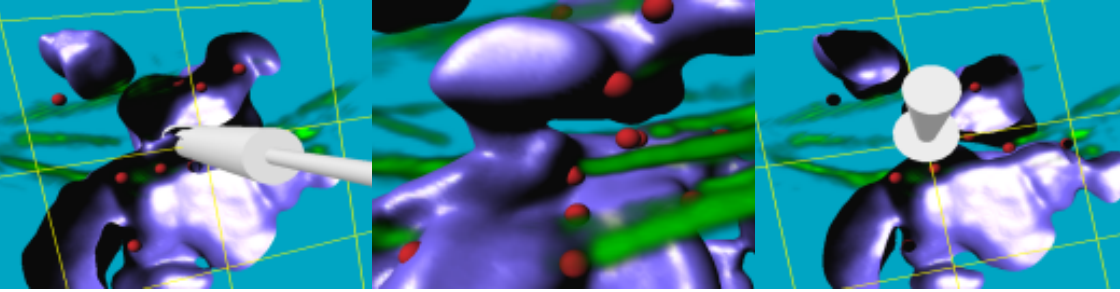

Quick Start Tutorials

Imaris 6.4

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BITPLANE
Scientific Software

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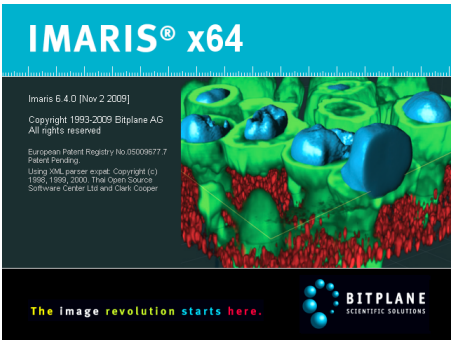
1 Introduction

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

Quick Start Tutorials is the fastest way to understand and master Imaris. It offers as a series of examples and supporting commentaries designed to quickly acquaint new user with the structure, possibilities and the most compelling features of the Imaris, furthermore it may show yet unrecognized new features of the software to the advanced user.
In the Quick Start Tutorials, you will follow a series of straightforward, step-by-step instructions. The tutorial is organized into 11 lessons. Each lesson introduces you to several of the unique features of Imaris.The tutorials are designed to be followed sequentially, but if you are already familiar with Imaris the basic lessons may be skipped. The tutorials are cross-referenced by hyperlinks highlighted in blue underlined font. The Table of Contents and the Index provide further support for navigation in the tutorials. At any time during the tutorial, you can access Reference Manual for additional information and an in-depth explanation of specific features of Imaris.
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.
When you finish the tutorial, you'll have a greater understanding of how to implement Imaris in your research and be ready benefiting from of user friendly, fast, and powerful Imaris in processing microscopic images.



Imaris Start Screen

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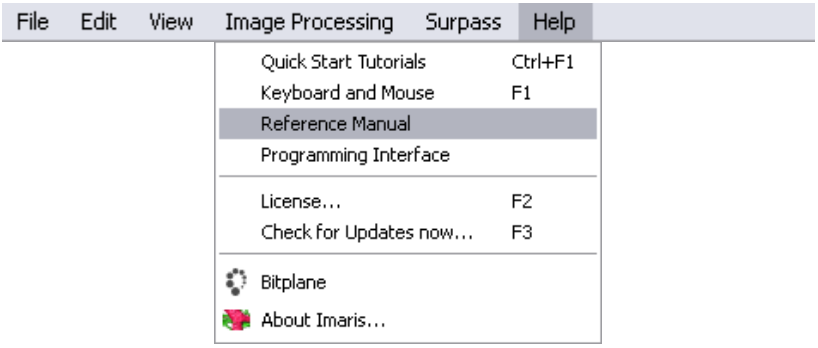
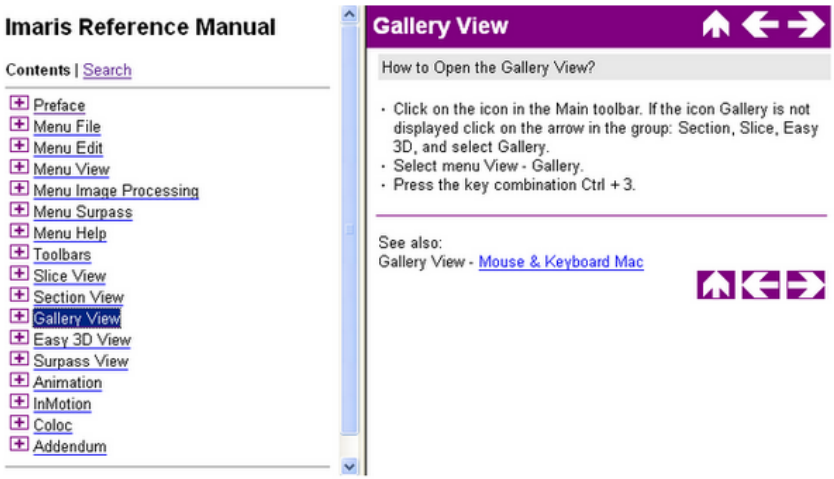
Quick Start Tutorials V 6.4

1.1 Reference Manual

The 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**.



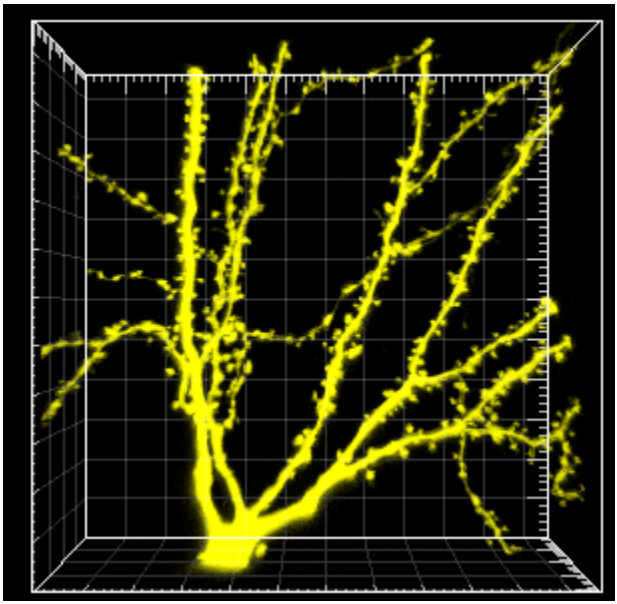
The Imaris Reference Manual displays.



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

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 7 to create a 3D volume visualization. In the chapter Select and Navigate 14 you will learn about the two different mouse pointer modes in the Surpass view. You will learn how to manipulate the view by Rotating 15, Translating 16 or Scaling 17 the image. Finally, you will save the file for later use. At the end of this tutorial you will also learn how to change the Imaris Background Color 108.



Visualized Data Set, Pyramidal Cell

User Level: Beginner
Module: Imaris

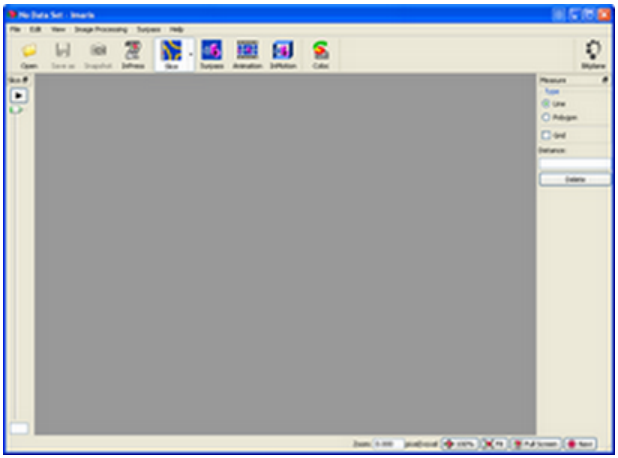
2.1 Imaris Main Screen

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.

After installation, upon the first program start Imaris is in the Surpass view.

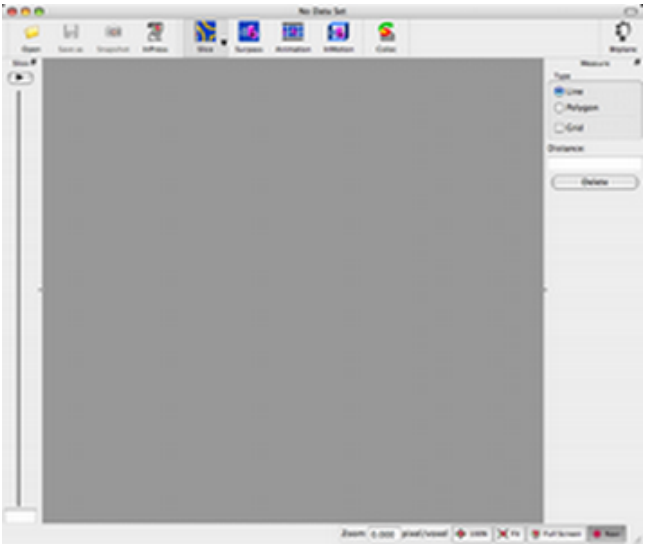
Switch to the Slice view by clicking on the **Slice** button.

PC Main Screen



This is the Imaris Slice view on a PC.

Mac Main Screen



This is the Imaris Slice view on a Mac.

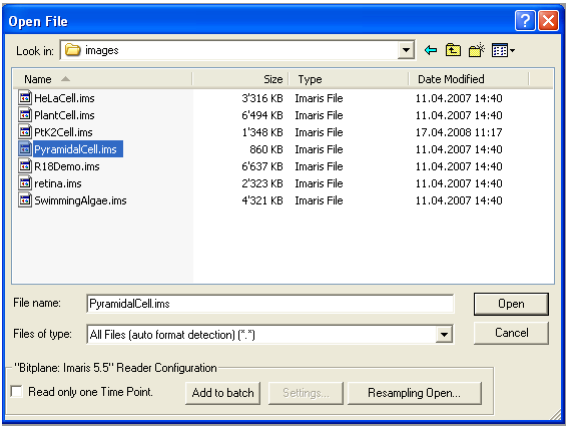
See also: Slice view 9 and Surpass Tree 11

2.2 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.

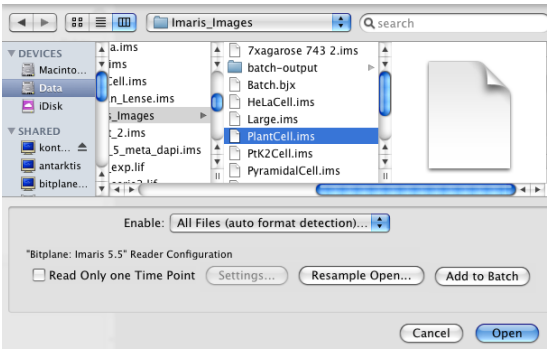
Open a Data Set in the Slice View

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 displays on the screen.



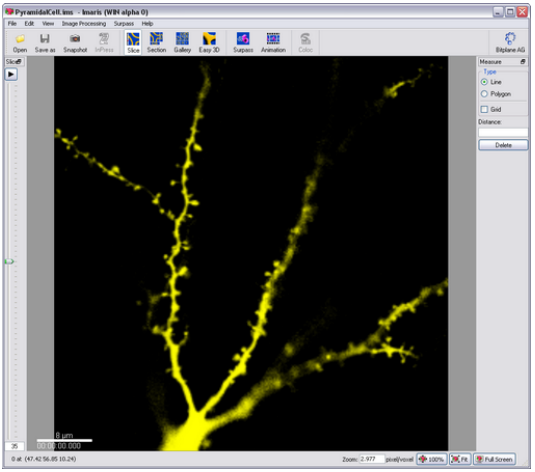
Select the data set Pyramidal cell and click on **Open**.
\ProgramFiles\Bitplane\Imaris\images\PyramidalCell.ims

This is the Mac **File Open** dialog:



Select the data set Pyramidal cell and click on **Open**.
/Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims

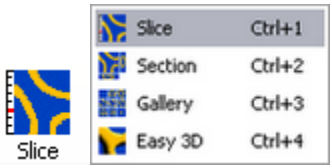
The Slice View



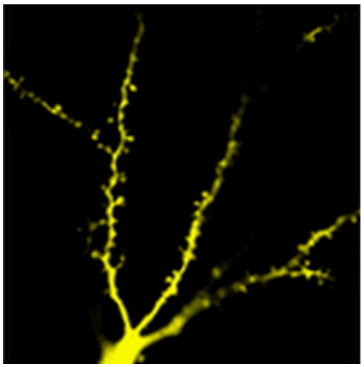
The Slice view is a 2D display mode, in the viewing area the middle slice (number 35) of the Pyramidal cell displays. You scroll the slices along the z-axis by dragging the slider handle in the left control bar up and down.

2.3 Slice View

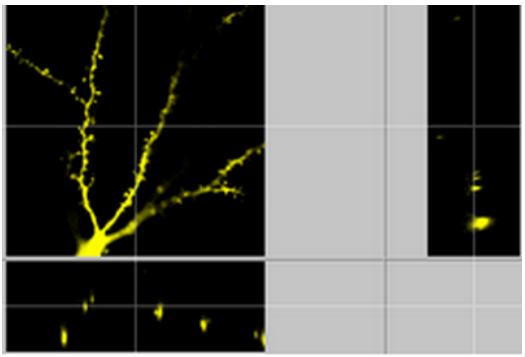
In **Slice** the following standard views: **Slice, Selection, Gallery** and **Easy 3D** are provided**.**



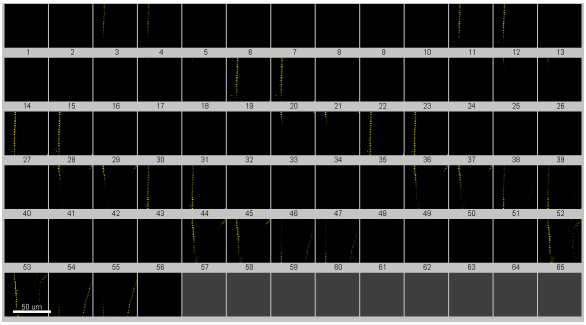
Slice view for individual slices:



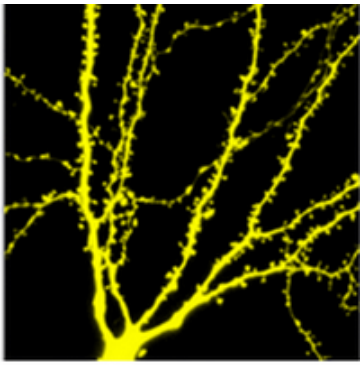
Selection view for XY; XZ; YZ views and navigation to any position in 3D:



Gallery view for viewing individual slices simultaneously:

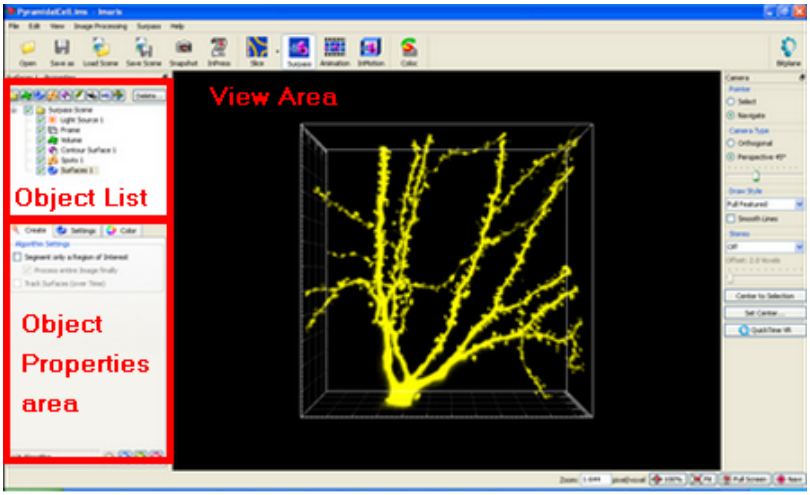


Easy 3D for 3D projection along Z



2.4 Surpass View

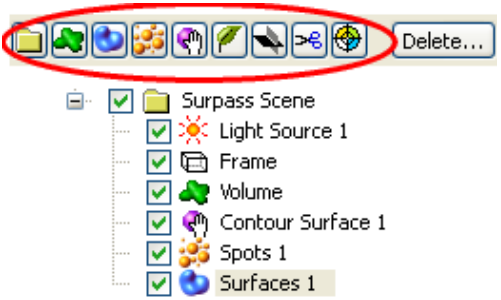
The Imaris is built around the three-panel workspace: **View Area**, **Object List** and **Object Properties Area**.



View Area is a display area for an image presentation and facilitate image navigation, selection and interaction.

Object List

Simultaneous combination of various visualization techniques is achieved by **Object List** making understanding of the complex data easier.



The default name of the folder is **SurpassScene**. The Scene includes the standard Light Source 1, Frame and the additional Objects. The Object List displays a list of all Surpass objects you added to the viewing area. To build a surpass scene click on the Creating buttons and follow creation process. The list is automatically updated when you add or delete an object. Each object has a check box. Check the box to make the object visible in the viewing area or un-check it to make the object invisible. The currently active object is highlighted in the Object List and the appropriate properties are displayed in the **Object Properties Area**.

Object Properties Area

In the **Object Properties Area** you can find all parameters for the selected Object.

See also: Design Mixed Model Rendering -Volume Rendering 41, Add Surface 45 and Analyze Neuron 78

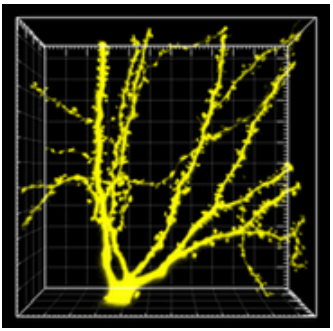
2.5 Volume

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

Select Volume in the Surpass View

Click on **Surpass** in the main toolbar of Imaris.

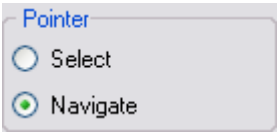
The Pyramidal cell displays in the viewing area.



See also: Design Mixed Model Rendering -Surpass View 11, Add Volume 41 and Analyze Neuron 78

2.6 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?

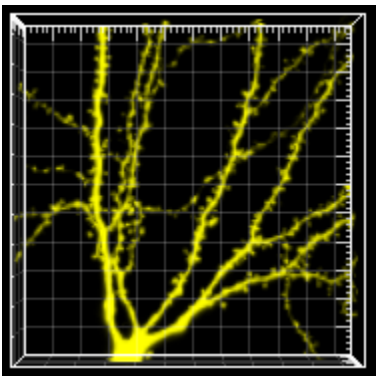
You use the pointer mode **Navigate** to move or rotate the image in the viewing area.

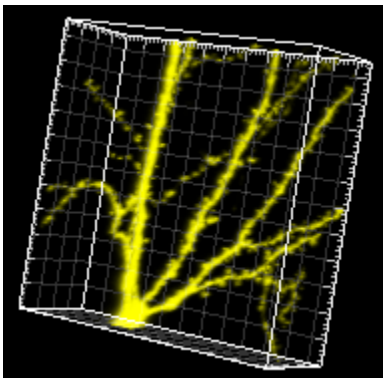
See also: Visualize Data Set - Rotate Image 15

2.7 Rotate Image

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

How to Rotate an Image?





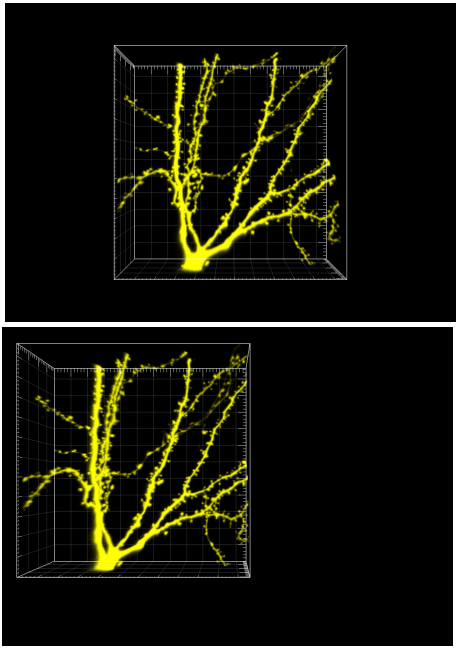
1. Choose the mouse pointer mode **Navigate**.
2. Click with the mouse in the image and hold the button down while moving the mouse (hold + drag). The image on screen is rotated towards the direction the mouse is dragged. Be sure to hold the mouse button down during the whole rotation.
3. Stop moving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

How to Keep the Image Continuously Rotated?

