Object Creation Buttons)
Menu Surpass - Delete Selected Objects...
Surpass View - Overview - Properties Area
Surpass View - Group
Surpass View - IsoSurface - Tab Settings - Split

## 13.1.3 Scene File Concept

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

### Save Scene File

The Scene File can be written as follows:

- Select Menu File - Export Scene as … in the Imaris menu bar.

The Save Imaris Scene window is displayed. Select the directory and enter the Scene File name and click OK. The Surpass Tree Items are saved as Imaris Scene File with the extension *.imx.

Load Scene File

Any Scene File with the file extension *.imx can be loaded into the Surpass View for further viewing.

- Select Menu File - Load Scene... in the Imaris menu bar.

The Load Surpass Scene window is displayed. Select the directory and requested file to be loaded, and click OK. The Scene File is loaded.

---

See also:
Menu File - Load Scene...
Menu File - Export Scene as...
Surpass View - Overview - Surpass Tree
Surpass View - Group

### 13.1.4 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. Click at the same time with the left and middle mouse button in the image, hold down the left and the middle mouse button and drag the mouse. Moving the mouse down will zoom in, moving the mouse up will zoom out. The second variation is to hold down the Ctrl-key and the middle mouse button and drag the mouse.

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

| | |
|---|---|
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |

On a Mac with a one-button mouse:

| | |
|---|---|
| Shift + Ctrl + click & drag | Move up: zoom out<br>Move down: zoom in |

Using the Buttons in the Status Bar

**Zoom ... pixel/voxel**
Enter the zoom factor.

**Button 100%**
Click on this button to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

**Button Fit**
Click on this button to pan the position to best fit in the window and adjust the 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.

---

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

## 13.1.5 Camera Toolbar

Pointer

**Select**
The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set some Measurement Points on the object surface.

**Navigate**
The cursor becomes two turning arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

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.

Camera Type

**Orthogonal**
Orthogonal display using parallel lines.

**Perspective X°**
Perspective projection is a type of drawing, or rendering, that graphically approximates on a planar (two-dimensional) surface. If you select Perspective the slider (see below) is active.

**Slider X°**
If you select as camera type Perspective X° (see above) the slider is active. Drag the slider to adjust the vertical aperture angle of the camera.

Draw Style

Select the draw style of the object from the drop-down list.

**Full Feature**
Shows all objects as they are.

**Wireframe**
Draws Surface objects, IsoSurfaces, Spots, Filaments, and Measurement Points as red colored wireframe models.

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

Please note: Set Volume and OrthoSlicer objects invisible before selecting Hidden Lines.

**No Texture**
Draws objects without textures. Only relevant for Topography objects.

**Bounding Box**
Shows only the boxes surrounding the objects.

**Wireframe Overlay**
Lays a red wireframe model over Surface objects, IsoSurfaces, Spots, Filaments, and Measurement Points.

**Low Resolution**
Not yet implemented.

**Points**
Draws IsoSurfaces as a point model.

**Smooth Lines**
Check the box to freeze the object in the view area and to prevent movement.

Stereo

**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.

**Offset**
Display of the offset (0...5).

**Slider**
Adjust the offset to get an optimized 3D effect. Use a small offset if you are far away from the screen, use a big offset, if you are close to the screen. Click on the slider handle and move it to the desired position.

**Button Set Center...**
Use this button to select a new center of rotation. Click on the button Set Center... and then onto the Surpass Scene to define the new center on which the camera zooms in.

Please note: Rotation centers can be set on IsoSurfaces, Contour Surface, Ortho Slices, External objects, and Topographies.

**Button Quick Time VR**
Please refer to the next chapter: Surpass View - Overview - QuickTimeVR.

---

## 13.1.6 QuickTimeVR

You find the Quick Time VR button in the Camera toolbar on the right hand side of the Surpass View.

**Button Quick Time VR**
QuickTimeVR animations show a display rotating around the horizontal or vertical axis, allowing a choice of the direction and the angle of rotation. Click on this button to open the Save As Movie window.

Save as Movie Window

**File name**
Enter a file name.

**Save as type**
The file type is QuickTimeVRMovie (*.mov).

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. The quality of the animation depends directly on the number of frames presented in the rotation. More frames result in a slower and more fluid movement of the object. Rendering is a time-consuming process and requires about 1 minute per frame, depending on your computer and the complexity of the image. We recommend checking how long it takes to render a few images before specifying a large number of frames.

**Add to ImageAccess database**
Check the box to add the file to the ImageAccess database.

**Play Movie with default Media Player when finished**
Check the box to play the movie with the default media player when finished.

QTVR Settings

Here you define the degrees between two frames.

**Total Angle Horizontally**
Defines the angle of the horizontal movement, the default setting is 360.

**Total Angle Vertically**
Defines the angle of the vertical movement, the default setting is 180.

---

See also:
Toolbars - Time Bar (Play and Record Time Series)
Surpass View - Overview
Surpass View - Overview - Camera Toolbar
Animation - Animation Window
Animation - Save Animation

## 13.1.7 Mouse & Keyboard PC

General Mouse & Keyboard Functions in the Surpass View

| | |
|---|---|
| Ctrl + 5 | Activates the Surpass View |
| Esc | Switch mouse mode Navigate/Select |

In the Surpass Tree

| | |
|---|---|
| Arrow up | Move Surpass Tree selection up |
| Arrow down | Move Surpass Tree selection down |

Mouse in Select Mode

| | |
|---|---|
| Arrow left | Go to the next time point |
| Arrow right | Go to the previous time point |
| Alt | Temporary activate Navigate mode (until key is released) |
| Left-click | Select object |
| Ctrl + left-click | Add object to selection/remove object from selection |

Mouse in Navigate Mode

| | |
|---|---|
| S | Set center (on IsoSurfaces, Contour Surface, Ortho Slices, External objects, and Topographies) |
| Arrow left | Go to the next time point |
| Arrow right | Go to the previous time point |
| Left-click & drag | Rotate image (scene) |
| Right-click & drag | Pan image |
| Middle-click & drag | Move up: zoom out<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>Move down: zoom in |

---

See also:
Addendum - Mouse & Keyboard PC

## 13.1.8 Mouse & Keyboard Mac

General Mouse & Keyboard Functions in the Surpass View

| | |
|---|---|
| Command + 5 | Activates the Surpass View |
| Esc | Switch mouse mode Navigate/Select |

In the Surpass Tree

| | |
|---|---|
| Arrow up | Move Surpass Tree selection up |

| | |
|---|---|
| Arrow down | Move Surpass Tree selection down |

Mouse in Select Mode

| | |
|---|---|
| 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 |

Mouse in Navigate Mode

| | |
|---|---|
| S | Set center (on IsoSurfaces, Contour Surface, Ortho Slices, External objects, and Topographies) |
| Arrow left | Go to the next time point |
| Arrow right | Go to the previous time point |

With a one-button mouse:

| | |
|---|---|
| Shift + Ctrl + click & drag | Move up: zoom out<br>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<br>Move down: zoom in |
| Right-click & drag | Pan image |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.2 Clipping Plane

The Clipping Plane cuts away objects on one side of the plane. 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 white double rod in the middle.

### How to Add a new Clipping Plane?

- Select Menu Surpass - Clipping Plane.

The Surpass Tree list is automatically updated and in the Clipping Plane - Properties the Settings parameter are displayed. The Clipping Plane is displayed in the viewing area.

To clip another object, this object must be placed “behind” the clipping plane within the Surpass Tree. It must be in the same group as the Clipping Plane and it must be placed below the Clipping Plane.

### Change Position of Clipping Plane

The position of the Clipping Plane can be changed. Choose the pointer mode Select, highlight the Clipping Plane in the viewing area and left-click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Clipping Plane to the required position.

Rotate Clipping Plane around Axes

The Clipping Plane can be rotated around the x-, y-, and the z-axes. In Select mode left-click the smaller diameter rod. It will turn colored as it is selected, move it to rotate the Clipping Plane.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

### 13.2.1 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.

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.

Perpendicular Movement

By pressing Ctrl and the left mouse button on the bigger diameter rod, the Clipping Plane can be moved in the directions parallel to the plane. The pointer must be in the pointer mode Select.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

### 13.2.2 Mouse & Keyboard PC

Clipping Plane Mouse & Keyboard Functions

| | |
|---|---|
| Left-click on plane & drag | Move plane to desired position |
| Left-click on the bigger diameter rod & drag | Move plane to desired position |
| Left-click on the smaller diameter rod & drag | Rotate plane to desired position |

---

See also:
Addendum - Mouse & Keyboard PC

### 13.2.3 Mouse & Keyboard Mac

Clipping Plane Mouse & Keyboard Functions

| | |
|---|---|
| Click on plane & drag | Move plane to desired position |
| Click on the bigger diameter rod & drag | Move plane to desired position |
| Click on the smaller diameter rod & drag | Rotate plane to desired position |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.3 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 thresholding does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

It is recommended to un-check any other object in the Surpass Tree before drawing the contour, so that only the contour is visible in the viewing area.

How to Add a new Contour Surface?

- Select Menu Surpass - Contour Surface.

The Surpass Tree list is automatically updated and in the Clipping Plane - Properties the Settings parameter are displayed. A drawing plane is displayed in the viewing area. The z-direction is suggested by default. The object properties area shows the position of the plane relative to the data set.

---

### 13.3.1 Tab Settings

A drawing plane is displayed in the viewing area. The z-direction is suggested by default. The object properties area shows the position of the plane relative to the data set.

Draw. Board/Size

**YZ X**
**XZ Y**
**XY Z**
Select the plane of the Contour Surface. 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. The size of the working channel is displayed in the corresponding field.

Drawing Mode

You can draw the contours in different draw styles. Please refer to chapter Surpass View - Contour Surface - Drawing Mode.

Visibility

You can choose Visibility options in order to display contours previously drawn:

**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.

### Change Position of the Drawing Plane

You can either use the slider, enter the position of the plane or change the drawing plane interactively in the viewing area.

**Slider**
**Slice (data field)**
Choose the slice for the first contour by moving the Slider or by entering the position of the plane in the Slice data field.

**Mouse Interaction**
The position of the drawing plane can also be changed in the viewing area. Switch to the pointer mode Select and move the drawing plane by means of the mouse pointer. The border of the active drawing plane is shown in your selected color, thus clearly indicating the Contour Surface currently in use. If more than one Contour Surface has been created in the Surpass Tree, the Contour Surfaces not currently in use have a gray border.

### Add Contour Surface

You can draw as many contours on a plane as required. All contours drawn on a plane will become part of the same object. Use the Slider to select the Slice for the next contour, or move the drawing plane in the viewing area (see above). Draw contours on as many slices as required for the Contour Surface.

**Button Copy**
To copy a polygon in a plane click on the button Copy.

**Button Paste**
To paste a polygon in click on the button Paste.

**Button Calculate Contour Surface**
When all contours on different planes have been drawn click on this button to start the calculation. The surface based on the contours is generated and the result is displayed in the viewing area.

### Number of Triangles

The number of triangles is displayed.

**Button Reedit**
To recalculate the contour if necessary click on the button Reedit.

**Create Surface Object**
To create a Surface object out of the Contour object click on the button Surface object. The Surface object is added in the Surpass Tree and displayed in the viewing area.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)
Surpass View - Contour Surface - Drawing Mode

## 13.3.2 Drawing Mode

You can draw the contours in different draw styles.

**Click**
Hold down the Shift-key and click with the mouse on the contour to insert a point, a so-called vertex.

**Time**
Hold down the Shift-key, click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms) for the next point, specified in the corresponding field. The interval can be changed by manually specifying the required value. This draw style allows you to draw a structure that contains fine details.

**Dist.**
Hold down the Shift-key, click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (mm) for the next point, specified in the corresponding field. To change the interval, specify the required value in the object properties area. This draw style is convenient if the structure is smooth.

Edit Vertices

Please return to the Slice on which the vertex is drawn.

Move node
To move a node you can click on the node while holding down the Shift-key and simultaneously drag the mouse.

Insert node
Hold down the Shift-key and click on the line between two nodes.

Delete node on PC
Hold down the Ctrl-key and double-click with the left mouse button on the node.

Delete node on Mac
Hold down the Command-key and double-click on the node.

Delete polygon on PC
Hold down the Ctrl-key and double-click with the left mouse button on a line between two nodes.

Delete polygon on Mac
Hold down the Command-key and double-click on a line between two nodes.

Size of Vertices and Lines
In the drawing mode, the size of the vertices as well as the connecting lines can be increased or decreased by repeatedly pressing the + key or – key on the numerical keypad.

