

Imaris

Quick Start Tutorials



1 Introduction

Why should you read and practice the Imaris Quick Start Tutorials?

They 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 open Imaris on 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. If you prefer to use a printout, a copy of this document is also provided in PDF format.

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www.bitplane.com or contact support@bitplane.com.

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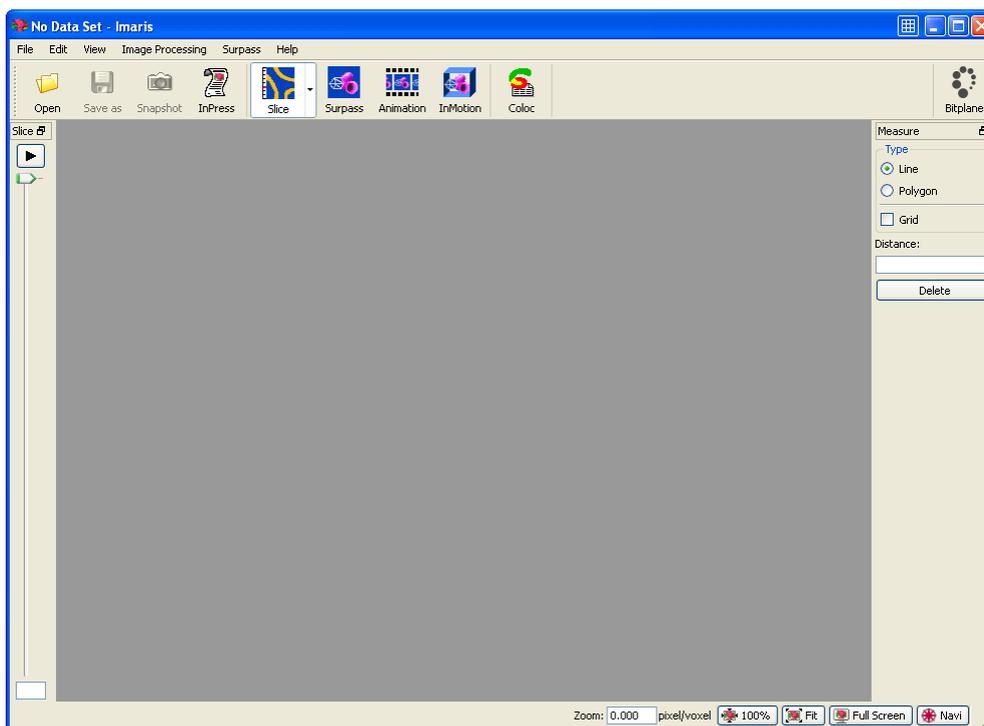
© 2007, Bitplane AG, Zürich
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Quick Start Tutorials V 5.7.0

1.1 Imaris Main Screen

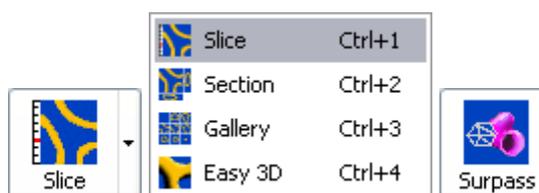


PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

PC Main Screen

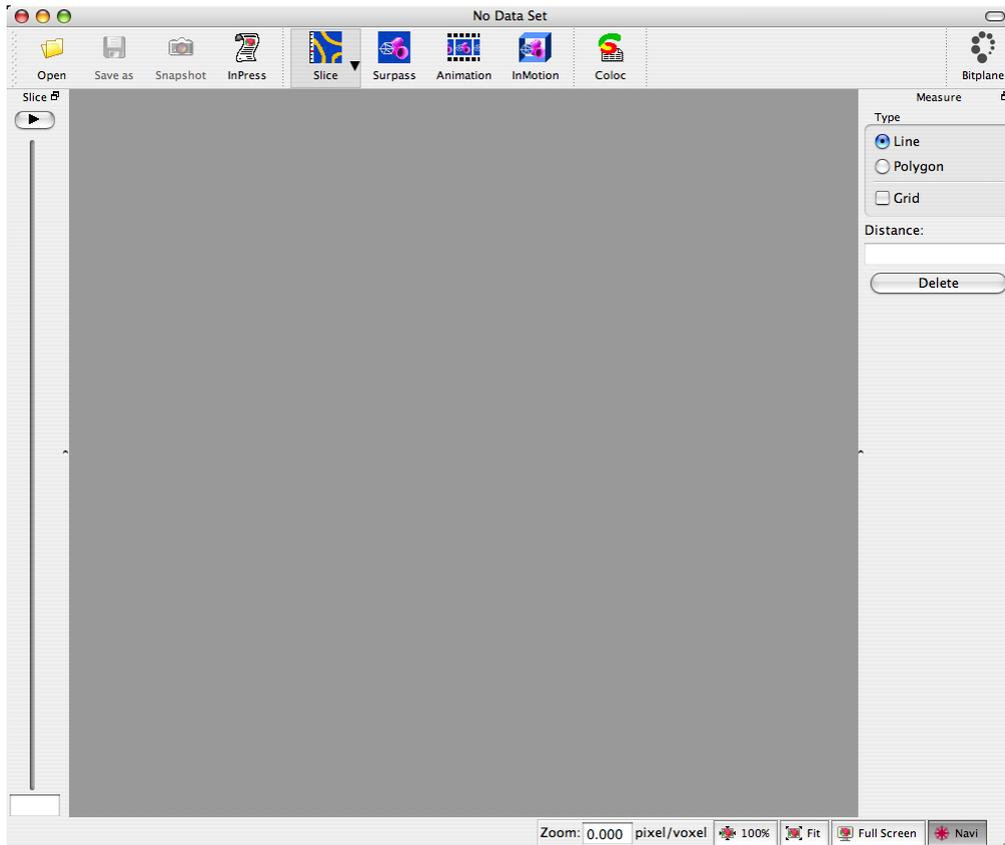


Upon the first program start after installation Imaris is in the Slice view.



There are three types of 2D display modes: **Slice**, **Section** and **Gallery**. There are two types of 3D display modes: **Easy 3D** and **Surpass**.

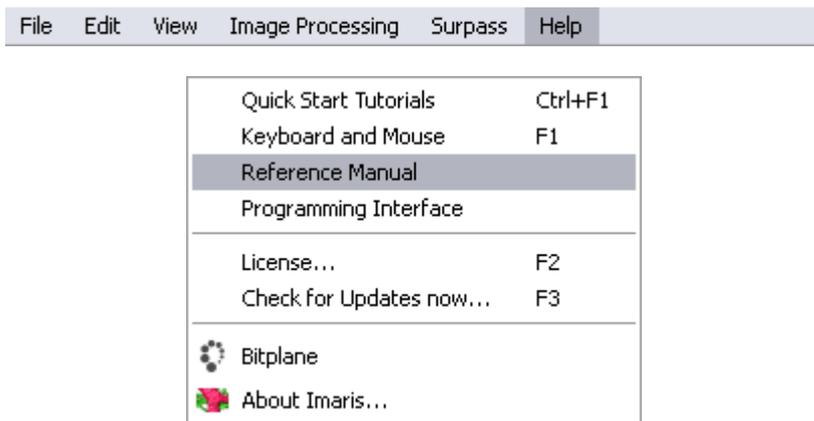
Mac Main Screen



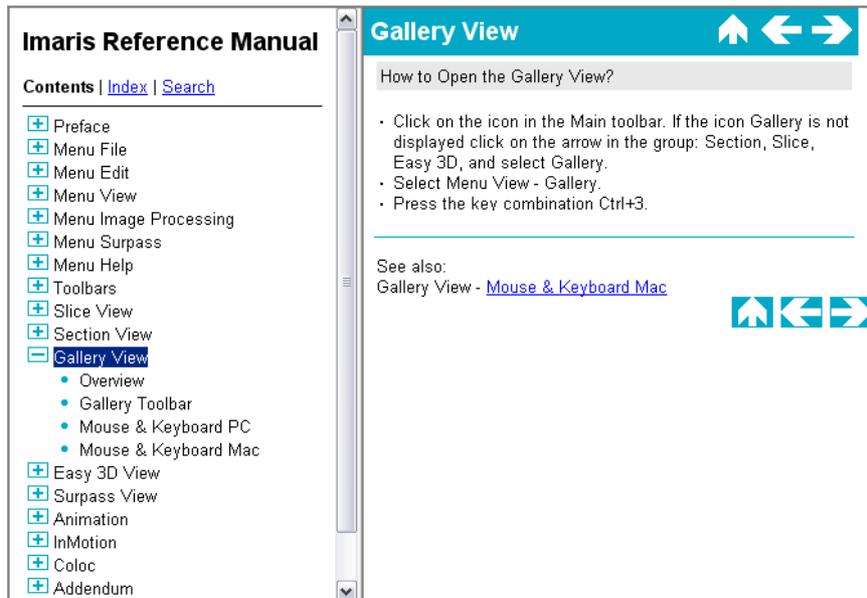
Upon the first program start after installation Imaris is in the Slice view.

1.2 Reference Manual

This Reference Manual provides a description of all menu entries, display modes, functions and parameters. To open the online Reference Manual click on the menu **Help** and select **Reference Manual**.



The Imaris Reference Manual displays.

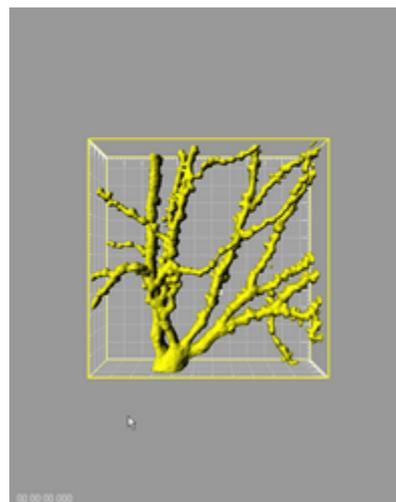


Imaris Reference Manual, Chapter Gallery View

On the left hand side, click on a chapter in the Table of Contents to display a list with the available subchapters.

2 Visualize Data Set

In this first Tutorial you will learn the Imaris basic working steps. The demo image is a Pyramidal Cell, which you will [Open](#) to create a 3D visualization by an [IsoSurface](#). In the chapter [Select and Navigate](#) you will learn about the two different mouse pointer modes in the Surpass view. You will learn how to manipulate the view by [rotating](#), [translating](#) or [scaling](#) the image. Finally, you will save the view to disk as [Scene File](#) for later use. At the end of this Tutorial you will also learn how to change the Imaris [Background Color](#).



Visualized Data Set, Pyramidal Cell

User Level: Beginner
 Module: Imaris

2.1 Open Data Set

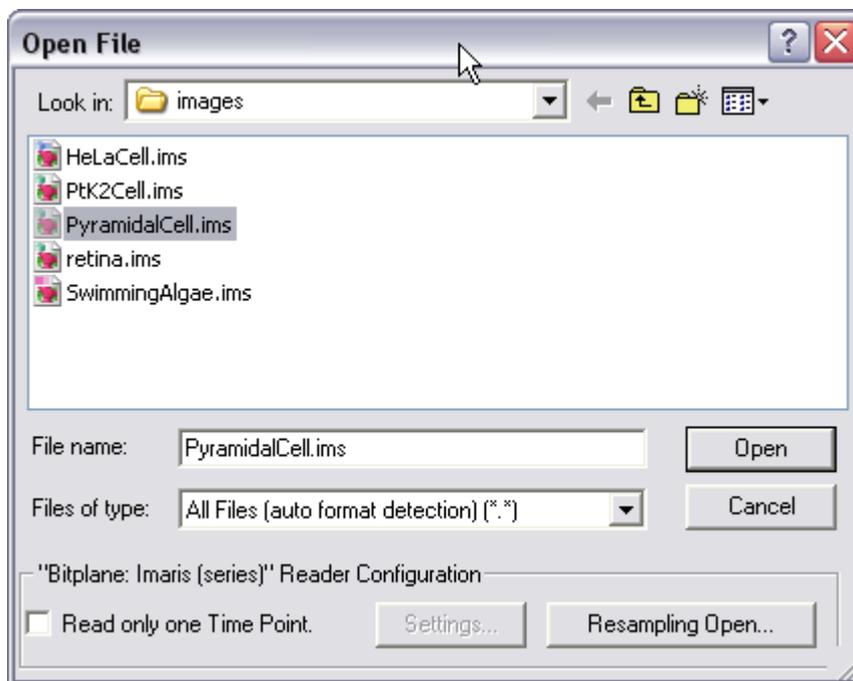


PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open a Data Set in the Slice View



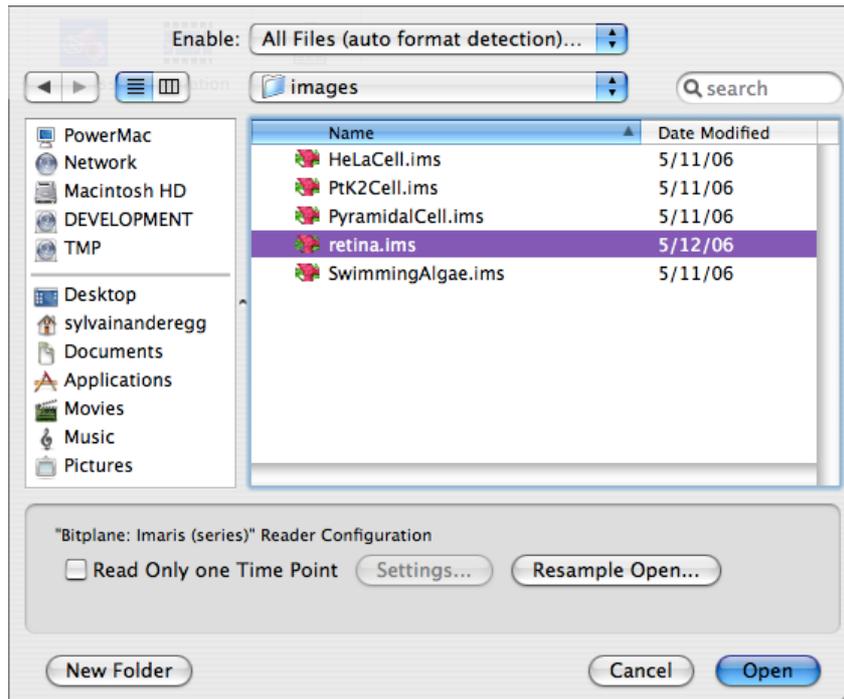
Switch to the Slice view by clicking on the **Slice** button in the main tool bar of Imaris and then click on **Open**. The **Open File** selection window is displayed on screen.



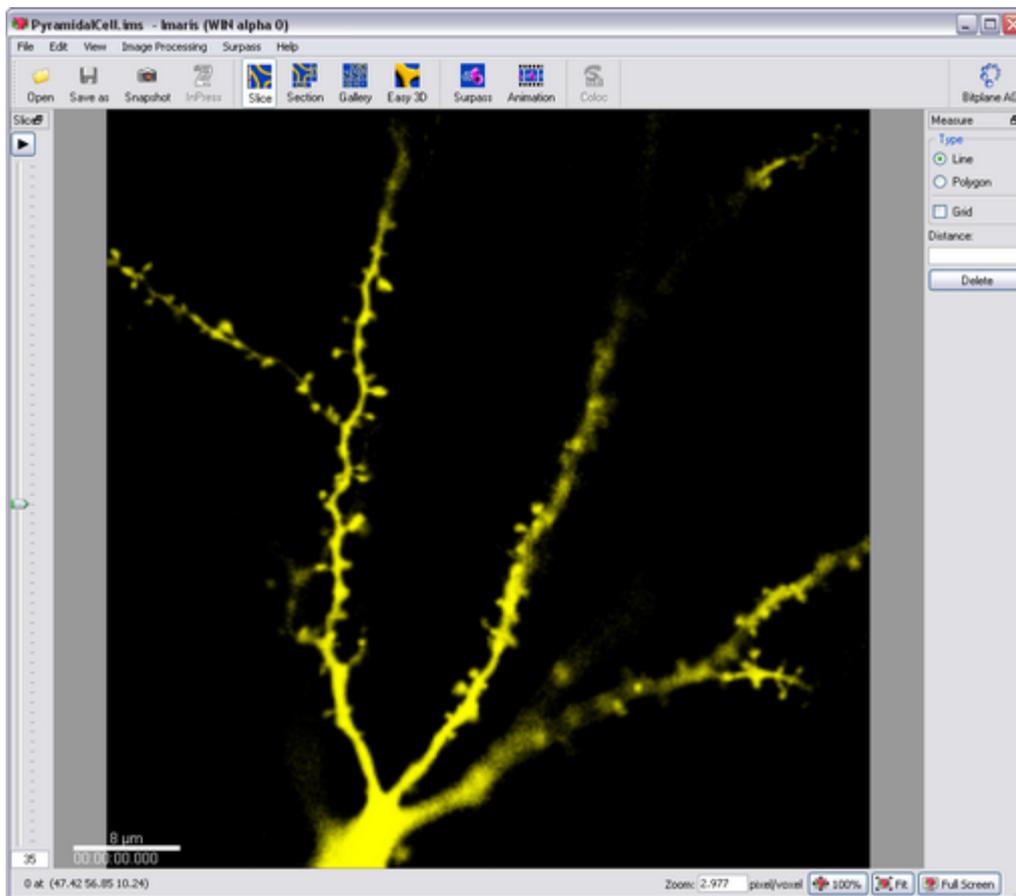
Select the data set Pyramidal Cell and click on **Open**.

PC file path \\ProgramFiles\\Bitplane\\Imaris\\images\\PyramidalCell.ims
Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims

This is the Mac File Open dialog:



The Slice View



The Slice view is a 2D display mode, in the viewing area the middle slice (number 35) of the Pyramidal Cell is displayed. You scroll the slices along the z-axis by dragging the slider handle in the left control bar up and down.

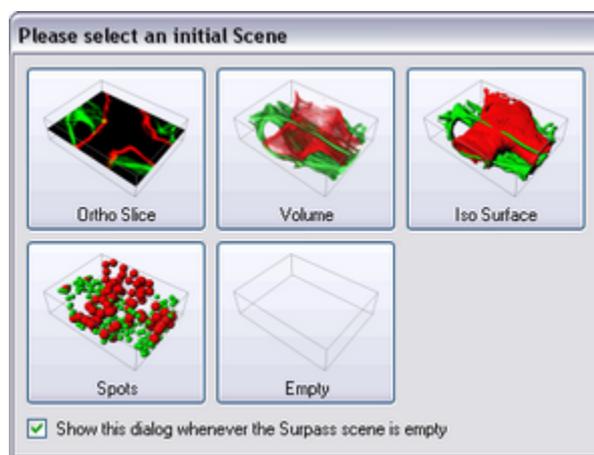
2.2 Create IsoSurface

Now you will create a 3D reconstruction of the Pyramidal Cell.

Select IsoSurface in the Surpass View



Click on **Surpass** in the main toolbar of Imaris. The following window is displayed in the viewing area.

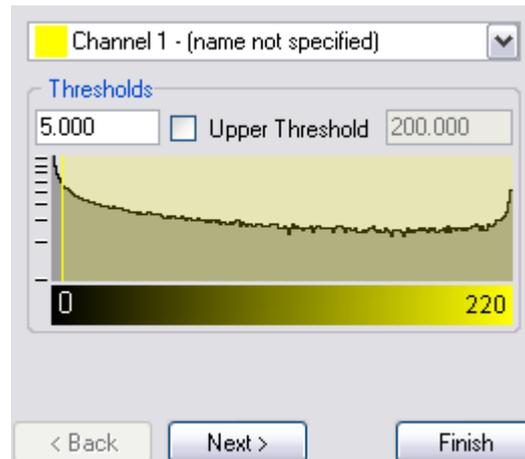


Select **IsoSurface**.

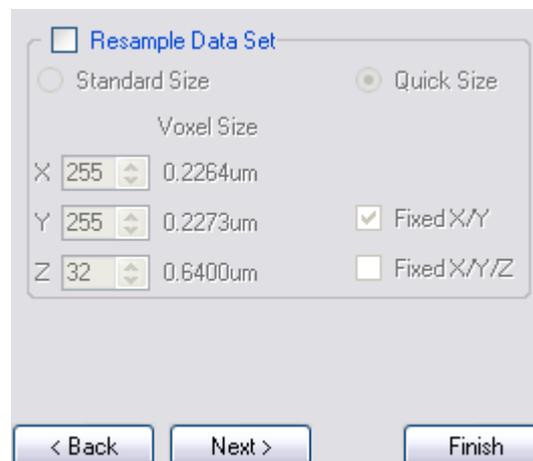


Adjust the IsoSurface Properties

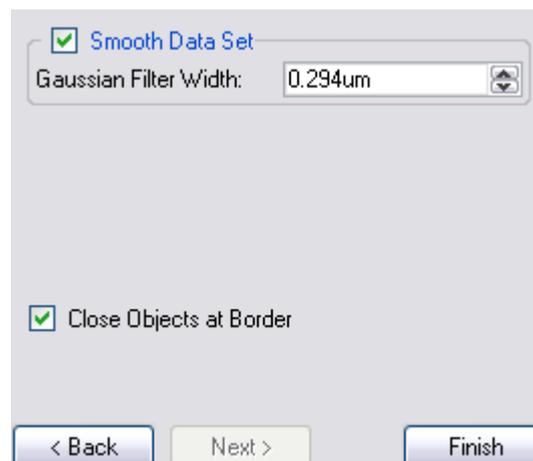
On the left hand side the **IsoSurface Properties** are displayed.



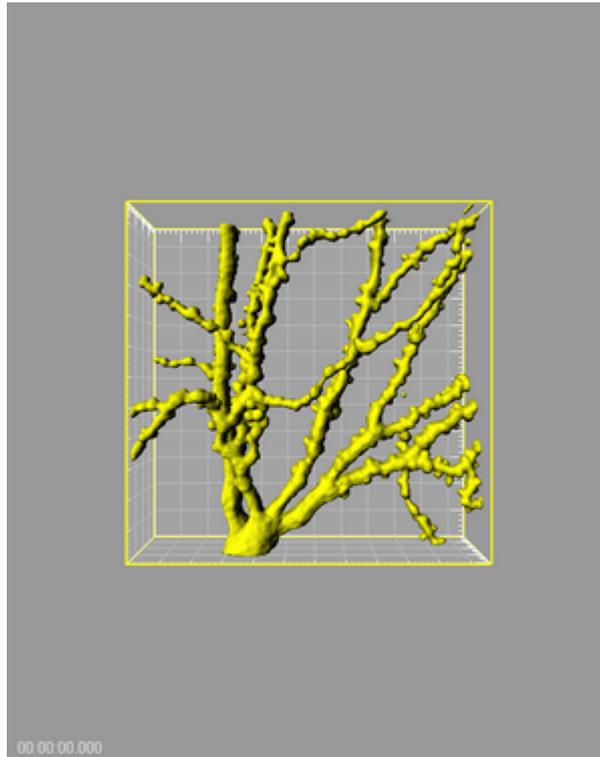
Change the **Threshold** to **5.000** and click on **Next >**.



Deselect the check box **Resample Data Set** and click on **Next >**.



Select **Smooth Data Set** and **Close Objects at Border** and click on **Finish**.



The IsoSurface reconstruction of the Pyramidal Cell is displayed in the viewing area.

See also:

[Add IsoSurface](#)
[Analyze Neuron](#)

2.3 Select and Navigate

In the Surpass view of Imaris there are two different mouse **Pointer** modes - **Select** and **Navigate**.



You select the respective mode in the **Pointer** selection on the right hand side of the screen.

Appearance of the Mouse Pointer

Depending on the pointer mode the symbol on screen changes.



Select



Navigate

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.

When to Use Select?

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.

When to Use Navigate?

You use the pointer mode **Navigate** to move or rotate the image in the viewing area.

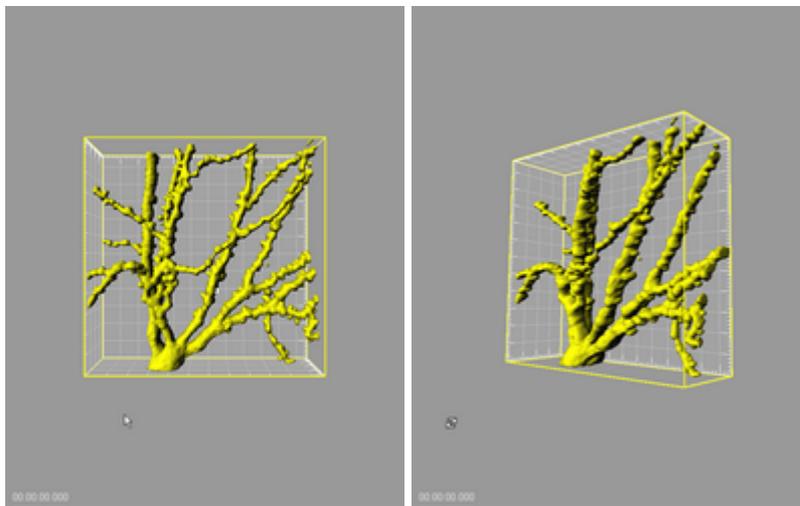
See also:

[Rotate Image](#)

2.4 Rotate Image

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

How to Rotate an Image?



