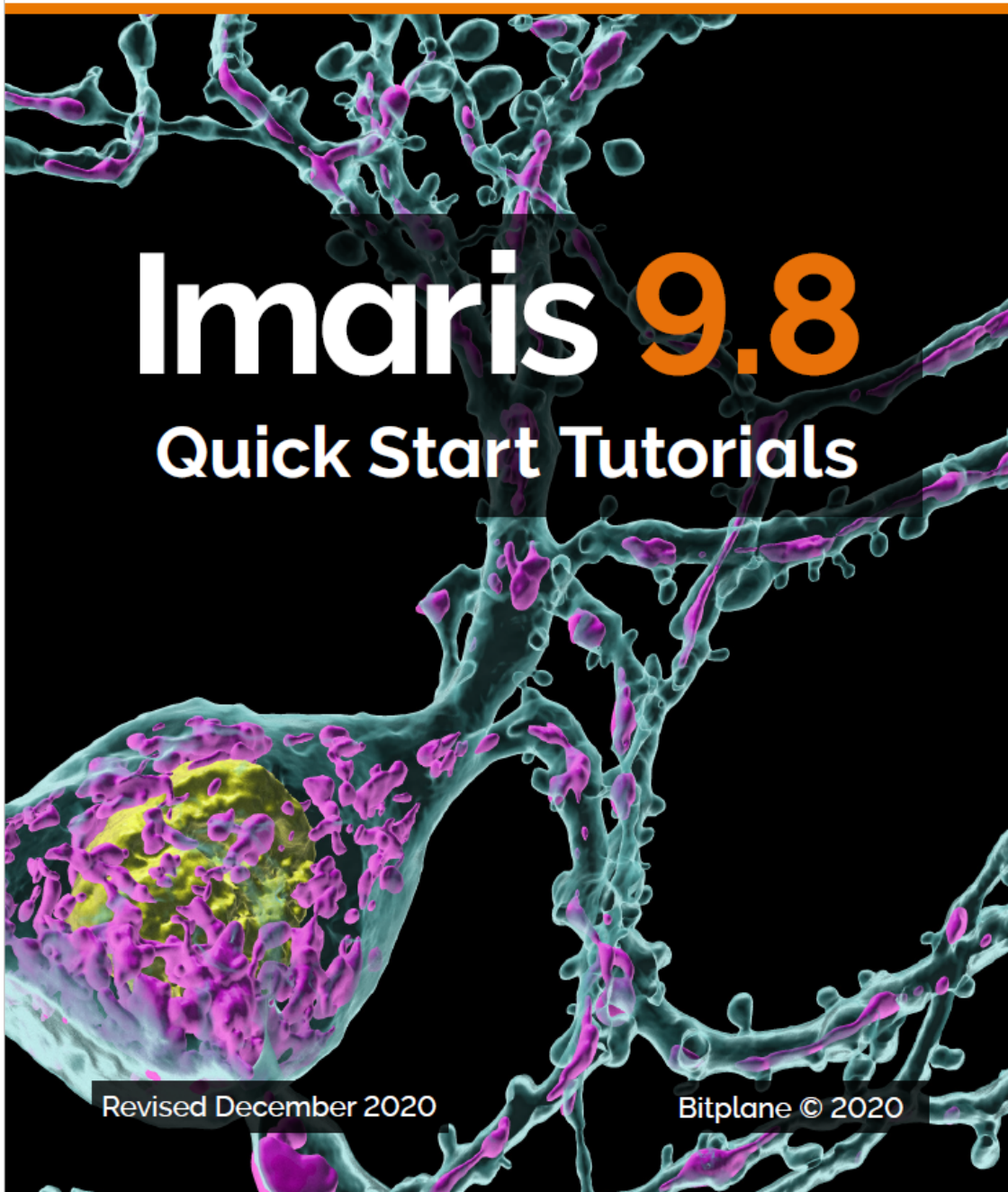
OXFORD INSTRUMENTS | IMARIS



Imaris 9.8

Quick Start Tutorials

Revised December 2020

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Section 1 Introduction

Imaris is a full program for visualizing and analyzing multidimensional microscopy images, which is available with an appropriate license. Imaris Viewer is a free program for visualizing multidimensional microscopy images. Download at www.imaris.com/viewer.

Imaris (left) and Imaris Viewer (right) Start Screens

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

Quick Start Tutorials are the fastest way to understand and master Imaris. The Quick Start Tutorials offer a series of examples and supporting commentaries designed to quickly acquaint the new user with the structure, possibilities and the most compelling features of Imaris. Furthermore it may show previously unrecognized new features of the software to the advanced user. Please note that some features described in the documentation are only available with the full version of Imaris and are not always applicable for Imaris Viewer.

In the Quick Start Tutorials, you will follow a series of straightforward, step-by-step instructions. The tutorial is organized into a number of lessons with each lesson introducing 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 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 tutorials, you'll have a greater understanding of how to implement Imaris in your research and be ready to benefit from the user friendly, fast, and powerful Imaris for processing your microscopy images.

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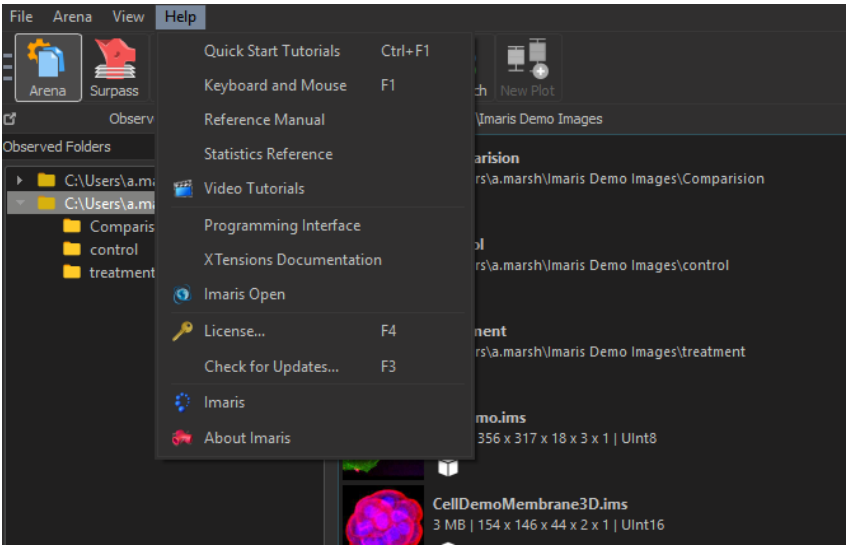
Quick Start Tutorials 9.8

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 in Imaris, click on the **Help** menu and select **Reference Manual**.



The Imaris Reference Manual displays. Click on a chapter in the Table of Contents to display a list with the available sub-chapters. Please note you can right click on any dialogue and click on help to be directed to the corresponding page in the reference manual.

Imaris Reference Manual

Contents | Search

- Preface
- Menu File
- Menu Edit
- Menu View
- Menu Image Processing
- Fiji
- Menu Surpass
  - Vantage Menu
- Menu Help
- Toolbars
- Slice View
- Section View
- Gallery View
- Easy 3D View
- Surpass View
- Vantage
- Animation
- InMotion
- Coloc
- XTensions
- Addendum

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Preface

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

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Imaris Reference Manual v 8.0

Section 2 Arena

In this tutorial, you will familiarize yourself with Arena, which is your starting point when Imaris is opened. Arena lets you organize and manage your image files and also provides "batch" processing capabilities so you can efficiently handle the analysis of a high volume of image data. This is explained in the Batch Analysis topic.

The first Arena topic provides an overview of Arena and its structure.

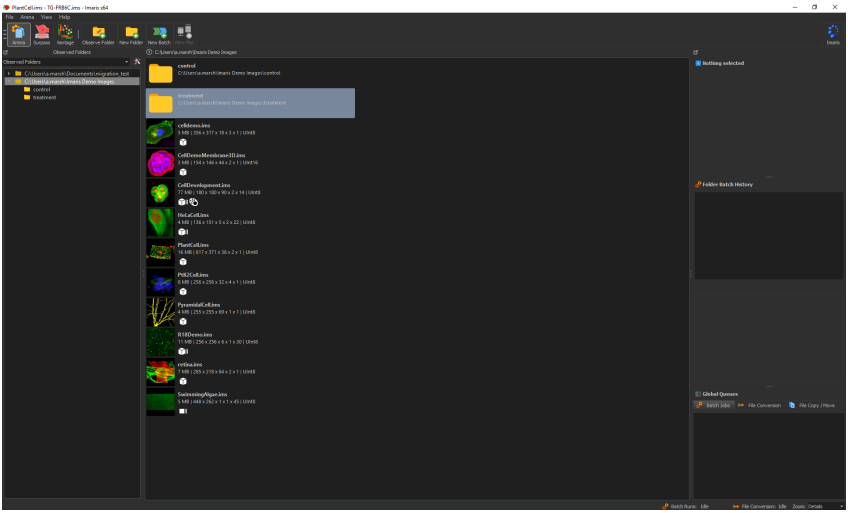
2.1 Arena Structure

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

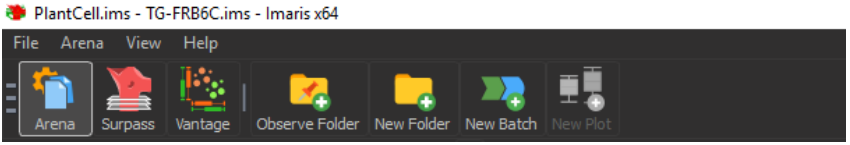
The Arena view appears once Imaris opens and presents you with the interface shown below. Arena View consists of a number of elements:

The left hand side of the Arena displays the current folder location and contents. The top right hand section of the Arena view displays additional information concerning the currently selected image.

Within the Arena view, all images are represented as thumbnails while each Arena item has a characteristic icon.



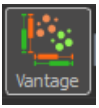
The top pane of the Arena now includes:



Clicking on the Surpass icon allows the user to access Surpass view, which provides extended functions for visualization, including:

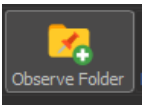
- A large viewing area with numerous tools for data preparation, presentation and manipulation.
- A selection of different types of data display as well as any combination of them.
- The possibility of loading additional External objects for comparison.
- Surpass Grouping functions.

Clicking on the Vantage icon allows the user to prepare Vantage plots. Vantage plots are the most informative and flexible way to visualize and generate a batch report. It provides a statistical data-visualization of the currently selected collection(s) of Batch Collection data. It facilitates the analysis of the collection results and illustrates patterns and trends that are not immediately apparent by just looking at the raw or segmented data.

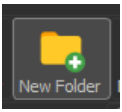


Clicking on the Observe Folder icon allows the user to select a new folder

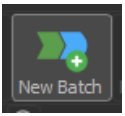
from a selected file path to be observed in the Arena. It should be noted that this does not create a new folder.



Clicking on the New Folder icon allows the user to create a new folder to the Arena and on the file system.

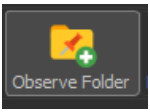


Clicking on the New Batch icon allows the user to create a new batch pipeline. Batch processing lets you apply the same creation parameters to all images within the selected Group, sparing you repetitive work.



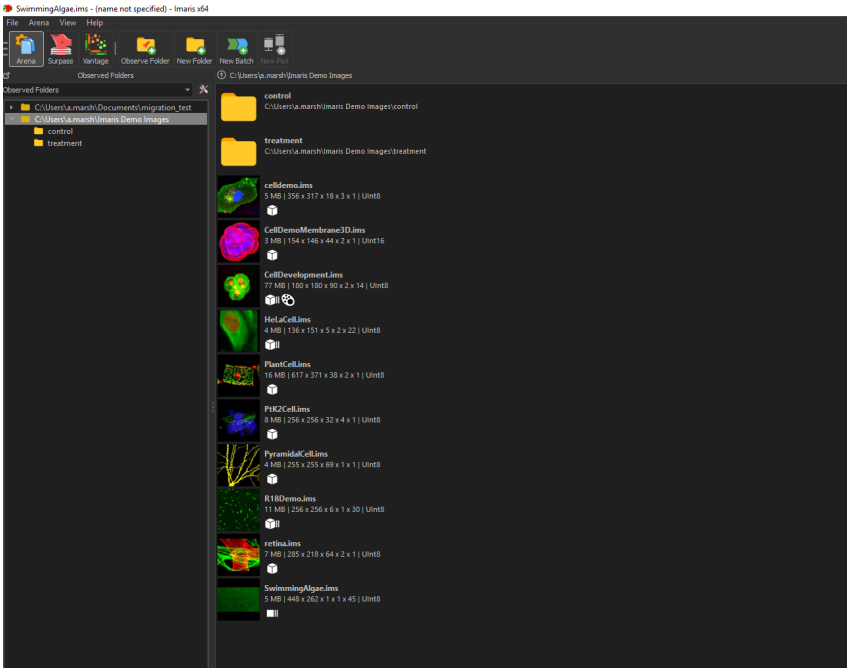
2.2 Observing a Folder

The Arena Tree is used to organize all the items within the Arena in a hierarchical structure. The Arena tree provides direct access to all the components of your experimental data.

To start working with Imaris, you can use the Demo images provided with the Imaris Quick Start Tutorials, or you can observe a folder containing images that you already have on your computer by selecting the observe folder icon. 

2.3 Opening files

Available files are shown by thumbnails within the Arena view. Go to Imaris Demo Images to view a selection of demo images which will be used in the following tutorials.



To select a file for processing and analysis, double-click its thumbnail.

Image files will open in Surpass.

Refreshing Arena and Finding Files in Explorer (or Finder)

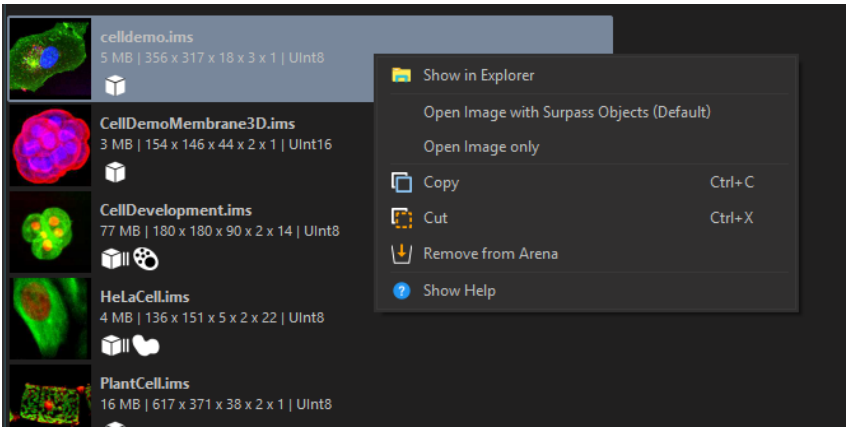
The contents of the open folder in Arena can be refreshed using F5.

To find a file in explorer (or finder on Mac) right click to the right of the image and select Show in Explorer. An explorer window (or finder window

on mac) will open to the correct location.

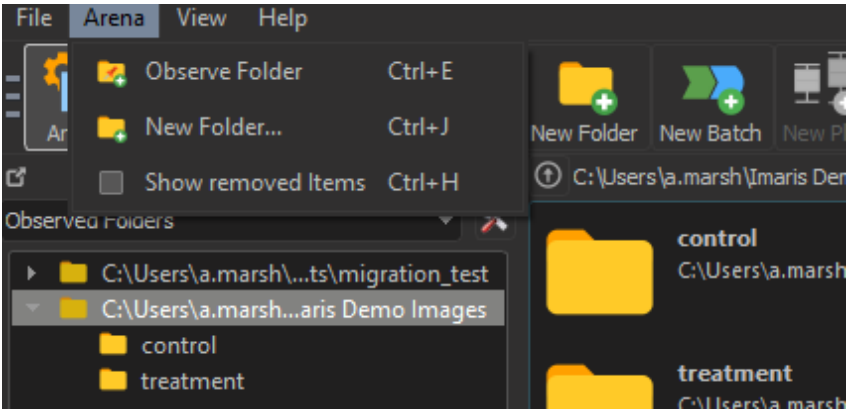
Removing and Re-adding Files to Arena

Files can be removed from the Arena (but remain in the folder on your file system), right click on the file you want to remove and click on Remove from Arena.



To re-add a removed file to the Arena go to the Arena menu select Show removed Items. The removed files will then be shown in the folders which they were removed from and can be restored to the Arena by right

clicking on the file and clicking Restore to Arena.



2.4 Save and Save as

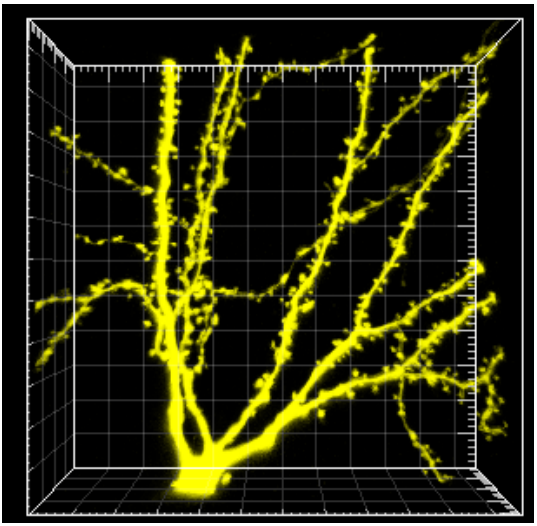
When in Surpass or Vantage view the created items should be saved within the Surpass or Vantage view prior to switching back to Arena view.

Save
Selecting this option saves the image data and created objects in the open file.

Save as
This option enables you to save the changes made to the image in a different file whilst maintaining the original file unchanged. Selecting this option allows you to modify the name of the image and creates a new image item within the Arena view. The selected folder now contains one original image file, which remains unchanged, and the new image file with the additionally segmented object.

Section 3 Visualize Data Set

In this tutorial you will learn the basic working steps in Imaris. The demo image is a Pyramidal cell, which you will Open to create a 3D volume visualization. In the chapter Select and Navigate you will learn about the two different mouse pointer modes in the Surpass view and how to manipulate the view by Rotating, Translating or Scaling 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 Background Color.
In this tutorial, select the Pyramidal cell data set from the Imaris Demo Images folder. The image automatically opens in the Surpass view.

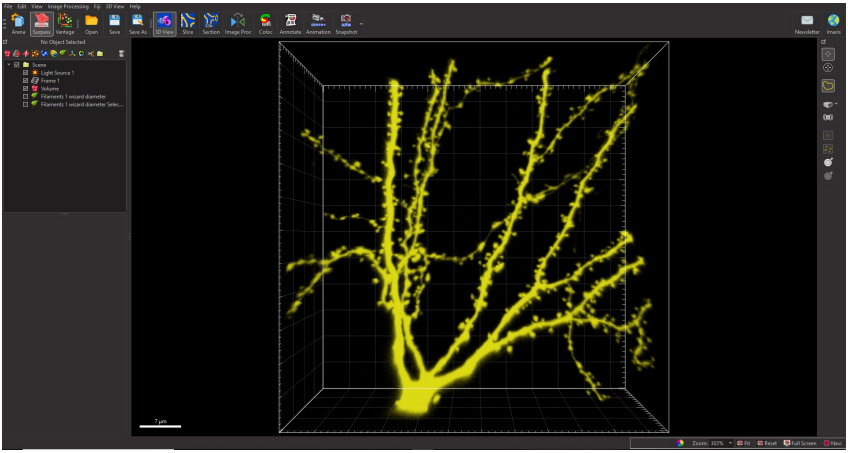


Visualized Data Set, Pyramidal Cell

User Level: Beginner
Module: Imaris

3.1 Surpass View

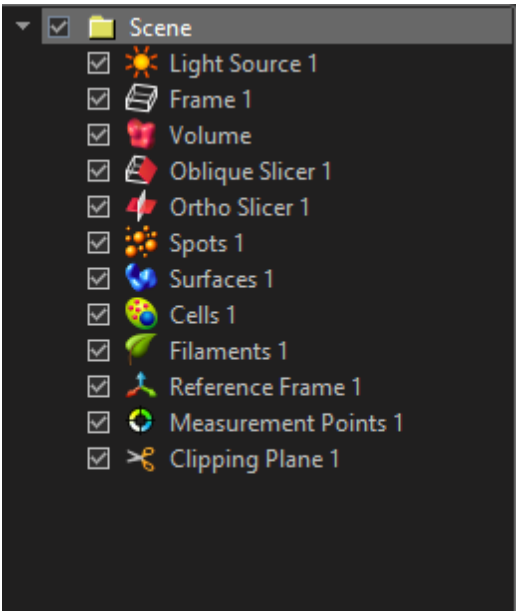
Imaris is built around a three-panel workspace: **View Area**, **Object List** and **Object Properties Area**.



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

Object List

Simultaneous combination of various visualization techniques is achieved by **Object List** making understanding of the complex data easier. The Surpass tree is used to organize all the Surpass objects in a hierarchical structure.



The default name of the folder is **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 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**.

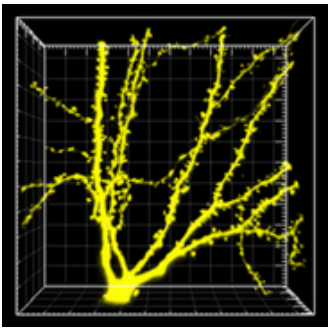
Object Properties Area

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

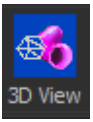
3.2 3D View

Now you will create a 3D reconstruction of the Pyramidal cell. Select the Pyramidal cell image and click on Surpass view.

Surpass view defaults to the interactive 3D volume visualization on opening. The 3D volume reconstruction of the Pyramidal cell is displayed in the view area.



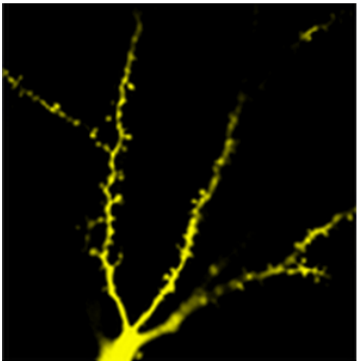
The 3D view can also be displayed by clicking on the 3D View icon when you are in other viewing modes.



3.3 Slice View

The Slice view allows you to quickly inspect an image. Switch to the Slice view by clicking on the Slice button.

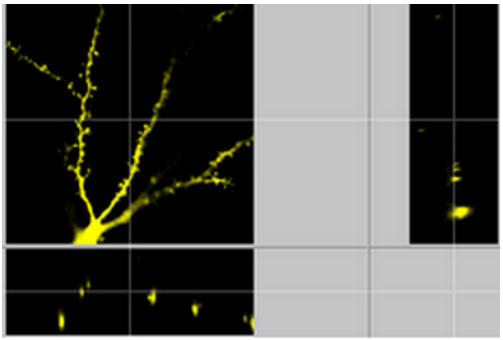
Slice view for individual slices:



3.4 Section View

The Section view allows you to quickly inspect an image. Switch to the Section view by clicking on the Section button.

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



3.5 Select and Navigate

In the Surpass view of Imaris there are two different mouse Pointer modes - **Pointer Selection Mode or Circle Selection Mode.** 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.

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

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

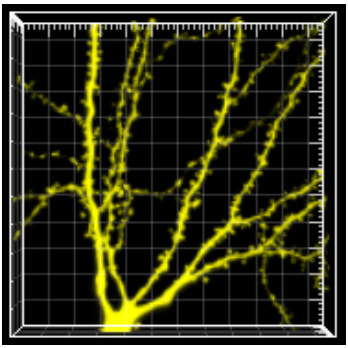
The shortcut keys 1 and 2 (or esc) let you quickly switch between Pointer Select Mode and Circle Selection mode respectively.

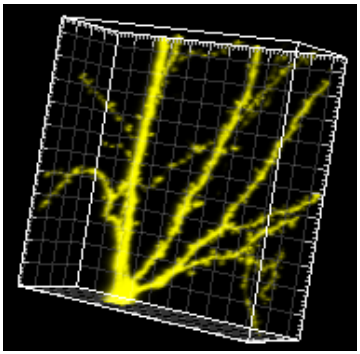
Navigate
Once either the Pointer Select Mode or the Circle Selection Mode have been selected, by dragging the cursor across the screen, the cursor becomes two turning arrows and you can use either pointer to navigate, move, rotate or zoom the image in the viewing area.

3.6 Rotate Image

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

How to Rotate an Image?



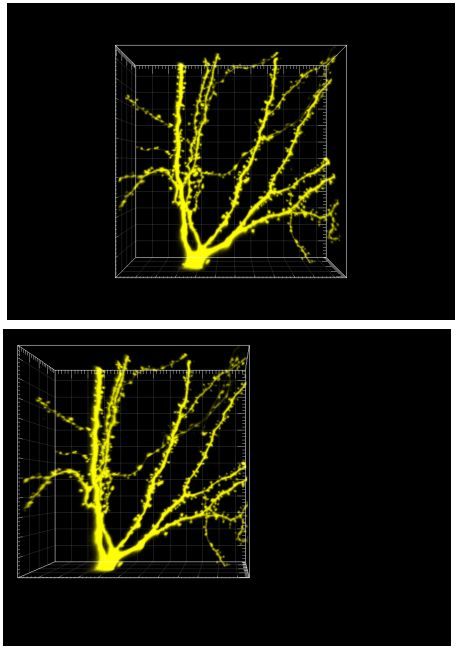


1. Click with the mouse in the image and hold the button down while moving the mouse (hold + drag). The image on the screen rotates in the direction the mouse is dragged (be sure to hold the mouse button down during the whole rotation).
2. Stop moving the mouse and release the mouse button to stop the rotation.

How to Keep the Image Continuously Rotating?

1. Click with the mouse button in the image and hold the button down while you move the mouse (hold + drag). The image on screen rotates towards the direction the mouse is dragged.
2. Release the mouse button while still dragging the mouse (the speed used to drag the mouse determines the speed of rotation of the image). The image now rotates continuously.
3. To stop the continued rotation re-click anywhere in the image area.

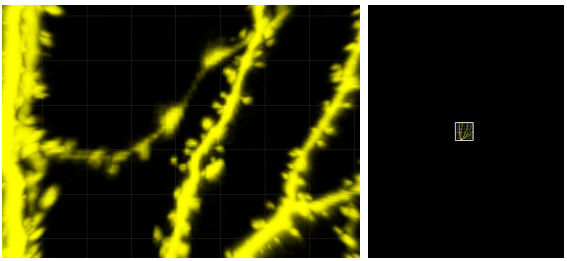
3.7 Translate Image



1. 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).
2. Release the right mouse button to place the image.

3.8 Scale Image

In Surpass view you can scale the image, either using the mouse or selecting one of the buttons at the bottom of the screen.



Using the Mouse

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

Using the Buttons at the Bottom of the Screen

Zoom	Enter the required zoom magnification %.
100%	Rotates the image to original position, centers in the middle and sets the zoom factor to one pixel per voxel.
Fit	Pan position to best fit in the window and adjust the zoom factor.
Full Screen	Maximizes the viewing area to the full size of the monitor.
Navi	Toggles display of navigation window (upper right corner of the viewing area).

3.9 Save as...

The standard way to save your data and Imaris scene on your local storage space is to choose the **Save as**

icon from the Main toolbar (you can also choose Save as from the file menu). Selecting this option allows you to modify the image name and creates a new image file. The file extension is *.ims and the created Imaris file contains both the image and the scene data. Select the folder where you want to save the file to and give it an appropriate name.

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.

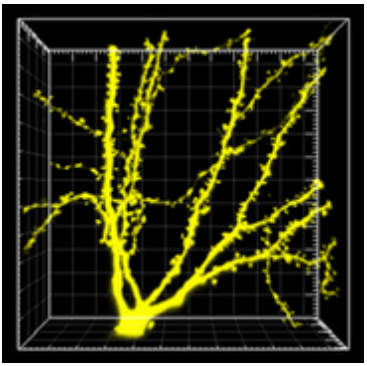
In this tutorial enter the name as **PyramidalCellA.**

3.10 Practice Makes Perfect

Navigating the image with the mouse in the viewing area needs some practice but once you master it, it is a powerful tool to analyze your data. Start with a central position, take some time to practice moving, rotating and panning the image.

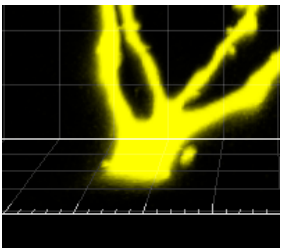
Home Position

Use the buttons at the bottom of the viewing area, click **Reset**.



The Pyramidal cell displays centered in the viewing area. **Tip**: Whenever you lose orientation, re-center the view to the Home Position by clicking **Reset** button again.

Rotate Backwards



Try rotating the image backwards. Click on the base of the Pyramidal cell, hold the button down while you drag the mouse upwards, stop the mouse movement and release the mouse button.

Move the Image Clockwise

Try rotating the image clockwise. Hold the button down while you move the mouse to the left, stop the mouse movement and release the mouse button.

3.11 Change Background Color

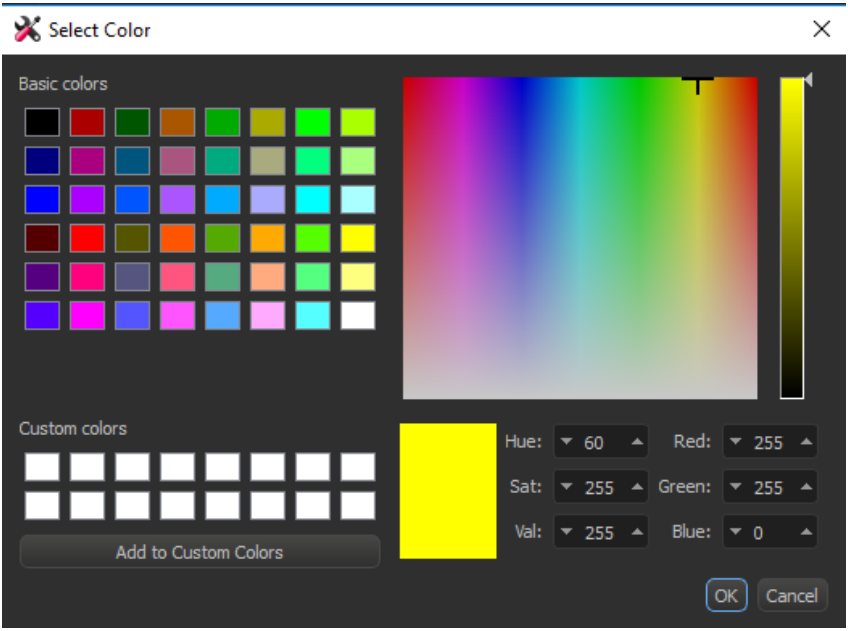
To change the background color go into the Imaris Display settings.

Open the Preferences - Display Window

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

Change Background Color

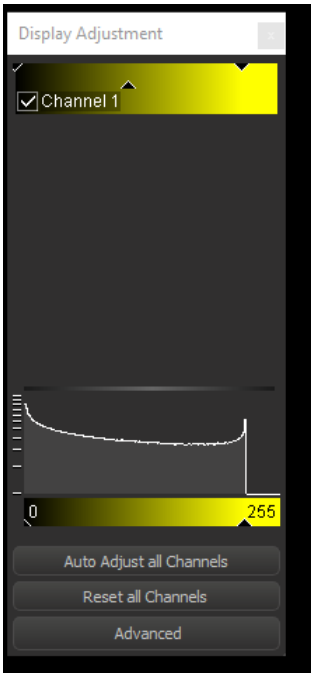
To change the **Background Color** click on the color wheel button to open the **Select Color** window.



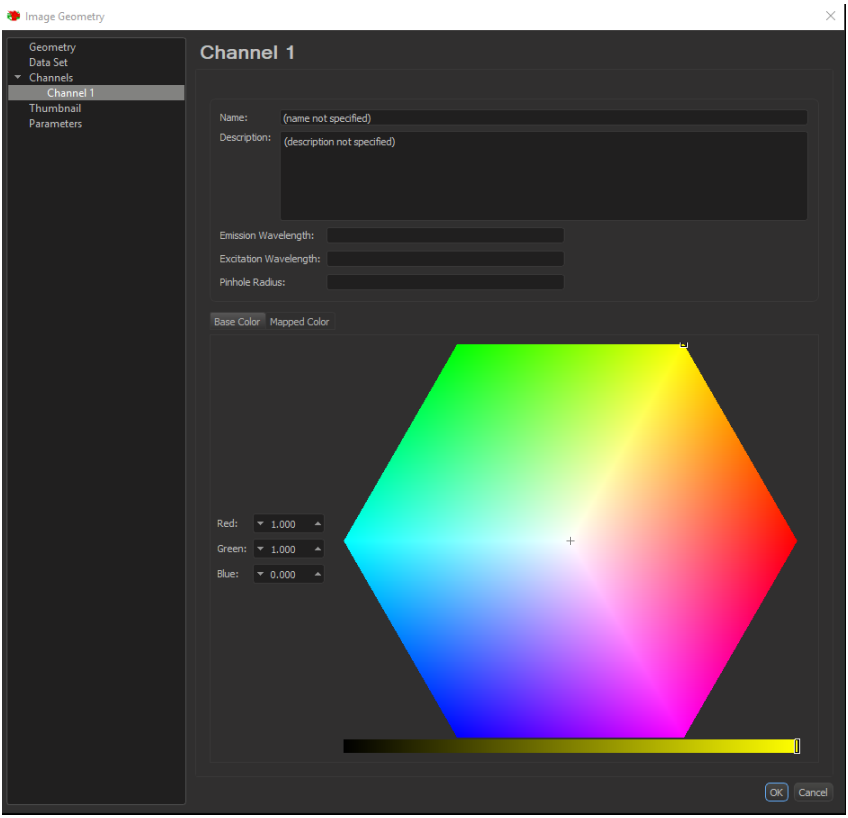
Select the desired color and click on **OK**. In the window **Preferences - Display** click on the **OK** button. The background color changes accordingly.

3.12 Change Channel Color

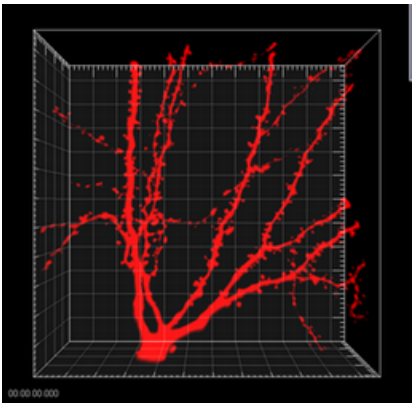
Now change the color of the display Channel 1 from yellow to red. To open the **Display Adjustment** window select in the **Edit** - **Show Display Adjustment** option.



