

Quick Start Tutorials

Imaris 7.3

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1 Introduction

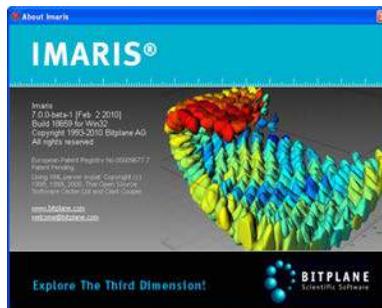
Why should you read and practice the Imaris Quick Start Tutorials?

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 12 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 the [Reference Manual](#)^[3] 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

1 - Introduction

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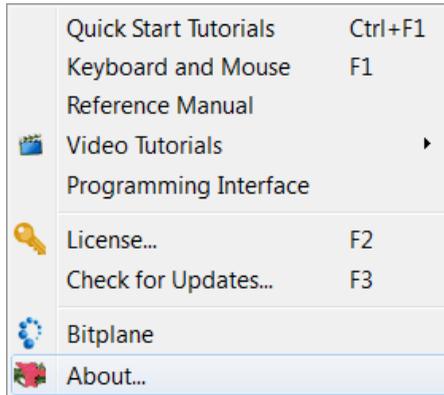
For further questions or suggestions please visit our web site at: www.bitplane.com or contact eusupport@bitplane.com.

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1.1 Help Menu

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



The Imaris Reference Manual displays.

Imaris Reference Manual

Contents | [Search](#)

- [+ Preface](#)
- [+ Menu File](#)
- [+ Menu Edit](#)
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- [+ Section View](#)
- [+ Gallery View](#)
- [+ Easy 3D View](#)
- [+ Surpass View](#)
- [+ Animation](#)
- [+ InMotion](#)
- [+ Coloc](#)
- [+ Addendum](#)

Gallery View

How to Open the Gallery View?

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

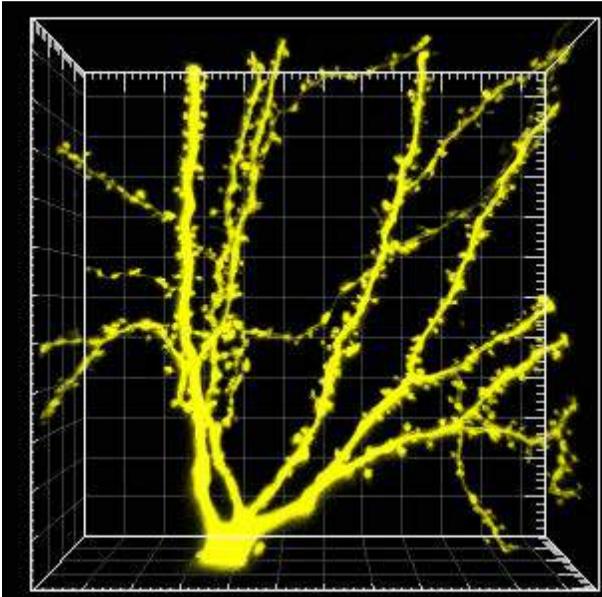
See also:
 Gallery View - [Mouse & Keyboard Mac](#)

1 - Introduction

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](#)^[8] to create a 3D volume visualization. In the chapter [Select and Navigate](#)^[15] you will learn about the two different mouse pointer modes in the Surpass view. You will learn how to manipulate the view by [Rotating](#)^[16], [Translating](#)^[17] or [Scaling](#)^[18] 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](#)^[11].



Visualized Data Set, Pyramidal Cell

User Level: Beginner

Module: Imaris

2 - Visualize Data Set

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.

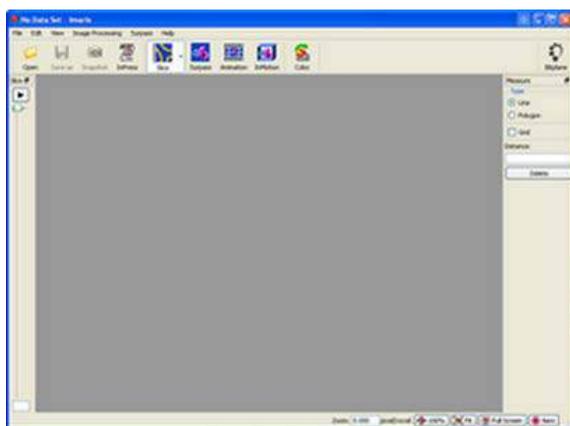
After installation, upon the first program start Imaris is in the Surpass view.



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



PC Main Screen



This is the Imaris Slice view on a PC.

Visualize Data Set - 2

Mac Main Screen



This is the Imaris Slice view on a Mac.

2 - Visualize Data Set

2.2 Open Data Set



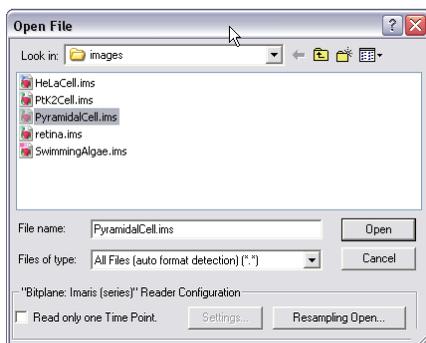
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

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

Open a Data Set in the Slice View



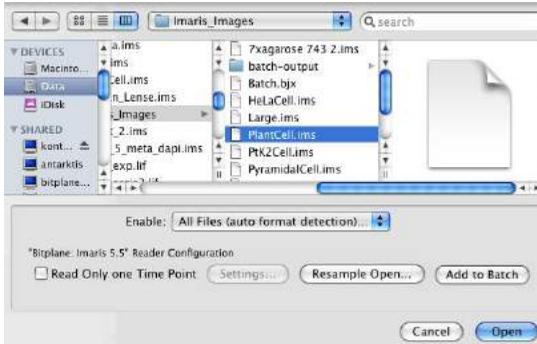
Switch to the Slice view by clicking on the **Slice** button in the main tool bar of Imaris and then click on **Open**. The **Open File** selection window displays on the screen.



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

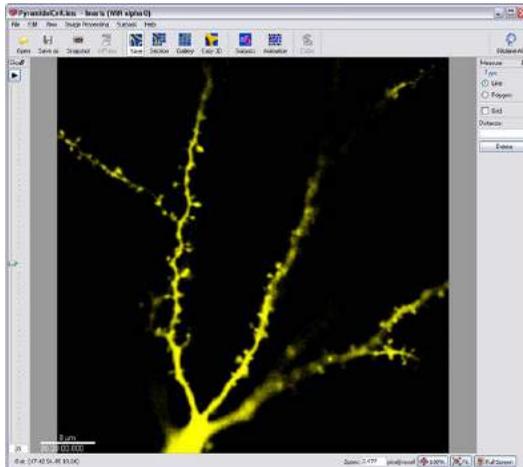
This is the Mac **File Open** dialog:

Visualize Data Set - 2



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

The Slice View



2 - Visualize Data Set

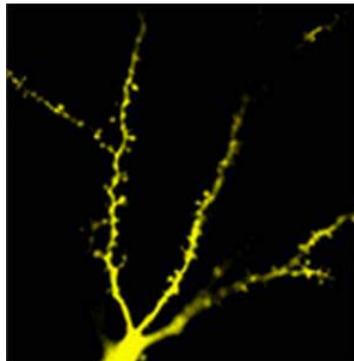
2.3 Slice View

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

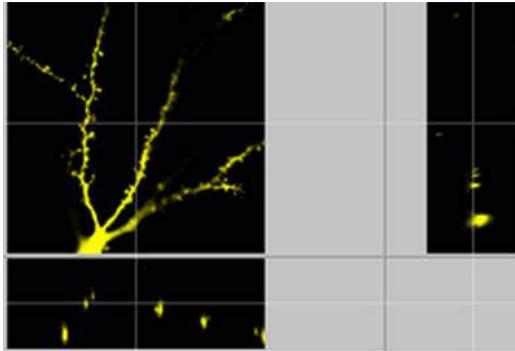


	Slice	Ctrl+1
	Section	Ctrl+2
	Gallery	Ctrl+3
	Easy 3D	Ctrl+4

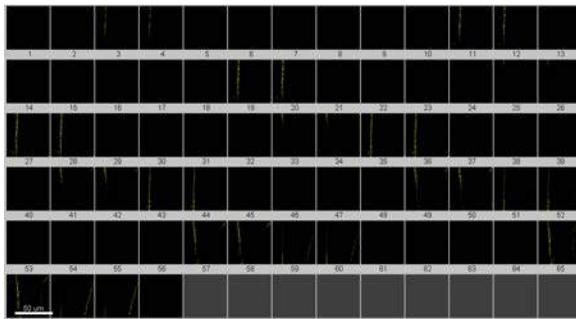
Slice view for individual slices:



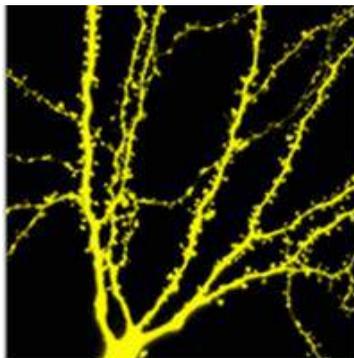
Selection view for XY; XZ; YZ views and navigation to any position in 3D:



Gallery view for viewing individual slices simultaneously:



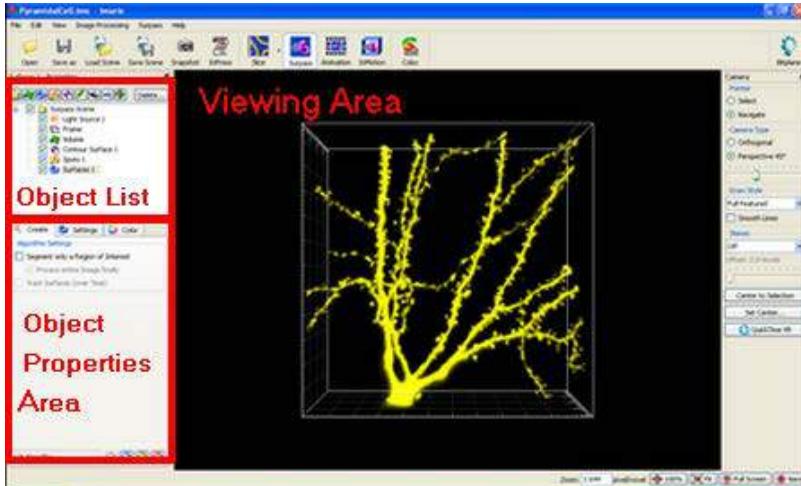
Easy 3D for 3D projection along Z



2 - Visualize Data Set

2.4 Surpass View

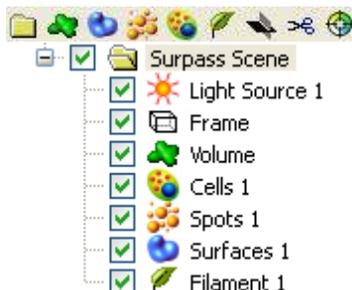
The Imaris is built around the three-panel workspace: **View Area**, **Object List** and **Object Properties Area**.



Viewing 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 **Surpass Scene**. 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 Creation 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.

2 - Visualize Data Set

2.5 Volume

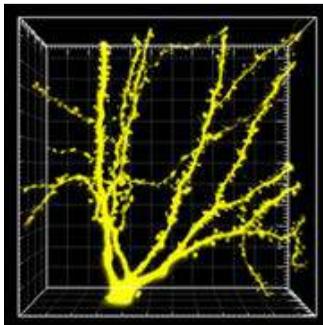
Now you will create a 3D reconstruction of the Pyramidal cell.

Select Volume in the Surpass View

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



The Pyramidal cell displays in the viewing area.



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.

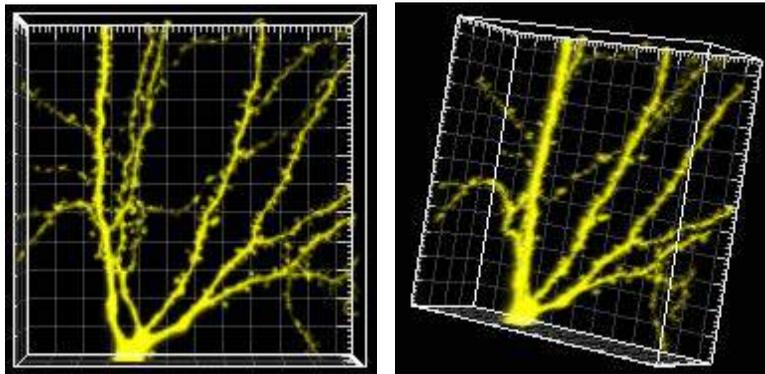
When to Use Navigate?

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

2.7 Rotate Image

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

How to Rotate an Image?

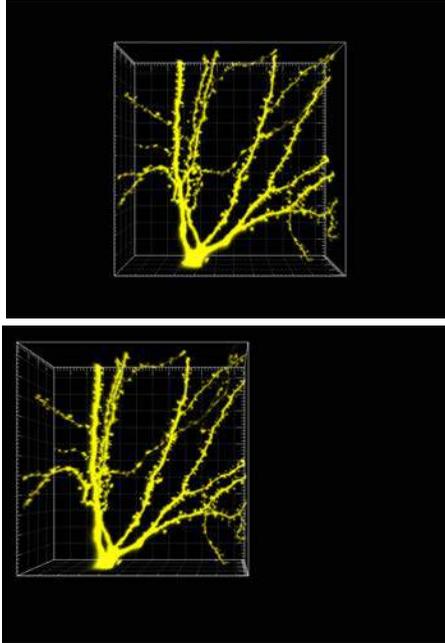


1. Choose the mouse pointer mode **Navigate**.
2. Click with the mouse in the image and hold the button down while moving the mouse (hold + drag). The image on screen is rotated towards the direction the mouse is dragged. Be sure to hold the mouse button down during the whole rotation.
3. Stop moving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

2.8 Translate Image

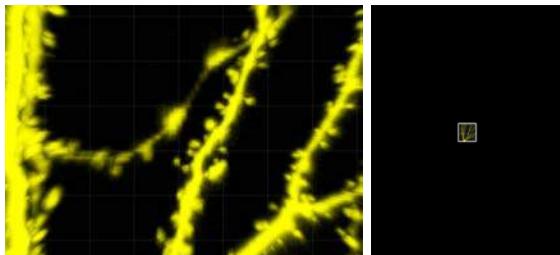


1. Choose the mouse pointer mode **Navigate**.
2. To move the image within the Surpass view (pan the object) hold the right mouse button and drag the image to the chosen location (for Mac users: ctrl + click & drag to pan).
3. Release the right mouse button to place the image.

2 - Visualize Data Set

2.9 Scale Image

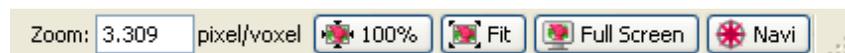
In the Surpass view you zoom the image either by using the mouse or by selecting one of the buttons at the bottom of the screen.



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

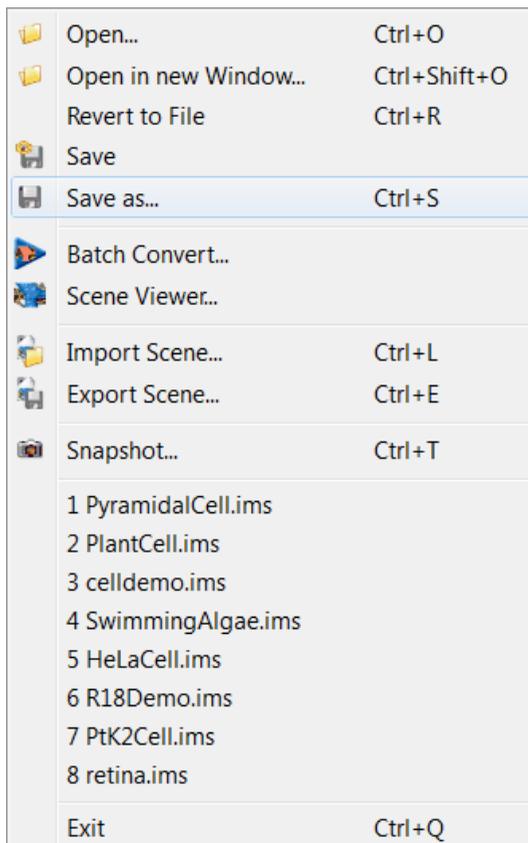


- | | |
|--------------------|-----------------------------------------------------------------------------------------------------|
| Zoom | Enter the zoom factor. |
| 100% | Rotate image to original position, center in the middle and set zoom factor to one pixel per voxel. |
| Fit | Pan position to 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). |

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**.



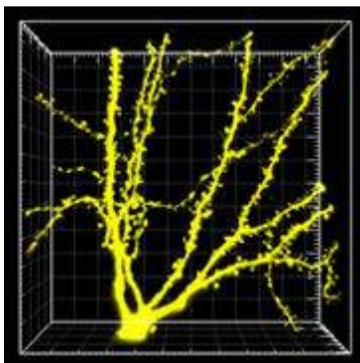
2 - Visualize Data Set

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, then rotate the image backwards and then rotate it clockwise.

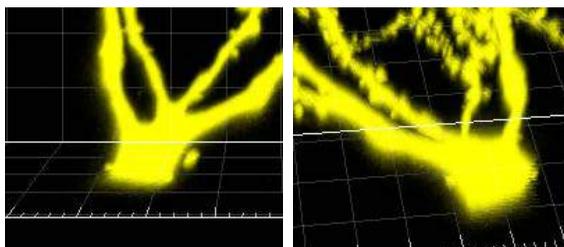
Home Position

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.

Rotate Backwards

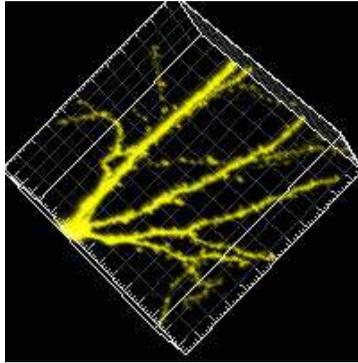


Now rotate the image backwards. Choose the mouse pointer mode

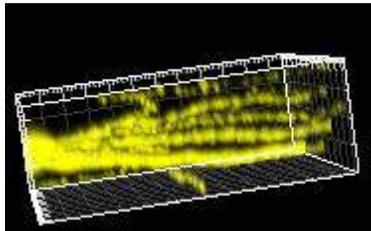
Visualize Data Set - 2

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.

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.

