

Imaris V 6.4

Reference Manual

1 Preface

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

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

Bitplane AG
Badenerstrasse 682
8048 Zurich
Switzerland

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Imaris Reference Manual V 6.4

1.1 Getting Familiar

Today, all optical microscopes commercially available can record several channels simultaneously to produce multi-channel images. Imaris is an application designed to visualize such microscopic data. Imaris uses a special file format to store images with parameters and can incorporate image files for all major microscopes and image acquisition systems. The images can be viewed in several different ways and processed to provide the optimum amount of information from 2D or 3D still images, time series, and animations.

Once a data set has been loaded into Imaris, individual parameters such as channel colors, geometrical settings or voxel sizes can be adjusted. Imaris has a variety of tools available, such as cropping, threshold cutting and filters for processing the images to bring out the required details.

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

- A [Slice](#) viewer.

- A [Section](#) viewer for simultaneous viewing along three coordinate axes.

- A [Gallery](#) viewer for slice image overview and selection.

- The [Easy 3D](#) viewer provides a quick image view.

- The [Surpass](#) viewer, which offers numerous tools for data preparation, presentation and manipulation of different types of data display as well as any combination of them and the ability to define, combine and group an arbitrary number of objects out of a set of viewing objects.

- [Animations](#) can be created from the Slice and 3D modes, or with the key frame animator in Surpass.

- [InMotion](#) is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view.

- A viewer for [Colocalization](#) computation.

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

1.2 Getting Started

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

Minimum hardware/software requirements are:

- Windows NT 4.0, or a more recent version, Windows 2000, XP, Vista, MacOS X 10.5.0 (Leopard) or higher
- CD-ROM
- Graphics card with 3D accelerator
- Network facilities for image import from the microscope
- 512 MB RAM (> 1 GB recommended)

Bitplane also recommends:

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

Installation

To install the software, please proceed as follows:

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

Licensing

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

Starting Imaris

Imaris can be started by one of the following methods:

- Double-click on the Imaris icon (we recommend copying the icon to the desktop).
- Drag the icon of an image or a file to the Imaris program icon.
- Imaris can be started directly from the Image Access database.

The software opens with the main screen.

Supported File Formats

Imaris (as of version 6.0.0) can read the following file formats, i.e. it can read the image and the parameters.

- Andor: Multi-TIFF series (*.tif, *.tiff)
- Applied Precision, Inc: DeltaVision (*.i3d, *.dv)
- Biorad: MRC-600, MRC-1024 (*.pic)
- BioVision: IPLab Mac (*.ipm)
- Bitplane: Imaris 5.5 (*.ims)
- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 Classic/Old (*.ims)
- Bitplane: Imaris Scene File (*.imx)
- Carl Zeiss: LSM 510 (*.lsm)
- Carl Zeiss: LSM 410, LSM 310 (*.tif, *.tiff)
- Carl Zeiss: Axiovision (*.zvi)
- Image Cytometry Standard: ICS - used by Nikon, Huygens, and others (*.ics, *.ids)

Leica: TCS-NT (*.tif, *.tiff)
Leica: LCS (*.lei, *.raw, *.tif, *.tiff)
Leica: series (*.inf, *.info, *.tif, *.tiff)
Leica: Image File Format (*.lif)
Molecular Devices: Metamorph STK (series) (*.stk)
MRC - primarily electron density volumes as in cryo-EM (*.mrc, *.st, *.rec)
Olympus: FluoView TIFF (*.tif, *.tiff)
Olympus: FluoView 1000 OIF (*.oif)
Olympus: FluoView 1000 OIB (*.oib)
Olympus: Cell^R 1.1/standard (*.tif, *.tiff)
Open Microscopy Environment XML (*.ome)
Open Microscopy Environment TIF (*.tif, *.tiff)
Perkin Elmer: UltraView (*.tim, *.zpo)
Scanalytics: IPLab (*.ipl)
TILL Photonics: TILLvisION (*.rbinf)

Plus it can read general TIFF series (or BMP series) of the format aaaNNN.tif (where a is a character and N is a number).

2 Menu File

Open...	Ctrl + O
Revert to File	Ctrl + R
Save as...	Ctrl + S

[Batch Convert...](#)

Import Scene...	Ctrl + L
Export Scene...	Ctrl + E

Snapshot...	Ctrl + T
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Exit	Ctrl + Q
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See also:
Addendum - [Mouse & Keyboard Mac](#)

2.1 Open

Data sets can be loaded from various file formats.