The process of drawing the contour can be interrupted and continued later. To change the position of the object while drawing the contour, switch the pointer to Navigate mode, move to the required location, and switch the pointer back to Select.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)
Surpass View - Contour Surface - Mouse & Keyboard PC
Surpass View - Contour Surface - Mouse & Keyboard Mac

### 13.3.3 Tab Statistics

Contour Surface statistics are automatically computed for each Contour Surface.

For the statistical values please refer to chapter Menu Edit - Preferences... - Statistics - Contour Surface.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:

Menu Edit - Preferences... - Statistics - Contour Surface.

### 13.3.4 Tab Color

To change the color use the color wheel or type in the color combination.

---

### 13.3.5 Mouse & Keyboard PC

Contour Surface Mouse & Keyboard Functions

| | |
|---|---|
| Left-click plane & drag | Change slice position |
| Arrow up | Change slice position up |
| Arrow down | Change slice position down |
| Shift + left-click line | Insert node into polygon |
| Ctrl + left-double-click node | Delete node |
| Ctrl + left-double-click line | Delete polygon |
| Num plus | Make contour lines bigger |
| Num minus | Make contour lines smaller |

In the Drawing Mode Click

| | |
|---|---|
| Shift + left-click | Add new polygon node |
| Shift + left-click node & drag | Move node |

In the Drawing Mode Time and Distance

| | |
|---|---|
| Shift + left-click plane & move mouse | Add new polygon nodes |
| Shift + left-click node & drag | Move node |

---

See also:
Surpass View - Contour Surface - Tab Settings (Drawing Mode)
Addendum - Mouse & Keyboard PC

### 13.3.6 Mouse & Keyboard Mac

Contour Surface Mouse & Keyboard Functions

| | |
|---|---|
| Click plane & drag | Change slice position |
| Shift + click line | Insert node into polygon |
| Command + double-click node | Delete node |
| Command + double-click line | Delete polygon |
| Num plus | Make contour lines bigger |
| Num minus | Make contour lines smaller |

In the Drawing Mode Click

| | |
|---|---|
| Shift + click | Add new polygon node |
| Shift + click node & drag | Move node |

In the Drawing Mode Time and Distance

| | |
|---|---|
| Shift + click plane & move mouse | Add new polygon nodes |
| Shift + click node & drag | Move node |

---

See also:
Surpass View - Contour Surface - Tab Settings (Drawing Mode)
Addendum - Mouse & Keyboard Mac

## 13.4 External Object

Surpass provides the ability to import External objects and display them concurrently with a Surpass object for comparison.

For example, previously calculated IsoSurfaces, which have been exported as Inventor files, can be imported to another data set.

How to Add a new External Object?

- Select Menu Surpass - External Object.

The Surpass Tree list is automatically updated and in the External object - Properties the Settings parameter are displayed.

---

See also:
Menu Surpass - Export Selected Objects ...

### 13.4.1 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 to transform/distort the object in the x-, y-, and z-direction (Fix Ratio not possible).

**Trackball**
The object shows surrounding circular lines. These lines allow rotation of the External object in any direction (Fix Ratio not possible).

**File Path Field**
Display of the file path (to load the object, see below).

**Button Load...**
Click on the button Load... and the Load External object File box is displayed. Enter the path and the requested File name and/or File type for the object to be loaded and click Open. The selected file is loaded and displayed as an External object.

**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.

See also:
Menu Surpass - Export Selected Objects ...

### 13.4.2 Tab Color

To change the color use the color wheel or type in the color combination.

### 13.4.3 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

### 13.4.4 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

## 13.5 Filament

The Imaris FilamentTracer offers features to segment, track, edit, draw, display, and measure Filaments. The FilamentTracer processes one channel (color) at a time and extract objects that qualify as Filaments (e.g length and average thickness comply with criteria).

After an automatic Filament extraction (see Create Wizard) you can launch an editor operation to delete Filaments, connect Filaments, or draw new Filaments. You may also skip the automatic Filament generation and process the data with semi-automatic or manual segmentation and Filament generation (see tab Draw).

Generated Filament objects will be visualized in the context of Surpass. 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.

Properties and measures of entire Filaments or selected segments will be generated automatically and can be exported in tabular format or directly to MS Excel.

How to Add a new Filament?

- Select Menu Surpass - Filament.

The Surpass Tree list is automatically updated and you start in the first window of the Create Wizard (see below).

See also:

Surpass View - Filament - Create Wizard (for automatic Filament extraction)
Surpass View - Filament - Tab Draw (for interactive Filament tracing method)

### 13.5.1 Data Input and Filtering

The FilamentTracer is designed to operate on 3D images, which provides sufficient resolution to resolve the Filaments to be studied in all three spatial directions. 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 (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 in the fluorescent case and it will be more difficult to automatically segment the Filament. Deconvolution can also be applied to transmission images although - theoretically - its performance is weaker than in the fluorescent case.

Filtering

You will filter the original images in such a way that noise is suppressed as good as possible and the image becomes optimally suited for the next step - the automatic graph compilation.

**Gaussian Filtering**
The staining, the illumination, and the photon detection have strong influence on the amount of noise present in the image. A gaussian filter is provided in the segmentation interface to reduce that noise. The gaussian filter is quite a fast operation taking only a few seconds on a 512x512x40 image. Images that have been deconvolved prior to tracing do not require any additional filter.

**Connective Baseline**
Simple thresholding methods classify voxels purely based on a single intensity value. This approach often does not lead to good results because the staining, the illumination, and the detection operate within a precision range. The connective thresholding scheme takes care of this and searches for object voxels using an intensity range rather than a single intensity.

You must provide two threshold values. The baseline (lower threshold) is the intensity below which a voxel is recognized as part of the background, the threshold (upper threshold) is the minimal intensity for a voxel to be part of the Filament object. Voxels with an intensity value between the two thresholds are treated as part of the Filament object if and only if they are connected to a voxel within intensity above the upper threshold ("double thresholding" with "feature-AND").

The result of the segmentation process described above is a clean image where the non-zero voxels are describing the objects (Filaments and some non-filamentous structures). Because such a description of the data is not suitable for morphometric analysis the structures are converted. Graph compilation is a process involving several image processing operations such as skeletonization, erosion, dilation and the creation of a vector definition for the Filaments. The result of the process is a database (kept in memory) describing the Filaments as a set of segments that may contain branches.

---

See also:
Menu Image Processing - Smoothing - Gaussian Filter
Menu Image Processing - Thresholding - Connective Baseline

### 13.5.2 Create Wizard

The automatic graph compilation (creation) is guided by a wizard-like user interface and consists essentially of four steps.

- Select the appropriate algorithm (or quit the automatic filament creation and draw the filaments interactively).
- Select source channel and do some preprocessing.
- Segmentation to specify filamentous and non filamentous parts.
- Graph compilation and finishing.

The Creation Wizard allows to go forth and back until the optimal parameters are found.

## First Window of the Create Wizard

In this first window of the Create Wizard you decide, if you want to go for an fully automated filament creation using one of the two predefined algorithms (AutoPath no loops or Threshold loops, see below); or if you want to draw the filaments interactively (click on the button Cancel and select the parameters on the tab Draw, see below).

### Select Algorithm

**AutoPath (no loops)**
**The automated AutoPath Algorithm:**
- **Produces a tree-like filament**
- **Is based on local intensity contrast**
- **Connects large start- and small end-points**

If you select this algorithm please refer to chapter AutoPath (no loops).

**Threshold (loops)**
**The Threshold based Algorithm:**
- **Produces a filament with loops**
- **Is based on an absolute intensity threshold**
- **Thinning to a skeleton**

If you select this algorithm please refer to chapter Threshold (loops).

**Button Next**
Press Next to proceed with the second window of the Create Wizard.

**Button Finish**
Press Finish and based on the selected algorithm (see above) Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

---

See also:
Surpass View - Filament - Create Wizard - AutoPath (no loops)
Surpass View - Filament - Create Wizard - Threshold (loops)
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

#### 13.5.2.1 AutoPath (no loops)

## Second Window of the Create Wizard

### Select Source Channel

Select the source channel.

### Starting Point Diameter (Largest Branch Diameter)

Enter the diameter of the starting point.

End Point Diameter (Thinnest Branch Diameter)

Enter the diameter of the end points.

**Optimize for Highly Branched Structure**
Check this box if you have a highly branched structure.

**Button Back**
Click on this button to switch back to the first window of the Create Wizard.

**Button Next**
Press Next to proceed with the third window of the Create Wizard.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

## Third Window of the Create Wizard

Starting Points Threshold =

Display of the starting points threshold and the respective histogram. Adjust the threshold by entering a new value in the data field or moving the colored threshold line (click and drag) in the histogram. The updated points are visualized in the viewing area.

End Points Threshold =

Display of the end points threshold and the respective histogram. Adjust the threshold by entering a new value in the data field or moving the colored threshold line (click and drag) in the histogram. The updated points are visualized in the viewing area.

**Button Back**
Click on this button to switch back to the second window of the Create Wizard.

**Button Next**
Press Next to proceed with the fourth window of the Create Wizard.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

## Fourth Window of the Create Wizard

Finish

**Calculate Diameter from Image**

Check this box and the Filament diameter is calculated based on the image data. Un-check the box and the Filament diameter will decrease from starting point to the end points.

Please note: The effect is only visible if you check as Style - Cone (on Surpass View - Filament - Tab Settings).

**Build Filaments for all Time Points**
Only available if you work with a time data set. Check this box if you want to create a filament over all time points.

**Button Back**
Click on this button to switch back to the third window of the Create Wizard.

**Button Next**
This button is grayed and not available.

**Button Finish**
Press Finish to calculate and display the Filaments in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

---

See also:
Surpass View - Filament - Tab Settings
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

#### 13.5.2.2 Threshold (loops)

## Second Window of the Create Wizard

Source Channel

Select the source channel.

Feature Preprocessing

**Enable Feature Preprocessing**
Check the box and the following parameters are adjustable. Un-check the box and the following parameters are grayed. Preprocessing is not necessary if the data is already deconvolved or if the noise has already been removed by a gaussian filter. The preprocessing will be applied to the source channel and the result will be added as new Filament Working channel in the Display Adjustments.

**Appr. Filament Diameter**
An approximate minimal Filament diameter has to be provided.

**Preserve Edges**
You can also enable an edge preserving preprocessing to maintain filamentous structures.

Before continuing you can inspect the result of the previous step with any viewer of Imaris: Slice, Section, Gallery, Easy3D, IsoSurface etc.

**Button Back**
Click on this button to switch back to the first window of the Create Wizard.

**Button Next**
Press Next to proceed with the third window of the Create Wizard.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation

Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

## Third Window of the Create Wizard

**Fill Cavities (fully enclosed volumes)**
Check this box to avoid hollow regions. These regions would be enclosed by a Filament mesh, which is not desired in most of the cases.

**Connective Baseline**
Key in two values for the lower threshold (colored line) and upper threshold (red line). You can also click into the histogram. Left mouse button for lower, right mouse button for upper threshold. Or you can click and drag the respective line.
To display only the Filament Working channel in the viewing area switch off all other channels in the Display Adjustment window. In the Filament Working channel there are three regions. The black region below the lower threshold is background. The gray region above the lower threshold is part of the Filament, but only under the condition that a white region (upper threshold) is included by this gray region (all the gray regions which do not contain a white region will be background).

**Button Back**
Click on this button to switch back to the second window of the Create Wizard.

**Button Next**
Press Next to proceed with the fourth window of the Create Wizard.

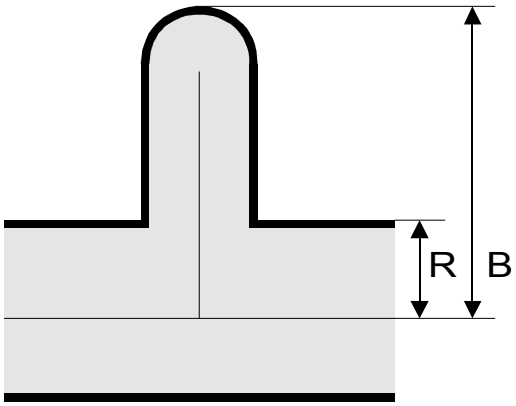
**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

## Fourth Window of the Create Wizard

Graph compilation

**Minimal Ration of Branch Length to Trunk Radius**
Enter a minimal ratio of branch length to trunk radius (see figure below). Branches shorter than this value will be removed.




*Ratio of branch length (B) to trunk radius (R).*

**Find Root Point**
A root point has to be specified for specific statistic values (e.g. tree order). The point is selected from the trunk position with the biggest radius.