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:



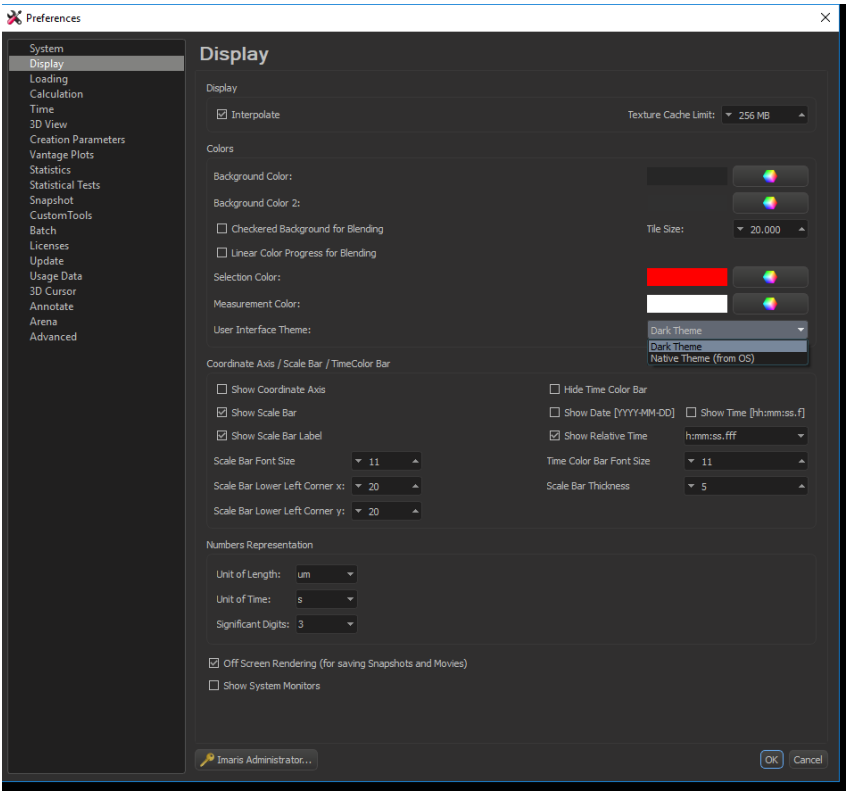
MIP Display, Channel Color Red, Pyramidal Cell

Please close Imaris before you start with the next tutorial.

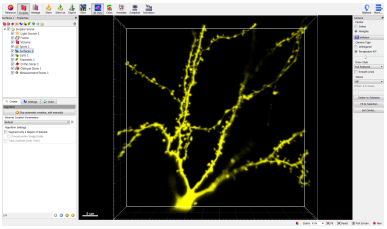
3.13 Change the User Interface Theme

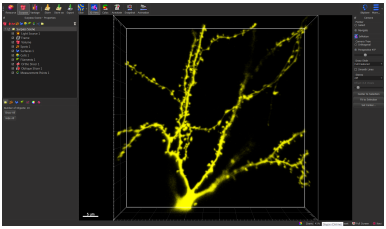
You may also choose to customize the user interface theme. To do so, select the **File - Preferences ... - Display** option (Mac: Imaris-Preferences ...).
First change the Selection Color from yellow to red by clicking on the color wheel button and selecting a red color. By changing this preference histograms are now presented in red.

You can either choose the **Native** theme (from OS) or select the **Dark** theme. Click OK.



The display changes instantaneously.

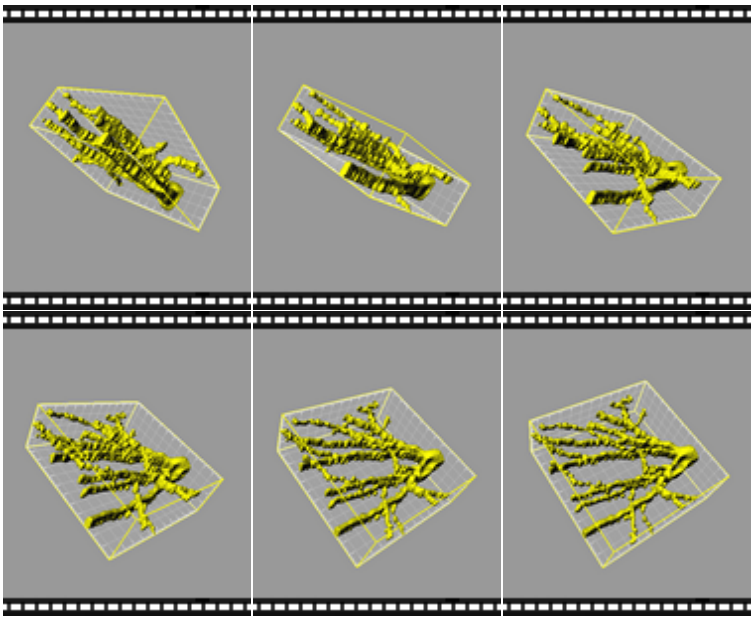




Throughout the remaining chapters of the QuickStart Tutorial the user interface is displayed using the dark theme and histograms displayed in red. However, feel free to adapt your Imaris interface to your personal preferences at anytime.

Section 4 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, Load the File of the processed data set generated in the previous tutorial Visualize a Data Set. In this tutorial you will learn the Key Frame Animation functions, practice to subsequently Shoot, Play, and learn how to record an animation as a movie file using the Save a Movie options.



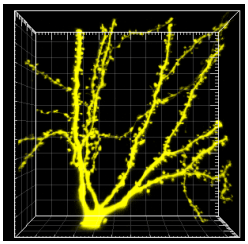
Movie Sequences, Pyramidal Cell

4.1 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.

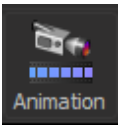
To make a movie please open the file PyramidalCell (double-click the thumbnail to open the file).

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



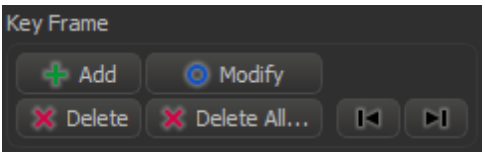
4.2 Key Frame Animation

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.



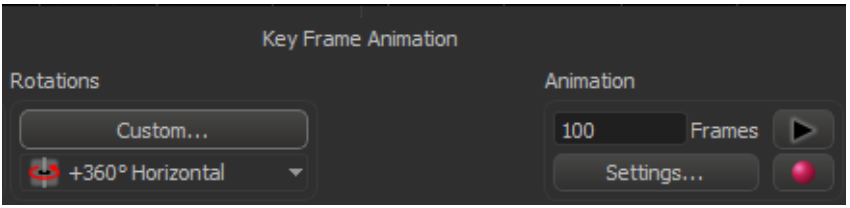
Click on the **Animation** icon in the toolbar. You find the **Key Frame Animation** window at the bottom of the screen. It is divided into five parts, the **Key Frame** window, the **Rotations** window, the **Animation** window, the **Play Back State** window, and the **Strip** window.

The Key Frame Window



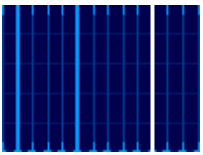
In the **Key Frame** window you can define and manage the key frames of your movie. The three buttons in the first row are to **Add**, **Modify** or **Delete** key frames in the movie strip. Use the **Delete All** button to delete all key frames. With the **Arrow** buttons you step to the **Previous** (arrow to the left) or **Next** (arrow to the right) user-defined key frame.

The Animation Window



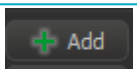
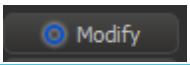
In the **Animation** window you can enter the number of movie **Frames**. There is the button to open the Animation **Settings...** as well as the buttons to **Play** (arrow to the right) and **Record** (red dot) the movie. To pause the animation re-click on **Play. Rotation** panel allows you to scroll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

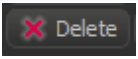
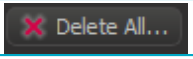
The Film Strip Panel



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.
Press the Add button and the image presented on the screen will appear as a key frame.

Buttons Overview

	**Add -** Addition of new key frame
	**Modify -** Modification of highlighted frames in the film strip

	**Delete** active key frame
	**Delete All** key frames
	Play to preview of animation
	Pause to pause animation
	Record animation to movie file
	Go to **Previous** key frame
	Go to **Next** key frame

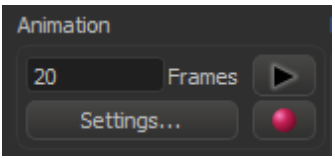
4.3 Shoot and Play

In this section you will learn how to:

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

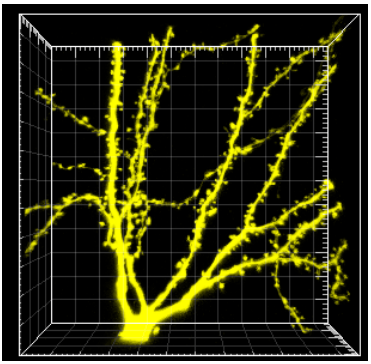
Please remember: Each time you click on the button +Add in the Key Frame window the camera captures the next key frame of your movie.

Movie Length



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

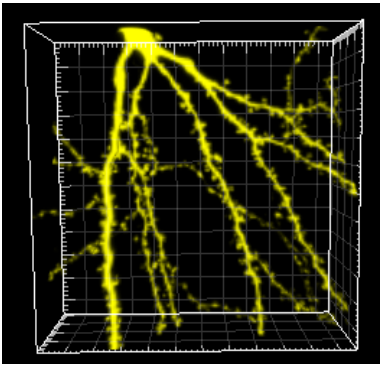
Adjust and Capture the First Key Frame



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

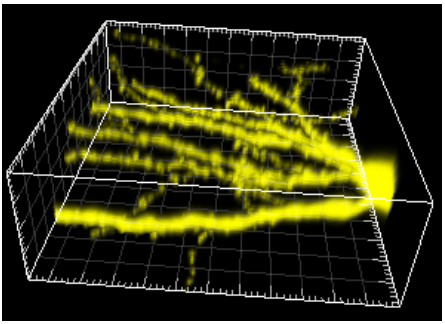
Add Additional Key Frames

Move the image to the next viewing position. Click on the base of the Pyramidal cell, hold the button down while you move the mouse upwards, stop the mouse movement and release the mouse button. The view should be similar to 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, Visualize Data Set - Rotate Image

4.4 Save Movie

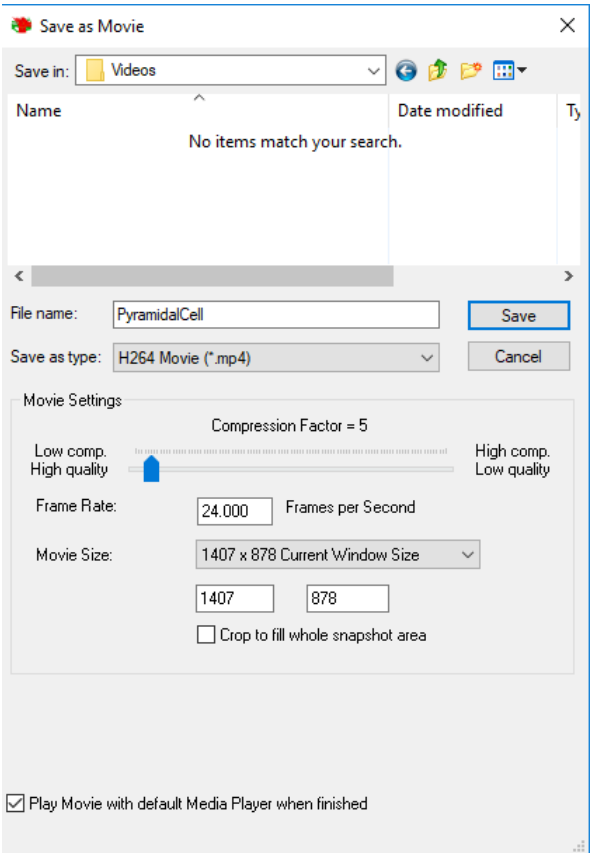
There are two possibilities to save the movie. Either as an Imaris File, or as a movie-file.

In Imaris as a Scene File

Whenever you save the file in Imaris, the actual animation is saved automatically as part of it. Click on **Store As** and enter a name for the new file. The animation is now saved and added to Arena.

Record the Animation as a Movie

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

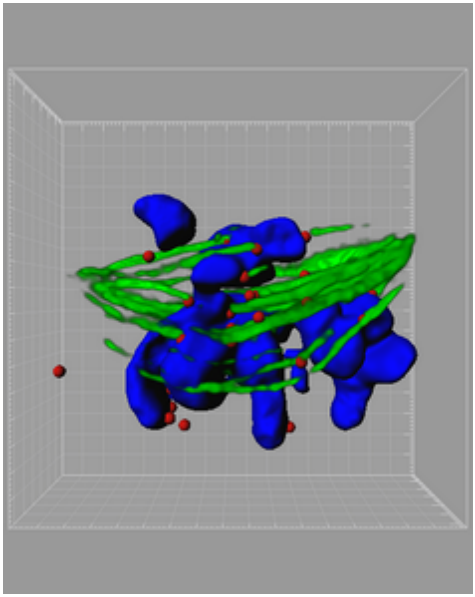


As **File name** type in the corresponding File name, use the default **Movie Settings** and click on **Save**.

Please close Imaris before you start with the next tutorial.

Section 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 objects. The PtK2 cell Demo Image is particularly suitable for a mixed model rendering. You will start with a Volume rendering for all channels and then you will adjust the contrast, brightness and transparency in the Display Adjustment window. The microtubules remain Volume rendered, but for the chromosomes you will choose the Surfaces mode in Imaris. For the kinetochores you select the Spots. The Object List displays a list of all objects in the viewing area and is the ideal instrument to handle the Final Image.



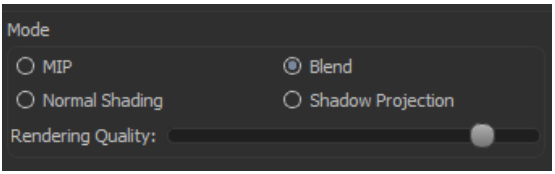
Mixed Model Rendering, PtK2 Cell

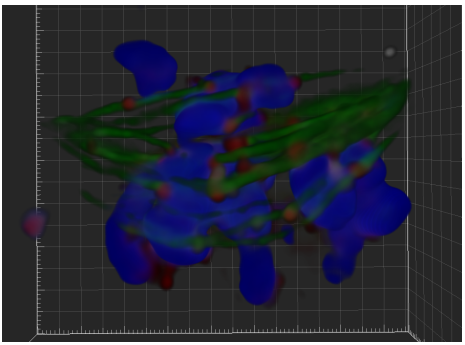
5.1 Add Volume

Double-click on the **PtK2 cell image** thumbnail to open it in the Surpass view.

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.

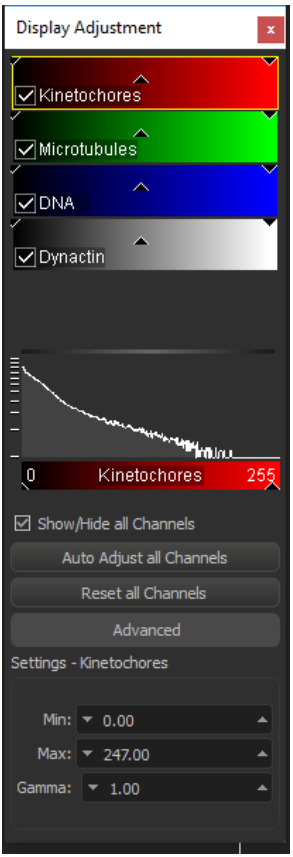




This is the PtK2 cell displayed in the viewing area.

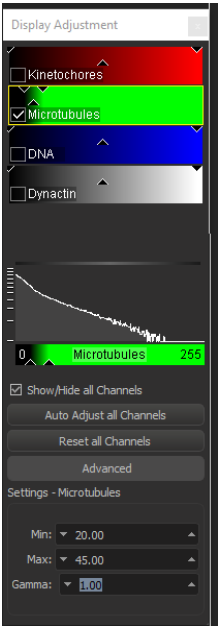
5.2 Display Adjustment

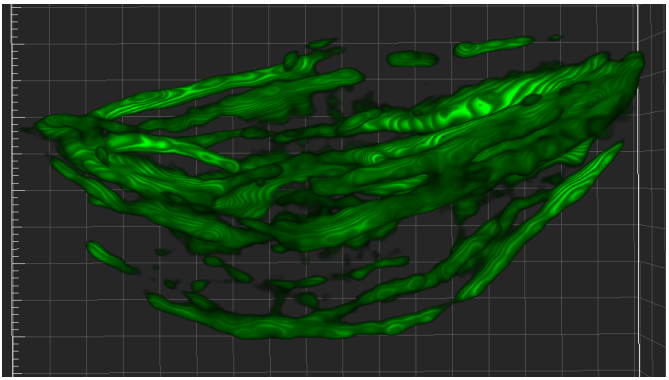
The objects rendered as Volume are usually quite dark and have a lot of noise. To optimize **Volume** rendering the image contrast, brightness and blend opacity will be adjusted. These settings are in the **Display Adjustment** window. The Display Adjustment function lets you interact with each channel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



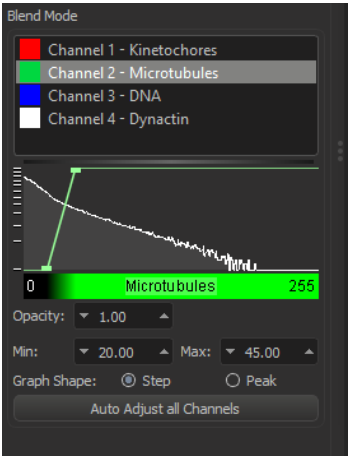
Adjust Individual Channel

To tune the green channel (microtubules) individually, un-check the red, blue and grey channels. Selecting or de-selecting channels controls their visibility. In the viewing area only the green channel is now displayed. Select the green channel by clicking on the channel bar. Click the Advanced Button and set the **Range - Microtubules Min: 20.000** and **Max: 45.000**.

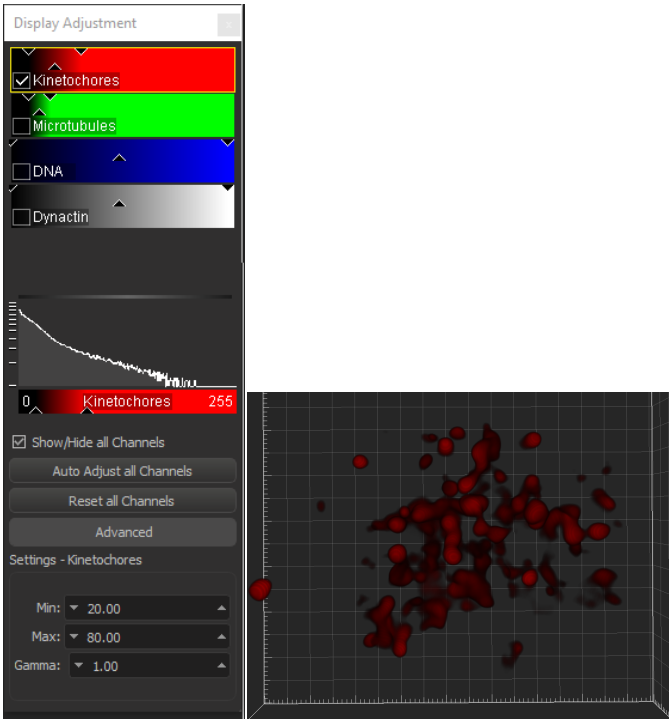




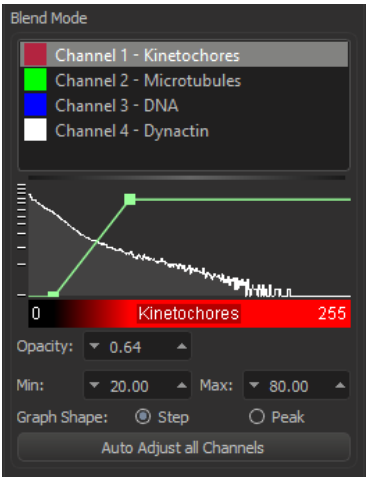
Set the **Opacity to 1** on the left hand side panel.



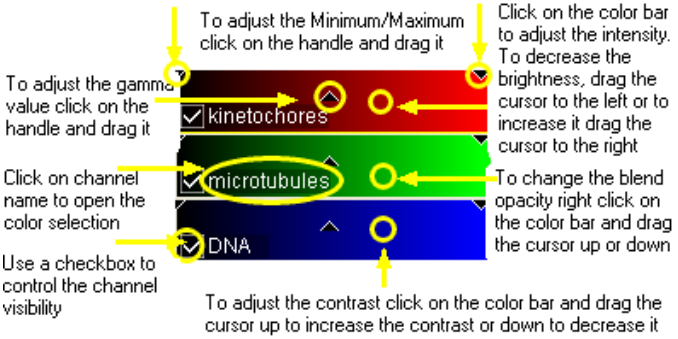
Now the red channel will be tuned. To focus on the red channel (kinetochores) un-check the box for the green and enable the red channel checkbox. In the viewing area only the red channel histogram is displayed. Change for the selected red channel the value **Min** to **20.000** and the value **Max** to **100.000**.



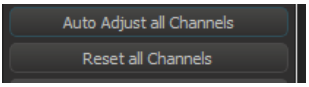
Adjust the **Opacity** to **0.64**.



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

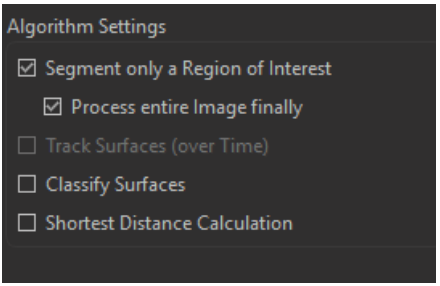


5.3 Add Surfaces

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.

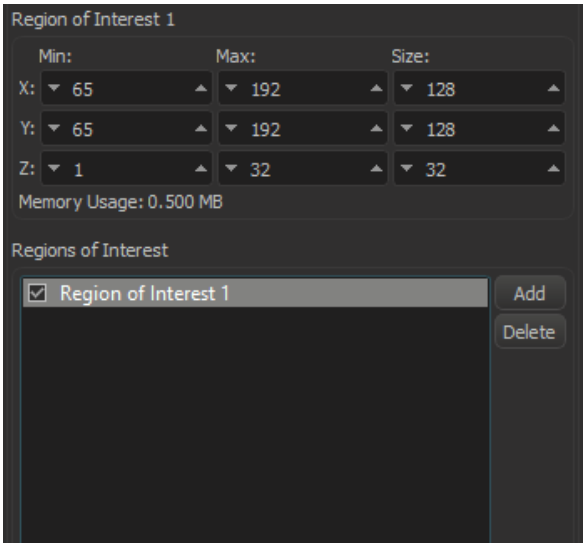
Creation Wizard - 1/6 Algorithm



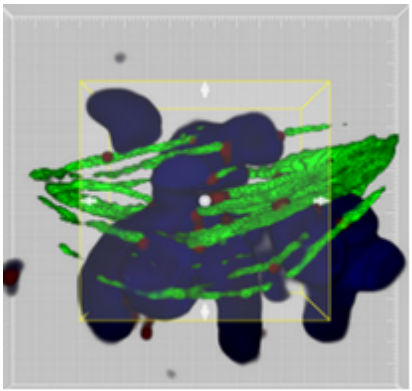
Select segment a **Region of Interest (ROI)** and **Process entire Image finally,** ensure all other boxes are unchecked and click on (Next).

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. By specifying only a part of the image this can speed up processing time and once parameters have been set for the ROI they can then be used to detect features across the entire image.



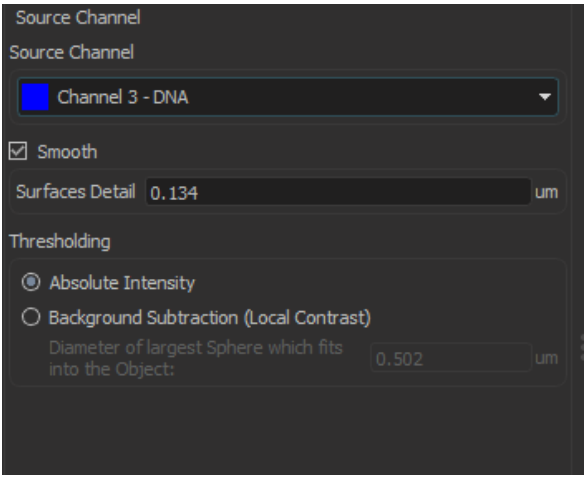
In a viewing area a rectangle-bordered section overlaid on the image represents the Region of Interest (ROI). Entering the values in the corresponding x-, y-, and z- fields (min/max) modifies the size and the position of the ROI.



The size and position of the ROI can also be modified directly by clicking on the arrows in the preview rectangle (cursor - Select mode).

Click on (Next).

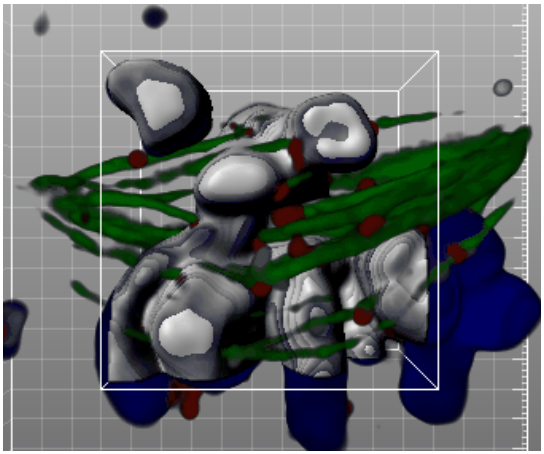
Creation Wizard - 3/6 Source Channel



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.



Click on (Next).

Creation Wizard - 5/6 Filter Surfaces

On the **Filter Surfaces** tab you can filter the resulting Surface by various criteria. In this example, filtering is not necessary, so delete all **Filters** by clicking on the **Delete** Button. Click on (Next).

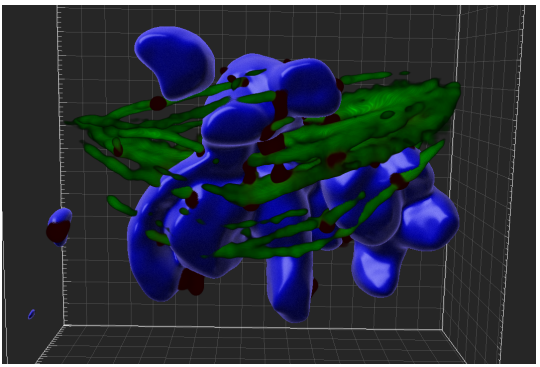
Creation Wizard - 6/6 Complete ROI

To complete Surface creation click on (Finish).

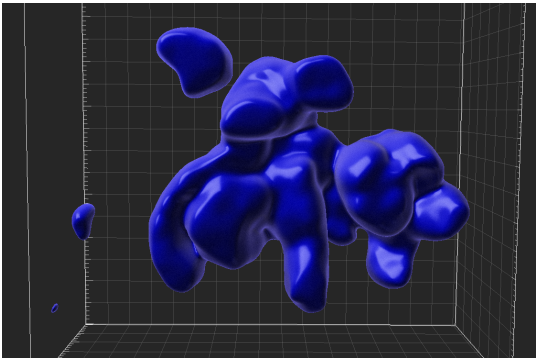
Region of Interest

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.

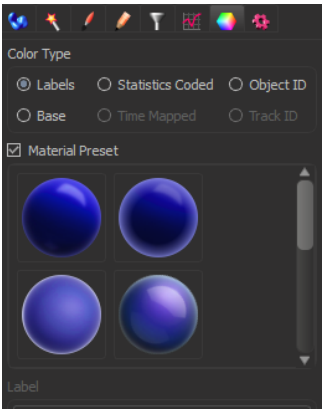


In the Object List now un-check the box for the item Volume and new created surface is displayed in viewing area.



The rendering of the Surface object can be adjusted in the color tab under

the material preset.



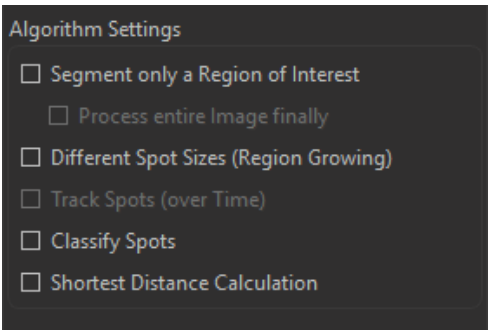
Note that the parameters of this tutorial are specific to this data set and to show how they are used. Your data will require its own specific parameters.

5.4 Add Spots

The kinetochores are small, globular and fairly spherical, and are ideal to show how Imaris detects 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.

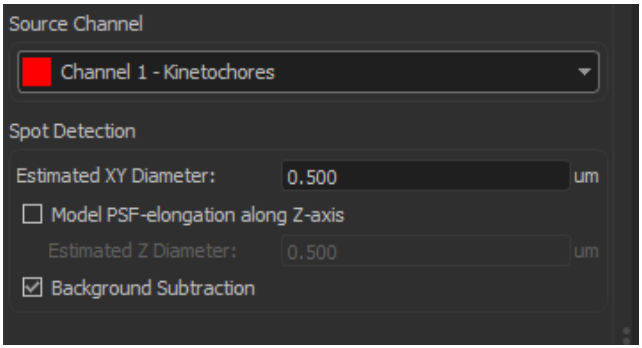
Creation Wizard - 1/3 Algorithm



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. Also ensure all other check boxes are unselected.

Click on (Next).

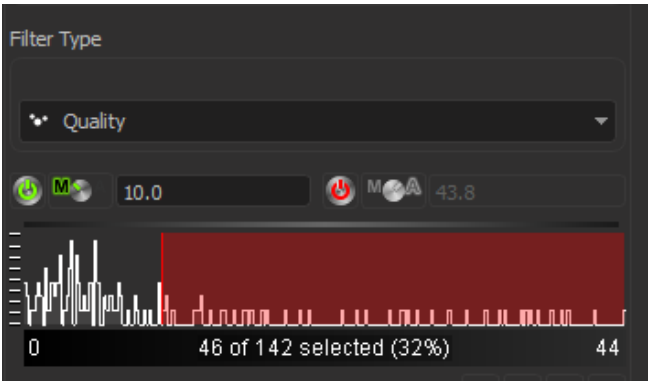
Creation Wizard - 2/3 Source Channel



Select as **Source Channel** the **Channel 1 - (kinetochores)** and set the **Estimated XY Diameter** to **0.500 um.**
Leave the **Model PSF-elongation along Z-axis** option unselected and select the **Background Subtraction** options.
To define the minimum spot diameter please use the Measure Structures features - Line and Polygon.
Click on (Next).

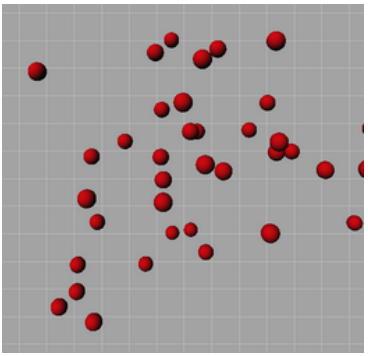
Creation Wizard - 3/3 Filter Spots

Imaris detects an automatic threshold at which to insert the Spots. In the View 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.



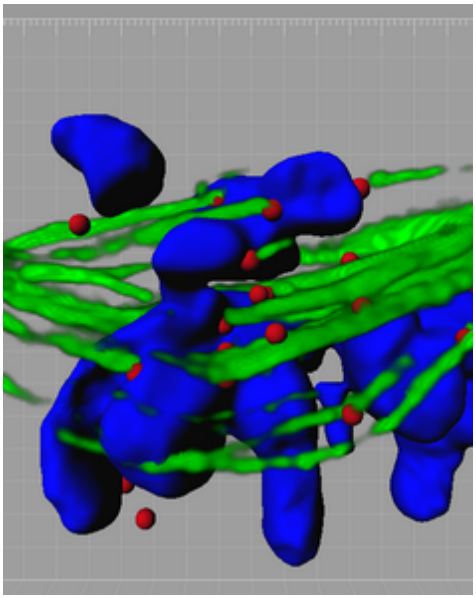
In this example select the **Manual** option and type in **10.000**. Click on (Finish).

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.



5.5 Final Image

The purpose of this tutorial is to highlight the different rendering possibilities of Imaris. To display the composed image check all items in the Object List. In the **Display Adjustment** window check only the green channel.

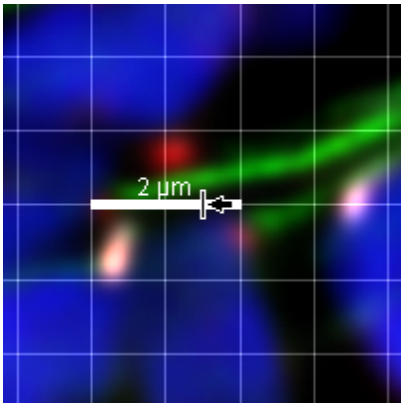


The microtubles display in green as Volume rendering, the chromosomes in blue as Surfaces and the kinetochores in red as Spots.

Please close Imaris before you start with the next tutorial.

Section 6 Measure Structures

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



Grid and Scale Bar Display, PtK2 Cell

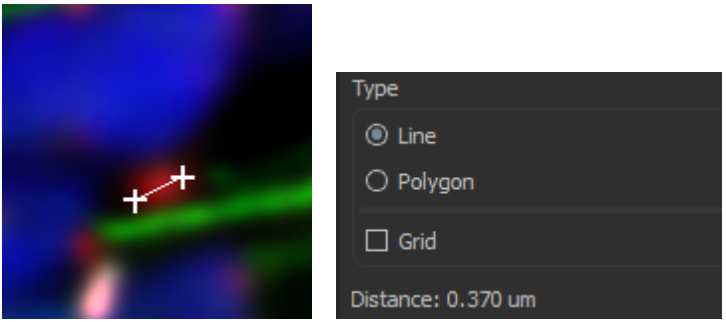
6.1 Line or Polygon

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.
Double click on the **PtK2 cell image** thumbnail to open it in the Surpass view.

Open the image **PtK2 cell** in the **Slice** view.
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

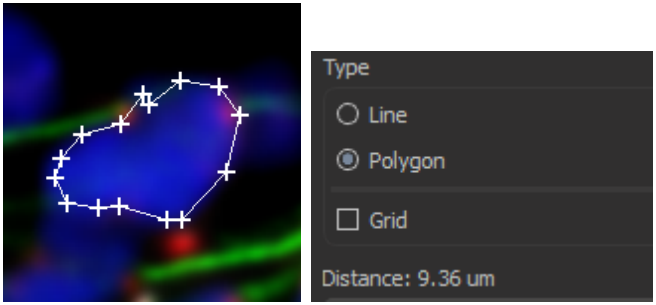
In the PtK2 cell the kinetochores are labeled in red. You will measure the diameter of a kinetochore using the point to point measurement function.



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 is displayed in the status field **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.

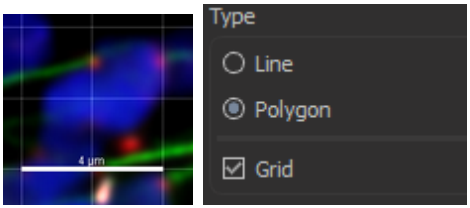


Choose the measurement **Type Polygon**. All measurement points are consecutively connected by lines and the distance is the sum of the distances between the points. Draw a polygon around the DNA like in the image above. The result is displayed in the status field **Distance**. Click on **Delete** to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

6.2 Grid and Scale Bar

Display a Grid

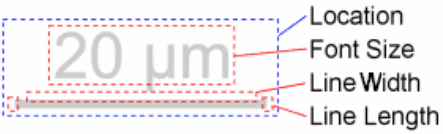
One way to easily estimate distances in the image is to use the grid display.