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

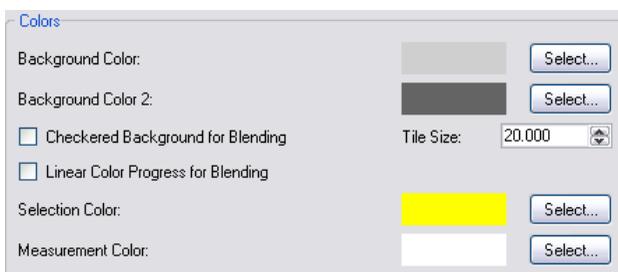
2 - Visualize Data Set

2.12 Change Background Color

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

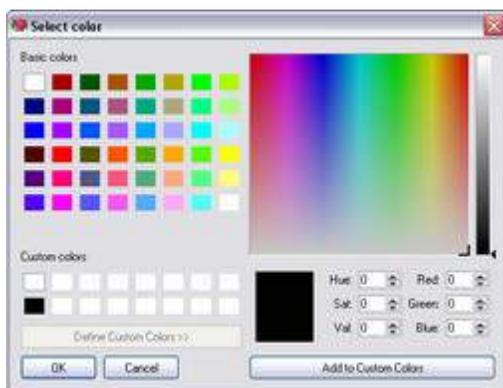
Open the Preferences - Display Window

Select the menu **Edit - Preferences ... - Display (Mac: Imaris-Preferences ...)**. On the right hand side you find the **Colors** dialog.

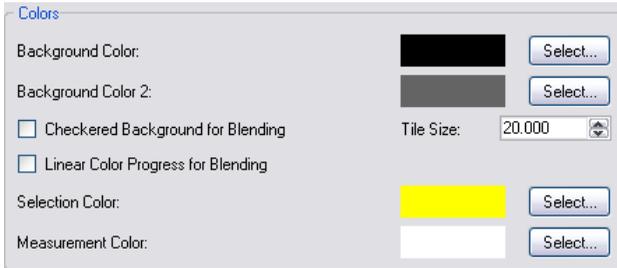


Change Background Color

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



Select the desired color and click on **OK**.



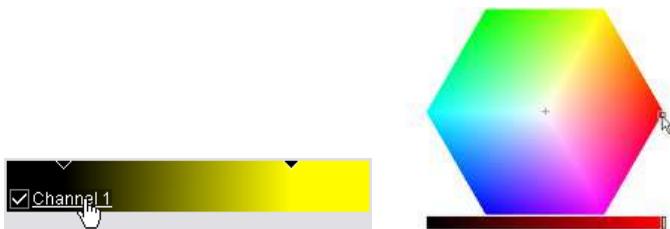
In the window **Preferences - Display** click on **Ok**.

The background color changes accordingly.

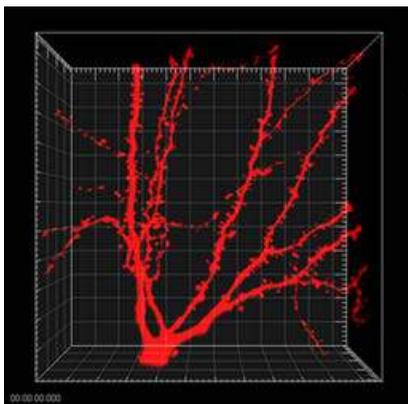
2 - Visualize Data Set

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:

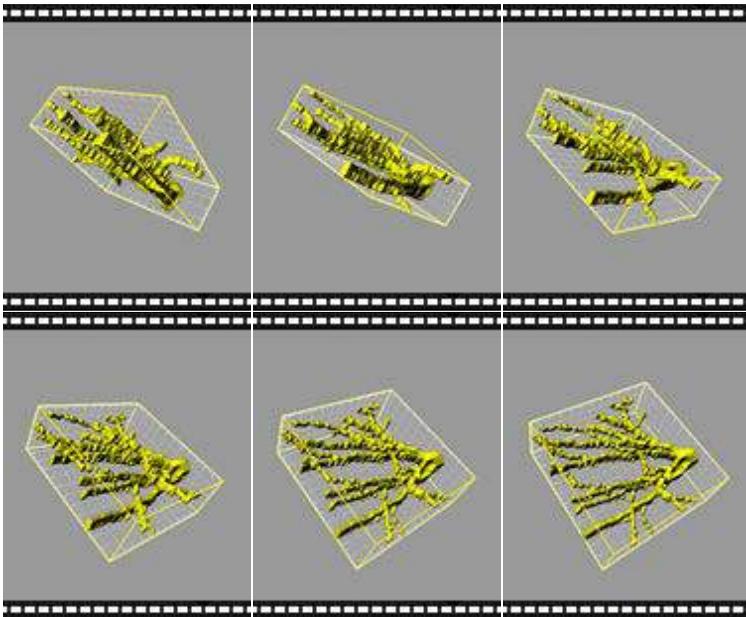


MIP Display, Channel Color Red, Pyramidal Cell

Please close Imaris before you start with the next tutorial.

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](#)^[5]. In this tutorial you will learn the [Key Frame Animation](#)^[27] functions, practice to subsequently [Shoot](#)^[29], [Play](#)^[29], and learn how to record an animation into a movie file using the [Save a Movie](#)^[32] options.



Movie Sequences, Pyramidal Cell

3 - Generating Movies

3.1 Load File

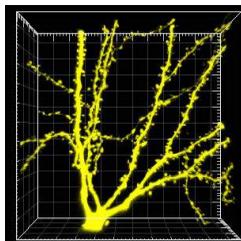


PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

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.



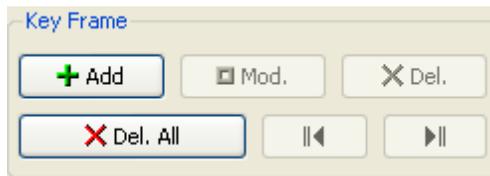
3.2 Key Frame Animation

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.



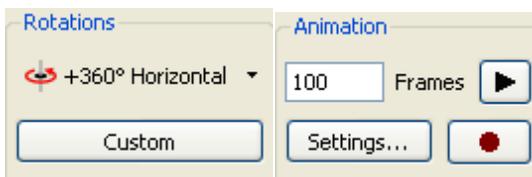
Click on the icon **Animation** in the toolbar. You find the **Key Frame Animation** window at the bottom of the screen. It is divided in five parts, the **Key Frame** window, the **Rotations** window, the **Animation** window, the **Play Back State** window, and the **Strip** window.

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.

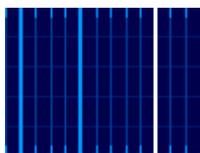
The Animation Window



3 - Generating Movies

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

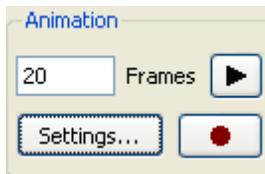
3.3 Shoot and Play

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.

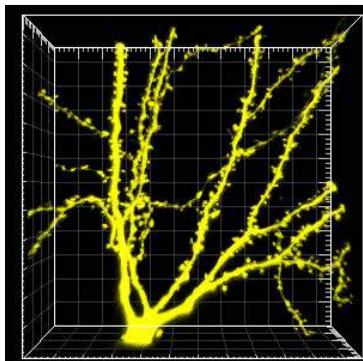
Please remember: Each time you click on the button **+Add** in the **Key Frame** window the camera captures the next key frame of your movie.

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

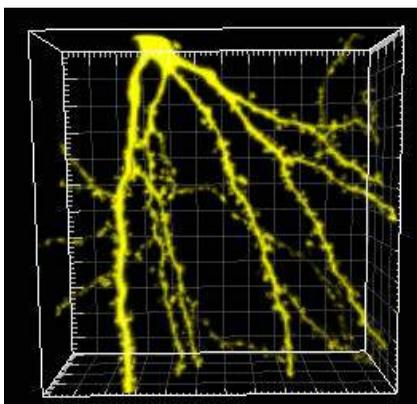


3 - Generating Movies

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.

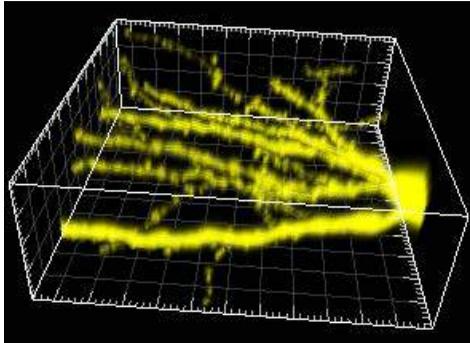
Add Additional Key Frames

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 the picture below:



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](#)^[16]

3 - Generating Movies

3.4 Save Movie

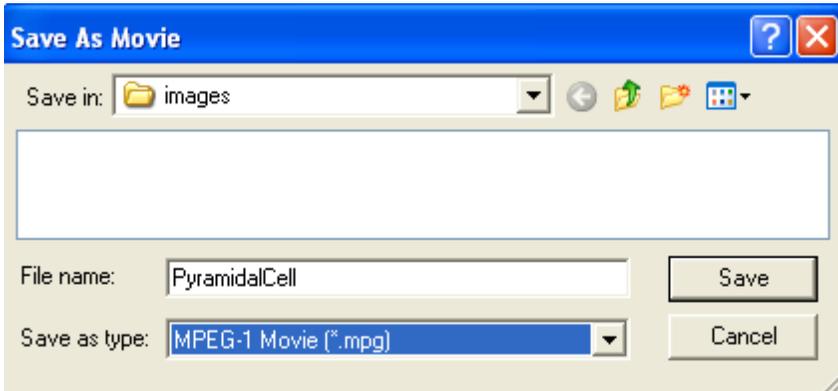
There are two possibilities to save the movie. Either as an Imaris File or as a movie-file.

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 an Movie

To save the animation as movie click in the **Animation** window on the button **Record** (●). The **Save As Movie** dialog window displays on screen.

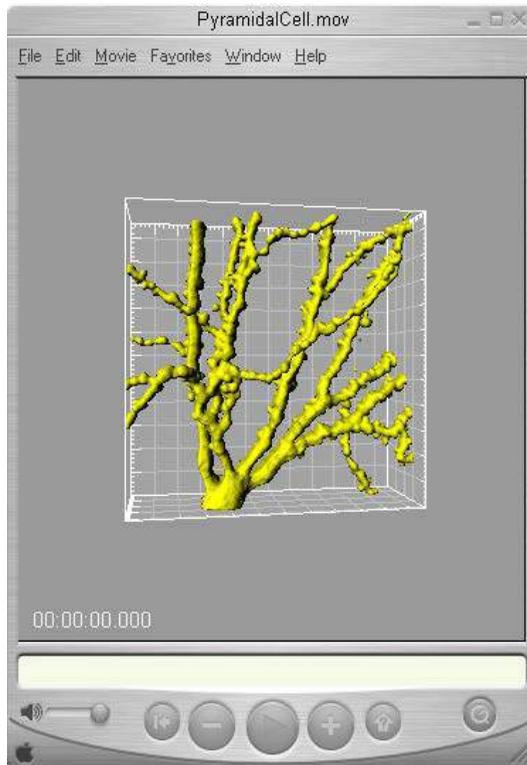


As **File name** type in the corresponding File name, use the default **Movie Settings** and click on **Save**.

Please close Imaris before you start with the next tutorial.

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

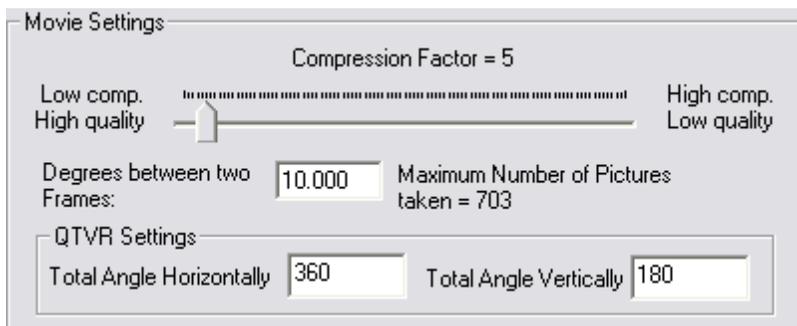
4 - Imaris and QuickTimeVR

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.

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



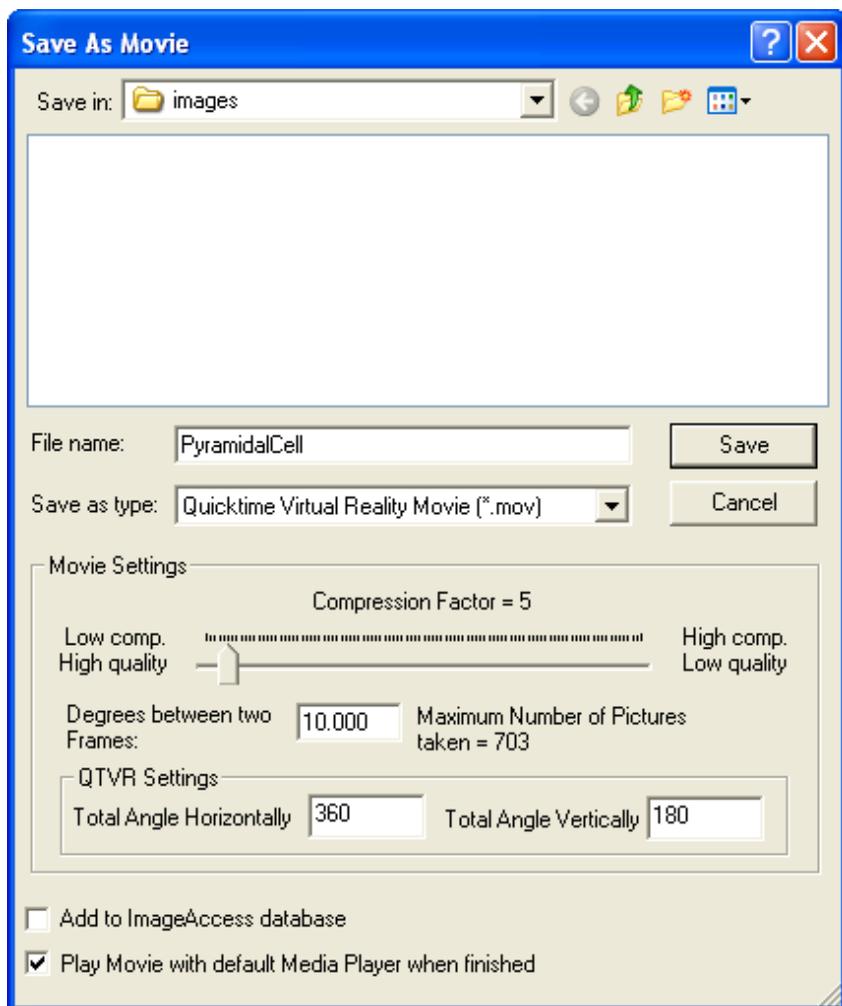
In the Surpass view click on the icon **QuickTimeVR** on the right hand side of the screen to open the **Save as Movie** dialog window.

Type in the **File name**, use the default **Movie Settings** and click on **Save**.

What Happens in the Imaris Viewing Area?

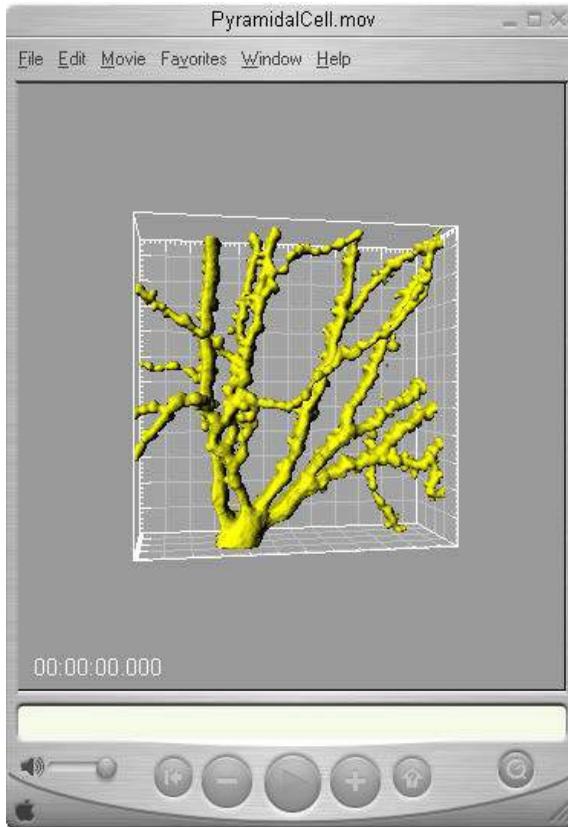
Automatically Imaris starts to turn around the image in the viewing area and takes a snapshot of each image position. The degree between two frames is 10, the angle horizontally is 360 and the angle vertically 180. Down to the right you find a progress bar. Depending on the settings, this procedure will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

4 - Imaris and QuickTimeVR



4.3 Interactive Display

Double-click on your QuickTime 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

In this chapter you will learn how to how to optimize the animation setting. With the optimized setting you can save a movie that can be either used for presentation or published over the Internet.

If you increase the degree between two frames less images are stored in the file. As 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.

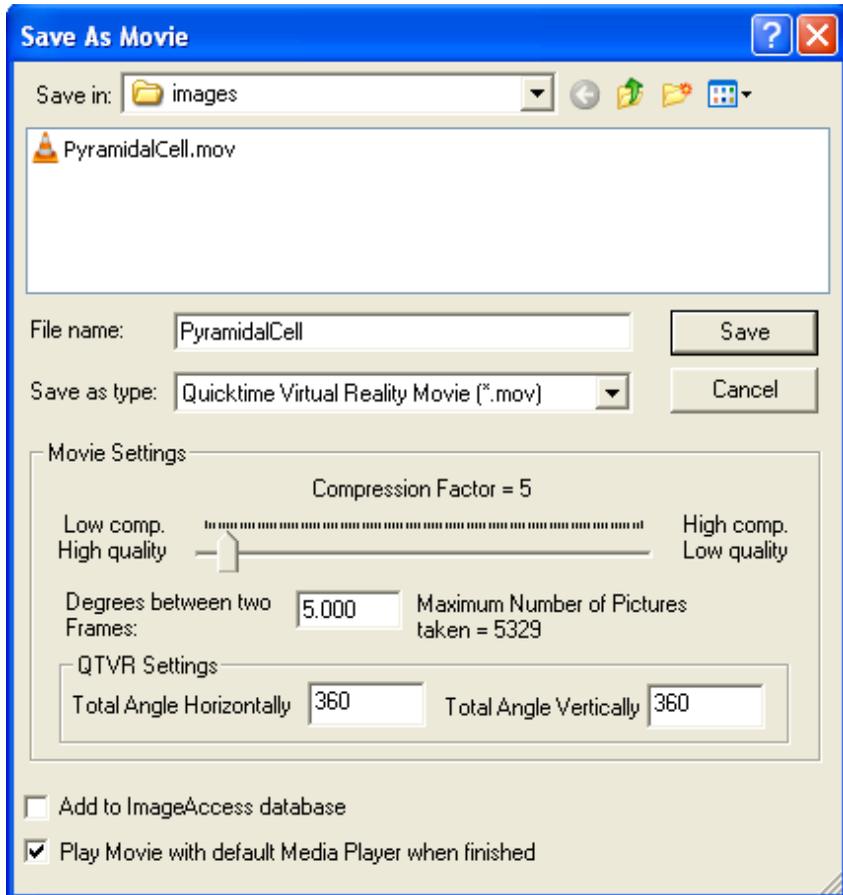
Adjust Settings for a Presentation

First adjust the size of the viewing area in Imaris, the display in QuickTimeVR is 1:1. Than 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.