Image File Series

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

In the menu select File – Open.

Select file type from the Files of Type pull-down menu.

Select a file name from the list and click **Open** or double-click on the requested file entry.

The file is loaded.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - [Batch Convert ...](#)

2.1.1 Reader Configuration

Read only one Time Point

Loads a single time point of a time series.

Resampling Open...

The Resampling Open dialog box can be used as preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This can be particularly important when reading large data sets over a network.

The revision applies to all views in Imaris and in Surpass.

Select menu File - Open or click on the button **Open** in the Main toolbar.

Click on a file to highlight it and click on the button **Resampling File Open ...**.

Image Preview

The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

Original Size

Display of the original file size.

Subsampling Factor

X,Y,Z, Ch, T

You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

Crop Limits (Min/Max)

X, Y, Z, Ch, T, From, To

The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

Resampled Size

Display of the resampled file size.

Click on **Open** and the data set is cropped and resampled while loading.

See also:

Menu Edit - Image Properties... - [Geometry](#)

Menu Edit - [Crop Time...](#)

Menu Edit - [Resample Time...](#)

Menu Edit - [Crop 3D...](#)

Menu Edit - [Resample 3D...](#)

2.1.2 Settings

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

Leica LCS Settings

A LeicaLCS data set consists of a number of image stacks (or experiments). A dialog box can be opened to

select a specific image stack.

Select File - Open or click **Open** in the Main toolbar.

Select Files of Type: Leica LCS.

Click on the *.lei file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).

By checking the box **Thumbnail Preview** possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.

Select the required image stack on the left side of the dialog box.

Click **OK** to open the image.

Leica LIF Settings

A LeicaLIF data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

Select File - Open or click **Open** in the Main toolbar.

Select Files of Type: Leica LIF.

Click on the *.lif file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).

By checking the box **Thumbnail Preview** the possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.

Select the required image stack on the left side of the dialog box.

Click **OK** to open the image.

Adjustable Tiff Series Reader Settings

If the data set consists of a series of images, individual images can be sorted according to various dimensions (i.e., slices, channels, time points, dimension sequence). The selected sequence is shown in the File Arrangement panel. The reader can handle tiff series with single and multiple running numbers.

Select the menu File - Open or click **Open** in the Main toolbar.

Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.

Open the folder containing the series.

Click Settings, which is grayed out if the file type selector is on automatic or if the current directory does not contain a series.

Use Apply Automatic File Filter, Apply, Dimensions, Dimension Sequence, described as follows, to define the series.

Apply Automatic File

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

Button **Apply**

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

*.tif selects all files with the ending *.tif.

myfile*.tif selects all files that start with "myfile" and are followed by any letter or digit and by the extension *.tif.

myfile???.tif selects all files that start with "myfile" and are followed by two letters or digits and by the extension *.tif.

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

Dimensions

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

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

Dimension Sequence

Defines how the individual images, which are sorted alphabetically, are to be assigned to Slices (Z), Channels (Ch), and Time Points (T).

Click **OK** to return to the Open dialog window.

Click **Resampling Open** to open the Resampling dialog box or click **Open** to open the image.

2.2 Revert to File

Re-opens the actual data set.

2.3 Save as ...

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

The standard way to save your data and Imaris scene is simply to choose **Save as** from the File menu and give an appropriate name. The file extension, suggested as the default, is *.ims. The single created Imaris file contains both the image and the scene data.

You can save the file when you are done or if you want you can stop in the middle and resume work later. Simply, choose **Save as** from the File menu and give the file a name you will remember. If you then close the file and reopen it at a later time, you can resume work from where you left off.

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

Select the directory and enter the name for the file to be saved or confirm the suggestion.

Select the requested file format and click **OK**.

The data set is saved.

Available File Formats in Imaris

Bitplane: Imaris 5.5 (*.ims)

Bitplane: Imaris 3.0 (*.ims)

Bitplane: Imaris 2.7 (Classic)(*.ims)

Tiff (series)(*.tif *.tiff)

RGBA-Tiff (series)(*.tif *.tiff)

ICS file (*.ics *.ids)

Olympus: cell^R 1.1/standard (*.tif *.tiff)

Open Microscopy Environment XML (*.ome)

Open Microscopy Environment Tiff (*.tif *.tiff)

BMP (series)(*.bmp)

Move file (slice animation) (*.avi, *.mov, *.tif, *.tiff)

Export and Import Scene File

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

Overview - [Scene File Concept](#).