**Button Back**
Click on this button to switch back to the third window of the Create Wizard.

**Button Next**
Press Next to proceed with the fifth window of the Create Wizard.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

## Fifth Window of the Create Wizard

Finish

To validate the automatically generated graph and the prior segmentation, you can inspect the result together with numerous Surpass modules: Ortho Slicer, Volume, IsoSurface 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 other use. Principally it is not needed any more when finishing the Filament graph.

**Button Back**
Click on this button to switch back to the fourth window of the Create Wizard.

**Button Next**
This button is grayed and not available.

**Button Finish**

Press Finish to calculate and display the Filaments in the viewing area.

**Button Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

---

See also:
Menu Edit - Show Display Adjustment
Menu Image Processing - Thresholding - Connective Baseline
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

### 13.5.3 Filament Properties

When the creation process is finished, the tab Create disappears while some other property Tabs appear. They allow the manipulation of the filament graph:

**Tab Create**
Only appears when you Add a new Filament.

**Tab Settings**
Change Filament appearance and inspect selection statistics.

**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:
Surpass View - Filament (Add a new Filament)
Surpass View - Filament - Tab Settings
Surpass View - Filament - Tab Draw
Surpass View - Filament - Tab Edit
Surpass View - Filament - Tab Statistics
Surpass View - Filament - Tab Color

### 13.5.4 Tab Settings

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 Nodes

Special nodes are highlighted with color-coded spheres.

**Terminal**
Terminal points are green.

**Branch**
Normal branch points are red. Branch points with 4 or more joining segments are blue.

**Root**
The root point is cyan.

**Button Rebuild**
Rebuild starts a new Filament creation with the previous used parameters. The current Filament graph will be deleted.

Please note: With a rebuild you go through the whole Creation Wizard using your previous parameter settings as initial values. You can easily change just a few parameters without going in detail through all parameters again.

**Button Set Selection as Root Point**
The root point (anchor node) defines the order of other nodes within the graph.

Text Field

Report and measurement of the currently selected segment(s): Length, Average Diameter, Volume, Serial Resistance, Depth, Number of Branchings, Number of End Points, etc. See the tab Statistics for more information. If multiple parts of the Filament graph are selected, the measurements are summarized or averaged (the depth may appears as not defined).

---

See also:
Surpass View - Filament - Tab Statistics

### 13.5.5 Tab Draw

Method

**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 chapter AutoPath (Surpass View - Filament -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 chapter AutoDepth (Surpass View - Filament -Draw - AutoDepth).

**Manual**
The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. If you select this method please refer to chapter Manual (Surpass View - Filament -Draw - Manual).

Filament Diameter

Here you adjust the filament diameter.

**Button Set Starting Point**
If you select as Method AutoPath the button Set Starting Point is available.

Select a point and click on this button to define the starting point of the filament.

Slice

If you select as Method Manual the Slice parameter settings are available.

**YZ Plane**

**XZ Plane**
**XY Plane**
Select the plane orientation of the drawing board: yz, xz, xy.

**Automatic Placement**
When this parameter is activated, the position is set automatically to the location of the highest data intensity. In order to avoid unwanted horizontal or vertical drawing offsets, the camera is set to a perpendicular position with an orthogonal projection. In this case the camera can only zoom or pan but not rotate.

**x ... um**
Display of the Slice the position.

**Slider**
The position of the drawing board can be altered in perpendicular direction by the Slice slider. The same is achieved using the Arrow-keys of the keyboard (Arrow up, Arrow down).

---

See also:
Menu Edit - Show Display Adjustment
Surpass View - Overview - Camera Toolbar - Pointer
Surpass View - Filament -Draw - AutoPath
Surpass View - Filament -Draw - AutoDepth
Surpass View - Filament -Draw - Manual

### 13.5.5.1 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.

Drawing in AutoPath Mode

- 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.
- Place the mouse cursor at the ends of the structure. The computed path is displayed.
- Shift + left-click and the displayed path is definitely 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 starting point into volume |
| Set starting point, then move mouse | Display the path to the end point |
| Shift + left-click | The displayed path is definitely drawn |
| Mouse wheel | Change the "pencil" diameter for drawing the filament |

Mac Mouse & Keyboard Function Overview in the AutoPath Mode

| | |
|---|---|
| Shift + Ctrl + click | Set a starting point into volume |
| Set starting point, then move mouse | Display the path to the end point |
| Shift + click | The displayed path is definitely drawn |
| Mouse wheel | Change the "pencil" diameter for drawing the filament |

---

### 13.5.5.2 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).

Drawing in AutoDepth Mode

- 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. A 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 |
| Mouse wheel | Change the "pencil" diameter for drawing the filament |

Mac Mouse & Keyboard Function Overview in the AutoDepth Mode

| | |
|---|---|
| Shift + click & drag | Draw a filament into the volume |
| Mouse wheel | Change the "pencil" diameter for drawing the filament |

---

#### 13.5.5.3 Manual

The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. Essentially you draw the Filaments onto the image, which serves as a visual reference (template). Drawing is done in the xy-plane. For z-positioning in the image you can choose to change planes manually (Arrow-keys or mouse) or to have the system change the planes automatically in such a way that the center of the Filament is kept in focus.

An orthogonal section plane appears within the Surpass viewer. Use the channel visibility and the Display Adjustments to optimize the section viewing. In order to draw Filaments onto the drawing plane switch the mode for the mouse pointer from Navigate to Select.

If you select Manual the Slice parameter settings are available.

Drawing in Manual Mode

- Select Manual in the Draw box and press the Shift-key and hold down the left mouse button to begin drawing. The circle on the drawing plane indicates the Filament diameter.

The diameter can also be changed using the mouse wheel. To make a connection to an existing Filament, start painting on this Filament. If the filamentous structure runs perpendicular to the drawing plane, use the right mouse button to alter the plane orientation.

PC Mouse & Keyboard Function Overview in the 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 "pencil" diameter for drawing the filament |
| PageUp | Increase the "pencil" diameter for drawing the filament |
| PageDown | Decrease the "pencil" diameter for drawing the filament |
| 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 |

Mac Mouse & Keyboard Function Overview in the 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 |
| Ctrl + click plane | Change the plane orientation (from XY to YZ to XZ) |
| Mouse wheel | Change the "pencil" diameter for drawing the filament |
| PageUp | Increase the "pencil" diameter for drawing the filament |
| PageDown | Decrease the "pencil" diameter for drawing the filament |
| 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 |

### 13.5.6 Tab Edit

Up to this point in the analysis process most steps have been carried out automatically. You have influenced the tracing process by specifying a set of parameters. The graph editor allows you to make manual changes to the graph topology and the number of nodes and segments in the graph. You make these changes directly by operating on the 3D view of the graph.

#### Mouse Selects

Specify how to select parts of the Filament by a single mouse click onto the Filament graph.

**Point**
Point selects only the position near to the mouse pointer.

**Segment**
Segment selects the Filament between two branch points.

**Branch**
Branches selects the Filament starting from the clicked point to all connected end points (a root point should be defined for this mode).

#### 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 (see figure above).

**Button Abs. Branch**
Abs Branch selects all branches shorter than an absolute length B.

**Button Loops...**
Loops finds circular closed Filaments limited by a maximum length.

**Button Invert**
Invert selects all unselected parts of the graph and deselects all selected parts.

**Button Path**
Path requires at least two selected points from the Filament graph. The shortest path between these two selections will be calculated.

**Button Clear**
Clear removes the selection.

#### Process Sel.

The following buttons apply only to the previously selected parts (see above, Mouse Selects) of the Filament.

**Button Smooth**
Removes the roughness of the current selection.

**Button Diameter...**
Click on this button to recalculate the Filament diameter. The window Calculate Diameter opens.

Window Calculate Diameter

**Small Diameter**
Type in the smallest Filament diameter in the selected region.

**Large Diameter**
Type in the largest Filament diameter in the selected region.

**Contrast Threshold**
Type in the contrast threshold in the selected region.

**Button Delete**
Delete the current selection.

Process Filament

The following buttons apply to the entire Filament.

**Button Smooth**
Smooth removes roughness of 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 Diameter...**
Click on this button to recalculate the Filament diameter. The window Calculate Diameter opens.

Window Calculate Diameter

**Small Diameter**
Type in the smallest Filament diameter in the selected region.

**Large Diameter**
Type in the largest Filament diameter in the selected region.

**Contrast Threshold**
Type in the contrast threshold in the selected region.

**Button Merge...**
Separated Filaments can be merged using the Merge… button.

**Button Center**
Click on this button to recenter the filament.

**Button Export**
The whole Filament graph can be stored in the Neuron file format (*.hoc). The Neuron application is freeware and is able to do electrical simulations.

### 13.5.7 Tab Statistics

For the statistical values please refer to chapter Menu Edit - Preferences... - Statistics - Filament.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Filament

### 13.5.8 Tab Color

To change the color use the color wheel or type in the color combination.

---

### 13.5.9 Time Data (4D)

All segmentation steps and graph compilation is applied with the same set of parameters to each time point. There is not one track of Filaments over time, but a Filament graph for each time point. When a huge time data set is loaded, the graph generation maybe takes some time (you have to crop the data set yourself).

To change Settings or Color the parent group from the Surpass Tree can be selected. The Filament settings are available on the tab Filaments.

A Filament graph is only visible if the current Time Point is the same as the Time Point, where the Filament creation passes the graph compilation.

The selection within the graph remains, if the global Imaris Time Point changes.

---

### 13.5.10 Mouse & Keyboard PC

Filament Mouse & Keyboard Functions in the Tab Draw

AutoPath Mode

| | |
|---|---|
| Shift + right-click | Set a starting point into volume |
| Set starting point, then move mouse | Display the path to the end point |
| Shift + left-click | The displayed path is definitely drawn |
| 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

### 13.5.11 Mouse & Keyboard Mac

Filament Mouse & Keyboard Functions in the Tab Draw

AutoPath Mode

| | |
|---|---|
| Shift + Ctrl + click | Set a starting point into volume |
| Set starting point, then move mouse | Display the path to the end point |
| Shift + click | The displayed path is definitely drawn |
| Mouse wheel | Change the filament diameter (to visualize change to Style Cone - Tab Settings) |

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 |
| Ctrl + 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

## 13.6 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. Un-check the box to make the frame invisible.

How to Add a new Frame?

- Select Menu Surpass - Frame.

The Surpass Tree list is automatically updated and in the Frame - Properties the Settings parameter are

displayed.

### 13.6.1 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.

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

**Shadow**
The entire Surpass 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 good performance of the graphics board (depending on the complexity of the scene).

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

Grid and Tickmarks Settings

**Grid**
Check the box Grid to display a grid. The grid serves as a scale bar in 3D perspective views. Use the slider to adjust the grid width.

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

**Fix to Data Set**
Check Fix to Data Set to fix the x-, y-, and z-axes to the same side when navigating the object.

**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.

### 13.6.2 Tab Color

To change the color use the color wheel or type in the color combination.

---

## 13.7 Group

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 Surpass Scene. Additional Groups can be created in order to sort objects of the scene.

How to Add a new Group?

- Select Menu 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:
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

### 13.7.1 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 un-checked.

---

### 13.7.2 Tab Color

The color change applies to all Items in the Group.

---

### 13.7.3 Tab Sort

The tab Sort is available when a Group contains at least one Surpass Tree Item.

Highlight the Group and select the tab Sort. Adjust the parameters to sort the objects within the Group by statistical values.

Sort Objects by

**(Various sorting criteria)**

Select the desired sorting criteria.

**Ascending**
In the Surpass Tree the objects are arranged in ascending order.

**Show All**
In the Surpass Tree the objects are checked and displayed in the viewing area.

**Button Select Hidden**
All objects in the Surpass Tree that are un-checked (hidden) are highlighted at once. Use this function to move the objects in a different group or delete them at once.

Show Objects between two Values

**Enable**
Check this box to enable the function and enter the two values.

Histogram

The numerical range of the statistics value (e.g. Track length), and the number of objects (e.g. Tracks) depending on the statistics value is displayed. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects is visualized. The visualized objects are checked in the Surpass Tree.

---

## 13.8 IsoSurface

The IsoSurface 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 IsoSurface?

- Select Menu Surpass - IsoSurface.

The Surpass Tree list is automatically updated and the first window of the Create Wizard is displayed in the IsoSurface - Properties.

---

See also:
Surpass View - Overview - Surpass Tree
Surpass View - IsoSurface - Create Wizard

### 13.8.1 Create Wizard

**First Window of the Create Wizard**

Channel

Select the respective source channel, from which the IsoSurface is to be computed.

Thresholds

Define the requested threshold by clicking into the histogram or by entering the value directly in the data field. The initial threshold value is automatically computed using an algorithm based on [T.W. Ridler and S. Calvard, "Picture thresholding using an iterative selection method", IEEE Trans. System, Man and Cybernetics, SMC-8 (1978) 630-632].