1. Choose the mouse pointer mode **Navigate**.
2. Click with the mouse button in the image and hold the button down while you move the mouse (hold + drag). The image on screen is rotated towards the direction the mouse is dragged.
3. Release the 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: Visualize Data Set - Select and Navigate 14

2.8 Translate Image

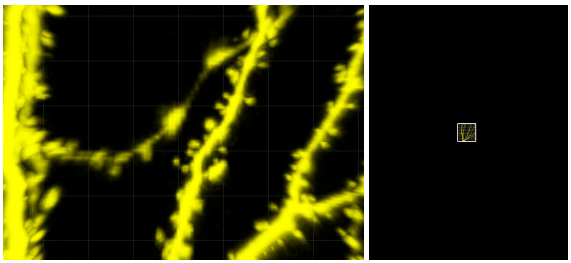


1. Choose the mouse pointer mode **Navigate**.
2. To move the image within the Surpass view (pan the object) hold the right mouse button and drag the image to the chosen location (for Mac users: ctrl + click & drag to pan).
3. Release the right mouse button to place the image.

See also: Visualize Data Set - Select and Navigate 14

2.9 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 in on the image hold the middle mouse button and drag it towards you (if you use a Mac with a one button mouse use: shift + ctrl + click & drag to zoom).
3. To zoom out from the image hold the middle mouse button and drag it away from you.

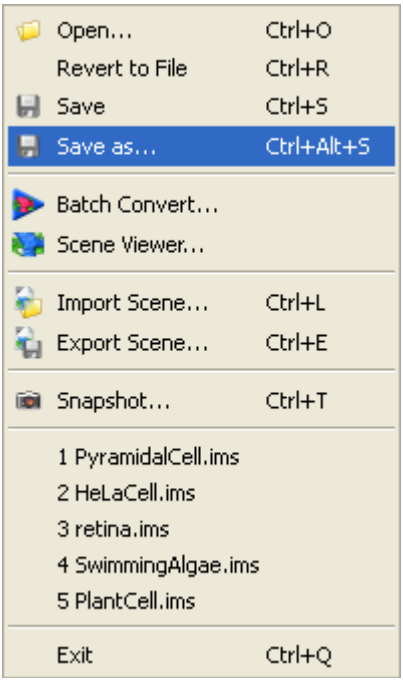
Using the Buttons at the Bottom of the Screen

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.
Navi	Toggle display of navigation window (upper right corner of the viewing area).

See also: Visualize Data Set - Select and Navigate 14

2.10 Save as...

The standard way to save your data and Imaris scene is simply to choose **Save as...** from the File menu and give an appropriate name. The filename extension is *.ims.The single created Imaris file contains both the image and the scene data.
You can save the file (the single file that contains the image and the scene) when you are done, or each phase of your work separately, so you can come back and look at it later. To save the file choose **Save as...** from the File menu and give the file a name you will remember. In this tutorial enter the name **PyramidalCellA.**



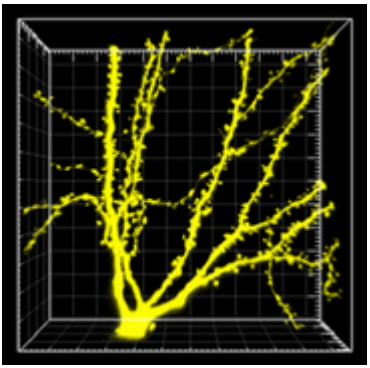
See also: Design Mixed Model Rendering - Surpass View 11,
Generating Movies - Load Scene File 26

2.11 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

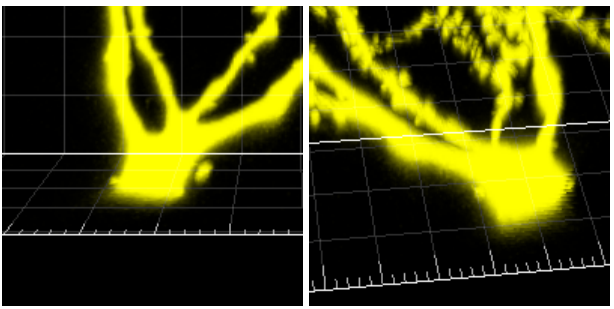
Use the buttons at the bottom of the viewing area, click first on **100%**, then on **Fit**.



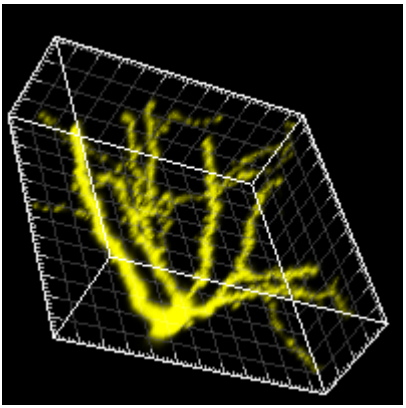
The Pyramidal cell displays centered in the viewing area.

Tip: Whenever you lose orientation, re-center the view to the Home Position by clicking the 100% and Fit buttons again.

Rotate Backwards

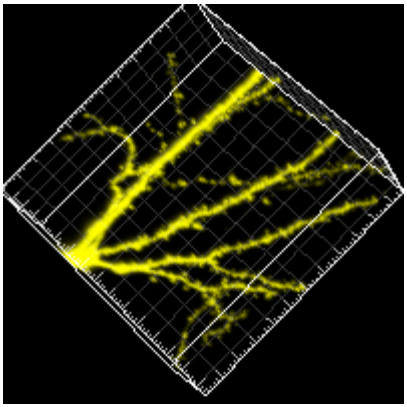


Now rotate the image backwards. Choose the mouse pointer mode **Navigate**. Click on the base of the Pyramidal cell, hold the button down while you move the mouse upwards, stop the mouse movement and release the mouse button.

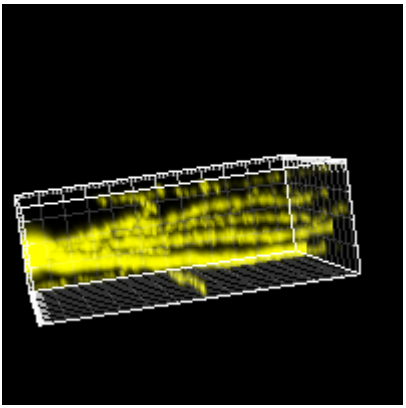


This is the result of the rotation.

Move the Image Clockwise



In the next step rotate the image clockwise. Click on the position shown above, hold the 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.

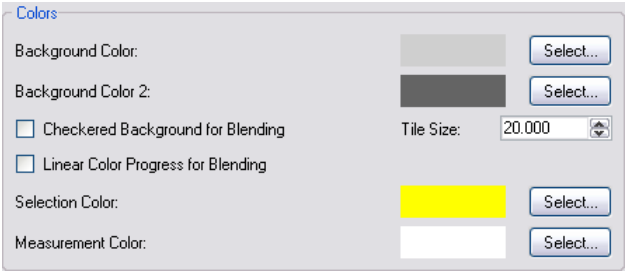
See also: Visualize Data Set - Select and Navigate 14, Visualize Data Set - Rotate Image 15, Visualize Data Set - Scale Image 17

2.12 Change Background Color

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

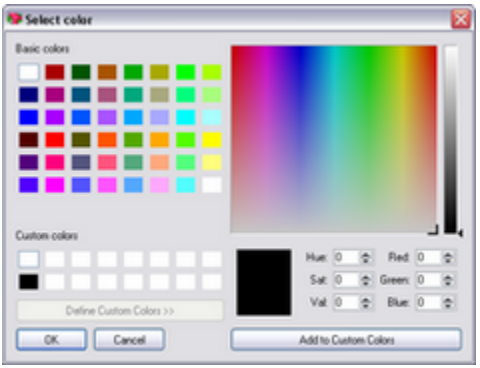
Open the Preferences - Display Window

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

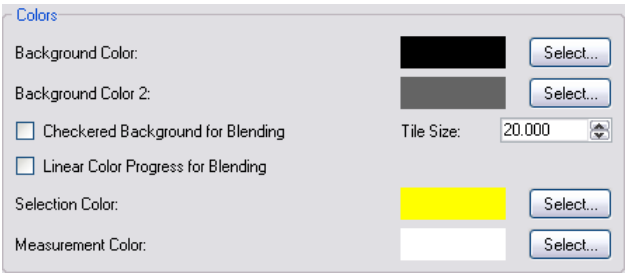


Change Background Color

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



Select the desired color and click on **OK**. The preview changes accordingly.

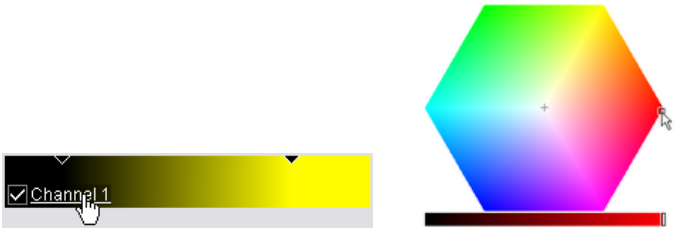


In the window **Preferences - Display** click on **Ok**.
The result is in the viewing area. Please close Imaris before you start with the next tutorial.

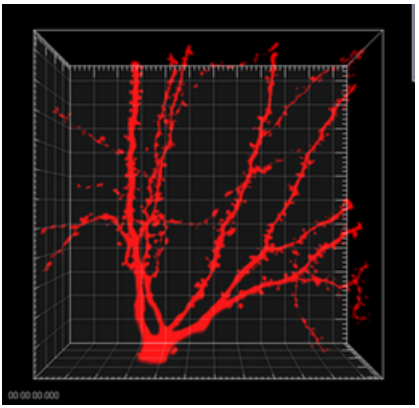
See also: Design Mixed Model Rendering - Change Spots Color 52

2.13 Change Channel Color

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



Click on **Channel 1** to open the color selection. In the color table select and adjust the color and click on **OK**. This is the result in the viewing area:



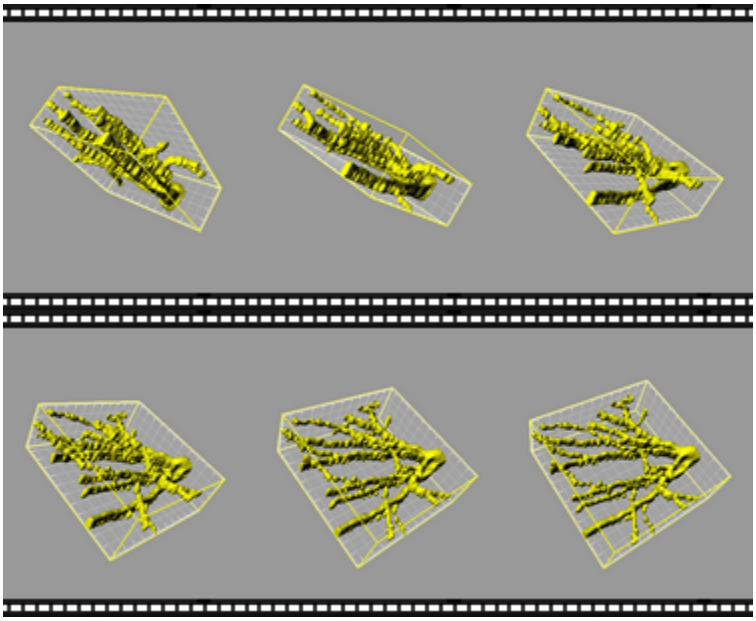
MIP Display, Channel Color Red, Pyramidal Cell

Please close Imaris before you start with the next tutorial.

See also: Design Mixed Model Rendering - Display Adjustment 42, Change Spots Color 52, Visualize Data Set - Change Background Color 22

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, you Load the File 26 of the processed data set generated in the previous tutorial Visualize a Data Set 4. This tutorial requires the understanding of the Key Frame Animation 27 function in Imaris. You will practice to subsequently Shoot 29, Play 29, and Save a Movie 32.



Movie Sequences, Pyramidal Cell

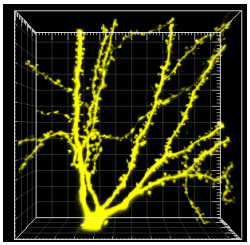
User Level: Beginner
Module: Imaris

3.1 Load 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 open the file PyramidalCellA. Click on the menu **File** and select **Open.**

The Pyramidal cell displays in the viewing area and in the properties area you find the corresponding Object List.



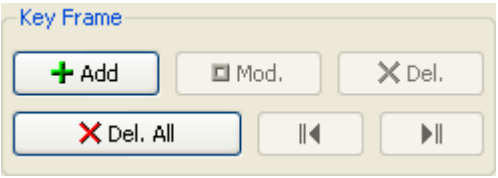
See also: Visualize Data Set - Save as 18..., Visualize Data Set - Surpass View 11

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.

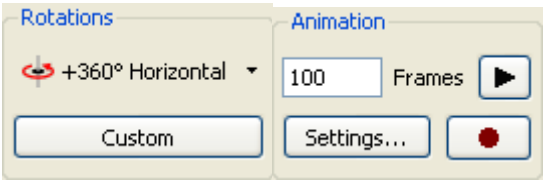
Click on the icon **Animation** in the toolbar. You find the **Key Frame Animation** window at the bottom of the screen. It is divided in five parts, the **Key Frame** window, the **Rotations** window, the **Animation** window, the **Play Back State** window, and the **Strip** window.

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 **Delete All** button to delete all key frames. With the **Arrow** buttons you step to the **Previous** (arrow to the left) or **Next** (arrow to the right) user-defined key frame.

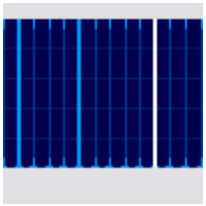
The Animation Window



In the **Animation** window you can enter the number of movie **Frames**.

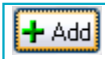
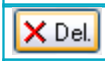
There is the button to open the Animation **Settings...** as well as the buttons to **Play** (arrow to the right) and **Record** (red dot) the movie. To pause the animation re-click on **Play. Rotation** panel allows scroll through the pre-set rotations and **Custom** button allows to insert you own degree of rotation in any direction.

The Film Strip Panel



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. A click on the blue line will display the image view corresponding to the view in Surpass view area.

Buttons Overview

	**Add**-addition of new key frame (the image presented on the screen will appear as a key frame)
	Modify-modification and adjustment of highlighted frames in the film strip
	**Delete** active key frame
	Delete All key frames
	Play/**Pause** to preview or to pause of animation
	Record animation to movie file
	Go to **Previous** key frame
	Go to **Next** key frame

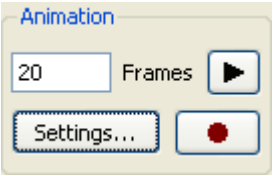
3.3 Shoot and Play

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.

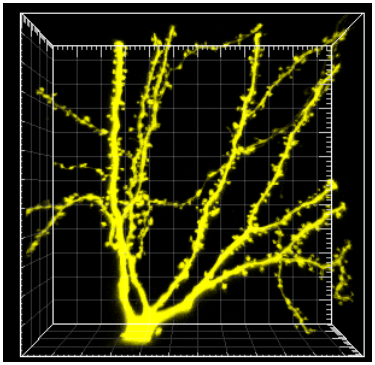
Please remember: Each time you click on the button **+Add** in the **Key Frame** window the camera captures the next key frame of your movie.

Movie Length



In the field **Frames** type in **20**. The strip will indicate these frames by 20 thin blue lines which will define the duration of animation.

Adjust and Capture the First Key Frame

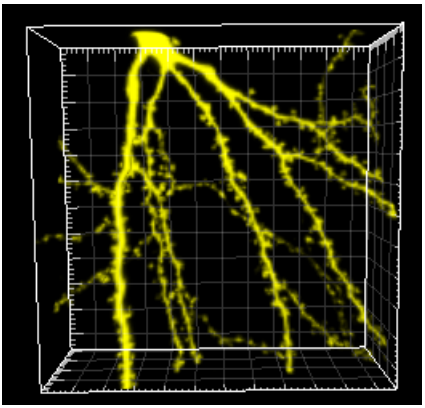


Turn the image view to the first position (click on the bottom of the

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.

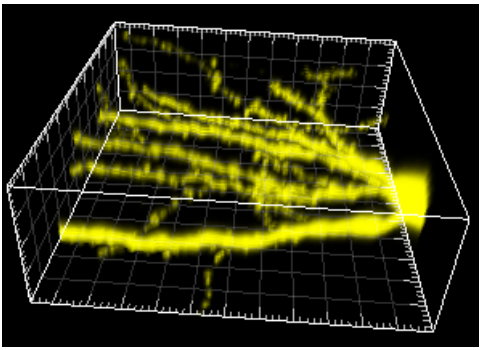
Add Additional Key Frames

Move the image to the next viewing position. Click on the base of the Pyramidal cell, hold the 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, hold the 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. The view should be similar to:



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 in the **Strip** window, hold the mouse button down and drag the mouse to the right or left, respectively. The corresponding image views display 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

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.** (Modify) to save the new image position.

See also: Generating Movies - Key Frame Animation 27, Visualize Data Set - Rotate Image 15, Visualize Data Set - Scale Image 17, Visualize Data Set - Practice Makes Perfect 19

3.4 Save Movie

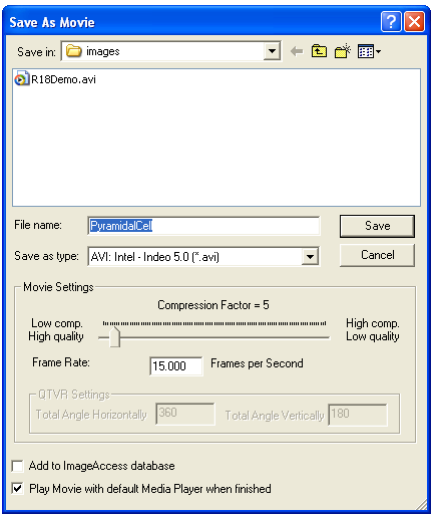
There are two possibilities to save the movie. Either as an Imaris File or as a movie-file (*.avi).

In Imaris as Scene File

Whenever you save the file in Imaris, the actual animation is saved automatically as part of it. Open the menu **File** and select **Save as...** 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** ( ). The **Save As Movie** dialog window displays on screen.

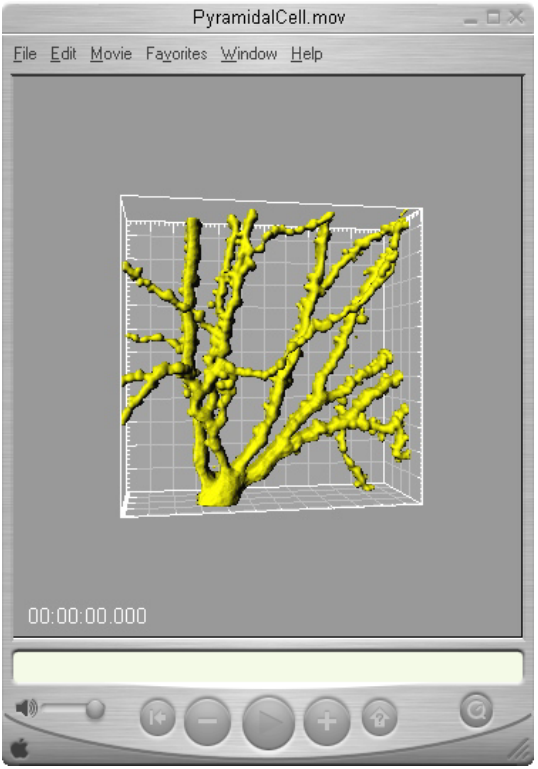


As **File name** type in the corresponding File name, use the default **Movie Settings** and click on **Save**. The file extension for the AVI movie is *.avi.
Please close Imaris before you start with the next tutorial.

See also: Visualize Data Set - Save as... 18, Generating Movies - Key Frame Animation 27, Imaris and QuickTimeVR 33

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 34 - the file generation mechanism and the default movie settings. Then you will Generate a QuickTimeVR File 35 in Imaris to Visualize the Results 37 interactively in QuickTimeVR. Optimized Settings 38 for a presentation and a web page are described in detail in the last chapter of this tutorial.