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - [Batch Convert...](#)

2.3.1 Advanced Save Options

Button **Format Settings...**

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

Window: Save Options

Bitplane: Imaris 5.5 (*.ims)

Enable Compression

Check the box to use compression.

Export Scene

Check the box to save the Imaris scene.

Bitplane: Imaris 3.0 (*.ims)

Time Series

Save as Single File

The time series are saved in a single file.

Save as Multiple Files

For each time point a new file is generated.

Compression

LZW Compression

Check the box to use an LZW compression.

Add to ImageAccess Database

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

2.4 Batch Convert ...

With the Imaris File Converter you can convert various image file formats to the Imaris file format *.ims. Select the menu entry Batch Convert... and the Imaris File Converter window displays. From the Windows Start menu select "ImarisFileConverter".

Input

Drag & Drop Files, or click the Button below to add Files.

Button **Add Files ...**

The window Select Files for Conversion displays. Choose the respective file and click on **Open**.

Thumbnail

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

Middle Slice

Thumbnail is the middle slice.

MIP

Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend

Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Output

Same Folder as Input

You find the converted image(s) in the same folder.

Special Folder

Here you can select another folder for the converted image(s). Either type in the respective path or use the button **Browse**.

Button **Browse**

Click on this button to browse for the special folder.

Format

The standard formats are:

Bitplane: Imaris 5.5 (*.ims)

Bitplane: Imaris 3.0 (*.ims)

Bitplane: Imaris 2.7 (Classic)(*.ims)

To add additional formats please refer to chapter Menu File - Batch Convert ... - [Preferences](#).

Input

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

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

Output

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

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

Clear Row

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

Button **Clear**

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

Button **Start**

Click on this button to start the conversion.

See also:

Menu File - Open - [Reader Configuration](#)

Menu File - Open - [Settings](#)

Menu File - Save as ... - [Advanced Save Options](#)

Menu File - Batch Convert ... - [Preferences](#)

2.4.1 Preferences

Button **Preferences**

Click on this button to open the Preferences window.

Window: Preferences

Data Cache

Imaris uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

Memory Limit (MB)

The value of Data cache limit controls the amount of data blocks Imaris will keep in memory at any time.

Cache File Paths:

Display of the cache file paths.

Button **Add**

Button **Remove**

You can use the buttons to add or remove file paths in the list.

Output Formats

If you want to extend the list of output formats you have to check the following parameter and enter a valid license in the next step. Find your licence number in Imaris as follows: Click on the menu Help, select the menu entry Licenses and copy the license number (in the License Path at the bottom of the window, next to last enter field).

All Imaris Output File Formats

Check this box to extend the output file formats.

License Location

Type in your license number or use the button **Browse**.

Button **Browse**

Click on this button and select the license path.

Button **OK**

Click on this button and all available Imaris output formats are available.

Standard formats:

Bitplane: Imaris 5.5 (*.ims)

Bitplane: Imaris 3.0 (*.ims)

Bitplane: Imaris 2.7 (Classic)(*.ims)

Additional formats:

Tiff (series)(*.tif *.tiff)

RGBA-Tiff (series)(*.tif *.tiff)

ICS file (*.ics *.ids)

Olympus: cell^R 1.1/standard (*.tif *.tiff)

Open Microscopy Environment XML (*.ome)

Open Microscopy Environment Tiff (*.tif *.tiff)

BMP (series)(*.bmp)

See also:

Menu File - [Save as ...](#)

2.5 Import Scene ...

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

Import Scene.....

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

See also:

Menu File - [Export Scene...](#)

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Overview - [Scene File Concept](#)

2.6 Export Scene

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

Save Imaris Scene

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

See also:

Menu File - [Import Scene... ..](#)

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Overview - [Scene File Concept](#)

2.7 Snapshot

Snapshot captures a 2D image of the 2D, 3D or 4D image data as it currently appears in the viewing area. It creates a new file containing your image as well any scale information, measurements data, and ROI that are displayed. The snapshots can be (1) stored in TIFF-Format, (2) saved as database records in ImageAccess in order to manage them more efficiently or else (3) copied to the clipboard.

The Image Size settings specify the size of a Snapshot. The snapshot size (in pixels) is equal to the settings in the width/height numerical fields.