Set the **Compression Factor to 5**. Enter **5** to the **Degrees between two Frames** field. Change the settings for the **Total Angle Horizontally** and the **Total Angle Vertically to 360**. Select the option **Play Movie with default Media Player when finished**. Finally click on the **Save** button.

Adjust Settings for a Web Page

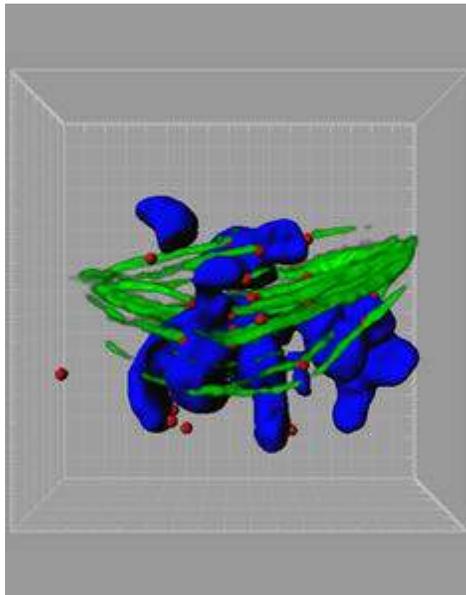
Adjust the size of the viewing area in Imaris and in the Surpass view click on the icon **QuickTimeVR** to open the **Save as Movie** dialog window. Alter the **Compression Factor to 50** and set the **Degrees between two Frames to 20**. Check **Play Movie with default Media Player when finished** and click on **Save**.



Please close Imaparis 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]. The [Object List](#)^[12] 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

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.

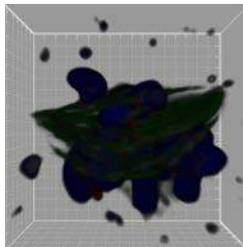
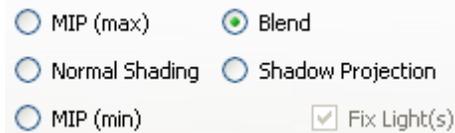
Open the demo image PtK2 cell.

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

Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PtK2Cell.ims

Volume

The image PtK2 cell is open. The image is presented is in **MIP** (Maximum Intensity Projection) Mode. Change display mode to **Blend**. The result of the Blend mode is a depth effect on the screen.



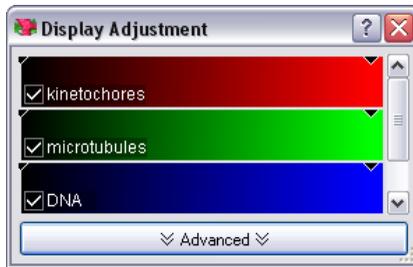
This is the PtK2 cell displayed in the viewing area.

5.2 Display Adjustment

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.

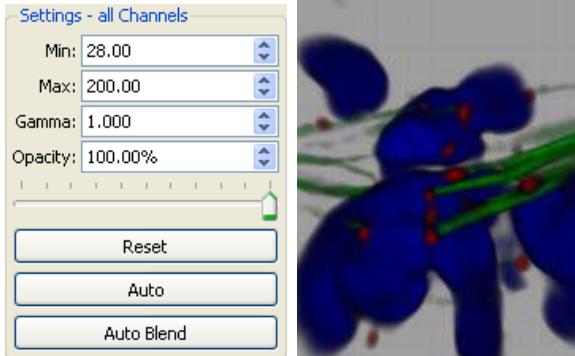
Adjust All Channels Simultaneously

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).

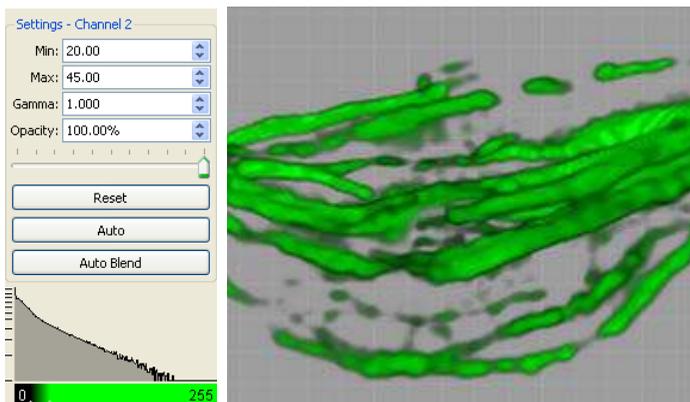
Design Mixed Model Rendering - 5



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, first un-check the box **Select all Channels**. Then un-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.



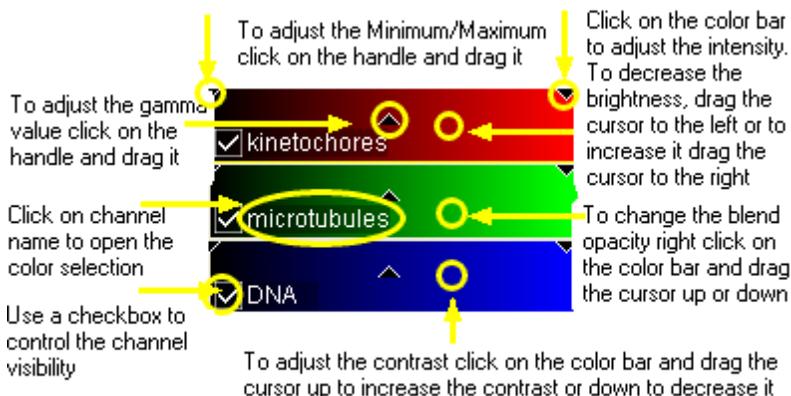
Set the **Range - Microtubules Min: 20.000** and **Max: 45.000**.

5 - Design Mixed Model Rendering

Now the red channel will be tuned. To focus on the red channel (kinetochores) un-check the box for the green and enable the red channel checkbox. 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.

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)

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

5 - Design Mixed Model Rendering

Region of Interest 1

	Min:	Max:	Size:
X:	65	192	128
Y:	65	192	128
Z:	1	32	32

Memory Usage: 0.500 MB

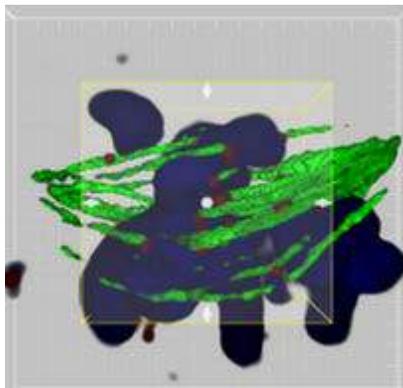
Regions of Interest

- Region of Interest 1

Add
Delete

Total Memory Usage: 0.500 MB

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 - Select mode).

Click on  (Next).

Creation Wizard - 3/6 Source Channel

Source Channel

Channel 3 - DNA

Smooth

Surface Area Detail Level μm

Thresholding

Absolute Intensity

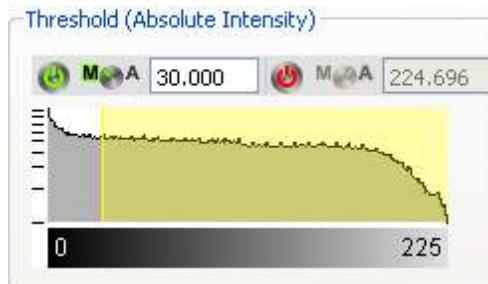
Background Subtraction (Local Contrast)

Diameter of largest
Sphere which fits into the μm
Object

As a Source Channel select the Channel 3 (DNA). Check the **Smooth** option to set up the smoothness of the resulting area. For the **Surface Area Detailed Level** accept the automatically generated value. For Threshold select the **Absolute Intensity** option.

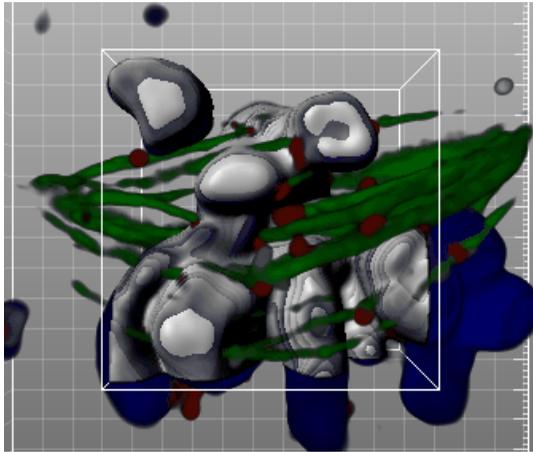
Click on  (Next).

Creation Wizard - 4/6 Threshold



For the Threshold adjustment select the Manual option and set a value to 30. In the viewing area a surface threshold preview is displayed in gray.

5 - Design Mixed Model Rendering



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 by clicking on the **Delete** Button. Click on  (Next).

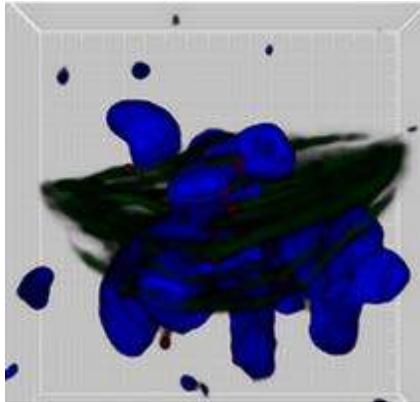
Creation Wizard - 6/6 Complete ROI

To complete Surface creation click on  (Finish).

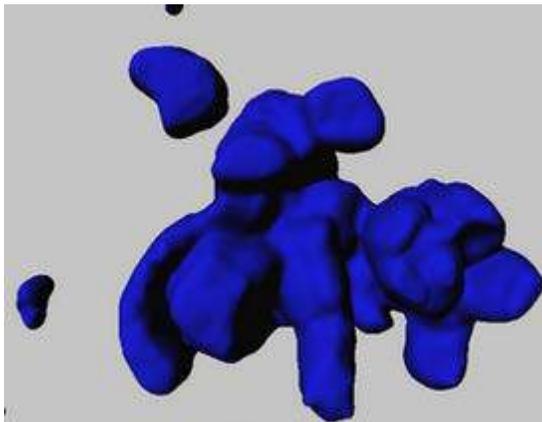
- Region of Interest

The parameters you have set up will now be used to compute Surfaces for the entire dataset. Depending on the size of the dataset this may take a while.

This is the result in the viewing area. In addition to the Volume rendered microtubules, the Surfaces reconstruction of the DNA is displayed.



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



Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

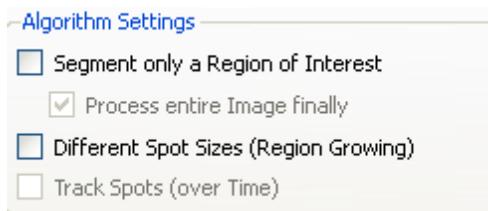
5 - Design Mixed Model Rendering

5.4 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. A new object, Spots, is added in the Object List and in the Object Properties Area the automatic Spots creation wizard is brought up.

Creation Wizard - 1/3 Algorithm



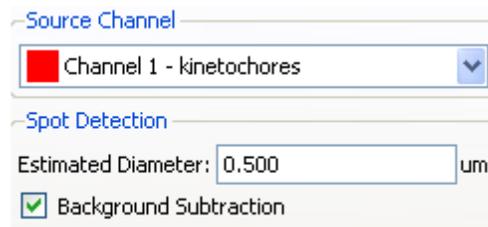
Algorithm Settings

- Segment only a Region of Interest
 - Process entire Image finally
- Different Spot Sizes (Region Growing)
- Track Spots (over Time)

In the first step, will process the whole image so leave the boxes **Segment only a Region of Interest** and **Different Spot Sizes (Region Growing)** un-checked.

Click on  (Next).

Creation Wizard - 2/3 Source Channel



Source Channel

 Channel 1 - kinetochores

Spot Detection

Estimated Diameter: um

- Background Subtraction

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

Subtraction option.

To define the minimum spot diameter please use Measure Structures features - [Line and Polygon](#)^[55].

Click on  (Next).

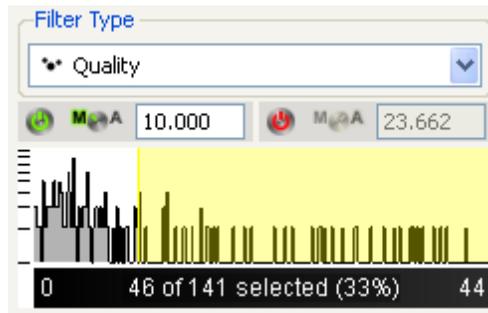
Creation Wizard - 3/3 Classify Spots

Imaris detects an automatic threshold at which to insert the Spots. In the Viewing Area you can see the gray created Spots objects and the Volume rendered red channel.

With the **Automatic** detection some Spots are not identified because they are under the threshold limit. To change the threshold, 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.

Moving the line to the left produces additional spots.

The effect is directly visible in the viewing area.

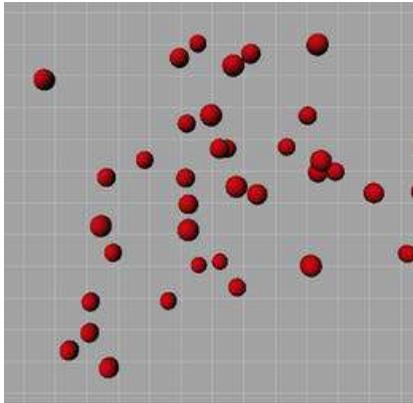


In this example select the **Manual** option and type in **10.000**.

Click on  (Finish).

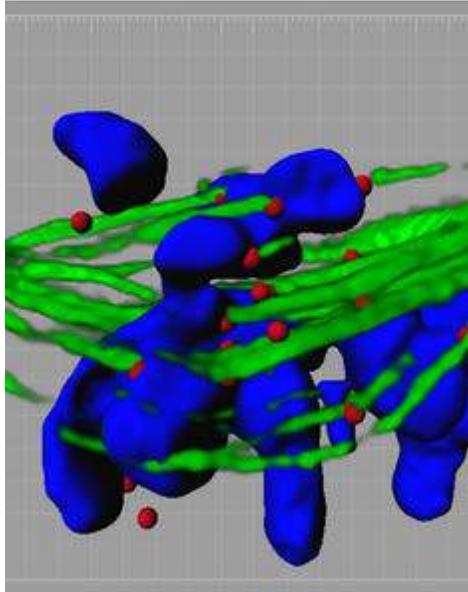
To see only the Spots object in the viewing area, in the Object List now un-check the box for the items Volume and Surface.

5 - Design Mixed Model Rendering



5.5 Final Image

The purpose of this 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.

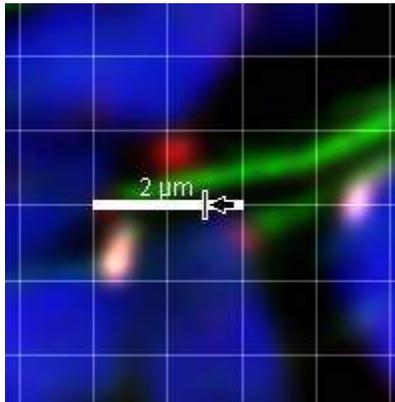


The microtubules display in green as Volume rendering, the chromosomes in blue as Surfaces and the kinetochores in red as Spots.

Please close Imaris before you start with the next tutorial.

6 Measure Structures

In each Imaris view there are several measurement options. This tutorial will focus on different measurement functions in the [Slice view](#)^[10]. 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

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 .

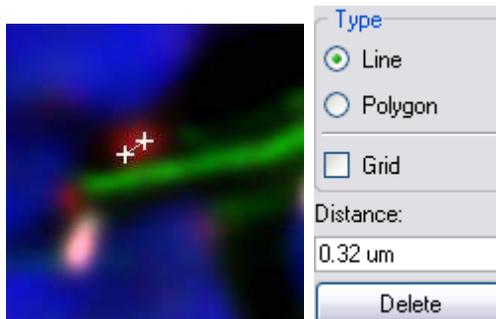
PC:\ProgramFiles\Bitplane\Imaris\images\PtK2Cell.ims

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.



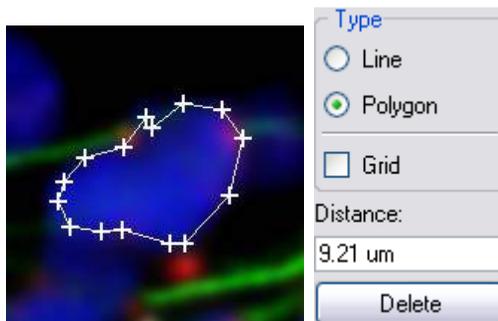
On the right hand side of the screen you find the **Measure** window. Choose the measurement **Type Line**. Set the two measurement points with two consecutive clicks. The result displays in the status field

6 - Measure Structures

Distance. 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.

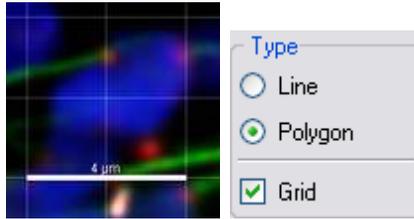


Choose the measurement **Type Polygon**. All measurement points are consecutively connected by lines and the distance is the sum of the distances between the points. Draw a polygon around the DNA like in the image above. The result displays in the status field **Distance**. Click on **Delete** to clear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

6.2 Grid and Scale Bar

Display a Grid

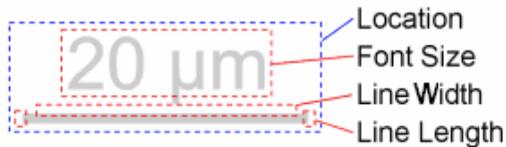
One way to estimate distances in the image is to use the grid display.