1. Choose the mouse pointer mode **Navigate**.
2. 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.
3. Stop moving the mouse and release the left mouse button to stop the rotation.

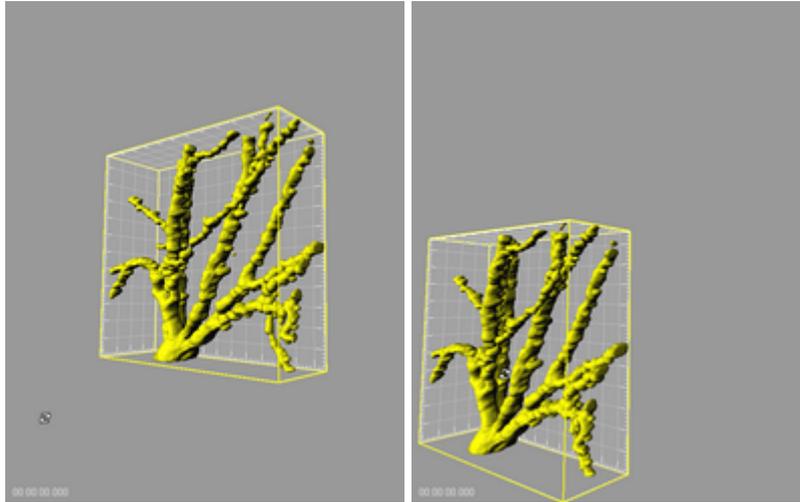
How to Keep the Image Continuously Rotated?

1. Choose the mouse pointer mode **Navigate**.
 2. 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.
 3. 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).
 4. To stop the continued rotation re-click in the image area.
-

See also:

[Select and Navigate](#)

2.5 Translate Image



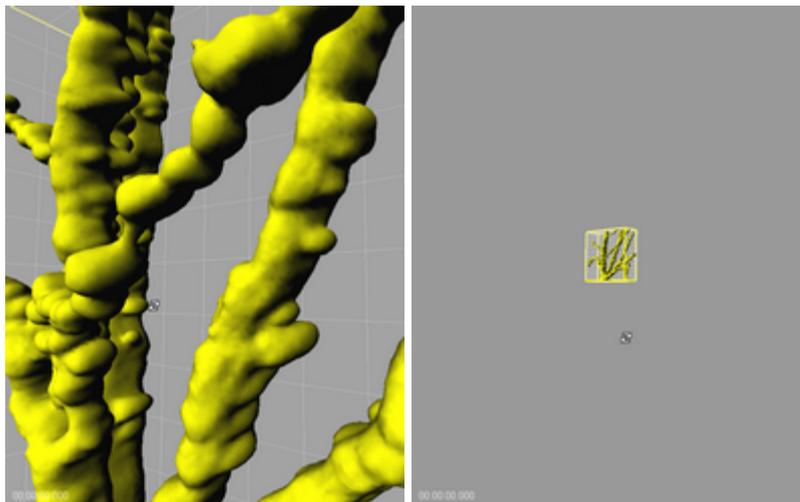
1. Choose the mouse pointer mode Navigate.
2. To move the image within the Surpass view (pan the object) click and hold the right mouse button while dragging the mouse.
3. Release the right mouse button to place the image.

See also:

[Select and Navigate](#)

2.6 Scale Image

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



Using the Mouse

1. Choose the mouse pointer mode Navigate.
2. To zoom the image within the Surpass view click and hold the middle mouse button while dragging the mouse.

Moving the mouse down will zoom in, moving the mouse up will zoom out.

Using the Buttons at the Bottom of the Screen



Zoom	Enter the zoom factor.
100%	Rotate image to original position, center in the middle and set zoom factor to one pixel per voxel.
Fit	Pan position to best fit in the window and adjust the zoom factor.
Full Screen	Maximize the viewing area to full size of the monitor.

See also:

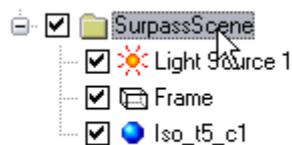
[Select and Navigate](#)

2.7 Save Scene File

You can store the actual configuration in a Scene File. After saving a Scene File, you can restore the configuration at any time by reloading the Scene File.

The Surpass Tree

On the left hand side of the screen in the objects area you find the Surpass Tree. The Surpass Tree displays a list of all Surpass objects you added to the viewing area. The list is automatically generated and updated when you add or delete an object.



The default name of the current folder is **SurpassScene**. The Scene includes the standard **Light Source 1**, the standard **Frame** display and the additional **IsoSurface** Iso_t5_c1.

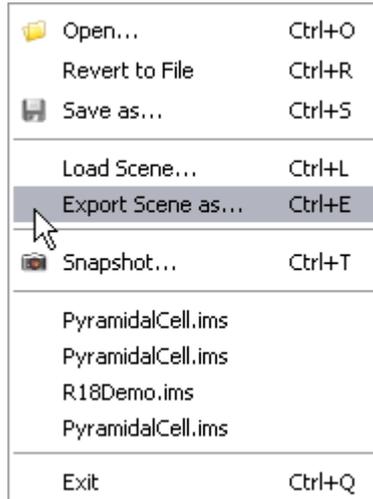
Rename the Scene

Double-click on the entry **SurpassScene** and enter the new name **PyramidalCellSceneA**.



Save the Scene as SceneFile.imx

To save the Scene File open the menu **File** and select **Export Scene as ...**



As name type in the new name of your Scene. The extension of an Imaris Scene File is *.imx. To save the Scene File PyramidalCellSceneA.imx click on **Save**.

See also:

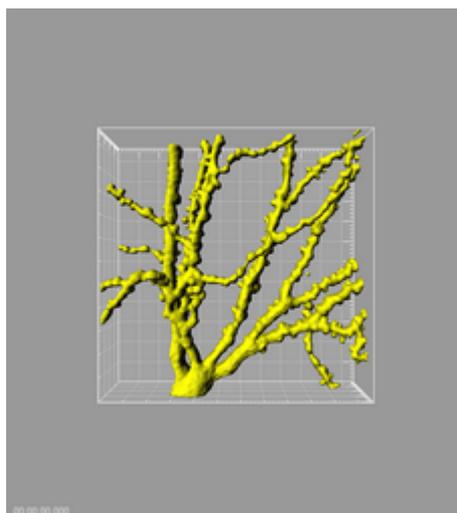
[Surpass Tree](#)

2.8 Practice Makes Perfect

The mouse handling to navigate the image in the viewing area needs some practice but once you master the mouse it is a powerful tool to analyze your data. Start with a center position (Home Position), then rotate the image backwards and then rotate it clockwise.

Home Position

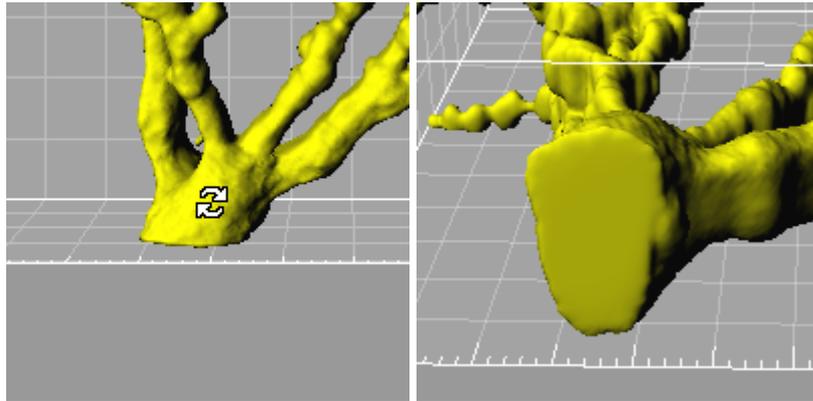
Use the buttons at the bottom of the viewing area, click first on **100%**, then on **Fit**.



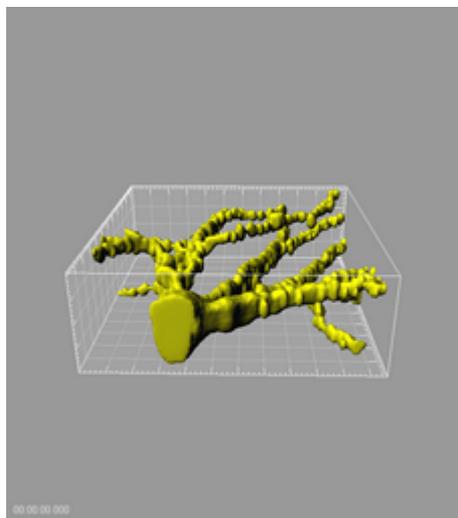
The Pyramidal Cell is displayed centered in the viewing area.

Tip: Whenever you lose orientation, re-center the view to the Home Position by clicking the two buttons again.

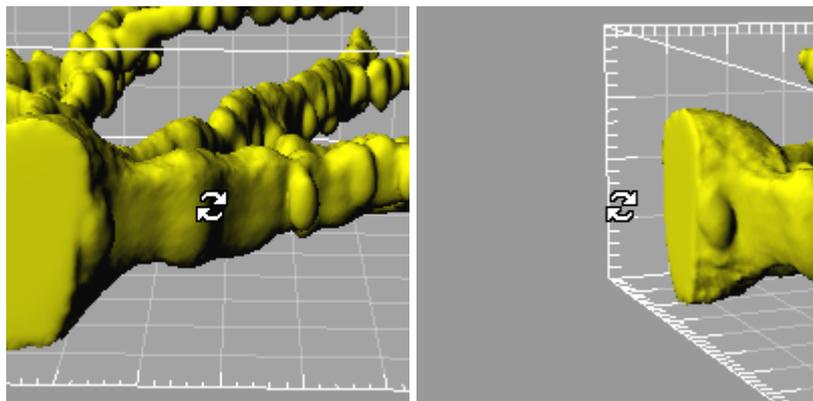
Rotate Backwards



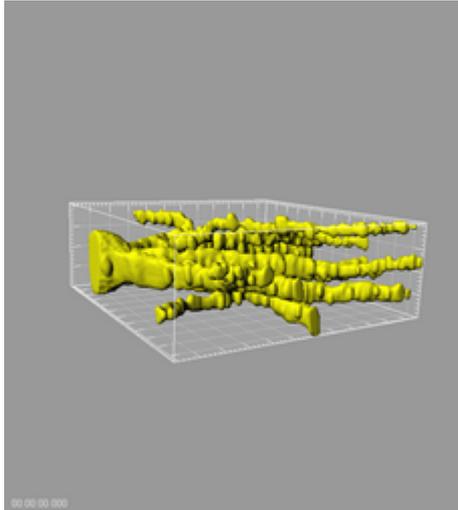
Now rotate the image backwards. Choose the mouse pointer mode Navigate. Click on the base of the Pyramidal Cell with a left-click, hold the left button down while you move the mouse upwards, stop the mouse movement and release the mouse button. This is the result of the rotation.



Move the Image Clockwise



In the next step rotate the image clockwise. Click on the position shown above with a left-click, hold the left button down while you move the mouse to the left, stop the mouse movement and release the mouse button. This is the result of the rotation.



See also:

[Select and Navigate](#)

[Rotate Image](#)

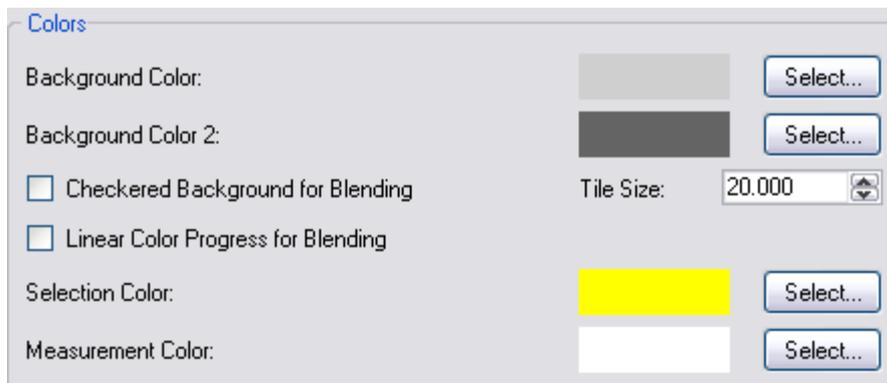
[Scale Image](#)

2.9 Change Background Color

The standard background color in Imaris is bright gray. To change the background color go into the Imaris Display settings.

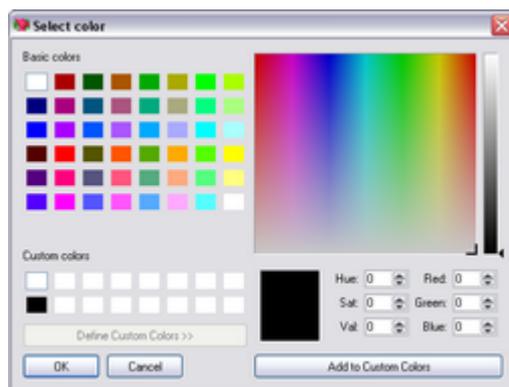
Open the Preferences - Display Window

Select the Menu **Edit - Preferences ... - Display**. On the right hand side you find the **Colors** dialog.

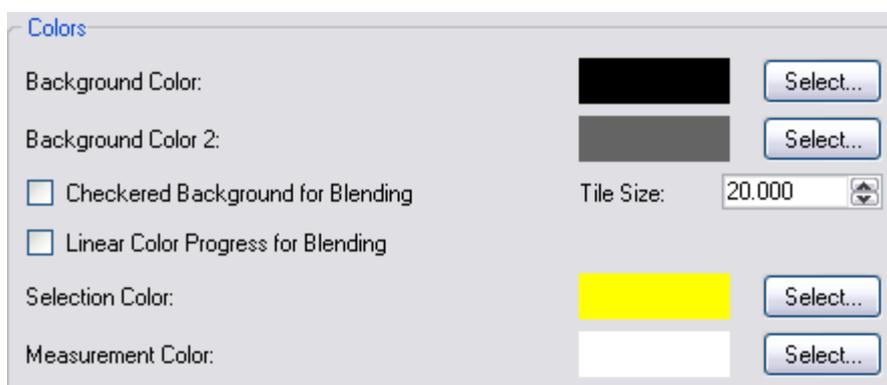


Change Background Color

To change the **Background Color** click on **Select...** to open the **Select Color** window.



Select the desired color (in this example black) and click on **OK**. The preview changes accordingly.



In the window **Preferences - Display** click on **Ok**.

Please reset the background color to bright gray and close Imaris before you start with the next Tutorial.

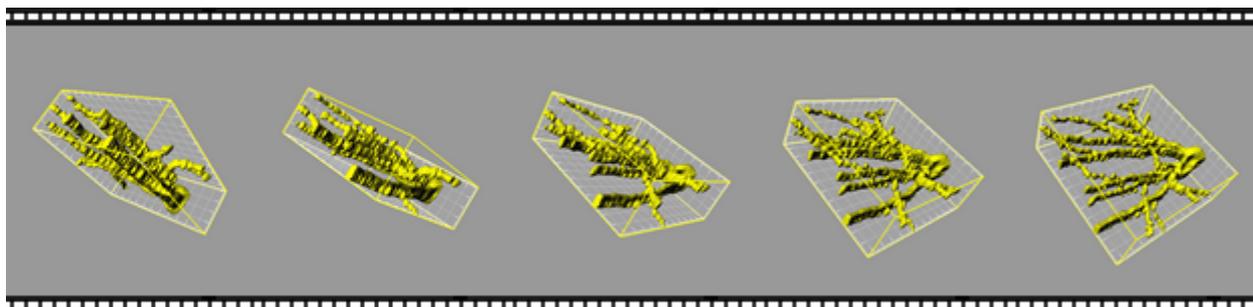
See also:

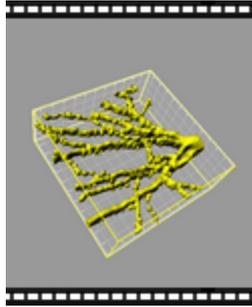
[Change Channel Color](#)

[Change Spots Color](#)

3 Generating Movies

Animated visualizations of three-dimensional data sets can be saved as animations in Imaris. They can also be exported as movies for convenient display in standard movie players. First of all, please [Load the Scene File](#) of the processed data set generated in the previous Tutorial [Visualize a Data Set](#). Generating movies requires understanding of the [Key Frame Animation](#) function in Imaris to subsequently [Shoot](#), [Play](#), and [Save the Movie](#).





Movie Sequences, Pyramidal Cell

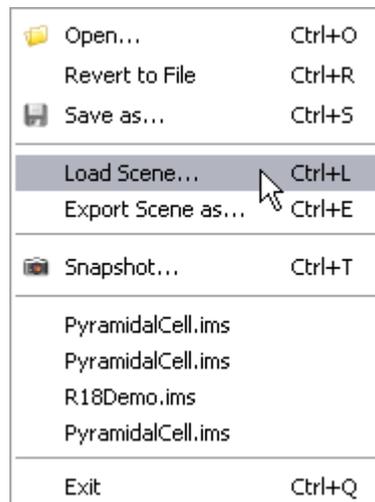
User Level: Beginner
Module: Imaris

3.1 Load Scene File

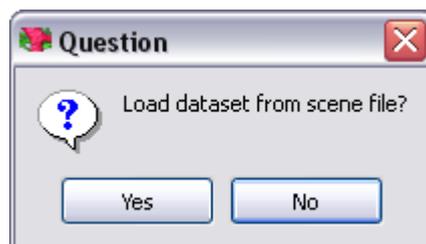


PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

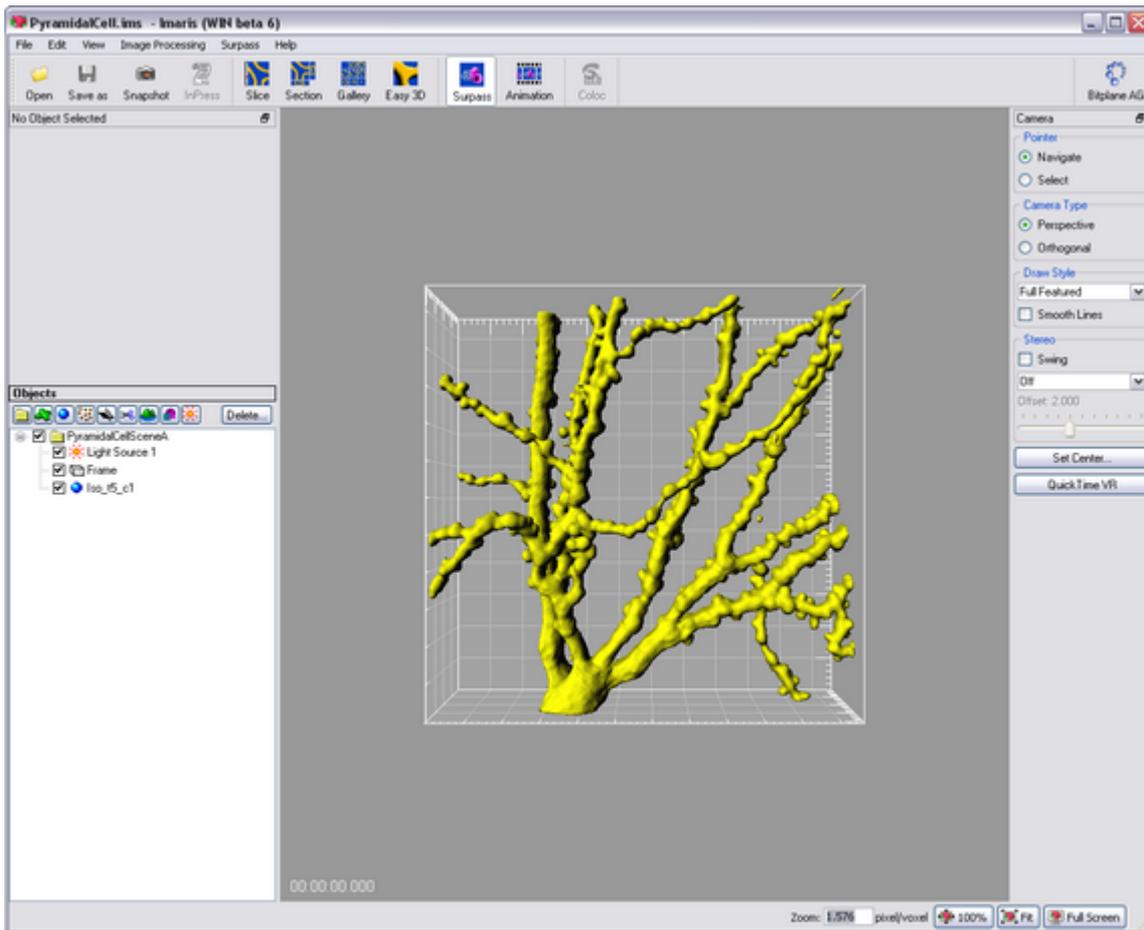
To make a movie please load the Scene File PyramidalCellSceneA. Click on the menu **File** and select **Load Scene ...**



In the dialog window select the file PyramidalCellSceneA.imx and click on **Open**.



Click on **Yes** to exactly reconstruct the Scene.



The Pyramidal Cell is displayed in the viewing area and in the objects area you find the Surpass Tree.

See also:

[Save Scene File](#)

[Surpass Tree](#)

3.2 Key Frame 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.



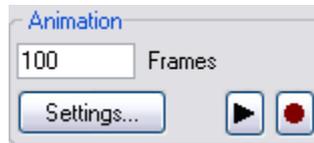
Click on the icon **Animation** in the toolbar. At the bottom of the screen the Key Frame Animation window is displayed. It is divided in three parts, the Key Frame window, the Animation window and the Strip window.

The Key Frame Window



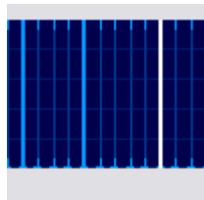
In the **Key Frame** window you define and manage the key frames of your movie. The three buttons in the first row are to **Add**, **Modify** or **Delete** key frames in the movie strip. Use the **Auto Distribute** button to set a constant speed throughout the entire movie. With the arrow buttons you step to the **Previous** (arrow to the left) or **Next** (arrow to the right) user-defined key frame.

The Animation Window



In the **Animation** window you can enter the number of movie **Frames**. There is the button to open the Animation **Settings...** as well as the buttons to **Play** (arrow to the right) and **Record** (red dot) the movie. To pause the animation re-click on **Play**.

The Strip Window



The **Strip** window provides the working area. A thick blue line indicates a user-defined key frame. A white line represents the active key frame and a thin blue line stands for an interpolated frame. Click on a line to display the corresponding image view in the viewing area.

Buttons Overview

	Add New Key Frame (after the Active Key Frame)
	Modify Active Key Frame
	Delete Active Key Frame
	Play/Pause Animation
	Record Animation to Movie File
	Go to Previous Key Frame
	Go to Next Key Frame

3.3 Shoot and Play

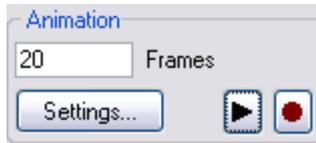
In this section you will learn how to:

- Shoot a movie with 20 frames.
- Add the first and last key frame to the movie strip.
- Insert additional key frames.
- Play and pause the movie.
- Modify a user-defined key frame.

Please remember: Each time you click on the button **+Add** in the Key Frame window the camera captures

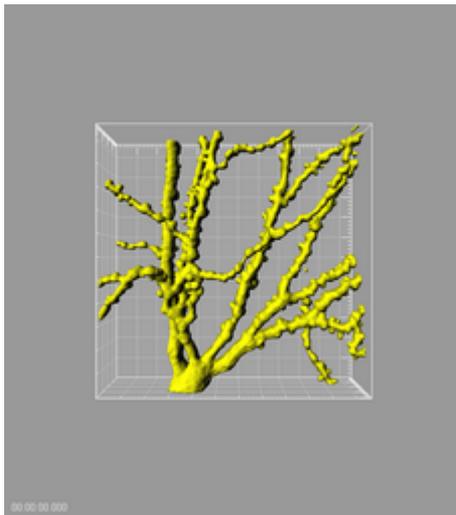
the next key frame of your movie.

Movie Length



In the field **Frames** type in **20**. The strip will indicate these frames by 20 thin blue lines.