QuickTimeVR Display, Pyramidal Cell

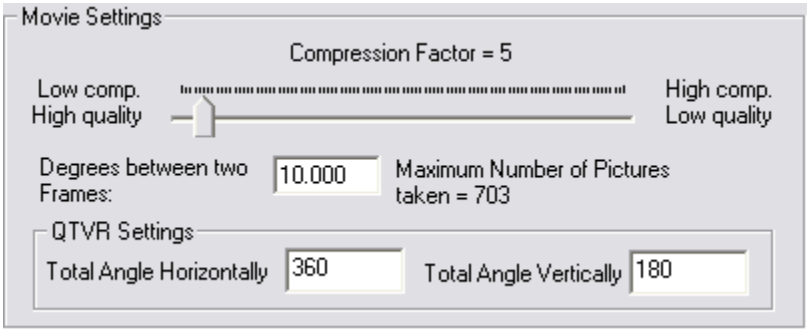
User Level: Beginner
Module: Imaris

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 allowing user to show complex scenes outside Imaris packing. 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: Imairs and Quick Time VR - Optimized Settings 38

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.

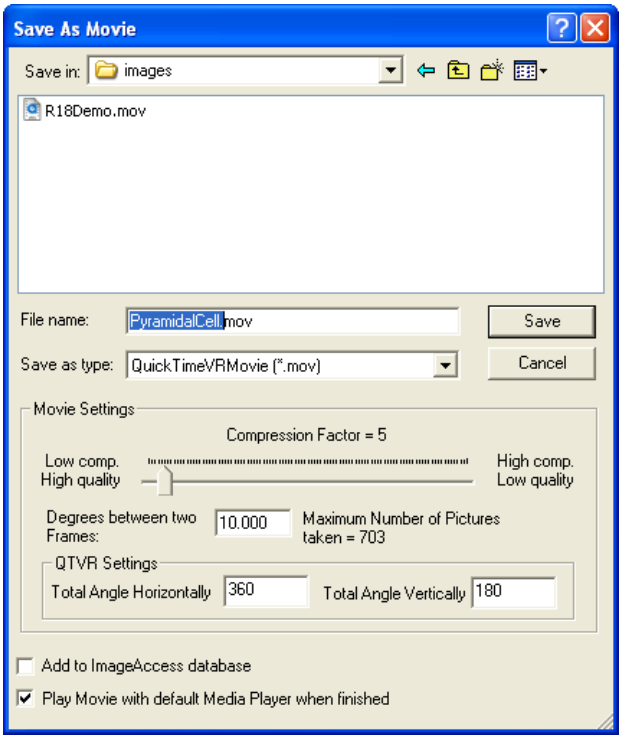
Load your data set (e.g. PyramidalCellSceneA).

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**.

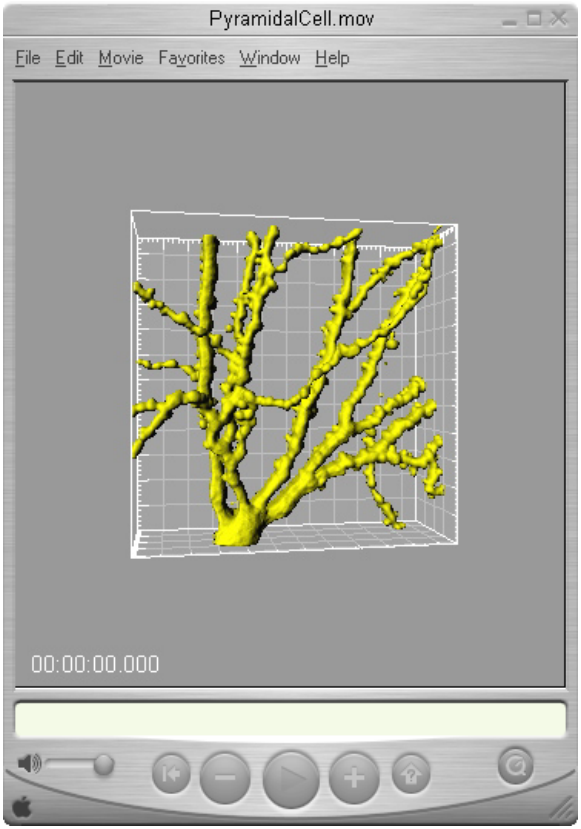
What Happens in the Imaris Viewing Area?

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: Visualize Data Set - Open Data Set 7, Generating Movies - Load File 18

4.3 Interactive Display

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



The image displays 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

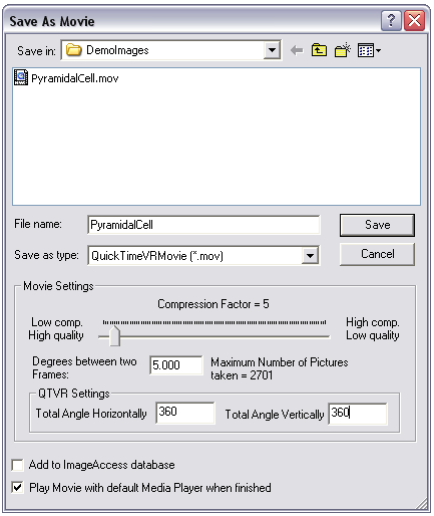
If you want to save a movie for a presentation on your computer the file size is according to experience is not the limitation. To put the movie on your web page the file should not be too large.

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.

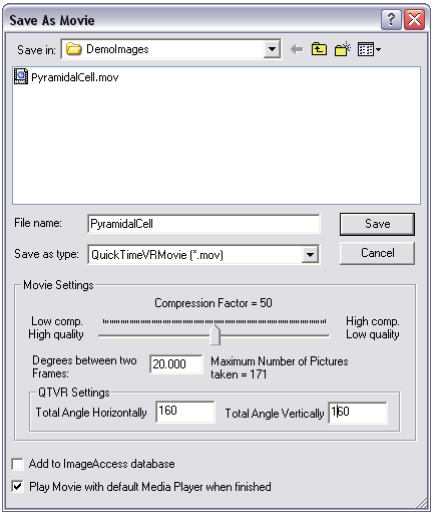


- Use the **Compression Factor 5**.
- Set the **Degrees between two Frames** to **5**.

- 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.

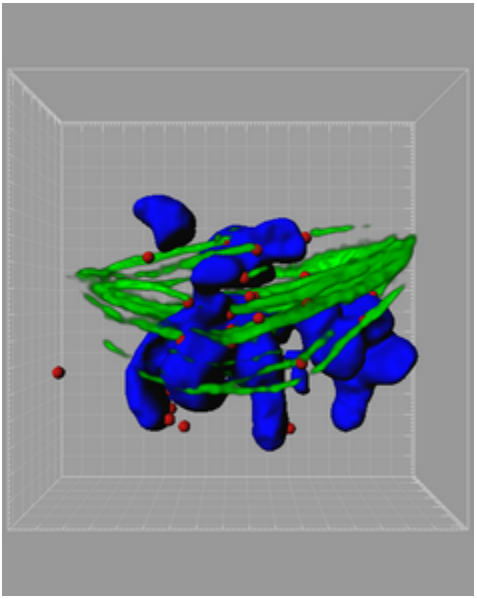


- Use the **Compression Factor 50**.
- Set the **Degrees between two Frames** to **20**.
- Set the **Total Angle Horizontally** to **160** and the **Total Angle Vertically** to **160**.
- Check **Play Movie with default Media Player when finished**.
- Click on **Save**.

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 41 rendering for all channels and you will adjust the contrast, brightness and transparency in the Display Adjustment 42 window. The microtubules remain Volume rendered but for the chromosomes you will choose the Surfaces 45 mode in Imaris. For the kinetochores you select the Spots 50 display and in the next step you Change the Spots Color 52. The Object List 11 displays a list of all objects in the viewing area and is the ideal instrument to handle the Final Image 53.



Mixed Model Rendering, PtK2 Cell

User Level: Beginner
Module: Imaris

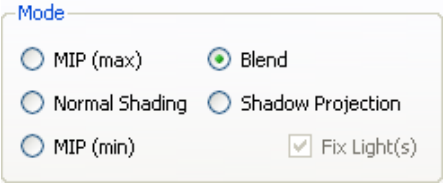
5.1 Add Volume

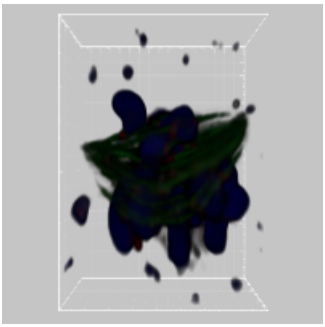
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image PtK2 cell.
PC:\ProgramFiles\Bitplane\Imaris\images\PtK2Cell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PtK2Cell.ims

Volume

The demo image PtK2 cell is open in the Surpass mode. In the **Object Properties Area** you find the **Mode** selection window. The image is presented is in MIP (Maximum Intensity Projection) Mode. Change display mode to **Blend**. In the Blend mode all values along the viewing direction including their transparency are used for the calculation.





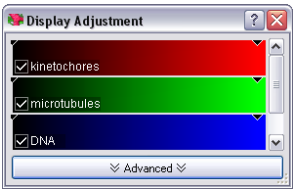
This is the PtK2 cell display in the viewing area.

5.2 Display Adjusment

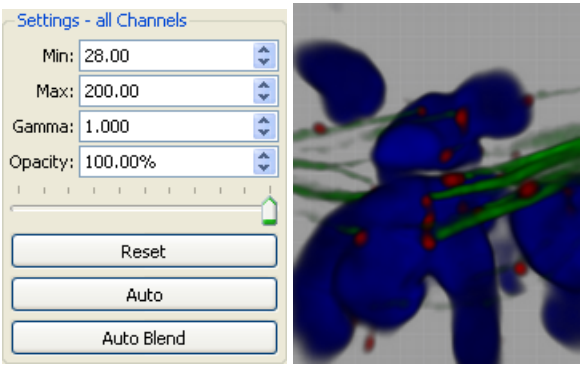
The objects rendered as Volume are usually quite dark and have a lot of noise. To optimize **Volume** rendering the image contrast, brightness and blend opacity will be adjusted. These settings are in the **Display Adjustment** window. The Display Adjustment function lets you interact with each channel individually, control Channel Visibility and Color, Intensity Range Min/Max, Blend Opacity and display Channel Intensity Histogram.

Adjust All Channels Simultaneously

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



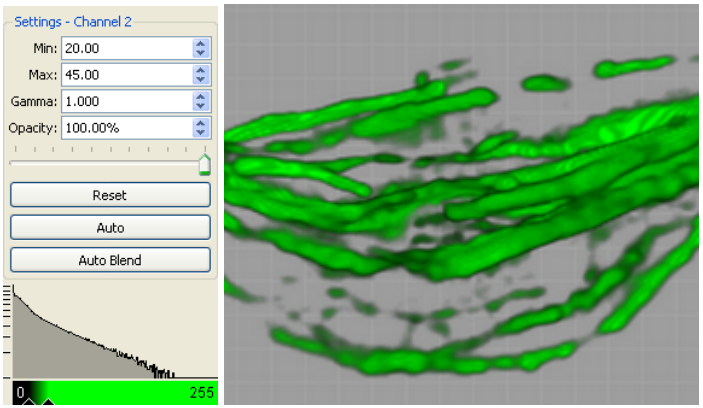
In the upper part of the window each channel is represented by a channel bar. In this case the red channel corresponds to the kinetochores, the green channel represents the microtubules and the blue channel is the DAPI stained DNA. Click on Advanced to open the advanced settings.Usually the color contrast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



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.

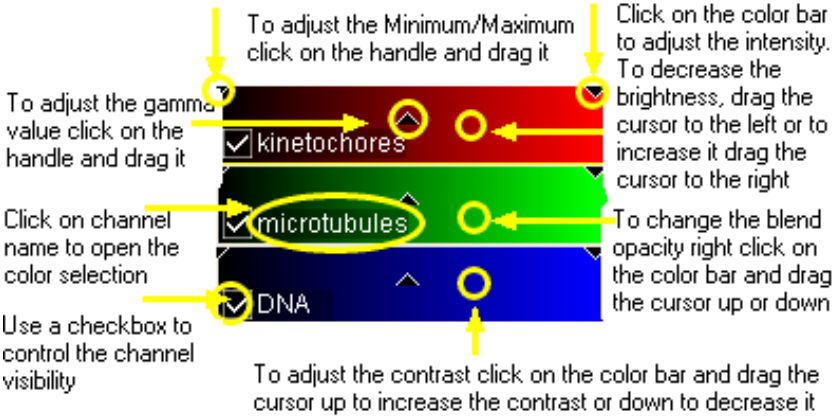
Adjust Individual Channel

To tune the green channel (microtubules) individually.To focus on the green channelun-check the red and blue channels. Selecting or de-selecting channels controls their visibility. Select the green channel by clicking on the channel bar. In the viewing area only the green channel is now displayed.



Set the **Range - microtubules Min: 20.000** and **Max: 45.000**.
To tune the red channel individually.To focus on the red channel (kinetochores) check the check box for the red channel and un-check the boxes for the green and blue channel to control channel visibility.In the viewing area only the red channel histogram is displayed. Change for the selected red channel the value **Min** to **20.000** and the value **Max** to **100.000**. The transparency of a channel is altered by adjusting the **Opacity** to **64%**.

The other option for Display Adjustment modification are available by clicking directly on the color channels. The effect of the change is immediately visible in the viewing area.

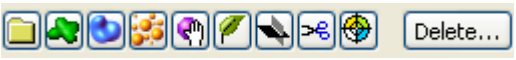


Clicking the **Reset** button resets the image to the default values
Clicking on **Auto** adjusts the minimum and max values in the data.

See also: Visualize Data Set - Open Data Set 7

5.3 Add Surfaces

You will now add to the Volume rendered microtubules a Surfaces reconstruction of the DNA.

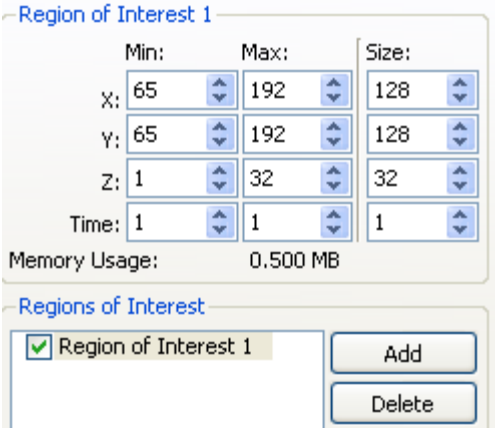


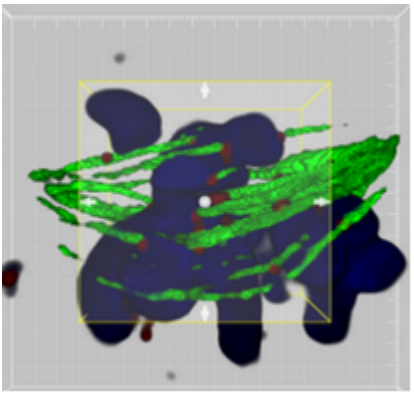
In the **Objects** toolbar of the Surpass view click on the icon to add a new Surfaces item.

Creation Wizard - 1/6 Algorithm

Select segment a **Region of Interest (ROI)** and **Process entire Image finally** and click on (Next).

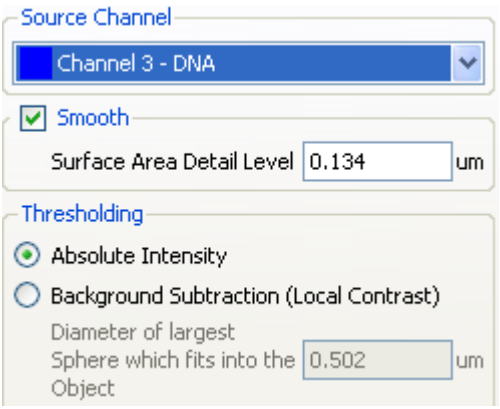
Creation Wizard - 2/6 Region of Interest (ROI)





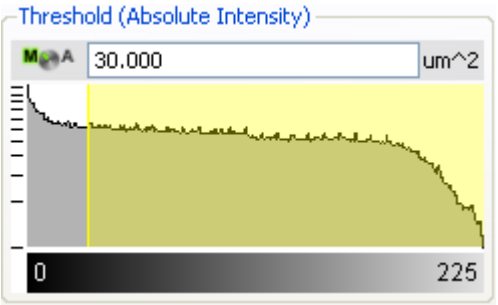
In a viewing area a rectangle-bordered section overlaid on image is representing the Region of Interest (ROI). Entering the values in the corresponding x-, y-, and z- fields (min/max) modifies the size and the position of the ROI. The size and position of ROI can be also modified directly by click on the arrows in the preview rectangle (cursor to Select mode). Click on (Next).

Creation Wizard - 3/6 Source Channel



Select as a Source Channel the Channel 3 (DNA) and Absolute Intensity as a option for Threshold. Click on (Next).

Creation Wizard - 4/6 Threshold



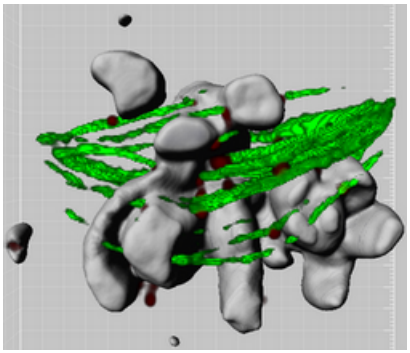
For the Threshold adjustment select the **Manual** option and set a value to **30**. Click on (Next).

Creation Wizard - 5/6 Classify Surfaces

On the tab **Classify Surfaces** you can filter the resulting Surface by various criteria. In this example, a filtering is not necessary, so delete all **Filters** and click on Next.

Creation Wizard - 6/6 Complete ROI

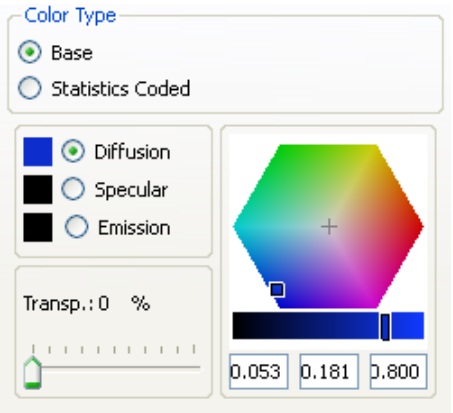
To complete Surface creation click on (Finish). Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters. This is the result in the viewing area.



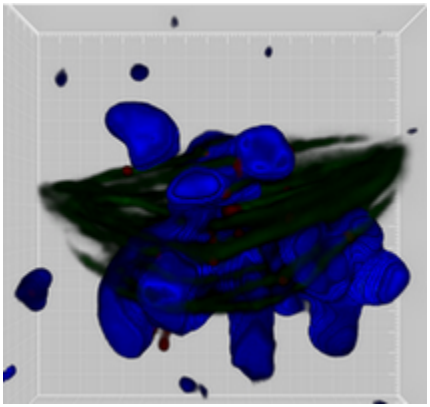
5.4 Change Surface Color

Color Properties

In the properties panel click on the tab **Color** (rightmost in the tab selection) to open the **Color Properties** dialog box. Change the color from gray to a dark blue by click on the color wheel. The Surface in the viewing area is displayed the chosen color.

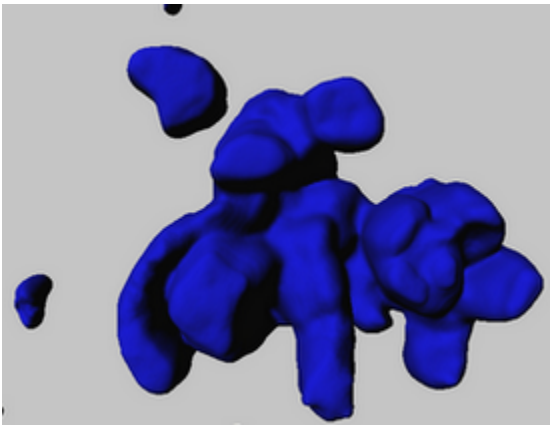


Additional to the Volume rendered microtubules the Surfaces reconstruction of the DNA displays in the viewing area of Imaris.



5 - Design Mixed Model Rendering

In the Object List now un-check the box for the item Volume and new created surface is displayed in viewing area.