**Upper Threshold**
If you want to set an upper threshold check this box and type in the desired value in the Upper Threshold filed. The histogram is updated accordingly.

**Button Next**

If you want to adjust parameters in the second window of the Creation Wizard click on Next.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the IsoSurface is displayed in the viewing area.

## Second Window of the Create Wizard

### Resample Data Set

Check the Resample Data Set check-box in order to reduce the amount of data before calculating the IsoSurface. Bitplane recommends resampling for all data sets. Resampling reduces the number of voxels in a grid but keeps the original relation between the voxels if specified by a Fixed ratio (see below, check-box Fixed).

If Resample Data Set is not checked, the respective fields show the size of the loaded data set and the parameters are grayed..

If Resample Data Set is selected, you can accept the defaults (see below, Standard Size and Quick Size) or customize the values for the voxel sizes.

**Standard Size**
**Quick Size**
You can use the two predefined standard sizes templates to resize your data set.

**Voxel Size**
**X**
**Y**
**Z**
You can customize the resampling of your data set. Enter the values for the x-, y-, or z-dimension directly in the respective fields.

**Fixed X/Y**
**Fixed X/Y/Z**
Check one of the Fixed ratio options to maintain the x/y- or x/y/z-ratio of the data set when entering values directly.

**Button Back**
If you want to go back in the first window of the Creation Wizard click on Back.

**Button Next**
If you want to adjust parameters in the third window of the Creation Wizard click on Next.

**Button Finish**
Press Finish and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the IsoSurface is displayed in the viewing area.

## Third Window of the Create Wizard

### Smooth Data Set

Check the Smooth Data Set option to smooth the structure of the object to obtain fewer triangles and therefore less data. Smoothing applies a Gaussian filter to the data set and requires a great deal of memory. Un-check the check-box if your system runs out of memory. Smoothing can result in the loss of small objects, so should not be used if the data set contains very small objects.

**Gaussian Filter Width**
The Gaussian filter width is calculated automatically from the voxel sizes (oriented at the largest voxel dimension) but can also be altered directly. The value is given in image coordinates and not in fraction of

voxels. The Gaussian filter is isotropic with respect to the image coordinates.

**Close Objects at Border**
If structures on the border of a data set are cut open, the volume is not calculated. Check Close Objects at Border to close these structures before calculating the IsoSurface object.

Note: If a new IsoSurface object is oversized and does not fit into the viewing area, select Menu View – Fit or click on the button Fit in the Status Bar on the lower right-hand side of the screen.

**Buttons Back**
If you want to go back in the second window of the Creation Wizard click on Back.

**Button Finish**
Press Finish and Imaris calculates the IsoSurface and displays the IsoSurface in the viewing area.

---

See also:
Menu View - Fit
Toolbars - Status Bar

## 13.8.2 Tab Settings

Time Data

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 an IsoSurface. Be sure that the Surpass Tree IsoSurface is highlighted and click on the tab Settings.

**Button Mask Channel...**
Click on the button to open the window Mask Channel.

Window Mask Channel

**Select Channel to mask with Surface**
Select the channel (destination channel) you want to mask with the IsoSurface (ROI).

**Duplicate Channel before applying Mask**
This parameter is grayed (only available for Surface objects).

**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 un-checked 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 IsoSurface 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.

**Buttons Cancel**
If you do not want to apply the mask click on Cancel.

Split the IsoSurface and Create Surface Objects

The number of unconnected Surface objects is displayed in the properties area (see below, Number of Surfaces).

**Button Split All...**
Click on the button and the window Split displays.

Window Split

**Create no more than ... Surface Objects**
Check Create no more than ... Surface Objects to specify the number of independent Surface objects created. The Surface objects are created beginning with the Surface object that contains the greatest number of triangles (depending on the shape it is possible that an object consisting of more triangles has a smaller volume than another one with less triangles). Remaining objects are grouped together in the final Surface object.

**Filter Objects with less than ... Triangles.**
Check Filter objects with less than ... triangles to eliminate very small objects which may not be relevant.

**Button Ok**
If you want to create Surface objects click on OK. Surface objects are created and displayed according to the setup parameters.

**Button Cancel**
If you do not want to create Surface objects click on Cancel.

Source

**Channel**
Displays the source channel for the mask function.

**Threshold**
Displays the threshold for the source channel.

**Button Rebuild**
Rebuild allows you to re-specify the settings for the selected IsoSurface. The main reason for performing a rebuild is when testing for the best threshold value, the calculated IsoSurface can be quickly replaced with a rebuild. The first window of the Creation Wizard is displayed in the properties area.

Please note: With a rebuild you go through the whole Creation Wizard using your previous parameter settings as initial values. You can easily change just a few parameters without going in detail through all parameters again.

Current Time Point

**Time Index (Point)**
Displays the actual time point.

**Number of Triangles**
Displays the number of Triangles of the IsoSurface.

**Number of Surface (Objects)**
Displays the number of unconnected Surface objects. If there are more than one unconnected Surface object (s) the button Split... is active.

**Button Split...**
If the number of unconnected Surface objects is more than one this button is active. Click on the button to open the window Split (see above).

---

See also:
Surpass View - IsoSurface - Create Wizard (First Window)
Surpass View - Surface Object - Tab Settings - Mask Channel

### 13.8.3 Tab Tracking

Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of the IsoSurfaces. Based on these predicted positions the best matches to candidate IsoSurfaces can be determined.

**Brownian Motion**
This algorithm models the motion of each IsoSurface as a Brownian motion. This model is appropriate, if your IsoSurfaces actually perform a Brownian motion.

**Autoregressive Motion**
This algorithm models the motion of each IsoSurface as an autoregressive process. This model is appropriate if your IsoSurfaces perform any kind of continuous motion.

**Autoregressive Motion Expert**
Select the expert mode to adjust additional parameters (see below).

**Connected Components**
This algorithm models the motion of continuous connected components.

Parameters

**Maximum Distance**
The parameter Maximum Distance disallows connections between an IsoSurface and a candidate match if the distance between the predicted future position of the IsoSurface 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).

**Maximum Gap Size**
It happens that the object segmentation fails for some time points. This can break a Track apart into two Tracks. The gapclosing algorithm tries to continue the movement of a disappearing object and connects the Tracks if it reappears. The number defines the maximum consecutive time points until the object re-appears.
The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

**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).

**Max Intensity Difference**
Maximum Intensity Difference between two successive objects in a track.
The parameter is only available if you select Autoregressive Motion Expert (see above).

**Filter Width**
If "Filter Width" is non-zero the predicted position of a particle for a future time point is computed based on all tracks within a neighborhood defined by "Filter Width".
The parameter is only available if you select Autoregressive Motion Expert (see above).

**Button Create Track**
Click on the button Create Track to start the calculation. After calculation the Surpass Tree in the objects area will contain a new group called Track Group containing one or several Track objects. Each Track in return contains several IsoSurfaces that have been linked into the same Track by the tracking algorithm.

Track Color

The different Tracks by default are assigned different colors in which they visualize their objects. This coloring serves to allow for an immediate visual inspection of the result.

Tab Tracks

After the tracking the Surpass Tree in the objects area will contain a new group called Track Group. Highlight the Track Group and select the tab Tracks to adjust the Tracks parameter (please refer to Surpass View - Track - Tab Settings).

---

See also:
Surpass View - Track - Tab Settings

### 13.8.4 Tab Statistics

IsoSurface statistics are automatically computed for each IsoSurface.

For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - IsoSurface.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - IsoSurface

### 13.8.5 Tab Color

To change the color use the color wheel or type in the color combination.

---

## 13.9 Light Source

A Light Source serves to illuminate objects in the viewing area.

How to Add a new Light Source?

- Select Menu Surpass - 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 move the Light Source in the viewing area, 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.

---

See also:
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

### 13.9.1 Tab Settings

**Fix to Data Set**
Check the box to fix the x-, y-, and z-axes to the same side when navigating the object. When the box is un-checked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

---

### 13.9.2 Tab Color

To change the color of the light source use the color wheel or type in the color combination.

---

### 13.9.3 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

### 13.9.4 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

## 13.10 Measurement Point

How to Add a new Measurement Point?

- Select Menu Surpass - Measurement Point.

The Surpass Tree list is automatically updated and in the Measurement Point - Properties the Edit parameters are displayed.

---

### 13.10.1 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.

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.

Please note: 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:
Surpass View - Measurement Point - Tab Color

## 13.10.2 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. For the rest the last parameter is crucial.

**First visible Channel**
In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this parameter and only the first visible channel is relevant for the placement of the Measurement Points.

**All visible Channels**
In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this parameter and only all visible channels are relevant for the placement of the Measurement Points.

**Solid Objects**
Check this box and the Measurement Points are placed on the surface of an object. Un-check the box and you can also place Measurement Points in an object.

**Button Delete All Points**
Click on this button to delete all Measurement Points. Alternatively click on the Delete-key on the keyboard.

Selected Point

Left-click to select an existing point. If no point is selected the following parameters are grayed. Re-left-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.

---

See also:
Menu Edit - Show Display Adjustment
Surpass View - Overview - Camera Toolbar (Pointer Select)

## 13.10.3 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:
Menu File - Save as... Snapshot

### 13.10.4 Tab Statistics

Measurement Point statistics are automatically computed for each Measurement Point. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Measurement Points.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Measurement Points

### 13.10.5 Tab Color

With this parameter set you can change the color of the Measurement Points and the connecting lines between the Measurement Points.

---

### 13.10.6 Mouse & Keyboard PC

Measurement Point Mouse & Keyboard Functions in 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<br>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 a lot in handling 3D data sets.

---

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard PC

### 13.10.7 Mouse & Keyboard Mac

Measurement Point Mouse & Keyboard Functions in 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 a lot in handling 3D data sets.

---

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard Mac

## 13.11 Oblique Slicer

The Oblique Slicer represents a plane that can be freely moved and rotated within the data set and allows you to look inside any object. The Oblique Slicer consists of a colored frame with a white double rod in the middle.

How to Add a new Ortho Slicer?

- Select Menu Surpass - 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 in the tab Settings).

Change Position of the Oblique Slicer

You can change the position of the Oblique Slicer. Choose the pointer mode Select, highlight the Oblique Slicer in the viewing area and left-click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Oblique Slicer to the required position.

Rotate Oblique Slicer around Axes

The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In Select mode left-click the smaller diameter rod. It will turn colored as it is selected, move it to rotate the Oblique Slicer.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)
Surpass View - Ortho Slicer - Tab Settings

### 13.11.1 Tab Settings

Position

**Button Recenter**
Click on this button to re-center the Slicer.

Orientation

**Button YZ Plane**
**Button XZ Plane**
**Button XY Plane**
Change the Slicer direction, if required, by selecting the adequate plane.

Camera

**Button Orthogonal View**
The camera is set to a position which is perpendicular to the Slicer.

**Show Frame**
Check the box to display a colored frame around the Slicer.

---

### 13.11.2 Mouse & Keyboard PC

Oblique Slicer Mouse & Keyboard Functions

| | |
|---|---|
| Left-click on the bigger diameter rod & drag | Move plane to desired position |
| Left-click on the smaller diameter rod & drag | Rotate plane to desired position |

---

See also:
Addendum - Mouse & Keyboard PC

### 13.11.3 Mouse & Keyboard Mac

Oblique Slicer Mouse & Keyboard Functions

| | |
|---|---|
| Click on the bigger diameter rod & drag | Move plane to desired position |
| Click on the smaller diameter rod & drag | Rotate plane to desired position |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.12 Ortho Slicer

Ortho Slices represent an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. When adding slices, Surpass suggests per default the directions z, x, and y as sequence for the first three and all subsequent slices.

How to Add a new Ortho Slicer?

- Select Menu Surpass - 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 in the tab Settings).

---

See also:
Surpass View - Ortho Slicer - Tab Settings

### 13.12.1 Tab Settings

Slice Orientation

**YZ Plane**
**XZ Plane**
**XY Plane**
Change the slice direction if required by selecting the adequate plane.

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).

---

### 13.12.2 Mouse & Keyboard PC

Ortho Slicer Mouse & Keyboard Functions

| | |
|---|---|
| Left-click plane & drag | Change slice position |
| Right-click plane | Change slice orientation |
| Num plus | Increase the extended section |
| Num minus | Decrease the extended section |
| Arrow up | Change slice position up |
| Arrow down | Change slice position down |

---

See also:
Addendum - Mouse & Keyboard PC

### 13.12.3 Mouse & Keyboard Mac

Ortho Slicer Mouse & Keyboard Functions

| | |
|---|---|
| Click plane & drag | Change slice position |
| Ctrl + click plane | Change slice orientation |
| Num plus | Increase the extended section |
| Num minus | Decrease the extended section |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.13 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 Surpass - Spots.