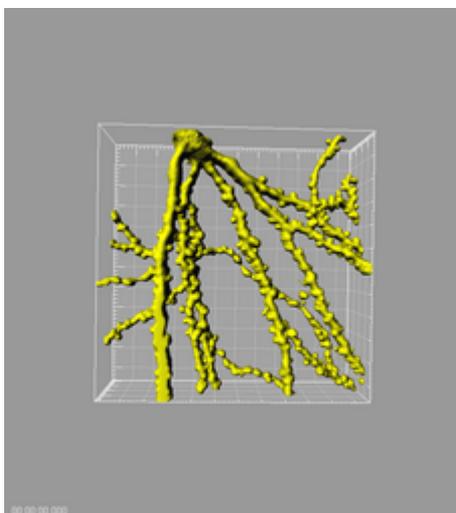
Adjust and Capture the First Key Frame



Turn the image view to the first position (click at the bottom of the screen on the button **100%**, then on the button **Fit**). Capture the first view with a click on the button **+Add** in the Key Frame window. By default, the actual image position is taken as first and last key frame. In the movie strip the first line turns white (active key frame), the last one turns thick blue (user-defined key frame) and the other lines stay thin blue.

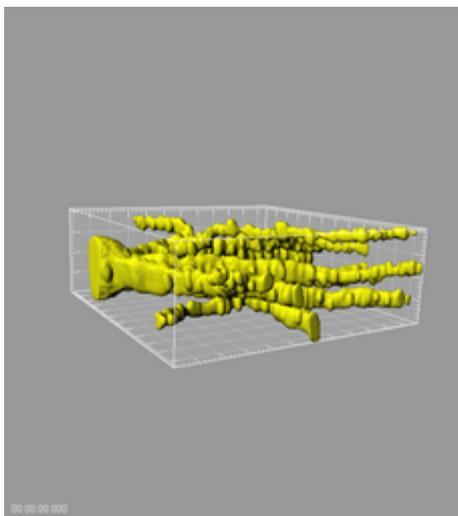
Add Additional Key Frames

Move the image to the next viewing position. Click on the base of the Pyramidal Cell with a left-click, hold the left button down while you move the mouse upwards, stop the mouse movement and release the mouse button. The view should be similar to:



Click on the button **+Add** in the Key Frame window. The first key frame turns thick blue (user-defined key frame). In the movie strip an additional white line (active key frame) is inserted on the right hand side of the last active key frame (in the example at position 10,5).

Move the image to the next position. Click on the base of the Pyramidal Cell with a left click, hold the left button down while you move the mouse downwards, and then move the mouse to the left hand side. Stop the mouse movement and release the mouse button:



Click on the button **+Add** in the Key Frame window. In the movie strip an additional white line (active key frame) is inserted on the right hand side of the last active key frame. All user-defined key frames are automatically distributed equally.

Tip: To get a first impression about the movie click with the left mouse button in the Strip window on the thin blue lines, hold the mouse button and drag the mouse to the right or left, respectively. The corresponding image views are displayed consecutively in the viewing area.

Play the Movie

Click in the Animation window on **Play** (arrow to the right) to display the movie in the viewing area. To pause the animation re-click on **Play** (arrow to the right).

Modify Key Frame

Left-click on any thick blue line in the strip to display the corresponding image view. Rotate the image in the viewing area to a new position and click in the Key Frame window on **Mod.** to save the new image position.

See also:

[Key Frame Animation](#)

[Rotate Image](#)

[Scale Image](#)

[Practice Makes Perfect](#)

3.4 Save Movie

There are two possibilities to save the movie. Either as an Imaris Scene File or as a movie-file (*.avi).

In Imaris as Scene File

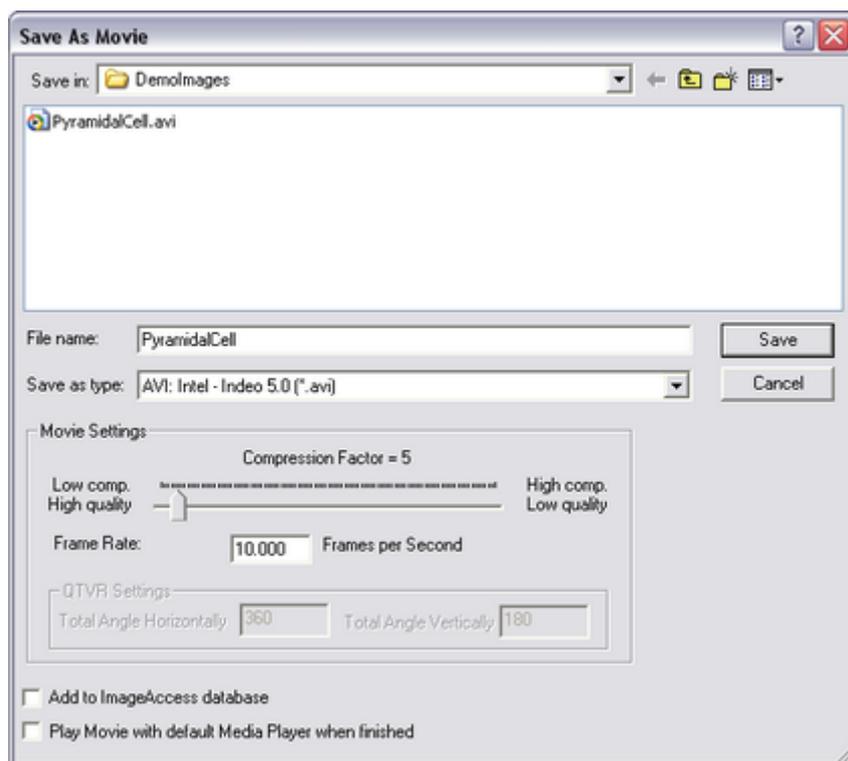
Whenever you save the Scene File in Imaris, the actual animation is saved automatically as part of the Scene File. Open the menu **File** and select **Export the scene as...** and click on **Save**.

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 the 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.

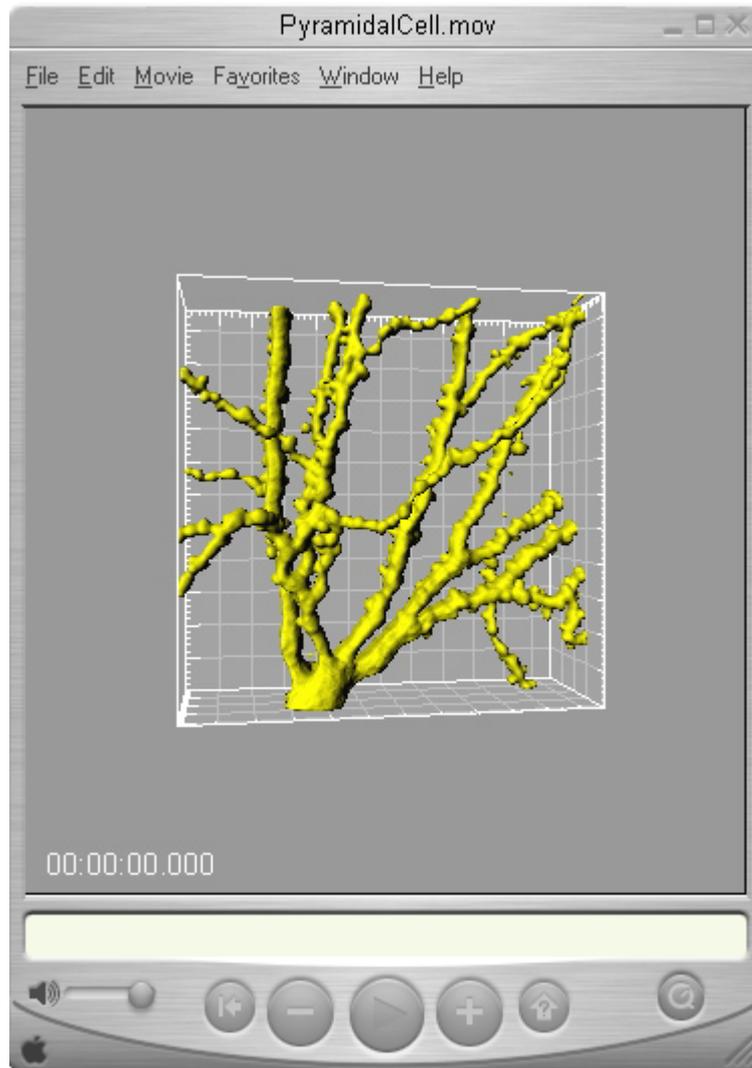
Please close Imaris before you start with the next Tutorial.

See also:

[Save Scene File](#)
[Key Frame Animation](#)
[Imaris and QuickTimeVR](#)

4 Imaris and QuickTimeVR

To export the visualized results of your investigation Imaris supports the QuickTimeVR file format. In the first section you will learn about the [Basic Principles of QuickTimeVR](#) - the file generation mechanism and the default movie settings. Then you will [Generate a QuickTimeVR File](#) in Imaris to [Visualize the Results](#) interactively in QuickTimeVR. [Optimized Settings](#) for a Presentation and a Web Page are described in detail in the last chapter of this Tutorial.



QuickTimeVR Display, Pyramidal Cell

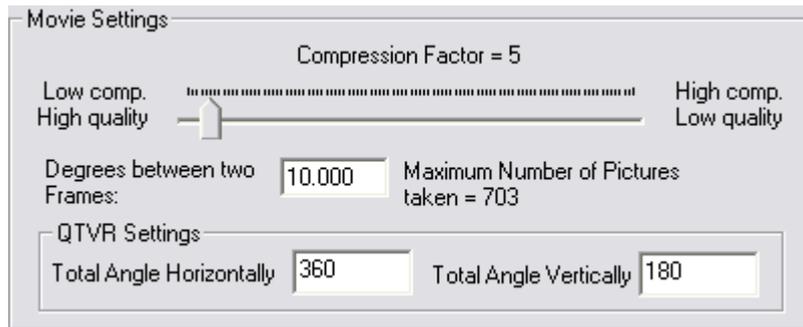
User Level: Beginner
Module: Imaris

4.1 Basic Principles

Sometimes it is very convenient to show the data visualized in Imaris in an alternative, independent program. Therefore Imaris supports the QuickTimeVR output format. Imaris automatically takes several snapshots of your image in the viewing area using different, predefined camera positions. Depending on the settings more or less snapshots are saved in the corresponding file (*.mov). The actual image size on the computer screen and the compression factor have an impact on the file size and the quality of your QuickTimeVR.

Please note: QuickTimeVR is not automatically installed on your Windows PC when you install Imaris. Please install QuickTimeVR before you continue this Tutorial.

QuickTime Default Movie Settings



Compression Factor: Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5.

Degrees between two Frames: Here you define the degrees between two frames, the default setting is 10.

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:

QuickTimeVR [Optimized Settings](#)

4.2 Generate File



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

Mac: In the folder Applications double-click on Imaris to open the program.

Load your data set or Scene File (e.g. PyramidalCellSceneA).

Adjust the Viewing Area

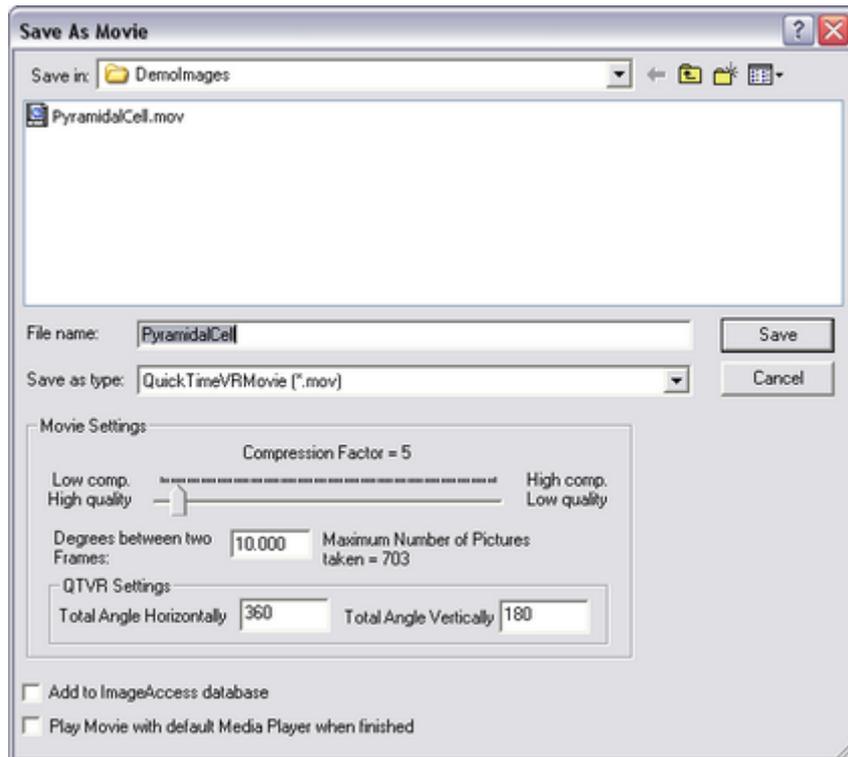


Adjust the size of the viewing area to the desired size of the QuickTimeVR viewing area, the display is 1:1.

Save the QuickTimeVR File



In the Surpass view click on the icon **QuickTimeVR** on the right hand side of the screen to open the **Save as Movie** dialog window.



Type in the **File name**, select **QuickTimeVRMovie (*.mov)**, use the default **Movie Settings** and click on **Save**.

What Happens in the Imaris Viewing Area?

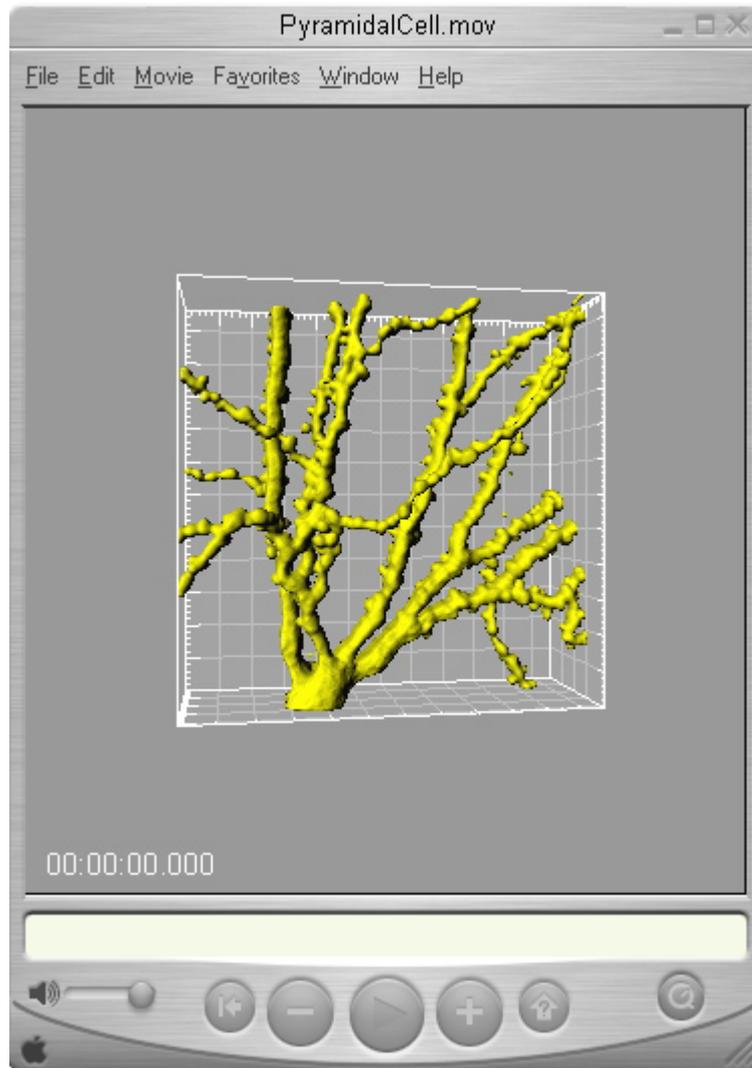
Automatically Imaris starts to turn around the image in the viewing area and takes a snapshot of each image position. The degree between two frames is 10, the angle horizontally is 360 and the angle vertically 180. Down to the right you find a progress bar. Depending on the settings, this procedure will take several seconds or even minutes.

See also:

[Open Data Set](#)
[Load Scene File](#)

4.3 Interactive Display

Double-click on your QuickTimeVR file to show the results.



The image is displayed in the QuickTimeVR viewing area. You navigate the Scene using the mouse. With the standard settings you rotate the object 360° horizontally and 180° vertically. The angle between two image frames is 10°.

4.4 Optimized Settings

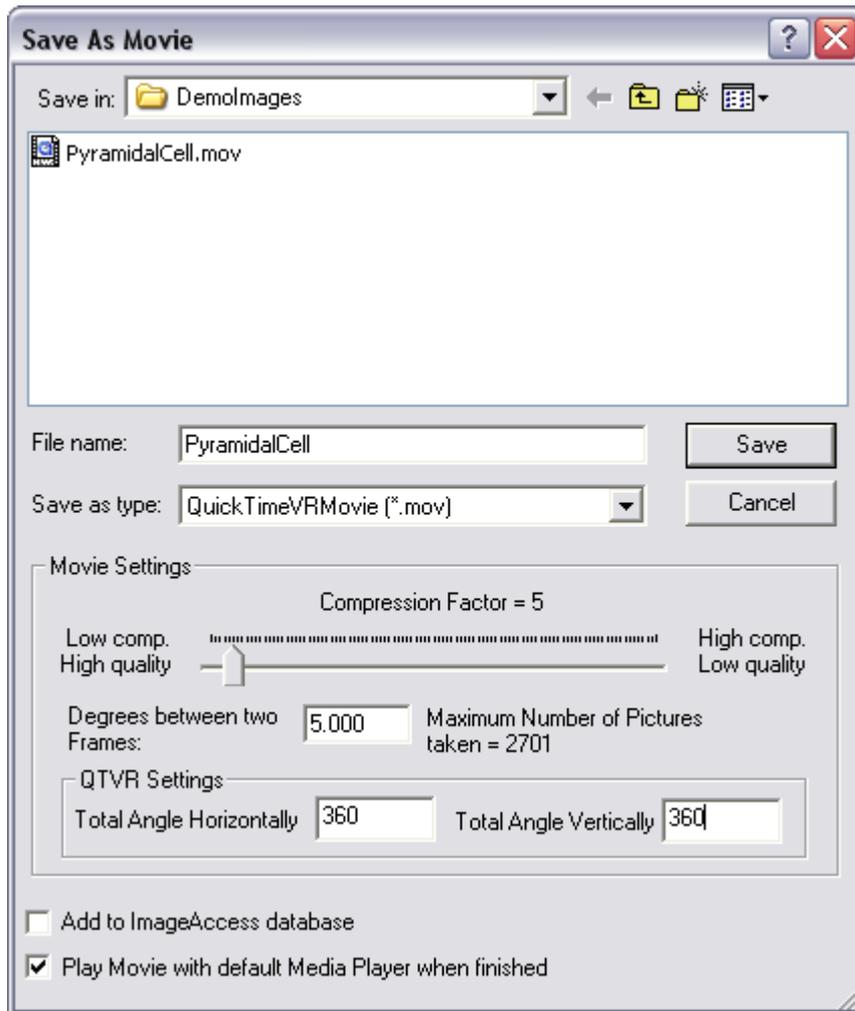
If you want to save a movie for a Presentation on your computer the file size is according to experience is not the limitation. To put the movie on your Web Page the file should not be too large.

Please note: If you increase the degree between two frames less images are stored in the file. The effect is directly visible during image navigation in QuickTimeVR. The step between two images is bigger, the navigation becomes fragmentary.

Please note: If you reduce the total angle horizontally from 360 to 160 it is no longer possible to turn the image around the axis.

Adjust Settings for a Presentation

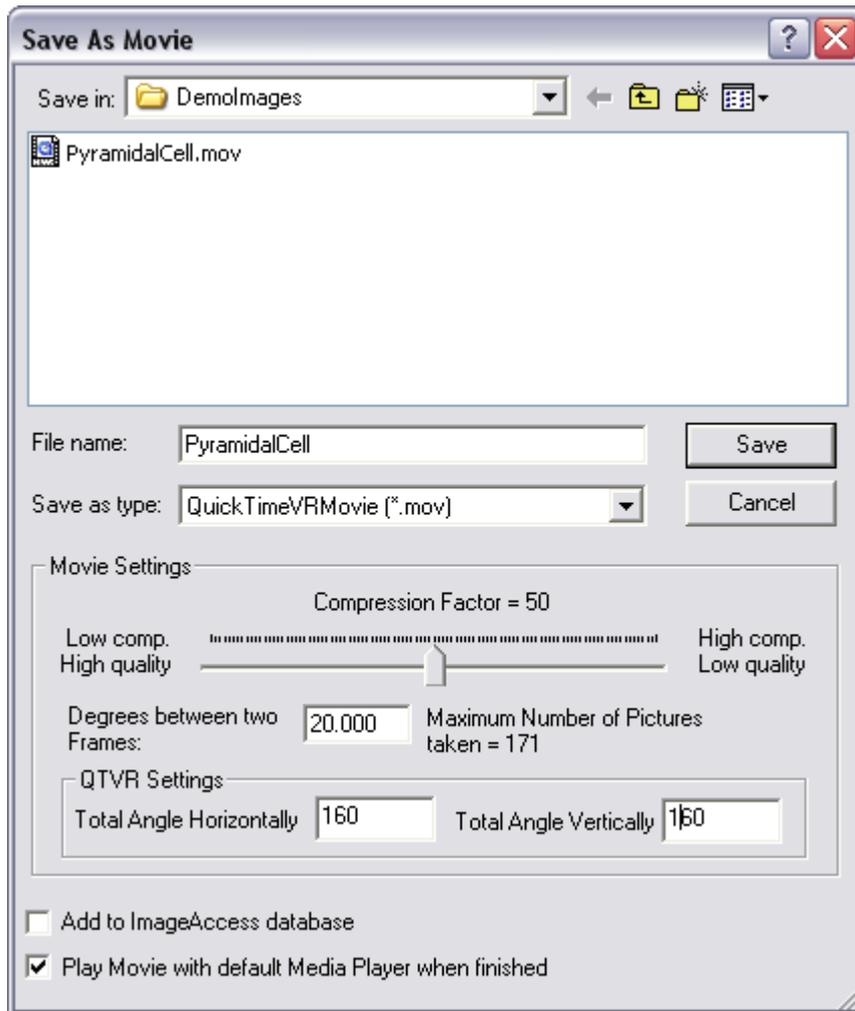
- Adjust the size of the viewing area in Imaris, the display in QuickTimeVR is 1:1.
- In the Surpass view click on the icon **QuickTimeVR** on the right hand side of the screen to open the **Save as Movie** dialog window.



- Use the **Compression Factor 5**.
- Set the **Degrees between two Frames to 5**.
- Set the **Total Angle Horizontally to 360** and the **Total Angle Vertically to 360**.
- Check **Play Movie with default Media Player when finished**.
- Click on **Save**.

Adjust Settings for a Web Page

- Adjust the size of the viewing area in Imaris, the display in QuickTimeVR is 1:1.
- In the Surpass view click on the icon **QuickTimeVR** on the right hand side of the screen to open the **Save as Movie** dialog window.

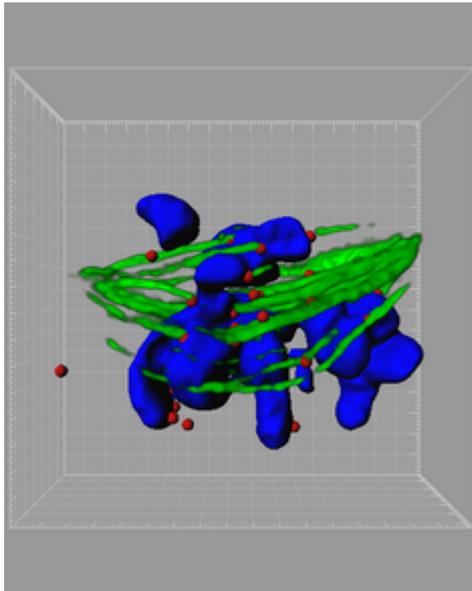


- Use the **Compression Factor 50**.
- Set the **Degrees between two Frames to 20**.
- Set the **Total Angle Horizontally to 160** and the **Total Angle Vertically to 160**.
- Check **Play Movie with default Media Player when finished**.
- Click on **Save**.

Please close Imaris before you start with the next Tutorial.

5 Design Mixed Model Rendering

This Tutorial is focused on the different visualization options in the Surpass mode of Imaris to gain visualization control of the objects. The Ptk2 Cell is particularly suitable for a mixed model rendering. You will start with a [Volume](#) rendering for all channels and you will adjust the contrast, brightness and transparency in the [Display Adjustment](#) window. The microtubules remain Volume rendered but for the chromosomes you will choose the [IsoSurface](#) mode in Imaris. For the kinetochores you select the [Spots](#) display and in the next step you [Change the Spots Color](#). The [Surpass Tree](#) displays a list of all objects in the viewing area and is the ideal instrument to handle the [Final Image](#).