See also: Visualize Data Set - Change Background Color 22

5.5 Add Spots

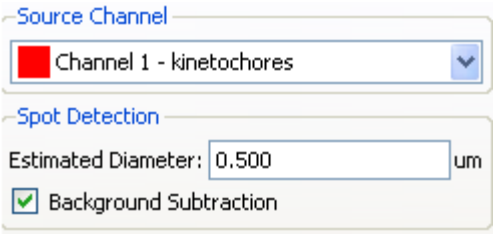
The kinetochores are small, globular and fairly spherical, and so ideal to show how Imaris detect Spots automatically. We will now add a Spots object to automatically segment the kinetochores.

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

Creation Wizard - 1/3 Algorithm

Leave the boxes **Segment only a Region of Interest** and **Different Spot Sizes (Region Growing)** un-checked and click on (Next).

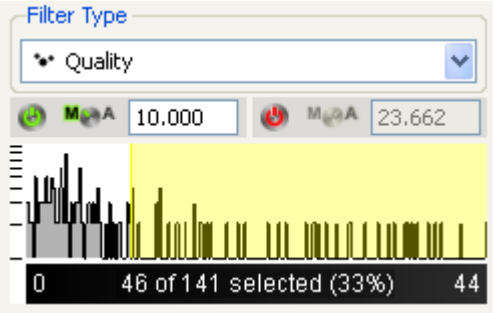
Creation Wizard - 2/3 Source Channel



Select as **Source Channel** the **Channel 1 - (kinetochores)** and set the **Estimated Diameter** to **0.500 um** and click on (Next).

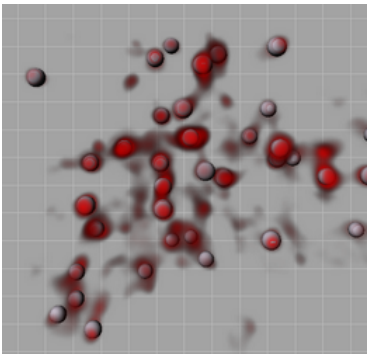
Creation Wizard - 3/3 Classify Spots

Imaris detects an automatic threshold at which to insert the Spots. You see the gray created Spots and the Volume rendered red channel. With the **Automatic** threshold detection some Spots are not identified because they are under the threshold limit. To change the lower threshold select the **Manual** option and type in **10.000**.



Alternatively you can left click on the yellow line and drag it. Moving the line to the right increases the threshold and removes some of the automatically created spots, while moving the line to the left produces additional spots. The effect is directly visible in the viewing area. Click on (Finish).

This is the result in the viewing area.



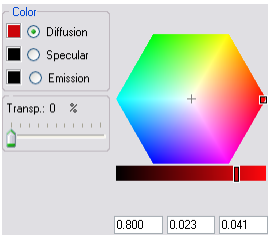
See also: Measure Sstructures - Line and Polygon 55 (to define the minimum spot diameter)

5.6 Change Spots Color

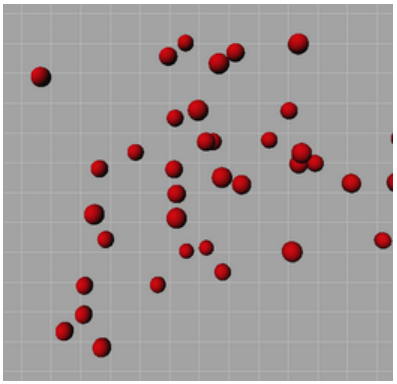
Color Properties

In the properties panel click on the tab **Color** (rightmost in the tab selection) to open the **Color Properties** dialog box.

Select the Tab Color and change the color from gray to a red by c lick on the color wheel. The Spots in the viewing area is displayed the chosen color.



Now un-check in the Object List the box for the item Volume.

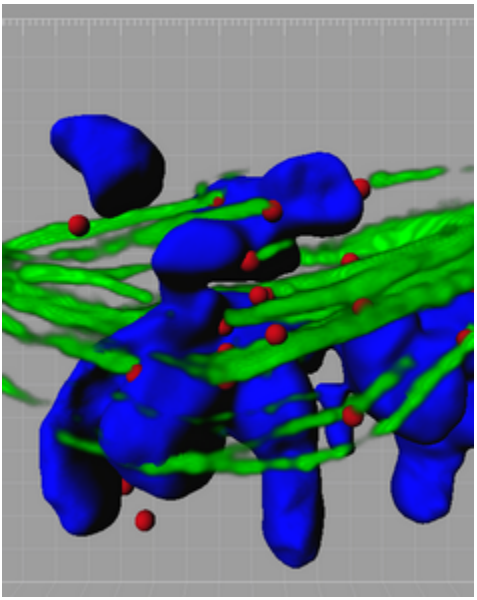


You see only the red Spots In the viewing area.

See also: Visualize Data Set - Change Background Color 22, Change Surface Color 48, Change Channel Color 24

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 Object List. In the **Display Adjustment** window check only the green channel.



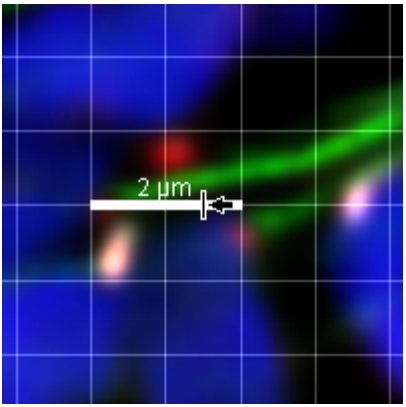
The microtubles display in green as Volume rendering, the chromosomes in blue as Surfaces and the kinetochores in red as Spots.

Please close Imaris before you start with the next tutorial.

See also: Visualize Data Set - Surpass View 11, Design Mixed Model Rendering - Display Adjustment 42

6 Measure Structures

In each Imaris view there are several measurement options. This tutorial will focus on different measurement functions in the Slice view 9. Depending on what you want to measure in the image you either choose as interactive measurement type Line 55 or Polygon 55. If you want to estimate distances in the image you can lay a Grid 57 over your image. The interactive Scale Bar 57 also helps in estimating sizes and distances. In the last chapter you will learn how to measure distances between different slices (3D Measurement 59).



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.

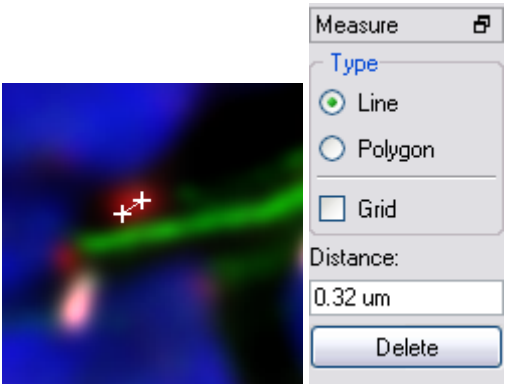
Open the demo image PtK2 cell in the Slice view.

PC:\ProgramFiles\Bitplane\Imaris\images\PtK2Cell.ims
Mac:/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

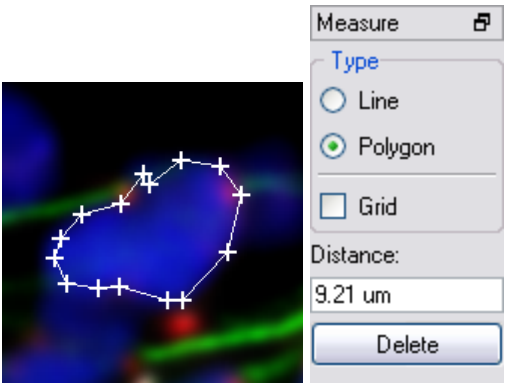
In the PtK2 cell the kinetochores are labeled in red. You will measure the diameter of a kinetochore using the point to point measurement function in Imaris.



On the right hand side of the screen you find the **Measure** window. Choose the measurement **Type Line**. Set the two measurement points with two consecutive clicks. The result displays 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.



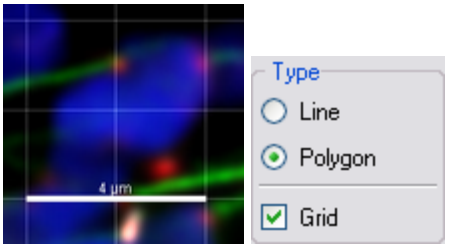
Choose the measurement **Type Polygon**. All measurement points are consecutively connected by lines and the distance is the sum of the distances between the points. Draw a polygon around the DNA like in the image above. The result displays in the status field **Distance 9.21 um**. Click on **Delete**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

See also: Visualize Data Set - Open Data Set 7

6.2 Grid and Scale Bar

Display a Grid

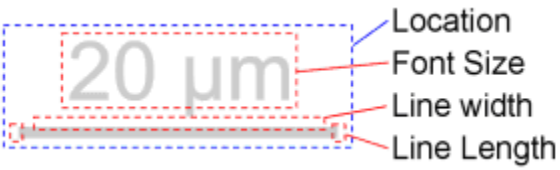
One way to estimate distances in the image is to use the grid display.



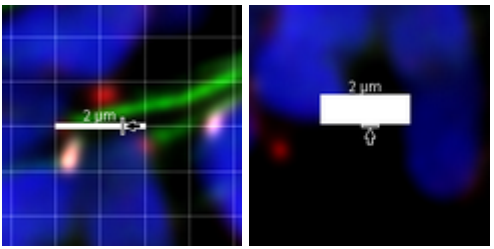
On the right hand side in the **Measure** window check the check box **Grid**. This is the result in the viewing area:

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.

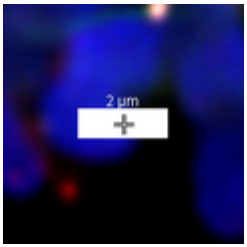


Line Length and Line Width



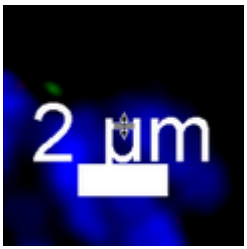
To adjust the length of the scale bar move the mouse to one end of the scale bar until the pointer symbol alters (vertical dash and arrow). Now click on the scale bar end and hold the button down while you drag the mouse to the left or right, respectively. The grid width changes accordingly. In the same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

Location



To translate the scale bar 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). 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

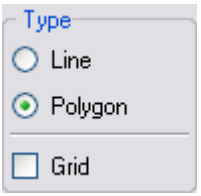
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on Imaris to open the program.

Open the demo image Pyramidal cell in the Slice view.

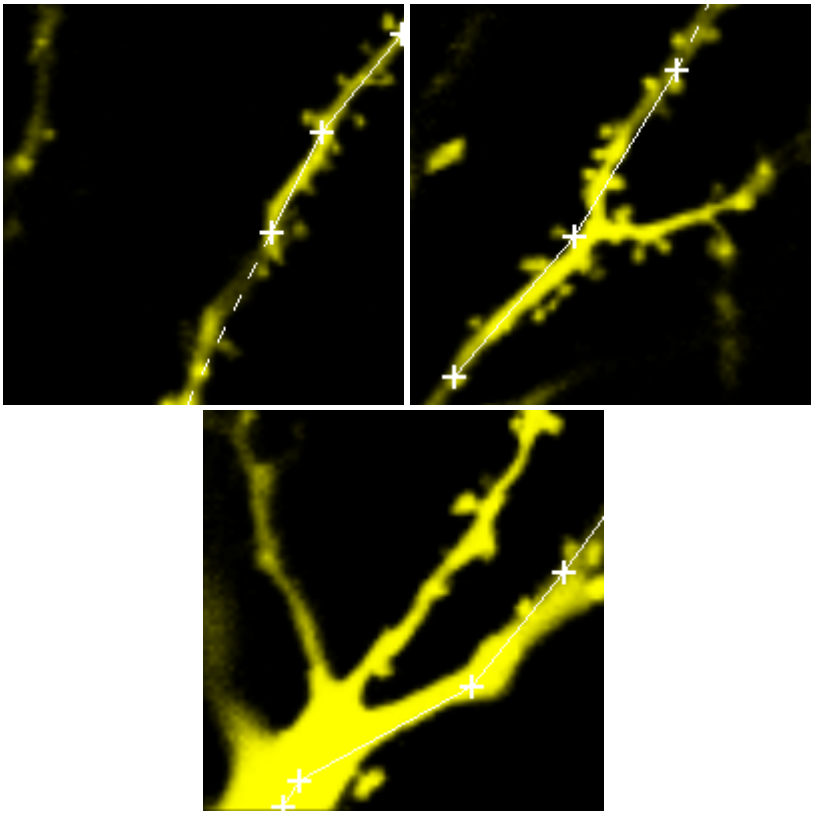
PC: \ProgramFiles\Bitplane\Imaris\images\PyramidalCell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims

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 as measurement type select the **Type Polygon**.



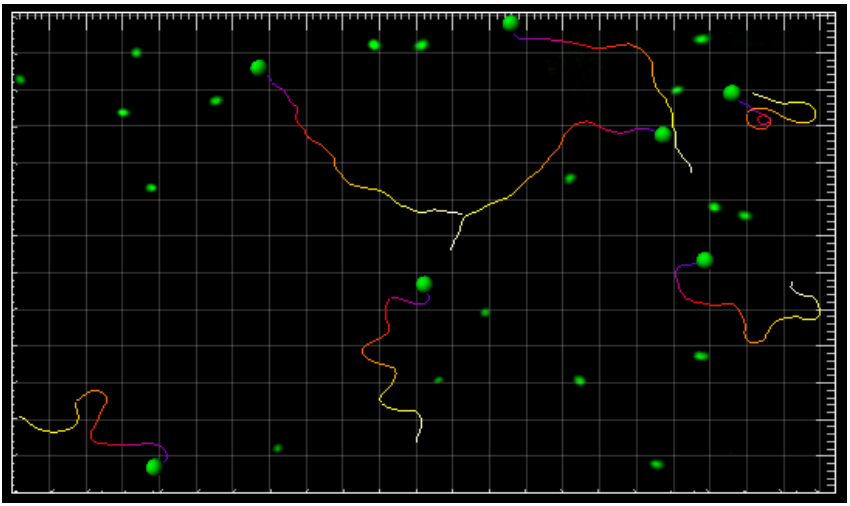
The first picture is slice number 21, the second picture is slice number 25 and the last picture is slice number 32.

Set the first point with a 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 clicks. Follow the filament through the slices. The dashed line indicates connecting lines between two slices. You find the distance between the first and the last measurement point 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 zoozpores*. The first step is the Visualization 62 of the data set and the automatic Spot Detection (Segmentation 64) over time. With the automatic Tracking 66 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 show the Track Displacement 68. Using the Filter 67 function you can group and analyze the Tracks depending on various criteria.



Spots & Tracks, Swimming Algae

User Level: Advanced
Module: Imaris, MeasurementPro, ImarisTrack

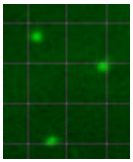
7.1 Visualization

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.
PC: \ProgramFiles\Bitplane\Imaris\images\SwimmingAlgae.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/SwimmingAlgae.ims

Add Volume

The demo image Swimming Algae is open in the Surpass mode in MIP (max) Mode.
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: 90** and **Max: 230**.



This is the result in the viewing area.

See also: Visualize Data Set - Open Data Set 7, Design Mixed Model Rendering - Add Volume 41

7.2 Time Concept

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 displays at the right hand of the time slider. You select images at different time points using the time slider.

Click slider + drag slider	Go to previous/next time point
Click slider (select) + arrow to the right on keyboard	Go to next time point
Click slider (select) + arrow to the left 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 displays (default setting). The arrow indicates the actual time point (in this example time point 7).

See also: Generating Movies - Save Movie 32, Imaris and QuickTimeVR 33

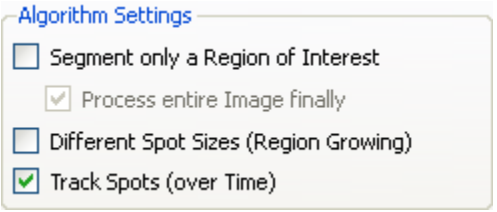
7.3 Segmentation

An automatic image segmentation and object tracking methods will be explained by analyzing image Swimming Algae. First you will identify and create spots. Then you will establish a time dependant relationship between spots (track them over time) and create Tracks 66. Finally you will sort the Tracks based on the Track duration 67 and visualize tracking 68 results.

In the **Objects** toolbar of the Surpass view click on the icon to add new Spots.
Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

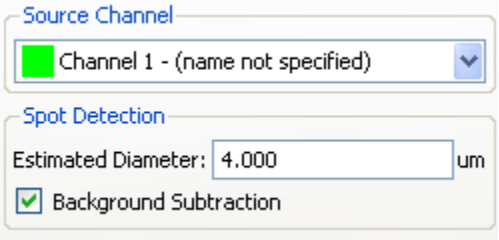
Creation Wizard - 1/6 Algorithm

In the Algorithm Setting check the **Track Spots (over Time).**



Click on (Next).

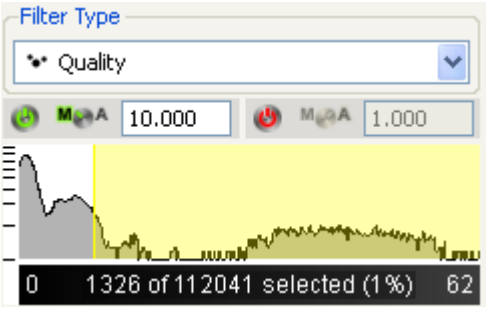
Creation Wizard - 2/6 Source Channel



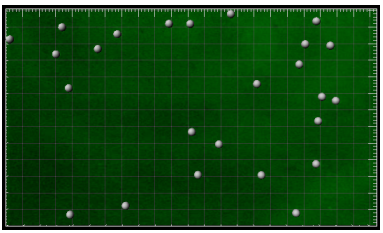
Select as **Source Channel** the **Channel 1** (green) and set the **Estimated Diameter** to **4.000.**Click on (Next).

Creation Wizard - 3/6 Classify Spots

As **Filter Type** select **Quality**, select the **Manual option** and set Lower threshold to **10.000.**



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



Click on (Next).

Creation Wizard - 4/6 Edit Spots

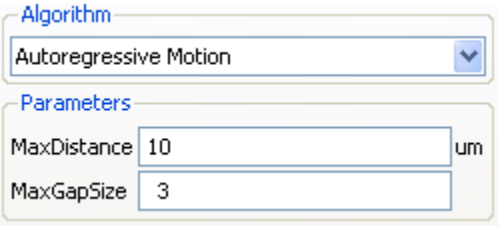
At this point of the algorithm you can manually add or delete spots.You will not do so in this example, so click on Next .

See also: Design Mixed Model Rendering - Add Spots 50, Design Mixed Model Rendering - Change Spots Color 52, Measure Structures - Line and Polygon 55 (to define the minimum spot diameter)

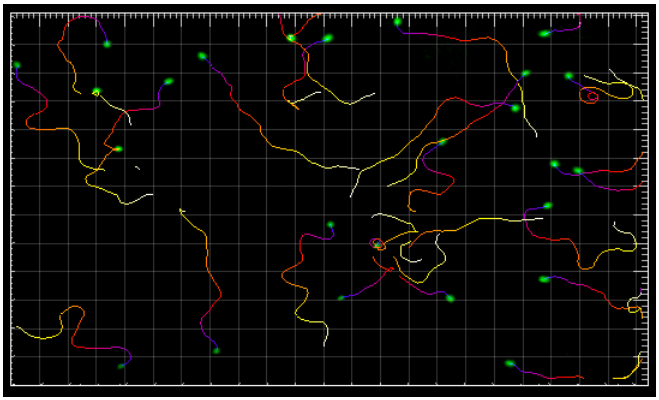
7.4 Tracking

Creation Wizard - 5/6 Tracking

A Track is a component within the Surpass view that describes the behavior of an object over time. (To automatically create all Tracks you did check the box **Track Spots (over Time)** on the first window of the Creation Wizard.)



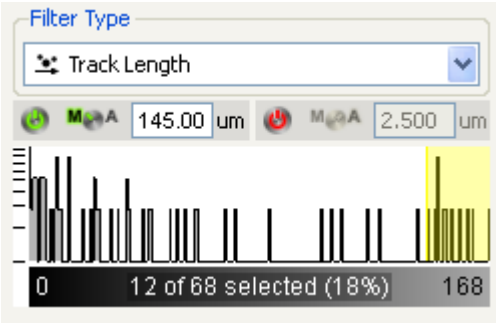
As **Algorithm** select **Autoregressive Motion** and as **MaxDistance** set **10.000** um, and for the **MaxGapSize** set **3**. Click on (Next).This is the result in the viewing area.