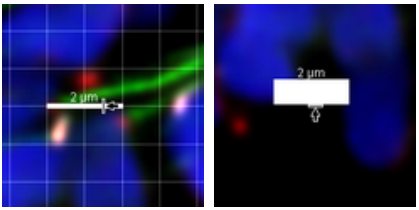
On the right hand side in the **Measure** window check the check box **Grid**. This is the result in the viewing area:

Adjust the Interactive Scale Bar

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

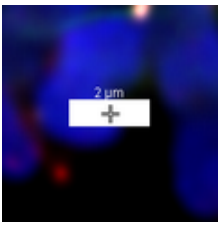


Line Length and Line Width



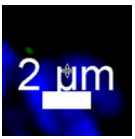
To adjust the length of the scale bar move the mouse to one end of the scale bar until the pointer symbol changes (vertical dash and arrow). Now click on the end of the scale bar and hold the button down while you drag the mouse to the left or right, respectively. The grid width changes accordingly.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

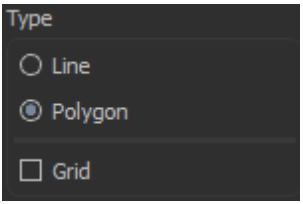
6.3 3D Measurement

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 **Slice** view.

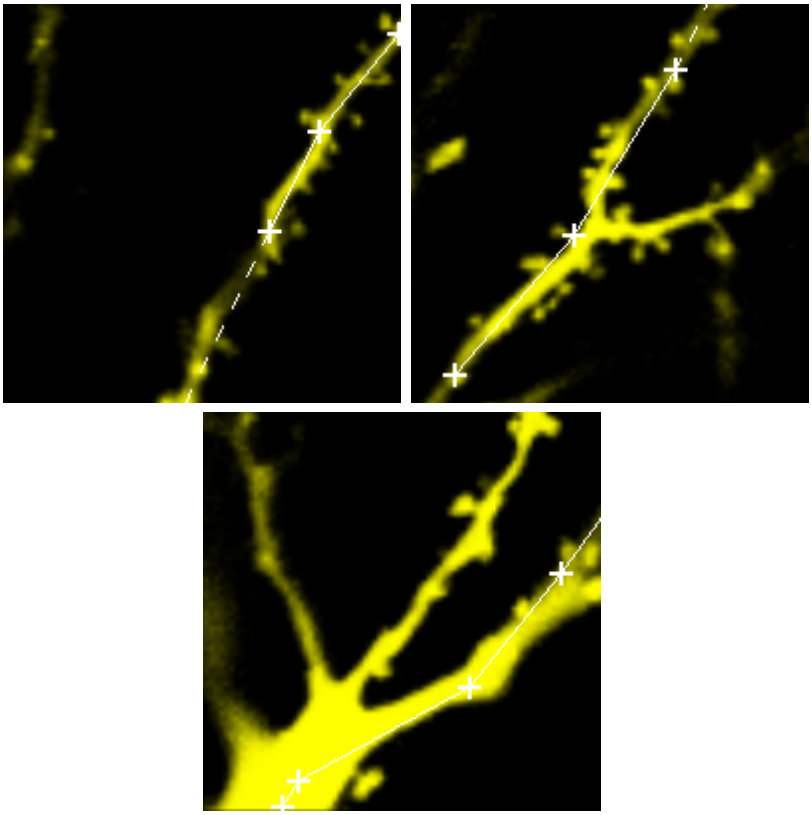
Measure Distance Between Different Slices

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. The distance between the first and the last measurement point in the status field **Distance** can be found on the right hand side of the screen.

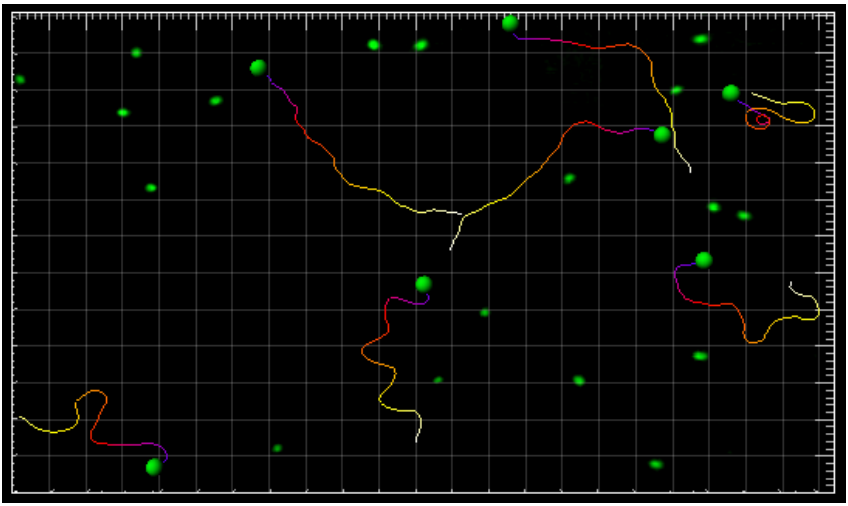


The first picture is slice number 21, the second picture is slice number 25 and the last picture is slice number 32.

Please close Imaris before you start with the next tutorial.

Section 7 Track Particles

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



Spots & Tracks, Swimming Algae

User Level: Advanced
Module: Imaris, MeasurementPro, ImarisTrack

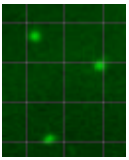
7.1 Visualization

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

Double-click on the **Swimming Algae image** thumbnail to open it in the Surpass view.

Add Volume

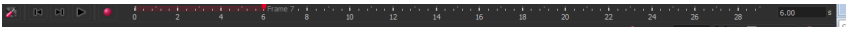
The demo image Swimming Algae is open in the Surpass mode in MIP (max) Mode.
To open the **Display Adjustment** window select in the **Edit** - **Show Display Adjustment** option. Click on **Advanced** to open the advanced settings. Set the threshold **Range** for **Channel 1** (green) to **Min: 90** 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 21. 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.

	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 line indicates the actual time point (in this example time point 7).

7.3 Segmentation

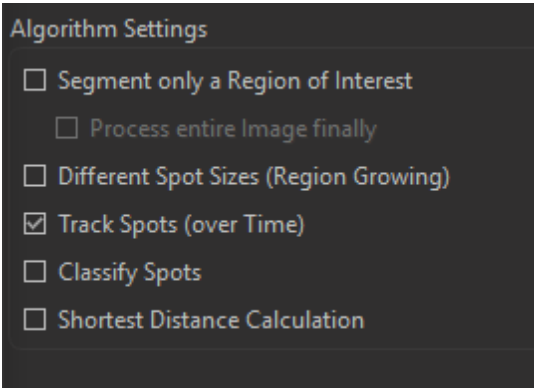
An automatic image segmentation and object tracking methods will be explained by analyzing image Swimming Algae. First you will identify and create spots. Then you will establish a time dependant relationship between spots (track them over time) and create Tracks. Finally you will sort the Tracks based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

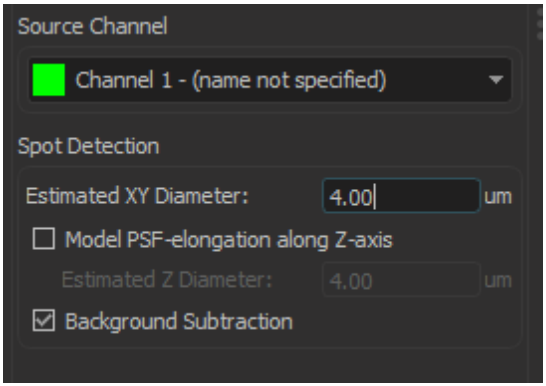
Creation Wizard - 1/6 Algorithm

In the Algorithm Setting check the **Track Spots (over Time).** Make sure no other check boxes are selected in the Algorithm Settings dialog.



Click on (Next).

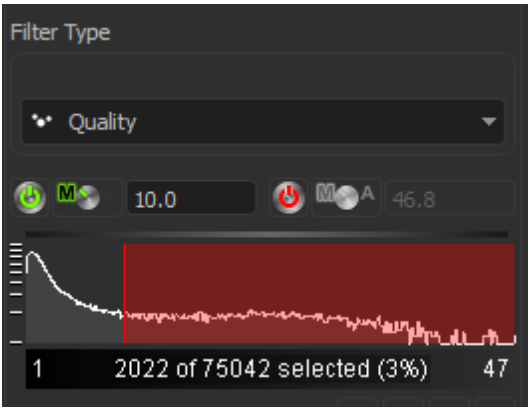
Creation Wizard - 2/6 Source Channel



Select as **Source Channel** the **Channel 1** (green) and set the **Estimated Diameter** to **4.000.** Leave the **Detect Ellipsoids** option unselected and select the **Background Subtraction** options. Click on (Next).

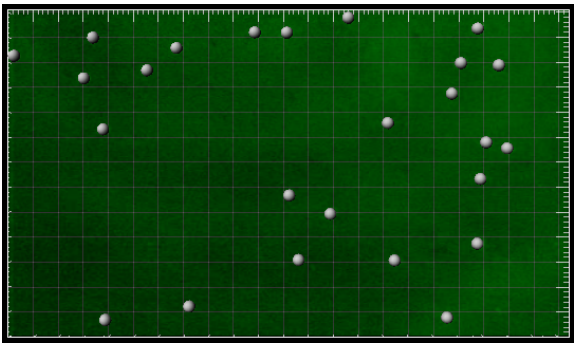
Creation Wizard - 3/6 Filter Spots

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



You will see the gray Spots and the Volume rendered green channel at

the same time.



Click on (Next).

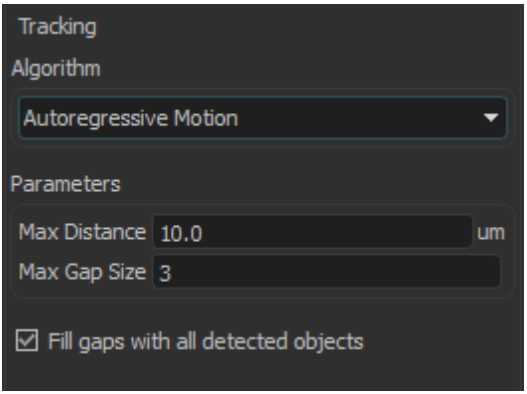
Creation Wizard - 4/6 Edit Spots

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 .

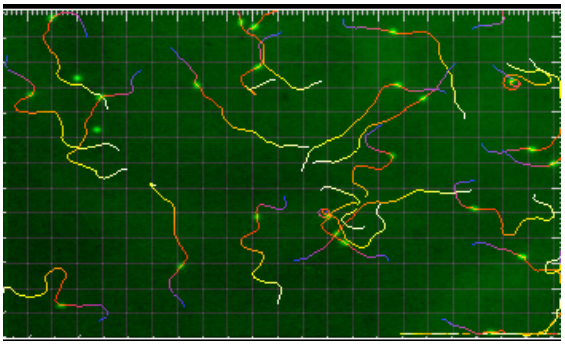
7.4 Tracking

Creation Wizard - 5/6 Tracking

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



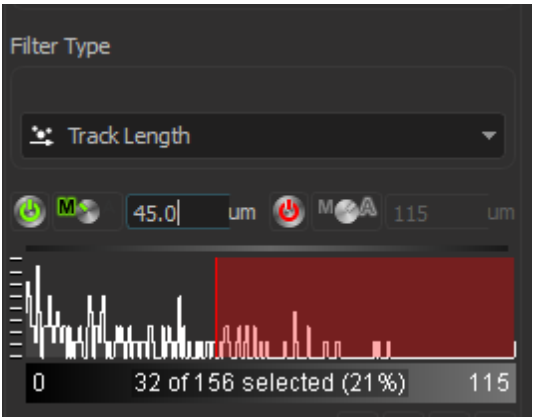
As **Algorithm** select **Autoregressive Motion** and as **MaxDistance** set **10.000** um, and for the **MaxGapSize** set **3** and select the **Fill gaps with all detected objects** option. Click on (Next). This is the result in the viewing area.



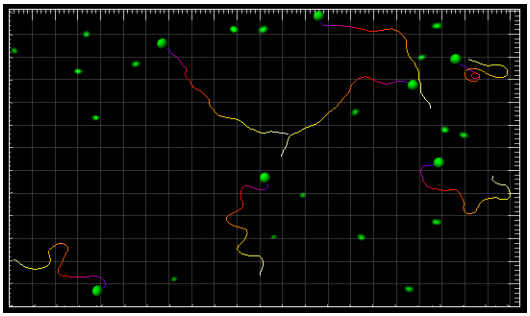
7.5 Filter Tracks

Creation Wizard - 6/6 Filter Tracks

Sorting the Tracks is essential for the further analysis. In the following you sort the Tracks by their length.



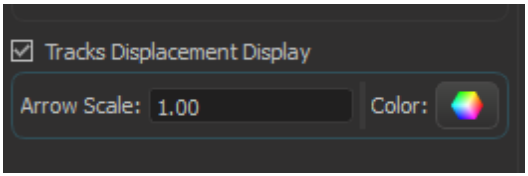
Choose **Filter Type: Track Length**. Select the **Manual** option and enter as value **45.000** um. Alternatively click on the yellow line and drag it. Moving the line changes the filter setting, thus removing or adding some of the tracks.The effect is directly visible in the viewing area. Click on (Finish).



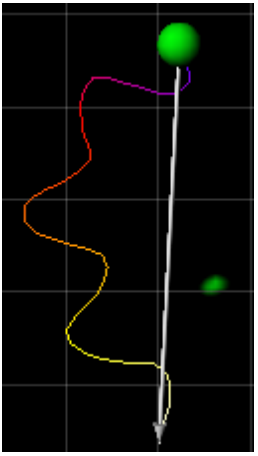
This is the Spots and Tracks display in the viewing area.

7.6 Displacement

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



In the **Tracks Style** adjustment window check the respective check box **Tracks Displacement Display**.



The displacement arrows display in the viewing area.

Please close Imaris before you start with the next chapter.

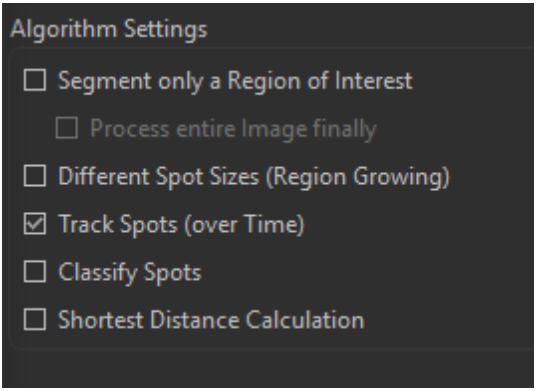
7.7 Additional Example

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

Double-click on the **R18Demo image** thumbnail to open it in the **Surpass** view.

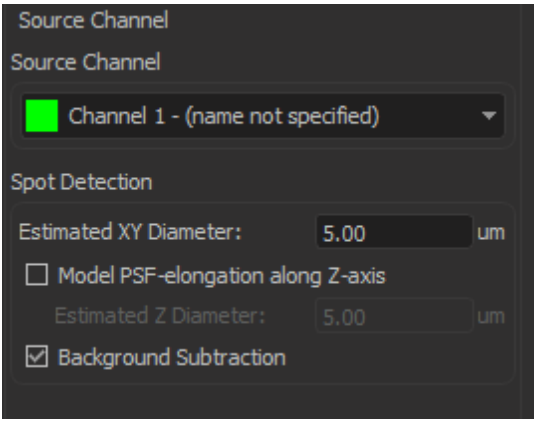
The R18Demo image is displayed.
In the Surpass view click on the icon to add new Spots.

Creation Wizard - 1/6 Algorithm



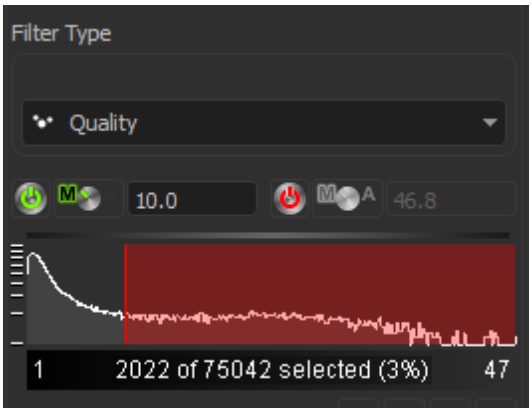
Check the **Track Spots (over Time).** Click on (Next).

Creation Wizard - 2/6 Source Channel



Select as **Source Channel** the **Channel 1** (green) and set the **Estimated Diameter** to **5.000.** Leave the **Detect Ellipsoids** option unselected and select the **Background Subtraction** options. Click on Next.

Creation Wizard - 3/6 Classify Spots



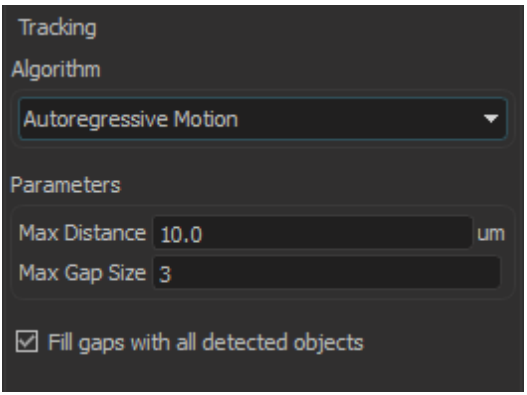
Select the parameter **Filter Type: Quality**, select the **Manual** option and set Lower Threshold to **10.000.** As an alternative left click on the red line and drag it. Moving the line to the right increases the threshold level

and as a result less spots are detected. Conversely moving the line to the left produces additional spots.
Click on Next .

Creation Wizard - 4/6 Edit Spots

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

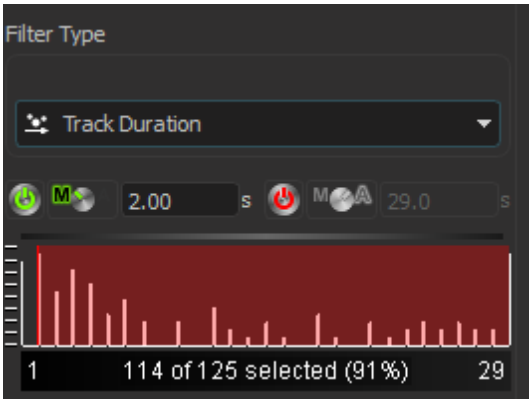
Creation Wizard - 5/6 Tracking



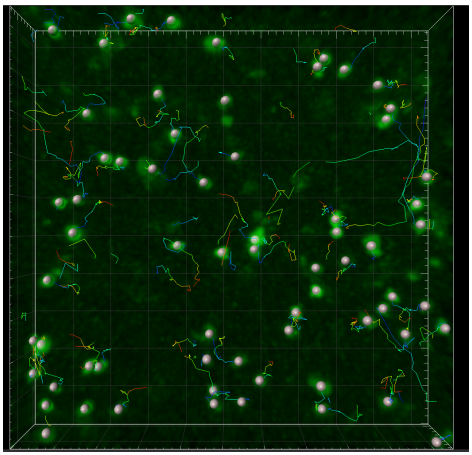
As **Algorithm** select **Autoregressive Motion** and as **MaxDistance** set **10.000** um, and as **MaxGapSize** set **3** and select the **Fill gaps with all detected objects** option.
Click on (Next).

Creation Wizard - 6/6 Filter Tracks

In this example your focus is on the track duration.



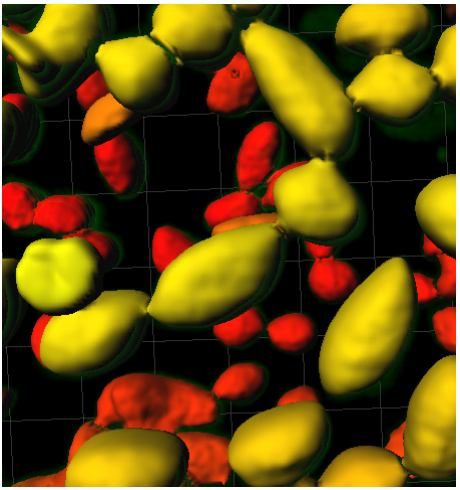
Select as the **Filter Type Track Duration**. Select the **Manual** option and set Lower Threshold to **2.000**. The effect is directly visible in the viewing area. Click on (Finish).
In the viewing area Spots and Tracks are shown.



Please close Imaris before you start with the next tutorial.

Section 8 Define Region Seed Points

In this tutorial you will get familiar with the region growing to segment touching objects. First you Visualize a plant cell with chloroplasts (green) and cell wall components (red), then detect the chloroplasts and define seed points for the Create Surfaces Seeded Region Growing. 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.

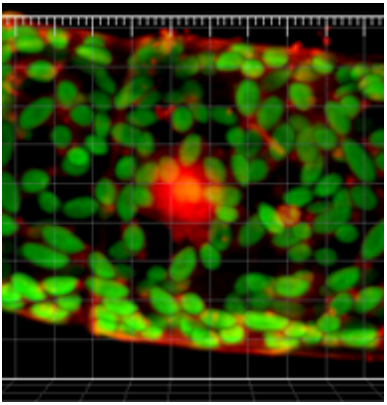


Surfaces, Region Growing, Plant Cell

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.
Double-click on the **PlantCell** image thumbnail to open it in the Surpass view.

Visualize Chloroplasts

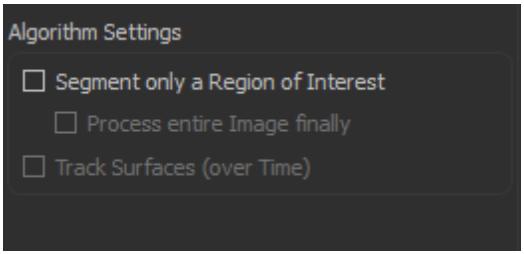


This is the volume rendering of the plant cell in the viewing area.

8.2 Create Surfaces and Region Growing

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.

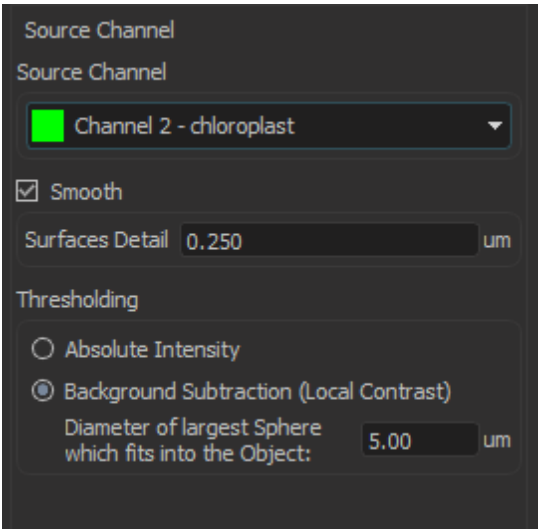
Creation Wizard - 1/5 Algorithm



In the first step, leave all boxes unchecked and click on Next.

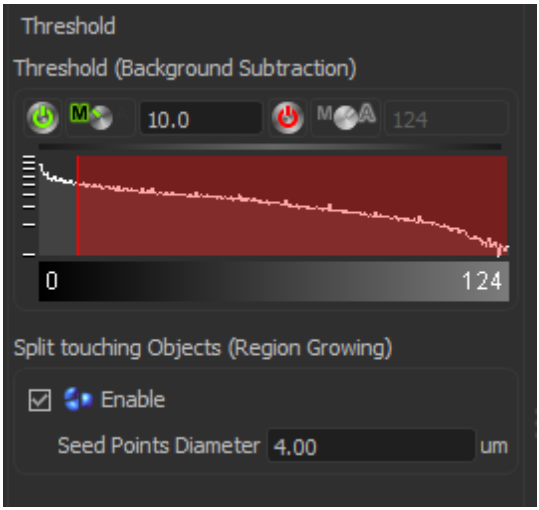
Creation Wizard - 2/5 Source Channel

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



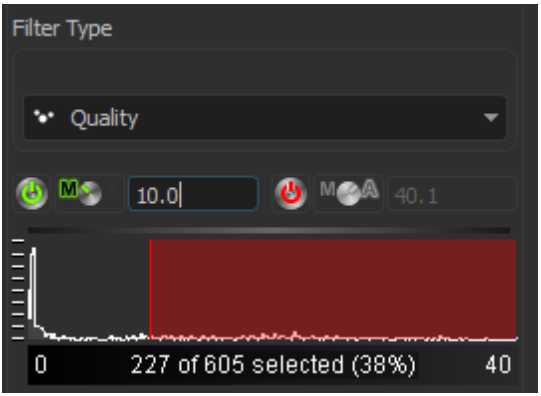
Creation Wizard - 3/5 Threshold

In the **Threshold** adjustment select **Manual,** set threshold to **10.000** and select the **Split touching Objects (Region Growing) Enable** option. Set the value of the **Estimated Diameter** to **4.0 um.** The number of steps increase from 4 to 5. Click on Next.

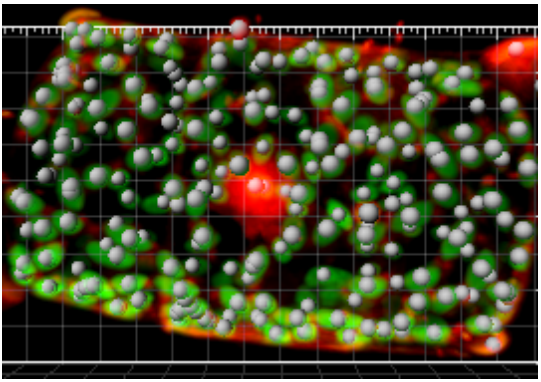


Creation Wizard - 4/5 Filter Seed Points

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

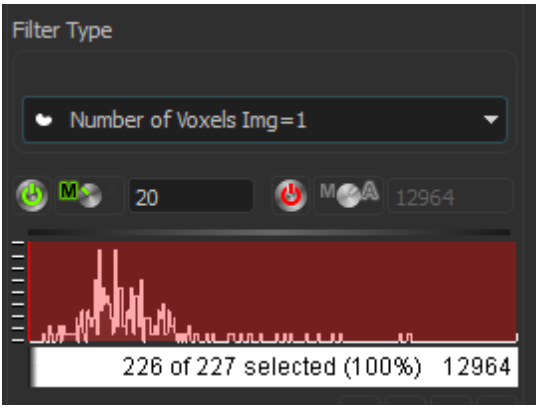


This is the result in the viewing area. Click on (Next).

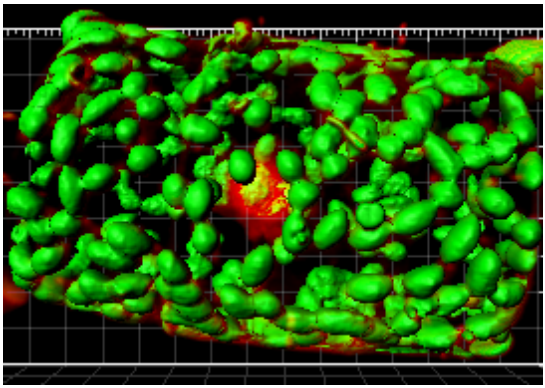


Creation Wizard -5/5 Fliter Surfaces

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

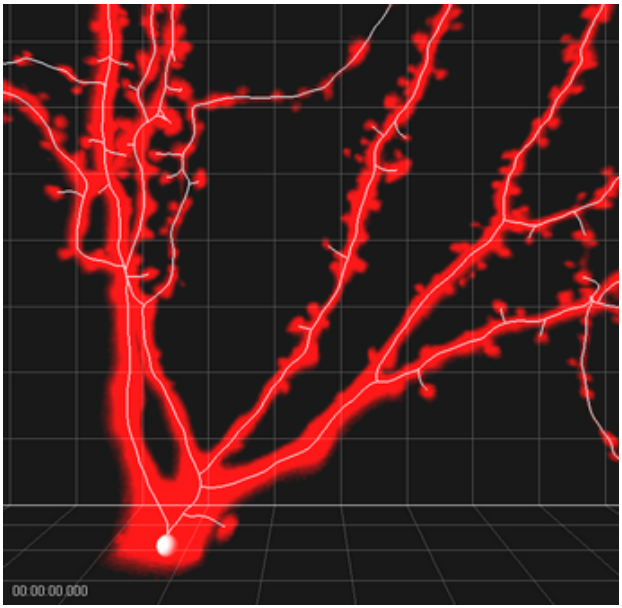


To complete Surface creation with Split touching objects selection click on (Finish). This is the result in the viewing area.



Section 9 Analyze Neuron

FilamentTracer is a separate Imaris module created to detect, visualize and measure filamentous structures. The demo image shows a hypocampal Pyramidal cell in an organotypic cell culture. The tutorial is focused on two ways of filament tracing. First you will learn how to automatically create filament structure in Automatic Detection and then the alternative semi-manual creation-AutoPath Mode is presented. In the last chapter Filament Tool Kit 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.

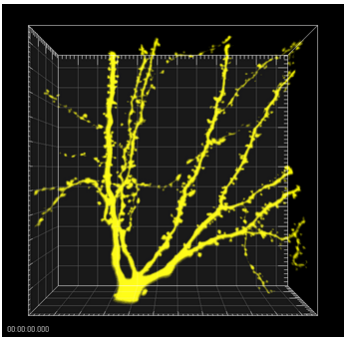


Filament Structure, Pyramidal Cell

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.
Double-click on the Pyramidal cell image thumbnail to open it in the Surpass view.

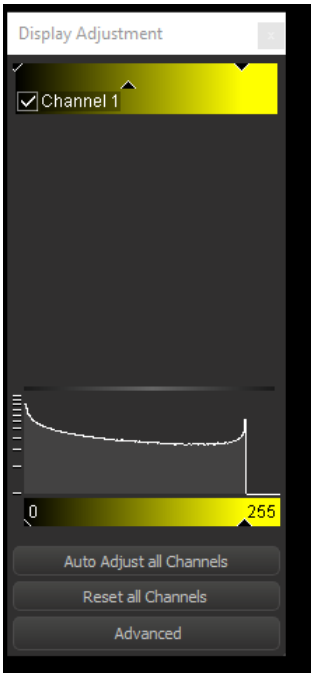
The Pyramidal Cell image is displayed.



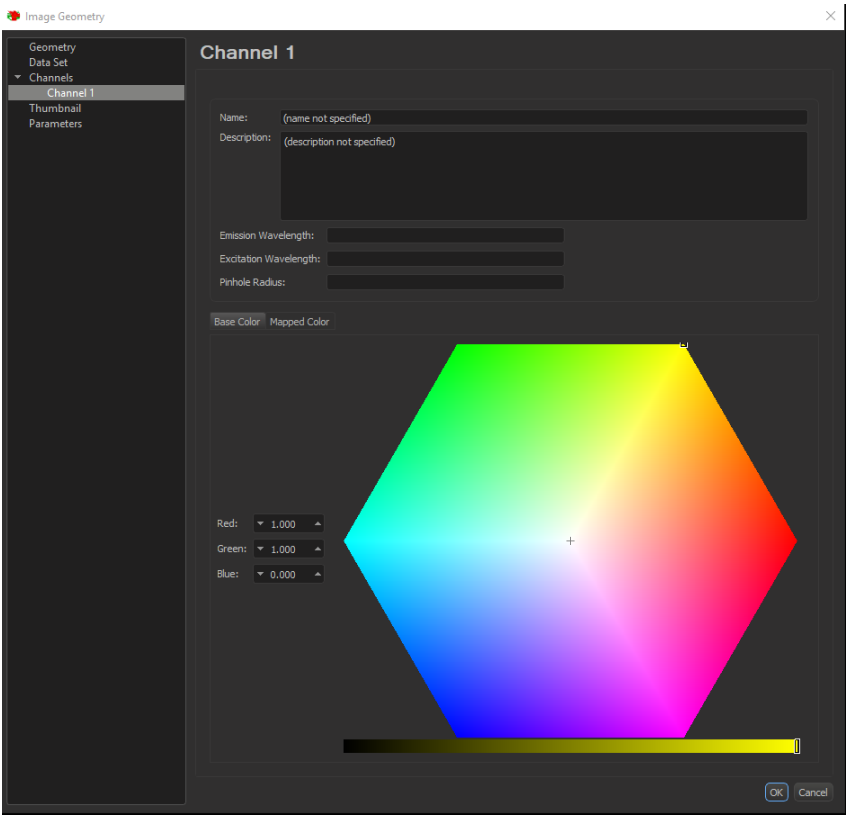
MIP Display, Pyramidal Cell

9.2 Change Channel Color

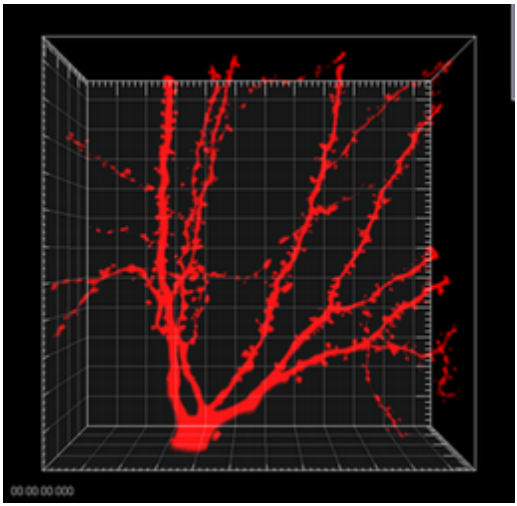
Now you change the color of the display Channel 1 from yellow to red. To open the **Display Adjustment** window select in the **Edit - Show Display Adjustment** option.



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

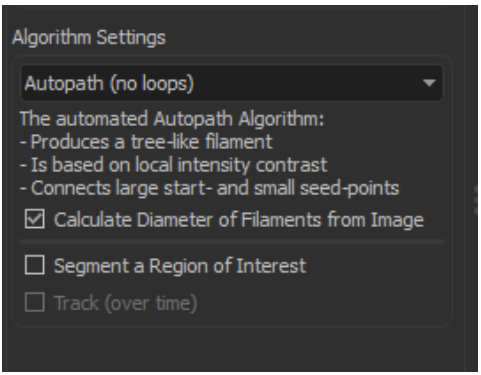
9.3 Automatic Detection

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 Filament icon to add a new Filament. The Creation Wizard appears automatically in the properties area.