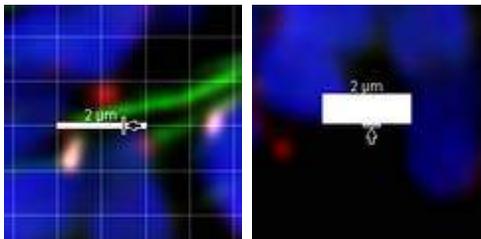
On the right hand side in the **Measure** window check the check box **Grid**. This is the result in the viewing area:

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.



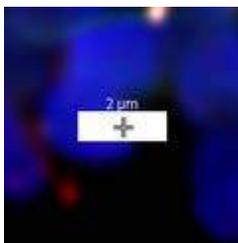
Line Length and Line Width



6 - Measure Structures

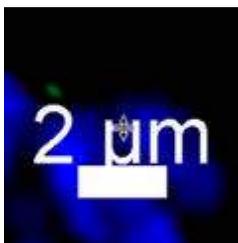
To adjust the length of the scale bar move the mouse to one end of the scale bar until the pointer symbol alters (vertical dash and arrow). Now click on the scale bar end and hold the button down while you drag the mouse to the left or right, respectively. The grid width changes accordingly. In the same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

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.



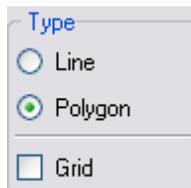
Slice

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).

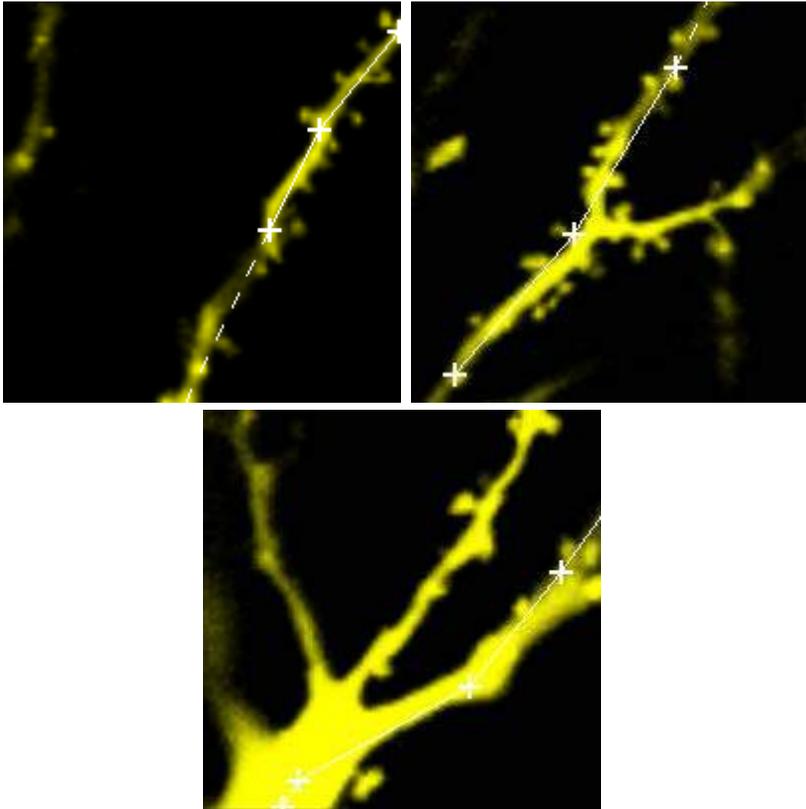


On the right hand side in the **Measure** window as measurement type select the **Type Polygon**.

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

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right hand side of the screen.

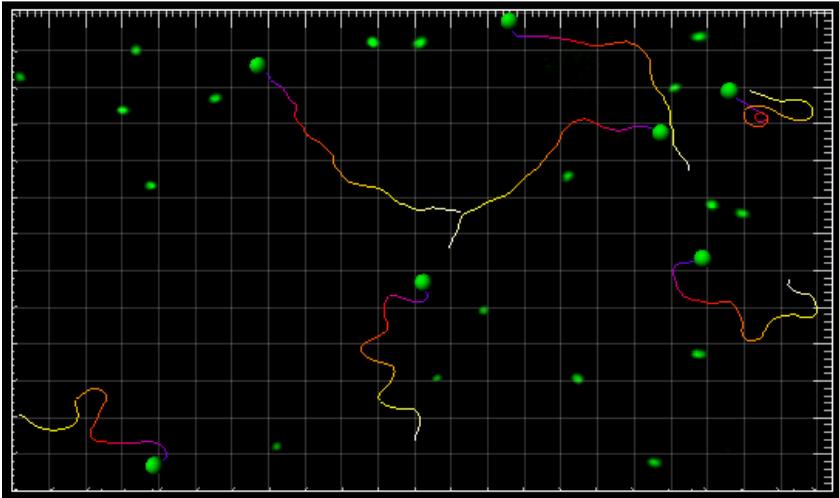


The first picture is slice number 21, the second picture is slice number 25 and the last picture is slice number 32.

Please close Imaris before you start with the next tutorial.

7 Track Particles

ImarisTrack is a separate Imaris module to follow 3D-objects over time, display their paths and analyze their movements. The demo image shows the movement of the algae *chlamydomonas reinhardtii* zoospores. The first step is the [Visualization](#)^[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 - Track Particles

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.

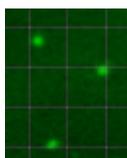
PC: \ProgramFiles\Bitplane\Imaris\images\SwimmingAlgae.ims

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.

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).

7 - Track Particles

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)**.

Algorithm Settings

- Segment only a Region of Interest
- Process entire Image finally
- Different Spot Sizes (Region Growing)
- Track Spots (over Time)

Click on  (Next).

Creation Wizard - 2/6 Source Channel

Source Channel

 Channel 1 - (name not specified) 

Spot Detection

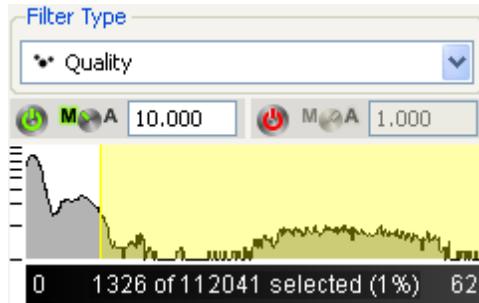
Estimated Diameter: um

Background Subtraction

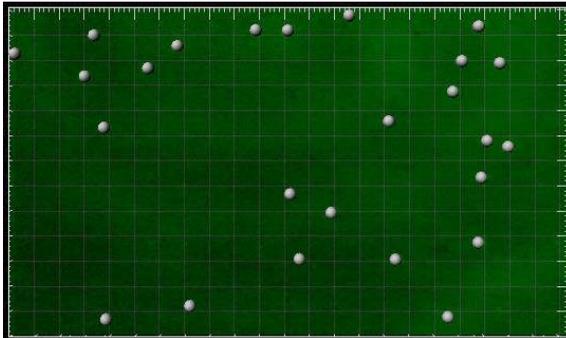
Select as **Source Channel** the **Channel 1** (green) and set the **Estimated Diameter** to **4.000**. Click on  (Next).

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  .

7 - Track Particles

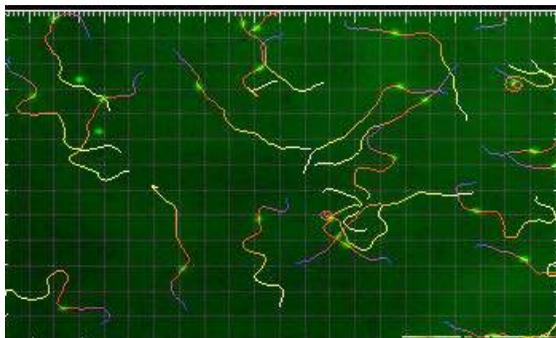
7.4 Tracking

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.)

Algorithm	
Autoregressive Motion ▼	
Parameters	
MaxDistance	10 um
MaxGapSize	3

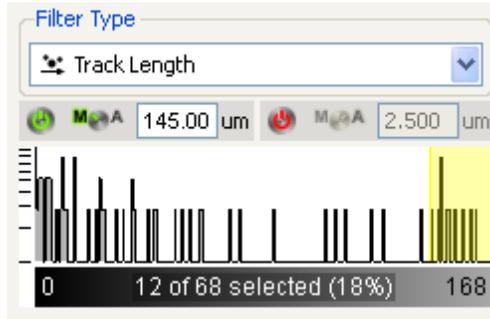
As **Algorithm** select **Autoregressive Motion** and as **MaxDistance** set **10.000 um**, and for the **MaxGapSize** set **3**. Click on  (Next). This is the result in the viewing area.



7.5 Filter Tracks

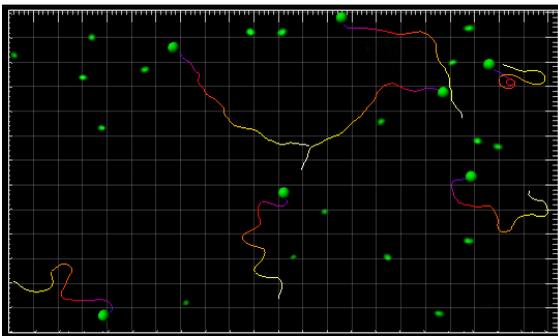
Creation Wizard - 6/6 Classify Tracks

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).

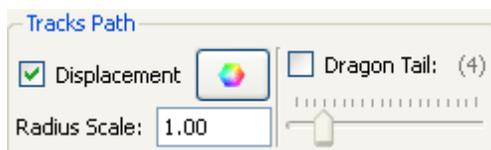


This is the Spots and Tracks display in the viewing area.

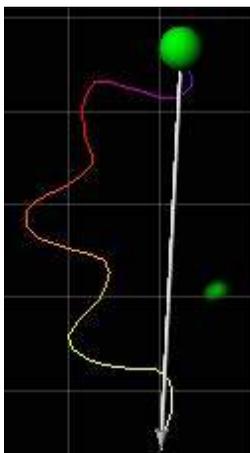
7 - Track Particles

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 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 Surpass view click on the icon  to add new Spots.

Creation Wizard - 1/6 Algorithm

Algorithm Settings

- Segment only a Region of Interest
 - Process entire Image finally
- Different Spot Sizes (Region Growing)
- Track Spots (over Time)

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

Creation Wizard - 2/6 Source Channel

Source Channel

 Channel 1 - (name not specified) 

Spot Detection

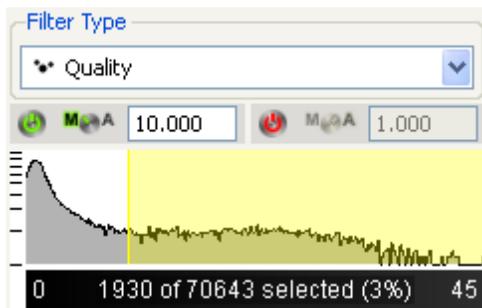
Estimated Diameter: um

- Background Subtraction

7 - Track Particles

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

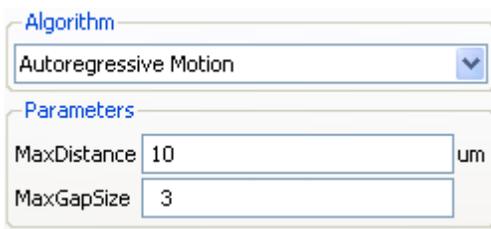


Select for the parameter **Filter Type: Quality**, select the **Manual** option and set Lower Threshold to **10.000**. As an alternative left click on the yellow line and drag it. Moving the line to the right increases the threshold level and as a result less spots are detected. Conversely moving the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



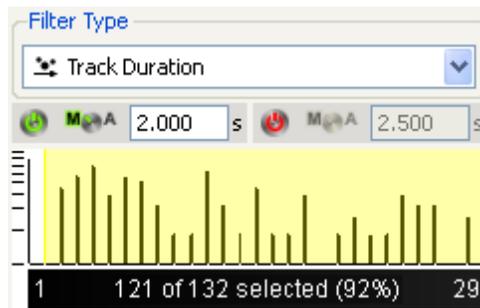
Track Particles - 7

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

Click on  (Next).

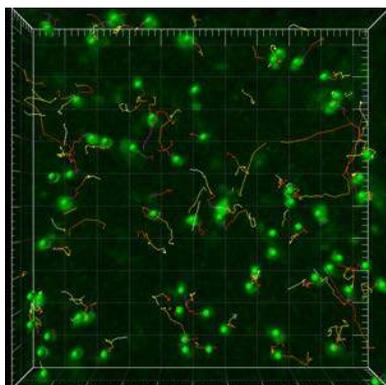
Creation Wizard - 6/6 Classify Tracks

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).

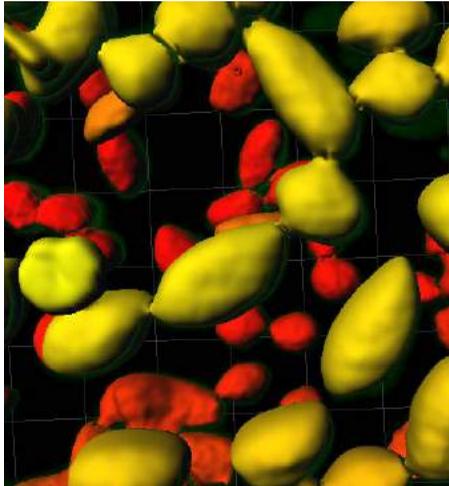
In the viewing area Spots and Tracks are shown.



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

8.1 Visualization



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

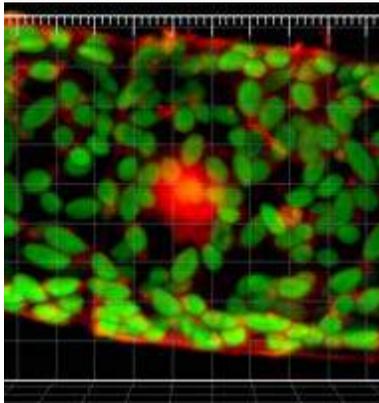
Mac: In the folder Applications double-click on Imaris to open the program.

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 - Define Region Seed Points

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

Algorithm Settings

Segment only a Region of Interest

Process entire Image finally

Track Surfaces (over Time)

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

Creation Wizard - 2/6 Source Channel

Region of Interest 1

	Min:	Max:	Size:
X:	155	462	308
Y:	94	278	185
Z:	1	38	38

Memory Usage: 2.065 MB

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**

Define Region Seed Points - 8

largest Sphere which fits into the Object value of 5um. Click on Next.

Source Channel

Channel 2 - chloroplast

Smooth

Surfaces Area Detail Level um

Thresholding

Absolute Intensity

Background Subtraction (Local Contrast)

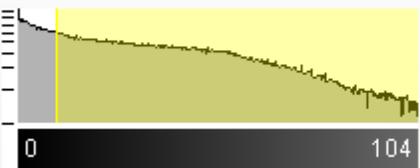
Diameter of largest Sphere which fits into the um Object

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.

Threshold (Background Subtraction)

M A M A



0 104

Split touching Objects (Region Growing)

Enable

8 - Define Region Seed Points

8.3 Define Region Growing and Display Surfaces

Creation Wizard - 5/8 Region Seed Points

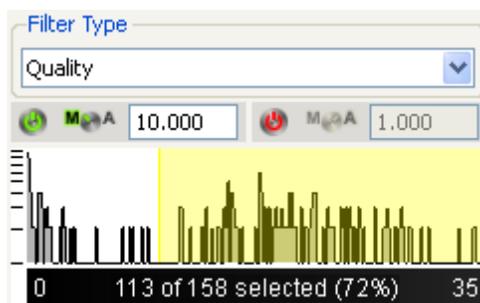
Seed Detection

Estimated Diameter: um

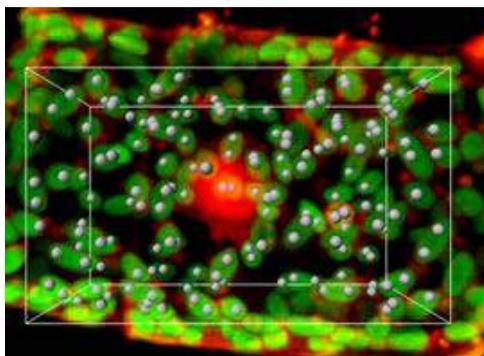
Set as **Estimated Diameter** value of **4.0 um**. Click on  (Next).

Creation Wizard - 6/8 Classify Seed Points

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



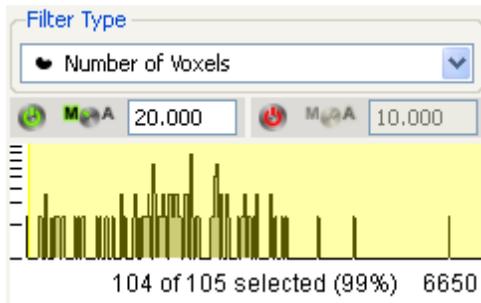
This is the result in the viewing area. Click on  (Next).



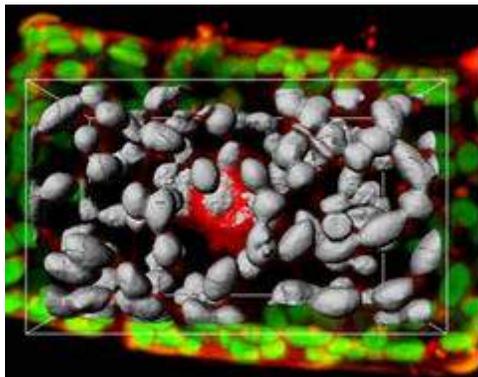
Define Region Seed Points - 8

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).