The Surpass Tree list is automatically updated and the first window of the Create Wizard is displayed in the Spots - Properties.

---

### 13.13.1 Create Wizard

The properties area displays the first window of a creation dialog. Fill in the appropriate values:

**First Window of the Create Wizard**

Source Channel

Select the respective source channel, from which Spots are to be computed.

Spot Detection

**Minimum Diameter**
Spots with diameter smaller than this are not detected. This is also the diameter of the inserted Spots.

**Background Object Subtraction**
If checked, the background will be removed prior to spot detection. Technically background subtraction smoothes the image with a Gaussian filter (sigma = Object Diameter / 2 ) and subtracts this filtered image from the original.

### Buttons Back
This button is grayed in the first window of the Create Wizard.

### Buttons Next
Press Next to proceed with the second window of the Create Wizard.

### Button Cancel
If you want to manually point out all the Spots click on the Cancel button. The parameters on the tab Settings are displayed in the properties area.

## Second Window of the Create Wizard

The properties area displays the second window of the creation dialog and the viewing area displays Spots as spheres of the specified diameter.

### Threshold

**Spot Quality Threshold**
The threshold in the creation dialog is initially set to such a value that most of the detected Spots are visible. By increasing the threshold “insignificant” Spots can be discarded. The viewer immediately reacts to a change in threshold and displays only the remaining Spots. Select the threshold such that the result optimally fits the data. It is helpful to view the data as a Volume and adjust the settings of the Volume such that both the Volume and the Spots are visible.

**Interpolate Positions**
If you check this box the Spot position can lay between two voxels.

**Region Growing**
Check this box if you want to use the seeded region growing algorithm and the corresponding parameter sets in Imaris. If you check this box the parameter set Region Method on the third window of the Create Wizard (see below) gets activated. If you check this box the button Next gets activated and you can switch to the third window of the Creation Wizard.

### Buttons Back
With Back you return to the first window of the Create Wizard.

### Buttons Next
If you check the box Region Growing this button gets activated. Click on Next to switch to the third window of the Creation Wizard.

### Button Finish
Click on Finish to calculate the desired Spots and display the Spots in the viewing area.

## Third Window of the Create Wizard

### Region Method

The region method for segmentation in Imaris starts with defined seed points. You can come to this third window of the Create Wizard in the Spots section of the Surpass view from two different sides:

- You come from the Spot detection, follow the instructions in the Creation Wizard and check the box Region Growing on the third window of the Create Wizard (see above).
- You finish a Spot detection without using the region growing parameters (un-checked box Region Growing). Then you edit the Spots (e.g. delete some Spots, add some Spots or rearrange Spots). Then you click on the tab Settings on the button Region Growing.

In both cases 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.

**Local Contrast Threshold**

**Source Channel Threshold**
You can set the border using the Local Contrast Threshold or the Source Channel Threshold as criteria. In both cases as next step a working channel is displayed in the viewing area (see fourth window of the Create Wizard).

**Buttons Back**
With Back you return to the second window of the Create Wizard.

**Buttons Next**
Press Next to proceed with the fourth window of the Create Wizard.

**Button Cancel**
If you want to manually point out all the Spots click on the Cancel button. The parameters on the tab Settings are displayed in the properties area.

## Fourth Window of the Create Wizard

### 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 (Local Contrast Threshold or Source Channel Threshold) refer to Local Contrast Threshold or Source Channel Threshold.

**Local Contrast Threshold**
In the working channel the regions with a local contrast brighter than the background are displayed in white. Now you adjust the threshold value of the local contrast channel. Edit the data field or adjust the threshold manually using the mouse (left-click and drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

**Source Channel Threshold**
In the working channel a copy of the intensity histogram of the source channel is displayed. Regions above the threshold are displayed in white. Now you adjust the threshold of the working channel. Edit the data field or adjust the threshold manually using the mouse (left-click and drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

Please note: To display the Working Channel in the viewing area you need a Volume Item in the Surpass Tree and the Item has to be checked on.

**Remove Overlapping Spots**
To remove overlapping Spots check this box.

### Region Growing and Output

Select from the drop-down list the output of the region growing. (If you already had some Spots and started the Region Growing from the tab Settings the Spots will also be recalculated.)

**Radius from Distance to Border**
The radius of the calculated Spot(s) is the smallest distance from the each Spot center to the region border.

**Radius from Region Volume**
The Spot(s) radius is calculated from the region volume (the region volume and the Spot volume are equal).

**Radius from Region Volume + Region Channel**
Additional to the Spots the Region Channel is displayed. Each region has a different color.

**Radius from Region Volume + SurfaceObjects**
Additional to the Spots colored Surface objects are displayed.

**Buttons Back**
With Back you switch to the third window of the Create Wizard.

**Button Finish**
Click on Finish to calculate the desired Spots and display the detected regions. The default color table for the Regions channel is a colorful Mapped Color template.

---

See also:
Menu Edit - Image Properties... - Channel 1...n - Tab Mapped Color

## 13.13.2 Tab Settings

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.

**Radius Scale**
The initial Spots diameter is the minimum diameter in the creation process. With this parameter you can change the size of the Spots.

Rendering Quality

You can adjust the rendering quality to produce faster performance of the display when the number of Spots is very large.

**Button Region Growing** (Radii from Regions)
Click on this button to switch directly to the third window of the Create Wizard.

---

See also:
Surpass View - Spots - Create Wizard (Third Window of the Create Wizard)

## 13.13.3 Tab Edit

Method

There a two different methods to add or delete spots. Switch to the mouse pointer mode Select. To add a Spot Shift + left-click in the image. To delete a Spot Shift + left-click on the Spot.

**AutoDepth**
The Auto. Depth function will automatically set the Spot depth where the object intensity is at a maximum depending on the current viewing angle (e.g. if you are looking at the xy-plane of your picture, the Spot z-coordinate will be automatically computed). If you check this box the parameter set Slice (see below) is not available.

**Manual**
A slicer appears in the viewing area and the Slice parameters are available (see below). To add a Spot fully manually, move the slicer to the desired position and Shift + left-click onto the slicer.

Tip: Adding Spots is easier when using a Volume object in MIP mode at the same time.

Diameter

The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

**Apply to All**
If you check this box the diameter above is applied to all existing and new Spots.

Slice

If the check-box Manual in the Method window (see above) is activated the Slice parameters are available.

**YZ Plane**
**XZ Plane**
**XZ Plane**
Here you can select the slicer orientation.

**Position**
**Slicer**
In the position box the slicer position in um is displayed. There are three possibilities to move the slicer:

- Type in the desired position.
- Move the slicer using the slider.
- Click in the image on the slicer and move the slicer to the desired position.

---

See also:
Surpass View - Overview - Camera Toolbar (Pointer Select)
Surpass View - Volume

## 13.13.4 Tab 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.

**Brownian Motion**
This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate, if your Spots actually perform a Brownian motion.

**Autoregressive Motion**
This algorithm models the motion of each Spot as an autoregressive process. This model is appropriate if your Spots perform any kind of continuous motion.

**Autoregressive Motion Expert**
Select the expert mode to adjust additional parameters (see below).

**Connected Components**
This algorithm models the motion of continuous connected components.

Parameters

**Maximum Distance**
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.
The parameter is only available if you select Brownian Motion, Autoregressive Motion or Autoregressive Motion Expert (see above).

**Maximum Gap Size**
It happens that the object segmentation fails for some time points. This can break a Track apart into two Tracks. The gapclosing algorithm tries to continue the movement of a disappearing object and connects the Tracks if it reappears. The number defines the maximum consecutive time points until the object re-appears.
The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

**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).

**Max Intensity Difference**
Maximum Intensity Difference between two successive objects in a track.
The parameter is only available if you select Autoregressive Motion Expert (see above).

**Filter Width**
If "Filter Width" is non-zero the predicted position of a particle for a future time point is computed based on all tracks within a neighborhood defined by "Filter Width".
The parameter is only available if you select Autoregressive Motion Expert (see above).

**Button Create Track**
Click on the button Create Track to start the calculation. After calculation the Surpass Tree in the objects area will contain a new group called Track Group containing one or several Track objects. Each Track in return contains several Spots that have been linked into the same Track by the tracking algorithm.

Track Color

The different Tracks by default are assigned different colors in which they visualize their objects. This coloring serves to allow for an immediate visual inspection of the result.

Tab Tracks

After the tracking the Surpass Tree in the objects area will contain a new group called Track Group. Highlight the Track Group and select the tab Tracks to adjust the Tracks parameter (please refer to Surpass View - Track - Tab Settings).

---

See also:
Surpass View - Track - Tab Settings

## 13.13.5 Tab Statistics

Spots statistics are automatically computed for each Spot. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Spots.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Spots

## 13.13.6 Tab Color

To change the color use the color wheel or type in the color combination.

---

## 13.13.7 Mouse & Keyboard PC

Spots Mouse & Keyboard Functions in the Tab Edit

| | |
|---|---|
| Shift + left-click | Add/delete spot |
| Mouse wheel | Change spot diameter |

---

See also:
Addendum - Mouse & Keyboard PC

### 13.13.8 Mouse & Keyboard Mac

Spots Mouse & Keyboard Functions in the Tab Edit

| | |
|---|---|
| Shift + click | Add/delete spot |
| Mouse wheel | Change spot diameter |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.14 Surface Object

Initial Objects for Surface Objects

As initial objects for Surface objects you can choose:

- IsoSurface - split the IsoSurface, the result are several Surface objects.
- Contour Surface - create a Contour Surface and then create the Surface object based on this Contour Surface.

---

See also:
Surpass View - IsoSurface - Tab Settings Button Split ...
Surpass View - Contour Surface - Tab Settings Button Create Surface Object

### 13.14.1 Tab Settings

**Time Index (Point)**
Displays the actual time point.

**Surface (Object) Triangles**
Displays the number of Surface object Triangles.

**Unconnected Surface (Objects)**
Displays the number of unconnected Surface objects. If there are more than one unconnected Surface objects the button Split... is active.

**Button Split...**
If you still have unconnected Surface objects (e.g. previous Split had a limited number of split objects) this button is activated. Click on the button to open the Split box.

**Create no more than ... Surface Objects**
Check Create no more than ... Surface objects to specify the number of independent Surface objects created. The Surface objects are created beginning with the Surface object that contains the greatest number of triangles (depending on the shape it is possible that an object consisting of more triangles has a smaller volume than another one with less triangles). Remaining objects are grouped together in the final Surface object.

**Filter Objects with less than ... Triangles**
Check Filter objects with less than ... Triangles to eliminate very small objects which may not be relevant.

**Button Ok**
If you want to create Surface objects click on OK. Surface objects are created and displayed according to the setup parameters.

**Button Cancel**
If you do not want to create Surface objects click on Cancel.

**Button Duplicate Surface (Object) to All Time Points**
Click on this button and the actual Surface object is copied to all time points in the data set.

**Button Mask Channel with Surface**
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 choose a Surface object. Be sure that the Surpass Tree Surface object is highlighted and click on the tab Settings. Click on the button Mask Channel.

Window Mask Channel

**Select Channel to mask with Surface**
Select the channel (destination channel) you want to mask with the Surface object (ROI).

**Duplicate Channel before applying Mask**
Check this box and an additional masking channel is added in the Display Adjustments.

**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 un-checked 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**
Check the box if you want to apply the mask to all time points.

**Button Ok**
**Button Cancel**
If you want to apply the mask click on OK. Otherwise click on Cancel.

---

See also:
Menu Edit - Show Display Adjustment

## 13.14.2 Tab Edit

Surface Scissors

Use the Surface Scissors to manually split two touching Surface objects (after IsoSurface segmentation).

- In the first step select the Surface object in the Surpass Tree.
- The selected Surface object is highlighted by a rectangle in the viewing area.
- Change to the pointer mode Select.
- Open the tab Edit and you are directly in the scissors mode to cut Surface objects.
- The scissors tool cuts the Surface object perpendicular.
- Hold the Shift-key and click with the left mouse button on the Surface object.
- A colored line indicates the cut in the viewing area.
- Rotate the view to take a look at the cut preview.

Selection

**Number of Cutted Triangles**
Displays the number of cut triangles in the preview.

**Number of Cutting Lines**
Displays the number of cutting lines in the preview.

Scissors Properties

**Close Borders**
Check this box to add a surface along the cutting line and close the surface.

**Button Cut**
Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects.

Surpass Tree Update

The Surpass Tree updates automatically. There are two separate Surface objects with automatically extended names in the Surpass Tree.