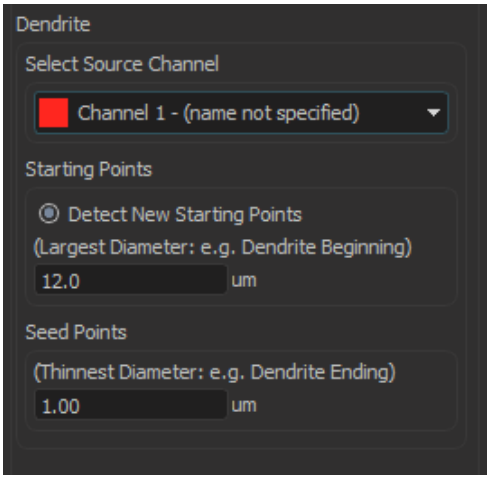
Creation Wizard - 1/7 Algorithm



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

Creation Wizard- 2/7 Dendrite Points Diameter

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

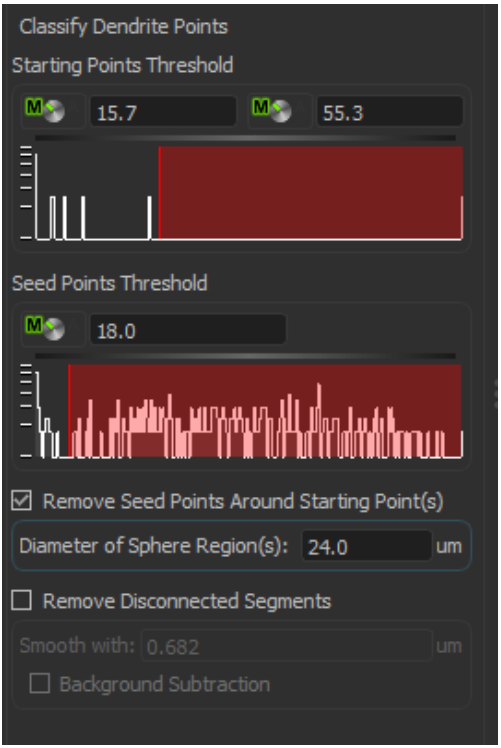


For the **Source Channel** of the dendrite select the **Channel 1.** The next parameter is the dendrite **Starting point** diameter. The maximum (largest) diameter of dendrite is the recommended value for the Starting point diameter. To define the starting and end point diameter please use Measure Structures features - Line and Polygon.
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.

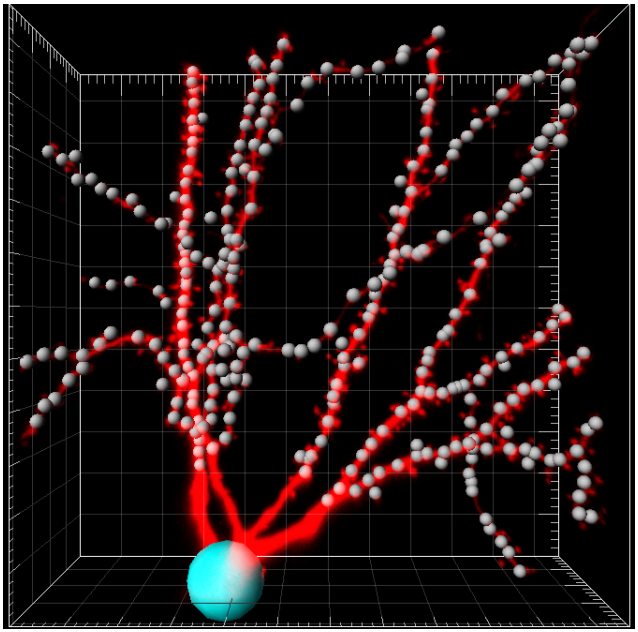
Creation Wizard - 3/7 Classify Dendrite Points

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 **15.7**. 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 removes all seed points located within the radius of the sphere centered at the starting point. The boundary of the sphere is defined by the value Diameter of the Sphere Region. Set this value to **24 um**.
At this point of the algorithm you can select the **Remove Disconnected Segments** option. You will not do so in this example, so leave the box unchecked.

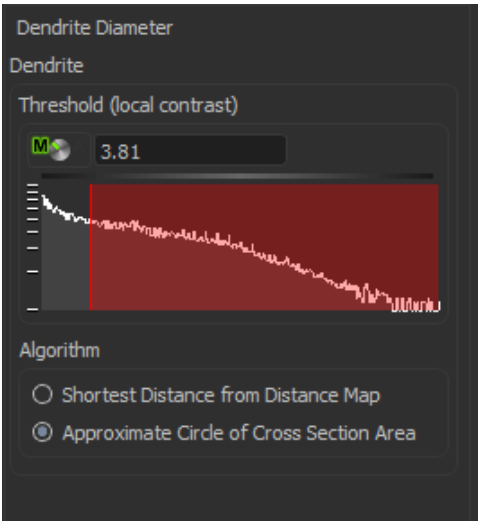


In the viewing area the detected dendrite's Starting point and seed points are displayed in the image by different colors.
Click on Next.

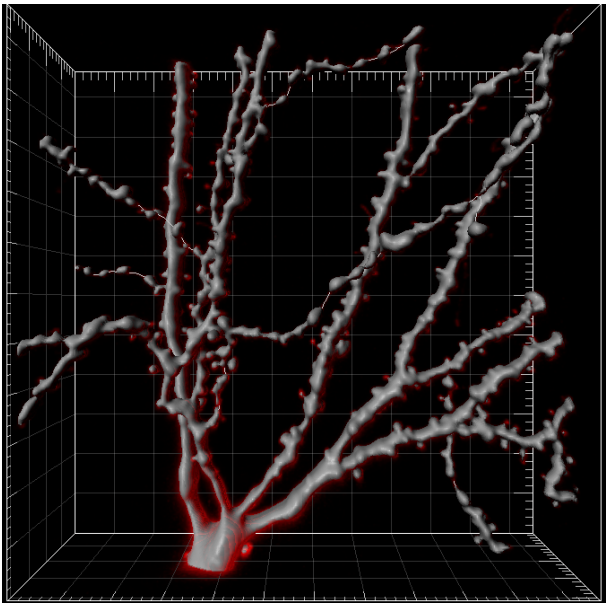


Creation Wizard - 4/7 Dendrite Diameter

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**.

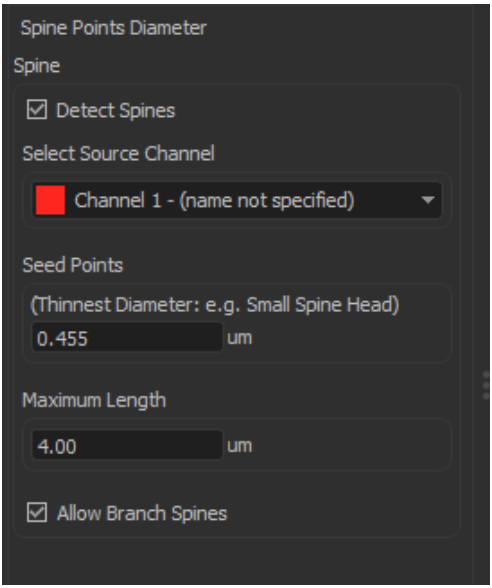


In the viewing area a threshold preview of the dendrite is displayed in gray. Click on Next.



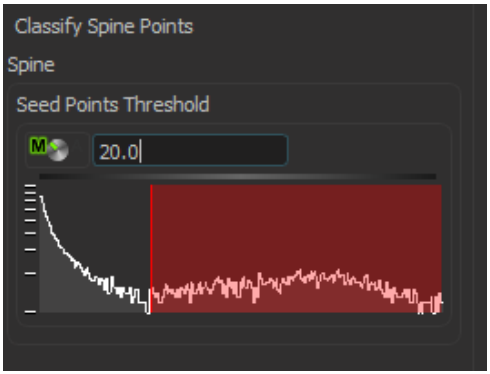
Creation Wizard - 5/7 Spine Points Diameter

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 um** and the **Maximum Length** to **4.00 um**. Select the option **Allow Branch Spines** and click on Next.

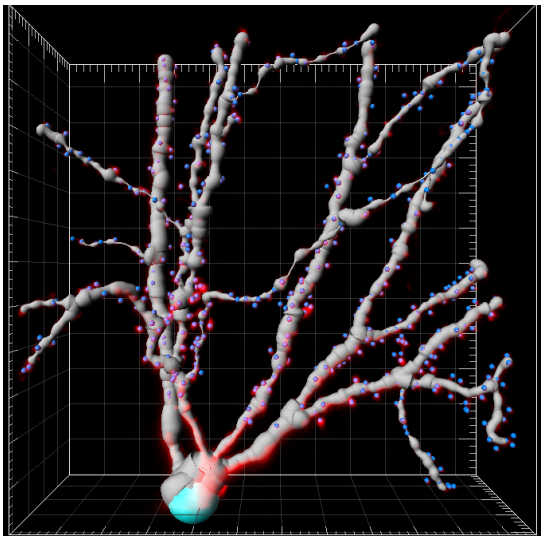


Creation Wizard - 6/7 Classify Spine Points

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



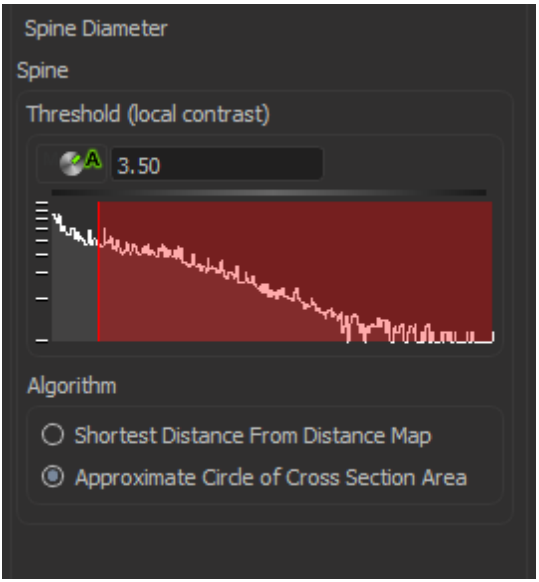
The threshold for the spine **Seed Point** is set to **20**. The detected spine Seed points are visualized in the image by different colors. Click on Next.



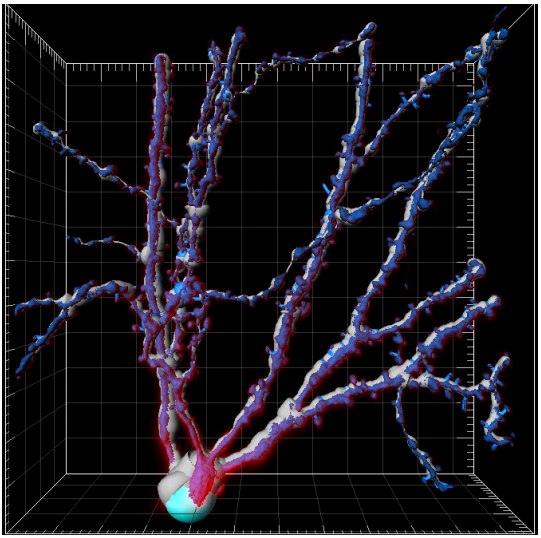
Creation Wizard -7/7 Spine Diameter

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.

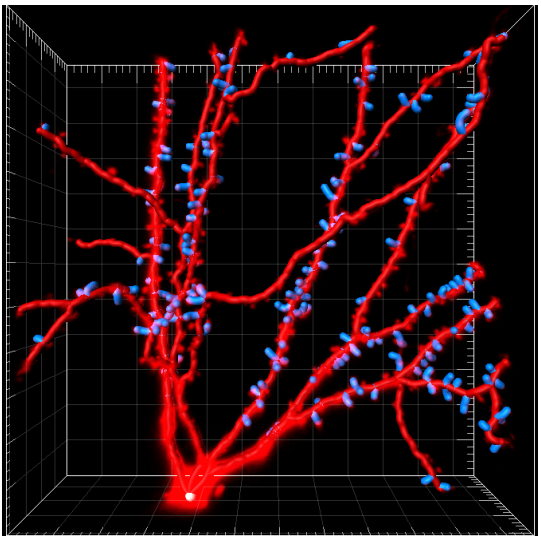
You can specify how the Spine diameters are calculated from the image. As an algorithm for the spine diameter calculation select the **Approximate circle of cross section area**.



In the viewing area a threshold preview 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.

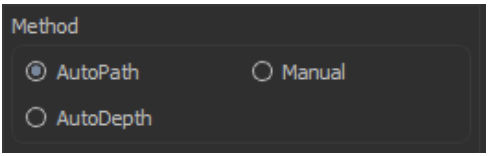


9.4 AutoPath Mode

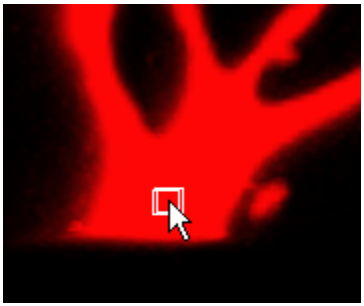
In the case that **Automatic Detection method** does not produce the desired results, filament structure can be created by the **AutoPath** method option. **AutoPath** method can be used for reselecting, redrawing and creating a new filament structure. In the **AutoPath** mode of the **FilamentTracer** 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

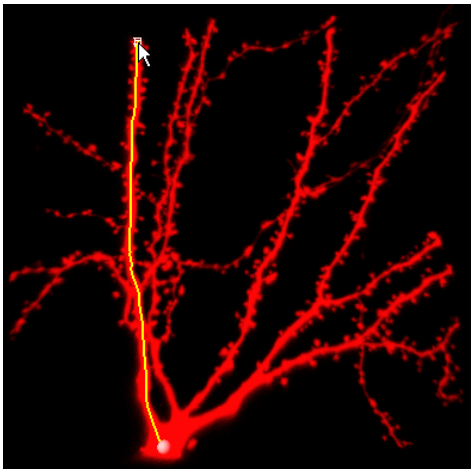
In the **Objects** toolbar of the Surpass view click on the icon to add a new Filament. In the **Filament - Properties** click on (Cancel) to stop the automatic detection of the start point and the end points. Click on the tab **Draw** .



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.)



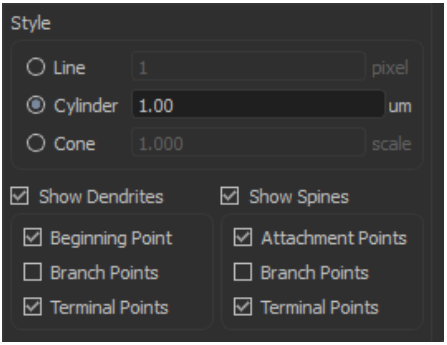
At the base of the cell you see the filament starting point. Now you move the mouse cursor to an end point of a filament. The filament is calculated automatically and displays on screen. With Shift + click you fix the filament. Move the mouse cursor to the end point of the next filament, press Shift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

9.5 Filament Tool Kit

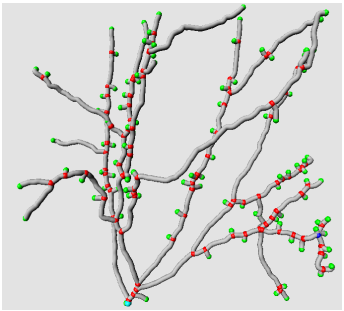
In this chapter you will learn the different visualization possibilities of FilamentTracer. A short, tabular overview concerning the three different interactive filament tracing methods is presented.

Filament Visualization

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



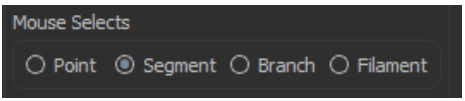
Un-check **Volume rendering** in Object List and new Filament Structure is displayed in viewing area.



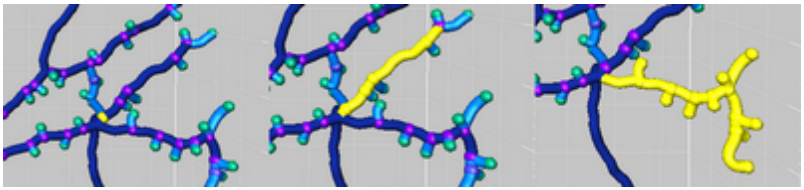
Additional filament visualization is achieved by selecting different styles

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

On the Edit tab selection and editing of filament structure can be achieved by selecting **Point**, **Segment, Branch or Filament**. 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 branch are selected.



Filament analysis and processing could be performed with automatic filament selection choosing the **Process Selection** option and then to examine the structural features of filament the **Process Filament** option.

The Mouse Select and Process Selection Window

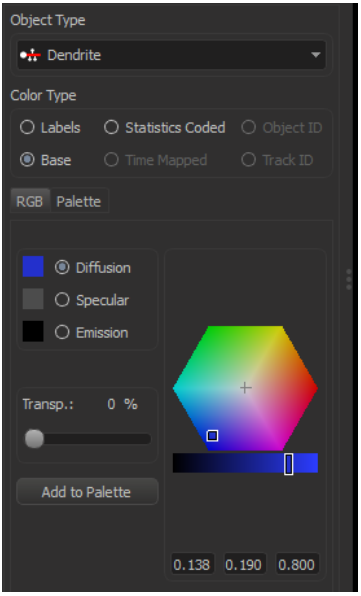
In the window **Process Selection** you find the **Delete** button, and other editing functions. These functions are applied only to the selected part(s) of the Filament.

9.6 Color Properties

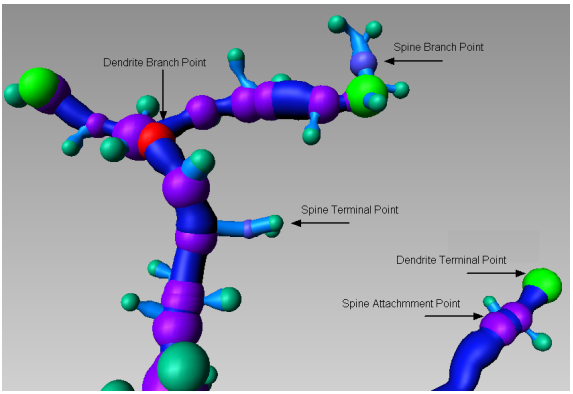
In the properties area click on the Color tab to open the Color Properties dialog box. Under the Tab Color you can edit, manage and assign material colors for dendrites, spines and each of their significant points.

Base

Select **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.

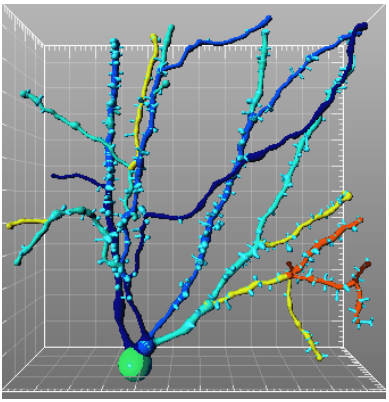


In the same way, you can adjust color for following items: Spines, Dendrite Beginning, Branching, Terminal points as well as Spine Attachments, Branch and Terminal points.
You can display Filament data in a numerous of different ways by using the Color tab options.



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.



9.7 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.

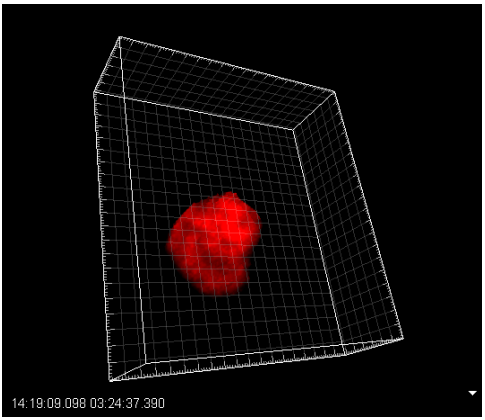
	PC:	Mac:
Draw the filament on the slicer	Shift + left-click + drag	Shift + click + drag
Stop drawing	Stop and release all buttons	Stop and release all buttons

Section 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.

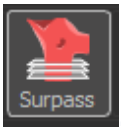
The demo data set is a time series of a HeLa cells going through all mitotic phases in the cell cycle. In the green channel, the mitotic spindle is visualized by an EGFP tagged alpha-tubulin. In the red channel, chromatin is visualized by a tagged core histone 2B.

You will start with the visualization of the data set. After that you draw the contour lines to outline the chromatin. Then you let the Imaris calculate the Contour Surface. This Surface object is your "cookie-cutter". Duplicate the original red channel and then use the "cookie-cutter" to lay a Mask to this channel. The focus of the last chapter is the display of the Masked Channel allowing you to concentrate your work on a specific ROI.



Masked Red Channel, HeLa Cell

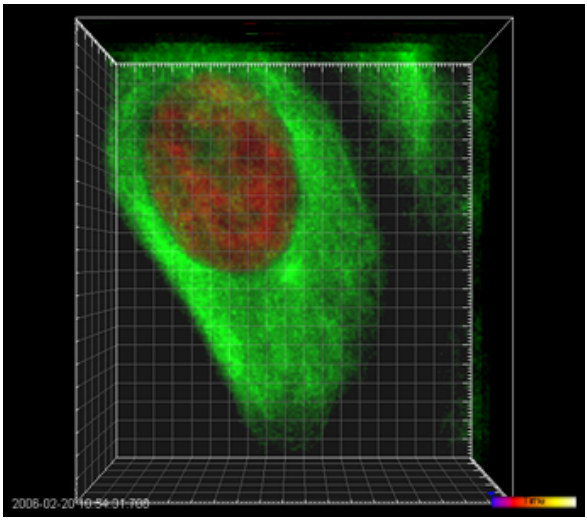
10.1 Visualization and Planes Verification

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.
Double-click on the HeLa cell image thumbnail to open it in the Surpass view 

.

To open the **Display Adjustment** window select in the **Edit** - **Show Display Adjustment** option. Click on **Advanced** to open the advanced settings. Set the threshold **Range** for **Ch1** (red) and **Ch2** (green) to **Min: 45** and **Max: 200**.

This is the volume rendering of the **HeLa Cell** in the viewing area in 3D view.

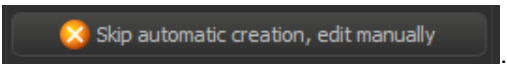


10.2 Add Contour Surface

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. Use 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

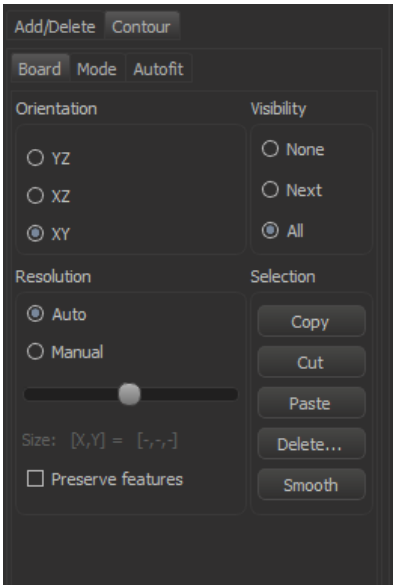
In the **Objects** toolbar of the Surpass view click on the icon to add a new Surfaces item.
To start the Contour surface creation first you have to cancel the automatic Surface creation wizard. You can do that either by clicking on the Cancel button or by selecting the Skip automatic creation, go to manual editing button.



In the Draw tab select the Contour tab.

Board -Orientation and Visibility

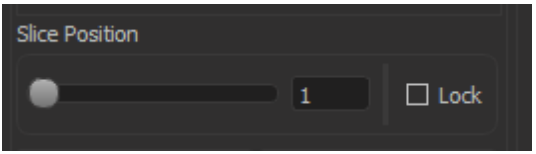
A drawing plane displays in the viewing area. To begin with the Contour surface creation process, adjust the **Orientation** and **Visibility** of the contour planes in the Board sub tabs.For the **Orientation** select **XY**, setting the orthogonal slicers of all other contours to the same position.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



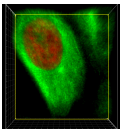
To start drawing contour lines select the Mode tab.

10.3 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.



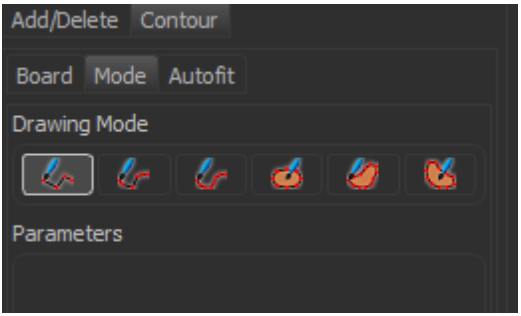
The border of the active drawing plane is shown in yellow color, thus clearly indicating the Contour Surface currently in use. This is the result in the viewing area.



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

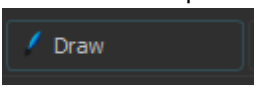
In the Mode tab several drawing mode tools are provided.

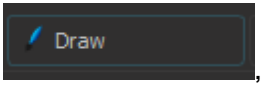


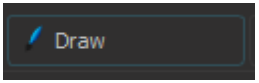
Select **Click** as the drawing mode tool, to create a selection that outlines the chromatin contour.

10.4 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 

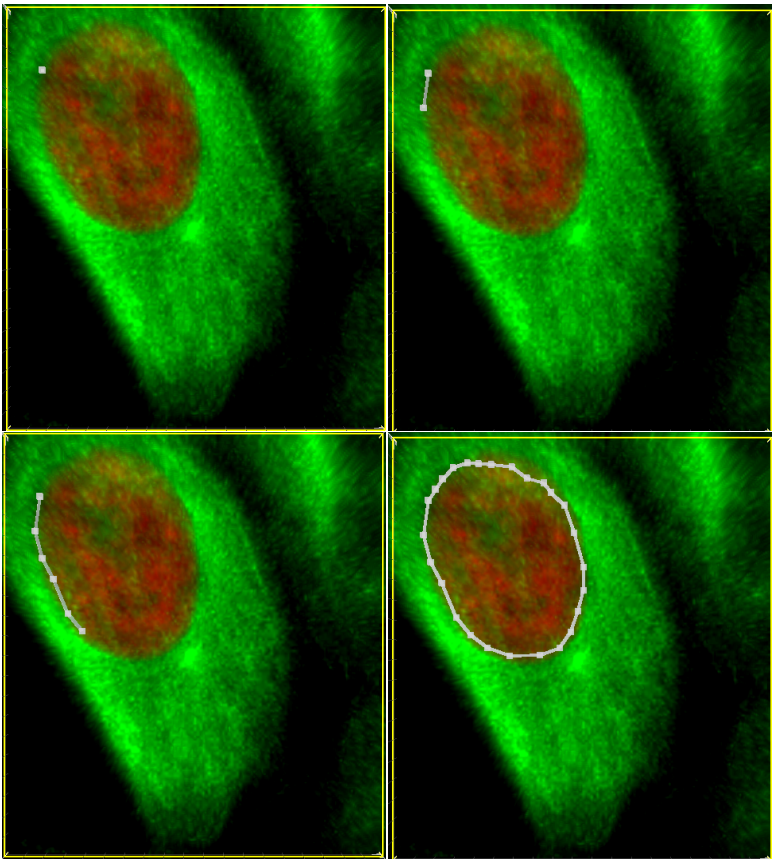
or by using the **Ctrl+Space** key on keyboard. The pointer appearance on the screen depends on the selected drawing mode. The symbol on the screen appears as an **arrow** in the **Inactive** drawing mode and as a **cross** in the **Active** drawing mode.
As you switch between the two drawing modes using 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 than press 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 outlines of chromatin boundaries in slice 1 of the HeLa cell image.

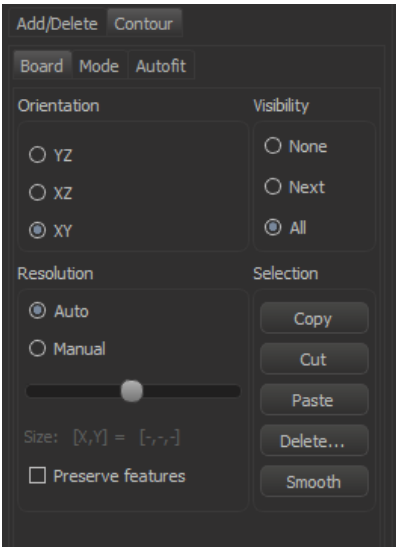


You can draw as many contours on a plane as required. All contours drawn on a plane will become part of the same object.
Change to the next drawing plane by moving the Slider to the next position. Repeat the process until the contours are created for all slices.

10.5 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.

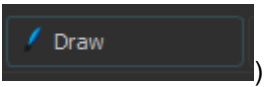


Once the contour is created, move to the next slice.

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.

10.6 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 Draw button 

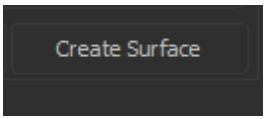
). Return to the slice on which the polygon is drawn and use the following shortcuts.

	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.

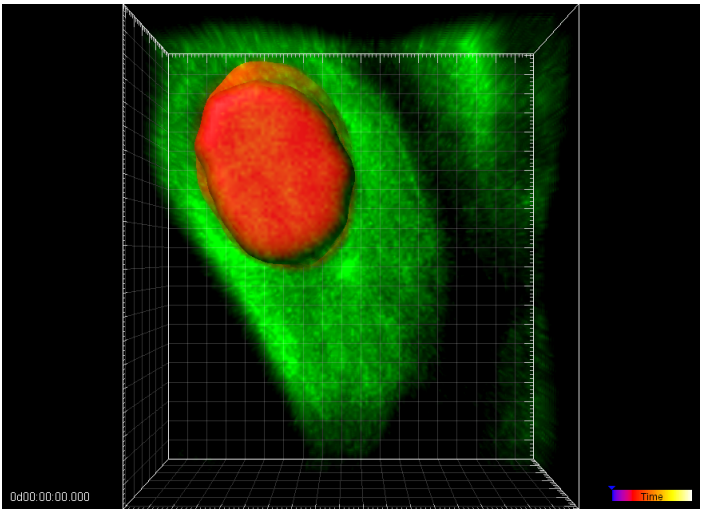
The 'Undo' function from the Edit is also available.

10.7 Create Surface Object

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

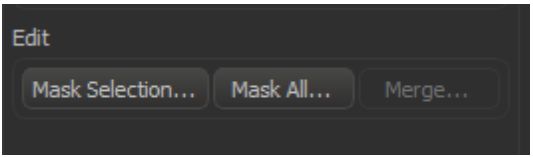


This is the result in the viewing area.



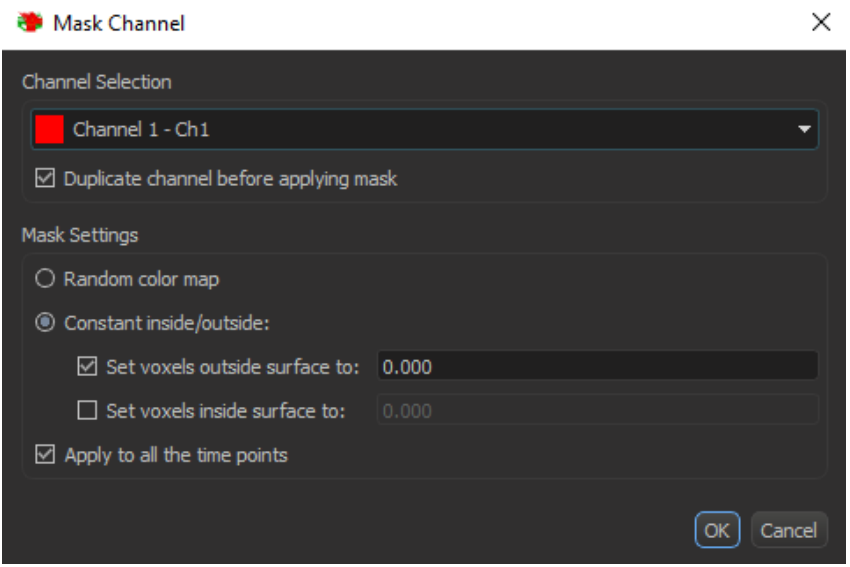
10.8 Mask Channel

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 Surfaces object. All voxels outside the "cookie-cutter" are excluded (set the voxels outside the surface to zero).



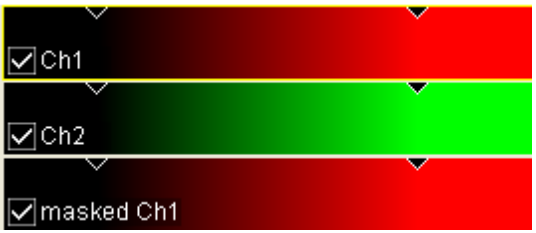
- Select the channel (**Channel 1 - Ch1,** the red channel) you want to mask with the Surface object (ROI).
- Check the box **Duplicate Channel before applying Mask** and an additional masking channel displays automatically in the **Display Adjustments** window.
- Select **Constant inside/outside** and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

10.9 Display Masked Channel

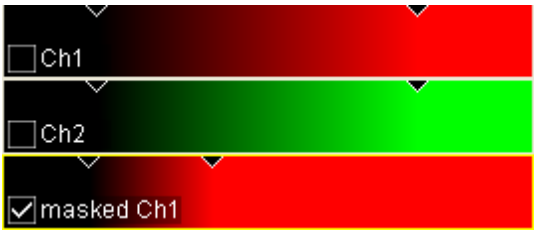
To display only the masked channel in the viewing area you have to adjust the channel display in the **Display Adjustment** window. Additionally you have to set up the visibility of the Object List items in the viewing area.

Display Adjustment Window

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



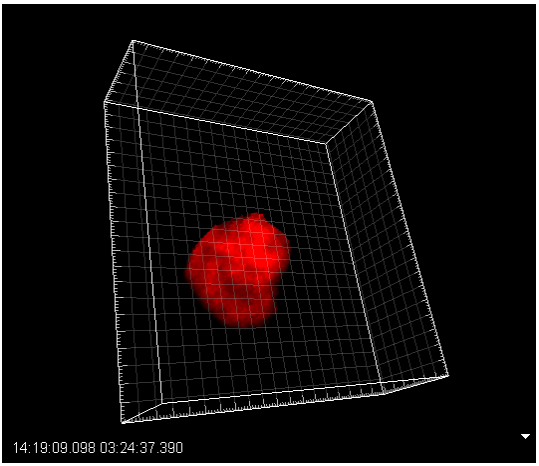
In the **Display Adjustment** window you can find the additional channel **masked Ch1**.



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**. 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.



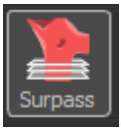
New, Modified Red Channel

Please close Imaris before you start with the next tutorial.

Section 11 Volume over Time

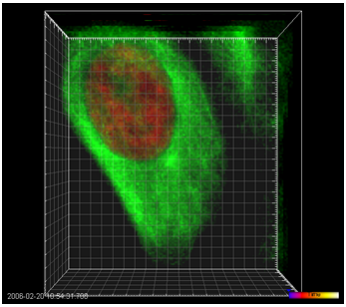
In this tutorial you will analyze the chromosome volume over time in a mitotic cell. The demo data set is a time series of a HeLa cell going through all mitotic phases in the cell cycle. In the green channel, the mitotic spindle is visualized by EGFP tagged alpha-tubulin. In the red channel, chromatin is visualized by tagged core histone 2B. You will start with the visualization of the data set.
First you Add a Volume reconstruction for both channels, then you generate an Surfaces reconstruction of the chromosomes. In the Time Concept chapter you will learn how-to-handle time series in Imaris. Then you Calculate and export statistical data for each time point. The focus of the last chapter is the Presentation of the Results 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.
Double-click on the HeLa cell image thumbnail to open it in the Surpass view 

.