Mixed Model Rendering, PtK2 Cell

User Level: Beginner
Module: Imaris

5.1 Add Volume



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

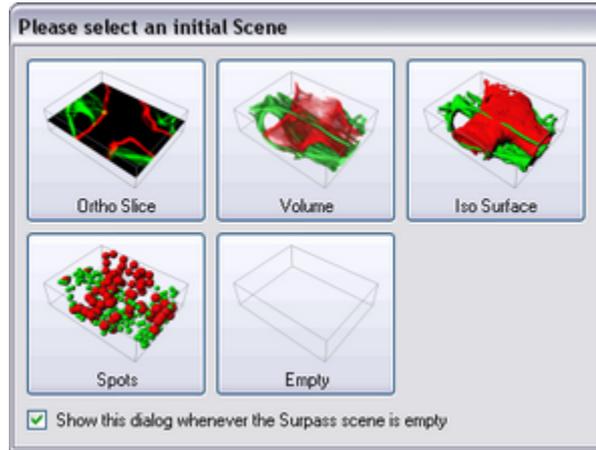
Open the demo image PtK2 Cell in the Slice view.

PC file path \\ProgramFiles\\Bitplane\\Imaris\\images\\PtK2Cell.ims
Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/PtK2Cell.ims

Select Volume Reconstruction in Surpass View



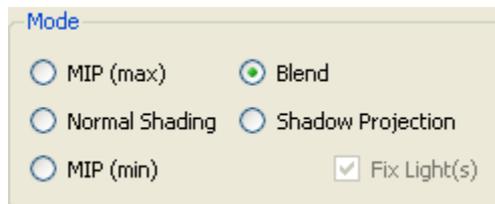
To create a Volume reconstruction of the data set select the Surpass mode. Click on the icon **Surpass** in the main toolbar of Imaris. The following window is displayed in the viewing area.



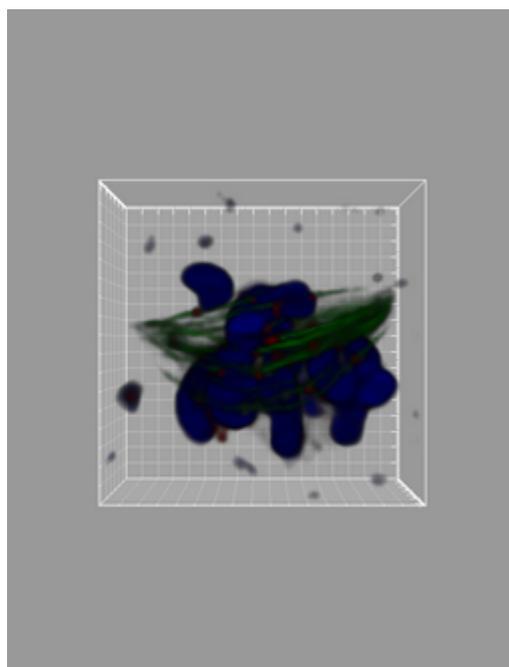
Select **Volume**.



On the left hand side in the **Volume - Properties** you find the **Mode** selection window.



Select as display mode **Blend**. In the blend mode all values along the viewing direction including their transparency are used for the calculation.



In the viewing area the PtK2 Cell is displayed.

See also:

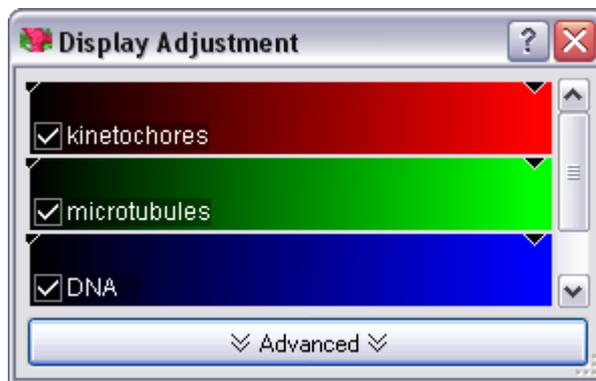
[Open Data Set](#)

5.2 Display Adjustment

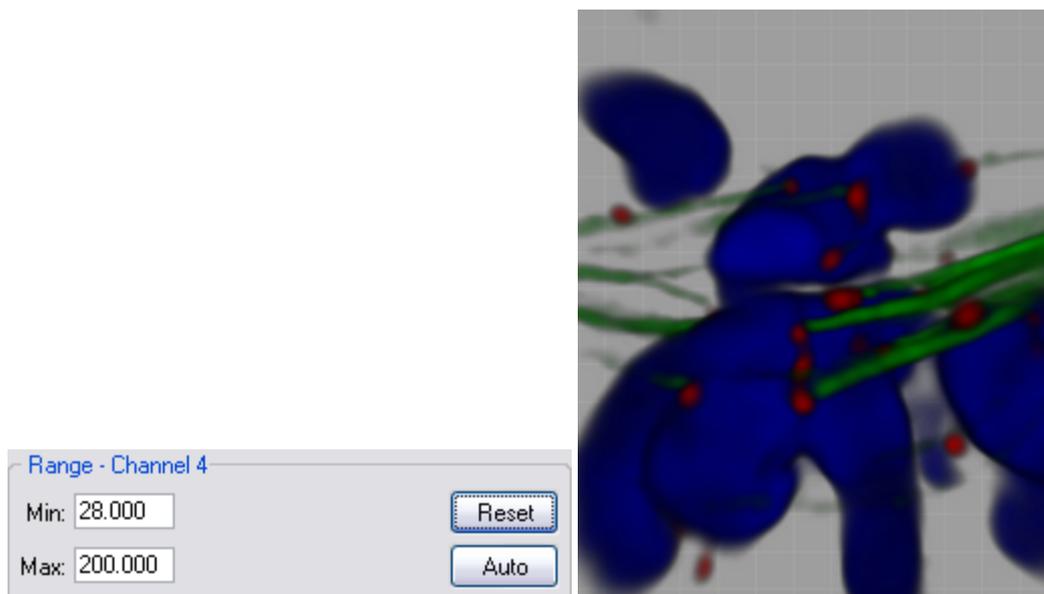
The objects rendered as Volume are usually quite dark and have a lot of noise. To optimize the Volume reconstruction you set the Range to adjust contrast and brightness. With the Blend Opacity you influence the transparency of a channel. You find all these settings in the Display Adjustment window.

Adjust All Channels Simultaneously

To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**.

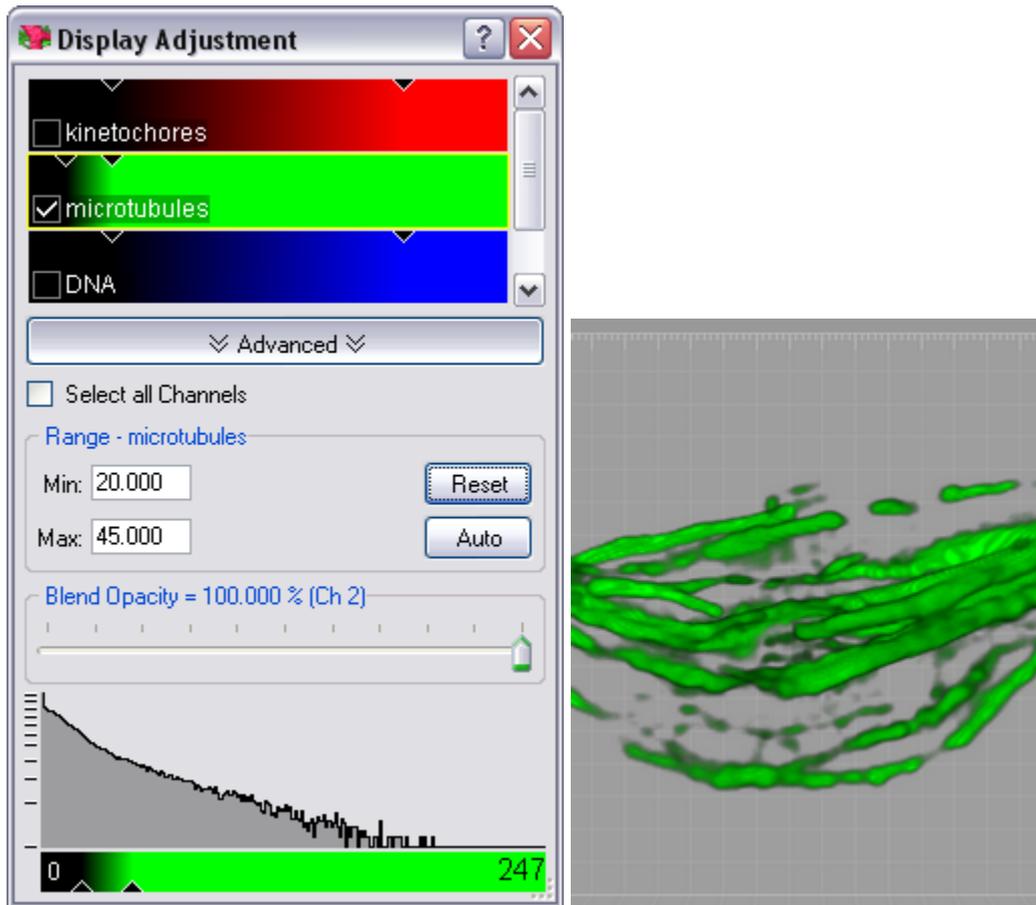


In the upper part the three channels red, green and blue are represented. In this case the red channel corresponds to the kinetochores, the green channel represents the microtubules and the blue channel is the DAPI stained DNA. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0-255). Click on **Advanced** to open the advanced settings.



In this multi channel dataset check the box **Select all Channels** and set **Min: 28.000** and **Max: 200.000**. The effect on all channels is directly visible in the viewing area.

Adjust Individual Channel



Now tune the green channel individually. To focus on the green channel (microtubules) check the check box for the green channel and un-check the boxes for the red and blue channel. The histogram for the green channel is displayed. Set the **Range - microtubules Min: 20.000** and **Max: 45.000**.

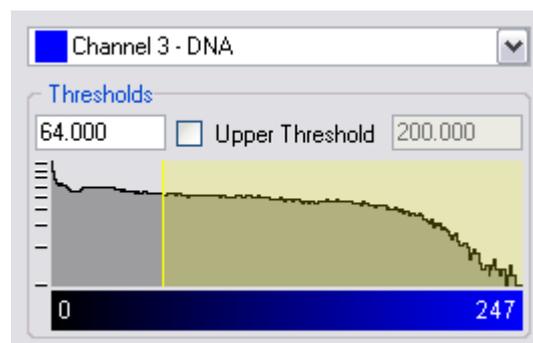
5.3 Add IsoSurface

You will now add to the Volume rendered microtubules an IsoSurface reconstruction of the DNA.



In the **Objects** toolbar of the Surpass view click on the icon  to add a new IsoSurface.

Adjust the IsoSurface Properties



To separate objects from the background adjust the threshold. Select **Channel 3 - DNA**, set the **Threshold** to **64.000** and click on **Next >**.

Resample Data Set

Standard Size Quick Size

Voxel Size

X 255 0.2264um

Y 255 0.2273um Fixed X/Y

Z 32 0.6400um Fixed X/Y/Z

< Back Next > Finish

Deselect the check box **Resample Data Set** and click on **Next >**.

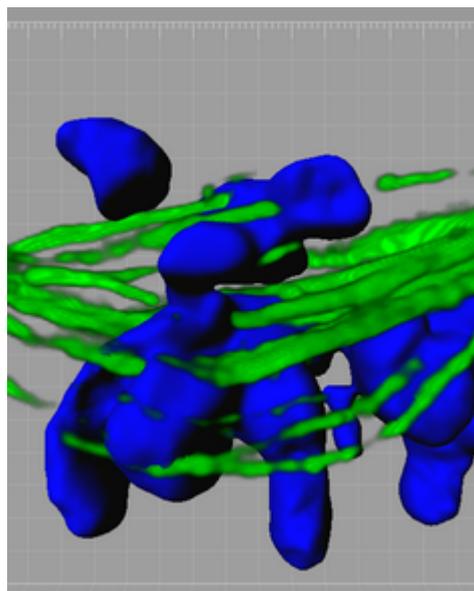
Smooth Data Set

Gaussian Filter Width: 0.294um

Close Objects at Border

< Back Next > Finish

Select **Smooth Data Set** and **Close Objects at Border** and click on **Finish**. Additional to the Volume rendered microtubules the IsoSurface reconstruction of the DNA is displayed in the viewing area of Imaris.



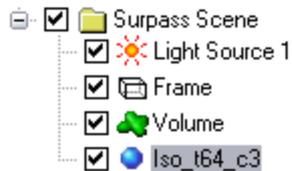
See also:

[Create IsoSurface](#)

5.4 Surpass Tree

On the left hand side of the screen in the objects area you can find the Surpass Tree. The Surpass Tree displays a list of all Surpass objects you added to the viewing area. The list is automatically generated and updated when you add or delete an object.

Surpass Tree Items

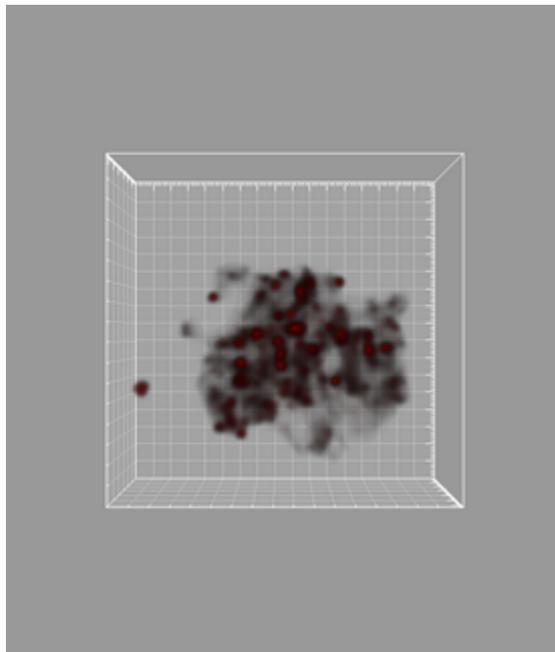


Each tree item includes a check box. Check the box to make the object visible in the viewing area. Un-check the box to make the object invisible in the viewing area. The currently active object is highlighted in the Surpass Tree and the appropriate properties are displayed.

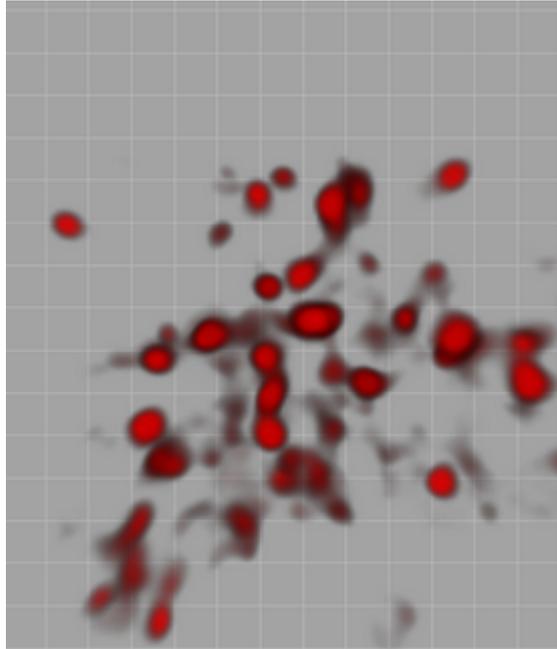
Adjust Color Channels

You will now learn how to display just the Volume rendered red channel in the viewing area and how to enhance the image. To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**.

- In the Surpass Tree switch off the tree item for the IsoSurface rendered blue channel (un-check the box Iso_t64_c3).
- In the Display Adjustment check the box for the red channel and un-check the boxes for the green and blue channels.



In the Display Adjustment window click on **Advanced** to open the advanced settings. Change for the selected red channel the value **Min** to **20.000** and the value **Max** to **100.000**. Adjust the **Blend Opacity** to **64%** and zoom the image.



See also:

[Display Adjustment](#)

[Scale Image](#)

5.5 Add Spots

The kinetochores are spot like very small objects and the ideal candidate to add a Spot component in the Surpass view, so let Imaris detect these Spots automatically.

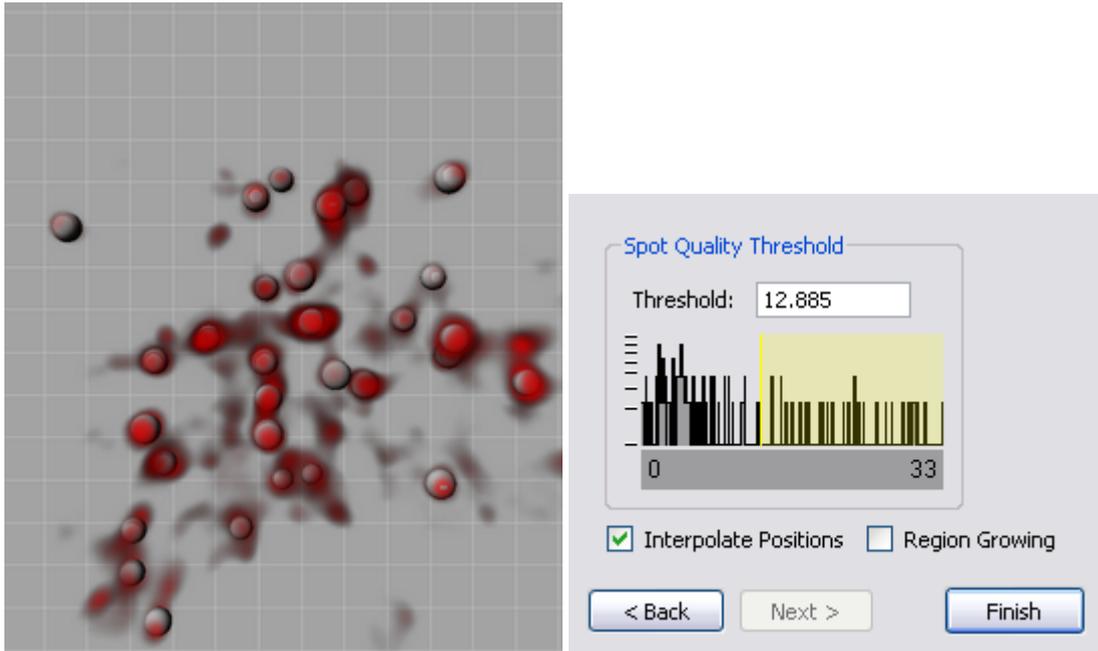


In the **Objects** toolbar of the Surpass view click on the icon  to add new Spots.

Adjust the Spots Properties

A screenshot of the "Spot Detection" dialog box in the Imaris software. The dialog has a "Source Channel" section with a dropdown menu showing "Channel 1 - kinetochores" with a red square icon. Below this is the "Spot Detection" section, which includes a "Minimum Diameter" input field set to "0.500" with "um" as the unit, and a checked checkbox for "Background Object Subtraction". At the bottom of the dialog are three buttons: "< Back", "Next >", and "Finish".

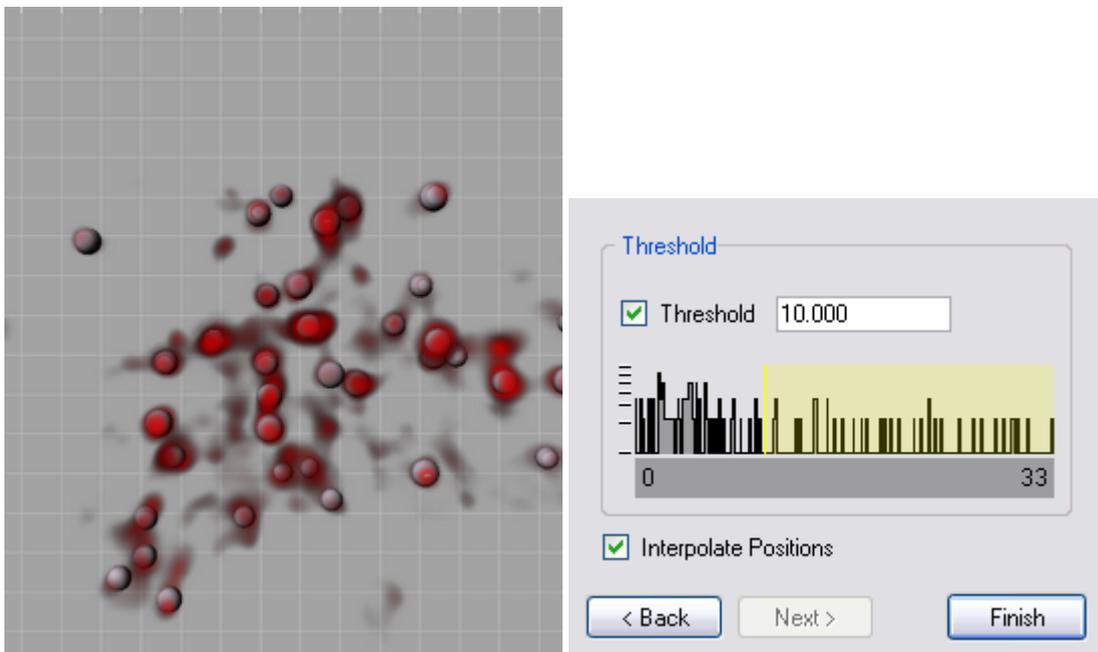
Select as **Source Channel** the **Channel 1 - kinetochores** and set the **Minimum Diameter** to **0.500** and click on **Next >**.



Imaris detects an automatic threshold to insert the Spots. You see at the same time the gray Spots and the Volume rendered red channel.

Verify the Spots

With the automatic threshold detection some Spots are not identified because they are under the threshold limit. To change the threshold click on the yellow line and drag the mouse. Move the line to the left to decrease the threshold and get additional objects with a low intensity value. The effect is directly visible in the viewing area.



Set the **Threshold** to **10** and click on **Finish**. Compared to the first image display (automatic threshold 12.885) there are additional Spots.

See also:

[Line and Polygon](#) to define the Minimum Spot Diameter
[Change Spots Color](#)

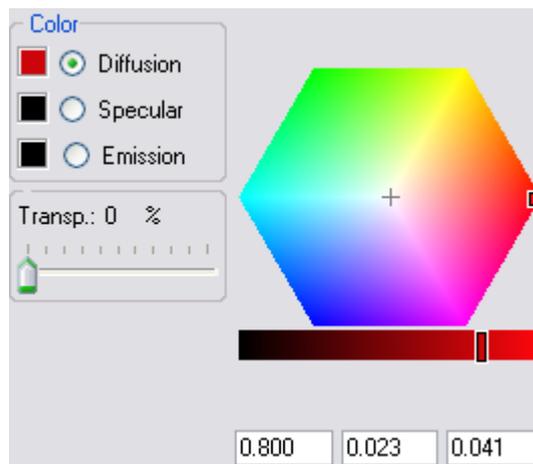
5.6 Change Spots Color

You will now change the Spots color.

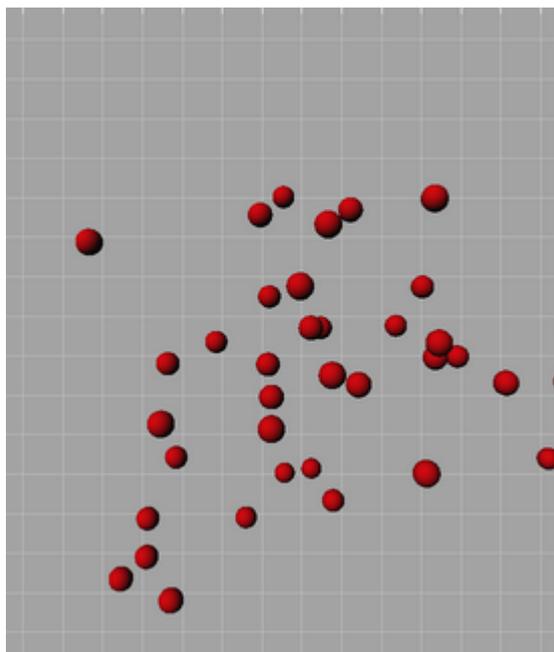
Open the Color Properties



In the properties area click on the tab **Color** (rightmost in the tab selection) to open the Color Properties Dialog Box.



Click on the color wheel to select a color. Choose a dark red and the Spots in the viewing area display the chosen color. Now un-check in the Surpass Tree the box for the item Volume. Just the red Spots are displayed in the viewing area:

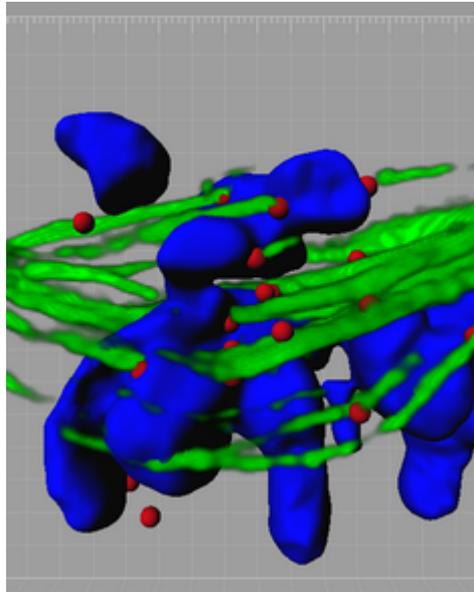


See also:
[Change Channel Color](#)

[Change Background Color](#)

5.7 Final Image

The purpose of this complex Tutorial is to get an idea about the different rendering possibilities in Imaris. To display the composed image check all items in the Surpass Tree. In the Display Adjustment check only the green channel.