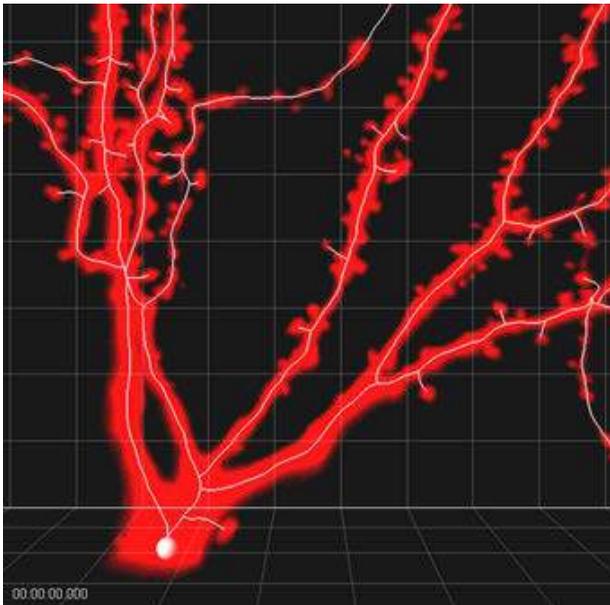
Creation Wizard -8/8 Surface computation for the entire dataset

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 created to detect, visualize and measure filamentous structures. The demo image shows a hippocampal 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](#)^[87] and then 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 FilamentTracer module.



Filament Structure, Pyramidal Cell

9.1 Visualization



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

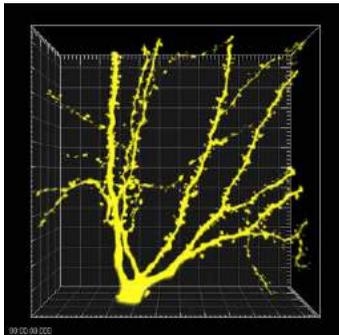
Mac: In the folder Applications double-click on Imaris to open the program.

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.

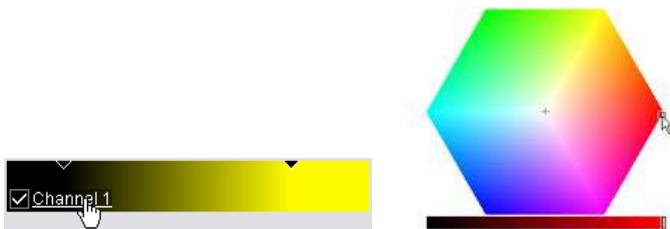


MIP Display, Pyramidal Cell

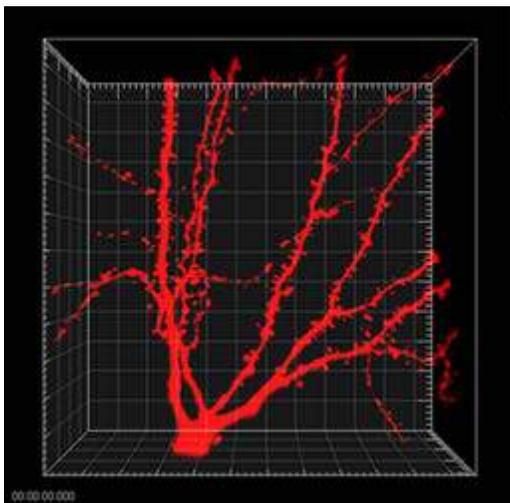
9 - Analyze Neuron

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

9.3 Automatic Detection

Automatic creation separates the dendrite and the spine detection, allowing a more sensitive detection of the 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 - 1/7 Algorithm

Algorithm Settings

Autopath (no loops) ▼

The automated Autopath Algorithm:

- Produces a tree-like filament
- Is based on local intensity contrast
- Connects large start- and small seed-points

Calculate Diameter of Filaments from Image

Segment a Region of Interest

Track (over time)

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

9 - Analyze Neuron

Creation Wizard- 2/7 Dendrite Points Diameter

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

Dendrite

Select Source Channel

Channel 1 - (name not specified)

Starting Points

Detect New Starting Points
(Largest Diameter: e.g. Dendrite Beginning)

12.000 um

Seed Points

(Thinnest Diameter: e.g. Dendrite Ending)

1.000 um

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. To define the starting and end point diameter please use Measure Structures features - [Line and Polygon](#)^[55].

In this example the starting point diameter is set to **12 um**. The value of the **Dendrite Seed Point** diameter is set to **1 um** to match the value of the smallest dendrite radius. Click on Next.

Creation Wizard - 3/7 Classify Dendrite Points

In this step, you can classify the detected points. The thresholds are set for Dendrite Starting Point and Seed Points. For the dendrite **Starting Point** select the **Manual** option for lower threshold value by clicking on the **M/A** button and enter **15.00**. For the higher threshold value accept the **automatically** calculated value. The threshold for the dendrite **Seed Point** is set the to **18.00**.

Select the **Remove Seed Point Around Starting Point** option. This

Analyze Neuron - 9

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 μm** .

Dendrite

Starting Points Threshold

M₀A 15.000 M₀A 50.726



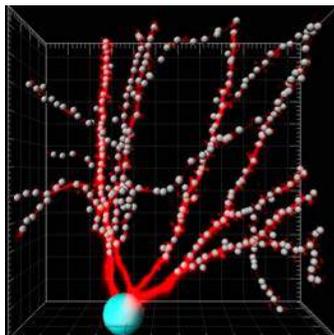
Seed Points Threshold

M₀A 18.000



Remove Seed Points Around Starting Point(s);
Diameter of Sphere Region(s): 24.000 μm

In the viewing area the detected dendrite's Starting point and seed points are displayed in the image by different colors. Click on Next.



9 - Analyze Neuron

Creation Wizard - 4/7 Dendrite Diameter

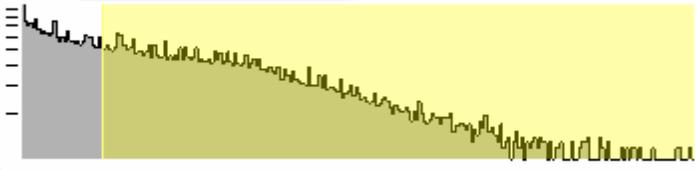
In the next step you can adjust the threshold value for the Dendrites Diameter. The threshold value is based on the local contrast. Accept **automatically** calculated values for the dendrite diameter.

You can specify how the dendrite diameters are calculated from the image. As an algorithm for the dendrite diameter calculation select the **Approximate circle of cross section area**.

Dendrite

Threshold (local contrast)

  3.814

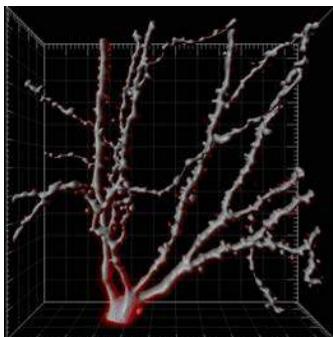


Algorithm

Shortest Distance from Distance Map

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 - 5/7 Spine Points Diameter

In this example we will detect the spines, so select the **Detect Spine** box. Then you can define the channel sources and diameters for the Spines. 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 μm** and the **Maximum Length** to **4.00 μm** . Select the option **Allow Branch Spines** and click on Next.

Spine

Detect Spines

Select Source Channel

 Channel 1 - (name not specified) ▼

Seed Points
(Thinnest Diameter: e.g. Small Spine Head)

μm

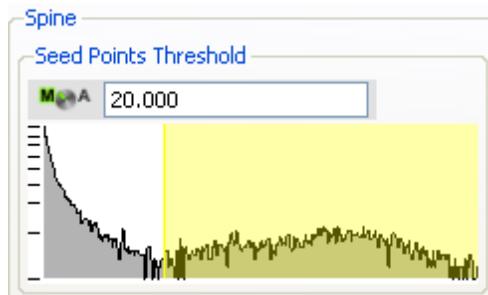
Maximum Length

μm

Allow Branch Spines

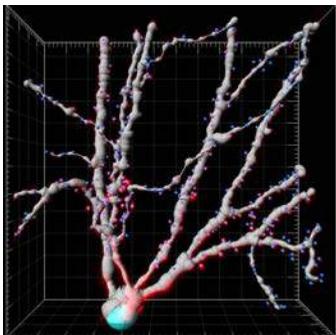
Creation Wizard - 6/7 Classify Spine Points

In this step, you can classify the number of Spine Spine Points by setting a threshold.



9 - Analyze Neuron

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



Creation Wizard -7/7 Spine Diameter

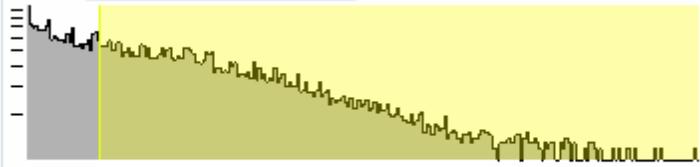
In the next step you can adjust the threshold value for the Spine Diameter. The threshold value is based on the local contrast. Accept **automatically** calculated values for the Spine diameter.

You can specify how the Spine diameters are calculated from the image. As an algorithm for the spine diameter calculation select the **Approximate circle of cross section area**.

Spine

Threshold (local contrast)

  3.509

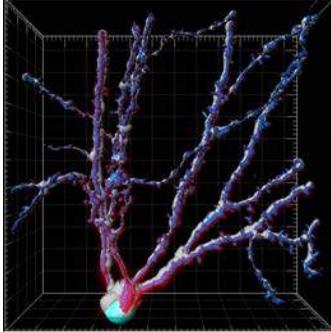


Algorithm

Shortest Distance From Distance Map

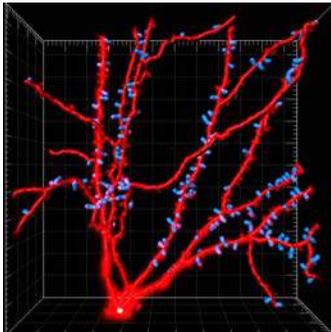
Approximate Circle of Cross Section Area

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



Click on Finish .

This is the result in the viewing area with the dendrite displayed in red and the spines are in blue.



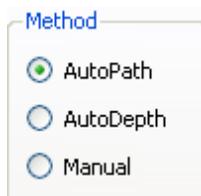
9.4 AutoPath Mode

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.

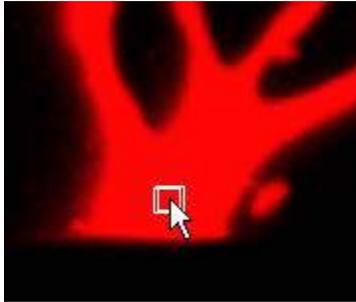
Add new Filament



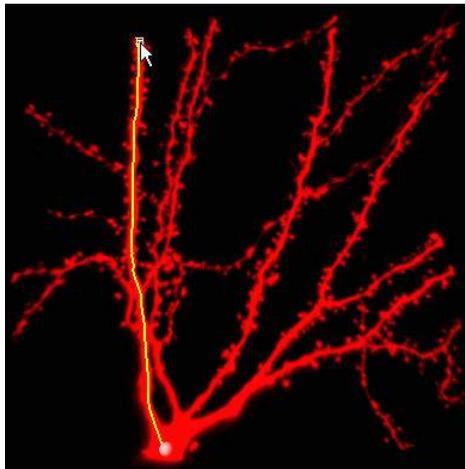
In the **Objects** toolbar of the Surpass view click on the icon  to add a new Filament. In the **Filament - Properties** click on  (Cancel) to stop the automatic detection of the start point and the end points. Click on the tab **Draw** .



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



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.

9.5 Filament Tool Kit

In this chapter you will learn different visualization possibilities of the FilamentTracer. A short, tabular overview concerning the three different interactive filament tracing methods is presented.

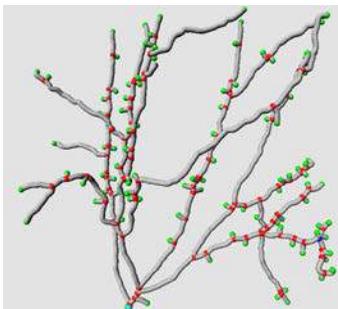
Filament Visualization

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

Style	
<input type="radio"/> Line	<input type="text" value="1"/> pixel
<input checked="" type="radio"/> Cylinder	<input type="text" value="1.000"/> um
<input type="radio"/> Cone	<input type="text" value="1.000"/> scale

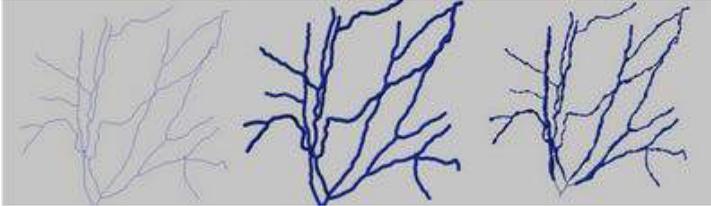
<input checked="" type="checkbox"/> Show Dendrites	<input checked="" type="checkbox"/> Show Spines
<input checked="" type="checkbox"/> Beginning Point	<input checked="" type="checkbox"/> Attachment Points
<input type="checkbox"/> Branch Points	<input type="checkbox"/> Branch Points
<input checked="" type="checkbox"/> Terminal Points	<input checked="" type="checkbox"/> Terminal Points

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

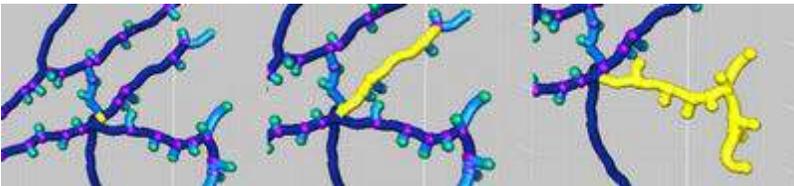
of filaments (**lines**, **cones** or **cylinders**).



On the Edit tab  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

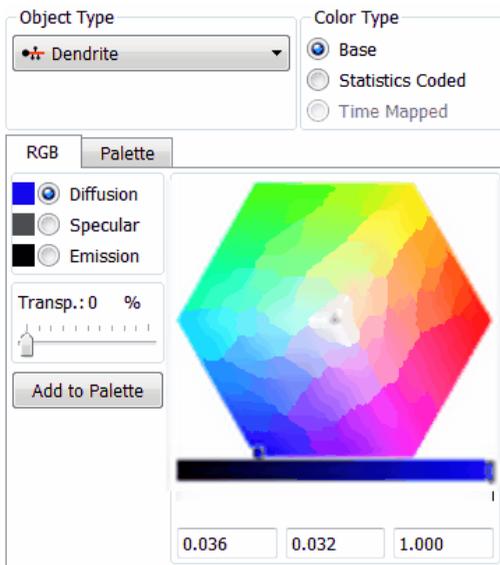
In the the window **Process Selection** you find the **Delete** button, and other editing functions. These functions are applied only to the selected part(s) of the Filament.

9.6 Color Properties

In the properties area click on the Color tab  to open the Color Properties dialog box. Under the Tab Color you can edit, manage and assign material colors for dendrites, spines and each of their significant points.

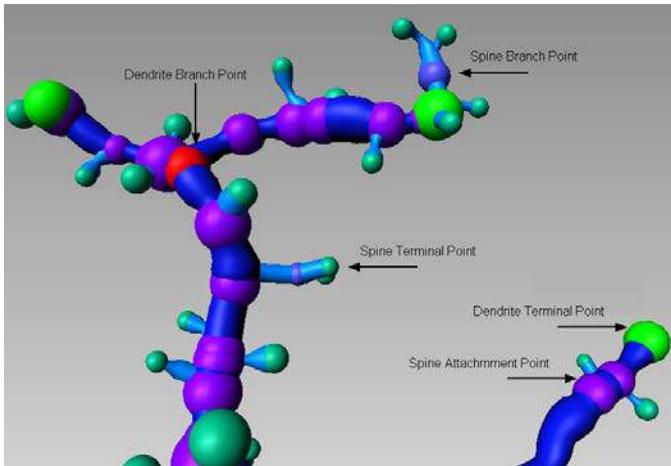
Base

Select the **Base** as an option for Color Type. Select the **Dendrite** from the **Object type** list. Change the color of the dendrite by clicking on the color wheel.



In the same way, you can adjust color for following items: Spines, Dendrite Beginning, Branching, Terminal points as well as Spine Attachments, Branch and Terminal points.

You can display Filament data in a numerous of different ways by using the Color tab options.



Statistical Color Coding

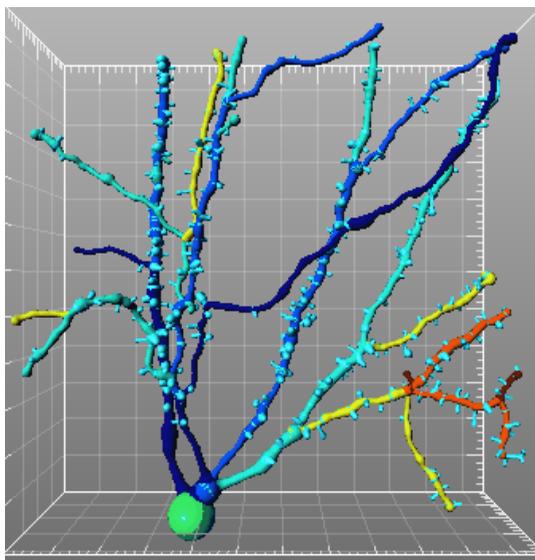
Under the Color tab, the **Statistical Color-Coding** is also available. Using this function, the selected objects are colored according to the value of the chosen statistical parameter.

In the Color type select the **Statistical Coded** option.

Then select the Object type-**Dendrite**. Depending on the object type selected, only the statistics appropriate for that object will be listed. Finally, select the statistical parameter of interest-**Dendrite Branch Level**.

As a result, in the viewing area, the objects are color-coded based on the statistical parameter selected.

9 - Analyze Neuron



9.7 Manual Filament Structure Creation

Click on the **Draw** tab . In the **Method** selection you can choose between **AutoPath**, **AutoDepth** and **Manual**.

AutoPath

The starting point and the end points are set manually. The filament structure is displayed automatically.

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

AutoDepth

Each filament is manually traced 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 and release all buttons	Stop and release all buttons

Manual

You draw each filament manually from starting point to end point. Hide the Volume object and draw in a single plane. The depth is not automatically computed.

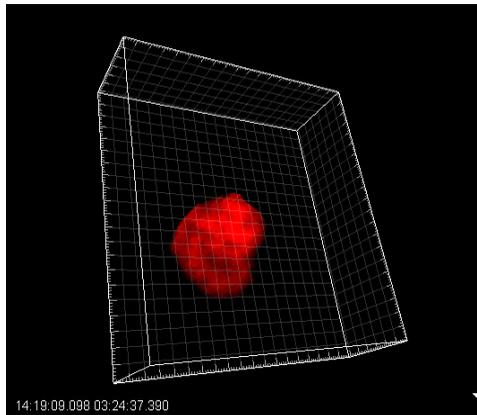
	PC:	Mac:
Draw the filament on the slicer	Shift + left-click + drag	Shift + click + drag
Stop drawing	Stop and release all buttons	Stop and release all buttons

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](#)^[97] of the data set. After that you [draw the contour lines](#)^[103] to outline the chromatin. Then you let the Imaris calculate the [Contour Surface](#)^[105]. This Surface object is your "cookie-cutter". Duplicate the original red channel and then use the "cookie-cutter" to lay a [Mask](#)^[106] to this channel. The focus of the last chapter is the [display of the Masked Channel](#)^[108] allowing you to concentrate your work on a specific ROI.