Please note: If the Surface object is not cut in two pieces the cutting is not directly visible in the viewing area or the Surpass Tree.

---

See also:
Menu Edit - Preferences... - 3D Cursor (here you can change the shape of the 3D cursor)

### 13.14.3 Tab Statistics

Surface object statistics are automatically computed for each selected Surface object.

For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Surface Object.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Surface Object

### 13.14.4 Tab Color

To change the color use the color wheel or type in the color combination.

---

## 13.15 Topography

Topography is a 3D relief representation, created from a 2D input file which contains intensity coded height data. This input file can be an ordinary TIFF file or a selected slice of a 3D data set.

Topography allows you to overlay an independent texture on the relief image to colorize the representation.

How to Add a new Topography?

- Select Menu Surpass - Topography.

The Surpass Tree list is automatically updated and in the Topography - Properties the Settings parameter are displayed.

An absolute topography of the file or of the slice 1 of channel 1 (default) of the loaded data set is created and displayed in the viewing area. The object properties area shows the suggested parameters for it.

---

## 13.15.1 Tab Geometry

**Data Set**
For the first Topography relief Imaris calculates per default the first slice in the loaded data set. To change the source slice adjust the parameters Data Set Options (see below).

**File ...**
To open another file for Topography check the File… field. The Open Topo File window is displayed. Select the requested file and click Open. The new Topography is displayed.

Data Set Options

To change the data set options adjust the following parameters.

**Channel**
Here you select the channel.

**Slice**
Here you select the slice.

Mask Volume

**Button Cut Above**
**Button Cut Below**
You can cut the volume above/below the topography line with a click on the button Cut Above/Cut Below.

Translation of Z

The topography can be moved in z-direction relative to the data set.

- In the pointer mode Select move the topography manually within the data set.
- Use the translation field (the first field) to specify the required value (z-position).

Scaling of Z

If necessary scale the height of the topographic representation.

**Step**
Adjust the scaling steps in um.

**Maximum Height**
The maximum height calibrates the geometric height (distance in z-direction) between the lowest and the highest gray value in the input slice.

**Button Auto Scale Z**
Clicking this button rescales the heights to a default value relative to the file's 2D extensions.

---

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

## 13.15.2 Tab Coloring

You can apply a color or a texture from different sources to the relief.

**Base Color**
If you select base color the next parameter set (Options) is grayed. To proceed click on the tab Base Color and select a color. The color is overlaid on the Topography relief.

**Altitude Weighted**

Check this box and the colors in the lower layers are black.

**Data Set**
The texture you apply to the relief is an image out of your actual data set. To specify the image proceed to the parameter set Options (see below).

**File ...**
The Open Texture File window opens. Select an image to overlay on the relief.

Options

If Data Set (see above) is selected, the parameter set Options is available.

**Channel**
Select one data set channel.

**Select All**
Check this box and all data set channels are selected. The Channel selection turns gray.

**Slider**
**X% Diffusion .... X% Emission**
100% diffusion: the light source is responsible for the illumination.
100% emission: the image shines by itself.

---

See also:
Surpass View - Topography - Tab Base Color

### 13.15.3 Tab Base Color

If you select Base Color on the tab Coloring the selected color is overlaid on the Topography relief.

To change the color use the color wheel or type in the color combination.

---

See also:
Surpass View - Topography - Tab Coloring - Base Color

### 13.15.4 Mouse & Keyboard PC

Topography Mouse & Keyboard Functions

| | |
|---|---|
| Left-click plane & drag | Change z position |

---

See also:
Addendum - Mouse & Keyboard PC

### 13.15.5 Mouse & Keyboard Mac

Topography Mouse & Keyboard Functions

| | |
|---|---|
| Click plane & drag | Change z position |

---

See also:
Addendum - Mouse & Keyboard Mac

## 13.16 Track

The Track Item in the Surpass Tree can be created directly as a starting point for a tracking session.

How to Add a new Track?

- Select Menu Surpass - Track.

The Surpass Tree list is automatically updated and in the Track - Properties the Settings parameter are displayed. The empty Track is displayed in the Surpass Tree.

### Manual Tracking

Tracks can be created or modified manually from existing Surface objects.

- Add a new, empty Track to the Surpass Tree.
- Move the desired Surface objects into the Track item.
- Then open the Track Editor Window (Surpass View - Track - Button Edit Track) and design the track.

### Initial Objects for Surface Objects

As initial objects for Surface objects you can choose:

- IsoSurface - split the IsoSurface, the result are several Surface objects (Surpass View - IsoSurface - Tab Settings Button Split...).
- Spots - create Spots, do an automated Tracking and then select the desired Spots for the manual Tracking (Surpass View - Spots - Tab Tracking).
- Contour Surface - create a Contour Surface and then create the Surface object based on this Contour Surface (Surpass View - Contour Surface - Tab Settings Button Create Surface Object).

---

See also:
Surpass View - Surface Object
Surpass View - Track - Button Edit Track

## 13.16.1 Tracking Overview

Given a time data set that describes several biological objects at several time points, the term tracking refers to a procedure that links objects of consecutive time points and results in a description like: “Object 3 at time 1 develops into object 7 at time 2, then into object 7 at time 3, then splits into objects 6 and 13 at time 4 …”.

In Surpass the objects that can be used for tracking are the Surface objects and Spots.

The result of tracking is a set of Tracks, each of which should describe a biological object (if tracking was successful). This has two important consequences. The different Tracks can be visualized separately or with different colors and statistics can be produced separately for each Track.

In the Surpass View tracking can be performed in several ways. Automatic tracking possibilities exist for Spots and Surface objects. For Spots several algorithms are provided that create Tracks without splits or merges. For IsoSurfaces a “threshold” tracking algorithm is available that generally produces Tracks with splits and merges. Furthermore a Track Editor allows to modify or create Tracks manually.

### Spot Tracking

To track Spots, it is necessary to create a Spots object and perform Spot detection as described in the section on Spots. Then you can select the tab Tracking in the properties window of the Spots object.

### Threshold Tracking for IsoSurfaces

In “threshold tracking” Surface objects are computed from an intensity threshold and linked into Track Groups. To perform “threshold tracking”, first create an IsoSurface object with settings that produce good segmentations of the biological objects. Then press the button Create Track in the Settings dialog of the IsoSurface.

After calculation the Surpass Tree in the objects area will contain a new group called Track Group containing one or several Track objects. Each Track in return contains several Surface objects that have been linked into the same Track by the tracking algorithm.

Besides the different Track objects, the Track Group generally contains a group of Surface objects that were not linked to other objects by the tracking algorithm.

Manual Tracking

Tracks can be created or modified manually from existing Surface objects. These Surface objects can come from either the IsoSurface, Spots or the Contour object.

Track Color

The different Tracks by default are assigned different colors in which they visualize their objects. This coloring serves to allow for an immediate visual inspection of the result.

---

See also:
Surpass View - Spots - Tab Tracking
Surpass View - IsoSurface - Tab Tracking

## 13.16.2 Tab Settings

**Button Edit Track**
The Edit Track button displays (or hides) the Track Editor window (please refer to chapter Surpass View - Track - Button Edit Track ...).

**Button Correct Drift**
If you have an object drift (e.g. a cell drift) during image acquisition you can use this button to apply a drift correction for the whole data set.

- Select a reference Track and highlight the Track in the Surpass Tree.
- A click on the button Correct Drift will translate the entire image in such a way that the selected object remains stationary in the corrected image.
- The result is directly visible in the viewing area.

Please note: The drift correction changes the data set.

Show Object Shape

When Show Object Shape is ticked, the Surface objects/Spots of the Track are visible in the viewing area. Otherwise, they are invisible.

Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

**Off**
When Off is selected, no connections are drawn and the other settings within the box have no effect. The comment Track invisible is displayed.

**Cylinder**
**Line**
When Line or Cylinder is selected the connections are dawn as lines or cylinders.

**Volume**
When Volume is selected the connections are drawn as truncated cones with radii proportional to the volume of the objects at their end points.

**Track invisible**
When you select Off the comment Track invisible is displayed.

**Width**
**Diameter**

**Scale**
Depending on the previous selection you adjust the width of the line, diameter of the cylinder, or scale the volume.

**Render Quality**
When you select Cylinder or Volume the Render Quality is adjustable using the slider.

Style/Quality

The box Style/Quality Connections has settings that determine the display of connections between the Spots of a Track. When Off is selected, no connections are drawn and the other settings within the box have no effect. When Line or Cylinder (see above) is selected the connections are dawn as lines or cylinders. When Volume is selected (see above) the connections are drawn as truncated cones with radii proportional to the volume of the Spots at their end points. The slider allows adjusting the width of lines, cylinders or cones.

Path

**Time Color**
Selecting Time Color draws lines, cylinders or truncated cones in colors with a range from blue to white and dark to bright corresponding to the range of time points. Alternatively it is possible to assign a single color via the Color Editor. Deselect Time Color and click on the Color symbol to open the Color Editor.

**Color Editor**
Select a desired color for the Track display.

**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.

**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.

---

See also:
Surpass View - Track - Button Edit Track

## 13.16.3 Button Edit Track

Click on the button Edit Track ... to open the Track Editor window.

You find this button on the Tab(s):

- Highlight a Track in the Surpass Tree and select the tab Settings.
- Highlight a Track Group in the Surpass Tree and select the tab Tracks.

Track Editor Window

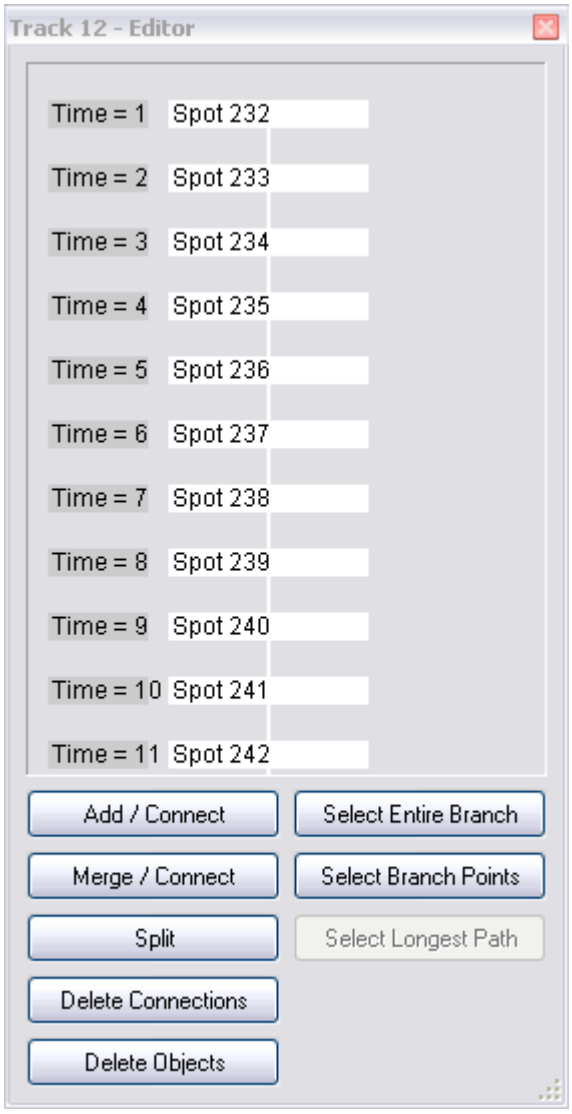
The Track Editor window displays an abstract view of all the Spots contained in a Track as well as their interconnections. It also allows to modify both the set of Spots as well as the connections. When the Track Editor window is displayed for a new Track that is to be created manually it is initially empty.

Please note: Tracks can be created or modified manually from existing Surface objects. These Surface objects can come from either the IsoSurface, Spots or the Contour object. You can manually copy Surface objects in the Track Item and manually edit a Track.
To create Surface objects of existing Spots use the functions on the Tab Tracking (Surpass View - Spots -

Tab Tracking). Then add a new, empty Track to the Surpass Tree and move the desired Surface objects into the Track item. Then open the Track Editor Window and design the Track.

### Example

The following figure displays a Track Editor window for a Track that contains one object per time point of the data set.




The Track Editor window allows the following operations on a Track:

### Select Spot

A Spot can be selected in the Track Editor window by left-clicking onto the corresponding box displaying its name. Using Shift + left-click it is possible to perform multiple selection. The Spots selected in the Track Editor window are also selected in the viewing area and in the Surpass Tree.

### Select Connections

A connection between two Spots can be selected by left-clicking onto the line corresponding to the connection in the Track Editor window. Using Shift + left-click it is possible to perform multiple selection.