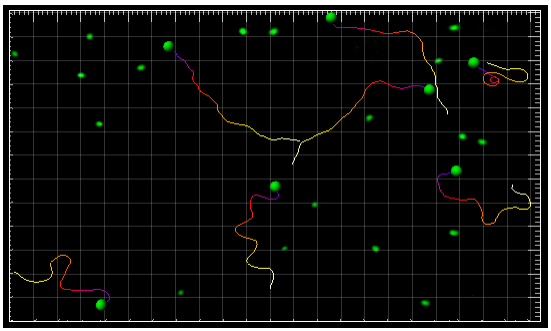
7.5 Filter Tracks

Creation Wizard - 6/6 Classify Tracks

The sorting of the Tracks is essential for the further analysis. In the following you sort the Tracks by their length.



Choose as **Filter Type: Track Length**. Select the **Manual** option and enter as value **145.000 um**. Alternatively click on the yellow line and drag it. Moving the line changes the filter setting, thus removing or adding some of the tracks.The effect is directly visible in the viewing area. Click on (Finish).

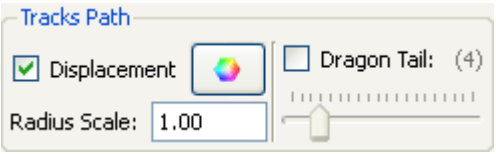


This is the Spots and Tracks display in the viewing area.

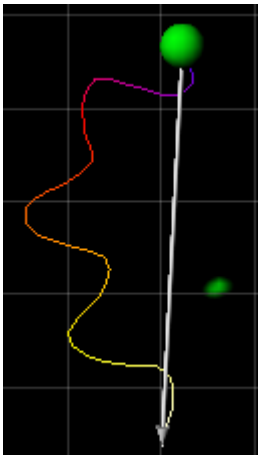
See also: Visualize Data Set - Surpass View 111, Volume over Time - Time Concept 113

7.6 Displacement

This visualization mode shows the displacement of a Track as an arrow. Select the tab **Settings** .



In the **Tracks Path** adjustment window check the respective check box **Displacement**.



The displacement arrows display in the viewing area.

Please close Imaris before you start with the next chapter.

7.7 Additional Example

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

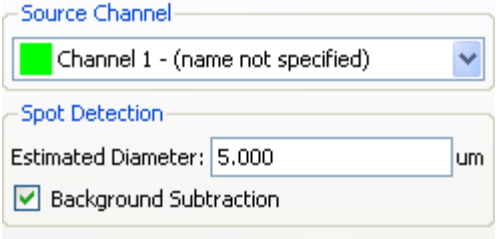
Open the demo image R18Demo.
PC: \ProgramFiles\Bitplane\Imaris\images\R18Demo.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/R18Demo.ims
The R18Demo image is displayed.

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

Creation Wizard - 1/6 Algorithm

Check the **Track Spots (over Time).** Click on (Next).

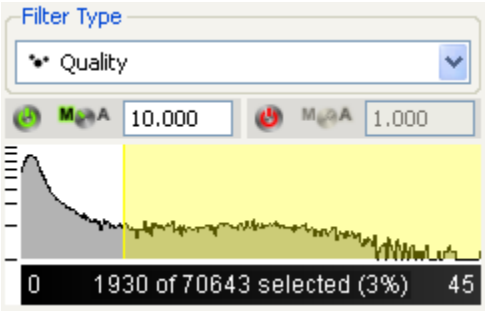
Creation Wizard - 2/6 Source Channel



Select as **Source Channel** the **Channel 1** (green) and set the

Estimated Diameter to **5.000.** Click on the box for the **Background Subtraction** option. Click on Next.

Creation Wizard - 3/6 Classify Spots



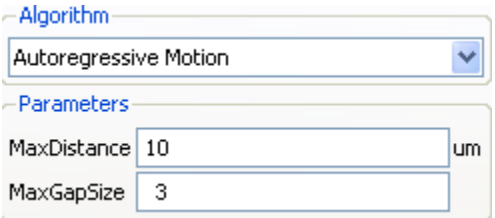
Select for the parameter **Filter Type: Quality**, select the **Manual** option and set Lower Threshold to **10.000.** As an alternative left click on the yellow line and drag it. Moving the line to the right increases the threshold level and as a result less spots are detected. Conversely moving the line to the left produces additional spots.

Click on Next .

Creation Wizard - 4/6 Edit Spots

In this example manual spot editing is unnecessary. Click on Next .

Creation Wizard - 5/6 Tracking

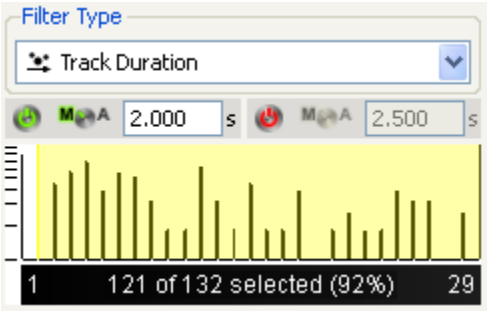


As **Algorithm** select **Autoregressive Motion** and as **MaxDistance** set **10.000** um, and as **MaxGapSize** set **3**.

Click on (Next).

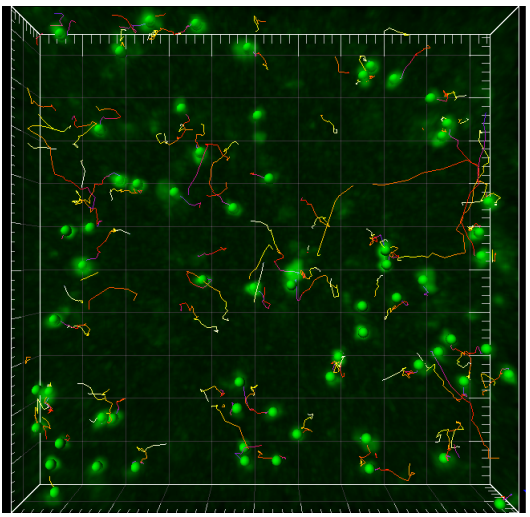
Creation Wizard - 6/6 Classify Tracks

In this example your focus is on the track duration.



Select as the **Filter Type Track Duration**. Select the **Manual** option and set Lower Threshold to **2.000**. The effect is directly visible in the viewing area.

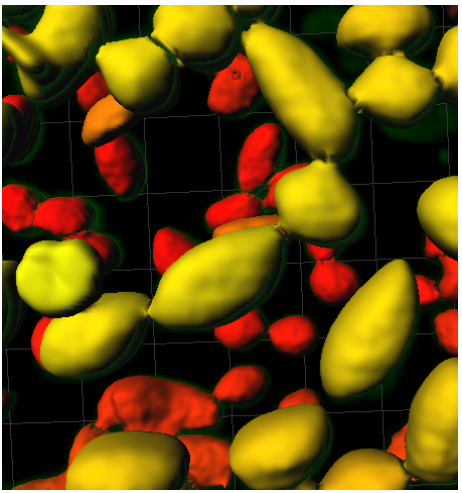
Click on (Finish).
In the viewing area Spots and Tracks are shown.



Please close Imaris before you start with the next tutorial.

8 Define Region Seed Points

In this tutorial you get familiar with the region growing to segment touching objects. First you Visualize 73 a plant cell with chloroplasts (green) and cell wall components (red). Then you detect the chloroplasts and define seed points for the Create Surfaces Seeded Region Growing 74. When you start the region growing a working channel displays in the viewing area. The seed point regions will grow until they reach the defined border. The result of the region growing in this example are Display Surfaces 76.



Surfaces, Region Growing, Plant Cell

User Level: Beginner
Module: Imaris

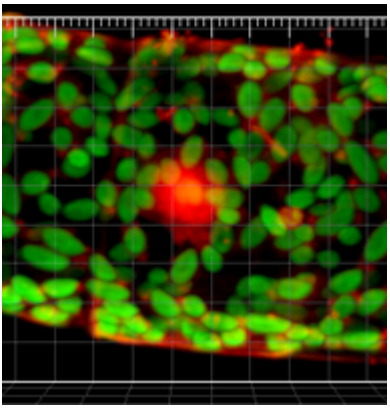
8.1 Visualization

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.

PC: \ProgramFiles\Bitplane\Imaris\images\PlantCell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PlantCell.ims

Visualize Chloroplasts



This is the volume rendering of the plant cell in the viewing area.

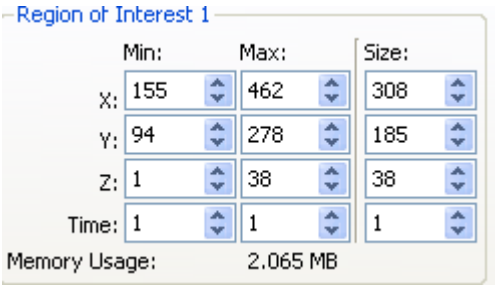
8.2 Create Surfaces

In the **Objects** toolbar of the Surpass view click on the icon to add new Surfaces.
Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

Creation Wizard - 1/6 Algorithm

Select segment only a **Region of Interest (ROI)** and **Process entire Image finally.** Click on Next.

Creation Wizard - 2/6 Source Channel



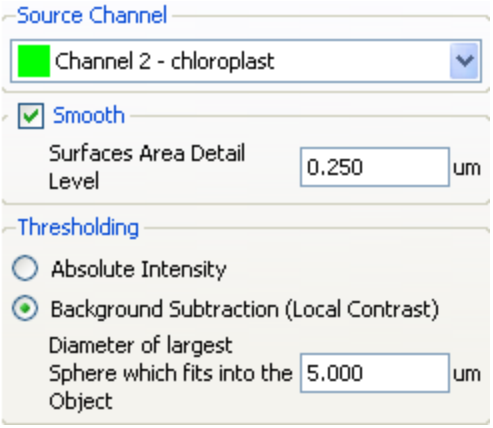
A rectangle-bordered section is overlaid on image, representing the Region of Interest (ROI). Entering the values in the corresponding x-, y-, and z- min/max fields or directly by click on the arrows to modify the ROI size and position. Click on Next.

Creation Wizard - 3/6 Source Channel

Select as **Source Channel** the **Channel 2** (chloroplast) and check the option **Smooth** and as **Surface Area Detail Level** use **0.250 um**. Check option **Background Subtraction** and set as **Diameter of largest Sphere which fits into the Object** value of **5um.** Click on

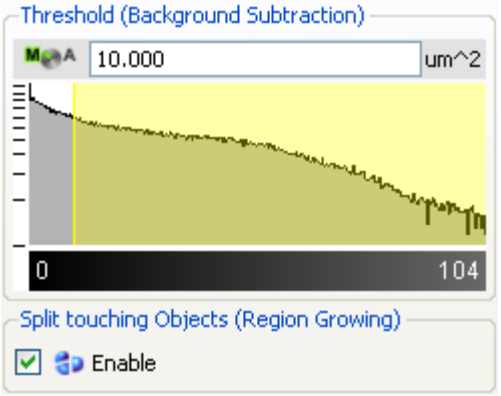
8 - Define Region Seed Points

Next.



Creation Wizard - 4/6 Threshold

In the **Threshold** adjustment select **Manual,** set threshold to **10.000** and select **Split touching Objects (Region Growing).** The number of steps increase from **6 to 8**. Click on Next.



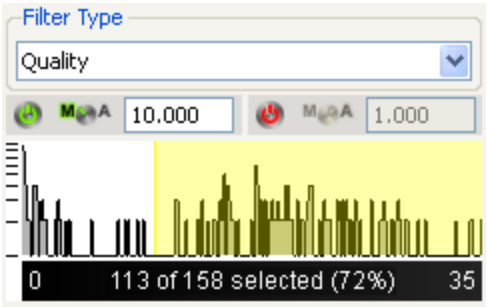
8.3 Define Region Growing and Display Surfaces

Creation Wizard - 5/8 Region Seed Points

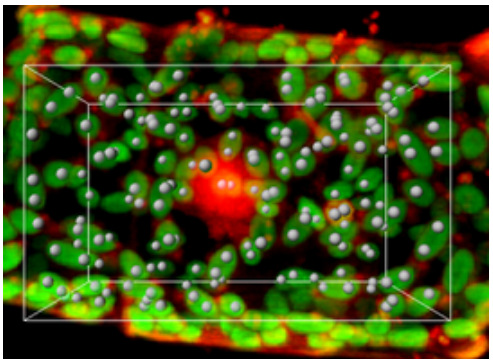
Set as **Estimated Diameter** value of **4.0 um**. Click on (Next).

Creation Wizard - 6/8 Classify Seed Points

Select as **Filter Type: Quality**, select **Manual**, set Lower Threshold to **10.000.**



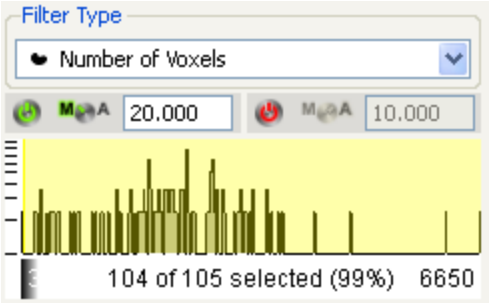
This is the result in the viewing area. Click on (Next).



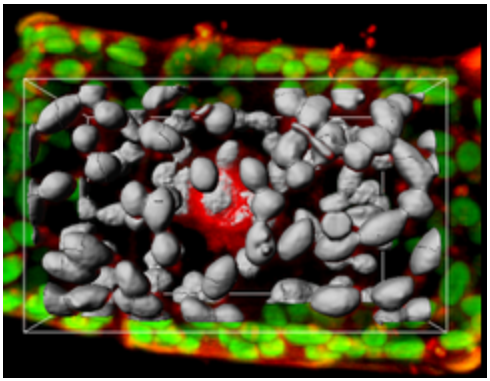
8 - Define Region Seed Points

Creation Wizard -7/8 Classify Surfaces

Select as **Filter Type: Number of Voxels**, select **Manual**, set Lower Threshold to **20.000**.



This is the result in the viewing area. Click on (Next).



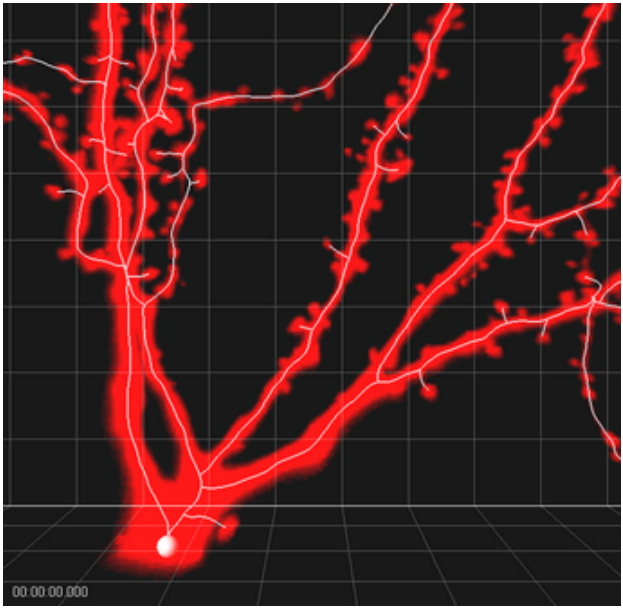
Creation Wizard -8/8 Surface computation for the entire dataset

To complete Surface creation with Split touching objects selection click on (Finish).

Please close Imaris before you start with the next tutorial.

9 Analyze Neuron

FilamentTracer is a separate Imaris module to detect, visualize and measure filamentous structures. The demo image shows a hypocampal Pyramidal cell in an organotypic cell culture. The tutorial is focused on two ways of filament tracing. First you will learn how to automatically create filament structure in Automatic Detection 81 and than the alternative semi-manual creation-AutoPath Mode 88 is presented. In the last chapter Filament Tool Kit 90 you will find numerous visualization filament possibilities and a short description where to find and how-to-handle the different tracing functions in the complex FilamentTracer module.



Filament Structure, Pyramidal Cell

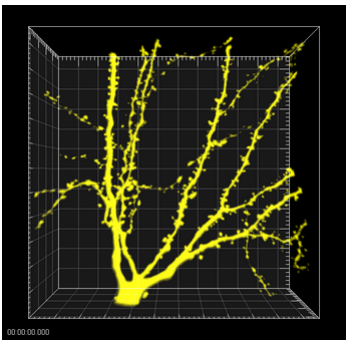
User Level: Advanced
Module: Imaris, FilamentTracer

9.1 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 Pyramidal cell.
PC: \ProgramFiles\Bitplane\Imaris\images\PyramidalCell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PyramidalCell.ims

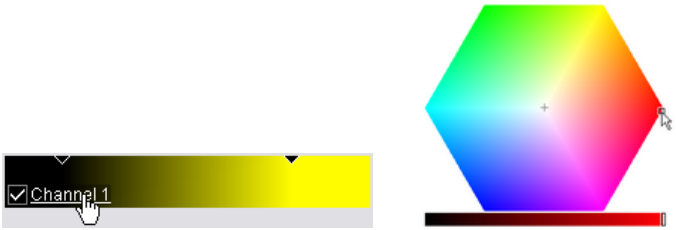
The Pyramidal Cell image is displayed.



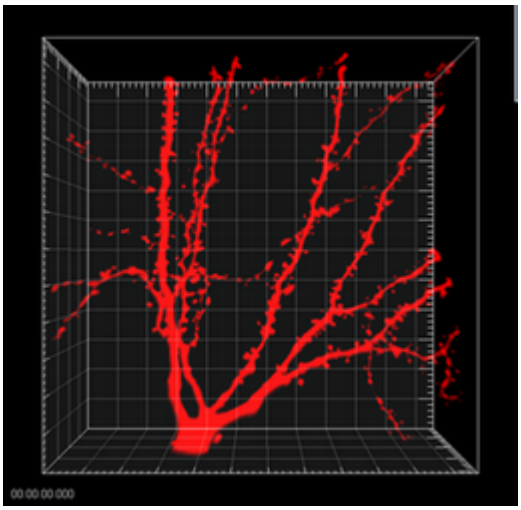
MIP Display, Pyramidal Cell

9.2 Change Channel Color

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



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: Design Mixed Model Rendering - Display Adjustment 42, Change Spots Color 52, Visualize Data Set - Change Background Color 22

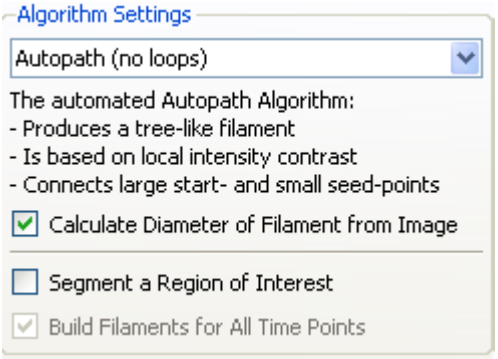
9.3 Automatic Detection

Automatic creation separates dendrite and spine detection, allowing a more sensitive detection of spines.
In the Automatic creation method of the FilamentTracer the start point and the end points are initially detected. Then these points are automatically connected with lines following the image intensity and the filament structure is created. For this neuron image there is only one start-point at the location of the soma, and many end-points located at the dendrites and/or the spines terminal point.
Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

Add new Filament

In the **Objects** toolbar of the Surpass view click on the icon to add a new Filament. The Creation Wizard appears automatically in the properties area.

Creation Wizard - 1/6 Algorithm



As **Algorithm setting** select **Autopath (no loops)** and check the **Calculate Diameter from Image** box. Click on (Next).