Masked Red Channel, HeLa Cell

10.1 Visualization



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

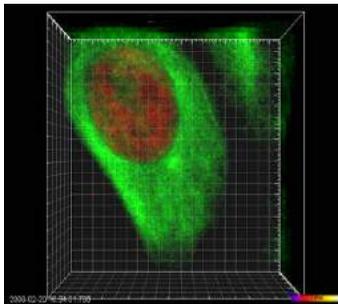
Mac: In the folder Applications double-click on Imaris to open the program.

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**.

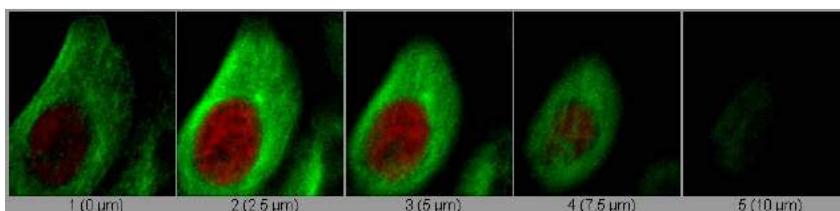


HeLa Cell Display in the Viewing Area

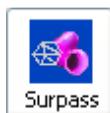
10 - Volume of Interest

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.

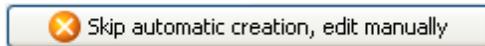
10.3 Add Contour Surface

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** .

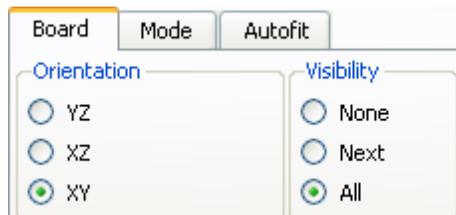
To start the Contour surface creation first you have to cancel the automatic Surface creation wizard. You can do that either by clicking on the Cancel button or by selecting the Skip automatic creation, go to manual editing button.



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.

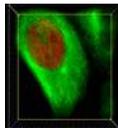
10 - Volume of Interest

10.4 Mode Tab

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.



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 pointer 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.



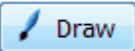
Select **Click**  as the drawing mode tool, to create a selection that outlines the chromatin contour.

10.5 Draw Modes

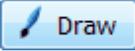
Drawing Modes

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.

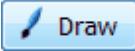
You can select the respective drawing mode by clicking on the Draw

button  or 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.

As you switch between the two drawing modes using the Draw button

, 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 then press

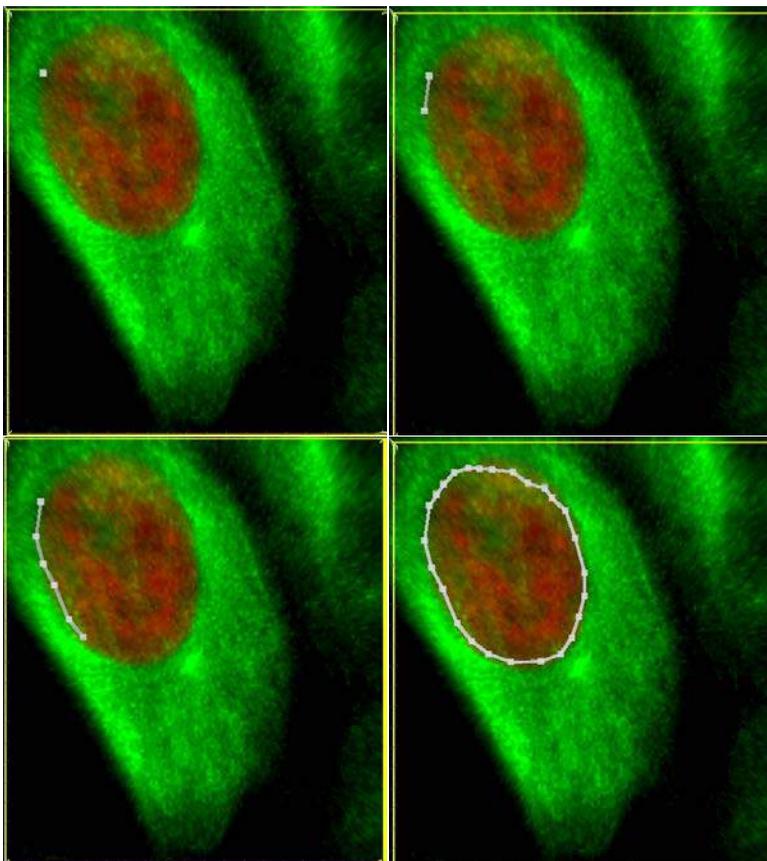
the the Draw button  to activate the drawing mode.

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.

10 - Volume of Interest



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

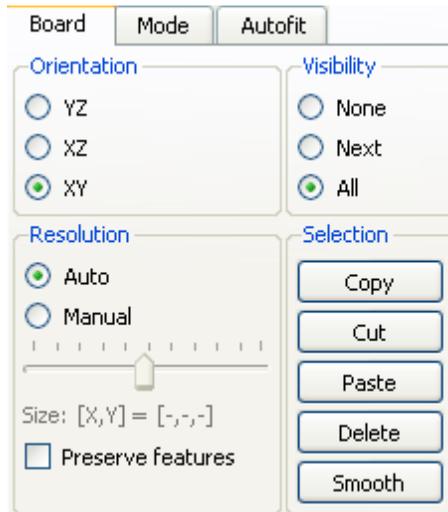
Change to the next drawing plane by moving the Slider to the next position. Repeat the process until the contours are created for all slices.

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.



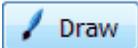
After the contour is created, move to the next slice.

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.

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 the

Draw button ). 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.

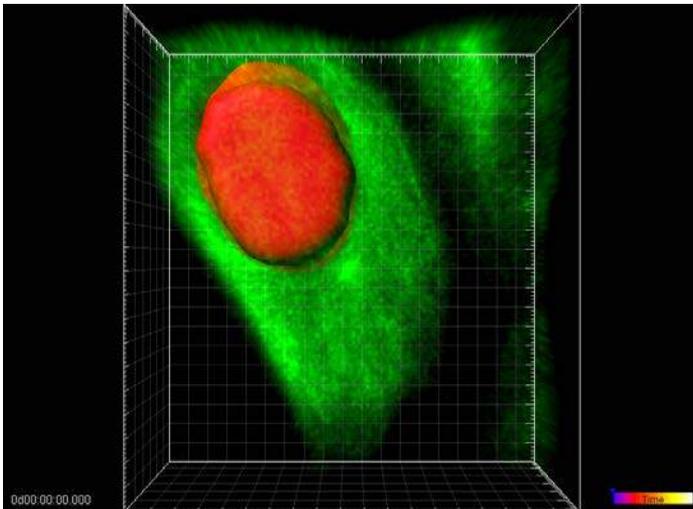
The 'Undo' function from the Edit menu is also available.

10.8 Create Surface Object

To create new Surface Object at each plane should contain a contour line. Then click on the button **Create Surfaces**.

Create Surfaces

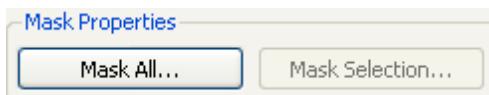
This is the result in the viewing area.



10 - Volume of Interest

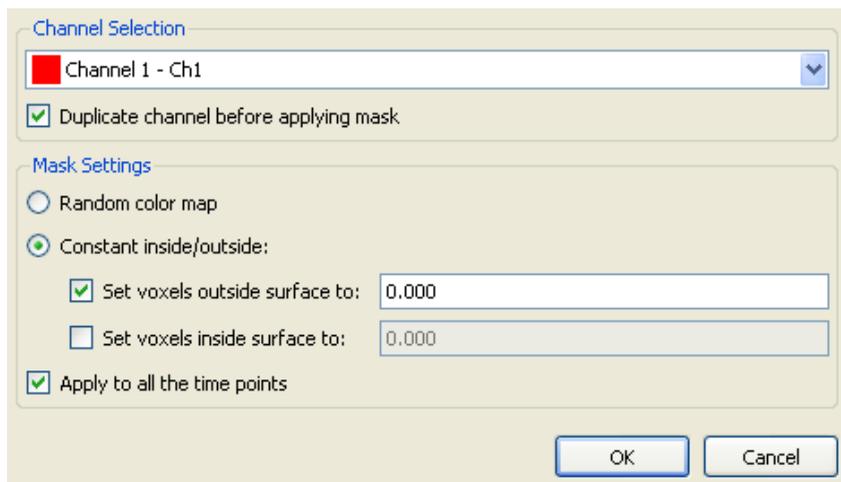
10.9 Mask Channel

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).



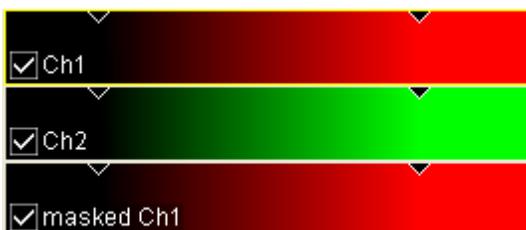
- Select the channel (**Channel 1 - Ch1**, the red channel) you want to mask with the Surface object (ROI).
- Check the box **Duplicate Channel before applying Mask** and an additional masking channel displays automatically in the **Display Adjustments** window.
- Select **Constant inside/outside** and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

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**.



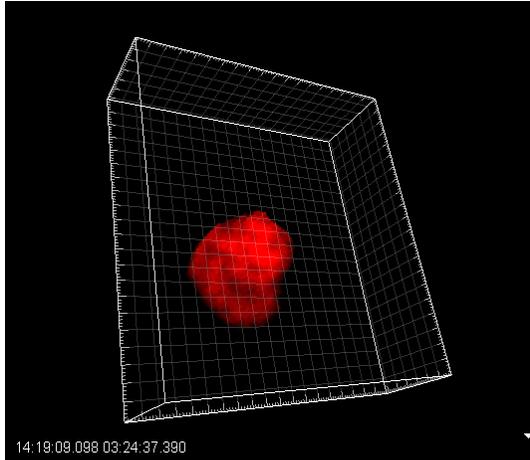
Un-check the original red and green channel. Adjust the upper threshold for the new channel **masked Ch1** (move the right arrow that represents the upper threshold to the left).

Adjust Object List

In the Object List check the item **Volume** and un-check the **Surfaces**.

Volume of Interest - 10

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.



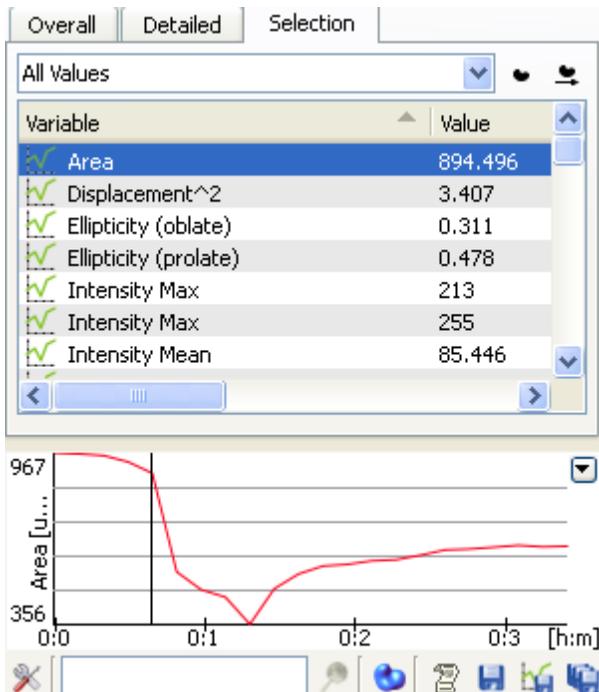
New, Modified Red Channel

Please close Imaris before you start with the next tutorial.

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](#)^[111] reconstruction for both channels, then you generate an [Surfaces](#)^[112] reconstruction of the chromosomes. In the chapter [Time Concept](#)^[110] you will learn how-to-handle time series in Imaris. Then you [Calculate](#)^[117] and export statistical data for each time point. The focus of the last chapter is the [Presentation of the Results](#)^[120] in MS Excel.



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.

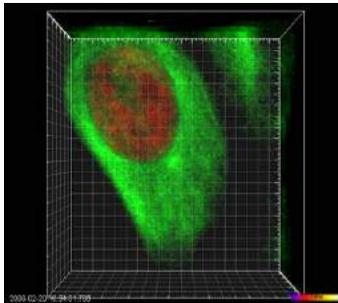
Open the demo image HeLa cell.

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.



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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

Algorithm Settings

- Segment only a Region of Interest
- Process entire Image finally
- Track Surfaces (over Time)

For algorithm setting select **Track Surfaces**.

Click on  (Next).

Creation Wizard - 2/7 Source Channel

Source Channel

 Channel 1 - Ch1

Smooth

Surfaces Area Detail Level um

Thresholding

Absolute Intensity

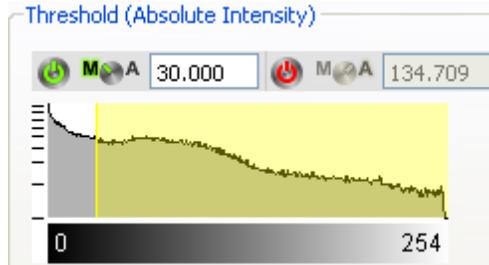
Background Subtraction (Local Contrast)

Diameter of largest Sphere which fits into the um Object

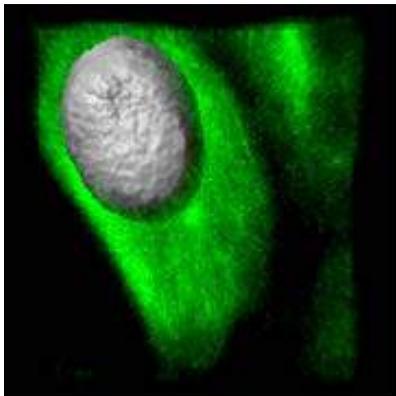
Select as **Source Channel** the **Channel 1** (red) and check the option **Smooth**. As **Surface Area Detail Level** use **0.250 um** and as a **Thresholding** option select **Absolute Intensity**. Click on  (Next).

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**. Click on  (Next).

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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).

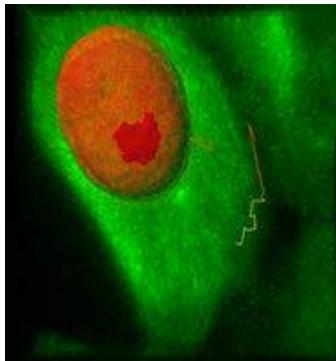
Creation Wizard - 6/7 Tracking

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

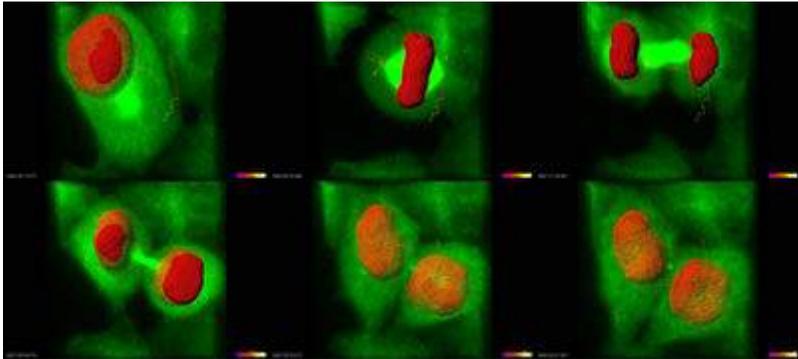
Algorithm	
Autoregressive Motion 	
Parameters	
MaxDistance	20 um
MaxGapSize	3

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).

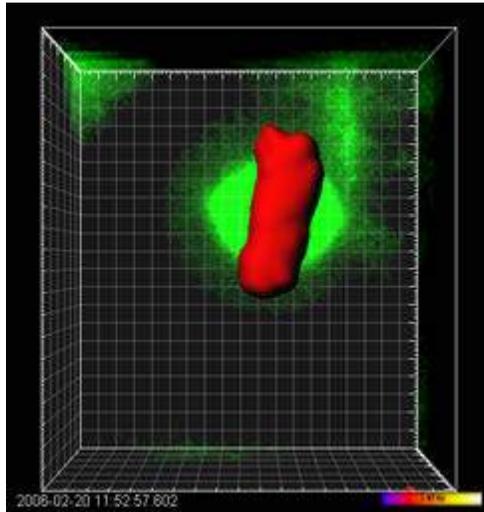


Additional to the Volume rendered tubulin proteins of the spindle the Surfaces reconstruction of the histone 2B proteins displays in the viewing area. In the Display Adjustment window now un-check the box for the Volume rendering of red channel. Newly created surface is displayed in viewing area. The pictures are representing frames numbers 4, 7, 10, 13, 17 and 20.



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

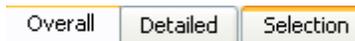
11.4 Calculate Statistics

Now you can evaluate statistical data for each Surface object. The statistical calculation is based on the selected Object List in the Surpass Tree. Select the **Surfaces**.



Show Results

To view statistical data select the **Statistics** tab . There are three statistical tabs **Overall, Detailed, Selection**.

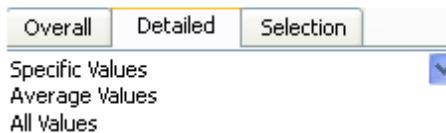


Overall

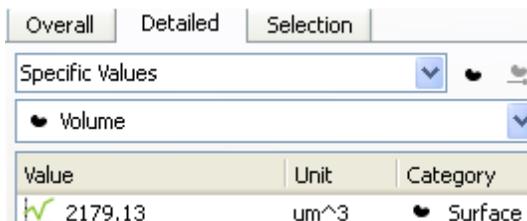
The output of the Overall statistics provides an overview of the image single determining variables.

Detailed

The pull down list provides three types of Detailed statistical calculations: **Specific Values, Average Values, All Values**.