The microtubules are displayed in green as Volume rendering, the chromosomes in blue as IsoSurface and the kinetochores in red as Spots.

Please close Imaris before you start with the next Tutorial.

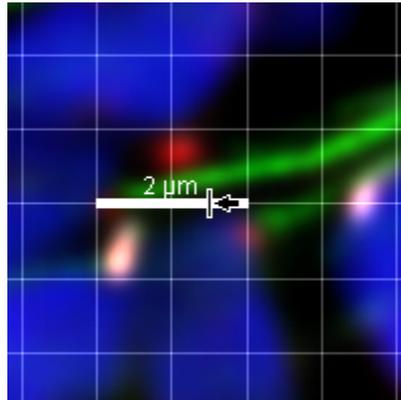
See also:

[Surpass Tree](#)

[Display Adjustment](#)

6 Measure Structures

In each Imaris view there are several measurement options. This Tutorial will focus on different measurement functions in the Slice view. Either you choose as interactive measurement type [Line](#) or [Polygon](#), depending on what you want to measure in the image. If you want to estimate distances in the image you can lay a [Grid](#) over your image. The interactive [Scale Bar](#) also helps in estimating sizes and distances. In the last chapter you will learn how to measure distances between different slices ([3D Measurement](#)).



Grid and Scale Bar Display, PtK2 Cell

User Level: Beginner
Module: Imaris

6.1 Line or Polygon



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

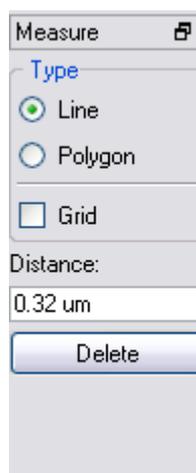
Open the demo image PtK2 Cell in the Slice view.

PC file path `\ProgramFiles\Bitplane\Imaris\images\PtK2Cell.ims`
Mac file path `/Applications/Imaris.app/Contents/SharedSupport/images/PtK2Cell.ims`

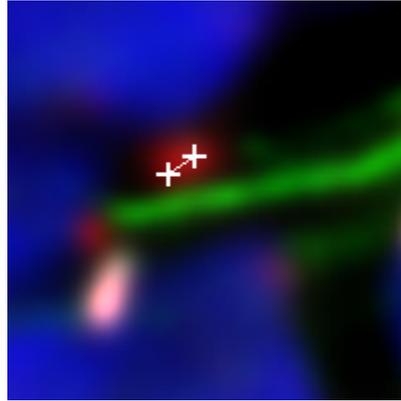
In the slice selection on the left hand side of the screen select slice number 17 (type in 17 and press Enter).

Point to Point Distance Measurement

In the PtK2 Cell the kinetochores are labeled in red. You will measure the diameter of a kinetochore using the point to point measurement function in Imaris.



On the right hand side of the screen the **Measure** window is displayed. Choose the measurement **Type Line**.



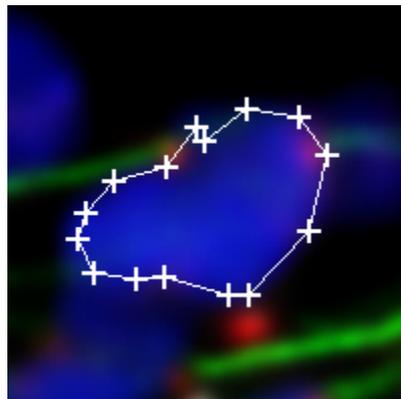
Set the two measurement points with two consecutive left-clicks. The result is displayed in the status field **Distance: 0.32 um**. Click on **Delete** to clear the status field and start a new measurement.

Measure Perimeter

Now you would like to know the perimeter of the blue labeled DNA.



Choose the measurement **Type Polygon**.



All measurement points are consecutively connected by lines and the displayed distance is the sum of the distances between the points. Draw a polygon around the DNA like in the image above. The result is displayed in the status field **Distance 9.21 um**. Click on **Delete** to clear the status field and start a new measurement.

See also:
[Open Data Set](#)

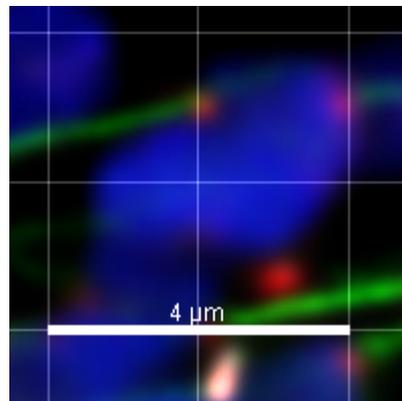
6.2 Grid and Scale Bar

Display a Grid

One way to estimate distances in the image is to use the grid display.

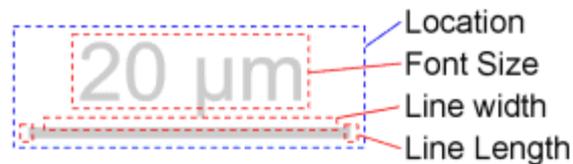


On the right hand side in the **Measure** window check the check box **Grid**. This is the result in the viewing area:

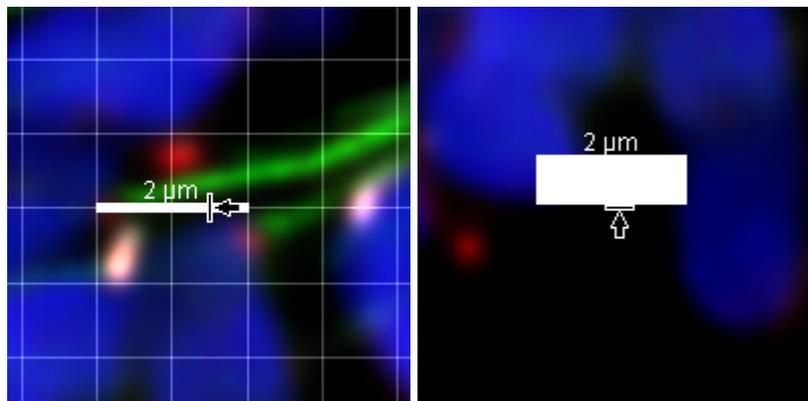


Adjust the Interactive Scale Bar

You can adjust the line length, line width, location and font size of the interactive scale bar. Move the mouse pointer over a drag region. The shape of the mouse pointer indicates the interaction.



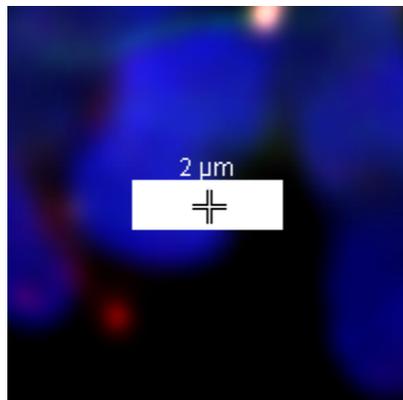
Line Length and Line Width



To adjust the length of the scale bar move the mouse to one end of the scale bar until the pointer symbol

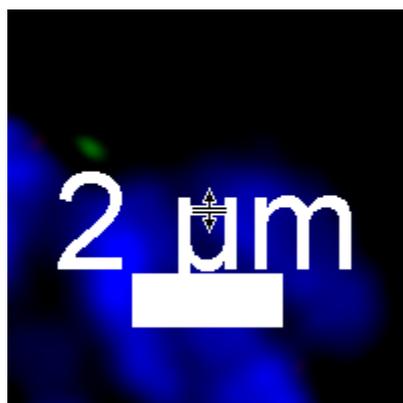
alters (vertical dash and arrow). Now left-click on the scale bar end and hold the button down while you drag the mouse to the left or right, respectively. The grid width changes accordingly. In the same way you adjust the thickness of the scale bar.

Location



To translate the scale bar left-click on it (pointer symbol cross), hold the button and drag the mouse.

Font Size



Move the mouse pointer over the legend until the pointer symbol alters (horizontal double dash and double arrow). Left-click and hold the button down while you drag the mouse up (enlarge size) or down (reduce size).

Please close Imaris before you start with the chapter 3D measurement.

6.3 3D Measurement



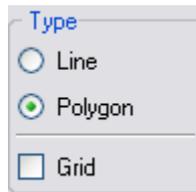
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image Pyramidal Cell in the Slice view.

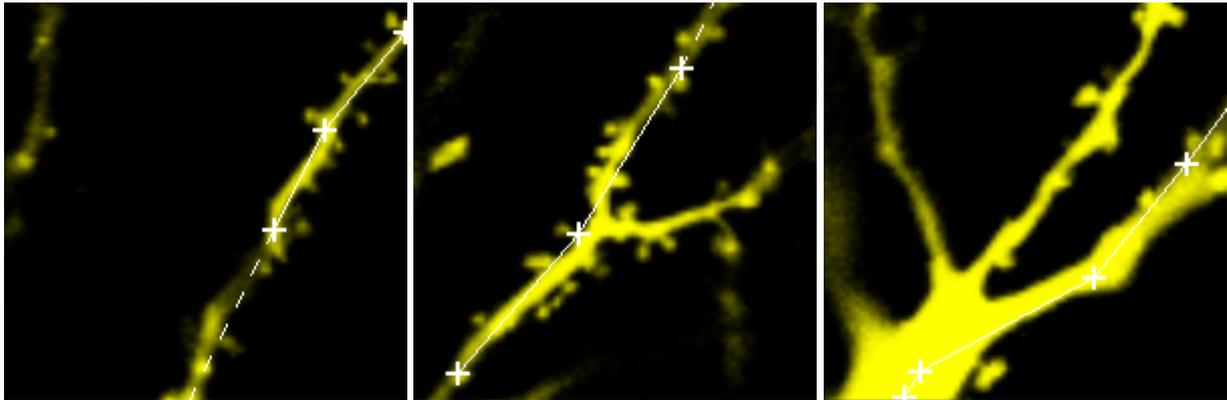
PC file path `\ProgramFiles\Bitplane\Imaris\images\PyramidalCell.ims`
Mac file path `/Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims`

Measure Distance Between Different Slices

In the slice selection on the left hand side of the screen select slice number 21 (type in 21 and press Enter).



On the right hand side in the **Measure** window select as measurement **Type Polygon**.



The first picture is slice number 21, the second picture is slice number 25 and the last picture is slice number 32.

Set the first point with a left-click on the upper right end of the filament. Change to the next slice number (edit the field or use the arrow keys on the keyboard) and set the next measurement points with consecutive left-clicks. Follow the filament through the slices. The dashed line indicates connecting lines between two slices. The distance between the first and the last measurement point is displayed in the status field **Distance** on the right hand side of the screen.

Please close Imaris before you start with the next Tutorial.

7 Track Particles

ImarisTrack is a separate Imaris module to follow 3D-objects over time, display their paths and analyze their movements. The demo image shows the movement of the algae *chlamydomonas reinhardtii* zoospores. The first step is the [Visualization](#) of the data set and the automatic Spot Detection ([Segmentation](#)) over time. With the automatic [Tracking](#) you link consecutive time points and the result is a colored Track - the motion path of a single object over time. You will learn how to display a [Single Track](#) and how to show the Track [Displacement](#). Using the [Sorting](#) function you can group and analyze the Tracks depending on various criteria.



Spot which Follows the Colored Track, Swimming Algae

User Level: Advanced

Module: Imaris, MeasurementPro, ImarisTrack

7.1 Visualization



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

Mac: In the folder Applications double-click on Imaris to open the program.

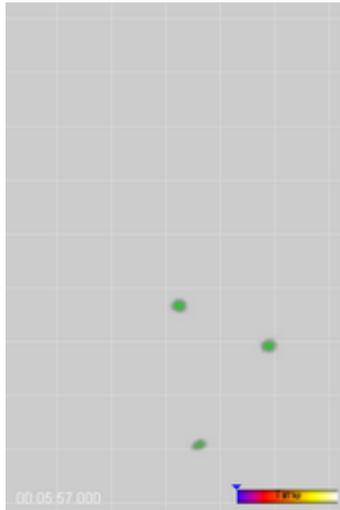
Open the demo image Swimming Algae in the Slice view.

PC file path \ProgramFiles\Bitplane\Imaris\images\SwimmingAlgae.ims

Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/SwimmingAlgae.ims

Add Volume

To create a Volume reconstruction of the data set select the **Surpass** mode. Click on the icon **Surpass** in the main toolbar of Imaris. As **Initial Scene** select **Volume**. On the left hand side in the **Volume - Properties** you find the **Mode** selection window. Select as display mode **Blend** (blending all values along the viewing direction and including their transparency). To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**. Click on **Advanced** to open the advanced settings. Set the threshold **Range** for **Channel 1** (green) to **Min:91** and **Max:230**.



This is the result in the viewing area.

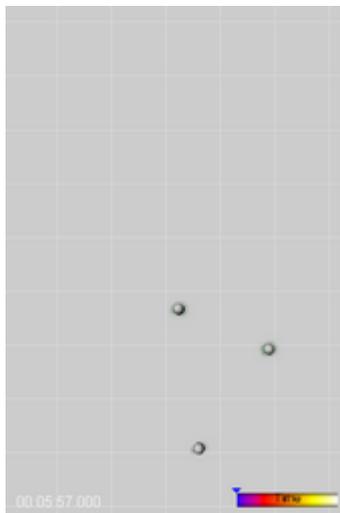
See also:

[Open Data Set](#)

[Add Volume](#)

7.2 Segmentation

In the **Objects** toolbar of the Surpass view click on the icon  to add new Spots. Select as **Source Channel** the **Channel 1** (green) and set the **Minimum Diameter** to **4.000** and click on **Next >**. Imaris detects an automatic threshold (19.065) to insert the Spots. Now click on **Finish**.



You see at the same time the gray Spots and the Volume rendered green channel.

See also:

[Add Spots](#)

[Change Spots Color](#)

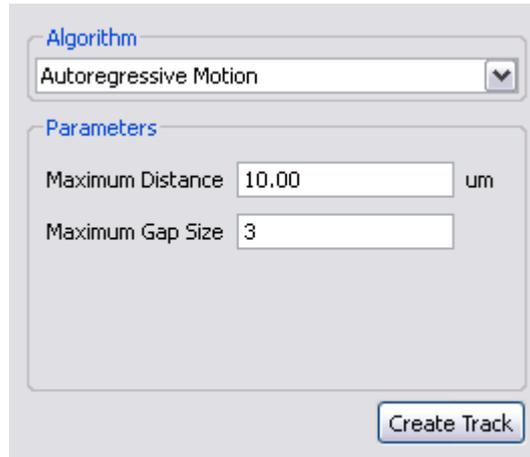
[Line and Polygon](#) to define the Minimum Spot Diameter

7.3 Tracking

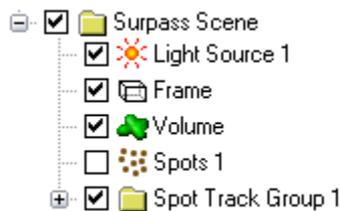
A Track is a component within the Surpass view that describes the behavior of an object over time.



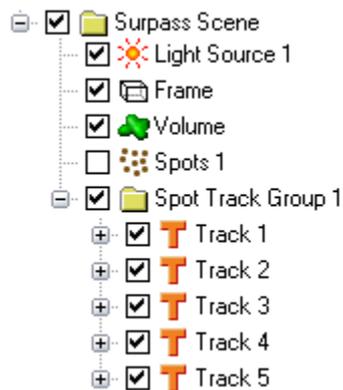
To automatically create all Tracks left-click on the tab **Tracking**.



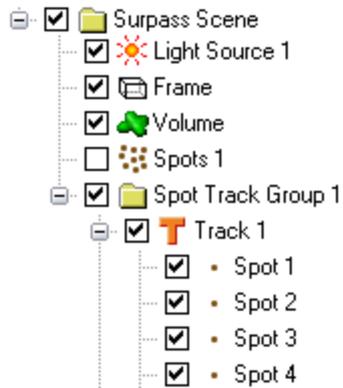
As **Algorithm** select **Autoregressive Motion** and as **Maximum Distance** set **10.000** um. Click on **Create Track**.



On the left hand side in the Surpass Tree you find the new **Spot Track Group 1**.



Click on  to display all Tracks in the Spot Track Group 1. Each Track has its own track number.



Click on  to display all Spots in a Track. Each Spot has its own Spot number.

Please note: The Tracks and Spots in Imaris are consecutively numbered. The sequence depends on the data set and the parameter settings. Due to this the Track numbers and the Spots numbers might not match between different Imaris sessions.



In the viewing area each Track is displayed as a line. The line color indicates the time point, corresponding to the Time Colorbar, down to the right.

See also:

[Time Concept](#)

[Surpass Tree](#)

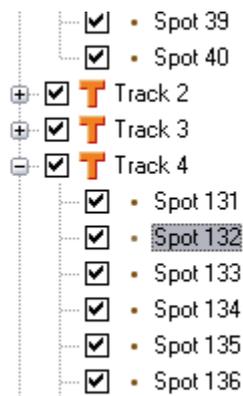
7.4 Single Track

There are various display options in the ImarisTrack module. This chapter will focus on the display of a single Track.

Select a Single Track



To select a single Track, choose the mouse pointer mode Select and left-click on a Spot in the image (or on a part of the Track). Please choose the Track in the image above, it has a distinct wave-form path and is located in the middle lower part of the image. Keep in mind the Track number, you will use this Track for a single Track display (in the section Display a Single Track, see below).

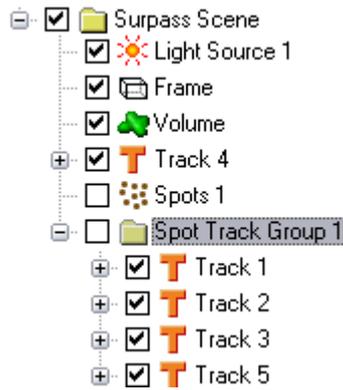


In the Surpass Tree automatically the corresponding Track is shown and all Spots belonging to this Track. The selected time Spot is highlighted.

At that time of the Tutorial and in this example the time Spots for Track 1 and Track 4 are displayed (please keep in mind, the numbering in your session could be different). Please click on  for the two Tracks to hide all Spots in the Surpass Tree before you start the display of a single Track.

Display a Single Track

To work with one single Track take the Track out of the Spot Track Group. In this example Track 4 will be displayed as Single Track. Please choose the Track you selected in the chapter above (Select a Single Track).



You select the Track in the Surpass Tree with a left-click and drag the Track to its new position. Un-check the check box Spot Track Group 1. This is the result in the viewing area:



Only one single Track is displayed in the viewing area.

See also:

[Select and Navigate](#) (mouse pointer mode)

[Surpass Tree](#)

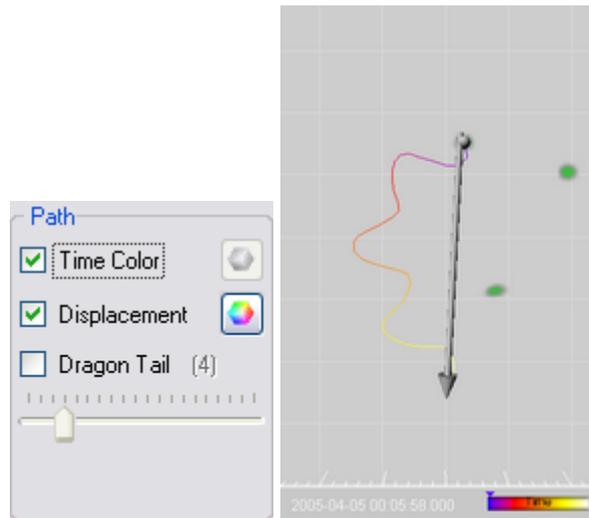
[Time Concept](#)

7.5 Displacement

This visualization mode shows the displacement of a Track as an arrow.

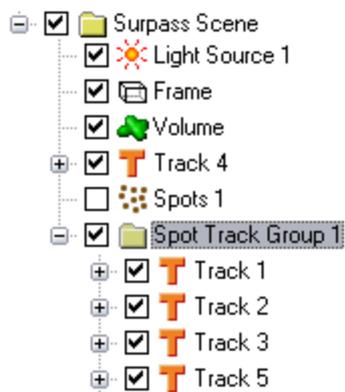
For a Single Track

You will now display for a single Track (choose the Track you used in the last section) the displacement arrow in addition to the colored Track. In the Surpass Tree left-click the Track (item is highlighted). Select the tab **Settings**. In the **Track x - Properties** you find the Path adjustment window.



Check the respective check box **Displacement** in the Path adjustment window. The arrow is displayed in the viewing area.

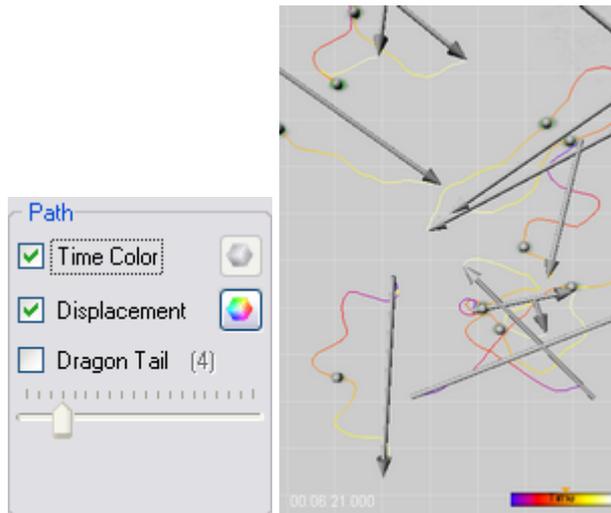
For all Tracks



Check the box Spot Track Group 1 in the Surpass Tree to display all Tracks in the image. Then left-click the item Spot Track Group 1 (item is highlighted).



Select the tab **Tracks**. In the **Spot Track Group 1 - Properties** you find the Path adjustment window.



Check the respective check box **Displacement** in the Path adjustment window. All arrows are displayed in the viewing area.

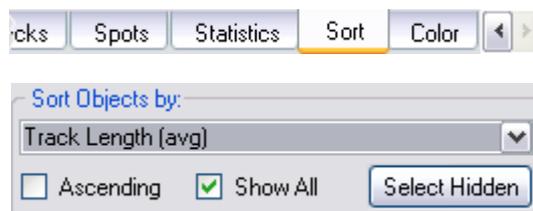
Please note: To open the Path adjustment window for a Spot Track Group select the tab **Tracks**. To open the Path adjustment window for a single Track select the tab **Settings** (the tab **Tracks** is only available if you work in a Spot Track Group).

Before you start with the sorting of the Tracks please move the Track above the Spot Track Group 1 (in this example Track 4) back in the Spot Track Group.

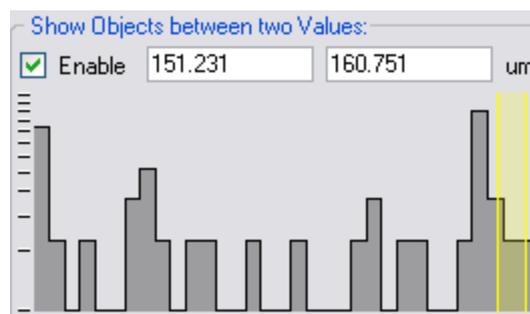
See also:
[Select and Navigate](#) (mouse pointer mode)
[Surpass Tree](#)
[Time Concept](#)

7.6 Sort Tracks

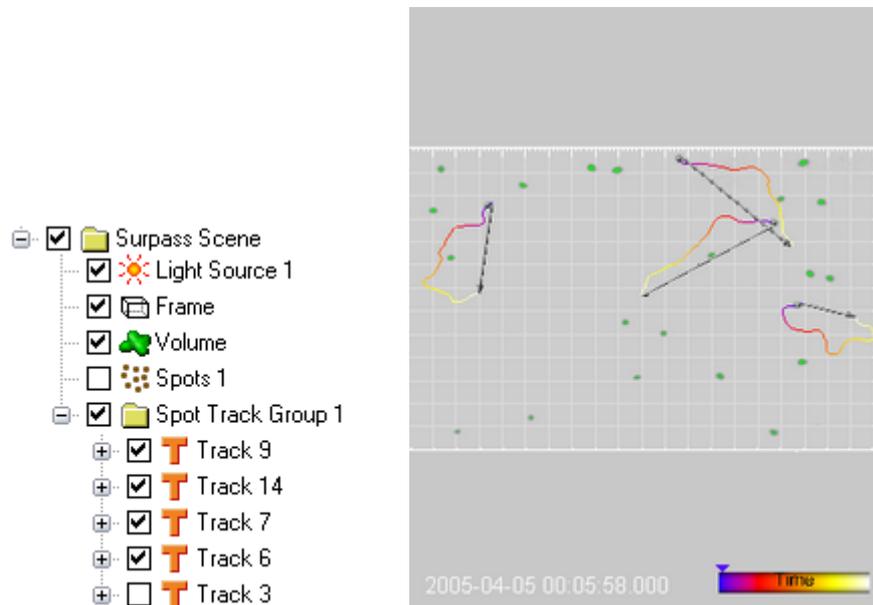
The sorting of the Tracks is essential for the further analysis. In the following you sort the Tracks by their length. In the Surpass Tree check the box Spot Track Group 1 and left-click on the item Spot Track Group 1 (item is highlighted).