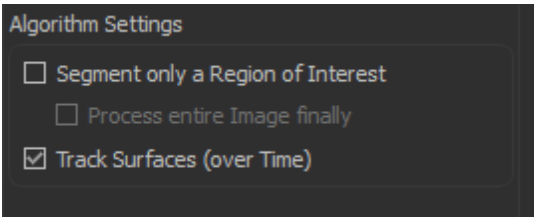
The demo image HeLa cell is open in the Surpass mode.
To open the **Display Adjustment** window select in the **Edit** - **Show Display Adjustment** option. Click on **Advanced** to open the advanced settings. Set the threshold Range for for **Ch1** (red) and **Ch2** (green) to **Min:45** and **Max:200**. The HeLa cell displays in the viewing area.



11.2 Add Surfaces

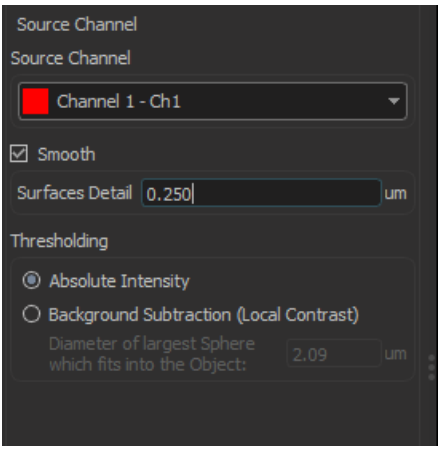
In the **Objects** toolbar of the Surpass view click on the icon to add a new Surfaces item.
Bear in mind that the parameters of this tutorial are specific to this data set. Your data will require its own specific parameters.

Creation Wizard - 1/7 Algorithm



For algorithm setting select **Track Surfaces.** Ensure no other check boxes are selected.
Click on (Next).

Creation Wizard - 2/7 Source Channel

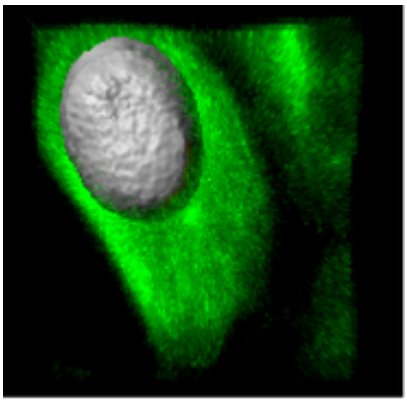


Select as **Source Channel** the **Channel 1** (red) and check the option **Smooth**. As **Surface Area Detail Level** use **0.250 um** and under the **Thresholding** select the **Absolute Intensity** option**.** Click on (Next).

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 Filter Surfaces

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

Creation Wizard - 5/7 Edit Surfaces

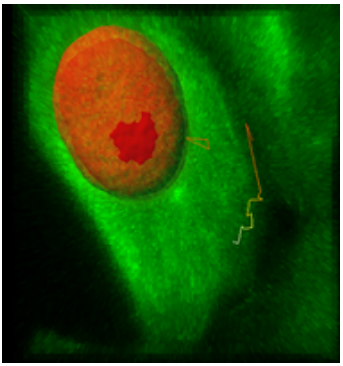
In the step **Edit Surfaces** you can edit the resulting Surfaces. In this example, editing is not necessary. Click on (Next).

Creation Wizard - 6/7 Tracking

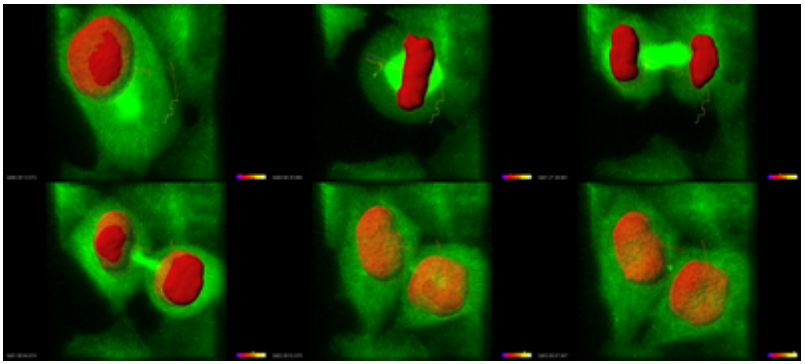
Select Autoregressive Motion as Algorithm and **MaxDistance** set **20.000** um, and for the **MaxGapSize** set **3**. Click on (Next).

Creation Wizard - 7/7 Filter Tracks

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

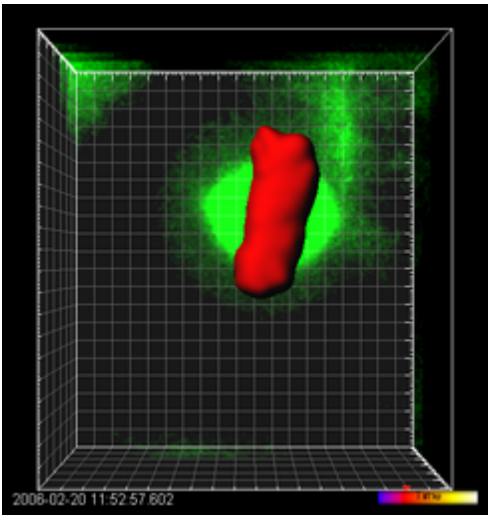


Additional to the Volume rendered tubulin proteins of the spindle the Surfaces reconstruction of the histone 2B proteins displays in the viewing area. In the Display Adjustment window now un-check the box for the Volume rendering of red channel. Newly created surface is displayed in viewing area. The 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 the Time Colorbar is displayed in the right hand corner. 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

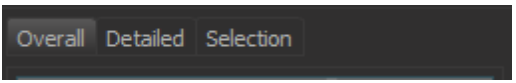
Now you can evaluate statistical data for each Surface object.

Note: The statistical calculation is based on the selected Object List in the Surpass Tree.

Select the **Surfaces**.

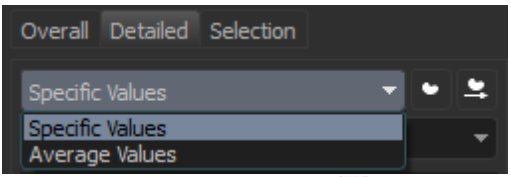
Show Results

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

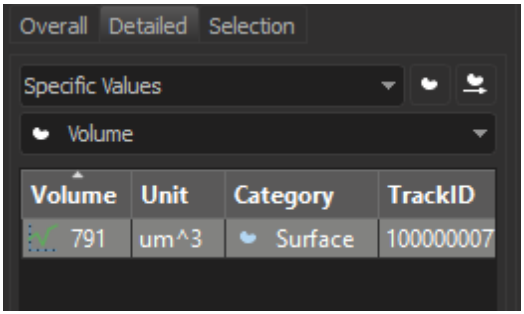


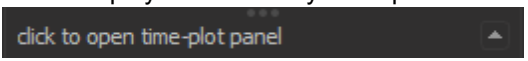
Overall
The output of the Overall statistics provides an overview of the image single determining variables.

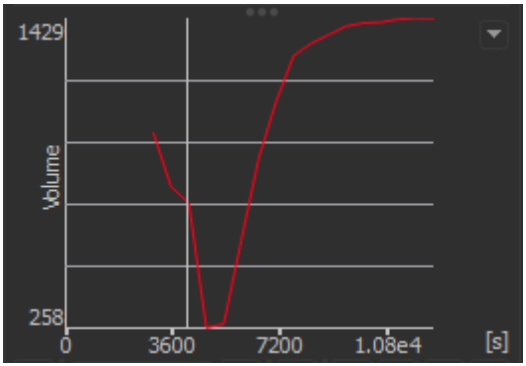
Detailed
The pull down list provides three types of Detailed statistical calculations: **Specific** Values, **Average** Values.



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



The time plot should display automatically if not please click to open time plot here. 



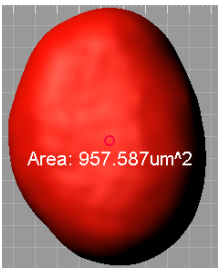
For further analysis the current selection can be saved to Excel by click on the single-disk-button .

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

Statistical Annotation

The Statistical tab provides the opportunity to 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 not show up, the respective statistical value is probably disabled. Click the button Settings on the Statistics tab 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

Beneath the time plot you can export the data presented in the time plot.

Export current selection of Statistical Tab.

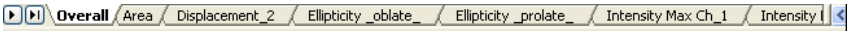
Export selected Data for plotting.

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.

11.5 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

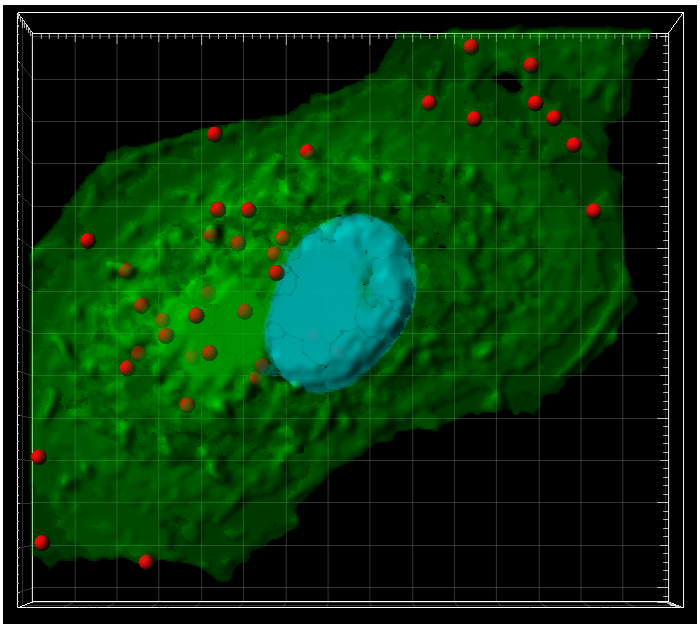
All time points and the corresponding Volume(s) are listed. In Excel the numbers are now sorted time-point (column "time") and then by track (column "parent"). In Excel image calculated statistical data can be represened using different charts modifications and adjustments.

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

Please close Imaris before you start with the next tutorial.

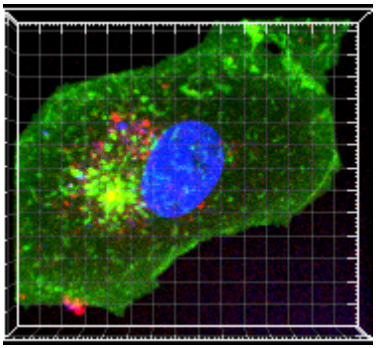
Section 12 Analyze Cells

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.



12.1 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.
Double-click on the cell demo image thumbnail to open it in the Surpass view.

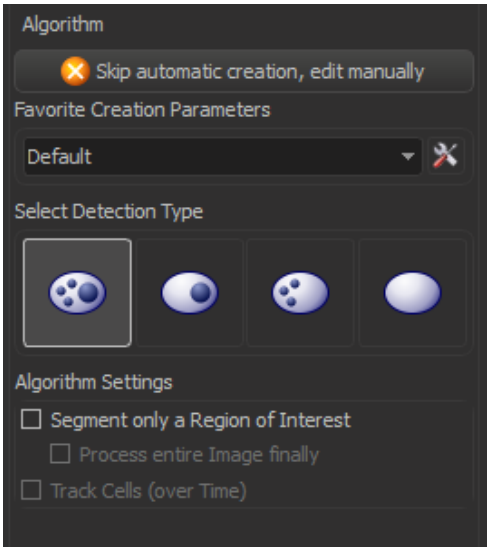


The demo cell image displays in the viewing area.

12.2 Cell Detection Type

In the Objects toolbar of the Surpass view click on the Cell icon to start the Cell Creation Wizard. In the Object Properties area, the Cells Creation Wizard appears automatically.

Creation Wizard - 1/9 Algorithm Settings



Imaris Cell lets you choose from four different detection types.

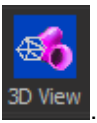
You can select to detect Nucleus, Cell and Vesicles, detect Nucleus and Cell, detect Cell and Vesicles or just to detect Cell.

In this example we will select the detect Nucleus, Cell and Vesicles algorithm. We'll process the whole image, so leave the other boxes un-checked. Click on the Next button.

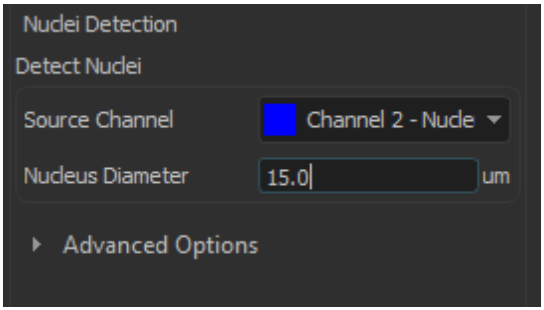
12.3 Nucleus Detection

Creation Wizard - 2/9 Nuclei Detection

In the next step you define the Nucleus object, its source channel and diameter.
If you are not sure of the size of your Nucleus object, click on the **Slice mode** icon

. Now you can look at the slices of your image. By two consecutive clicks measure the nucleus diameter. Now reselect the **3D view** icon 

.

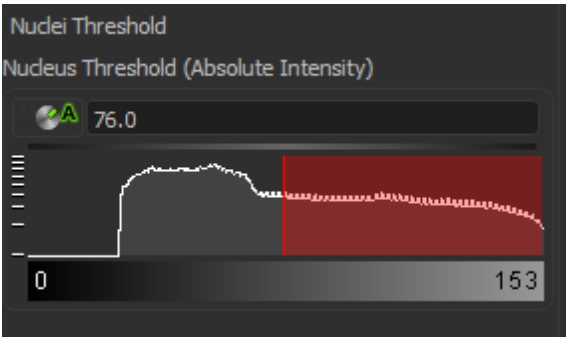


For Nuclei detection the **Channel 2** is preselected. If you are not sure of the size of your Nucleus object, you can measure it within the Slice mode. Use two consecutive clicks to measure the nucleus diameter. Switch back to the Surpass view with a click on the Surpass icon and for Nucleus diameter enter the value **15** um.

The advanced options lets you adjust the filter settings and seed points algorithm. Click on the Next .

Creation Wizard - 3/9 Nuclei Threshold

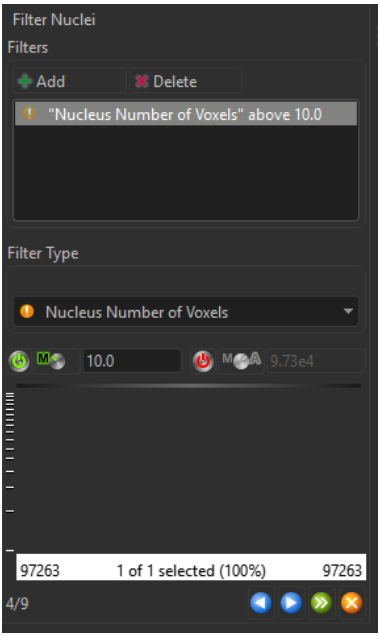
Now we will adjust the threshold value for the nucleus. To change the threshold, click on the histogram and drag the line. Alternatively, you can enter a value directly into the field. In this example, however, accept the automatically calculated value.



Click on the Next button .

Creation Wizard - 4/9 Filter Nuclei

In the nuclei filter step, one Nucleus object is automatically segmented, so click on Next .

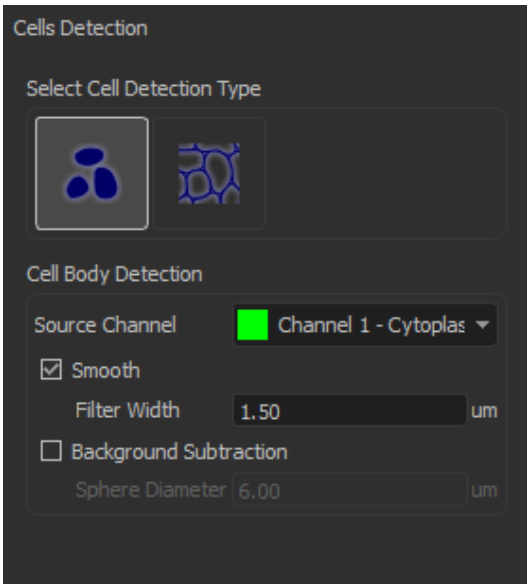

Filter Nuclei
Filters
Add
Delete
"Nucleus Number of Voxels" above 10.0
Filter Type
Nucleus Number of Voxels
10.0
9.73e4
97263
1 of 1 selected (100%)
97263
4/9

12.4 Cells Detection

Creation Wizard - 5/9 Cells Detection

Now we will identify Cell objects. Here you can choose from two different detection algorithms.
Depending on the cell staining techniques and sample preparation used, you can select either to detect the Cell Boundary from Cytoplasm or to detect the Cell Boundary from Cell Membrane.

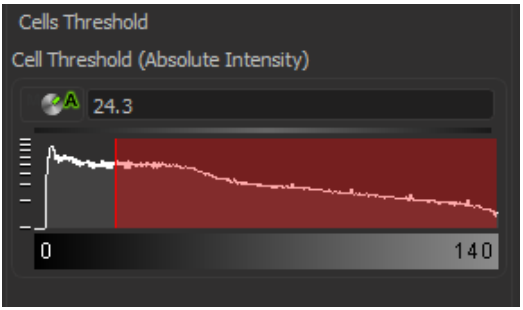
Select the Cell Boundary from Cytoplasm option. Then accept the automatically selected options for Source channel, Smooth and Filter Width. Click on Next.



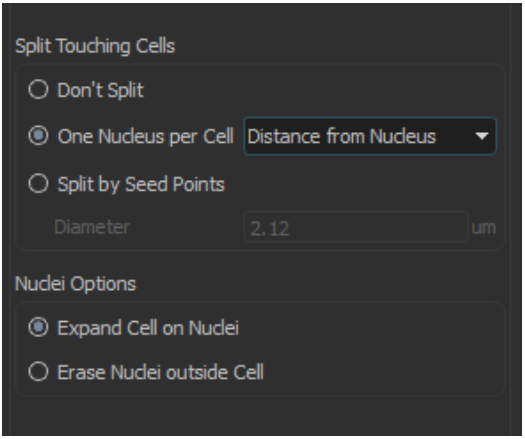
Creation Wizard - 6/9 Cells Threshold

Next you can adjust the Cell threshold value. In this example, accept the

automatically calculated value.



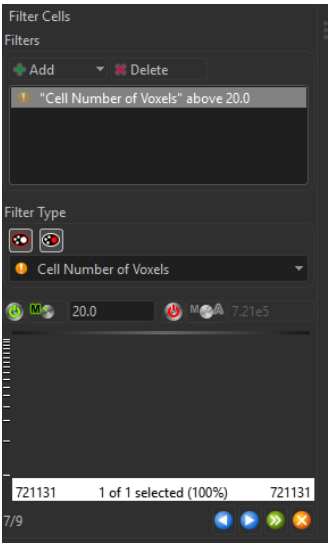
In this step further adjustments are available. Here you can also select several options that correspond to particular biological specification. Select the **One Nucleus per Cell** option and leave the automatically selected **Expand Cell on Nucleus** option unchanged.



Click on Next .

Creation Wizard - 7/9 Filter Cells

At this point of the algorithm you can filter your Cell object.

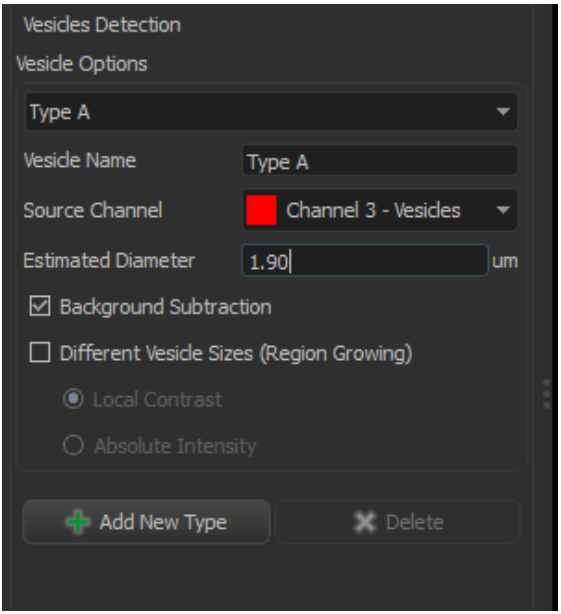


In this example one cell object is automatically segmented, so click on Next .

12.5 Vesicles Detection

Creation Wizard - 8/9 Detect Vesicles

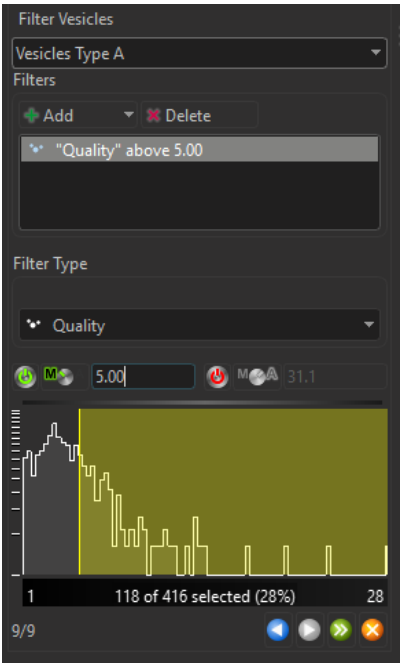
The next step is the automatic creation process for Vesicles.
The Vesicle Name field allows you either to enter the name for a newly added vesicle type or to edit a name already defined. If you want to add a new Vesicle type, click on the Add New Type button and enter a name in the Vesicle name field. However, in this example, no additional Vesicle types are necessary.
For Vesicle detection, **Channel 3** is automatically selected as the Source Channel, set the **Estimated Diameter** to **1.90** um and select the option Background Subtraction.



Click on the Next button .

Creation Wizard - 9/9 Filter Vesicles

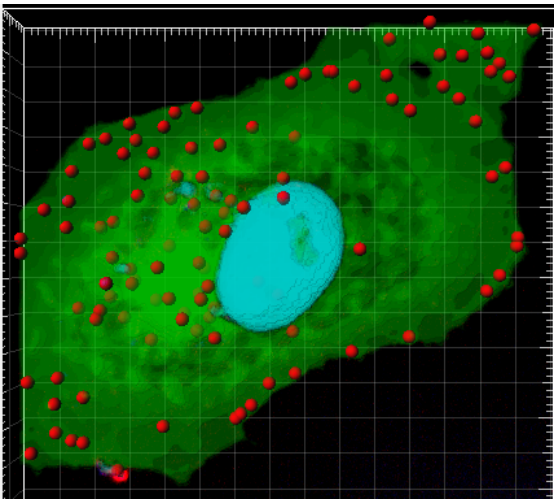
Select as the filter type **Vesicle Quality** and set the value to **5.00**.



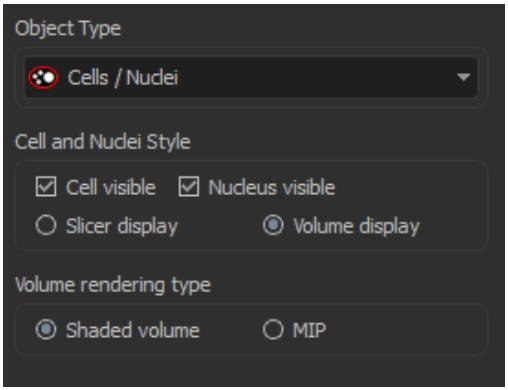
Click on Finish .

12.6 Analyze Cell Objects

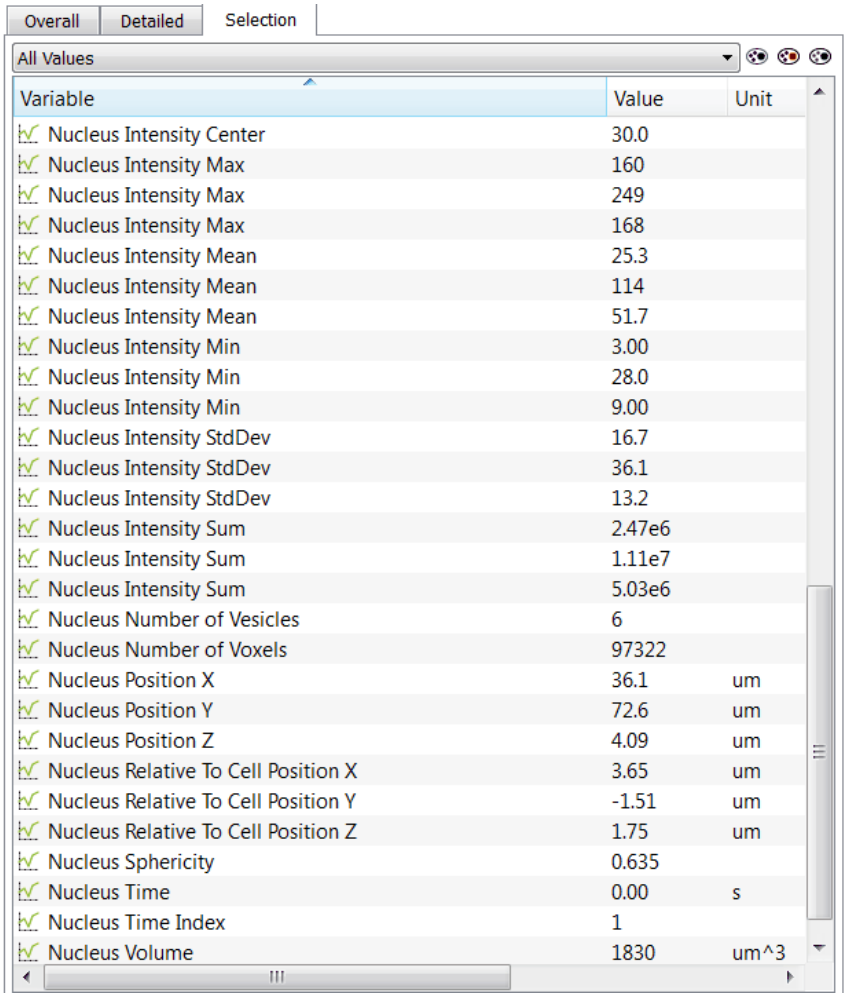
This is the result in the viewing area. In the Object List now un-check the box for the Volume and reselect Cells. The Cell object is displayed in green, blue is used for Nucleus, while the Vesicles are represented as red spheres.



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, the **Volume display** option is already preselected.



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.



Variable	Value	Unit
Nucleus Intensity Center	30.0	
Nucleus Intensity Max	160	
Nucleus Intensity Max	249	
Nucleus Intensity Max	168	
Nucleus Intensity Mean	25.3	
Nucleus Intensity Mean	114	
Nucleus Intensity Mean	51.7	
Nucleus Intensity Min	3.00	
Nucleus Intensity Min	28.0	
Nucleus Intensity Min	9.00	
Nucleus Intensity StdDev	16.7	
Nucleus Intensity StdDev	36.1	
Nucleus Intensity StdDev	13.2	
Nucleus Intensity Sum	2.47e6	
Nucleus Intensity Sum	1.11e7	
Nucleus Intensity Sum	5.03e6	
Nucleus Number of Vesicles	6	
Nucleus Number of Voxels	97322	
Nucleus Position X	36.1	um
Nucleus Position Y	72.6	um
Nucleus Position Z	4.09	um
Nucleus Relative To Cell Position X	3.65	um
Nucleus Relative To Cell Position Y	-1.51	um
Nucleus Relative To Cell Position Z	1.75	um
Nucleus Sphericity	0.635	
Nucleus Time	0.00	s
Nucleus Time Index	1	
Nucleus Volume	1830	um^3

Once the image analysis is completed, the statistical data can be exported to Excel using the Export button in the Statistics Tab. Please close Imaris before you start with the next tutorial.

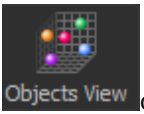
Section 13 Vantage

In this section, we will introduce ImarisVantage **1D View** and **Objects View**.

1D View

is the default view and provides a simple and effective set of options to view data:

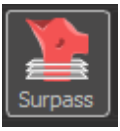
Select the data parameter type from the options available under the Settings tab.
Apply the required filter from the Filter tab.
Adjust the display options from the Frame and Source tabs.

Objects View 

can be used to create and analyze statistical values visually in colorful high-dimensional plots.

In this chapter we will look at the basic features of **Objects View** as well as step-by-step instructions for common work-flows of the ImarisVantage plot creation. You will learn how the properties such as color or object size can be used to represent numerical data, how to create scatter and time plots with multiple source objects.
This tutorial assumes that you know the concepts used in Imaris for object creation.
This tutorial will help you understand the possibilities of Imaris Vantage Objects View. It emphases the visual representation of the created object's statistical data. The tutorial focuses on how to create informative plots and present complex data sets visualized in Imaris.

We will start from the demo image CellDemomembrane3D
Double-click on the CellDemoMembrane3D image thumbnail to open it in

the Surpass view 

.

Add new surfaces and create surfaces according to the following parameters. See the Add Surfaces tutorial for an introduction to this topic if required.
Click on (Next) to proceed to step 2.

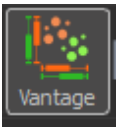
In step 2, set the source channel to Channel 2 - DNA and click on (Next).

Set the Threshold (Absolute Intensity) value to 1548
Enable Split touching Objects (Region Growing), in this example leave the seed Point diameter at the default value.
Click on (Next).

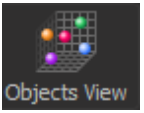
Set the Lower Threshold to 357.

To complete Surface creation click on (Finish).

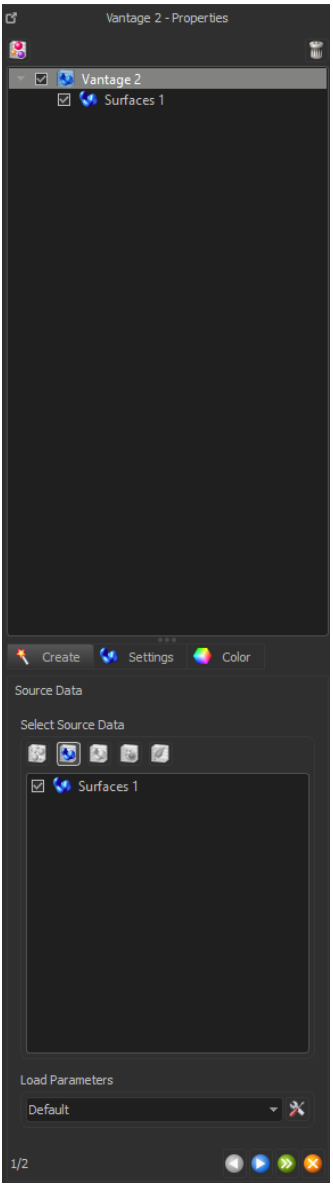
To start ImarisVantage click on the icon Vantage.



When the ImarisVantage opens click on Objects view.



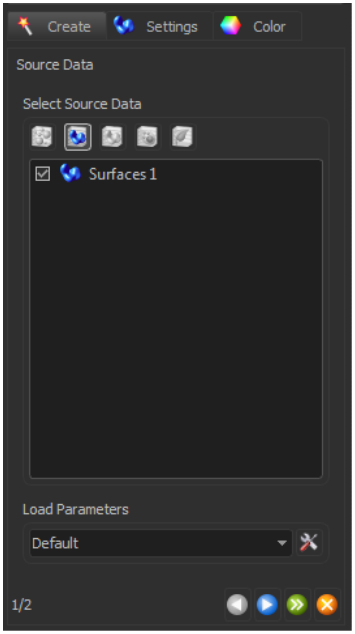
In the Properties Area the Creation Wizard appears.



13.1 Plot Creation Wizard

Source Data

The wizard shows a list of all available input components for ImarisVantage plots.

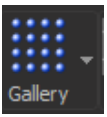


In the first step of the wizard select the Surfaces object and click on the Next icon .

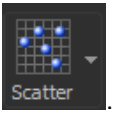
Plot Setup

Next you have to select the plot type you want to create. ImarisVantage lets you choose from four different plot types:

XYZ

Gallery 

Time

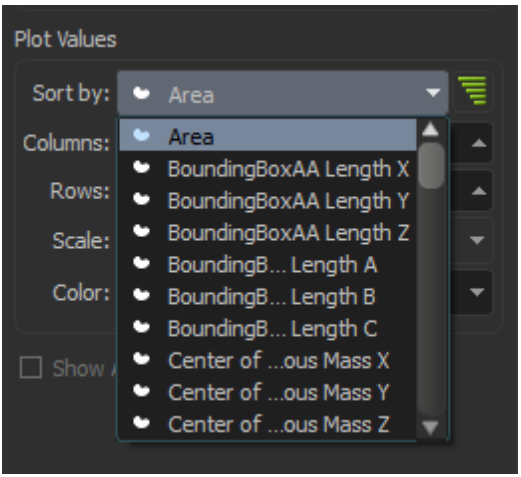
Scatter 

.

13.2 Gallery plot

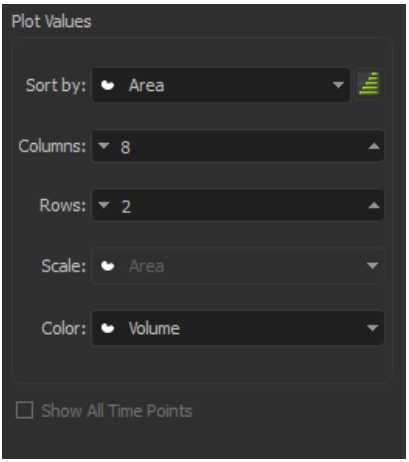
In this example, select the **Gallery** plot .
In a Gallery plot, the objects are sorted in ascending or descending order based on the value of the selected statistical variable.

To select a plot subtype, click on the icon . This icon indicates that the additionally assigned plot dimension is a **Color**.
Under the Plot Values, click on the **Sort by** list.This drop-down list offers a set of all available statistical variables for the selected object category. To select the statistical variable click on its name.
The selected statistical variables will be automatically assigned to the chosen plot dimension.
In this example, select the **Area**. This is the statistical variable that will be used in the Gallery plot for the objects sorting.

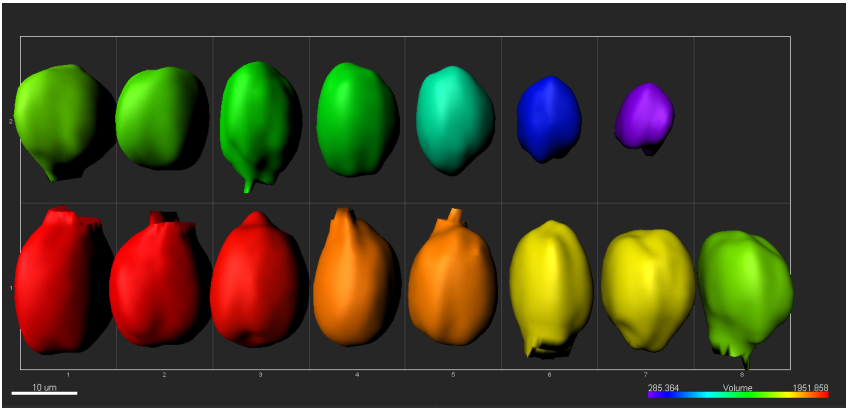


The objects are ordered in the Gallery panes according to the increasing value of the selected parameters. Now you should define the size of the Gallery plot by setting the number of columns and rows. For this example, leave these at the system calculated default values: the

columns enter **8** and for the rows **2**. Then select the statistical variable to be assigned to the Color plot dimension. In this example, select the **Volume**.