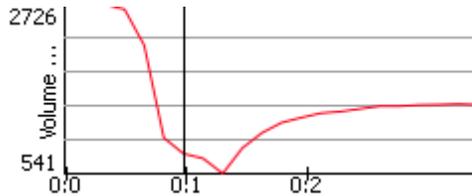


To display the statistical time plots select  Statistics tab **Detailed / Specific value / Volume**.



11 - Volume over Time

The time plot is automatically displayed.



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

Selection

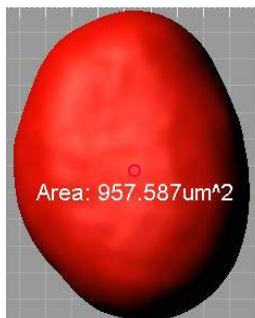
Tab Selection displays statistical values only for the selected object(s).

Statistical Annotation

The Statistical tab provides the opportunity annotate image objects with the statistical data. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area.

In the **Detailed** or **Selection Tab** a list offers **Specific Values** or **All values**. Select the object and select the statistical value - **Area**. Click on the Statistical Annotation button .

Click on this button automatically generates the statistical annotation. This is the result in the viewing area.



Configure List of Visible Statistical Values



Button **Settings**

Please note: If a statistical value does 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**. All generated statistical data are exported to MS Excel and sorted in different sheets. Excel starts automatically.

11 - Volume over Time

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 time-point (column "time") and then by track (column "parent"). In Excel image calculated statistical data can be represented using different charts modifications and adjustments.

	A	B	C	D	E	F
1	Value	Unit	Category	Time	Parent	ID
2	2725.06	um³	Surface	1	1000000000	0
3	2718.67	um³	Surface	2	1000000000	1
4	2699.41	um³	Surface	3	1000000000	2
5	2639.22	um³	Surface	4	1000000000	3
6	2179.13	um³	Surface	5	1000000000	4
7	997.84	um³	Surface	6	1000000000	5
8	795.65	um³	Surface	7	1000000000	6
9	734.89	um³	Surface	8	1000000000	7
10	541.74	um³	Surface	9	1000000000	8
11	886.98	um³	Surface	10	1000000000	10
12	1061.47	um³	Surface	11	1000000000	12
13	1192.56	um³	Surface	12	1000000000	15
14	1256.34	um³	Surface	13	1000000000	17
15	1313.07	um³	Surface	14	1000000000	19
16	1333.27	um³	Surface	15	1000000000	21
17	1368.34	um³	Surface	16	1000000000	23
18	1402.89	um³	Surface	17	1000000000	25
19	1402.58	um³	Surface	18	1000000000	27
20	1417.27	um³	Surface	19	1000000000	29
21	1421.42	um³	Surface	20	1000000000	31
22	1429.56	um³	Surface	21	1000000000	33
23	1420.49	um³	Surface	22	1000000000	35
24	260.44	um³	Surface	9	1000000001	9
25	273.35	um³	Surface	10	1000000001	11
26	590.15	um³	Surface	11	1000000001	13
27	901.70	um³	Surface	12	1000000001	14
28	1114.51	um³	Surface	13	1000000001	16
29	1286.90	um³	Surface	14	1000000001	18
30	1333.38	um³	Surface	15	1000000001	20
31	1366.22	um³	Surface	16	1000000001	22
32	1398.25	um³	Surface	17	1000000001	24
33	1410.88	um³	Surface	18	1000000001	26

Please close Imaris before you start with the next tutorial.

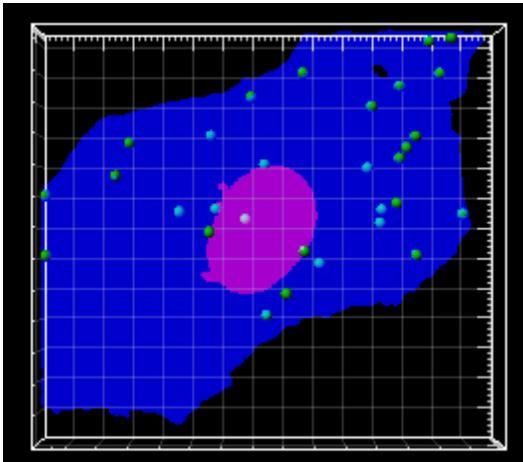
12 Analyze Cells

This tutorial is provided to demonstrate the functionality and operation of ImarisCell. ImarisCell is an Imaris module designed specifically to identify, visualize, track, measure and analyze cells in 2D and 3D time-resolved images.

The demo image analysis involves the following steps:

First, the image is segmented and all the cellular and subcellular components in the dataset are identified. Secondly, the segmented objects are classified based on their size and voxel quality. Finally, the last chapter reviews the topics related to the cell objects visualization options and the statistical data analysis.

Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.



12 - Analyze Cells

12.1 Visualization



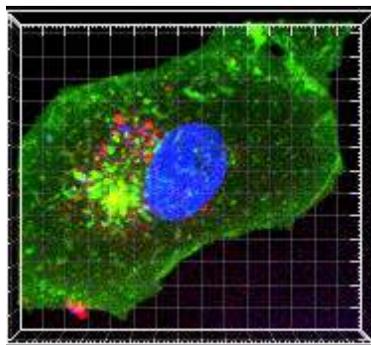
PC: Double-click on the Imaris shortcut on the desktop of your computer to open the program.

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

Open the cell demo image.

PC:\ProgramFiles\Bitplane\Imaris\images\celldemo.ims

Mac:/Applications/Imaris.app/Contents/SharedSupport/images/celldemo.ims



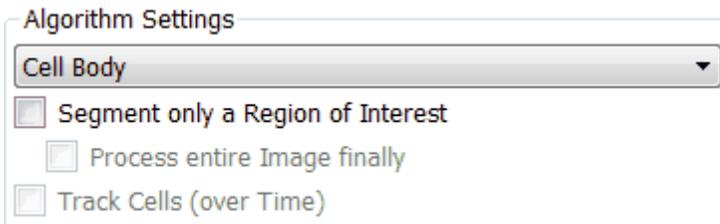
The demo cell image displays in the viewing area.

12.2 Cell Components Detection

In the Objects toolbar of the Surpass view click on the Cell icon  to start the Cell Creation Wizard.

Creation Wizard - 1/4 Algorithm

In the **Algorithm Setting** select the Cell Body algorithm. Leave other the boxes un-checked and click on the Next button .



Creation Wizard - 2/4 Detection

In the next step you define the objects, their source channels and the filter settings. First, we will identify Cell objects.

For the **Cell** object, as the source channel select **Channel 1 - Cytoplasm**. Select the **Smooth** option and accept the **automatically** calculated value for **Filter Width**.

For **Nuclei** detection select **Channel 2 -Nucleus**, select the **Smooth** option and enter the value **0.25 um** for **Filter Width**. Click on the Next button .

12 - Analyze Cells

Cell Body Detection

Source Channel  Channel 1 - Cytoplasm ▾

Smooth

Filter Width um

Background Subtraction

Sphere Diameter um

Detect Nuclei

Source Channel  Channel 2 - Nucleus ▾

Smooth

Filter Width um

Background Subtraction

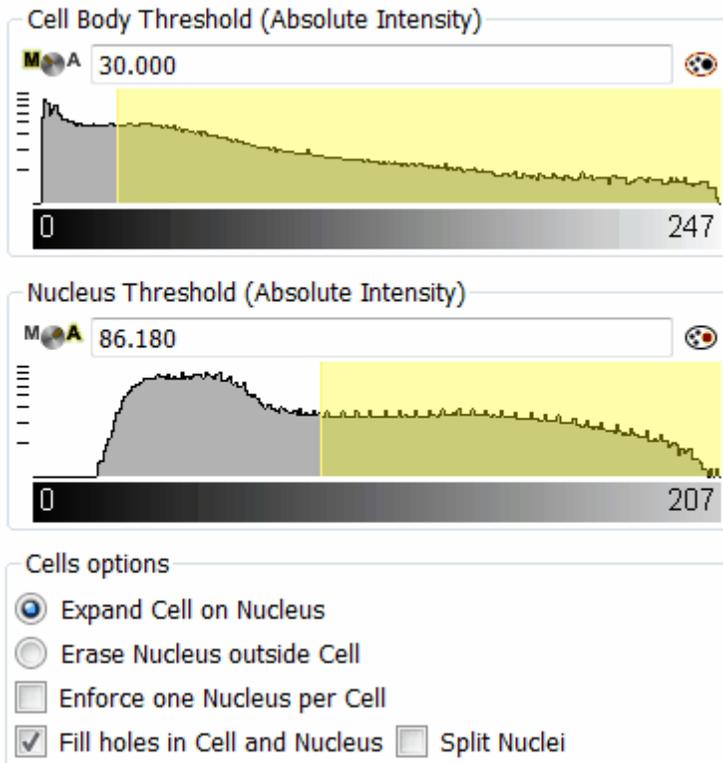
Sphere Diameter um

Creation Wizard - 3/4 Threshold

The image is segmented. For the Cell **Threshold** select the **Manual** option and set the value to **30**.

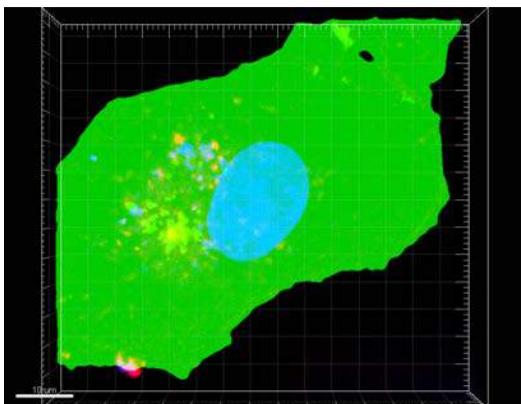
For the **Nuclei** Threshold accept the **Automatically** calculated value.

Further Cell Nucleus segmentation is available with the **Cell Options**. In this example, the Nucleus is detected within the Cell, so leave the automatically selected **Expand Cell on Nucleus** option unchanged. Select the option **Fill holes in Cell and Nucleus**.



In the viewing area a threshold preview of the Cell and Nucleus is displayed. Click on Next .

12 - Analyze Cells



12.3 Classification of Cell Objects

Creation Wizard - 4/4 Classify

Once you completed the segmentation step, you can classify any cell object according to discriminate characteristics.

The filter classifies objects into different groups according to the statistical value selected as the filter type. Any of the available measurements can be used, as the filter type, to classify the Cell/ Nucleus/Vesicles objects.

As a filter type for the Cell object select **Cell Area** and set the value above **1000 μm^2** .

For nuclei classification, select filter type **Nuclei Number of Voxels** and accept the **automatically** calculated value.

For Vesicle classification select as the filter type **Vesicle Quality** and set the value to **5.00**.

Filters

+ Add X Delete

"Cell Area" above 1000.000 μm^2

"Nucleus Number of Voxels" above 10.000

Filter Type

Cell Area

1000.000 μm^2 11389.835 μm^2

1 1 of 3 selected (33%) 11390

12 - Analyze Cells

12.4 Detect and Classify Vesicles

Detect Vesicles

Select the Detect Vesicle option. In this example, leave the automatically selected Vesicle name in the name field. For **Vesicle** detection select Channel 3 (red-Vesicles) as the Source Channel, set the **Estimated Diameter** to **1.9 um** and select the option **Background Subtraction**.

Detect Vesicles

Type A

Vesicle Name

Source Channel

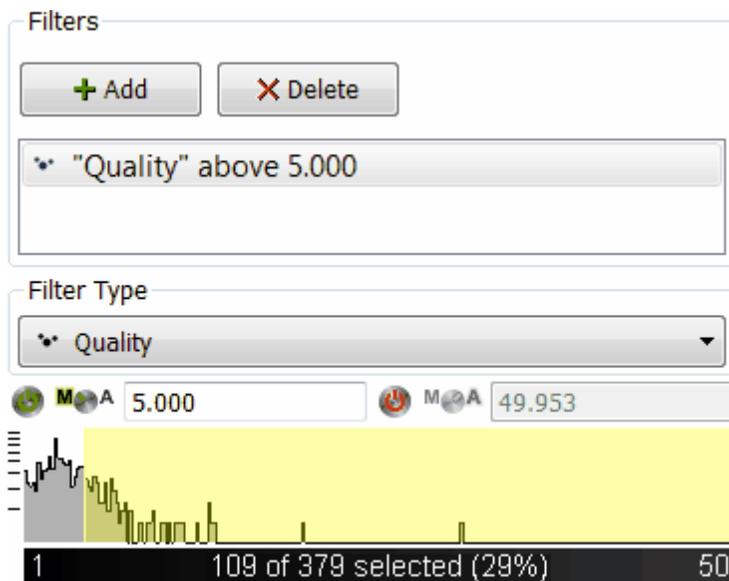
Estimated Diameter um

Background Subtraction

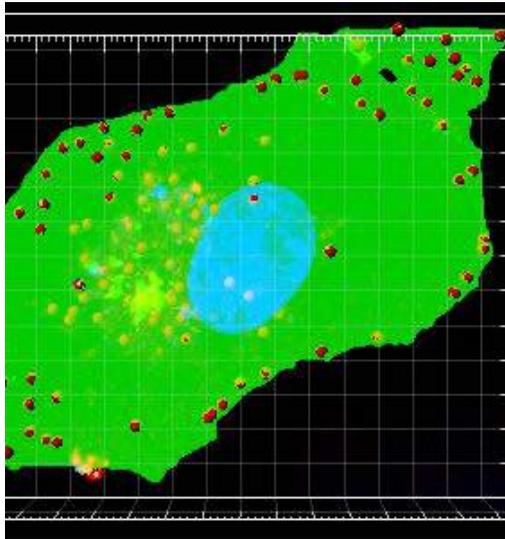
Classify Vesicles

For Vesicle classification select as the filter type **Vesicle Quality** and set the value to **5.00**.

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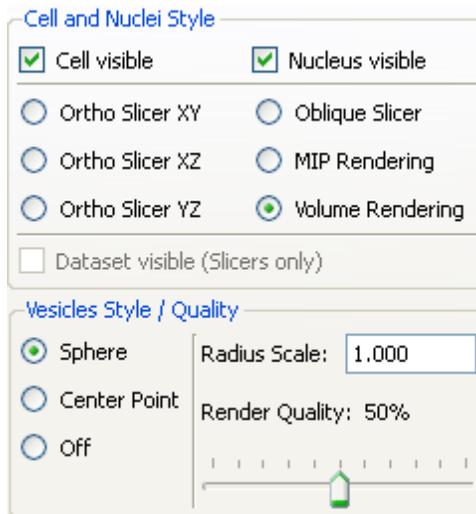
This is the result in the viewing area. The Cell object is displayed in green, blue is used for Nucleus, while the Vesicles are represented as red spheres.



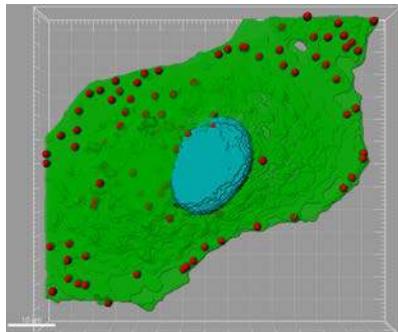
Click on Finish .

12.5 Analyze Cell Objects

In the Setting tab , several visualization options for **Cell**, **Nucleus** and **Vesicles** objects are available. To enhance 3D cell structures and visualize the spatial relationships of the cell objects, select the option **Volume Rendering**.

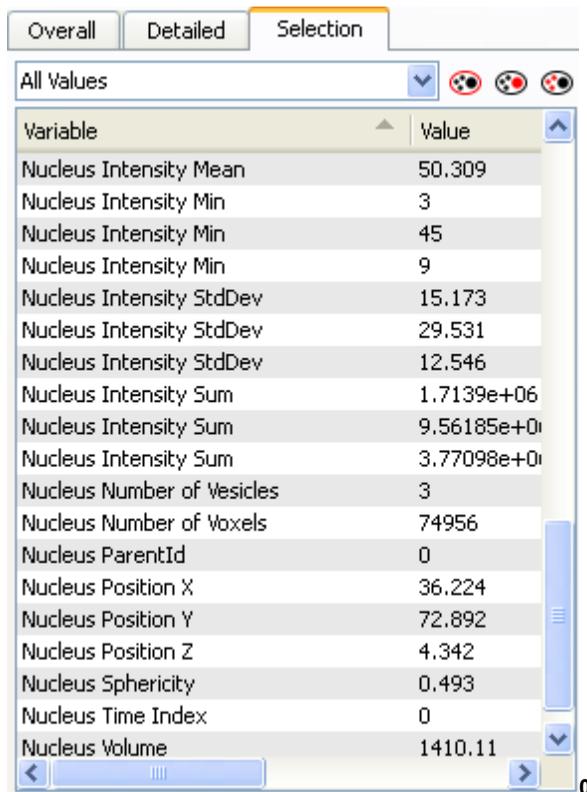


In the Object List now un-check the box for the item Volume. The created Cell objects are displayed in the viewing area.



Analyze Cells - 12

The measurements from the cell components are stored in a table found under the Statistics Tab . Select the **Selection** Tab. Change to the pointer mode **Select** and select the cell object, Nucleus. All Statistical values for the selected Nucleus are displayed. Once the image analysis is completed, the statistical data can be exported to Excel using the Export button  in the Statistics Tab.



The screenshot shows the 'Selection' tab of the Statistics window. A dropdown menu is set to 'All Values'. Below the menu is a table with two columns: 'Variable' and 'Value'. The table lists various statistical metrics for a nucleus, such as intensity mean, standard deviation, sum, and volume.

Variable	Value
Nucleus Intensity Mean	50.309
Nucleus Intensity Min	3
Nucleus Intensity Min	45
Nucleus Intensity Min	9
Nucleus Intensity StdDev	15.173
Nucleus Intensity StdDev	29.531
Nucleus Intensity StdDev	12.546
Nucleus Intensity Sum	1.7139e+06
Nucleus Intensity Sum	9.56185e+0i
Nucleus Intensity Sum	3.77098e+0i
Nucleus Number of Vesicles	3
Nucleus Number of Voxels	74956
Nucleus ParentId	0
Nucleus Position X	36.224
Nucleus Position Y	72.892
Nucleus Position Z	4.342
Nucleus Sphericity	0.493
Nucleus Time Index	0
Nucleus Volume	1410.11

Please close Imaris before you start with the next tutorial.

13 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.