Left-click on the tab **Sort** and choose **Track Length (avg)** as sorting criteria.



Adjust **Shown Objects between two Values** either by editing the status fields or by moving the thin yellow lines using the mouse. The effect is directly visible in the Surpass Tree and the viewing area.



On the left hand side in the Surpass Tree the visible Tracks are automatically checked, in the viewing area the corresponding Tracks and the displacement arrows are displayed. Please keep in mind, the Track numbering in your session could be different.

Please close Imaris before you start with the next chapter.

See also:

[Surpass Tree](#)
[Time Concept](#)

7.7 Additional Example

In this additional example for particle tracking you will analyze developing thymocytes in an intact thymic lobe. You will visualize and segment the cells. Then you will create the Tracks over time and sort the Tracks based on the Track duration. Then you will select and delete the short Tracks.



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

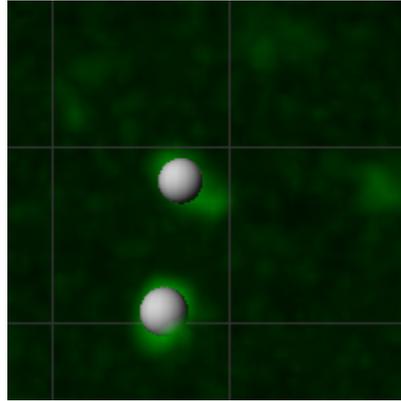
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image R18Demo in the Surpass view.

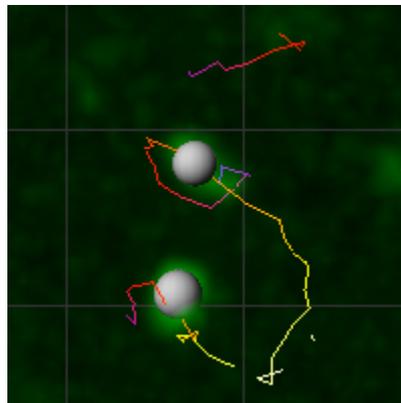
PC file path \\ProgramFiles\\Bitplane\\Imaris\\images\\R18Demo.ims

Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/R18Demo.ims

Visualize, Segment and Track the Cells

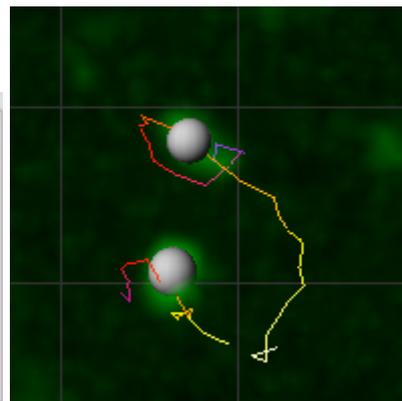
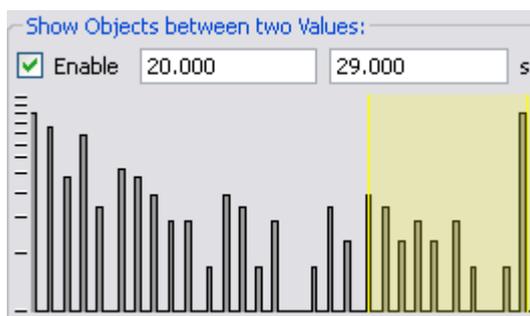


As **Initial Scene** select **Volume**. In the **Objects** toolbar of the Surpass view click on the icon  to add new Spots. Select as **Source Channel** the **Channel 1** (green) and set the **Minimum Diameter** to **5.000** and click on **Next >**. Change the threshold to **10.000** and click on **Finish**.



To automatically create all Tracks left-click on the tab **Tracking**. As **Algorithm** select **Autoregressive Motion** and as **Maximum Distance** set **15.000** um.

Track Duration as Sorting Criteria



Now you sort the Tracks. Left-click on the tab **Sort** and choose **Track Duration (avg)** as sorting criteria. Adjust the parameter **Shown Objects between two Values** either by editing the status fields or by moving the thin yellow lines using the mouse. The effect is directly visible in the viewing area. In the display detail above the two long Tracks remain visible in the viewing area.

Select and Delete Hidden Tracks

Select Hidden

In the Surpass Tree the Tracks between the Value 0 and 20.00 are not checked and invisible (hidden) in the viewing area. Press the button **Select Hidden** (in the Properties) and all hidden Tracks are selected at once.



Press the button **Delete ...** in the Objects Toolbar to delete the selected objects after confirmation.

Please close Imaris before you start with the next Tutorial.

See also:

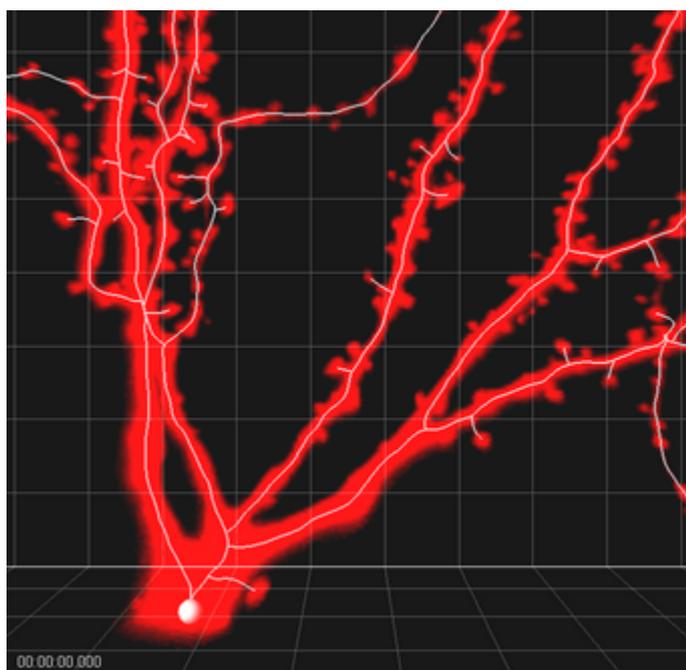
[Visualization](#)

[Segmentation](#)

[Tracking](#)

8 Analyze Neuron

FilamentTracer is a separate Imaris module to detect, visualize and measure filamentous structures. The demo image shows a hippocampal Pyramidal Cell in an organotypic cell culture. You will start with the [MIP](#) (Maximum Intensity Projection) rendering of the Pyramidal Cell and learn how to [Change the Channel Color](#) from yellow (original) to red. There are different ways of filament tracing, this Tutorial will focus on just two of them: The [Automatic Detection](#) of filaments and the [AutoPath Mode](#). In the last chapter [Filament Tool Kit](#) you will find a short description where to find and how-to-handle the different tracing functions in the complex FilamentTracer module.



Filament Structure, Pyramidal Cell

User Level: Advanced

Module: Imaris, FilamentTracer

8.1 MIP Display



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image Pyramidal Cell in the Slice view.

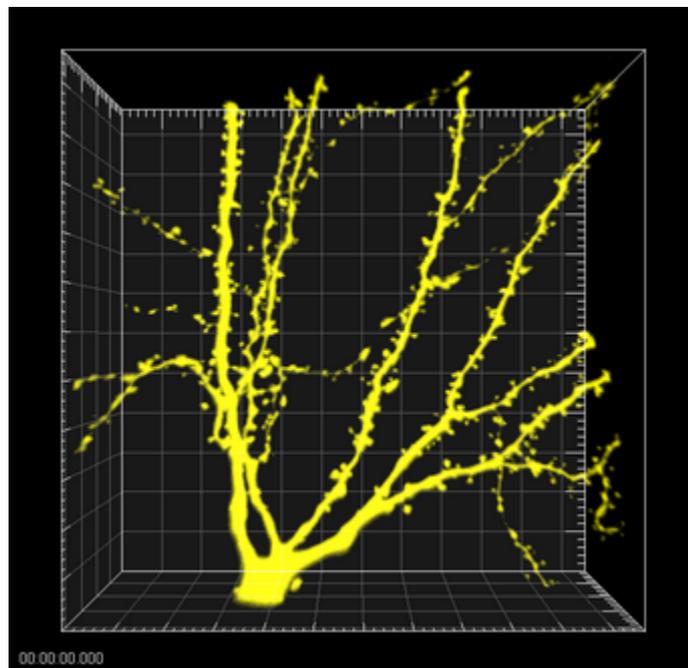
PC file path \\ProgramFiles\\Bitplane\\Imaris\\images\\PyramidalCell.ims
Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims

Add Volume

To create a Volume reconstruction of the data set select the Surpass mode. Click on the icon **Surpass** in the main toolbar of Imaris. As **Initial Scene** select **Volume**.

MIP (max.) Mode

In the **Volume - Properties** you find the **Mode** selection window. The display mode **MIP (max)** (Maximum Intensity Projection, maximum intensity of all layers along the viewing direction) shows always a black background. The Pyramidal Cell is displayed in the viewing area:



MIP Display, Pyramidal Cell

See also:

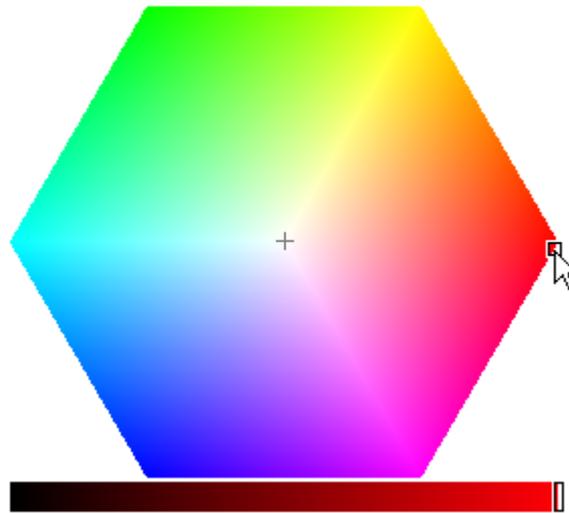
[Add Volume](#)

8.2 Change Channel Color

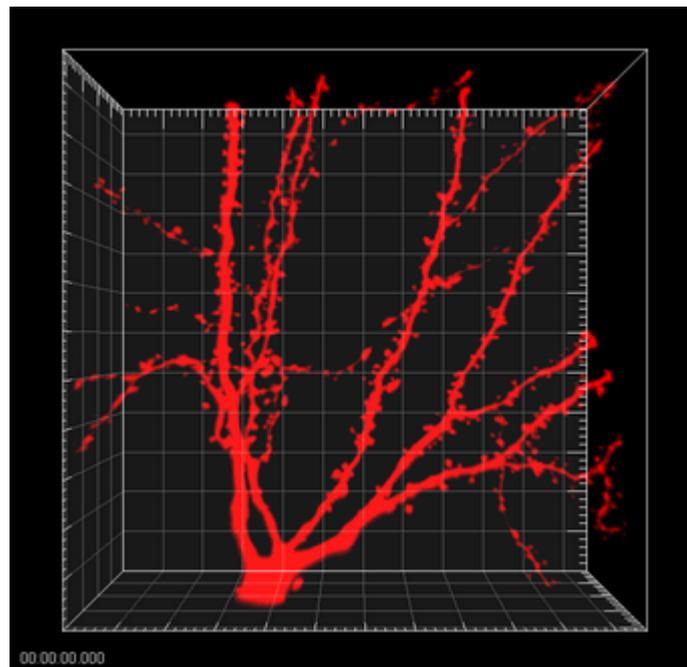
Now you change the color of the the display Channel 1 from yellow to red. To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**.



Left-click on **Channel 1** to open the color selection.



In the color table select red and click on OK. This is the result in the viewing area:



MIP Display, Channel Color Red, Pyramidal Cell

See also:

[Display Adjustment](#)

[Change Spots Color](#)

[Change Background Color](#)

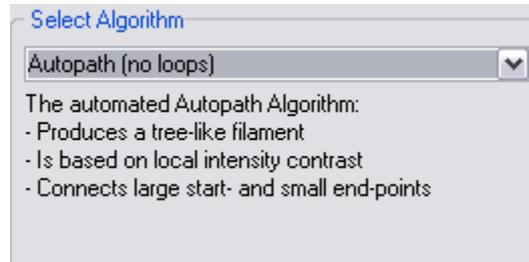
8.3 Automatic Detection

In the Automatic mode of the FilamentTracer the start point and the end points are calculated automatically. Based on the calculation the filament is built.

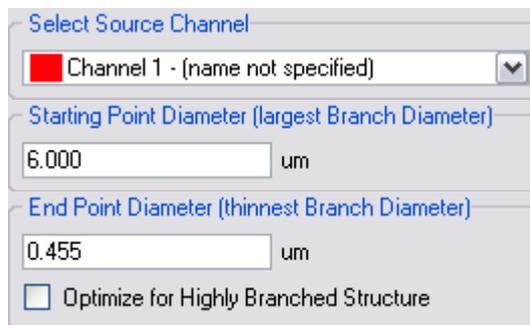
Add new Filament



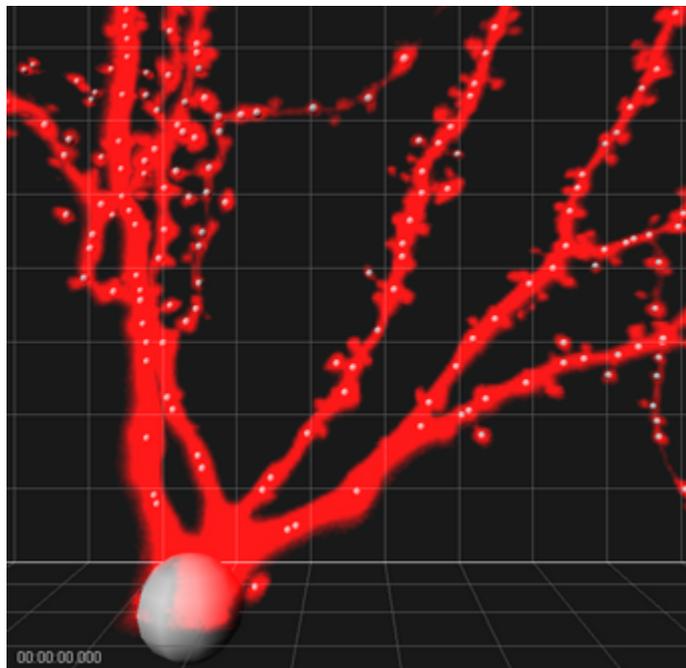
In the **Objects** toolbar of the Surpass view click on the icon  to add a new Filament.



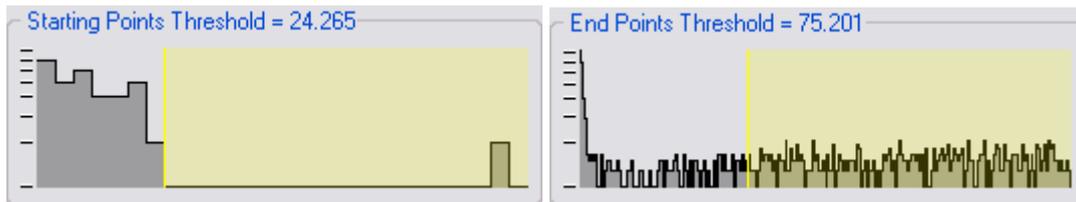
In the **Filament - Properties** select **Autopath (no loops)** and click on **Next>**.



The **Select Source Channel** is **Channel 1**, set the **Starting Point Diameter** to **6.000 um**, the **End Point Diameter** to **0.455 um**, and click on **Next>**.



The filament start point and the automatically detected end points are displayed in the image.



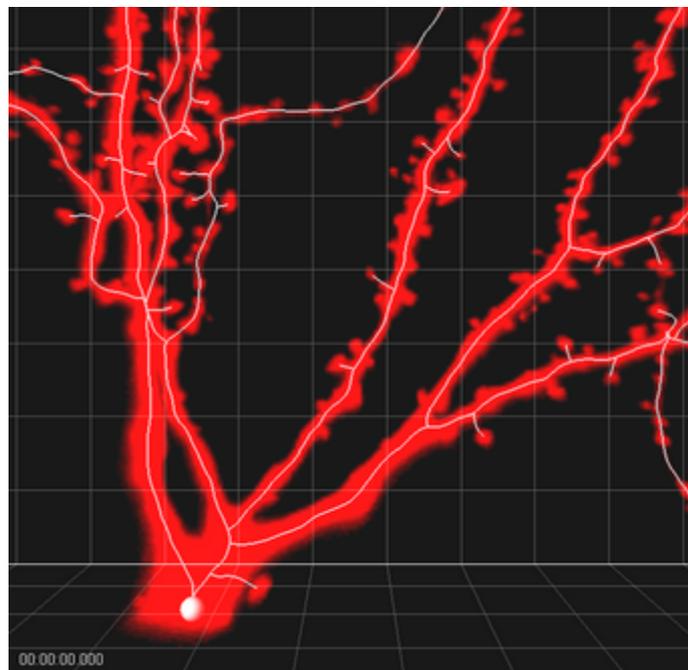
On the left hand side you find the calculated thresholds. Click on **Next>**.

Finish

Calculate Diameter from Image

Build Filaments for all Time Points

Leave the box **Calculate Diameter from Image** checked and click on **Finish**. This is the result in the viewing area:



All end points are automatically connected to the start point.

Switch off the item Filament 1 in the Surpass Tree (un-check the box) before you insert a new filament in the next chapter AutoPath.

See also:

[Line and Polygon](#) to define the Starting and End Point Diameter

8.4 AutoPath Mode

In the AutoPath mode of the FilamentTracer you define the start point and all the end points manually. Based on the position of the end point the AutoPath function automatically computes the path to the starting point.

Add new Filament



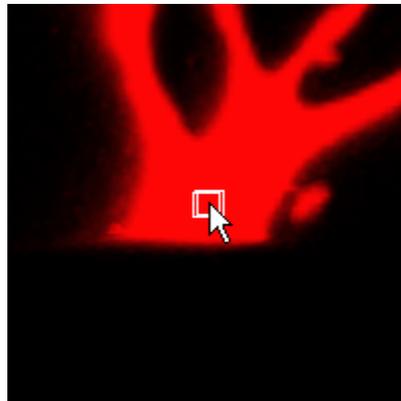
In the **Objects** toolbar of the Surpass view click on the icon  to add a new Filament. In the **Filament - Properties** click on **Cancel** to stop the automatic detection of the start point and the end points.



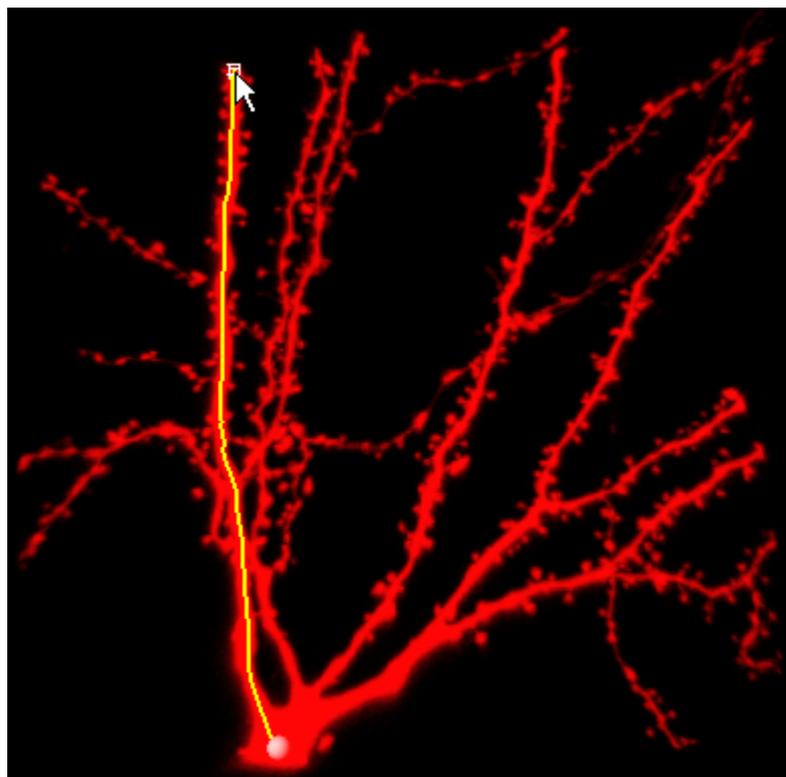
Select the tab **Draw** and the **Method** selection window is displayed:



Select **AutoPath** and change to the mouse pointer mode **Select**. A rectangle is displayed around the mouse pointer.



Press Shift and right-click on the base of the Pyramidal Cell to fix the starting point.



At the base of the cell you see the filament starting point. Now you move the mouse cursor to an end point of a filament. The filament is calculated automatically and displayed on screen. With Shift + left-click you fix the filament. Move the mouse cursor to the end point of the next filament, press Shift + left-click to fix the filament. In the same way set all the filament end points in the image.

See also:

[Select and Navigate](#) (mouse pointer mode)

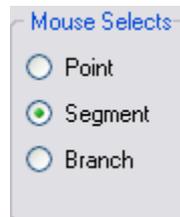
[Filament Tool Kit](#)

8.5 Filament Tool Kit

In this chapter you will learn where to find the different selection windows in the module FilamentTracer and you will find a short, tabular overview concerning the three different interactive filament tracing methods.

The Mouse Select and Process Selection Window

Left-click the tab **Edit**.



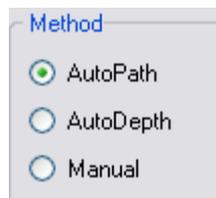
In the window **Mouse Selects** you can choose between **Point**, **Segment** (filament between two branch points) and **Branch** (starting from the clicked point to all connected end points).



In the the window **Process Selection** you find the **Delete** button, and other editing functions which can be restricted to only the part(s) of the Filament which are currently selected.

The Method Window

Left-click the tab **Draw**.



In the **Method** selection window you can choose between **AutoPath**, **AutoDepth** and **Manual**.

AutoPath

You set the starting point and the end points manually. The filament is calculated automatically and displayed on screen.

Please note: The button **Set Starting Point** is only available in the AutoPath mode.

Shift + Right-click	Set the Starting Point on a Volume object
Left-click on a Segment or Branch, then on 	Selected Segment or Branch is set as Starting Point
Move Cursor	Draw the Filament
Shift + Left-click	Fix the Filament
Left-click to Select, then on 	Deletes the Selected Point/Segment or Branch
Mouse Wheel	Change the "Pencil" Diameter for Drawing the Filament

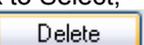
AutoDepth

You draw each filament manually from starting point to end point. The depth is automatically computed.

Shift + Left-click + Drag	Draw a Filament into the Volume
Stop Movement and Release all Buttons	Stop Drawing
Left-click to Select, then on 	Delete Selected Point/Segment or Branch
Mouse Wheel	Change the "Pencil" Diameter for Drawing the Filament

Manual

You draw each filament manually from starting point to end point. You hide the Volume object and draw in one drawing plane, the depth is not automatically computed unless Automatic Placement is enabled.

Shift + Left-click + Drag	Draw the Filament on the Slicer
Stop Movement and Release all Buttons	Stop Drawing
Left-click to Select, then on 	Delete Selected Point/Segment or Branch
Mouse Wheel	Change the "Pencil" Diameter for Drawing the Filament

Please close Imaris before you start with the next Tutorial.

See also:

[Imaris Keys Help](#)

9 Volume over Time

In this Tutorial you analyze the chromosome volume over time in a mitotic cell. The demo data set is a time series of a HeLa Cell going through all mitotic phases in the cell cycle. In the green channel, the mitotic spindle is visualized by EGFP tagged alpha-tubulin. In the red channel, chromatin is visualized by tagged core histone 2B. You will start with the visualization of the data set.

First you [Add a Volume](#) reconstruction for both channels, then you generate an [IsoSurface](#) reconstruction of the chromosomes. In the chapter [Time Concept](#) you will learn how-to-handle time series in Imaris. Then you [Separate the Objects](#) in order to [Calculate](#) and export statistical data for each time point. The focus of the last chapter is the [Presentation of the Results](#) in MS Excel.