**Button Add\Connect**

A single Spot

To add a new Spot to a Track, select this Spot either within the viewing area or in the Surpass Tree. Then press the Add\Connect button in the Track Editor window. As a result the selected Spot appears in the Track Editor window and it is moved from its original location in the Surpass Tree to become a child of the Track object to which the Track Editor window belongs.

**Button Add/Connect**

Multiple Spots

Several Spots can be added to the Track at once. To do so, use multiple selection to select several Spots. Then press the button Add/Connect in the Track Editor window. All selected Spots will appear in the Track Editor window and they all become children of the corresponding Track. In addition connections are automatically created between all the selected Spots, in the order in which they were selected (e.g. first to second, second to third, third to fourth).

**Button Add/Connect**

Create connections between Spots

If several Spots of the Track that is being edited are selected (they then appear highlighted in the Track Editor window) and the Add/Connect button is pressed the effect is that connections between the selected Spots are created, in the order in which they were selected (e.g. first to second, second to third, third to fourth).

**Button Merge\Connect**

A single Surface Object

If a Spot to be added to a Track is the child of another Track it may have connections to other Spots within that other Track. If this Spot is selected and you press the Merge\Connect button the Spot will be added to the Track and all other Spots to which it was previously connected in the other Track will also be added. Furthermore the connections between these Spots will be restored in the new Track exactly as they existed in the Track they came from.

**Button Merge\Connect**

Multiple Spots

Several Spots can be merged into a Track at once. To do so use multiple selection to select several Spots. Then press the Merge\Connect button in the Track Editor window. All selected Spots will be merged into the Track together with the Spots to which they were previously connected in another Track (if any). In addition connections are created between all selected Spots, in the order in which they were selected.

**Button Split**

A Track contains Spots and connections between these objects, both of which are visualized in the Track Editor window. It may happen that a Track can be split into two or more Tracks with exactly the same connections between Spots without having to delete any connections. If this is the case, the operation is performed by pressing the Split button. If it is not the case, pressing the Split button has no effect.

**Button Delete Connections**

A connection between two Spots can be selected by left-clicking onto the line depicting the connection within the Track Editor window. Pressing the Delete Connections button in the Track Editor window will delete all selected connections.

**Button Delete Objects**

Pressing the Delete Objects button in the Track Editor window will remove all selected Spots of that Track. The Spots are not actually deleted, but they are made invisible and moved into a group called Trash in the Surpass Tree.

**Button Select Entire Branch**

If a Spot or a connection is selected and you press the Select entire Branch button, all other Spots and connections of the Track will also be selected unless there is a branch between them and the originally selected Spots or connections.

**Button Select Branch Points**

By pressing the button Select Branch Points it is possible to select and thus highlight all Spots that have more than two connections.

**Button Select Longest Path**

Press this button to select the Longest Path.

---

## 13.16.4 Tab Statistics

The tab Statistics displays some statistics values of its children as well as several Track specific statistics values. The Track statistics are automatically computed for each Track.

For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Track.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Track

## 13.16.5 Tab Sort

Highlight a Track Group (or Track) and select the tab Sort. This tab allows sorting the objects within the Track or Track Group by any statistics value.

Sort Objects by

**(Various sorting criteria)**
Select the desired sorting criteria.

**Ascending**
In the Surpass Tree the objects are arranged in ascending order.

**Show All**
In the Surpass Tree the objects are checked and displayed in the viewing area.

**Button Select Hidden**
All objects in the Surpass Tree that are un-checked (hidden) are highlighted at once. Use this function to move the objects in a different group or delete them at once.

Show Objects between two Values

**Enable**
Check this box to enable the function and enter the two values.

Histogram

The numerical range of the statistics value (e.g. Track length), and the number of objects (e.g. Tracks) depending on the statistics value is displayed. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects is visualized. The visualized objects are checked in the Surpass Tree.

---

## 13.16.6 Tab Color

To change the color use the color wheel or type in the color combination.

---

# 13.17 Volume

How to Add a new Volume?

- Select Menu Surpass - 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.

---

## 13.17.1 Display Adjustments

To set the correct Display Adjustments for the Volume rendering of your data set please refer to chapter - Show Display Adjustment.

---

See also:
Menu Edit - Show Display Adjustment

## 13.17.2 Tab Settings

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 then 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.

**MIP (min)**
A Maximum Intensity Projection with minimum intensity of all layers along the viewing direction.

**Blend**
The result of the bland 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.

**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.

If Shadow Projection is selected, the parameter Fix Light(s) to Data Set is available.

**Fix Light(s) to Data Set**
Check the box to fix the x-, y-, and z-axes to the same side when navigating the object. When the box is un-checked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

This check-box is only available, if Shadow Projection is selected.

Rendering Quality

If the rendering quality is set to 1.000, then 1pixel represents 1 voxel. If you decrease the rendering quality the image 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

## 13.17.3 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 an IsoSurface, Spots, or Filament. (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 hallow spheres into the selected image channel.

- First select the desired channel and painter shape.
- Rotate the view to execute a vertical cut.
- Then customize the painter settings.
- Change to the pointer mode Select.
- Move the cursor to the desired position.
- Hold down the Shift-key and left-click to add the shape.
- In the viewing area you see a preview of the painter tool.
- To re-edit a shape use the point selection.
- Set intensity to 0 to erase a region, or set the intensity to 255 to fill a region.
- 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.

---

### 13.17.4 Tab Statistics

Volume statistics are automatically computed for each Volume.

For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Volume.

**Button Settings**
Click on the button Settings and you switch to the window Preferences - Statistics.

**Button Export CSV**
**Button Excel**
To do some further statistical analysis you can export the data either as CSV (Comma Separated Values, *. csv file) or directly into an MS Excel sheet. With a left-click on the button Excel automatically all generated statistical data is exported to MS Excel, and sorted in different sheets. Excel starts automatically and the sheets show up one after another (this will take several seconds).

---

See also:
Menu Edit - Preferences... - Statistics - Volume

# 14 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?

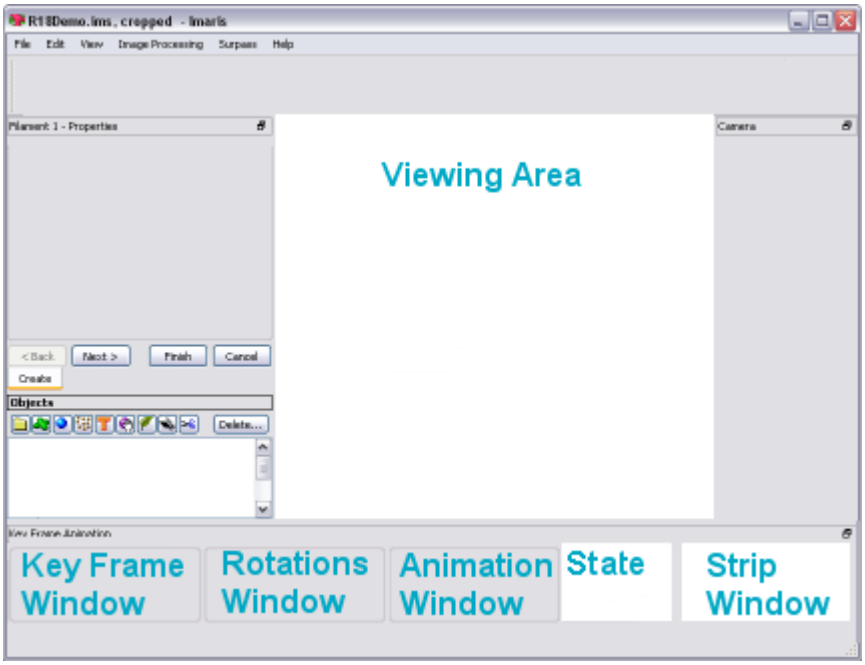
- Click on the icon in the Main toolbar.
- Select Menu View - Animation.
- Press the key combination Ctrl + 6.

---

See also:
Menu Edit - Preferences... - Surpass
Animation - Mouse & Keyboard Mac

## 14.1 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 Animation Window, the State selection, and the Strip Window.

## 14.2 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.

**Button Auto Distribute**
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 |
| **>** | **Play**/**Pause** animation |
| **Red Dot** | **Record** animation to movie file |

| | |
|---|---|
| **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 chapter Animation - Strip Window.

---

See also:
Animation - Create Animation

## 14.3 Rotations Window

Use the templates to quickly define standard camera motions for the key frame animation system.

**Templates**
Select one of the predefined templates. 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.

---

## 14.4 Animation Window

**Frames**
In the Animation window you can enter the number of movie frames.

**Button Settings ...**

To open and adjust the Key Frame settings click on this button. For details please refer to chapter Menu Edit - Preferences... - Surpass.

**Button >**
Click on this button (arrow to the right - Play/Pause) to start the movie. To pause the movie re-click on this button.

**Button O**
To record the movie click on this button (red dot - Record). The Save As Movie window is displayed (see below).

Save as Movie Window

**File name**
Enter a file name.

**Save as type**
Select the desired file type (QuickTimeMovie, 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.

QTVR Settings

The QuickTimeVR Settings are not available.

**Add to ImageAccess database**
Check the box to add the file to the ImageAccess database.

**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)
Surpass View - Overview - QuickTimeVR

## 14.5 Play Back State

In the State selection you can adjust the play back settings.

**Camera**
- Check: Play back the previously recorded Camera positions for each key frame.
- Un-check: 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.
- Un-check: 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.
- Un-check: 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.
- Un-check: 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.
- Un-check: 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.
- Un-check: 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.
- Un-check: 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

## 14.6 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 left-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: Left-click on a key frame to change the camera position in the viewing area accordingly. Right-click on a key frame and the view does not change.

Strip Window Overview

| | |
|---|---|
| **Left-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... - Surpass (default interpolated frame settings)

## 14.7 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 (IsoSurface, 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 chapter 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 un-check 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 un-check 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

## 14.8 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 (*.avi).

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. Open the menu File and select Export Scene as... and click on Save.

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. Open the menu File and select Export Scene as... . In the dialog window type in the new Scene File name (e.g. PyramidalCellSceneAMovie.imx) and click on Save.

Record the Animation as AVI Movie

To save the animation as AVI movie click in the Animation window on the button Record (red dot). The Save As Movie dialog window is displayed on screen. As File name type in the corresponding Scene File name, use the default Movie Settings and click on Save. The file extension for the AVI movie is *.avi.

---

See also:
Menu File - Export Scene as...
Surpass View - Overview - Scene File Concept
Surpass View - Overview - Quick TimeVR
Animation - Animation Window (Button Record)

## 14.9 Mouse & Keyboard PC

Animation Mouse & Keyboard Functions

| | |
|---|---|
| Ctrl + 6 | 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 |

---

## 14.10 Mouse & Keyboard Mac

Animation Mouse & Keyboard Functions

| | |
|---|---|
| Command + 6 | 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 |

# 15 InMotion

The function InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view. 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 Menu View - InMotion.
- Press the key combination Ctrl + 8.

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.

See also:
Toolbars - Main Toolbar
InMotion - Mouse & Keyboard Mac

## 15.1 Mouse & Keyboard PC

### InMotion Mouse & Keyboard Functions

| | |
|---|---|
| Ctrl + 8 | Activates the InMotion function |

## 15.2 Mouse & Keyboard Mac

### InMotion Mouse & Keyboard Functions

| | |
|---|---|
| Command + 8 | Activates the InMotion function |

# 16 Coloc

In fluorescent 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 every 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 ImarisSurpass.

Coloc is an optional module within the Imaris program and is not available as a standalone program.

How to Display the Coloc Main Screen?

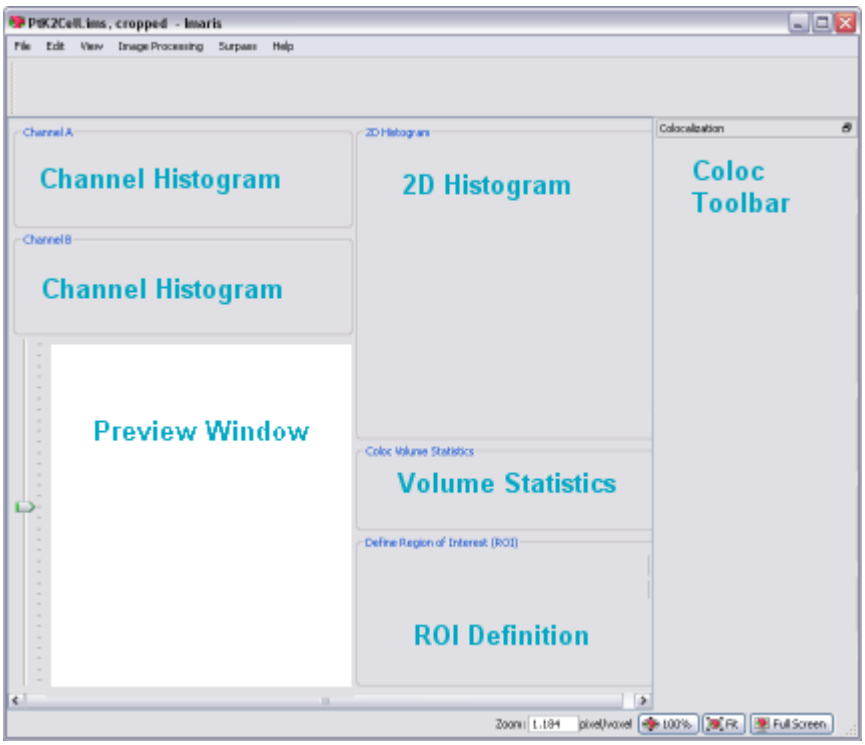
- Click on the icon in the Main toolbar.
- Select Menu View - Coloc.
- Press the key combination Ctrl + 7.