13.1 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

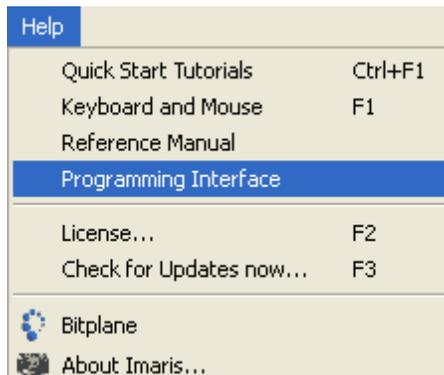
To use ImarisXT right out of the box, there is no need to write any code. However, you can include your own image processing and analysis functions and extend Imaris capabilities, using your favorite programming language (e.g. Matlab, Java, C, C++, and VisualBasic®).

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 (www.bitplane.com/products/imarisxt/doc/index.html) and in PDF formats.

To access the help, in Imaris from the **Help** menu, select **Programming Interface**.



Components

Minimum software requirements are:

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- 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 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
```

13.2 Programmable Interface

ImarisXT Programmable Interface consists of three major components:

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

Component Object Model (COM) - COM Interface

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

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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

Predefined Set of 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.

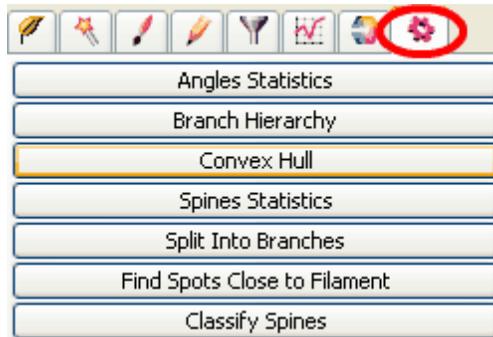
Through the course of the Imaris software development, Bitplane has also developed XTensions. Most of that predefined set of XTensions are MATLAB M-files, which are a series of MATLAB statements that implement specialized image processing algorithms. XTensions are modular and flexible, providing users 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.

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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.

13.3 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. 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.

```
vmarisApplication = actxserver('Imaris.Application');  
vmarisApplication.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 = vmarisApplication.mDataSet.GetDataSlice(32,0,0);
```

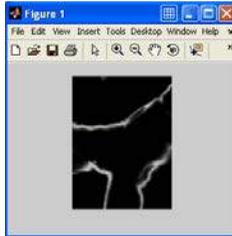
If you click on the Workspace tab of Matlab, you will now see a variable named vSlice.

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:

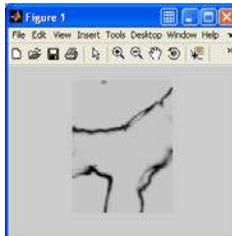
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Now you will invert the intensity data and send it back to Imaris. Matlab vector-arithmetic lets us process an image with a single command.

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

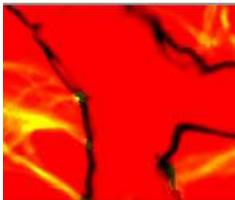
An image of the Retina.ims intensity-inverted slice 32 is now open in Matlab:



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:



13.4 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.

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

Please 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, vIndexC, vIndexT);
```

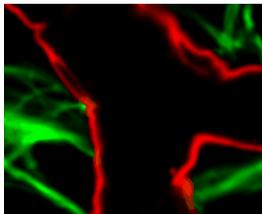
Save the file.

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);
```



13.5 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 `vmarisApplication` 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(almarisApplicationID)
if isa(almarisApplicationID, 'COM.Imaris_Application')
vmarisApplication = almarisApplicationID;
else
vmarisServer = actxserver('ImarisServer.Server');
vmarisApplication = vmarisServer.GetObject
    (almarisApplicationID);
end
```

file

% The lines below are the same as the first tutorial example M-

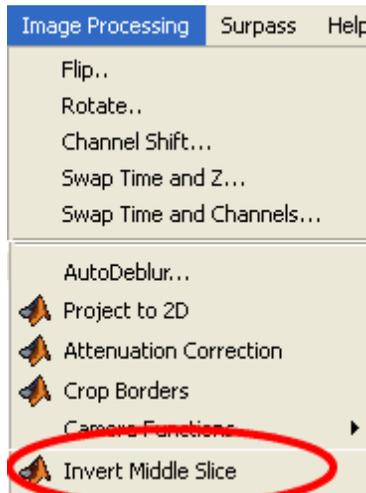
```
% Set up middle slice index
vIndexZ = vmarisApplication.mDataSet.mSizeZ / 2;
vIndexC = 0;
vIndexT = 0;
% Get slice, invert it, send back
```

```
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.

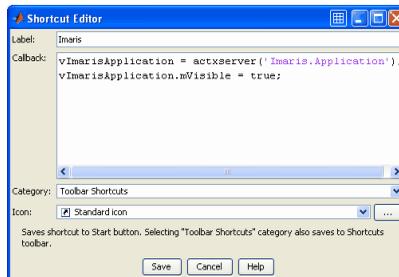


13.6 Matlab Shortcut to Start Imaris

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.

13.7 Similarity Statistic Values

This tutorial will illustrate an example of how to use the Similarity Statistic Values XT extension.

The Similarity Statistic Values is a XT extension that obtains the statistical characteristics of the selected object(s), creates amalgamate of the chosen values and identifies the potential similarity among the other objects within the image.

This tutorial first discusses creation of Similarity Statistic Values for the set of values, and then proposes a unified framework for extending existing functionality under the Filter, Statistical and Color tab.

The procedures discussed in this tutorial demonstrate how the creation of the similarity values helps in detecting new complex statistical properties of the image.

To demonstrate the use of the Similarity Statistic Values XT extension we will start with the demo image PlantCell.ims

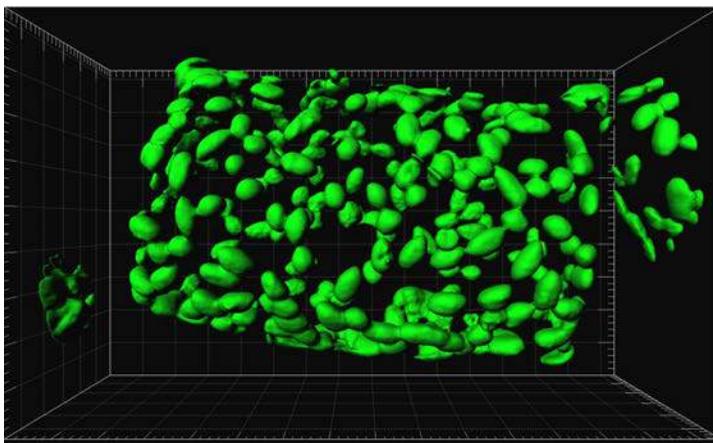
PC: \ProgramFiles\Bitplane\Imaris\images\PlantCell.ims

Mac:/Applications/Imaris.app/Contents/SharedSupport/images/PlantCell.ims

Following the example given in the [Define Region Seed Points](#)⁷² tutorial, complete the Surface creation wizard.

Under the Color tab change the color of the created surface from gray to green.

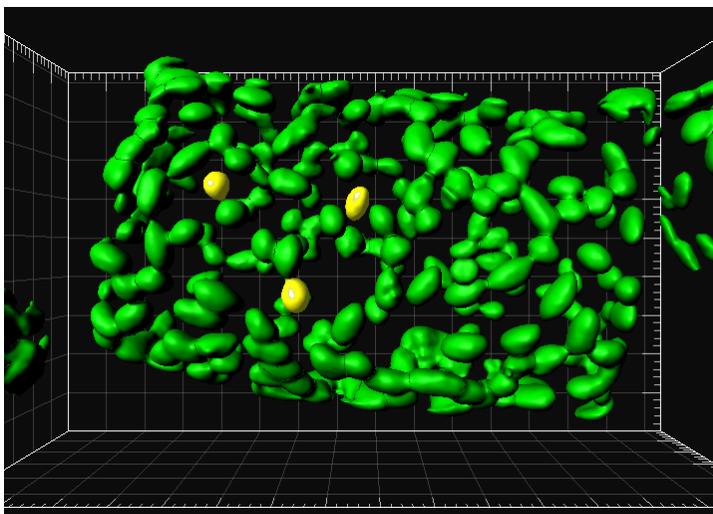
This is the result in the viewing area.



13.7. Tool tab

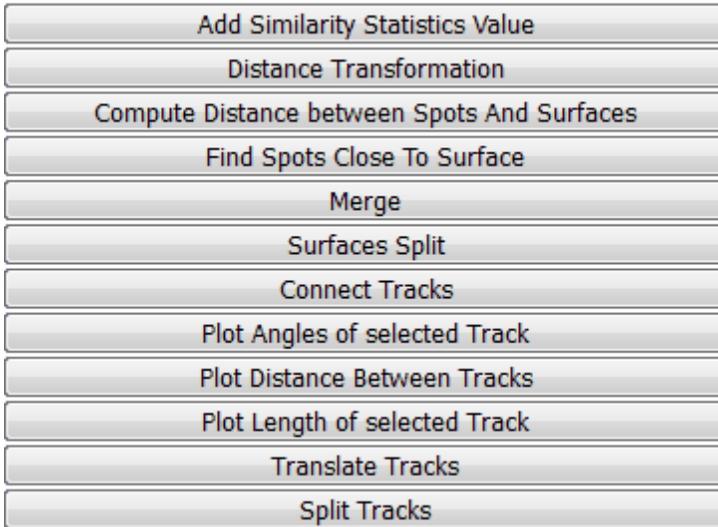
Under the Tool tab  the use Similarity Statistic Values XT extension is available.

First, you have to select the reference objects according to your requirements.

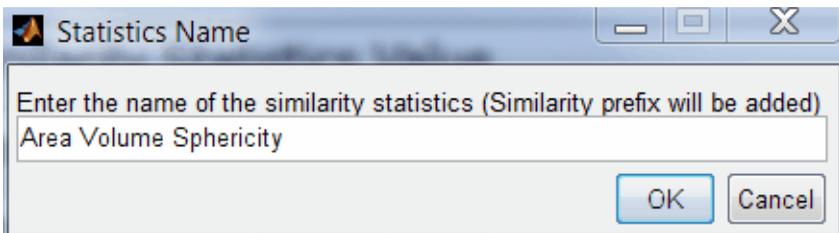


Then click on the button Add Similarity Statistical Value to launch the

Matlab and the Statistics name window appears.



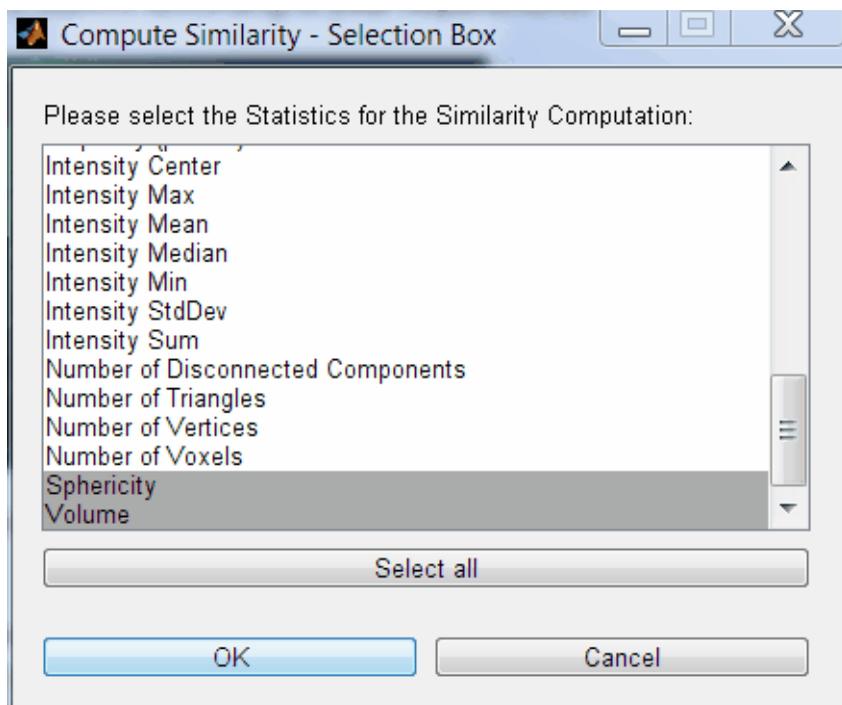
Enter an appropriate name for the new statistical value. In this example we will name the Similarity value: Area Volume Sphericity. Select OK.



To assist you in finding the added statistical value, the **Similarity** is automatically added to the name as a **prefix**.

The new Computer Similarity-Selection Box window appears. There you can select individually or in combination the statistical values from the list.

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In this example, we will select: Area, Volume, Sphericity. To select the Statistic, click on the name of the variable in the list. For multiple selection use Ctrl +click.

The newly created statistical value is based upon the aggregate weighted selected statistical values.

Confirm the selection with the OK button.

The final Matlab Info window confirms the creation of the new statistical value; Similarity Area Volume Sphericity.



"Similarity Area Volume Sphericity " statistics value has been added. It can be used in Filter, Statistics and Color coding tabs.

OK

The created statistical value can be used as any other Imaris statistical value.

13.7.:Filter tab

Select the Filter tab. Click on the Add button and choose **Similarity Area Volume Sphericity** from the Filter Types list.

The screenshot displays the ImarisXT Filter tab interface. It features a 'Filters' section with '+ Add' and 'X Delete' buttons. A filter entry is visible: "Similarity Area Volume Sphericity" between 0.021 and 0.121. Below this is the 'Filter Type' section, which includes a dropdown menu set to 'Similarity Area Volume Sphericity'. Two input fields are present for threshold values: '0.021 um' and '0.121 um'. At the bottom, a histogram shows a distribution of values with a yellow highlight on the left side, indicating the selected range. The histogram shows 27 of 194 objects selected (14%).

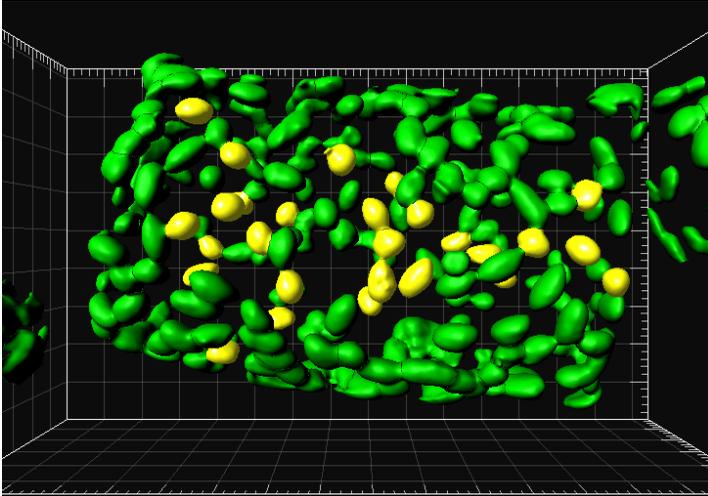
Similarity function requires the definition of a threshold value(s) in order to determine whether two different objects are similar.

Set the threshold values (lower, upper or both) to define the section of interest and identify the objects with similar statistical properties. Based on these limits all objects that have less similarity than the threshold

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value are filtered out.

The similar objects are instantly highlighted in the viewing area.



Objects that meet the similarity criteria may be duplicated to the new selection. The numerical range of the similarity value, the number and percentage of objects selected are displayed at the bottom of the threshold histogram.

13.7.:Statistic Tab

Under the Statistic Tab, the new similarity statistical value is added to the already existing statistical value list. It is available under the Detailed and Selection tab.

The similarity numerical values let you assign objects to groups based on their similarity and facilitates understanding of the complex relationships in large data sets.

Overall Detailed Selection

Specific Values

Similarity Area Volume Sphericity

Value	Unit	Category
0.305	um	Surface
1.812	um	Surface
0.356	um	Surface
0.77	um	Surface
0.257	um	Surface
0.476	um	Surface
0.373	um	Surface
0.761	um	Surface
0.883	um	Surface
0.337	um	Surface
1.064	um	Surface
0.695	um	Surface
1.472	um	Surface
0.534	um	Surface
0.635	um	Surface
0.634	um	Surface
1.159	um	Surface
0.288	um	Surface
0.172	um	Surface

✂ | | 🔍 | 🌐 | 📁 42 | 📄 | 🖨 | 📧

In the statistical table a single value represents the similarity between

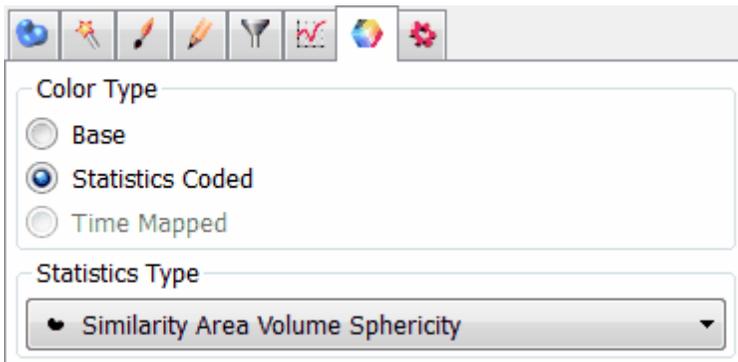
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the object within the image and selected reference objects.

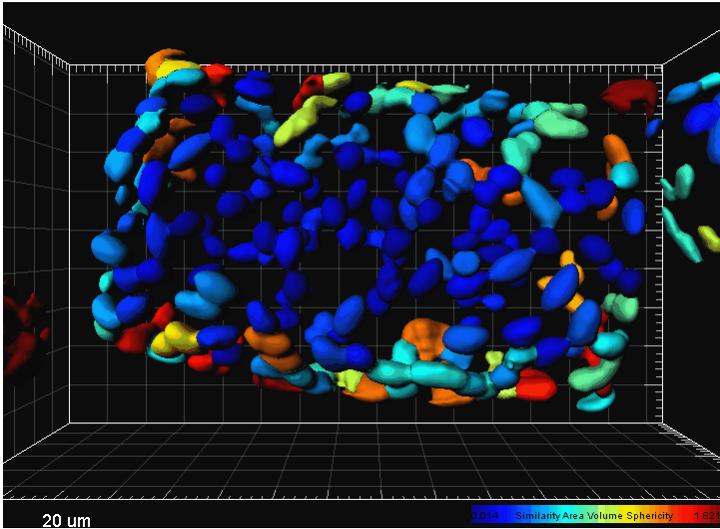
The ideal similarity between the selected object and the perfect match is described as a zero value in the statistical table. If more than one object is selected as a set of reference objects, the case perfect similarity is seldom. The similarity value increases, as the similarity between objects decrease.

13.7. Color tab

Under the Color tab Similarity statistical values can be applied in Statistics Color-coding.



Using the similarity function, the objects in the viewing area are colored according to the similarity value.



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