Variable	Value	Unit	Time
Sphericity (avg)	0.691		22
Volume (sum)	2356.639	3	1
Volume (sum)	2355.298	3	2
Volume (sum)	2348.335	3	3
Volume (sum)	2321.949	3	4
Volume (sum)	1858.878	3	5

Settings Export CSV Excel

Statistical Data, HeLa Cell

User Level: Advanced
Module: Imaris, MeasurementPro

9.1 Add Volume



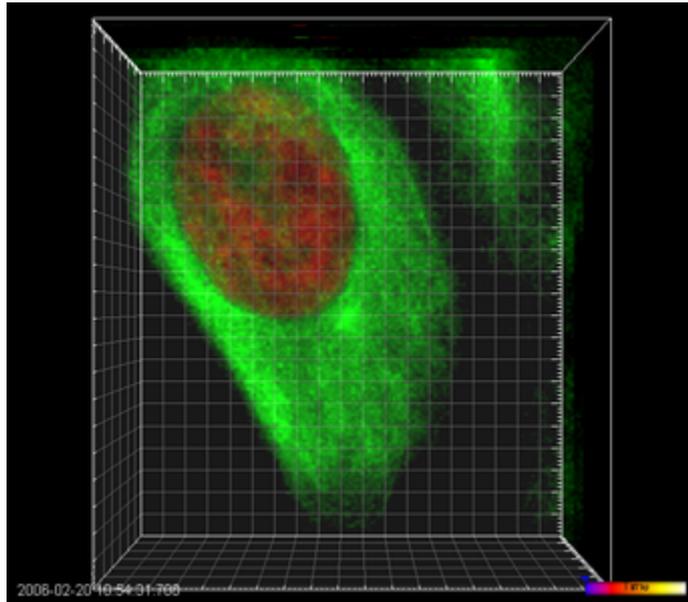
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image HeLa Cell in the Slice view.

PC file path \\ProgramFiles\Bitplane\Imaris\images\HeLaCell.ims
Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/HeLaCell.ims

Add Volume

To create a Volume reconstruction of the data set select the Surpass mode. Click on the icon **Surpass** in the main toolbar of Imaris. As **Initial Scene** select **Volume**. In the **Volume - Properties** you find the **Mode** selection window. The display mode **MIP (max)** (Maximum Intensity Projection, maximum intensity of all layers along the viewing direction) shows always a black background. To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**. Click on **Advanced** to open the advanced settings. Set the threshold **Range** for for **Ch1** (red) and **Ch2** (green) to **Min:45** and **Max:200**. The HeLa Cell is displayed in the viewing area.



See also:

[Add Volume](#)

[Display Adjustment](#)

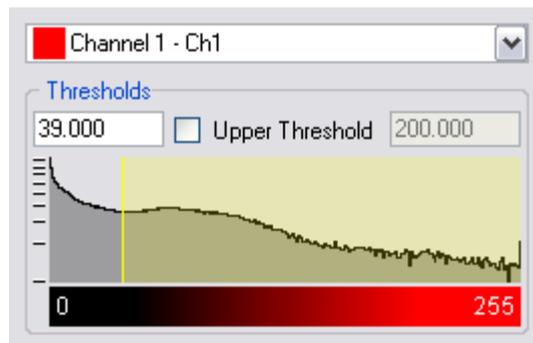
[MIP Display](#)

9.2 Add IsoSurface

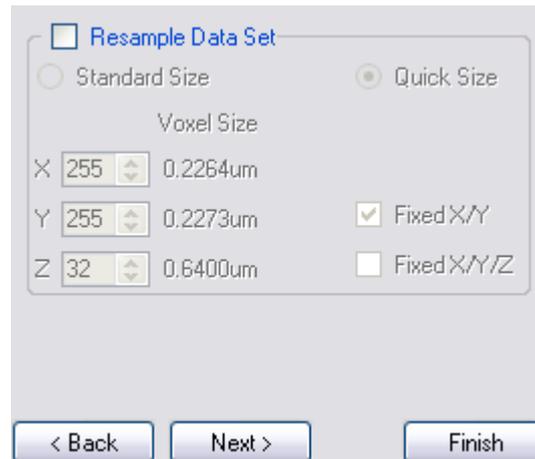


In the **Objects** toolbar of the Surpass view click on the icon  to add a new IsoSurface.

Adjust the IsoSurface Properties



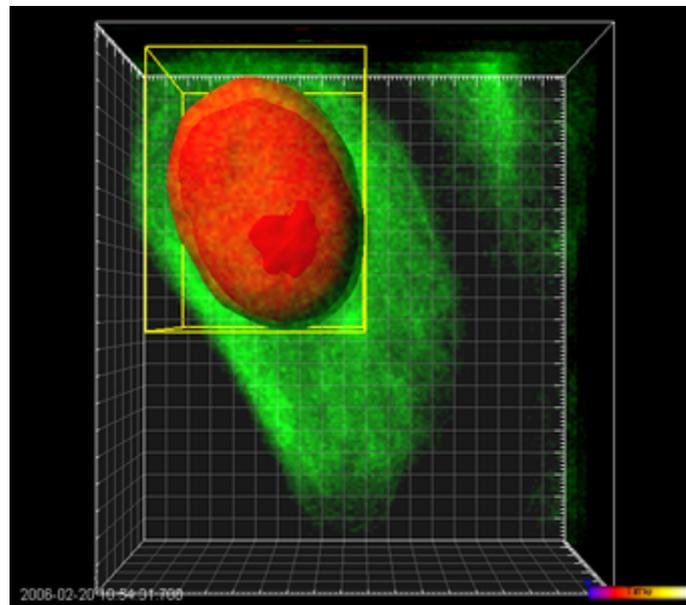
To separate objects from the background adjust the threshold. Select **Channel 1 - Ch1**, set the **Threshold** to **39.000** and click on **Next >**.



Deselect the check box **Resample Data Set** and click on **Next >**.



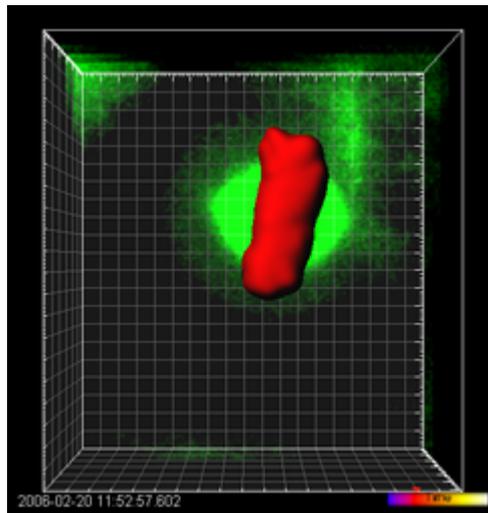
Select **Smooth Data Set** and **Close Objects at Border** and click on **Finish**.



Additional to the Volume rendered tubulin proteins of the spindle the IsoSurface reconstruction of the histone 2B proteins is displayed in the viewing area.

See also:
[Create IsoSurface](#)

9.3 Time Concept



HeLa Cell at Time Point 7

The time concept in Imaris is very intuitive to understand. Whenever you load a time series automatically the Time window is displayed at the bottom of the screen.

Time Window and Time Slider



On the left hand side you find the button for the **Time Settings...**, the **Play** button, and the **Movie** button. Each bar in the time slider represents one time point. In the example the slider is at time point 7. Additionally, the time point of the actual image is displayed at the right hand of the time slider. You select images at different time points using the time slider.

Left-click Slider + Drag Slider	Go to Previous/Next Time Point
Left-click Slider (Select) + Right Arrow on Keyboard	Go to Next Time Point
Left-click Slider (Select) + Left Arrow on Keyboard	Go to Previous Time Point
	Play/Pause through All Time Points
	Record Movie of All Time Points

Time Colorbar



In the viewing area down to the right the Time Colorbar is displayed (default setting). The arrow indicates the actual time point (in this example time point 7 out of 22).

See also:

[Save Movie](#)

[Imaris and QuickTimeVR](#)

9.4 Segment Objects

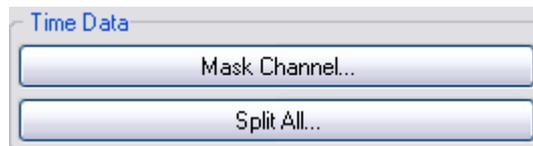
To analyze the IsoSurface object at different time points Imaris will generate one Surface object for each time point. You will find all new items in the automatically created new Group in the Surpass Tree.

Split the IsoSurface to Analyze Time Points

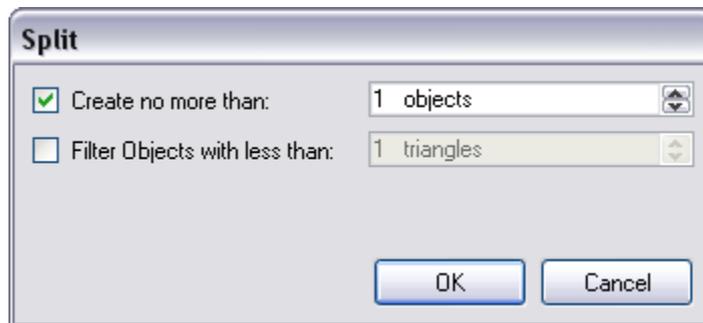
Set the time slider to time point 1.



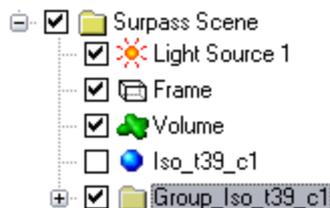
Highlight with a left-click the Surpass Tree item **Iso_t39_c1**.



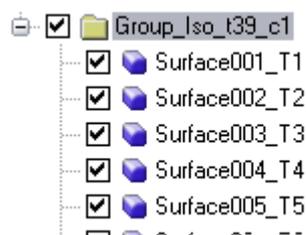
In the **Iso_t39_c1 - Properties - Time Data** click on **Split All...**



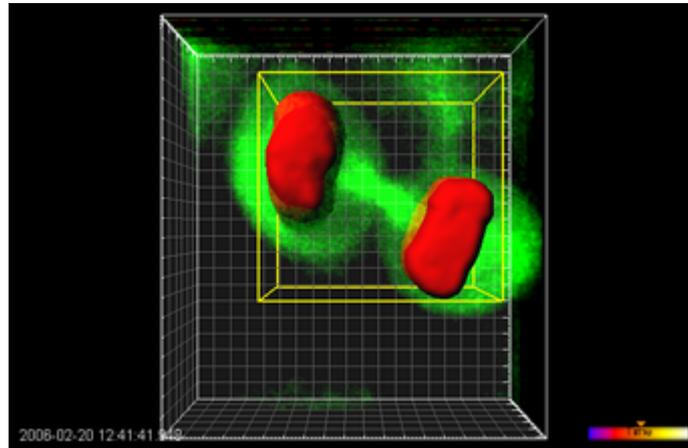
In the **Split** window check the box for **Create no more than: 1 objects** and click on **OK**.



In the Surpass Tree you find the new **Group_Iso_t39_c1**.



Click on  to display all Surface items in the Group. Highlight Surface012_T12 (in this example object 012 at time point 12) with a left-click and the Surface object 12 at time point 12 is displayed in the viewing area:



HeLa Cell at Time Point 12

Please note: "Surface001..." corresponds to the time point that the time slider is set to when you press the button Split All... . If your numbering is different you did not set the time slider back to time point 1 (first step in this section).

See also:

[Time Concept](#)

[Surpass Tree](#)

9.5 Calculate Statistics

Now you will evaluate statistically for each Surface object in the **Group_Iso_t39_c1** the volume for the related time point.

Please note: The statistical calculation is based on the selected Surpass Tree item. If the Group item is selected the statistics is done for all items in the Group.



Highlight with a left-click the Surpass Tree item **Group_Iso_t39_c1**.

Start the Calculation and Show Results



Left-click on the tab **Statistics** to start the calculation.

Variable	Value	Unit	Time
Sphericity (avg)	0.691		22
Volume (sum)	2356.639	3	1
Volume (sum)	2355.298	3	2
Volume (sum)	2348.335	3	3
Volume (sum)	2321.949	3	4
Volume (sum)	2295.563	3	5

Settings Export CSV Excel

The result of the calculation is displayed in the spreadsheet. You scroll the table by dragging the slider handles. In the example above the **Value** for the **Volume (sum)** for **Time Point 1** is highlighted. In the next row you see the Value for the next time point, and so on.

Please note: If the Volume calculation is not displayed, it is probably disabled. Click the Settings button of the Statistics tab, and scroll down to Surface object (not IsoSurface), and enable the desired calculations.

Export Statistics for Further Analysis

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.

Please note: MS Excel is not automatically installed on your computer when you install Imaris. Please install MS Excel before you continue this Tutorial.

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:
[Present Results](#)

9.6 Present Results

On your computer Imaris runs now in the background and Excel runs in the foreground. This chapter will focus on two display options in MS Excel. The statistical table and the graphical representation as vertical-bar chart. All statistical values of the Surface objects are sorted in different sheets. You find the Volume (sum) for the different time points in the sheet Volume.



On the bottom of the screen click on the tab **Volume**.

Statistical Table

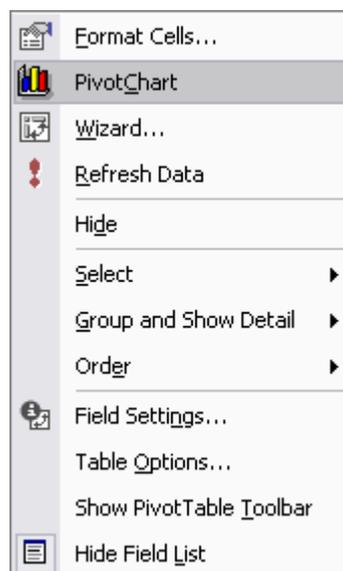
In the middle of the sheet Volume you find the Pivot Table:

Drop Page Fields Here		
Sum of Value		
Group	Time	Total
Group Iso_t39_c1	1	2356.638916
	2	2355.298096
	3	2348.334961
	4	2321.948975
	5	1958.969971
	6	866.6140137
	7	677.4110107
	8	624.9970093
	9	669.6110229
	10	969.2290039
	11	1413.689941
	12	1814.145996
	13	2044.300049
	14	2196.0979
	15	2223.133057
	16	2258.931885
	17	2286.948975
	18	2301.823975
	19	2304.13208
	20	2301.584961
	21	2321.394043
	22	2314.74292
Group Iso_t39_c1 Total		40929.97876
Grand Total		40929.97876

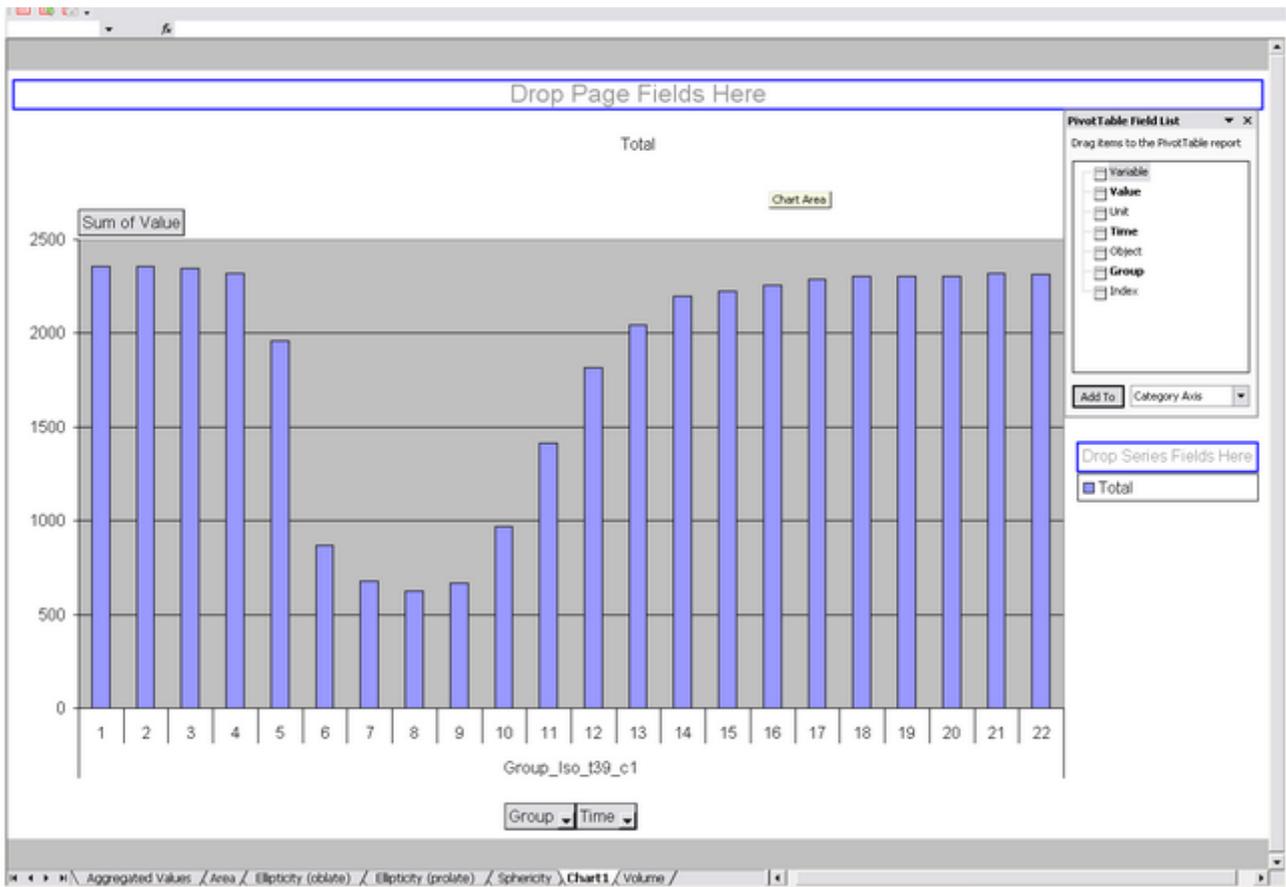
All time points (1 to 22) and the corresponding Volume (sum) are listed.

Graphical Representation

Now right-click in the field **Group** and select **PivotChart**.



This is the graphical representation of your statistical data as vertical-bar chart:



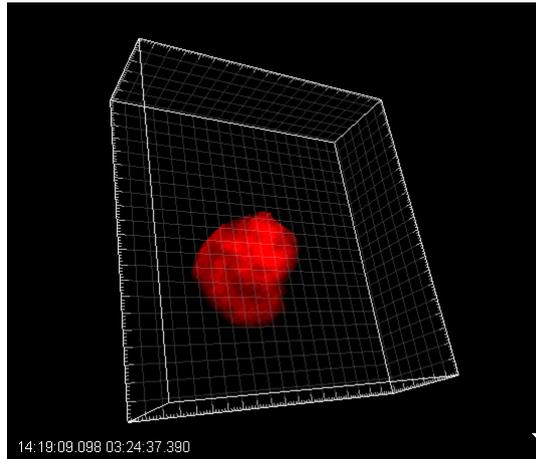
The volume over time for the chromatin, visualized by tagged core histone 2B, is decreasing from time point 4 until time point 8 (mitotic plate) and then increasing again.

Please close Imaris before you start with the next Tutorial.

10 Volume of Interest

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). In this Tutorial you define such a volume of interest. The demo data set is a time series of a HeLa Cell going through all mitotic phases in the cell cycle. In the green channel, the mitotic spindle is visualized by EGFP tagged alpha-tubulin. In the red channel, chromatin is visualized by tagged core histone 2B.

You will start with the visualization of the data set. First you [Add a Volume](#) reconstruction for both channels, then you manually generate a Contour Surface by [Drawing Border Lines](#) in your data set. Then you let Imaris calculate the complete [Contour Surface](#). Based on this surface, you [Create a Surface Object](#). This Surface object is your "cookie-cutter". Duplicate the original red channel and then use the "cookie-cutter" to [Lay a Mask](#) on this channel. The focus of the last chapter is the display of the [Masked Channel](#).



Masked Red Channel, HeLa Cell

User Level: Advanced
Module: Imaris, MeasurementPro

10.1 Add Volume



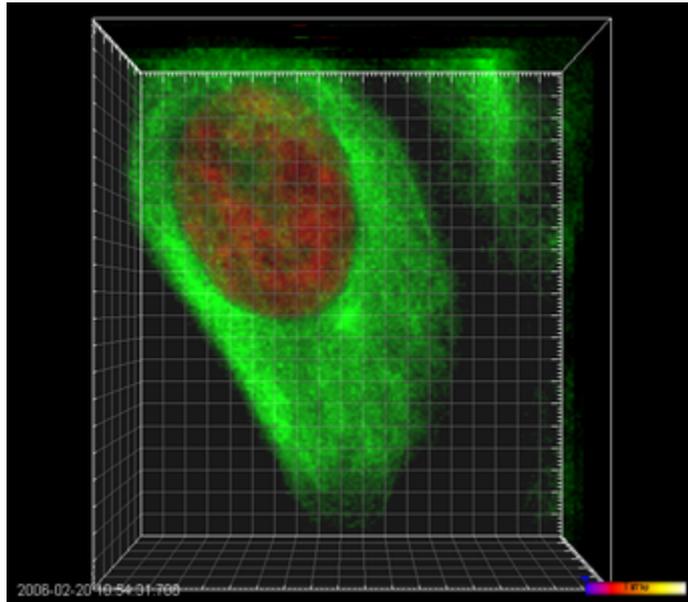
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image HeLa Cell in the Slice view.

PC file path \\ProgramFiles\\Bitplane\\Imaris\\images\\HeLaCell.ims
Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/HeLaCell.ims

Add Volume

To create a Volume reconstruction of the data set select the Surpass mode. Click on the icon **Surpass** in the main toolbar of Imaris. As **Initial Scene** select **Volume**. In the **Volume - Properties** you find the **Mode** selection window. The display mode **MIP (max)** (Maximum Intensity Projection, maximum intensity of all layers along the viewing direction) shows always a black background. To open the Display Adjustment window select in the Menu **Edit - Show Display Adjustment**. Click on **Advanced** to open the advanced settings. Set the threshold **Range** for for **Ch1** (red) and **Ch2** (green) to **Min:45** and **Max:200**. The HeLa Cell is displayed in the viewing area.



HeLa Cell Display in the Viewing Area

See also:

[Add Volume](#)

[Display Adjustment](#)

[MIP Display](#)

10.2 Add 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).

Add Contour Surface

In the menu **Surpass** select  **Contour Surface**. The new Contour Surface item displays in the Surpass Tree. All parameters on the tab **Settings** display in the Contour Surface Properties (on the upper left hand side of the screen). Adjust the following parameters and settings.

Select Draw Board and Drawing Mode

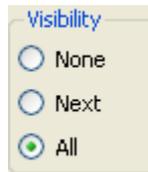
A drawing plane is displayed in the viewing area.

Draw. Board / Size		
<input type="radio"/> YZ	X	<input type="text" value="136"/>
<input type="radio"/> XZ	Y	<input type="text" value="151"/>
<input checked="" type="radio"/> XY	Z	<input type="text" value="5"/>

In the Contour Surface Properties select as **Draw Board XY**, that means you draw the first contour on the xy plane.

Drawing Mode	
<input checked="" type="radio"/> Click	
<input type="radio"/> Time	<input type="text" value="200"/> ms
<input type="radio"/> Dist.	<input type="text" value="5.63067"/>

You can draw the contours in different draw styles. As drawing mode select **Click**. How-to-draw a contour is explained in detail in the chapter Volume of Interest - [Draw Border Lines](#).



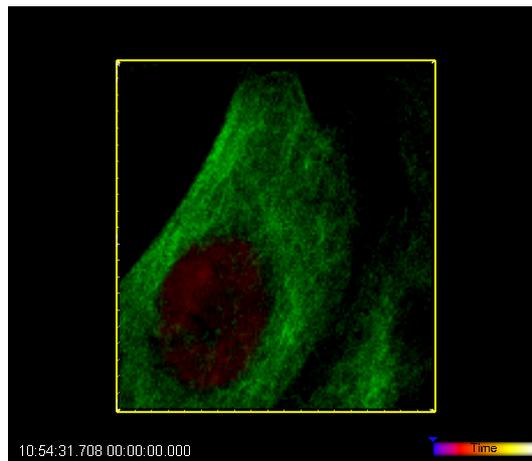
You can choose Visibility options to display previously drawn contours. Select **All** to show all contours.

Change Position of the Drawing Plane

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



Choose the slice for the first contour by moving the **Slider** to the first position. Alternatively you can change the position of the drawing plane by mouse interaction. 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.