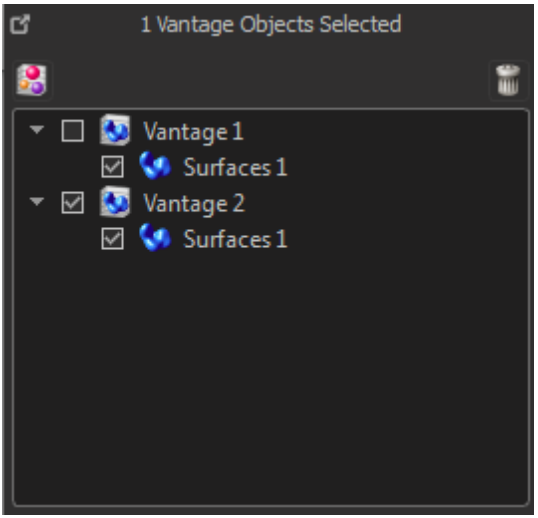
In the Gallery panels, all objects are colored according to the **Volume** size. Click on the Finish icon. This is the result in the viewing area. The objects are ordered based on the selected values for this tutorial i.e. by Area and then colored according to the size of object Volume.



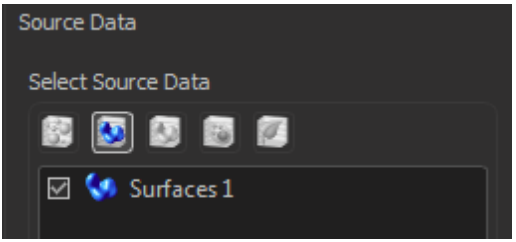
13.3 Scatter plot

For more information about CellDemomembrane 3D cell images an additional plot will have to be created. In the plot the mutual relationships of several statistical variables will be compared.
To create a new plot, click on the ImarisVantage icon in the Toolbar

.
The list is updated and a new Vantage plot is generated. To remove the Gallery plot from the Plot Main Display Area un-check the box next to the Vantage 1-Surfaces 1. Reselect the Vantage 2-Surfaces 1.



In the Plot Properties Area, a new creation wizard is started and the Surface object is selected. Click on Next .

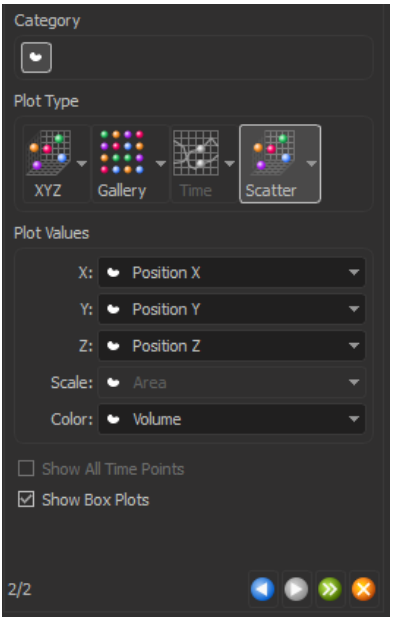


In this example, we will create the Scatter plot . To create a **3D scatter plot with the color** mapped as a 4th dimension select the icon .

Under the Plot Values, the available plot dimensions are listed. Assign one statistical variable for each plot dimension by clicking on its name. The selected statistical variable is automatically assigned to the chosen plot dimension.

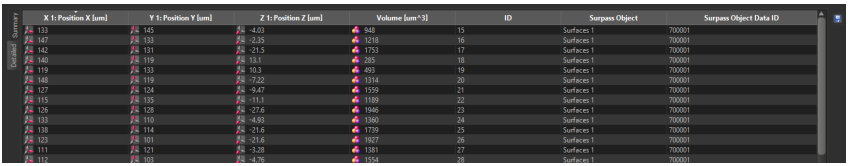
Many different combinations of variables are possible and will depend on the data and the relationships you want to investigate. In this example select the following:

For **X** select **Position X**
For **Y** select **Position Y**
For **Z** select **Position Z**
For **Color** dimension, select the **Volume** variable.



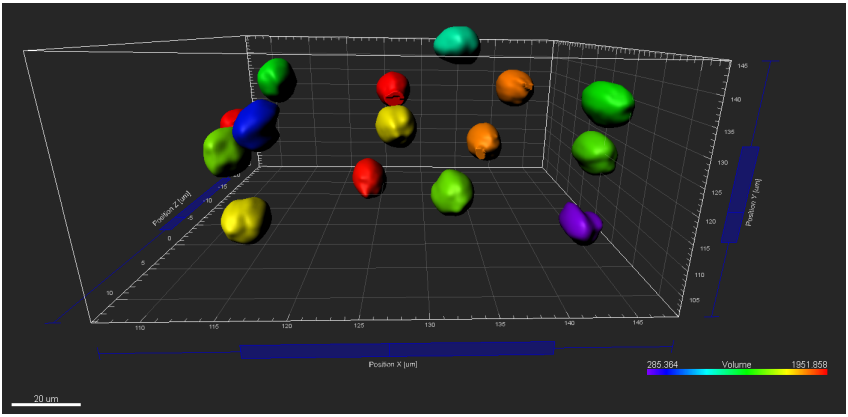
By default the **Show Box Plots** option is selected. This option controls the display of the box plot within the Viewing area.
Box plot are visually summarizing the data of the selected statistical variable to five numbers: the minimum, lower quartile, median, upper quartile, and maximum.

In the Plot Number Area two types of tables are available: **Detailed** and **Summary**.
The **Detailed** table contains all statistical variables values used for the plot creation.



The Summary table contains values determined on the basis of all values within a set of the selected variable. The table displays a five-number statistical summary of variables assigned to the plot dimensions: minimum, the lower quartile (Q1), Median, upper quartile (Q3) and maximum.

Click on the Finish icon and this is the result in the viewing area. The position of the objects within the plot indicates the values of the positions X, Y and Z. The objects Volume values are graded by color.



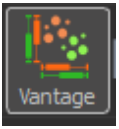
To further enhance the visualization, you can use scale and orientation options under the **View tab,** for example the **Align Objects** option. This may be useful for e.g. analysis of filaments. Please close Imaris before you start with the next tutorial.

13.4 Time plot

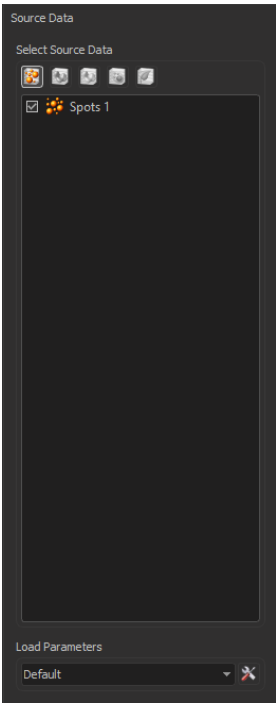
The next example shows how to create **Time plots**.
We will start from the R18Demo image. Double-click on the **R18Demo image** thumbnail to open it in the Surpass view.

In the Surpass view click on the icon to add new Spots . Follow the creation wizard with the parameters provided in the chapter Track Particles - Additional Example with a modification:
In the Classify Tracks step, select tracks with Track Displacement Length higher than 5 um. Click on Finish.

Start ImarisVantage by clicking on the icon.



Switch between 1D view to objects view.
Under the Setting tab , disable the visualization of the Tracks.
In the first step of the creation wizard, select the Spots object and click on the Next button.



In the Plot Setup step, select the **Time** plot by clicking on the icon .

We will create a **3D Time** plot with an additional statistical variable to map the objects **Scale** and **Color** .

The **X** Plot dimension is fixed and assigned to the **time**.

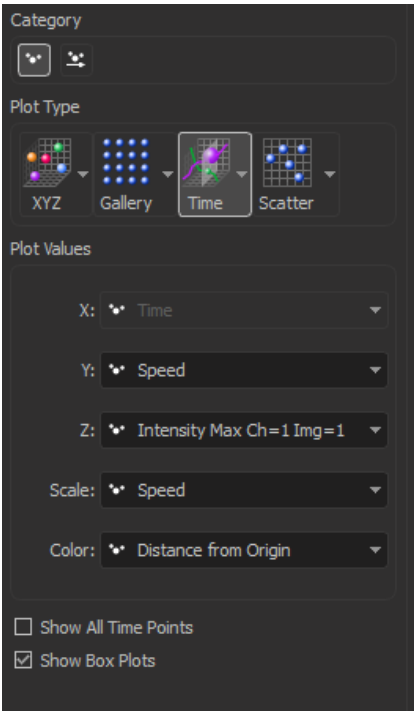
For the **Y** Plot dimension, select the **Speed** as a statistical variable.

For the **Z** Plot dimension, select the **Intensity Max Ch =1 Img = 1** variable.

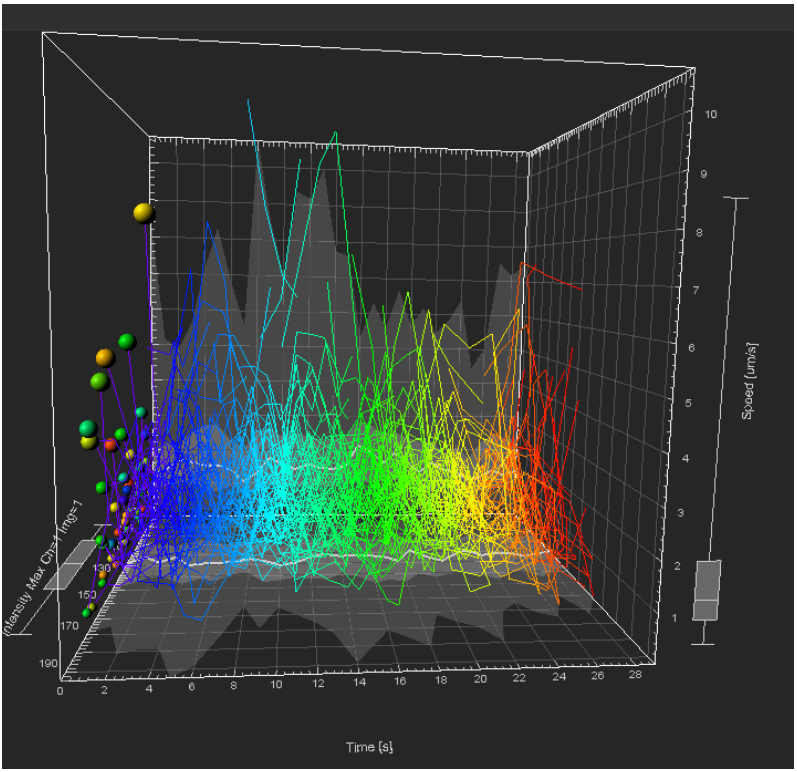
For **Scale** dimension, select the **Speed** variable. The values of the Speed are assigned to each Spot object providing a new dimension.

For **Color** dimension, select the **Distance from Origin** variable.

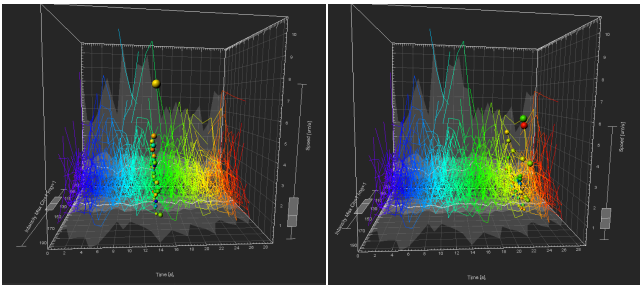
By default the **Show Box Plots** option is selected.



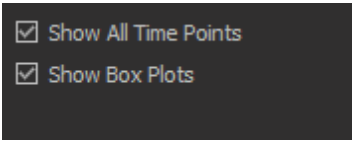
This is the result in the viewing area.



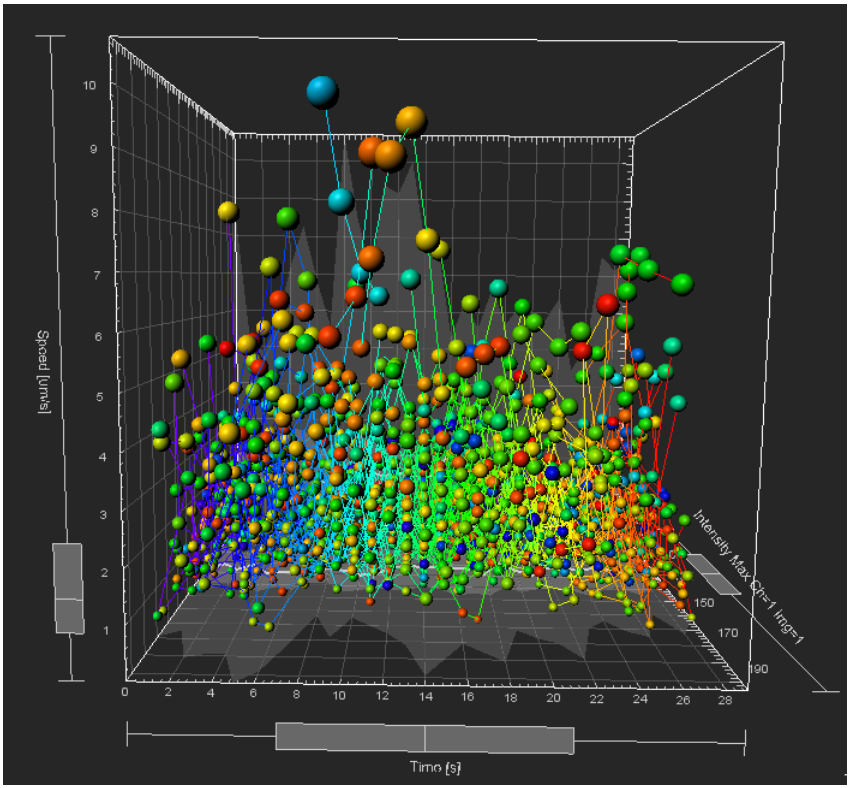
The Time Plot shows the time dependency between the **Speed** and **Maximal Intensity**. The size of each object corresponds to the value of the **Speed**, while color represents the **Distance from Origin** of the objects. The track is shown for each object.
The box plots represent a moving statistical summary and they change the position and shape at each time point press to move through the frames continuously or to move through frame by frame.
The following pictures are representing time frames 15 and 24.



When using the "Time" plot type Imaris displays the summary data as XY and XZ projections. In this example, the XY plane shows the five-number summary time dependency of the **Volume**. Similarly, the XZ plane shows the values of the statistical five-number summary for **Sphericity** as a function of time.
On the projection plots, a tick line indicates where the median value lies. The range between the lower and upper quartile values is shown with a less intensive shade, while the lightly colored area labels the minimum-maximum range. If you select the **Show All Time Points** box, the created objects are now shown for all time points.



When the **Show All Time Points** option is selected, the box plots represent the summary of all **Speed** and **Intensity** values for the whole time series.
This is the result in the viewing area.


Speed [units]
10
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Time [s]
Intensity Max_Ch=1 Img=1
150
170
190

13.5 Time plots - multiple objects

The next example shows how to create Time plots with two different Spots objects.

Double-click on the **R18Demo image** thumbnail to open it in the Surpass view.

In the Surpass view click on the icon to add new Spots.

Follow the creation wizard with the parameters provided in the chapter Track Particles - Additional Example with two modifications:

1. In the Classify Spots step, select spots that have an Intensity Max higher than 120
2. In the Classify Tracks step, select tracks Track Speed Max higher than 4 um/s

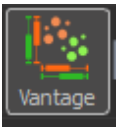
Click on **Finish**.

Under the Color tab select **Base** and change the Spots color to red.
From the same data set create second set of Spots objects, with lower intensity values and lower max speed.
Follow the creation wizard with the parameters provided in the Chapter Track Particles - Additional Example with two modifications:

1. In the Classify Spots step, select spots that have an intensity in the range of 60 to 110
2. In the Classify Track step, select tracks with Track Speed Max in the range of 1 um/s to 3.9 um/s
3. Under the Color tab select **Base** and change the Spots color to green.

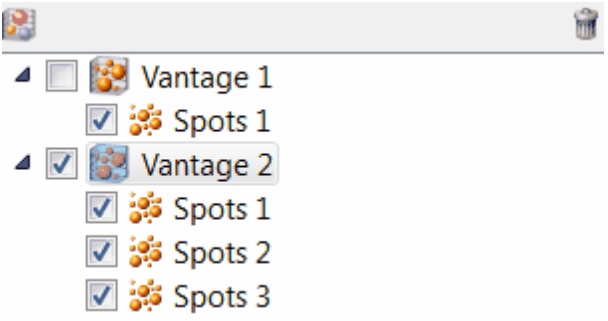
Click on **Finish**.

Click on the **Vantage** icon.

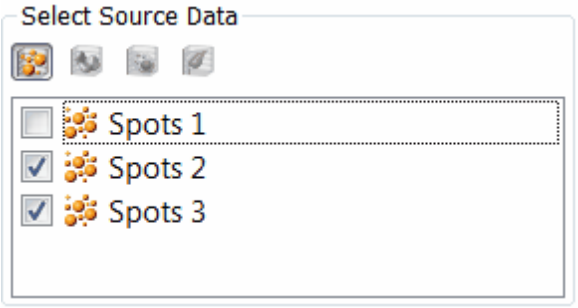


In the Plot Input Area (top left of the screen) a new Vantage plot is generated.
To remove the Vantage plot from the Plot Main Display Area un-check the box next to the Vantage 1 - Spots 1 and reselect the Vantage 2 plot.

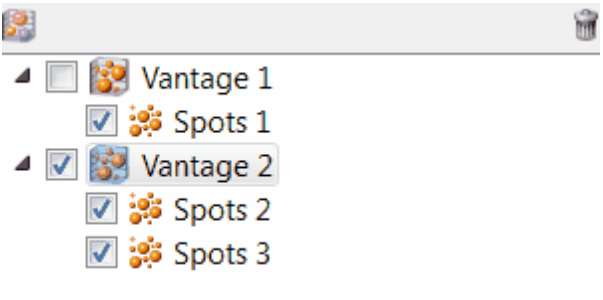
A Vantage tree lists all objects selected for a Vantage plot. A Vantage tree has the same characteristic as a Surpass tree.



In the Properties Area (bottom left of the screen), three Spots objects are listed. To remove an object data from the plot creation wizard, deselect the checkbox next to the object name. In this example, deselect the box next to the Spots 1.

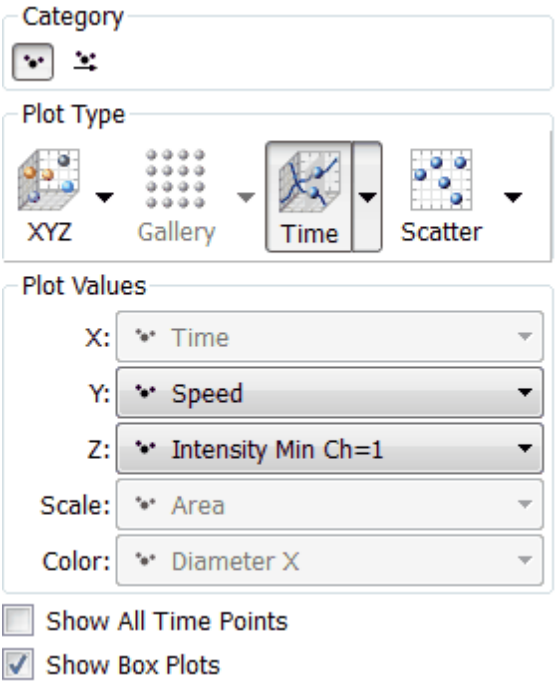


The Vantage tree is automatically updated when a source data object is added or deleted.

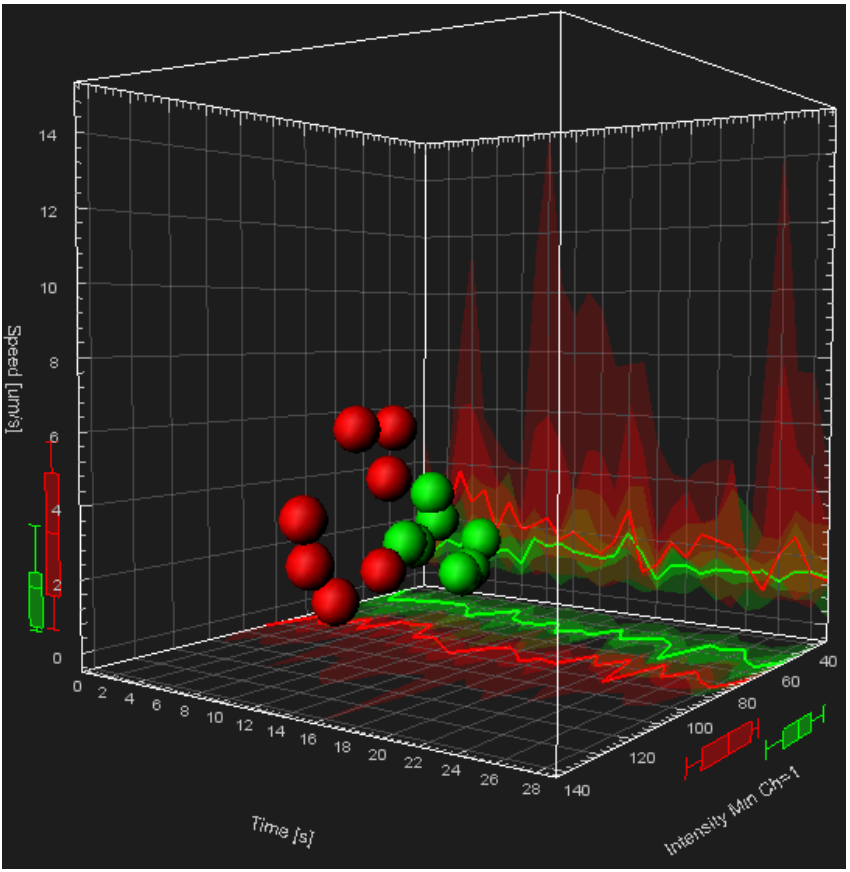


Click on Next .

In the next step, we will create the 3D Time plot .
The **X** Plot dimension automatically assigned to the **Time**.
For the **Y** Plot dimension, select the **Speed** as a statistical variable.
For the **Z** Plot dimension, select the **Intensity Min** variable.
The Show Box Plots option is already selected.
Click on **Finish**.



Under the **Setting tab** , disable the **visualization of the Tracks**.
In the viewing area, two groups of the Spots are shown with the separate box plots for each data set.
Box plot and object colors are matched to facilitate the comparisons between populations. The box plot summaries show the distribution of the selected statistical variables and provide an easy way to compare multiple object populations.
The summary values of Speed and Intensity are plotted as colored and shaded regions on the XY and XZ projections.



Please close Imaris before you start with the next tutorial.

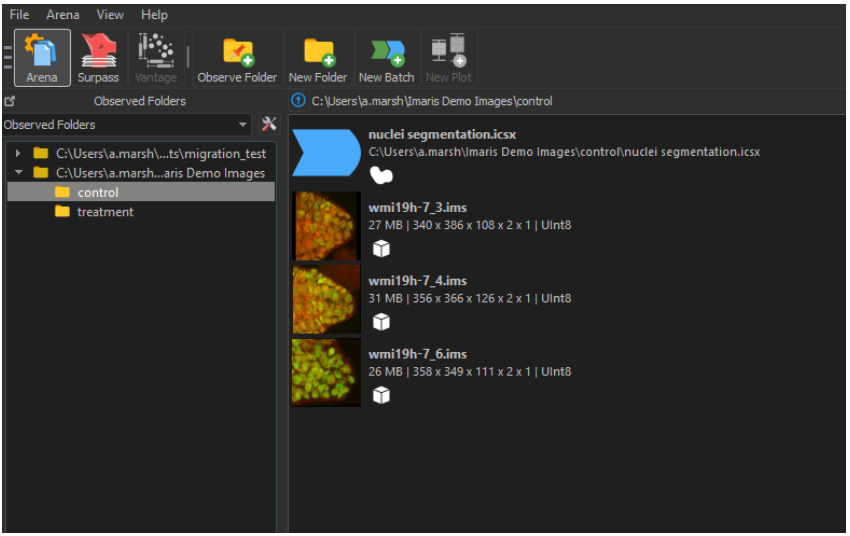
Section 14 Batch Analysis

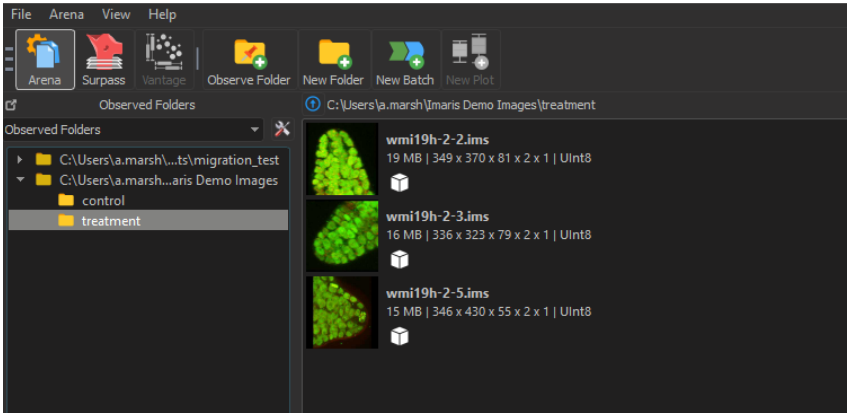
In this section we look at the batch analysis capabilities of Imaris. Image analysis often requires the application of the same creation parameters to any number of grouped images. Arena allows you to apply the same creation parameters to any number of grouped images. It facilitates running of batch job runs as well as the display of grouped images' segmentation and analysis of results.

This tutorial provides illustrative examples of how to set up the creation parameters, execute batch runs, and visualize and analyze the batch results.
This tutorial assumes that you know the concepts used in Imaris for object creation.

14.1 Control and Treatment Groups

Currently, the Arena view contains one Folder with Imaris demo images and two Folders of images called **Control** and **Treatment**. Within these Folders are images showing the immunostaining of plant tissue. The structures correspond to ovule primordia from Arabidopsis plants. The Control and Treatment Group differ by the genetic background of the Arabidopsis plants used to generate the images. In both groups the green signal shows a chromatin modification, while the red signal shows DNA staining.

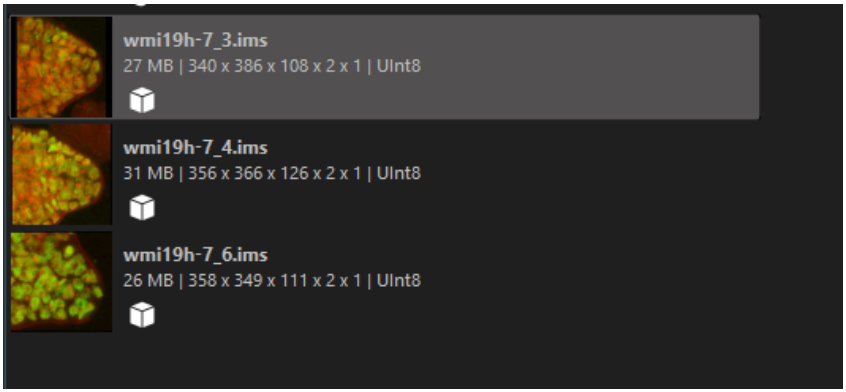



File
Arena
View
Help
Arena
Surpass
Vantage
Observe Folder
New Folder
New Batch
New Plot
Observed Folders
C:\Users\a.marsh\Imaris Demo Images\treatment
Observed Folders
C:\Users\a.marsh\...ts\migration_test
C:\Users\a.marsh...aris Demo Images
control
treatment
wmi19h-2-2.ims
19 MB | 349 x 370 x 81 x 2 x 1 | UInt8
wmi19h-2-3.ims
16 MB | 336 x 323 x 79 x 2 x 1 | UInt8
wmi19h-2-5.ims
15 MB | 346 x 430 x 55 x 2 x 1 | UInt8

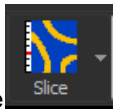
14.2 Batch Creation Parameters

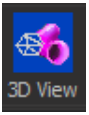
The first step in performing analysis for any Batch is setting up the **batch creation parameters**. Here we will introduce two different methods for setting batch creation parameters in Imaris 9.3. The first method is discussed below and the second method is introduced in Image Processing and Batch Creation Parameters and should be used to do image processing to a batch of images simultaneously.

Start by identifying the most representative image within the whole Control group. This image will be used to define the creation parameters and the entire batch settings.
In this tutorial select the **wmi19h-7_3 image**.



To start setting up of the creation parameters image, double-click on the thumbnail. The image is opened in the Surpass view

Within the Surpass view, inspect your image by using a combination of Slice 

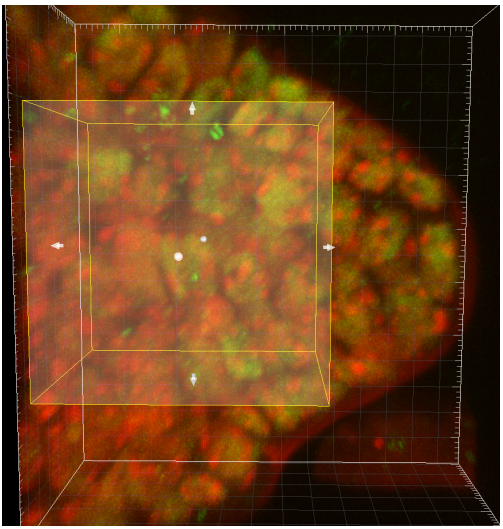
and 3D Views 

visualization options.

Based on your data set and according to your scientific requirements,

select an appropriate creation wizard. You can choose between Spots, Surfaces, Cells, and Filament creation wizards.

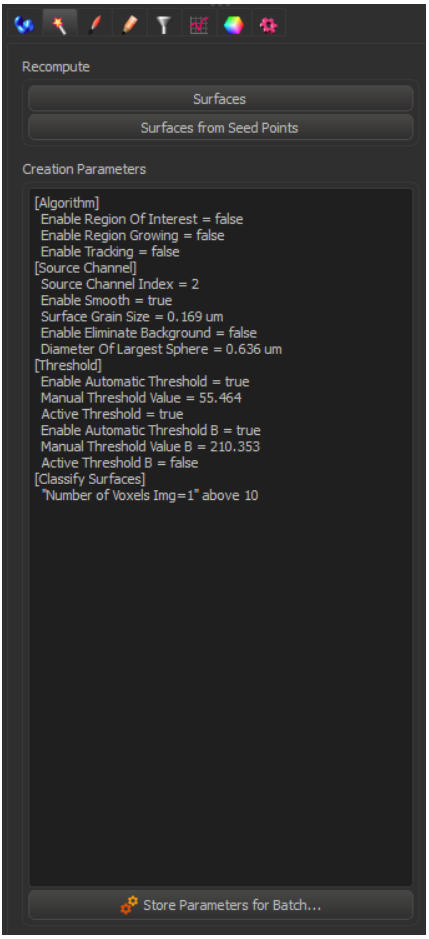
For this tutorial select and follow the Surface creation wizard. Complete the Surfaces Creation Wizard, use a region of interest with size x = 200, y = 200 and z = 108, as shown below and create the required objects using the Channel 1 as a source channel.



For details on the Surface creation wizards, please follow the instructions provided within Chapter 5 - Design Mixed Model Rendering or Chapter 11 Volume over Time.

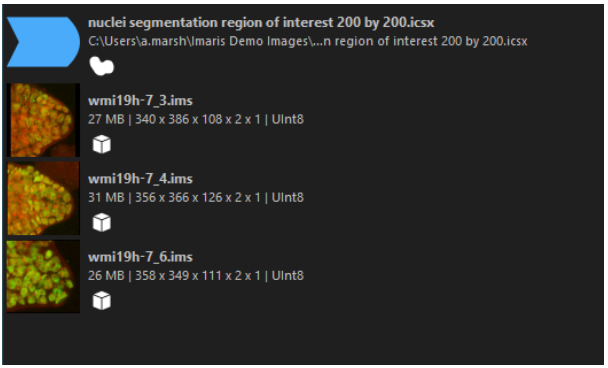
14.3 Creation Tab

Once you have completed an appropriate Creation Wizard and the desired objects are created, processing instructions and the parameter's value are listed under the Creation Tab.



The **Store Parameters for Batch button** allows you to save the complete set of the creation parameters and all relevant settings. In the

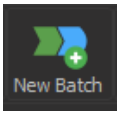
Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required storage location. If you select the Arena option and confirm it with the OK button, a new Arena item within the Arena – a Creation Parameter item is automatically created. In the Arena, within the Control group you can find **your batch creation parameters** item .



However for the purposes of this tutorial the optimal parameters for analysis have already been provided in the existing **nuclei segmentation** item in the control folder.

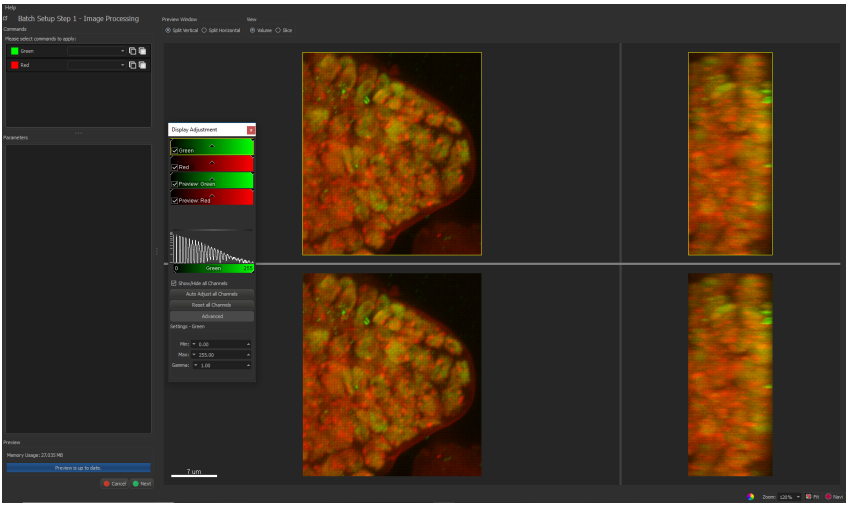
14.4 Image Processing and Batch Creation Parameters

Here we introduce another method for creating batch parameters which includes image processing.