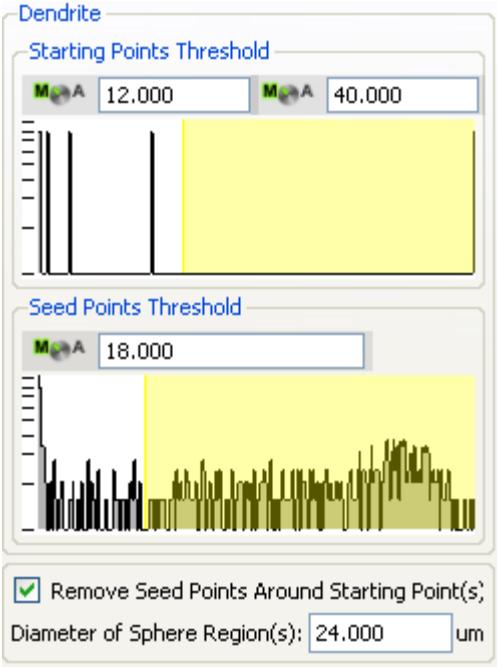
Creation Wizard- 2/6 Channel and Diameter Setting

In the step Points diameter you will define the channel sources and diameters for the Dendrite and the Spines.

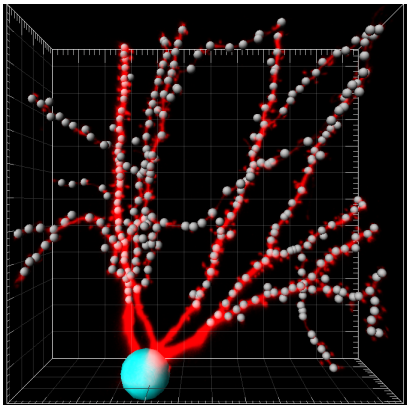
For the **Source Channel** of the dendrite select the **Channel 1.**
The next parameter is the dendrite **Starting point** diameter. The maximum (largest) diameter of dendrite is the recommended value for the Starting point diameter. In this example the starting point diameter is set to **12**.
The value of the **Dendrite Seed Point** diameter is set to **1** to match the value of the smallest dendrite radius.
In this example we will detect the spines, so select the **Detect Spine** box.
The **Source Channel** for spine detection is by default the same as for the dendrite, Channel 1.
The spine Seed point diameter is set to **0.455**. Click on Next.

Creation Wizard - 3/6 Classify Dendrite Points

In this step, you can classify the detected points. The thresholds are set for Dendrite Starting Point and Seed Points.



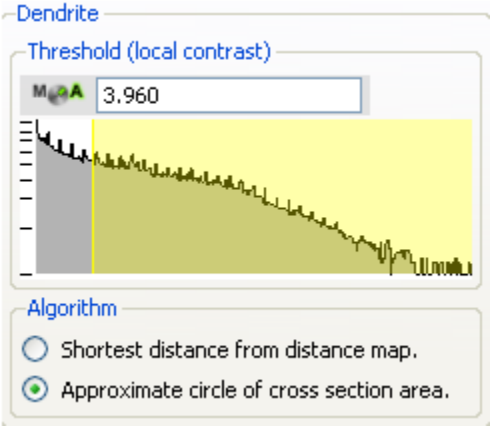
The threshold can be set manually or automatically. Select the **Manual** option by clicking on the **M/A** button.
As a lower threshold value for the dendrite **Starting Point** enter **12** and for the higher **40**.
The threshold for the dendrite **Seed Point** is set the to **18**.
Select the **Remove Seed Point Around Starting Point** option. This removes all seed points located within the radius of the sphere centered at the starting point. The boundary of the sphere is defined by the value Diameter of the Sphere Region. Set this value to **24** um.
The detected dendrite's Starting point and seed points are displayed in the image by different colors.



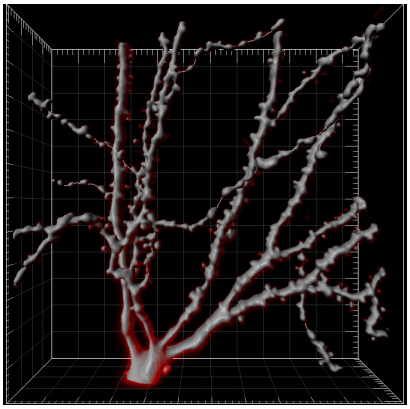
Click on Next.

Creation Wizard - 4/6 Dendrite Diameter

In the next step you can adjust the threshold value for the Dendrites Diameter. The threshold value is based on the local contrast. Accept automatically calculated values for the dendrite diameter.
You can specify how the dendrite diameters are calculated from the image. As an algorithm for the dendrite diameter calculation select the Algorithm of **Approximate circle of cross section area**.



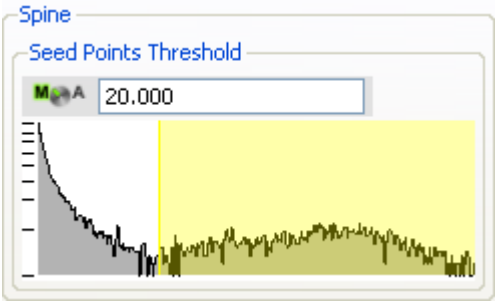
In the viewing area a threshold preview of the dendrite is displayed in gray.



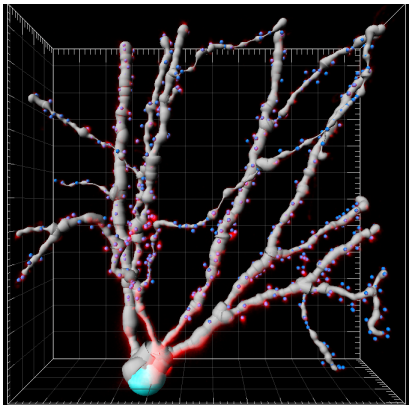
Click on Next.

Creation Wizard - 5/6 Classify Spine Points

In this step, you can classify the Spine points. The thresholds is set for Spine Seed Points.



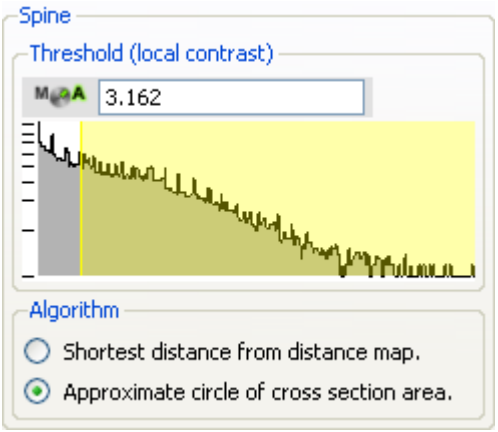
The threshold for the dendrite **Seed Point** is set the to 20.
The detected spine Seed points for dendrites are visualized in the image by different colors.



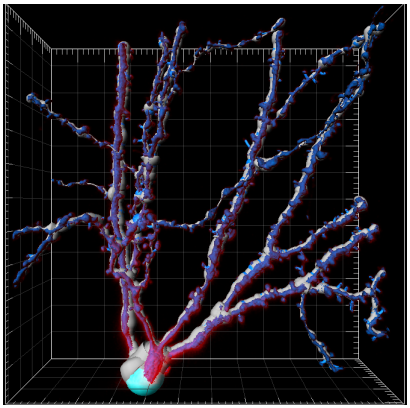
Click on Next.

Creation Wizard -6/6 Spine Diameter

In the next step you can adjust the threshold value for the Spine Diameter. The threshold value is based on the local contrast. Accept automatically calculated values for the Spine diameter.
You can specify how the Spine diameters are calculated from the image. As an algorithm for the dendrite diameter calculation select the Algorithm of **Approximate circle of cross section area**.

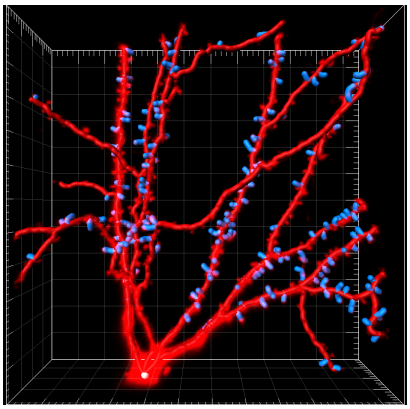


In the viewing area a threshold preview of the is displayed.



Click on Finish .

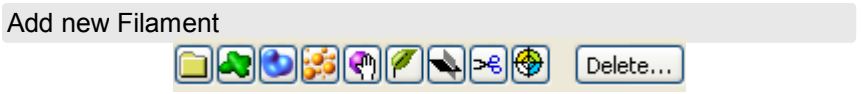
This is the result in the viewing area.



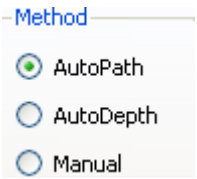
See also: Measure Structures - Line and Polygon 55 (to define the starting and end point diameter)

9.4 AutoPath Mode

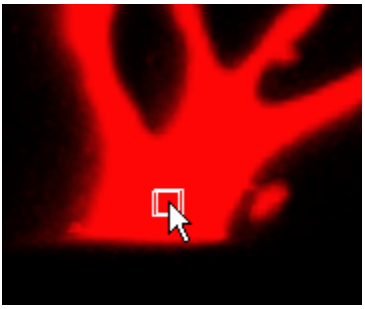
In the case that **Automatic Detection method** is not producing desired results, filament structure could be created by **AutoPath** method option. **AutoPath** method can be used for reselecting, redrawing and creating new filament structure. In the **AutoPath** mode of the **FilamentTracer** you define 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.



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. Click on the tab **Draw** .

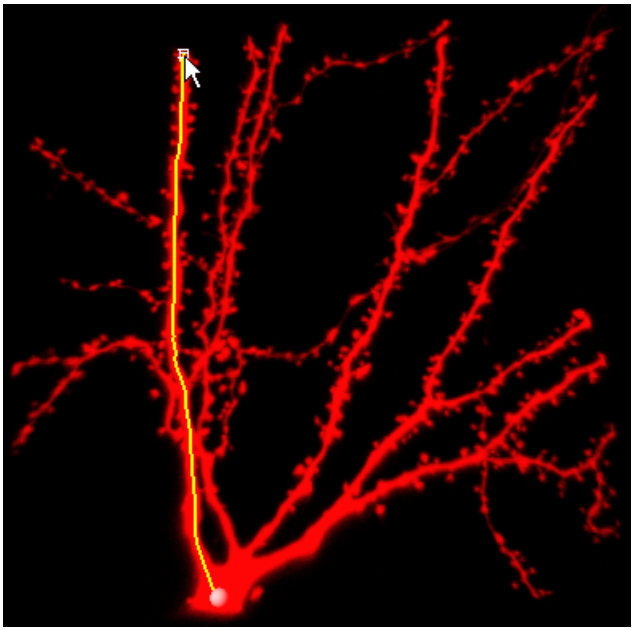


In the **Method** parameters select **AutoPath** and change to the mouse pointer mode **Select**. A rectangle displays around the mouse pointer.



9 - Analyze Neuron

Press Shift and right-click (if you work with a PC) on the base of the Pyramidal cell to fix the starting point. (If you work on a Mac set the starting point with: Shift + Ctrl + click.)



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 displays on screen. With Shift + click you fix the filament. Move the mouse cursor to the end point of the next filament, press Shift + click to fix the filament. In the same way set all the filament end points in the image.

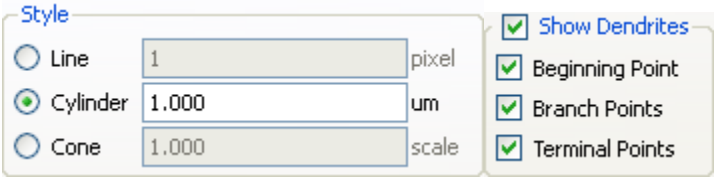
See also: Visualize Data Set - Select and Navigate 14 (mouse pointer mode), Analyze Neuron - Filament Tool Kit 90

9.5 Filament Tool Kit

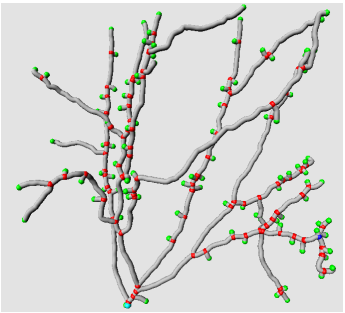
In this chapter you will learn different visualization possibilities and where to find the different selection windows in the module FilamentTracer. A short, tabular overview concerning the three different interactive filament tracing methods will be presented.

Filament Visualization

On Filament **Setting** Tab change the **Style** to **Cylinder.** Check boxes for **Show Dendrites Beginning, Branch and Terminal points** for Filament structure visualization.



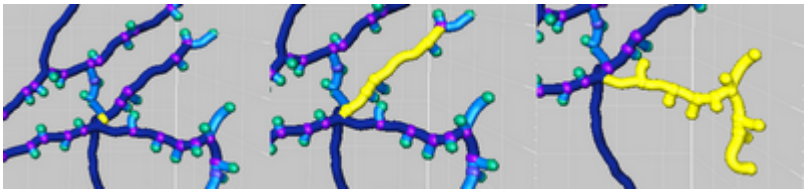
Un-check the Volume rendering in Object List and new Filament Structure is displayed in viewing area.



Additional filament visualization is achieved by selecting different styles of filaments (**lines**, **cones** or **cylinders**).

Selection and editing of filament structure can be achieved by selecting **Point**, **Segment** or **Branch**. Depending on selection process the different image parts in viewing area are highlighted.

This is the result in the viewing area if point, segment or brunch are selected.



Filament analysis and processing could be performed with automatic filament selection choosing option **Process Selection** and **Process Filament** for examination filament structure features.

The Mouse Select and Process Selection Window

In the the window **Process Selection** you find the **Delete** button, and other editing functions. These functions are applied only to the selected part(s) of the Filament.

9.6 Manual Filament Structure Creation

Click on 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. The button **Set Selection as Starting Point(s)** is only available in the AutoPath mode.

	PC:	Mac:
Set the starting point	Shift + right-click	Shift + Ctrl + click
Selected segment or branch as a starting point	Click on a segment or branch, then on the **Set Selection as Starting point** button	Click on a segment or branch, then on the **Set Selection as Starting point** button
Fix the filament	Shift + left-click	Shift + left-click

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

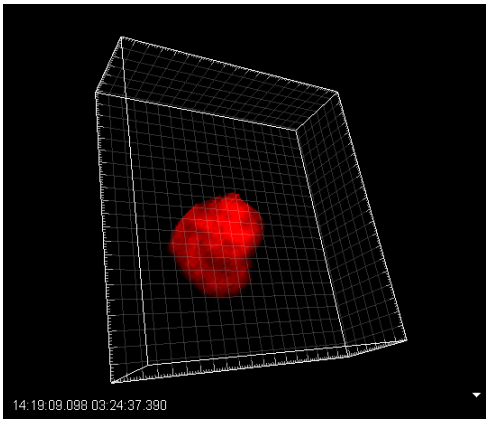
	PC:	Mac:
Draw a filament into the volume	Shift + left-click + drag	Shift + click + drag
Stop drawing	Stop movement and release all buttons	Stop movement and release all buttons

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.

	PC:	Mac:
Draw the filament on the slicer	Shift + left-click + drag	Shift + click + drag
Stop drawing	Stop movement and release all buttons	Stop movement and release all buttons

10 Volume of Interest

A useful approach to some images is to apply a certain function not on the entire image but only in a region of interest (ROI). In this tutorial you will define such a volume of interest by generating a surface object from manually created contours. A semi-automatic surface creation, based on a user-drawn contour, is helpful in visualization and measurement of structures, which cannot be easily segmented from the background or from neighboring structures.
The demo data set is a time series of a HeLa cells going through all mitotic phases in the cell cycle. In the green channel, the mitotic spindle is visualized by an EGFP tagged alpha-tubulin. In the red channel, chromatin is visualized by a tagged core histone 2B.
You will start with the visualization 94 of the data set. After that you draw the contour lines 100 to outline the chromatin. Then you let the Imaris calculate the Contour Surface 102. This Surface object is your "cookie-cutter". Duplicate the original red channel and then use the "cookie-cutter" to lay a Mask 103 to this channel. The focus of the last chapter is the display of the Masked Cannel 105 allowing you to concentrate your work on a specific ROI.



Masked Red Channel, HeLa Cell

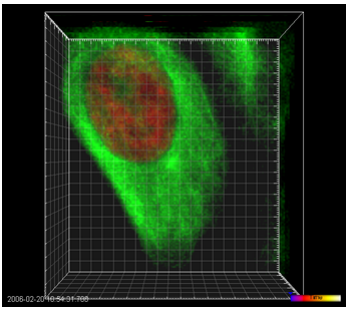
User Level: Advanced
Module: Imaris, MeasurementPro

10.1 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 HeLa cell.
PC: \ProgramFiles\Bitplane\Imaris\images\HeLaCell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/HeLaCell.ims

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 **Ch1** (red) and **Ch2** (green) to **Min:45** and **Max:200**.

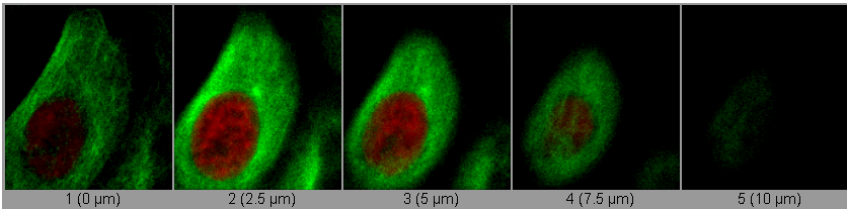


HeLa Cell Display in the Viewing Area

See also: Design Mixed Model Rendering - Add Volume 41 and - Display Adjustment 42, Analyze Neuron - MIP Display 79

10.2 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.

10.3 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).

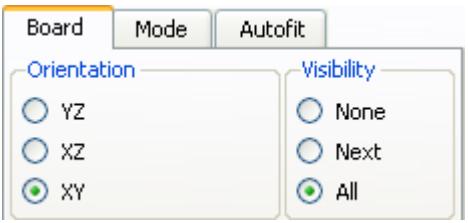
Create Contour Surface

In the menu Surpass select Add new Surface.
To start the Contour surface creation first you have to cancel the automatic Surface creation wizard. You can do that either by clicking on the Cancel button or by selecting the Skip automatic creation, go to manual editing button.

In the Draw tab select the Contour tab.

Board -Orientation and Visibility

A drawing plane displays in the viewing area. To begin with the Contour surface creation process, adjust the **Orientation** and **Visibility** of the contour planes in the Board sub tabs.For the **Orientation** select **XY**, setting the orthogonal slicers of all other contours to the same position. The **Visibility** option controls the display of previously drawn contours. Select **All** to show all contours as you move up and down the stack.

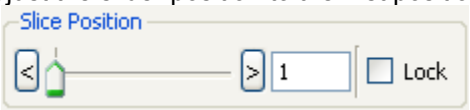


To start drawing contour lines select the Mode tab.

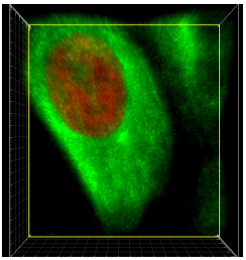
10.4 Mode Tab

Slice position

Before starting with the first contour line the position of the drawing plane must be adjusted. The respective slice number is displayed in the number field. Adjust the slider position to the first position.



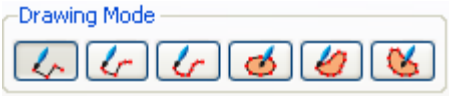
The border of the active drawing plane is shown in yellow color, thus clearly indicating the Contour Surface currently in use.This is the result in the viewing area showing, Slider in First Position.



There are several ways to move between slices and select individual slices to create a new contour.
The drawing plane can be interactively adjusted either by dragging with the slider, using the up or down arrow keys or by entering the number of the slice in the numerical field. The position of slices can be alternately changed in the viewing area. Switch the pointers to the Select mode and then simply click on the drawing plane and drag it to a new position.

Draw Modes

In the Mode tab several drawing mode tools are provided.



Select **Click** as the drawing mode tool, to create a selection that outlines the chromatin contour.