---

See also:
Coloc - Mouse & Keyboard Mac

## 16.1 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.

---

## 16.2 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

## 16.3 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

## 16.4 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. 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.

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 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, left-click and drag the vertex around to the desired location. The polygon changes its shape.

**Move Line**
Point on a line between two vertices, left-click and drag the line around to the desired location. The polygon changes its shape.

**Move Polygon**
Point somewhere beside of the polygon edge, left-click and drag the polygon around to the desired location.

**Delete Point**
Shift + left-click on a point to delete the point.

---

See also:
Coloc - Coloc Toolbar (selection Mode Threshold or Polygon)

## 16.5 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).

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 $N_{coloc}$.

**% of data set colocalized**
Percentage of total data set voxels colocalized.

$$\frac{N_{coloc}}{N_{dataset}} \cdot 100\%$$

**% of volume A above threshold colocalized**
Percentage of channel A voxels above threshold A colocalized.

$$\frac{N_{coloc}}{N_{objectA}} \cdot 100\%$$

**% of volume B above threshold colocalized**
Percentage of channel B voxels above threshold B colocalized.

$$\frac{N_{coloc}}{N_{objectB}} \cdot 100\%$$

**% of material A above threshold colocalized**
Percentage of channel A material above threshold A that is colocalized.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in objectA} SA_i} \cdot 100\%$$

**% of material B above threshold colocalized**
Percentage of channel B material above threshold B that is colocalized.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in objectB} SB_i} \cdot 100\%$$

**% of ROI colocalized**
Percentage colocalization of channel A and channel B volume inside the region of interest.

$$\frac{N_{coloc}}{N_{mask}} \cdot 100\%$$

**% of ROI material A colocalized**
Percentage colocalization of ROI channel A material.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in mask} SA_i} \cdot 100\%$$

**% of ROI material B colocalized**
Percentage colocalization of ROI channel B material.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in mask} SB_i} \cdot 100\%$$

**Channel correlation 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.

$$R_{AB,coloc} = \frac{\sum_{i \in coloc} \left(SA_i - SA_{avg,coloc}\right)\left(SB_i - SB_{avg,coloc}\right)}{\sqrt{\sum_{i \in coloc} \left(SA_i - SA_{avg,coloc}\right)^2 \sum_{i \in coloc} \left(SB_i - SB_{avg,coloc}\right)^2}}$$

**Channel correlation in data set volume**
Pearson correlation of channel A and channel B inside the entire data set volume.

$$R_{AB,dataset} = \frac{\sum_{i \in dataset} \left(SA_i - SA_{avg,dataset}\right)\left(SB_i - SB_{avg,dataset}\right)}{\sqrt{\sum_{i \in dataset} \left(SA_i - SA_{avg,dataset}\right)^2 \sum_{i \in coloc} \left(SB_i - SB_{avg,dataset}\right)^2}}$$

**Channel correlation in ROI volume**
Pearson correlation of channel A and channel B inside the region of interest.

$$R_{AB,mask} = \frac{\sum_{i \in mask} \left(SA_i - SA_{avg,mask}\right)\left(SB_i - SB_{avg,mask}\right)}{\sqrt{\sum_{i \in mask} \left(SA_i - SA_{avg,mask}\right)^2 \sum_{i \in mask} \left(SB_i - SB_{avg,mask}\right)^2}}$$

---

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)

## 16.6 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 Dataset**
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

## 16.7 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 bolded line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded 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 chapter Coloc - Coloc Toolbar - Automatic Thresholding.

### Histogram Mode

The histogram mode options are used to define the calculation basis for the colocalization within the data set.

**Single Slice**
The histograms are calculated and displayed for the current slice only.

**Single Time Point**
The histograms are 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 histograms are 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**
**Button Channel Statistics**
Please refer to chapter Coloc - Coloc Toolbar - Build Coloc Channel.

---

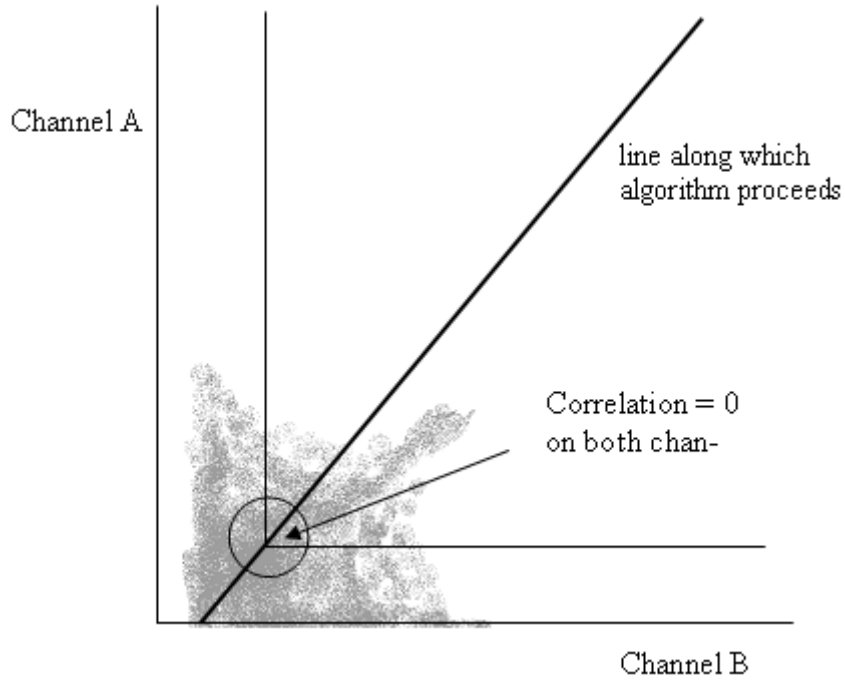
See also:
Coloc - Channel Histogram
Coloc - Preview Window
Coloc - 2D Histogram

## 16.7.1 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**
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

---

## 16.7.2 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.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File – Save As or click the Save as… button in the toolbar. The Save As 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 Channel Statistics button 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

## 16.8 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 bolded line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded 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 bolded 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 bolded 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 chapter 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 – Save As or click the Save as… button in the toolbar. The Save As 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.

---

### 16.8.1 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 un-check 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 an IsoSurface 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 IsoSurface button.
- In the IsoSurface 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 un-check 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.

## 16.9 Mouse & Keyboard PC

Coloc Mouse & Keyboard Functions

| | |
|---|---|
| Ctrl + 7 | 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<br>Move down: zoom in |
| Shift + right-click & drag | Move up: zoom out<br>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 |
| Ctrl + left-click | Add multiple new points |
| Left-click & drag (on point or line) | Move point or line |
| Left-click & drag (outside polygon) | Move polygon |
| Delete | Delete single point |

## 16.10 Mouse & Keyboard Mac

Coloc Mouse & Keyboard Functions

| | |
|---|---|
| Command + 7 | 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<br>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 |

# 17 Addendum

In this addendum you find the Imaris global Mouse & Keyboard PC and Mouse & Keyboard Mac functions, tips and tricks how to customize your Imaris Interface in daily routine, and a Terminology table with frequently used technical terms and specific image processing terms.

## 17.1 Mouse & Keyboard PC

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

| | |
|---|---|
| Ctrl + 1 | Activates the Slice View |
| Ctrl + 2 | Activates the Section View |
| Ctrl + 3 | Activates the Gallery View |
| Ctrl + 4 | Activates the Easy 3D View |
| Ctrl + 5 | Activates the Surpass View |
| Ctrl + 6 | Activates the Key Frame Animation |
| Ctrl + 7 | Activates the Coloc View |
| Ctrl + 8 | Activates InMotion |
| | |
| Ctrl + A | Sets zoom factor to 1 pixel per voxel and 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 Surpass scene file |
| Ctrl + F | Refreshes the textures of all views |
| Ctrl + I | Allows to inspect and edit the image properties |
| Ctrl + L | Opens Surpass 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 + P | Allows to edit figures and annotations |
| | |
| F1 | Opens Reference Manual |
| F2 | 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<br>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 chapter in this Reference Manual for details.

| | |
|---|---|
| Menu Edit - | Show Display Adjustments - Mouse & Keyboard PC |
| Slice View - | Mouse & Keyboard PC |
| Section View - | Mouse & Keyboard PC |
| Gallery View - | Mouse & Keyboard PC |
| Easy 3D View - | Mouse & Keyboard PC |
| Surpass View - | Overview - Mouse & Keyboard PC |
| | Clipping Plane - Mouse & Keyboard PC |
| | Contour Surface - Mouse & Keyboard PC |
| | External Object - Mouse & Keyboard PC |
| | Filament - Mouse & Keyboard PC |
| | Light Source - Mouse & Keyboard PC |
| | Measurement Point - Mouse & Keyboard PC |
| | Oblique Slicer - Mouse & Keyboard PC |
| | Ortho Slicer - Mouse & Keyboard PC |
| | Spots - Mouse & Keyboard PC |
| | Topography - Mouse & Keyboard PC |
| Animation - | Mouse & Keyboard PC |
| InMotion - | 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 massage the Reference Manual opens in the actual browser and displays the corresponding page.

---

## 17.2 Mouse & Keyboard Mac

In the following you find the global Mouse & Keyboard functions.

### Global Mouse & Keyboard Functions

| | |
|---|---|
| Command + 1 | Activates the Slice View |
| Command + 2 | Activates the Section View |
| Command + 3 | Activates the Gallery View |
| Command + 4 | Activates the Easy 3D View |
| Command + 5 | Activates the Surpass View |
| Command + 6 | Activates the Key Frame Animation |
| Command + 7 | Activates the Coloc View |
| Command + 8 | Activates InMotion |

| | |
|---|---|
| Command + A | Sets zoom factor to 1 pixel per voxel and 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 Surpass scene file |
| Command + F | Refreshes the textures of all views |
| Command + I | Allows to inspect and edit the image properties |
| Command + L | Opens Surpass 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 + P | Allows to edit figures and annotations |

| | |
|---|---|
| F1 | Opens Reference Manual |
| F2 | 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<br>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<br>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 chapter in this Reference Manual for details.

| | |
|---|---|
| Menu Edit - | Show Display Adjustments - Mouse & Keyboard Mac |
| Slice View - | Mouse & Keyboard Mac |
| Section View - | Mouse & Keyboard Mac |
| Gallery View - | Mouse & Keyboard Mac |
| Easy 3D View - | Mouse & Keyboard Mac |
| Surpass View - | Overview - Mouse & Keyboard Mac |
| | Clipping Plane - Mouse & Keyboard Mac |
| | Contour Surface - Mouse & Keyboard Mac |
| | External Object - Mouse & Keyboard Mac |
| | Filament - Mouse & Keyboard Mac |
| | Light Source - Mouse & Keyboard Mac |
| | Measurement Point - Mouse & Keyboard Mac |
| | Oblique Slicer - Mouse & Keyboard Mac |
| | Ortho Slicer - Mouse & Keyboard Mac |
| | Spots - Mouse & Keyboard Mac |
| | Topography - Mouse & Keyboard Mac |
| Animation - | Mouse & Keyboard Mac |
| InMotion - | 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 massage the Reference Manual opens in the actual browser and displays the corresponding page.

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## 17.3 Customize Interface

In this chapter 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.
- Left-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 un-dock the toolbars and dock them all at the same side. Especially in combination with InPress, this saves a lot of screen area.

Example: In the Section View you can bring the toolbar Figures (InPress), 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 - Surpass - 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 view (Extended) and in the Easy 3D view. Any change in the Display Adjustment window in the parameter Blend Opacity is only visible, if a Blend Mode is selected.

---

## 17.4 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.<br><br>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±2. In this case 100% of the values in the population are at one standard deviation of the mean.<br><br>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.<br><br>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. |