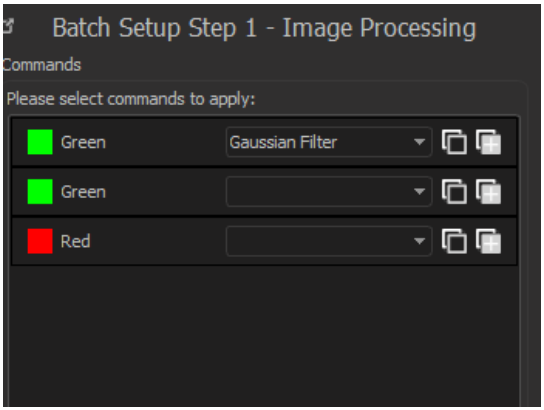
Open the control folder, select wmi19h-7_3.ims, click on the new batch icon 

in the top toolbar.

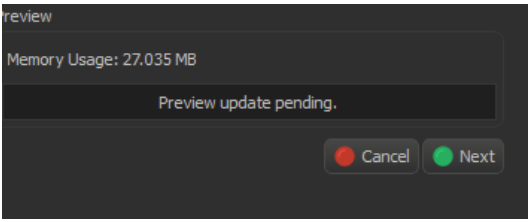
A screen will open like below.



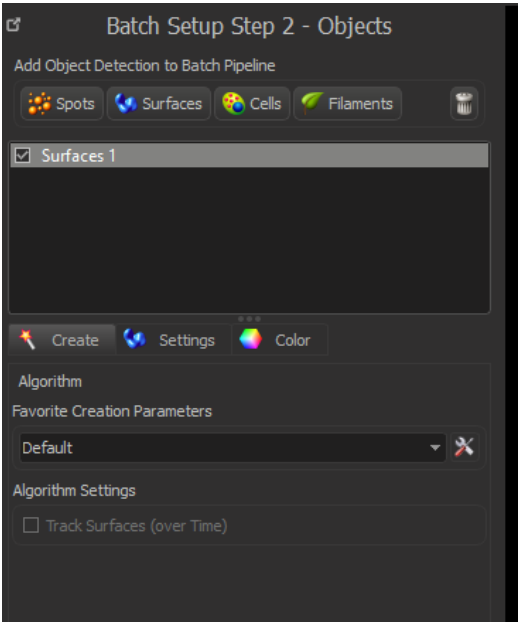
Next, select Gaussian Filter for image analysis in the example discussed in this tutorial. Alternative image analysis methods can be chosen to appropriately process images from your experiments.



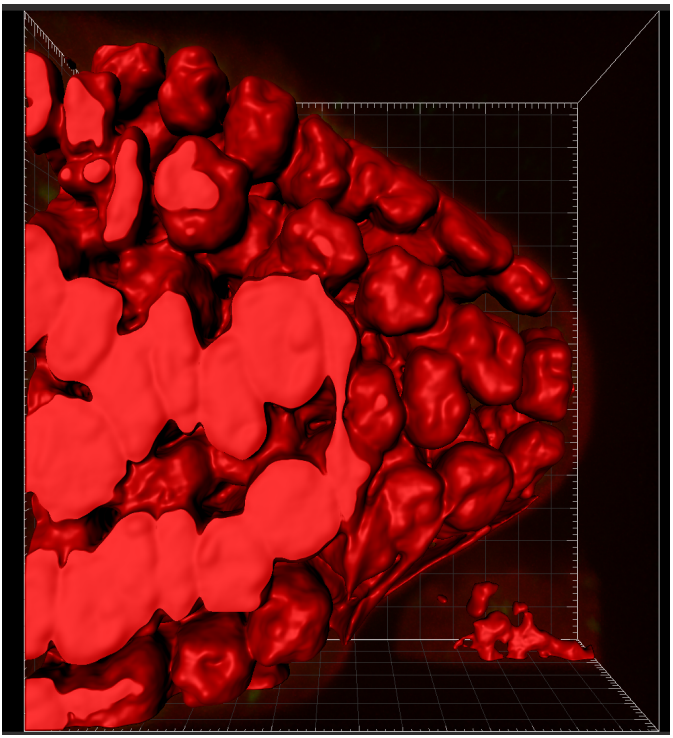
Then press next at the bottom of the page.



In Batch Setup Step 2 set objects, here select surface. Press next.



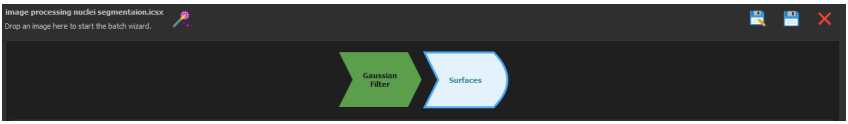
Set source channel to Channel 2, press green arrow to end.



Select Ok. you will be prompted to name your new Batch. Save it with the name image processing nuclei segmentation. Your new Batch will be saved as a new Arena item.

You can edit the properties of your batch creation parameters by selecting the batch item you wish to edit then clicking on the pencil icon in the top right hand corner of the Arena.

The following will appear at the bottom of the Arena.

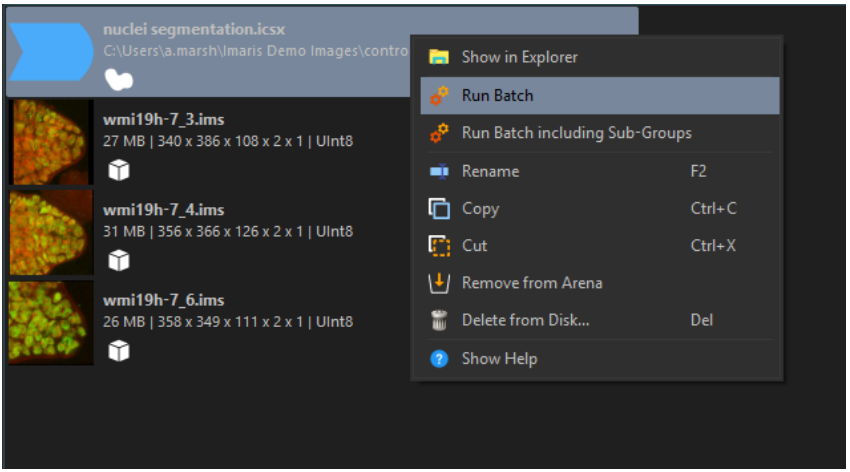


Click on if you want to edit your batch creation parameters .

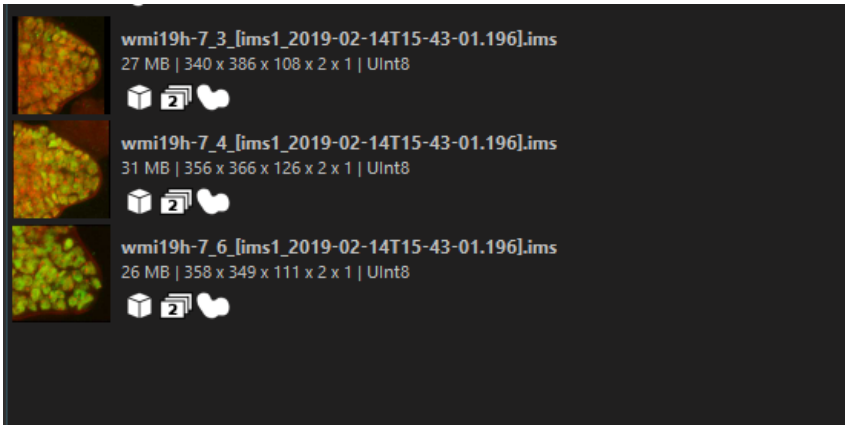
This section has demonstrated how to prepare Batch Creation Parameters which include image processing. For the purposes of demonstrating Batch Creation Parameters in the remainder of this tutorial we will use the **nuclei segmentation** batch creation parameters provided with the tutorial in the control folder.

14.5 Run Batch Job

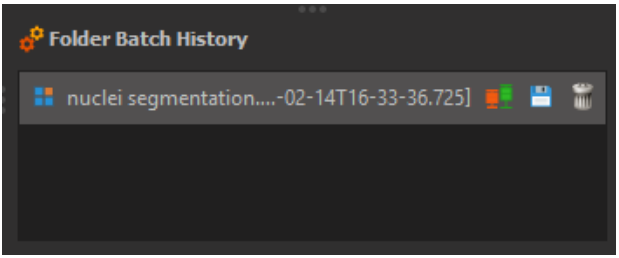
In this section we will run the nuclei segmentation creation parameters provided with the tutorial.
To sequentially apply the nuclei segmentation creation parameters to all images within the Control Group, right click on the creation parameters item and select the Run Batch option.



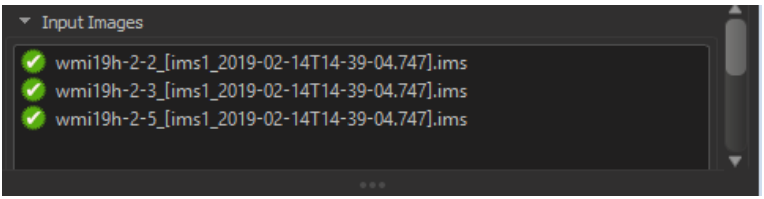
When you press Run Batch the results are directly stored in each file and the images in the folder processed using the nuclei segmentation parameters will show icons beneath the file dimensions. In this example the following icons appear underneath the file dimensions , because there is an image stack size of two and a surface object is created in the nuclei segmentation creation parameters.



A new Batch Collection item is created to the right of the Arena in the Folder Batch History.

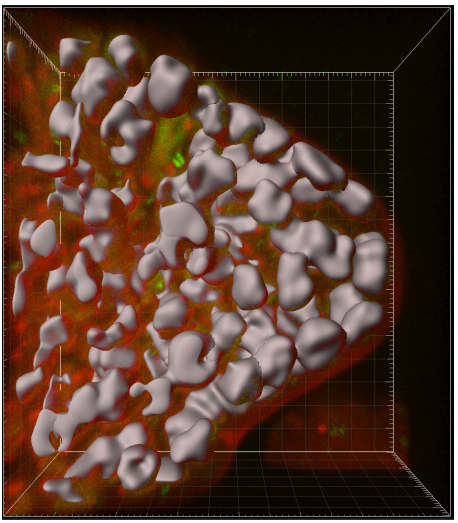


The icon in the bottom right corner of below the Folder Batch History indicates when the batch job is completed for each image.

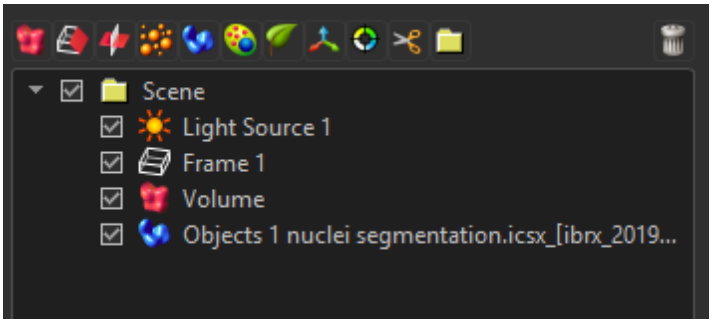


14.6 Batch Results Validation

The next step is to take a look at the Batch Collection results. It is always recommended to verify the batch segmentation results before trying to analyze them.
To do so, double click on any image thumbnail to **Open with Surpass.** The segmented image is now open within the Surpass view.

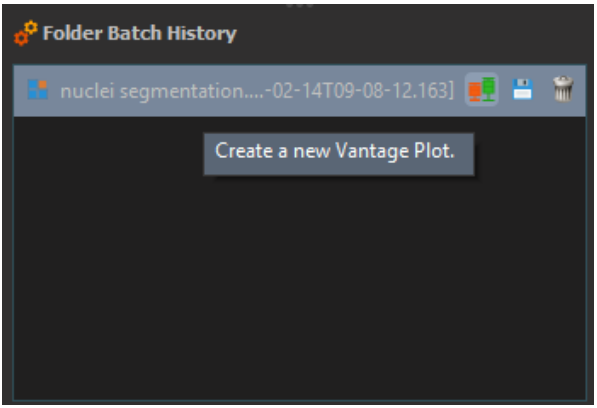


The Surpass tree is automatically updated and the Surface object is added.



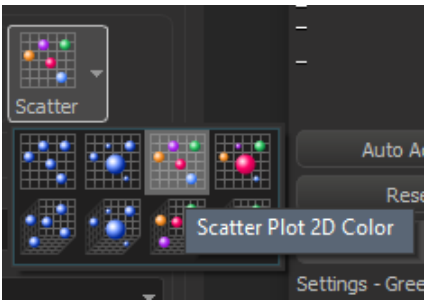
14.7 Analyze the Batch Results

The second stage of this tutorial is to analyze the Batch results. To visualize the Batch results right go to the right-hand side of the arena window click on to create a new vantage plot of your selection. As soon as you select this option the Vantage view is opened.



For details on the Vantage plot wizards, please follow the instructions provided within Chapter 12 - Vantage.

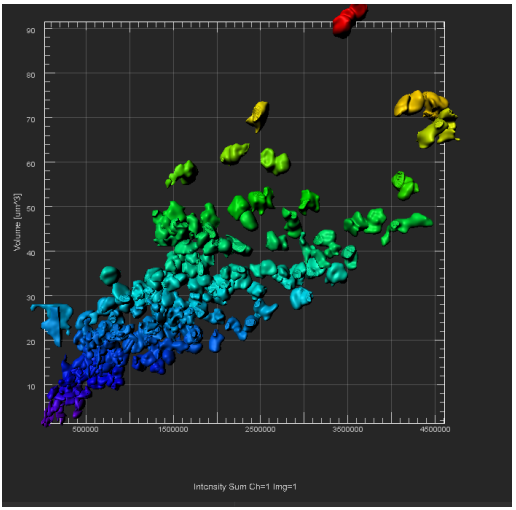
In this tutorial, we will create the **2D Scatter plot with the color**.



For **X dimension** select **Intensity Sum of Ch = 1 Img = 1.** For **Y and**

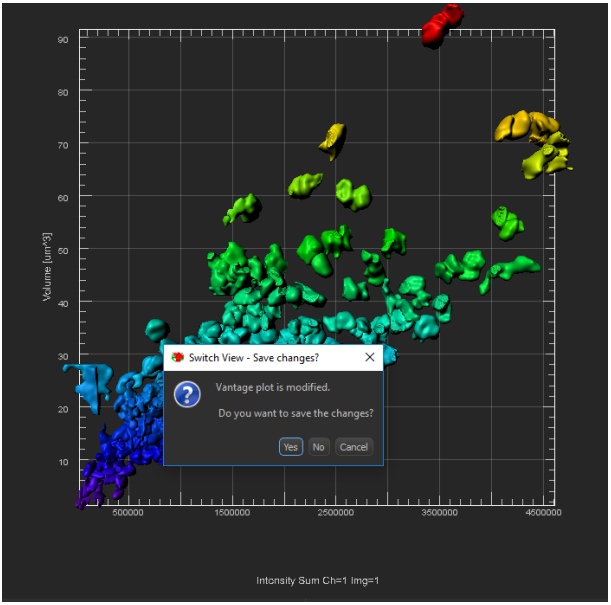
Color select **Volume**.

This is the result in the viewing area. A scatter plot shows the dependency between the Intensity sum of Channel 1 and Volume. To enhance the data visualization, the created Surfaces are color coded based on the Volume values.

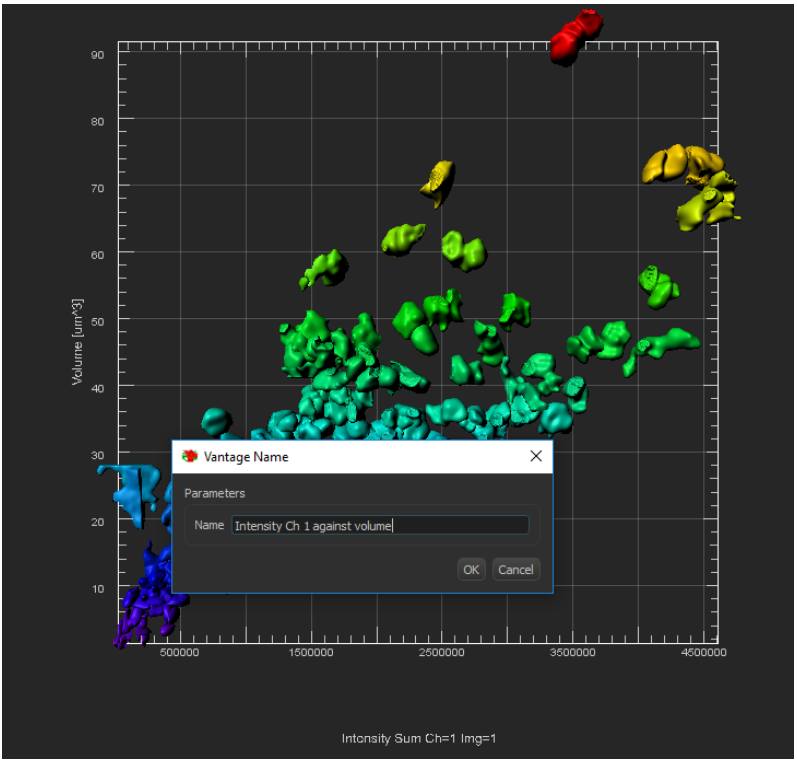


14.8 Saving Vantage Plots with Batch Processing

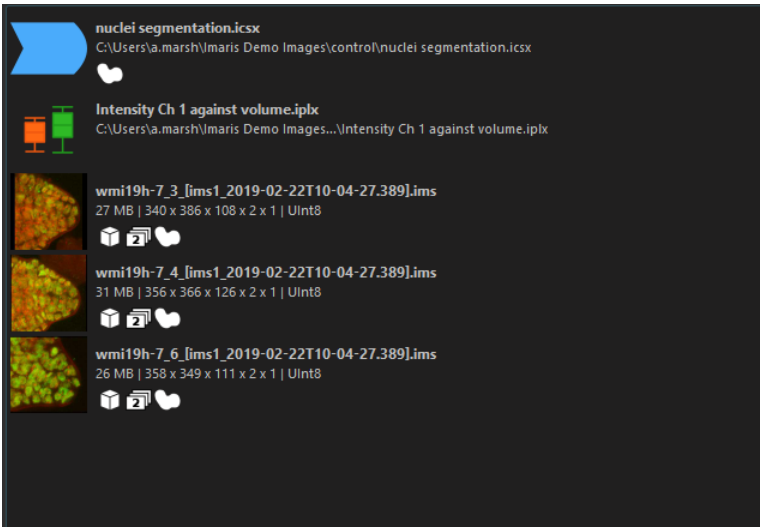
Click the Arena icon to leave Vantage view, you will be prompted to save your vantage plot:



Select yes, and add an appropriate name when the following pop up window appears, select ok.



Now return to Arena view and your plot will be saved in the control folder with your images.

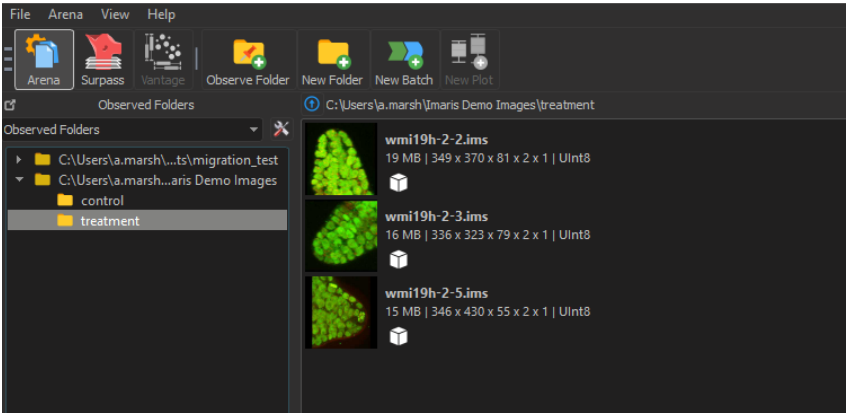

nuclei segmentation.icsx
C:\Users\a.marsh\Imaris Demo Images\control\nuclei segmentation.icsx
Intensity Ch 1 against volume.iplx
C:\Users\a.marsh\Imaris Demo Images...\Intensity Ch 1 against volume.iplx
wmi19h-7_3_[ims1_2019-02-22T10-04-27.389].ims
27 MB | 340 x 386 x 108 x 2 x 1 | UInt8
wmi19h-7_4_[ims1_2019-02-22T10-04-27.389].ims
31 MB | 356 x 366 x 126 x 2 x 1 | UInt8
wmi19h-7_6_[ims1_2019-02-22T10-04-27.389].ims
26 MB | 358 x 349 x 111 x 2 x 1 | UInt8

Section 15 Comparison Between Folders

A common procedure in scientific analysis is the comparison of two experiments. This comparison could be of two different treatments or the comparison of a treatment to a control group.
This tutorial describes how you can process different image groups within Imaris by applying the same Batch creation parameters. In addition it shows, how to compare the groups' Batch Results.
This tutorial uses the Control folder Batch results that we created in the previous tutorial.

15.1 Copying the Creation Parameters item

To apply the same creation parameters on the images within the Treatment group, the nuclei segmentation creation parameters item should be added within the Treatment group.

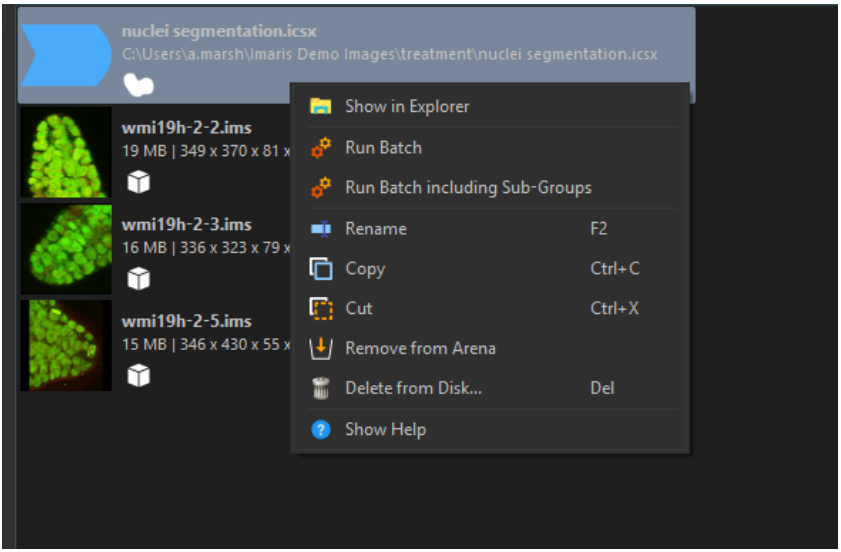


There are several ways to add a creation parameters item within the Folder.
You can use the **drag and drop** method to add a Creation Parameters item within the Folder. To copy an item to a new location, select the item within the Arena and then simply drag and drop the item into another folder. Alternatively you can **copy and paste** the batch creation parameters you would like to use into the treatment group folder.

In this example, select the **nuclei segmentation** item **from the Control group** and drag it **to the Treatment group**. The item's icon shows a plus sign, indicating the drag and drop mode. The Treatment Group content within the Arena view is updated, displaying the **nuclei segmentation** Creation Parameters item.

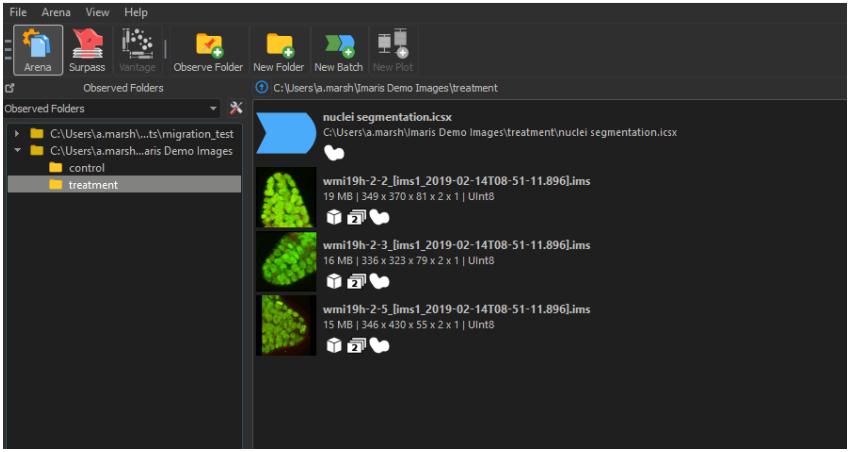
15.2 Batch Processing Treatment Group

Right-click on the nuclei segmentation creation parameters item and select the Run Batch option. The same creation parameters used in the Control group are applied to the images within the Treatment group.



When you press run batch the results are stored directly into the files and in this example the following icons display beneath the image size information.

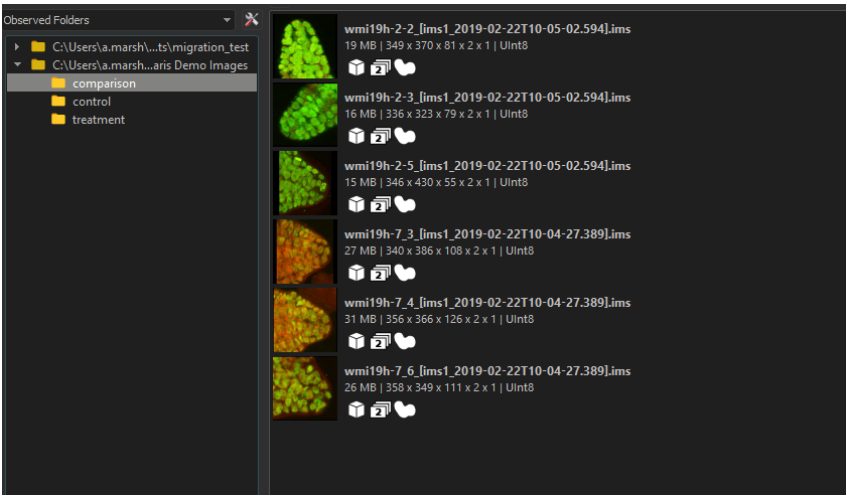
15 - Comparison Between Folders



15.3 Vantage Plot Item

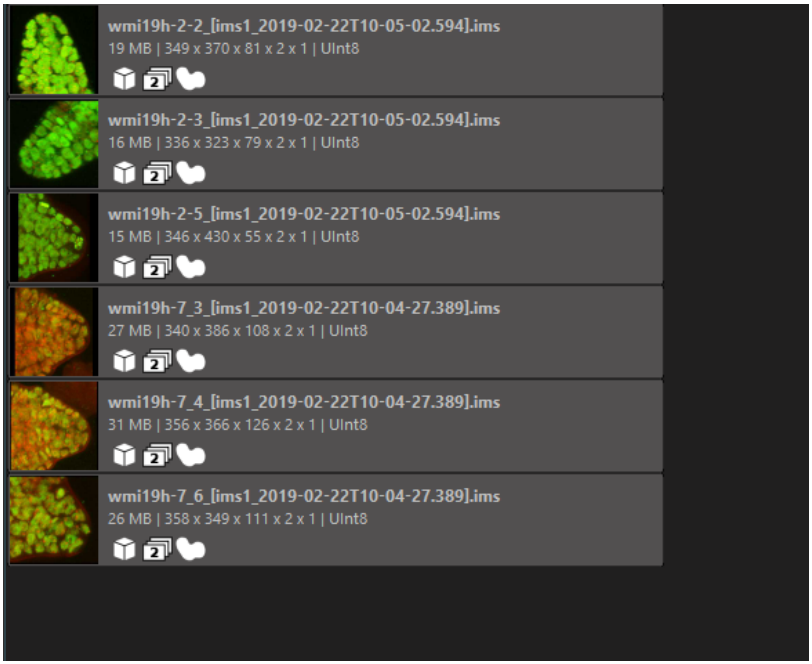
In order to compare batch image files from different folders you must first create a new folder and name the folder comparison.

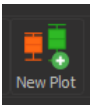
Copy and paste or **drag and drop** images into the new **comparison folder** in this example we use all of the images analyzed so far shown below.

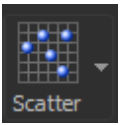


15.4 Comparison Visualization

Vantage plot is the most informative and flexible way to visualize and generate a batch report. It provides a statistical data-visualization of the currently selected Batch Collection data. Select the images you would like to view:

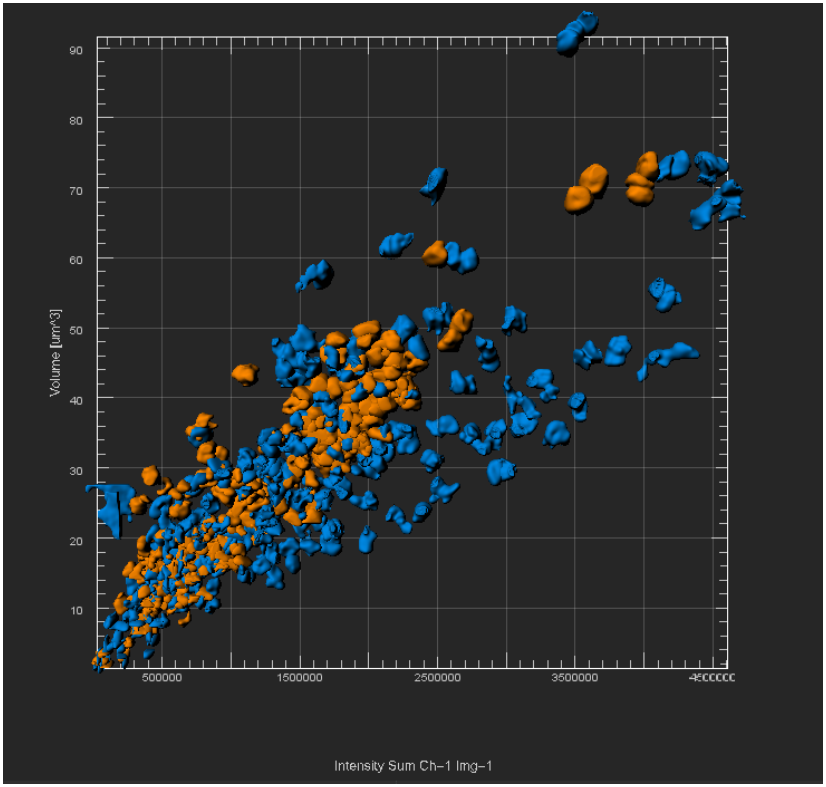


Select new plot in the top toolbar. 

The Vantage view is instantly opened. Select **Scatter** 

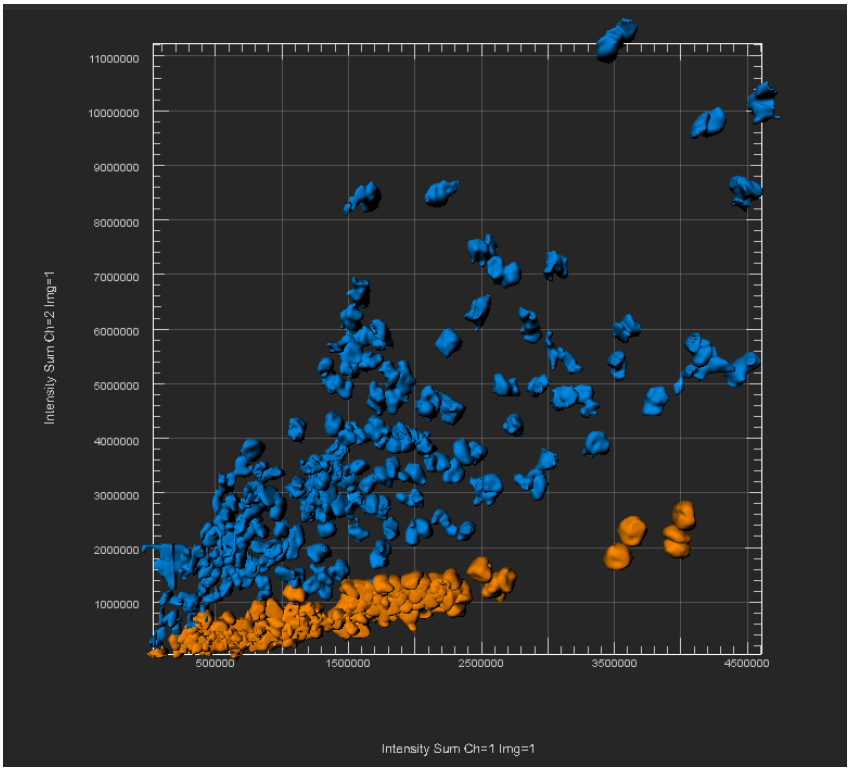
and select **x as volume and y as Intensity Sum Ch1 = 1 Img = 1**.

Batch Collection results of the Control and the Treatment group are summarized into a Vantage plot with a separate Vantage component created for each group. The Vantage plot enables the analysis of the results and illustrates patterns and trends that are not immediately apparent by just looking at the raw or segmented data.



15.5 Comparison Analysis

To compare the levels of chromatin modification (Channel 1) relative to the DNA content (Channel 2) in each Group, the **Intensity Sum Ch1 = 1 Img = 1** and **Intensity Sum Ch2 = 1 Img = 1** are selected for the X and Y plot dimensions, respectively. In the Vantage plot each group is represented by a different color, Control in blue and Treatment in yellow.



This is the result in the viewing area showing differences in chromatin modification levels between the two data sets.

Section 16 ImarisXT

The ImarisXT Programmable Interface is a part of Imaris. It enables 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.

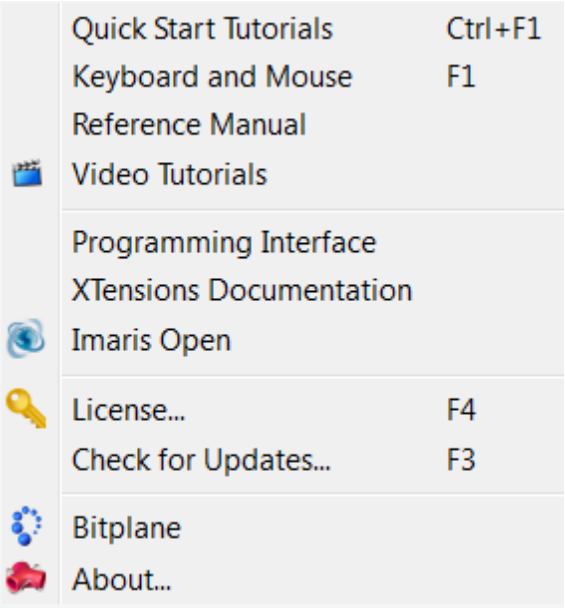
By providing interfaces known to both parties, the ImarisXT Programmable Interface facilitates the communication between the biologists and computer scientists. Design and development of specific applications that fit individual projects, while meeting the scientific community's requirements and the software developer's conditions are achievable through the highly configurable and extensive ImarisXT Programmable Interface. The ImarisXT Programmable Interface extends the user interface beyond the standard functionality.

16.1 ImarisXT Features and Advantages

ImarisXT is a multi-functional two-way interface from Imaris to classic programming languages, Matlab and other imaging software programs. So to use ImarisXT, there is no need to write any code. However, you can include your own image processing and analysis functions and extend Imaris capabilities further, using other programming languages.
ImarisXT lets you turn your ideas into advanced software options. It helps you target your specific applications and suit your own preferences. By creating your own custom routines and integrating them in Imaris, you may explore the limitless possibilities of image processing and analysis.