The default setting for snapshot size is the Snapshot size from window size option. To modify and customize snapshot dimensions you can either select the predefined sets from the drop-down list or manually adjust the **Width** and **Height** values.

Adjusting the width or height settings for the snapshot changes the number of pixels in the snapshot, by resampling and/or cropping/expanding the image in the viewing area to match the specified pixel dimensions.

The placement of the snapshot borders (cropping or expansion) depends on the aspect ratio of the viewing area compared to the snapshot aspect ratio, and the setting called Crop to fill whole snapshot area (see below).

Changing the snapshot dimensions scales the input image, so that none of its dimensions are greater than the corresponding snapshot (output image) dimension. The scaled image is centered within the new snapshot size. In other words, the zoom of the input image is automatically scaled to better fit the available pixels in the snapshot. Depending on the settings used, some of the background color might also be considered to be part of the input image.

When the aspect ratio of the viewing area does not match the aspect ratio of the snapshot, the snapshot is surrounded with bars in the color of the Imaris screen background. There are two options allowing the user to adjust the content of the snapshot and to eliminate the Imaris screen background. The image can be either scaled to fit the selected snapshot dimensions (by adjusting the zoom in the viewing area) or else the Crop to fill whole snapshot area option can be used.

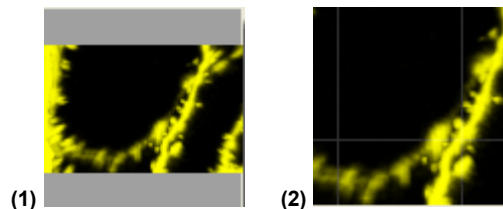
Snapshot preview

Snapshot preview offers a view of the snapshot content, as it will look when finalized.

Crop to fill whole snapshot area

This option crops the image to fit the snapshot specified Width and Height values. The option sets an image so that one dimension fits the snapshots viewing area, while the overflow of the other dimension is cut off, respecting image aspect ratio.

The images below illustrate Pyramidal cell image Snapshots taken with the various settings. (1) Disparity between image size and snapshot intended dimensions, (2) Crop to fill whole snapshot area.



The option is only available if the option Snapshot from window size is not selected.

Image size

Specifies the size (in pixels) of the Snapshot area.

The size of the Snapshot can be chosen from the drop-down list. The drop-down list offers a predefined set of numerical parameters defining image dimensions. The last choice in the drop-down list with the suffix dataset is a choice that represents the XY dimensions of the original dataset.

Width

Defines the Snapshot width in pixels.

Height

Defines the Snapshot height in pixels.

Lock Aspect Ratio Button

This setting locks the aspect ratio when the image width/height is manually changed. It changes the height and width settings in relation to one another so that the ratio of the image's width and height remains constant.

Resolution

Resolution (dpi) controls the default size of the Snapshot when it is prepared for printing in an external program. For example, if the snapshot width and height are 512 x 512 pixels, and the resolution value is set to 100 dpi, then the default print size will be 5.12 inches x 5.12 inches when the snapshot is loaded into an external application to prepare the image for printing.

Zoom in Snapshot

The Zoom in Snapshot value displays the zoom value in the finished snapshot.

Note: The Zoom in Snapshot value is set to 1 if the option 100% Snapshot button is activated.

Snapshot size from window size

When this option is selected, the dimensions of snapshot equal the dimensions of the viewing area. (The snapshot width/height in pixels corresponds exactly to the viewing area width/height). Resizing the view area alters the snapshot dimensions.

Manually changing the Image dimensions will disable the Snapshot size from window size option.

Re-Selecting this option changes the size of the Snapshots accordingly.

100% Snapshot button

Use of the 100% Snapshot button is an easy way to ensure that multiple snapshots have the same scale factor (pixel/voxel ratio), as it always results in snapshots with the zoom factor set to 1 pixel per voxel. By selecting the 100% Snapshot button, the width and height of the snapshot are adjusted (while maintaining the aspect ratio) to set the Zoom in the snapshot equal to 1.

This standardized snapshot scaling provides the opportunity to handle, compare and superimpose different snapshots. It can also be a useful guideline for creating snapshots where the number of pixels is neither too

many nor too few relative to the detail contained in the original dataset.

Image Output

To create the snapshot, first the Image Output format must be selected. Select the Snapshot Format from Image Output type boxes. Multiple selections are possible.