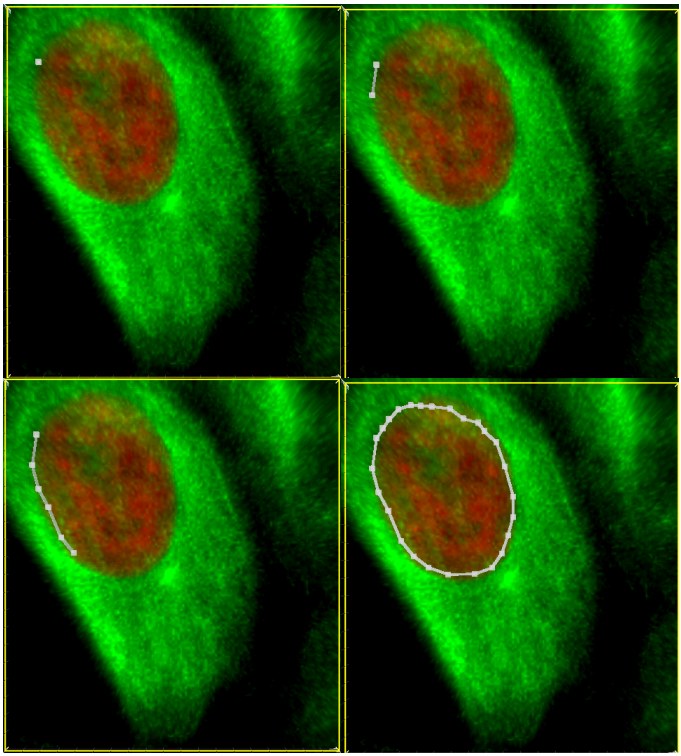
10.5 Draw Modes

Drawing Modes

In the contour line there are two different drawing modes: **Active** and **Inactive**. The main function of the active drawing mode is to create contour lines. The Active drawing mode can also be used to insert new vertices into existing contour lines. In the Inactive drawing mode the existing contour lines can be modified or deleted.
You can select the respective drawing mode by using the **ctrl+space** key on keyboard. The pointer appearance on the screen depends on the selected drawing mode. The symbol on the screen appears as an **arrow** in the **Inactive** drawing mode and as a **cross** in the **Active** drawing mode.
Tip: You can easily switch between the two drawing modes using the **ctrl+space-key**. The effect is directly visible on screen by the mouse's altered pointer appearance.
Change to the pointer mode **Select** (press the ESC-key) and than press the **ctrl+space key** to activate the drawing mode.

Draw Contour Lines

Drawing the contour with a **Click** drawing mode involves a series of clicks and drags to set the points and to define the contour. Click on the desired first position to insert the first point. Move the mouse to the next point and click. Each new click creates a connected line to the previous point. To finish the contour and ''close" the path move the cursor to the original point and click.
This is the result in the viewing area showing the outlines of the chromatin boundaries in the slice 1 of the HeLa cell image.

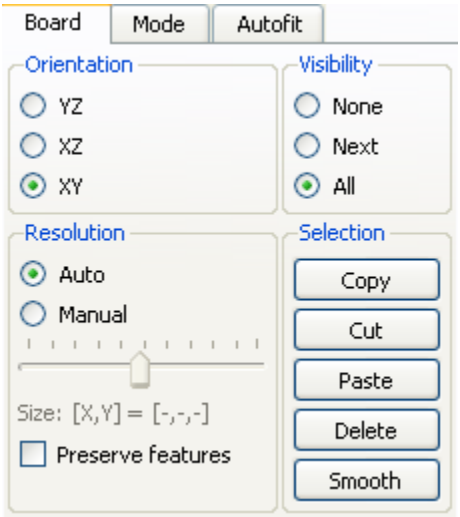


You can draw as many contours on a plane as required. All contours drawn on a plane will become part of the same object.
Change to the next drawing plane by moving the Slider to the next position. Repeat the process until the contours are created for all slices.

10.6 Copy Contour Line

Copy the Contour Line

On the next plane you can either draw an individual polygon line as described at the beginning of this chapter or you can **Copy** the contour from the previous slice.
If you want to copy the same polygon to the next image plane do the following:Switch to the **Board** tab and click on the **Copy** button and you can copy the contour line.
Change to next plane by moving the Slider to the next position.With a click on the **Paste** button the same contour line is placed to the second plane.



After the contour is created, move to the next slice, to create the next contour.

Color of the Contour Line

It is possible to change the color of a contour line. This can be achieved by selecting the desired color in the Color tab. It is generally wise to select a bright color (for gray scale images) or black or white (for color images) so that the marks are easily visible.

10.7 Edit Polygon

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.If at any time you want to edit the contour that you placed select the **Inactive** drawing mode (press the **ctrl+space**-key). Return to the slice on which the polygon is drawn and use the following shortcuts.

	PC:	Mac:
Move node	To move a node you can click on the node while holding down the T-key and simultaneously drag the mouse.	To move a node you can click on the node while holding down the T-key and simultaneously drag the mouse.
Delete node	Hold down the D-key and left-click with the left mouse button on the node.	Hold down the D-key and left-click on the node.
Delete polygon	Hold down the D-key and left-click with the left mouse button on a line between two nodes.	Hold down the D-key and double-click on a line between two nodes.
Change vertices/line size	The size of the vertices and the connecting lines can be increased or decreased by pressing the + key or – key on the numerical keypad.	The size of the vertices and the connecting lines can be increased or decreased by pressing the + key or – key on the numerical keypad.

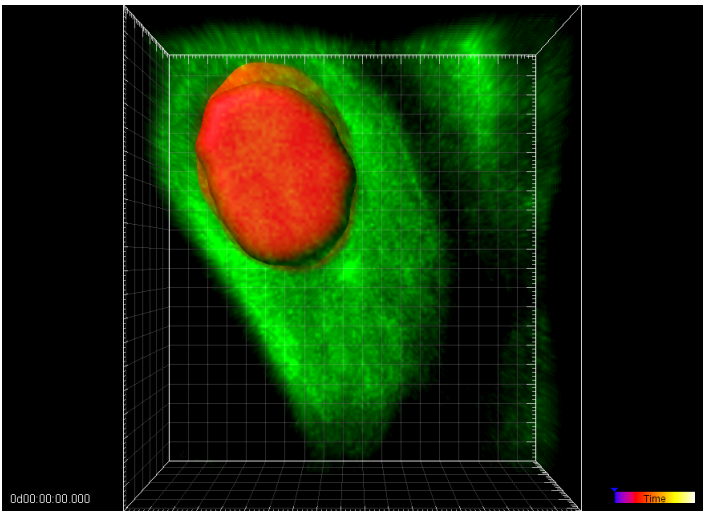
The 'Undo' function from the Edit menu is also available.

See also: Visualize Data Set - Select and Navigate 14

10.8 Create Surface Object

When at each plane there is a contour line, select the Calculate Surface button to create new Surface Object. Click on the button **Create Surfaces**.

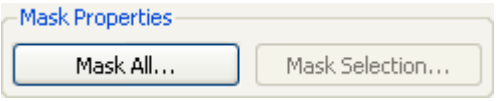
This is the result in the viewing area.



See also: Visualize Data Set - Select and Navigate 14

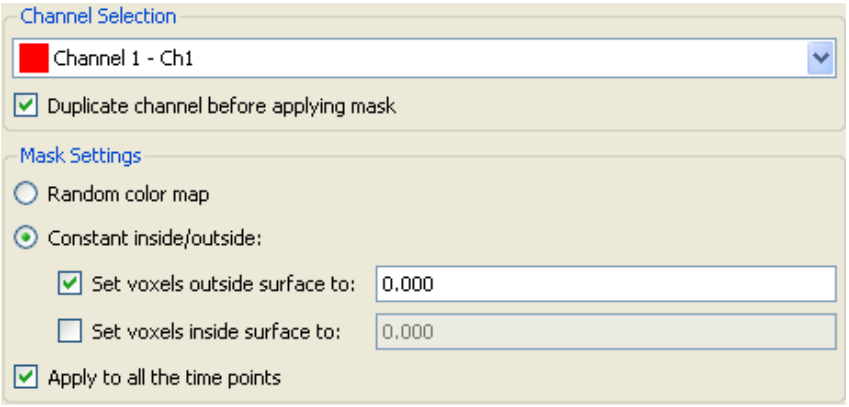
10.9 Mask Channel

To apply a certain function not on the entire image but only in a region of interest (ROI) you will use the Mask Channel option. In this tutorial a volume of interest is the Surface object generated from manually created contours. Be sure that in the Object List the Surfaces object is highlighted and click on the tab **Edit** . Click in the **Mask Properties** on the button **Mask All ...** .



Window Mask Channel

The Surfaces object is your "cookie-cutter", the selected channel your "cake mixture". First you **duplicate** the original red **channel**. Then you use Surface object as the ROI ("cookie-cutter") to mask this channel. In the following you will cut out the red area to see only the inside of the the Surfaces object. All voxels outside the "cookie-cutter" are excluded (set the voxels outside the surface to zero).



- Select the channel (**Channel 1 - Ch1,** the red channel) you want to mask with the Surface object (ROI).
- Check the box **Duplicate Channel before applying Mask** and an additional masking channel displays automatically in the **Display Adjustments** window.

- Select **Constant inside/outside** and check the box **Set voxels Outside Surface to:** and set the default value to **0.000**. So, no voxels outside the ROI are displayed.
- Leave the **Set voxels Inside Surface to:** box un-checked. The original channel intensities inside the region of interest are displayed.
- Check the box **Apply to all time points**.
- Click on the button **OK** to apply the mask.

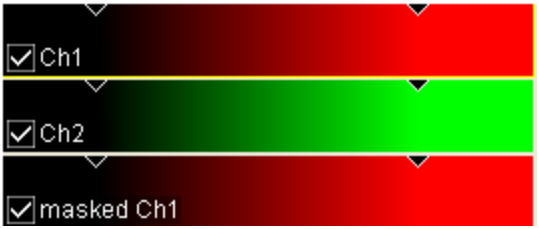
See also: Design Mixed Model Rendering - Display Adjustment 42

10.1 Display Masked Channel

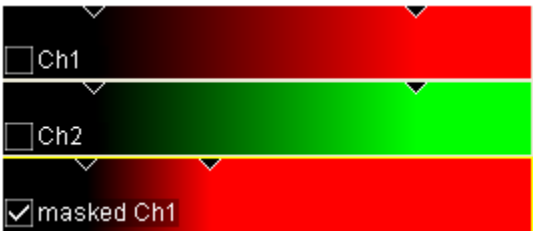
To display only the masked channel in the viewing area you have to adjust the channel display in the **Display Adjustment** window. Additionally you have to set up the visibility of the Object List items in the viewing area.

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**.

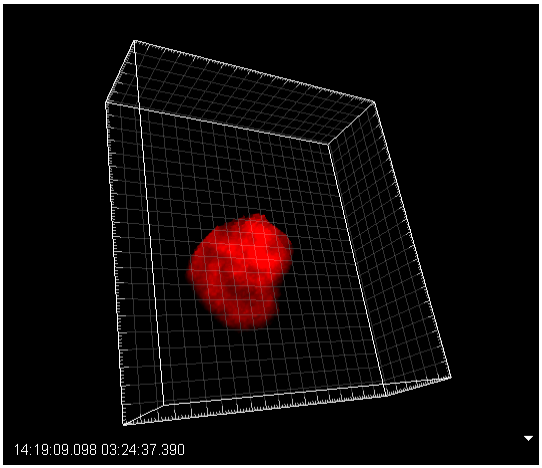


Un-ckeck the original red and green channel. Adjust the upper threshold for the new channel **masked Ch1** (move the right arrow that represents the upper threshold to the left).

Adjust Object List

In the Object List check the item **Volume** and un-check the **Surfaces**. 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 Surface object. Then you use this Surface object as the region of interest ("cookie-cutter") for the masking.



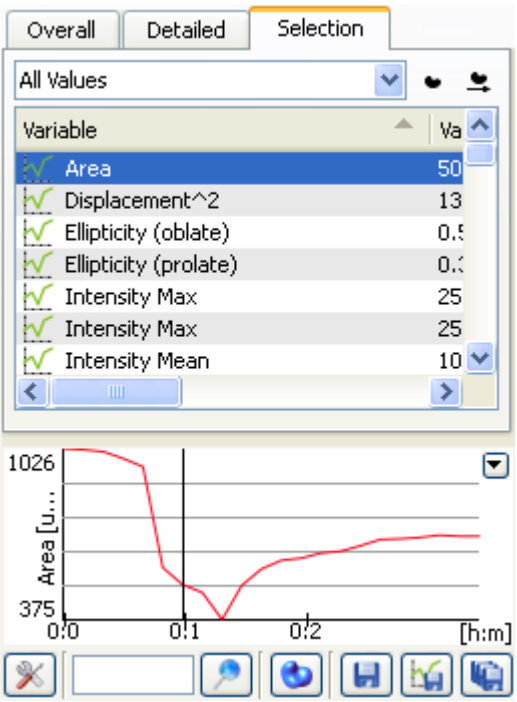
New, Modified Red Channel

Please close Imaris before you start with the next tutorial.

See also: Design Mixed Model Rendering - Surpass View 11 and Display Adjustment 42

11 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 108 reconstruction for both channels, then you generate an Surfaces 109 reconstruction of the chromosomes. In the chapter Time Concept 113 you will learn how-to-handle time series in Imaris. Then you Calculate 114 and export statistical data for each time point. The focus of the last chapter is the Presentation of the Results 117 in MS Excel.



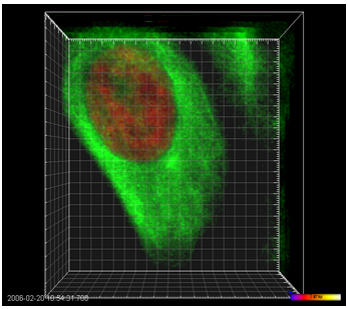
User Level: Advanced
Module: Imaris, MeasurementPro, Tracking

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 HeLa cell.
PC: \ProgramFiles\Bitplane\Imaris\images\HeLaCell.ims
Mac:/Applications/Imaris.app/Contents/SharedSupport/images/HeLaCell.ims

The demo image HeLa cell is open in the Surpass mode.
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 displays in the viewing area.



See also: Design Mixed Model Rendering - Add Volume 94, Design Mixed Model Rendering - Display Adjustment 42, Analyze Neuron - MIP Display 79

11.2 Add Surfaces

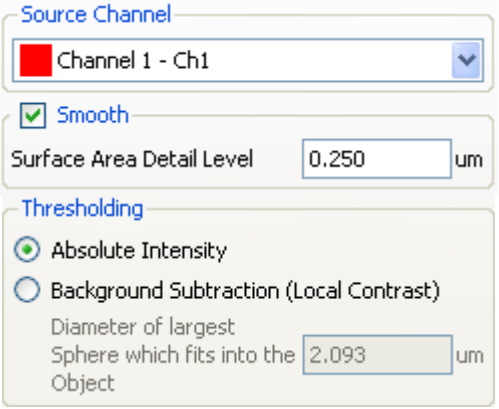
In the **Objects** toolbar of the Surpass view click on the icon to add a new Surfaces item.
Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

Creation Wizard - 1/7 Algorithm

For algorithm setting select **Track Surfaces.**

Click on (Next).

Creation Wizard - 2/7 Source Channel

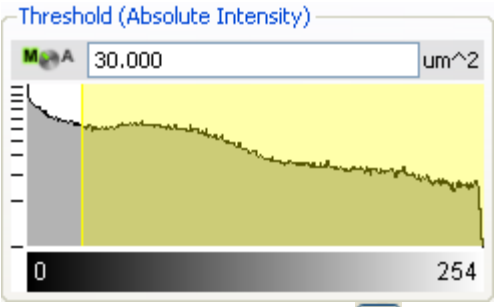


Select as **Source Channel** the **Channel 1** (red) and check the option **Smooth**. As **Surface Area Detail Level** use **0.250 um and as a Thresholding** select option **Absolute Intensity.**

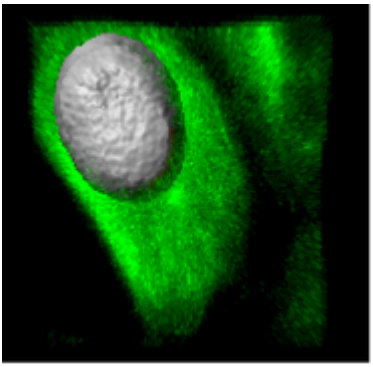
Click on (Next).

Creation Wizard - 3/7 Threshold

For the **Threshold** adjustment select **Manual**, and set as threshold **30.000**.



This is the result in the viewing area.Click on (Next).



Creation Wizard - 4/7 Classify Surfaces

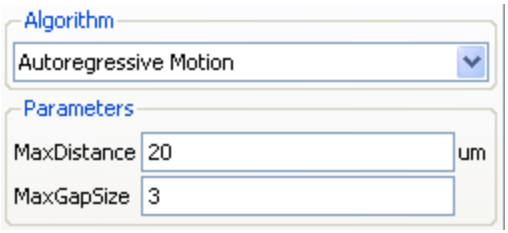
On the tab **Classify Surfaces** you can sort and filter the resulting Surfaces by various filter criteria. In this example, a sorting is not necessary. You can delete all **Filters** and click on (Next).

Creation Wizard - 5/7 Edit Surfaces

In the step **Edit Surfaces** you can edit the resulting Surfaces. In this example, an editing is not necessary. Click on (Next).

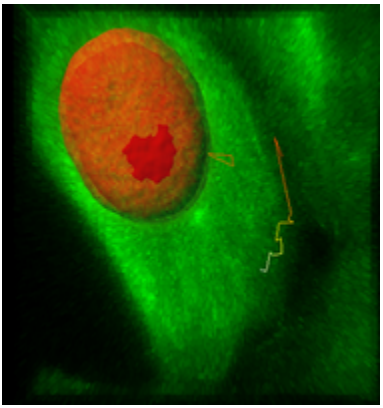
Creation Wizard - 6/7 Tracking

Select Autoregressive Motion as Algorithm and MaxDistance set 20.000 um, and for the MaxGapSize set 3. Click on (Next).



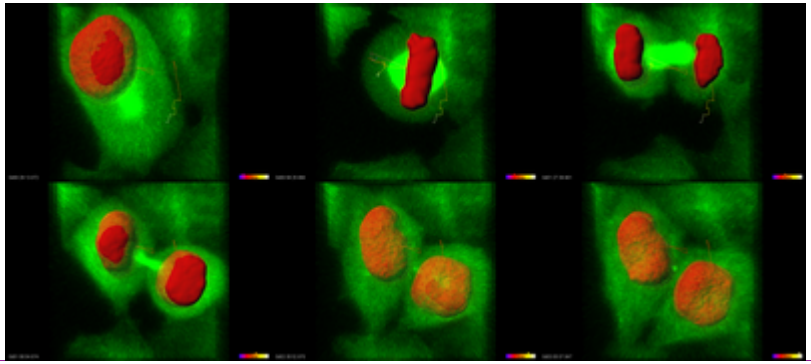
Creation Wizard - 7/7 Classify Tracks

On the tab Classify Track you can sort and filter the resulting Tracks by various filter criteria. In this example, a sorting is not necessary. You can delete all Filters and click on (Next). To complete Surface creation click on (Finish).



Additional to the Volume rendered tubulin proteins of the spindle the

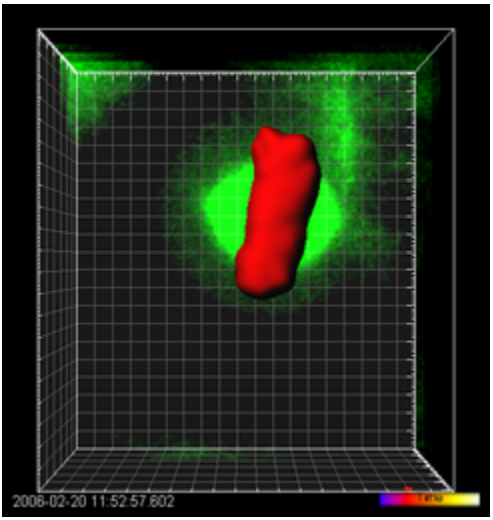
Surfaces reconstruction of the histone 2B proteins displays in the viewing area. In the Display Adjustment window now un-check the box for the Volume rendering of red channel. New created surface is displayed in viewing area. In the viewing area time depended movement of newly created surfaces represented in film frames numbers 4, 7, 10, 13, 17 and 20.



See also: Visualize Data Set - Create Surface 45

11.3 Time Concept

The time concept in Imaris is very intuitive to understand. Whenever you load a time series automatically the **Time** window displays at the bottom of the screen. In the viewing area down to the right the Time Colorbar displays. The arrow indicates the actual time point (in this example time point 7 out of 22).



HeLa Cell at Time Point 7

11.4 Calculate Statistics

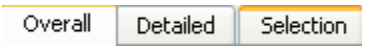
Now you will evaluate statistically for each Surface object the volume for the related time point.

Please note: The statistical calculation is based on the selected Object List item.