Documentation

The XTensions documentation is available online as HTML files (http://open.bitplane.com/) and in PDF formats.
To access the help, select the **Programming Interface** option under the **Help** menu.



Minimum software requirements:

1. Imaris version 7.3 or higher.
2. ImarisXT
3. XTensions installed with Imaris (MatLab) have to be complied, or downloaded from the website (http://open.bitplane.com/)
4. XTensions written in an environment supporting the Java based interface or Python written XTensions

16.2 Programmable Interface

ImarisXT Programmable Interface consists of three main components:

- Java based Interface
- Expandable User Interface
- Predefined Set of XTension

Java based Interface

Java based interface is used to help interprocess communication and dynamic object creation in a large selection of programming languages.
Imaris XT incorporates a Java based interface that provides a method for sharing code between Imaris and different programming languages and/or other imaging software programs. The Java based interface contains more than 300 functions that stand for and can perform some of the tasks available in Imaris. Java based interface technology supports easy integration of newly developed XTensions directly into Imaris, expanding its functionality.

The Java based interface has two principal functions:

1. The Java based interface provides communication between Imaris and other programming languages.
It allows querying information from and sending commands to Imaris.

2. The Java based 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

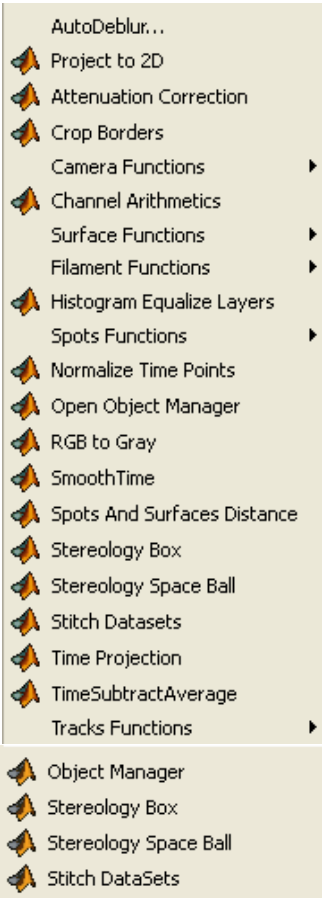
The Expandable user interface accommodates expansion of the Imaris interface beyond the predefined configuration that comes with the software. It allows you to add interface elements that access your personal custom Imaris XTensions, right along-side the built-in elements

that launch core features of Imaris. To integrate your individually developed XTension into Imaris, you are not required to modify, or even access Imaris code. The ImarisXT expandable user interface handles all details of incorporating, executing and presenting the results of this new XTension. It also provides a method for new XTensions to be called by the touch of a single button.
There is no limitation to the number of external functions that can be integrated into Imaris.
Integration of new XTensions into Imaris is available in two locations of the Imaris user interface:

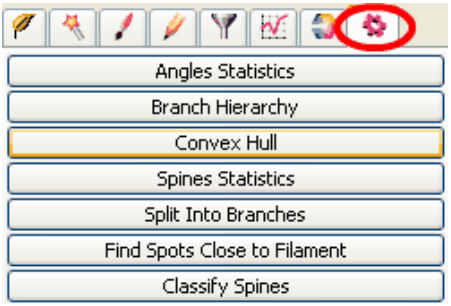
1. Image Processing Menu
3. Surpass Tools Tab

Predefined Set of XTensions

XTensions are a collection of supplemental utility functions that you set up and 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 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 custom routines as M-files, or to combine one with other XTensions. We hope that this set of predefined XTensions will lead the Imaris user community to establish a common repository for external programs to be accumulated and freely shared.
The individual Matlab files (.m files) are stored within the folder called XT/matlabor (or XT/rtmatlab) (default folder name).
The XTensions are available in the **Image Processing** menu.



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



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

16.3 InvertMiddleSlice

If you are comfortable with Imaris performing the analysis and processing of your images without knowing what goes on behind the curtain, feel free to skip this tutorial. To 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 the introduction of some basic image processing concepts. The example starts by reading an image into the Matlab workspace. Then an InvertMiddleSlice function is performed on the image. The example also provides a guided step-by-step procedure how to embed the InvertMiddleSlice Function as a XTension into Imaris.

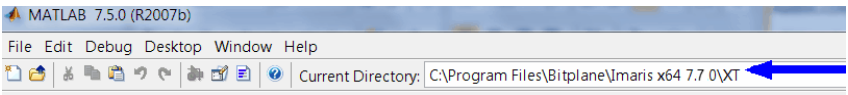
Start MATLAB

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

```
close all
```

Open Imaris and open the dataset retina.ims.

Open Matlab. Set the Current Directory to the Imaris XTensions folder C:\Program Files (x86)\Bitplane\Imaris 8.4\XT\matlab. (note: If your software is not Imaris 8.4, replace this with your version of Imaris)



In Matlab, activate the java library for the communication between Matlab and Imaris (ImarisLib.jar):

```
javaaddpath ImarisLib.jar
vImarisLib = ImarisLib;
```

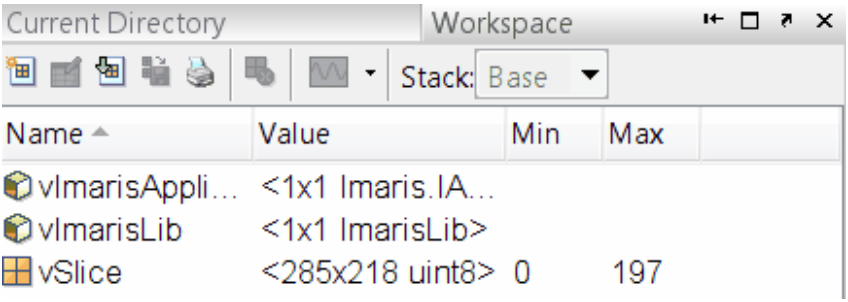
Now get in contact with Imaris. The index 0 provided as argument indicates that we want to get in contact with the first instance of Imaris. If there are multiple instances of Imaris open, please provide the index corresponding to the desired instance.

```
vImarisApplication = vImarisLib.GetApplication(0);
```

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

```
vSlice                                                    =
uint8(vImarisApplication.GetDataSet.GetDataSliceShor
ts(32, 0, 0))
```

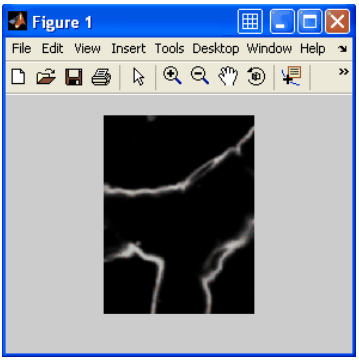
If you click on the Workspace tab of Matlab, you will now see a variable named vSlice.



The next command is to show that slice as an image in Matlab.

```
imshow(vSlice);
```

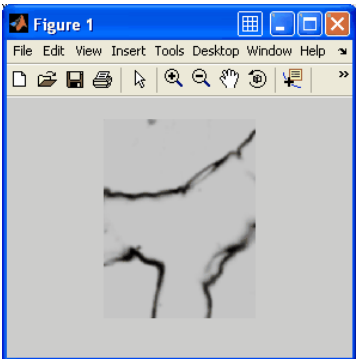
As a result Retina slice 32 is open in Matlab:



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

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

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

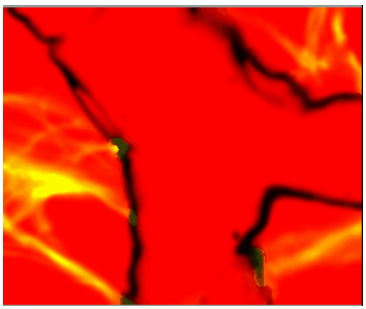


To copy the inverted Slice and send it back to Imaris, write the command:

```
vImarisApplication.GetDataSet.SetDataSliceBytes(vSli
```

```
ceInverted, 32, 0, 0)
```

This is the result in the Imaris viewing area:



16.4 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:

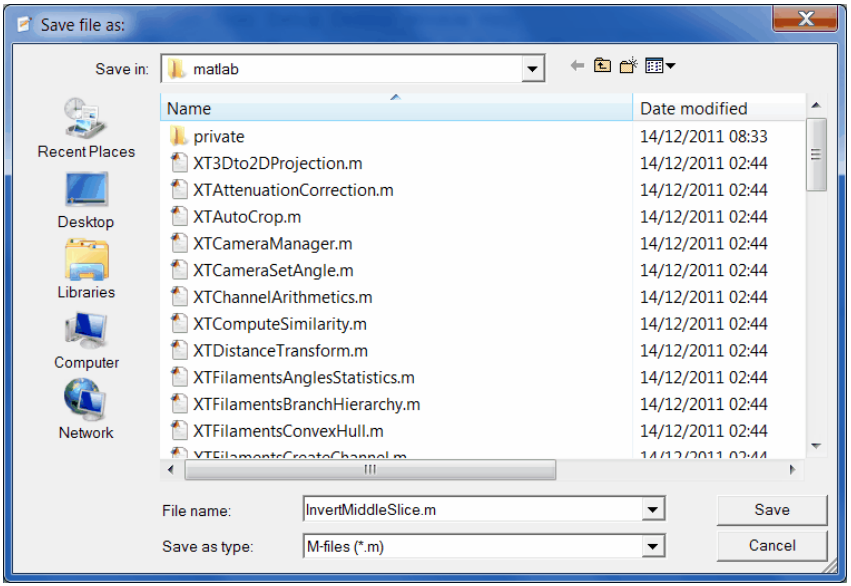
- **M-files are saved in the Work folder in the MATLAB program files subdirectory**
- **Variable names are case-sensitive.**

From the Matlab **File** menu select **/ New / M-File**, and a new Editor window appears.

In the Editor window write this code:

```
% This function inverts the middle slice
function InvertMiddleSlice(vImarisApplication)
vDataSet = vImarisApplication.GetDataSet;
% Set up middle slice index
vIndexZ = vDataSet.GetSizeZ / 2;
vIndexC = 0;
vIndexT = 0;
% Get slice, invert it, send back
vSlice =
uint8(vImarisApplication.GetDataSet.GetDataSliceShor
ts(vIndexZ, vIndexC, vIndexT));
vSliceInverted = max(vSlice(:)) - vSlice;
vImarisApplication.GetDataSet.SetDataSliceBytes(vSli
ceInverted, vIndexZ, vIndexC, vIndexT);
```

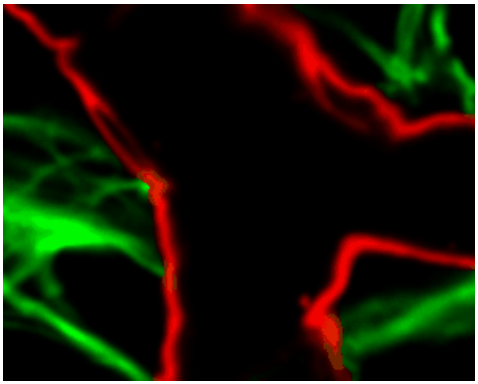
Save the file.



Tip: 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);
```



16.5 Embed the new Function as XTension

Embed the new Function as a 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 need to change the first few lines of the M-file: the function line where the new command is defined, and several lines that connect to the Java based interface of Imaris and define the vImarisApplication variable differently than the earlier example.

```
% <CustomTools>
% <Menu>
% <Item name="Invert Middle Slice" icon="Matlab">
% <Command>MatlabXT::XTInvertMiddleSlice(%i)</
Command>
% </Item>
% </Menu>
% </CustomTools>

function XTInvertMiddleSlice(aImarisApplicationID)

if ~isa(aImarisApplicationID,
'Imaris.IApplicationPrxHelper')
javaaddpath ImarisLib.jar
vImarisLib = ImarisLib;
if ischar(aImarisApplicationID)
aImarisApplicationID =
round(str2double(aImarisApplicationID));
end
vImarisApplication =
vImarisLib.GetApplication(aImarisApplicationID);
else
vImarisApplication = aImarisApplicationID;
end
```

The lines bellow are the same as the first content of the tutorial example InvertMiddleSlice

```
vDataSet = vImarisApplication.GetDataSet;
% Set up middle slice index
vIndexZ = vDataSet.GetSizeZ / 2;
vIndexC = 0;
vIndexT = 0;
% Get slice, invert it, send back
vSlice =
uint8(vImarisApplication.GetDataSet.GetDataSliceShor
ts(vIndexZ, vIndexC, vIndexT));
vSliceInverted = max(vSlice(:)) - vSlice;
vImarisApplication.GetDataSet.SetDataSliceBytes(vSli
ceInverted, vIndexZ, vIndexC, vIndexT);
```

Save the M-file, and copy it to the special folder:" **C:\Program Files \Bitplane\Imaris 8.4\XTensions".** (note: shown for Imaris 8.4, replace with your version of Imaris if required)
Close Matlab and restart Imaris (Imaris checks the XTensions folder when it starts).
The new created XTensions will appear in the Image Processing menu.

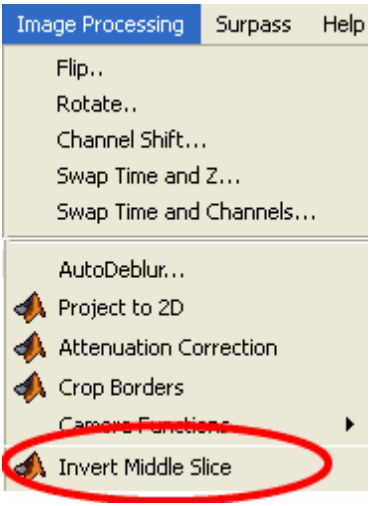
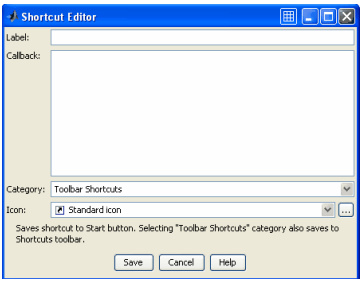


Image Processing
Surpass
Help
Flip..
Rotate..
Channel Shift...
Swap Time and Z...
Swap Time and Channels...
AutoDeblur...
Project to 2D
Attenuation Correction
Crop Borders
Invert Middle Slice

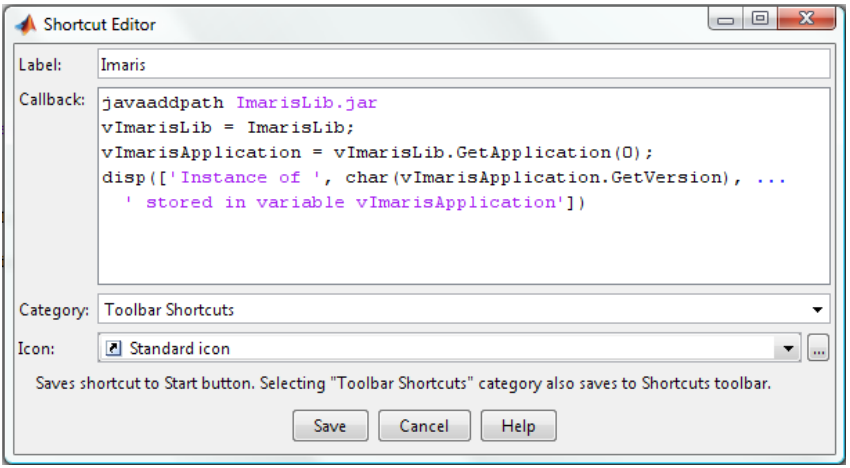
16.6 Matlab Shortcut to Start Imaris

This tutorial shows 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.** To create a New Shortcut follow the instructions. A new shortcut editor window will appear.

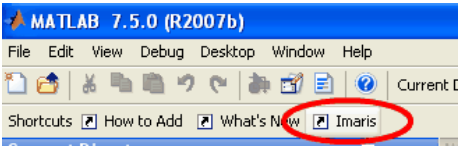


In the **Label** field enter **Imaris**. In the Callback field enter:

```
javaaddpath ImarisLib.jar
vImarisLib = ImarisLib;
vImarisApplication = vImarisLib.GetApplication(0);
disp(['Instance of ',
char(vImarisApplication.GetVersion), ...
' stored in variable vImarisApplication'])
```



Click **Save.**



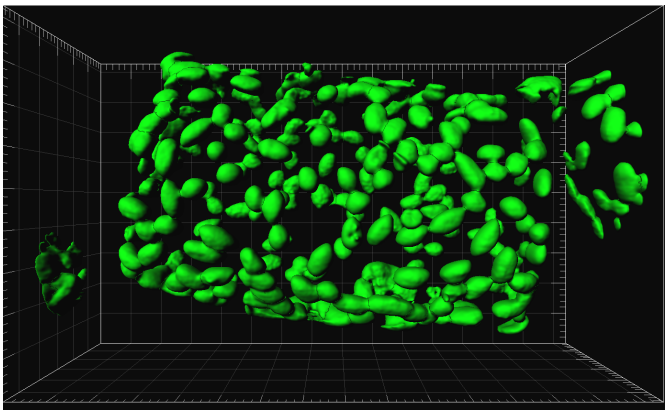
Clicking on the shortcut icon takes you directly to Imaris.

Section 17 Similarity Statistic Values

This tutorial will illustrate an example of how to use the Similarity Statistic Values XTension.
The Similarity Statistic Values is a XTension 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.
It demonstrates 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 XTension we will start with the demo image PlantCell.ims

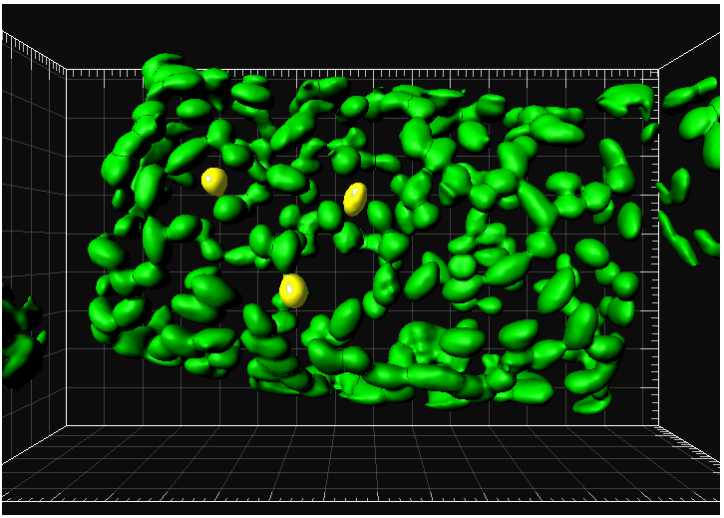
Double-click the demo image, **Plant Cell image** in Arena to open it in Surpass.

Following the example given in the Define Region Seed Points 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.

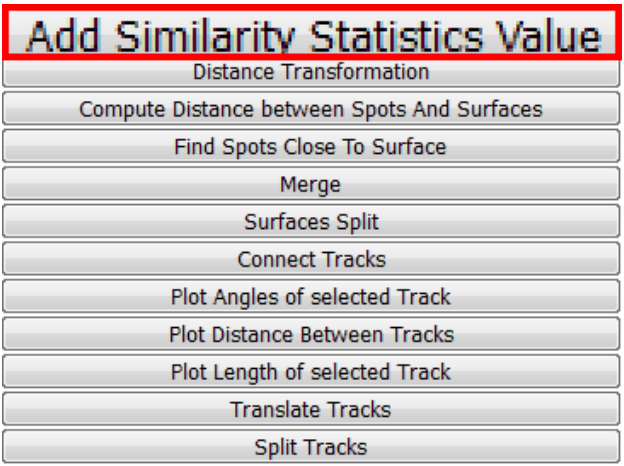


17.1 Tool tab

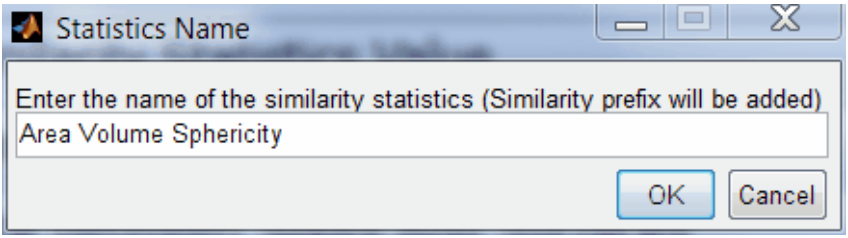
Under the Tool tab the use Similarity Statistic Values XT extension is available.
First, you have to select the reference objects according to your requirements.



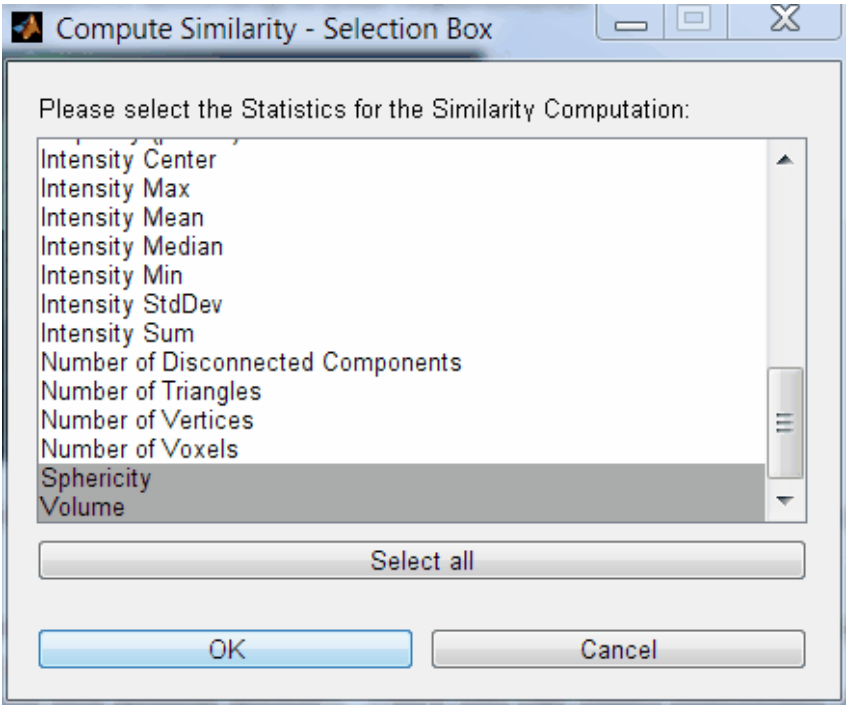
Then click on the button **Add Similarity Statistical Value** to launch the Matlab and the Statistics name window appears.



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



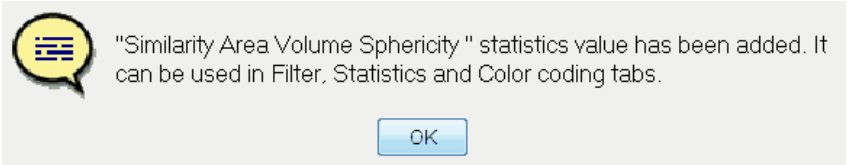
To assist you in finding the added statistical value, the **Similarity** is automatically added to the name as a **prefix**.
The new Computer Similarity-Selection Box window appears. There you can select individually or in combination the statistical values from the list.



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 Crtl +click.

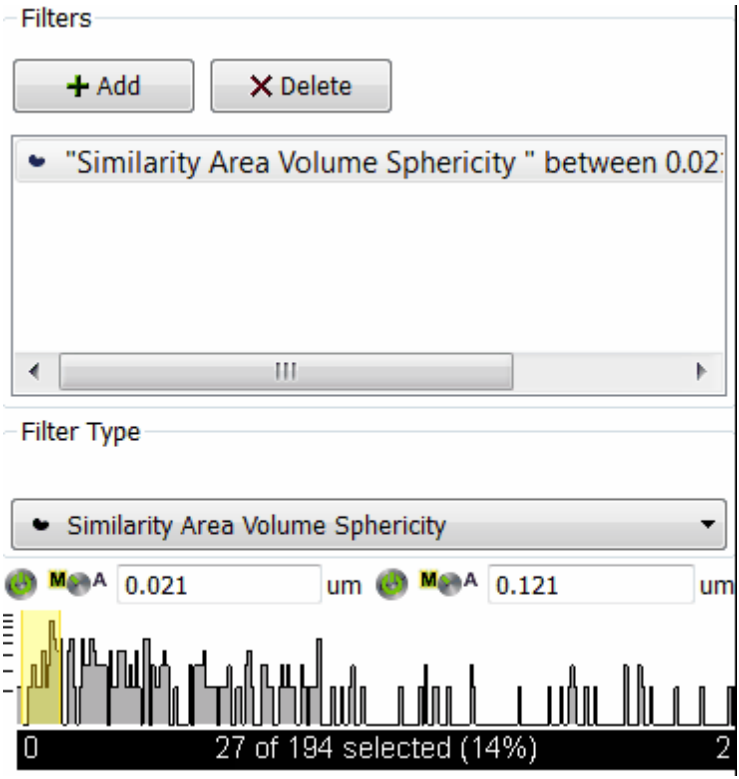
The newly created statistical value is based upon the aggregate weighted selected statistical values. Confirm the selection with the OK button.

The final Matlab Info window confirms the creation of the new statistical value; **Similarity Area Volume Sphericity**. The created statistical value can be used as any other Imaris statistical value.

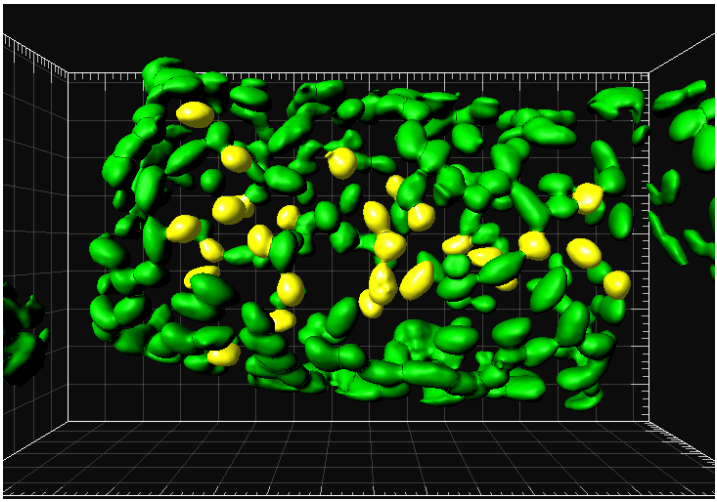


17.2 Filter tab

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



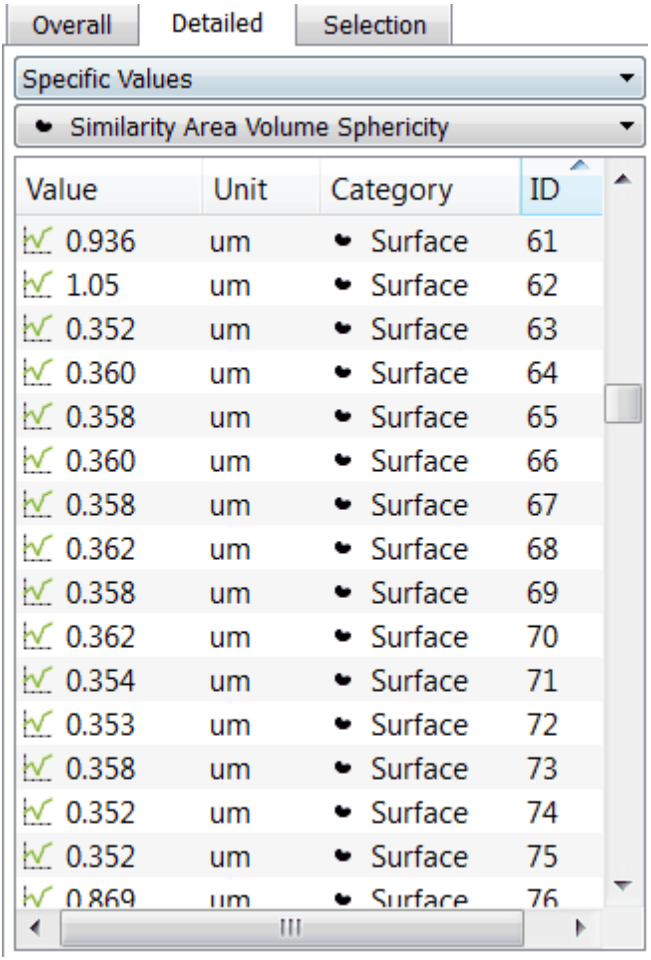
Similarity function requires the definition of a threshold value(s) in order to determine whether two different objects are similar.
Set the threshold values (lower, upper or both) to define the section of interest and identify the objects with similar statistical properties. Based on these limits all objects that have less similarity than the threshold 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.

17.3 Statistic Tab

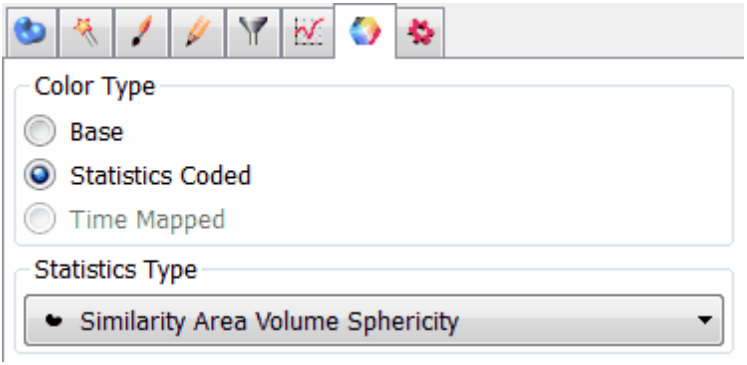
Under the Statistic Tab, the new similarity statistical value is added to the already existing statistical value list. It is available under the **Detailed and Selection** tab.
The similarity numerical values let you assign objects to groups based on their similarity and facilitates understanding of the complex relationships in large data sets.



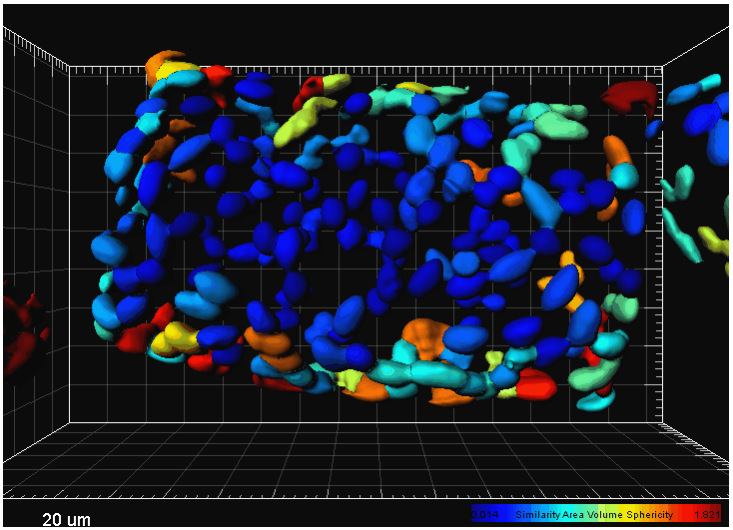
In the statistical table a single value represents the similarity between the object within the image and selected reference objects. Values are always in range from 0 to 1, 0 is indicating no similarity and 1 complete similarity. If more than one object is selected as a set of reference objects, the case perfect similarity is seldom.

17.4 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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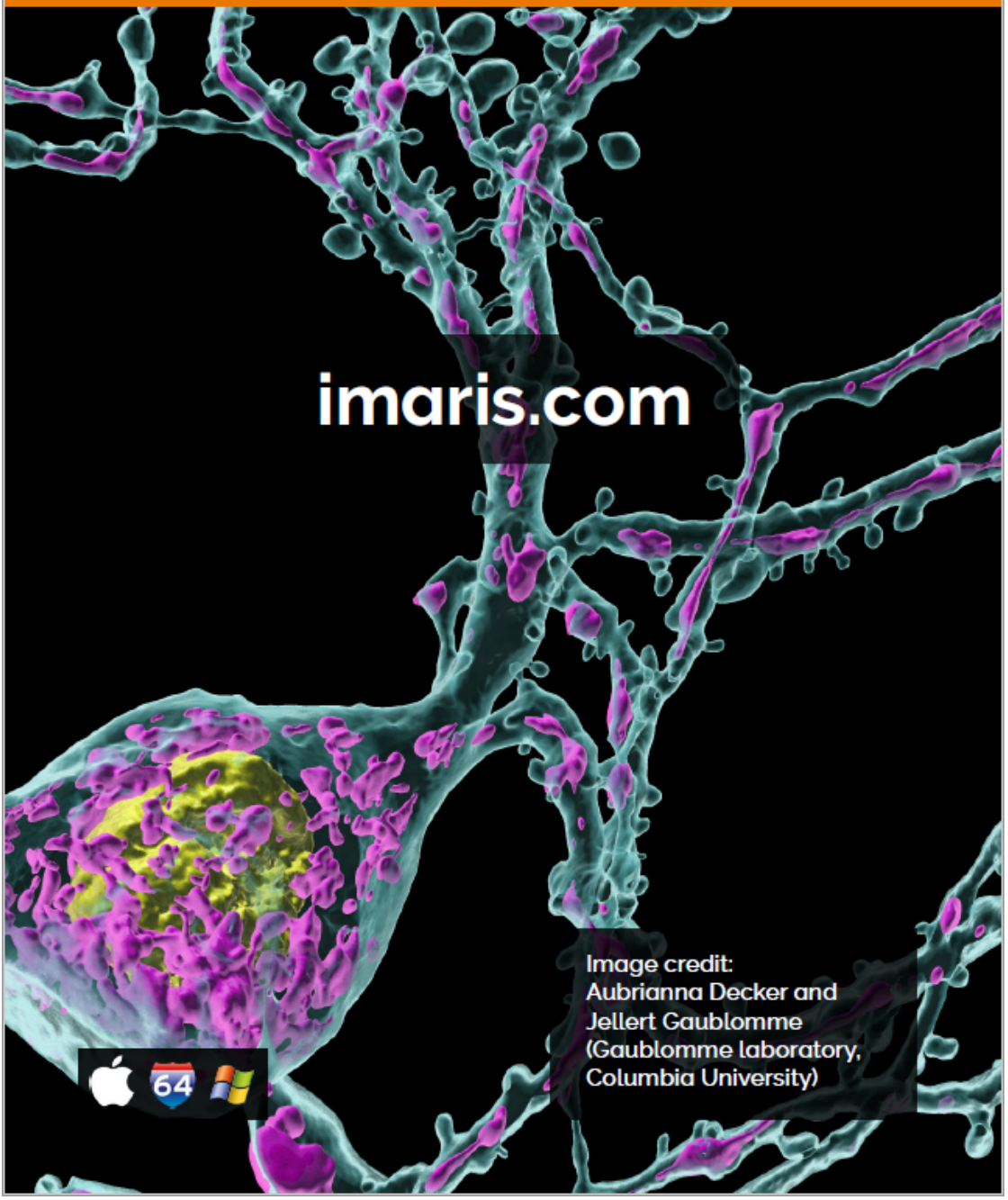
OXFORD INSTRUMENTS

IMARIS