Save to File

When this option is selected, clicking on the Do Snapshot! button saves the snapshot to the file and folder specified in the snapshot name field. You can either accept the automatic naming suggestion or enter a new name for the image the name field.

Copy to Clipboard

When this option is selected, clicking on the **Do Snapshot!** button copies the snapshot to the clipboard. Open another application and use the paste function.

Add to ImageAccess Database

Make sure that the database ImageAccess is started. In ImageAccess, select the requested directory and enter a name for the image. Then in Imaris click on the **Do Snapshot!** button and the image is saved on the disk and an entry is added to the database.

Snapshot name

This field specifies under which folder and filename the snapshot will be saved. Initially, the automatic naming suggestion is displayed. Snapshots are automatically placed in the same directory with the same name as the original dataset and a sequential number is inserted at the end of the name. This 3-digit trailing number will automatically increment by one each time you click Do Snapshot!, to save the user the trouble of manually typing a new filename each time.

Do Snapshot! button

Click on the Do Snapshot! button to create the image snapshot.

See also:

Menu Edit - [Copy Snapshot Image](#)

Menu Edit - Preferences - Display - [Off Screen Rendering](#)

Toolbars - Main Toolbar - [Snapshot](#)

2.8 Exit

Terminates Imaris and returns to the desktop.

3 Menu Edit

Undo	Ctrl + Z
Copy Snapshot Image	Ctrl + C
Image Properties...	Ctrl + I
Show Display Adjustment	Ctrl + D
InPress	Ctrl + Shift + P
Add Time Points...	
Delete Time Points...	
Add Channels...	Ctrl + Shift + A
Delete Channels...	Ctrl + Shift + B
Add Slices...	
Delete Slices...	
Crop Time...	

[Resample Time...](#)
[Crop 3D...](#) Ctrl + W
[Resample 3D...](#)
[Change Data Type...](#)

[Preferences...](#) Ctrl + P
[File Type Associations...](#)

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

3.1 Undo

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

3.2 Copy Snapshot Image

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

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

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

See also:
Menu File - [Snapshot](#)

3.3 Image Properties...

[Geometry](#)
[Data Set](#)
[Channels](#)
[\(Channel 1...n\)](#)
[Thumbnail](#)
[Parameters](#)

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

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

3.3.1 Geometry

The geometrical settings of the actual data set are displayed.

Type

Data Type

Display of the image type.

Size

Size X, Y, Z, T

Display of the image size.

Coordinates

Voxel Size, X, Y, Z

The voxel sizes directly influence the views because they control the height of the image relative to its width. Check the parameters and adjust the **Voxel Size** and/or other settings if necessary.

Min, X, Y, Z

The minimum value of the coordinate axes.

Max, X, Y, Z

The maximum value of the coordinate axes.

Unit, nm, um, mm, m, unknown

Here you can select the unit.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Time Point

First Box

Select the time point.

Date

Enter the collection data.

Time

Enter the collection time.

Button **All Equidistant...**

If the data set is a time series, enter the date/time for each time point or click **All Equidistant** to open the Set Equidistant Time Points dialog box.

Enter the **Start Date** and **Start Time** and the **Time Interval**.

Click **OK** when finished. Imaris will calculate the time for each time point in the series.

The data set must be saved to retain the changes. Click **OK** when finished or select another heading for further adjustments.

Button **OK**

To apply the changes click on **OK**.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.3.2 Data Set

Name

Data field to type in a data set name.

Description

Data field to type in a data set description.

Numerical Aperture (N.A.)

Reads out the numerical aperture (as defined in the menu Edit - Image Properties... - Parameters).

Log

Display of processing steps.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.3.3 Channels

There is no parameter on this card.

3.3.4 Channel 1...n

In the Index of the Image Properties box click the **Channel** entry (Channel 1, Channel 2 etc.) to select the required channel.

Name

Data field to type in the channel name.

Description

Data field to type in a channel description.

Emission Wavelength

Reads out the emission wavelength.

Excitation Wavelength

Reads out the excitation wavelength.

Pinhole Radius

Reads out the pinhole radius.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.3.4.1 Tab Base Color

Red, Green, Blue

Reads out the assigned color. To change the color either adjust the values or move the square in the color circle.

To apply the changes click on **OK**.

3.3.4.2 Tab Mapped Color

Selected Color

Button **Edit...**

Click on a square in the grid and click on the button **Edit...** . Select a color and click on **OK**. The selected color is displayed in the square.