Highlight with a click the Object List item **Surfaces**.

Start the Calculation and Show Results

Click on the tab **Statistics** to start the calculation. There are three statistical tabs **Overall, Detailed, Selection.**

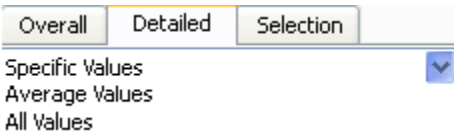


Overall

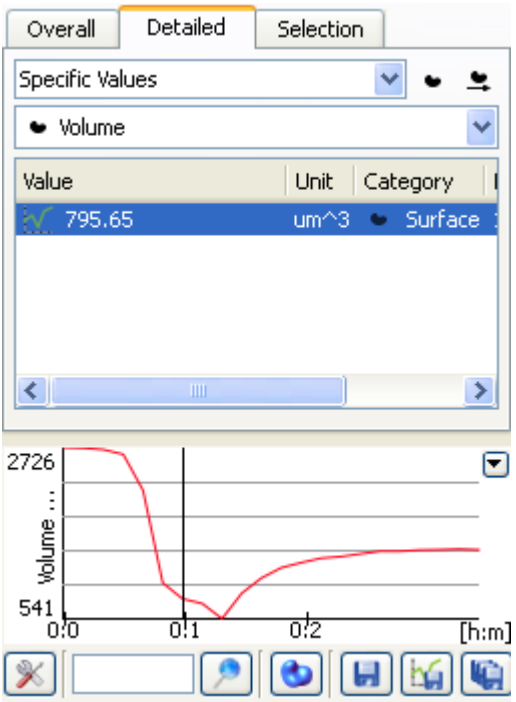
The output of the Overall statistics provides an overview of the image single determining variables.

Detailed

Pull down list is availed offering three types of Detailed statistical calculations: Specific Values, Average Values, All Values.



To display the statistical time plots: choose "Statistics tab Detailed / Specific value / Volume". Select the value in the list, and the time plot will be displayed.



For further analysis the current selection can be exported to Excel by click on the single-disk-button .

Specific values

In the Specific Values second pull down list at the top of the window present available specific statistical values.

Configure List of Visible Statistical Values

Button **Settings**

Please note: If a statistical value does not not show up, the respective statistical value is probably disabled. Click the button **Settings** on the tab **Statistics** to enable the value.

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.

Export all Statistics to File

Export current selection of Statistical Tab.

Export selected Data for plotting.

Export all statistical files.

Click on the button **Export**. If you select 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: Volume over Time - Present Results 117

11.5 Present Results

On your computer Imaris runs now in the background and Excel runs in the foreground. This chapter will focus on the statistical table in MS Excel. All statistical values of the Surfaces are sorted in different sheets. You find the **Volume** for the different time points in the sheet Volume.

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

Statistical Table

All time points and the corresponding Volume(s) are listed. In Excel the numbers are now sorted by and then by time-point (column "time") and than by track (column "parent"). In Excel image calculated statistical data can be represent using different charts modifications and adjustments.

	A	B	C	D	E	F
1	Value	Unit	Category	Time	Parent	ID
2	2725.06	um^3	Surface	1	1000000000	0
3	2718.67	um^3	Surface	2	1000000000	1
4	2699.41	um^3	Surface	3	1000000000	2
5	2639.22	um^3	Surface	4	1000000000	3
6	2179.13	um^3	Surface	5	1000000000	4
7	997.84	um^3	Surface	6	1000000000	5
8	795.65	um^3	Surface	7	1000000000	6
9	734.89	um^3	Surface	8	1000000000	7
10	541.74	um^3	Surface	9	1000000000	8
11	866.98	um^3	Surface	10	1000000000	10
12	1061.47	um^3	Surface	11	1000000000	12
13	1192.56	um^3	Surface	12	1000000000	15
14	1256.34	um^3	Surface	13	1000000000	17
15	1313.07	um^3	Surface	14	1000000000	19
16	1333.27	um^3	Surface	15	1000000000	21
17	1368.34	um^3	Surface	16	1000000000	23
18	1402.89	um^3	Surface	17	1000000000	25
19	1402.58	um^3	Surface	18	1000000000	27
20	1417.27	um^3	Surface	19	1000000000	29
21	1421.42	um^3	Surface	20	1000000000	31
22	1429.56	um^3	Surface	21	1000000000	33
23	1420.49	um^3	Surface	22	1000000000	35
24	260.44	um^3	Surface	9	1000000001	9
25	273.35	um^3	Surface	10	1000000001	11
26	590.15	um^3	Surface	11	1000000001	13
27	901.70	um^3	Surface	12	1000000001	14
28	1114.51	um^3	Surface	13	1000000001	16
29	1286.90	um^3	Surface	14	1000000001	18
30	1333.38	um^3	Surface	15	1000000001	20
31	1366.22	um^3	Surface	16	1000000001	22
32	1398.25	um^3	Surface	17	1000000001	24
33	1410.88	um^3	Surface	18	1000000001	26

Please close Imaris before you start with the next tutorial.

12 ImarisXT

The ImarisXT Programmable Interface is a part of Imaris that will enable the Imaris user to meet their evolving needs by developing their own algorithms, incorporating them into Imaris, and as a result enhance Imaris image processing and analysis capability.
By providing interfaces known to both parties, the ImarisXT Programmable Interface facilitates the communication between the biologists and computer scientists. Design and development of specific applications that fit individual projects, while meeting the scientific community's requirements and the software developer's conditions are achievable through the highly configurable and extensible ImarisXT Programmable Interface. The ImarisXT Programmable Interface extends the user interface beyond the customary boundaries.

12.1 ImarisXT Features and Advantages

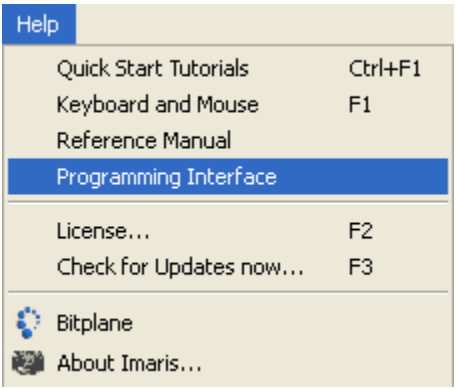
ImarisXT is a multi-functional two-way interface from Imaris to classic programming languages, Matlab and other imaging software programs.

Features and Advantage

To use ImarisXT right out of the box, there is no need to write any code. However, you can include your own image processing and analysis functions and extend Imaris capabilities, using your favorite programming language (e.g. Matlab, Java, C, C++, and VisualBasic®). ImarisXT lets you turn your ideas into advanced software options to target your specific applications and suit your own preferences. By creating your own custom routines and integrating them in Imaris, you are pursuing the limitless potential of image processing and analysis.

Documentation

The XTensions documentation is available online as HTML files and in PDF formats. To access the help, in Imaris from the **Help** menu, select **Programming Interface.**



Components

Minimum software requirements are:

- Imaris version 4.1.2 or higher.
- ImarisXT

- XTensions installed with Imaris (MatLab), or downloaded from the website (ImageJ); or

XTensions written in a development environment supporting the COM interface such as Matlab, C++, C#, Java.

MATLAB code

The MATLAB code for all functions could be viewed by using the link:
http://www.bitplane.com/go/products/imarisxt/xtensions/matlab
In Matlab from the menu bar of the Matlab desktop, select **Help / Matlab Help**" or <**F1**>.
Alternatively write in the Command Line

```
>> doc actxserver
```

12.2 Programmable Interface

ImarisXT Programmable Interface consists of three major components:

- COM Interface
- Expandable User Interface
- Predefined Set of XTension

Component Object Model (COM)

Component Object Model (COM) is an interface standard for software componentry introduced by Microsoft in 1993. It is used to enable interprocess communication and dynamic object creation in a large range of programming languages.
Imaris XT incorporates a COM interface that provides a method for sharing code between Imaris and different programming languages and/or other imaging software programs. The COM Interface contains more than 300 functions that represent and can set off some of the tasks available in Imaris. COM interface technology supports easy integration of newly developed XTensions directly into Imaris, expanding its functionality.

The COM interface has two major functions:

1. The COM interface provides communication between Imaris and other programming languages.
It allows querying information from and sending commands to Imaris.
2. The COM interface facilitates image data exchange.
Data can be image raw data with voxel or color description but can also be data with segmentation results (coordinates of Surfaces, Spots, Tracks, Filament, Measurement Points etc.)

Expandable User Interface

The Expandable user interface accommodates expansion of the Imaris interface beyond the predefined configuration that comes with the software. It allows you to add interface elements that access your own custom Imaris XTensions, right along-side the built-in elements that launch core features of Imaris. To incorporate your individually developed XTension into Imaris, you are not required to modify, or even access Imaris code. The ImarisXT expandable user interface handles all details of incorporating, executing and presenting the results of this new XTension. It also provides a method for new XTensions to be

called by the touch of a single button.
There is no limitation to the number of external functions that can be integrated into Imaris.
Integration of new XTensions into Imaris is available in three locations of the Imaris user interface:

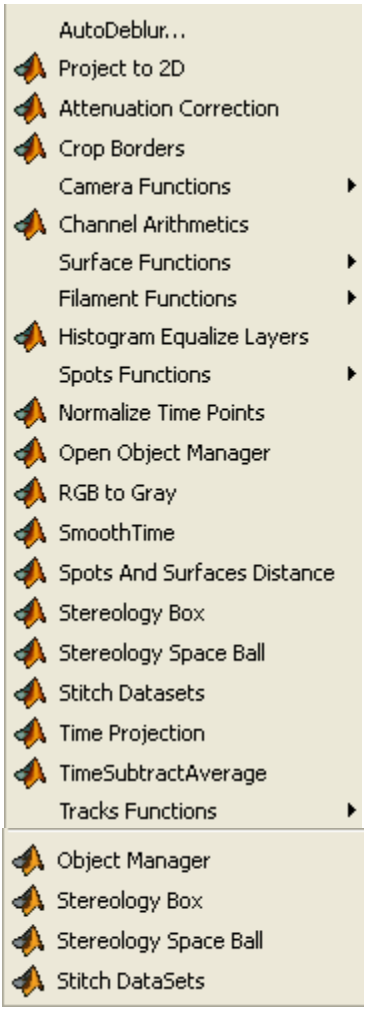
1. Image Processing Menu
2. Surpass Tool Bar (which is also reflected by the Surpass main menu)
3. Surpass Tools Tab

XTensions

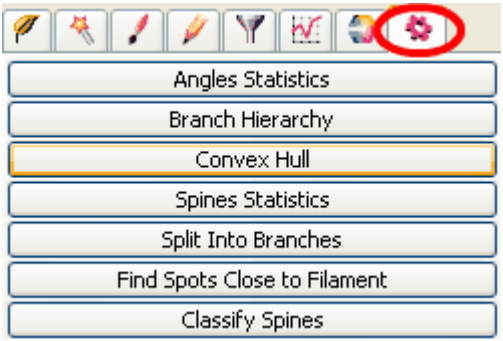
XTensions are a collection of supplemental utility functions that you set up to extend the capabilities of Imaris by adding custom commands and specialized features. You can program a new XTension using any of many different programming languages to fulfill your individual requirements and suit your own preferences.

Predefined Set of XTensions

Through the course of the Imaris software development, Bitplane has also developed XTensions. Most of that predefined set of XTensions are MATLAB M-files, which are a series of MATLAB statements that implement specialized image processing algorithms. XTensions are modular and flexible, providing users with an excellent starting point to extend the capabilities of the predefined Imaris XTensions by writing your own custom routines as M-files, or to combine one with other XTensions.
The individual Matlab files (.m files) are stored within a unique folder called XTensions (default folder name).
The XTensions are available in the menu Image Processing and in the menu Surpass.



Additionally the XTensions are available on the Tools tab of the Surpass object that they operate on.



Requirements: To use the XTensions shown here you need ImarisXT and Matlab running on the same computer. Some XTensions also require the Matlab Image Processing Toolbox.

ImageJ

ImarisXT has a special XTension in the form of a plug-in for **ImageJ** that combines the capabilities of Imaris with the available functions in ImageJ.

It is hoped that this set of predefined XTensions will prompt the Imaris user community to establish a common repository where external programs are accumulated and freely shared and that this repository be expanded to encompass all areas of image processing and analysis.

12.3 InvertMiddleSlice

If you feel comfortable that Imaris helps you analyze and process your images without knowing what goes on behind the curtain, feel free to skip this tutorial. To fully benefit from this example, you should be comfortable with Matlab and be familiar with basic computer programming. On the other hand, if your field of expertise is not computer programming, the purpose of this tutorial may well be to initiate your interest in the wide possibilities offered by MATLAB.
The tutorial includes complete example applications written in Matlab with introduction of some basic image processing concepts. Programmers who are familiar with COM should have no problems following and translating the code. The example starts by reading an image into the MATLAB workspace and then performs an InvertMiddleSlice function on the image. The example also provides a guided step-by-step procedure how to embed the InvertMiddleSlice Function as an XTension into Imaris.

Start MATLAB

Clear the MATLAB workspace of any variables and close open figure windows.

```
close all
```

Start Imaris from the Matlab Command Line.
First you will open Imaris from Matlab.

```
vImarisApplication = actxserver('Imaris.Application');
vImarisApplication.mVisible = true;
```

Imaris is launched.
In Imaris load the dataset retina.ims using the menu "File / Open".

Switch back to MATLAB.

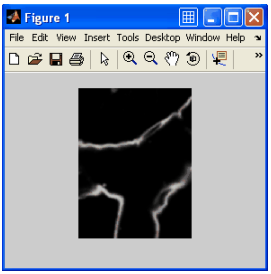
Now you will make a copy of slice 32 (first channel, first time point), and name it "vSlice".

```
vSlice = vImarisApplication.mDataSet.GetDataSlice(32,0,0);
```

If you click on the Workspace tab of Matlab, you will now see a variable named vSlice.
The next command is to show that slice as an image in Matlab.

```
imshow(vSlice);
```

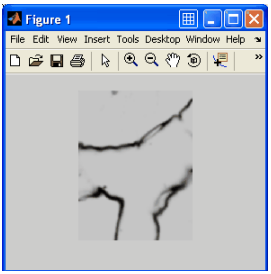
As a result Retina slice 32 is open in Matlab:



Now you will invert the intensity data and send it back to Imaris.
Matlab vector-arithmetic lets us process an image with a single command.

```
vSliceInverted = max(vSlice(:)) - vSlice;
imshow(vSliceInverted);
```

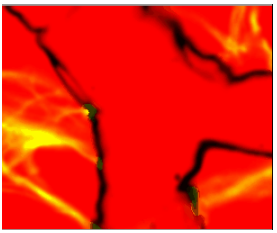
An image of the Retina.ims intensity-inverted slice 32 is now open in Matlab:



To copy the inverted Slice and send it back to Imaris, write the command:

```
vImarisApplication.mDataSet.SetDataSlice(vSliceInverted,32,0,0);
```

This is the result in the Imaris viewing area:



12.4 Create the M-File"InvertMiddleSlice.m"

The following example will help you create the M-File "InvertMiddleSlice.m". M-scripts and Functions must be written in separate files.

Note: M-files are saved in the Work folder in the MATLAB program files subdirectory

Note: Variable names are case-sensitive.

From the Matlab menus select **File / New / M-File**, and a new Editor window appears. Write in the Editor window this code:

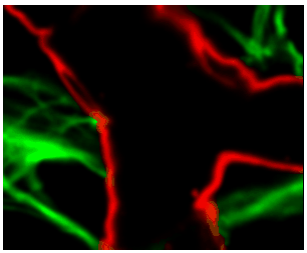
```
% This function inverts the middle slice
function InvertMiddleSlice(vImarisApplication)
% Set up middle slice index
vIndexZ = vImarisApplication.mDataSet.mSizeZ / 2;
vIndexC = 0;
vIndexT = 0;
% Get slice, invert it, send back
vSlice =
vImarisApplication.mDataSet.GetDataSlice(vIndexZ,vIndexC,vIndexT);
vSliceInverted = max(vSlice(:)) - vSlice;

vImarisApplication.mDataSet.SetDataSlice(vSliceInverted,vIndexZ,vInd
exC,vIndexT);
```

Save the file.

If there are any errors in your code, they will show up as messages in red text in the prompt window.

Now you can try the new **InvertMiddleSlice** Function.
To run the new function on the Matlab command line, call:

```
InvertMiddleSlice(vImarisApplication);
```



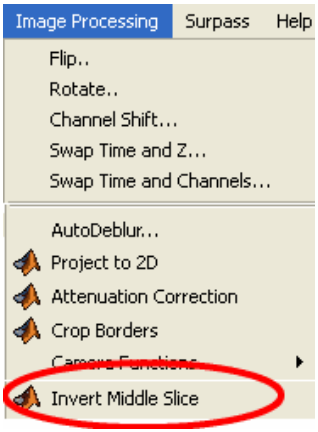
12.5 Embed the new Function as XTension

Embed the new Function as an XTension into Imaris and change the description of created M file.
By adding a special header to the M-file, and copying the M-file to the special XTensions folder of the Imaris Program Files path, a user-written command can be given point-and-click access within Imaris. Because we are no longer launching the function from within the Matlab interface, you will also need to change the first few lines of the M-file: the function line where the new command is defined, and also several lines that connect to the COM interface of Imaris and define the vImarisApplication variable differently than the earlier example.

```
% <CustomTools>
% <Menu>
% <Item name="Invert Middle Slice" icon="Matlab" tooltip="This
Inverts!">
% <Command>Matlab::InvertMiddleSlice(%i)</Command>
% </Item>
% </Menu>
% </CustomTools>

function InvertMiddleSlice(aImarisApplicationID)
if isa(aImarisApplicationID, 'COM.Imaris_Application')
vImarisApplication = aImarisApplicationID;
else
vImarisServer = actxserver('ImarisServer.Server');
vImarisApplication =
    vImarisServer.GetObject(aImarisApplicationID);
end

% The lines below are the same as the first tutorial example
M-file
% Set up middle slice index
vIndexZ = vImarisApplication.mDataSet.mSizeZ / 2;
vIndexC = 0;
vIndexT = 0;
% Get slice, invert it, send back
vSlice =
vImarisApplication.mDataSet.GetDataSlice(vIndexZ,vIndexC,vIndexT);
vSliceInverted = max(vSlice(:)) - vSlice;
```

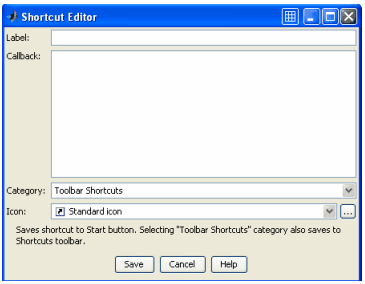
```
vImarisApplication.mDataSet.SetDataSlice(vSliceInverted,vIndexZ,vIndexC,vIndexT);
```

Save the M-file, and copy it to the special folder:" **C:\Program Files\Bitplane\Imaris 6.2\XTensions".**
Close Matlab and restart Imaris (Imaris checks the XTensions folder when it starts).
The new created XTensions will appear in the menu Image Processing.



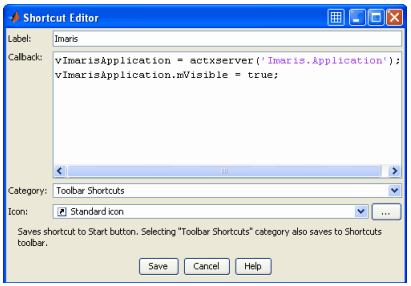
12.6 Matlab Shortcut to Start Imaris

This tutorial will show you the code you need to write to have Imaris shortcuts created in Matlab. At the top of the Matlab window right click on the **Shortcuts toolbar** and choose New Shortcut. A new shortcut editor window will appear.

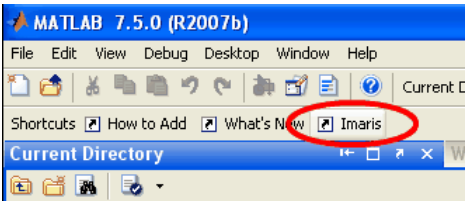


In the **Label** field enter **Imaris**. In the Callback field enter:

```
vImarisApplication = actxserver('Imaris.Application');
vImarisApplication.mVisible = true;
```



Click "**Save**".



Clicking on the shortcut icon takes you directly to Imaris.

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