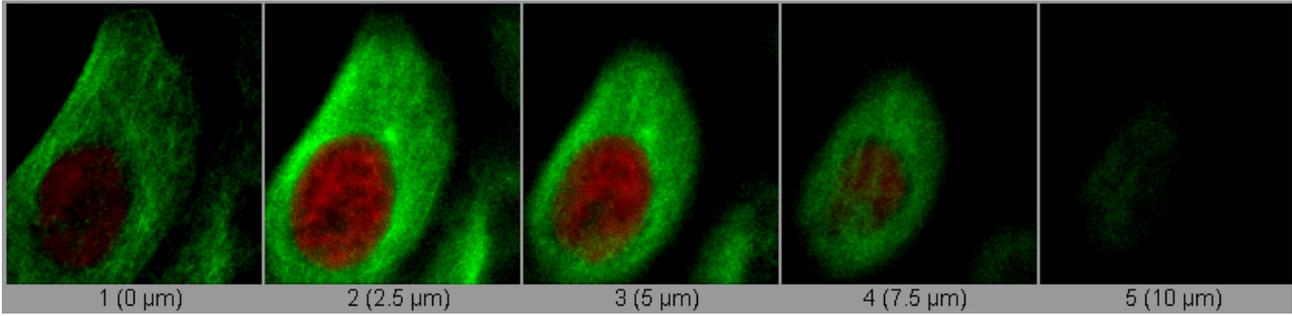
This is the Surpass Viewing Area, Slider in First Position

See also:
Volume of Interest - [Draw Border Lines](#)

10.3 Verify Planes



To verify the image planes you can switch to the Slice view and the Gallery view by clicking on the respective icons in the main toolbar.



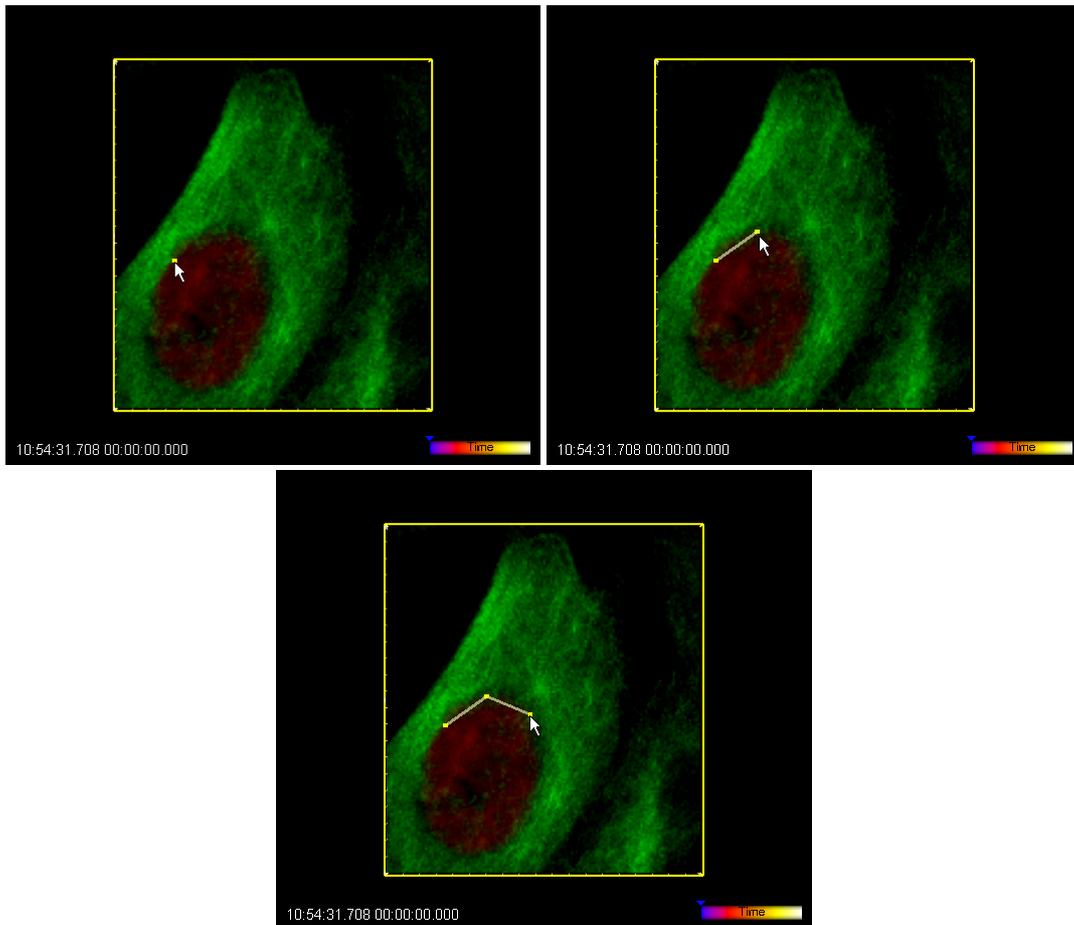
This is the Image Plane Overview in the Gallery View



Switch back to the Surpass view with a click on the icon Surpass.

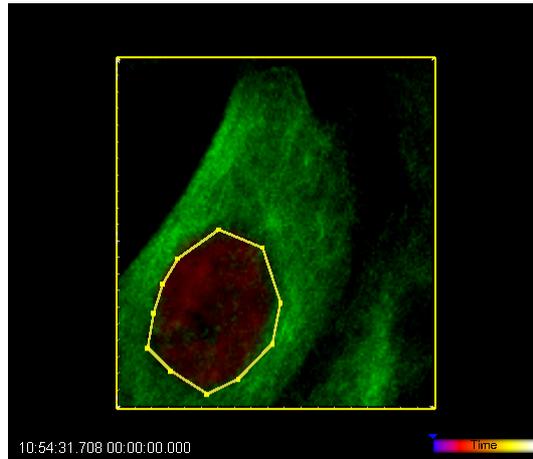
10.4 Draw Border Lines

You can draw as many contours on a plane as required. All contours drawn on a plane will become part of the same object.



Change to the pointer mode Select (press the ESC-key), hold down the Shift-key and click with the mouse on the desired first position to insert the first point. Move the mouse to the next point of the polygon, hold down

the Shift-key and click with the mouse to insert the next point, and so on.



Polygon Line on the First Plane

Copy and Paste Polygon

Copy or draw on each image plane a border line. If you want to copy the same polygon to the next image plane do the following:



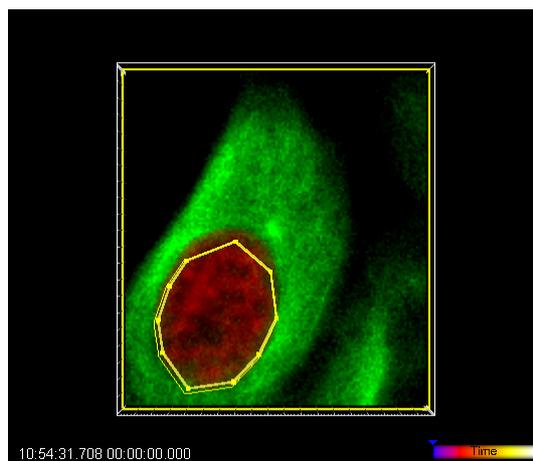
With a click on the button **Copy** you can copy the polygon line.



Change to next plane by moving the **Slider** to the next position.



With a click on the button **Paste** the same polygon line is placed to the second plane.



Polygon Line on the First and Second Plane

Change to the Next Plane



Change to next plane by moving the **Slider** to the next position. On the next plane you can either paste the same polygon line again, or you draw an individual polygon line, as described on top of this chapter. Go on with the next plane, and so on until on each plane there is a border line.

See also:

[Select and Navigate](#)

10.5 Edit Polygon

To edit the polygon lines return to the Slice on which the polygon 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.
Move 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. 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. on Mac: Hold down the Command-key and double-click on a line between two nodes.
Change Size	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:

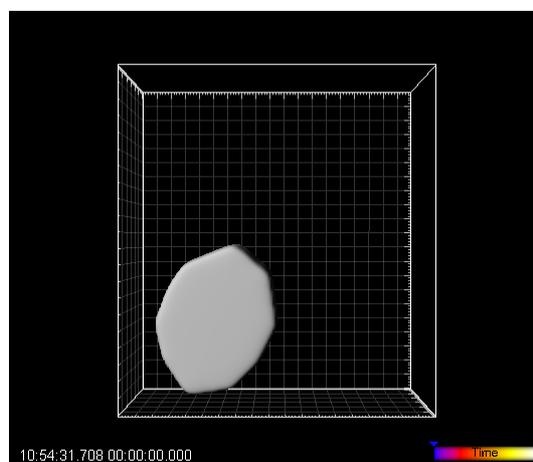
[Select and Navigate](#)

10.6 Calculate Contour Surface

In the next step you calculate the new Contour Surface.

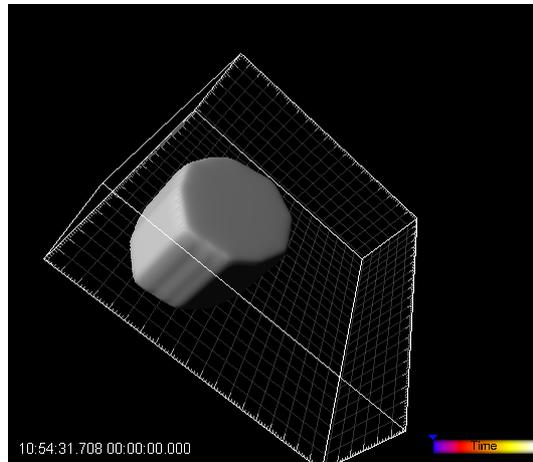


Click on the button **Calculate Contour Surface**.



This is the New Contour Surface

You can change to the pointer mode Navigate (press the ESC-key) and move the Contour Surface to any desired position.



Rotate Contour Surface in Viewing Area

See also:

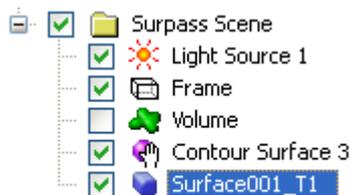
[Select and Navigate](#)

10.7 Create Surface Object

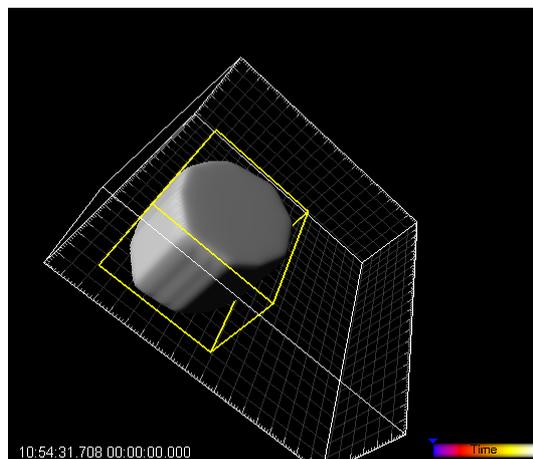
In the next step you create a Surface object.



Click on the button **Create Surface Object**.



Highlight the **Surface** in the SurpassTree.



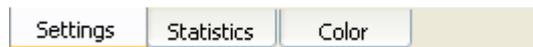
You can change to the pointer mode Navigate (press the ESC-key) and move the Contour Surface to any desired position.

See also:

[Select and Navigate](#)

10.8 Mask Channel

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 take the Surface object.



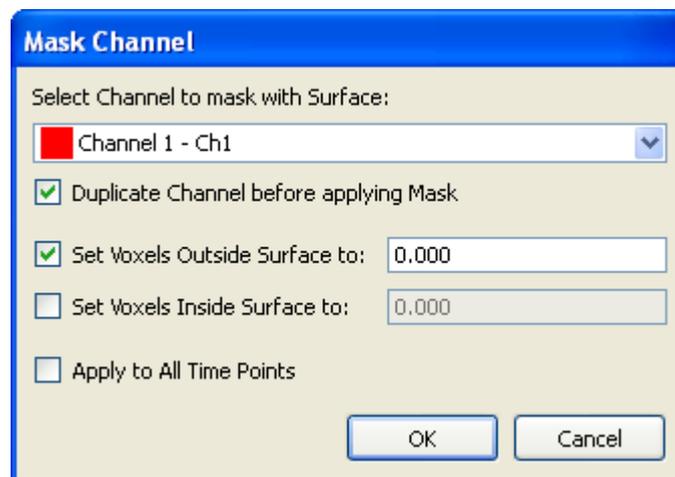
Be sure that in the Surpass Tree the Surface object is highlighted and click on the tab **Settings**.



Click on the button **Mask Channel with Surface...**

Window Mask Channel

The Surface object is your "cookie-cutter", the selected channel your "cake mixture". You duplicate the original red channel and then you use the "cookie-cutter" to mask this channel. In the following you will cut out the red area to see only the inside of the the Surface object. To get this result you will exclude all voxels outside the "cookie-cutter" (set the voxels outside the surface to zero).



- Select the channel (destination channel) you want to mask with the Surface object (ROI). In this case you select the red channel, **Channel 1 - Ch1**.
- Check the box **Duplicate Channel before applying Mask** and an additional masking channel displays automatically in the Display Adjustments.
- Check the box **Set voxels Outside Surface to:** and use the default value **0.000**. This means, that no voxels outside the region of interest are displayed.
- Leave the next box un-checked (**Set voxels Inside Surface to:**). The original channel intensities inside the region of interest are displayed.
- Click on the button **OK** to apply the mask.

See also:

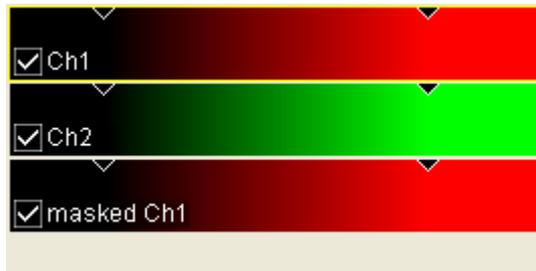
[Display Adjustment](#)

10.9 Display Masked Channel

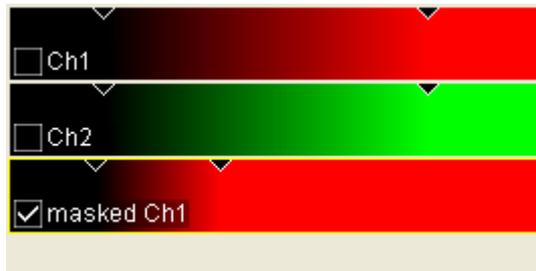
To display only the masked channel in the viewing area you have to adjust the channel display in the Display Adjustment Window. Additionally you have to set up the visibility of the Surpass Tree items in the viewing area.

Display Adjustment Window

To open the Display Adjustment window select the Menu **Edit - Show Display Adjustment**.



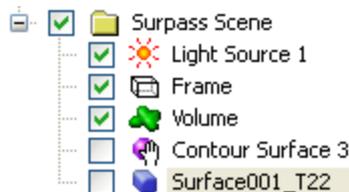
In the Display Adjustment Window you can find the additional channel **masked Ch1**.



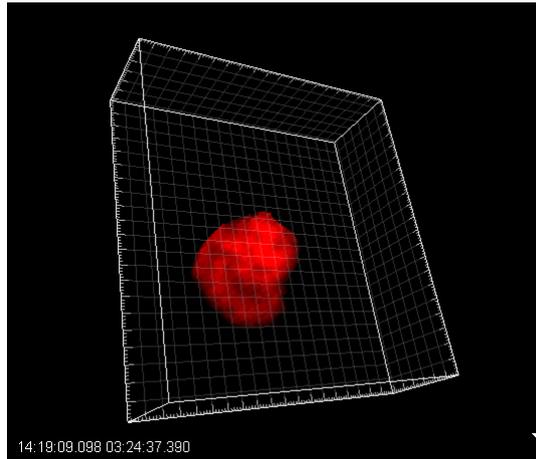
Un-check the original red and green channel. Adjust the upper threshold for the new channel **masked Ch1** (move the right arrow that represents the upper threshold to the left).

Adjust Surpass Tree

Each item in the Surpass Tree 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 is highlighted in the Surpass Tree.



In the Surpass Tree check the item **Volume** and un-check the **Contour Surface** and the **Surface**. This is the display of the new, modified red channel in the viewing area.



As the result of the masking everything outside the surface is cut away and only the inside voxels are visible. The initial object for the masking is the Contour object. Based on this Contour object you create a Surface object. Then you use this Surface object as the region of interest ("cookie-cutter") for the masking.

Please close Imaris before you start with the next Tutorial.

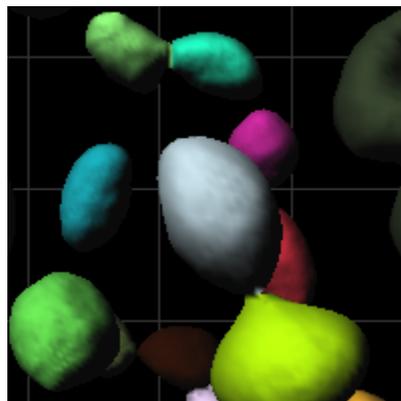
See also:

[Surpass Tree](#)

[Display Adjustment](#)

11 Region Growing

In this Tutorial you get familiar with the region growing to segment touching objects. First you [Visualize](#) a plant cell with chloroplasts (green) and cell wall components (red). Then you detect the chloroplasts with an automatic spot detection. In the next step you check and edit the spots. Now you can use these spots as [Seed Points](#) for the region growing. When you start the region growing a [Working Channel](#) is displayed in the viewing area and in the display adjustments. The seed point regions will grow until they reach the defined border. The result of the region growing in this example are Sport [Surfaces](#).



Spot Surfaces, Region Growing, Plant Cell

User Level: Beginner
Module: Imaris

11.1 Visualization



PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

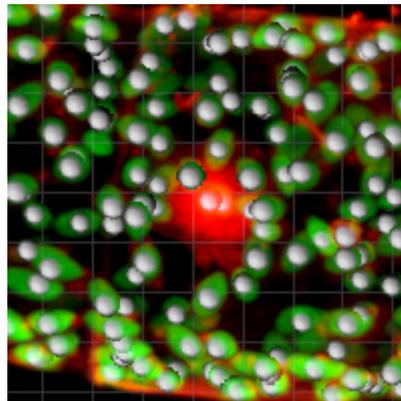
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image PlantCell.ims in the Surpass view.

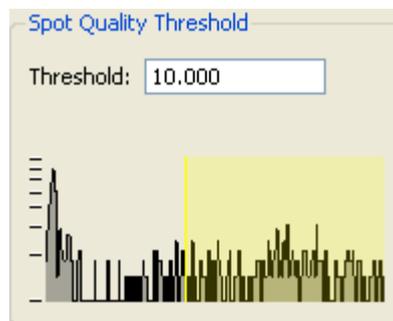
PC file path \ProgramFiles\Bitplane\Imaris\images\PlantCell.ims

Mac file path /Applications/Imaris.app/Contents/SharedSupport/images/PlantCell.ims

Visualize and Segment Chloroplasts



As **Initial Scene** select **Volume**. In the **Objects** toolbar of the Surpass view click on the icon  to add new Spots. Select as **Source Channel** the **Channel 2** (green) and set the **Minimum Diameter** to 4.000 and click on **Next >**.

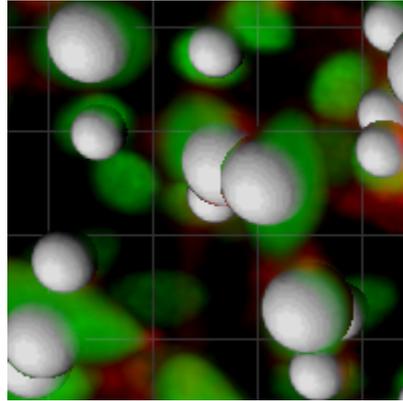


Change the **Threshold** to **10.000** and click on **Finish**.

11.2 Edit Seed Points

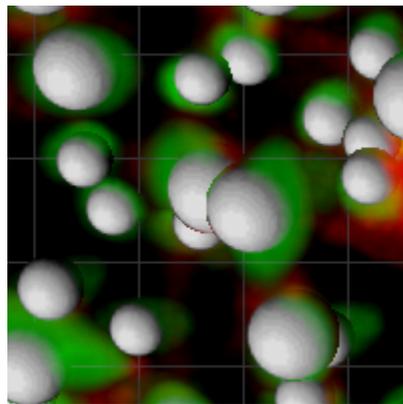
Now you can check and edit the Spots.

Edit Seed Points



To add a missing Spot click on the Tab **Edit**. Switch to the mouse pointer mode Select.

Tip: You can easily switch between the two pointer modes using the ESC-Key. The effect is directly visible on screen by the altered mouse pointer display.



To add a Spot Shift + left-click in the image. To delete a Spot Shift + left-click on the Spot.

See also:

[Select and Navigate](#) (mouse pointer mode)

11.3 Start Growing

The region growing method for segmentation starts with defined seed points. You use Spots as seed points for the region growing that is performed in the next steps. During the region growing process around each seed point the region grows until a border stops the growing.



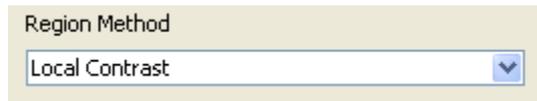
Select the Tab **Settings**.



Click on the button **Region Growing**.

Select Region Method

You can use the Local Contrast (Threshold) as border criteria for the growing of the seed points.



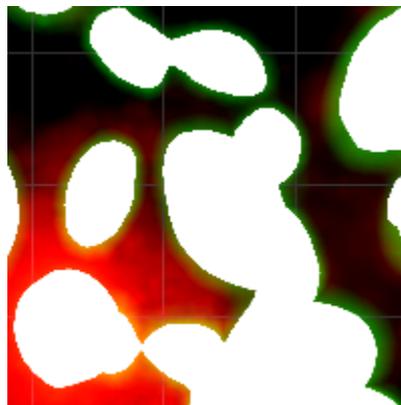
Select as **Region Method** the **Local Contrast** and click on **Next >**.

11.4 Working Channel

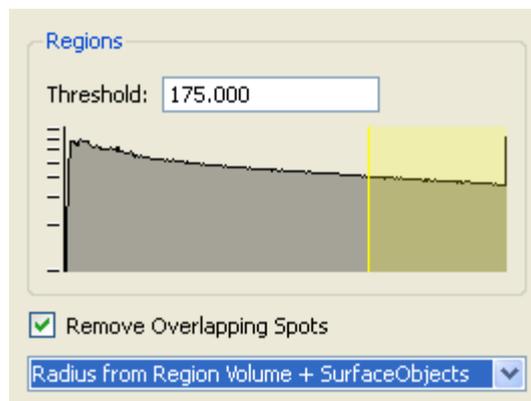
The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black.

Adjust the Local Contrast Threshold

In the working channel the regions with a local contrast brighter than the background are displayed in white.



Now you adjust in the **Spots Properties** the **Threshold** value of the local contrast channel.

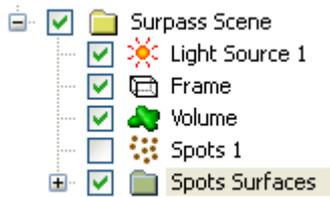


Set the **Threshold** to **175.000** and select **Radius from Region Volume + SurfaceObjects** from the drop-down list. Click on **Finish**.

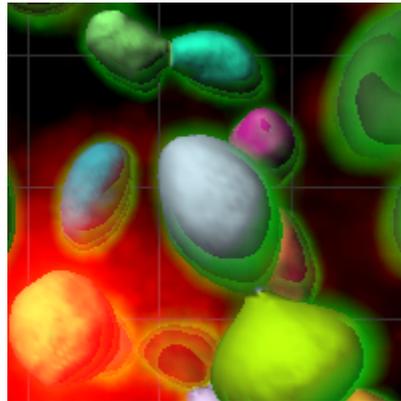
Please note: To display the Working Channel in the viewing area you need a Volume Item in the Surpass Tree and the Item has to be checked on.

11.5 Display Surfaces

The regions will grow until they fill the white regions and reach the border.

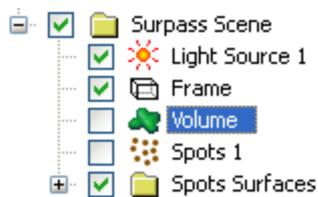


On the left hand side in the Surpass Tree list you find the new item **Spots Surfaces**. The **Spots** are switched off automatically.

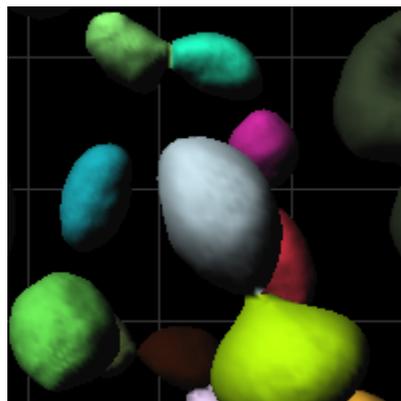


The **Volume** and the colored **Spots Surfaces** are displayed.

Switch the Volume Item Off



Now switch off the **Volume** item in the Surpass Tree.



As result of the region growing the colored Spots Surfaces are displayed in the viewing area.