Button **Copy**

Click on a square in the grid and then on the button **Copy** to copy the color.

Button **Paste**

Click on a square in the grid and then on the button **Paste** to paste the color.

Interpolation

Colorspace

RGB

The RGB color model is an additive model in which red, green and blue are combined in various ways to reproduce other colors.

HSV

The HSV (Hue, Saturation, Value) model, defines a color space in terms of three constituent components.

Hue, the color type.

Saturation, the vibrancy of the color.

Value, the brightness of the color.

Button **Interpolate**

Select two squares in the grid and click on the button **Interpolate**. The colors between the two selected squares are interpolated.

Color Table File

Button **Import ...**

Imaris comes with a set of pre-defined color tables. Click on **Import...** to open the Import Color Table File window. Select a color table file and click on **OK**.

Button **Export...**

Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

To apply the changes click on **OK**.

3.3.4.3 Tab Coloc Statistics

The tab Coloc Statistics is available when a data set contains a Coloc channel and the Coloc channel is selected in the channel selection of the Image Properties.

All statistics about the resulting colocalized volume are displayed. Definitions of the displayed values and further information are given in chapter Coloc - Volume Statistics.

Button **Export...**

Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

See also:

Coloc - [Volume Statistics](#)

3.3.5 Thumbnail

Type

None

Select None if no thumbnail should be displayed.

Middle Slice

Thumbnail is the middle slice.

MIP

Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend

Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Preview

Displays a preview of the thumbnail image.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.3.6 Parameters

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

Button **Add Group...**

Opens a dialog to add a new group.

Button **Delete Group...**

Deletes a group.

Button **Add Parameter...**

Opens a dialog to add a new parameter to the group.

Button **Delete Parameter...**

Deletes a parameter.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.4 Show Display Adjustments

The Display Adjustment function lets you choose the channel visibility as well as improve the image display by concentrating on a limited color contrast range of voxels. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0 - 255).

The Display Adjustment function lets you set an upper limit for maximum color and a lower limit for minimum color (i.e., black). The range between these two limits is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.

Display Adjustment Dialog (one for each channel)

Switch the individual channels on or off.

Check or un-check the required channel check-box to switch the channel visibility.

Change the channel parameters such as name, color and description.

Click on the channel name to open the Image Properties. For a detailed description please refer to chapter Menu Edit - Image Properties - [Channels 1...n](#).

Button **Advanced**

Click on the button to open the Advanced settings (see below).

Select all Channels

Check Select all Channels to apply the settings to all channels.

Advanced Settings

Min

Max

Enter direct values in the Min (lower limit for minimum color) and Max (upper limit for maximum color) fields.

Alternatively drag in the display adjustment dialog the upper or lower handle of the adjustment line to adjust the Min and Max limits.

The effect of the change can be seen on the channels (channels appear brighter or darker).

Gamma

The default value of the gamma correction is 1 (the range between lower and upper limit is extrapolated in a linear mode to the full data set range). Enter a value below 1 and the linear mode is transferred to a nonlinear mode, the lower intensities appear brighter. The effect of the change is directly visible in the viewing area.

Enter the value in the respective field.

Alternatively click onto the middle triangle in the display adjustment dialog and drag it to the left to increase brightness/to the right to decrease brightness.

Opacity

The blend opacity adjustment allows you to change the opacity in real-time in blend projections in Section, Full 3D, and Surpass Volume views.

Drag the blend opacity slider bar to adjust the blend opacity.

Alternatively right-click in the display adjustment dialog and drag the mouse to adjust the values.

The effect of the change can be seen on the channels (channels appear more or less transparent).

List of Shortcuts in the Display Adjustment Dialog

Please refer to chapter Menu Edit - Show Display Adjustments - [Mouse & Keyboard PC](#).

Please refer to chapter Menu Edit - Show Display Adjustments - [Mouse & Keyboard Mac](#).

Button **Reset**

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

Button **Auto**

When clicking the button **Auto** the system detects the real high and low values (e.g. 10 - 150) and sets the Max. and Min. limits automatically to these values.

If you check the parameter Select all Channels (see above) all channels are calculated consecutively.

Button **Auto Blend**

This button is useful if you display your data in the Blend mode (item Volume - tab Settings - Mode Blend).

Click on this button and Imaris automatically calculates the optimized Min. and Max. limits. A good portion of the selected image channel becomes transparent.

If you check the parameter Select all Channels (see above), all channels are calculated consecutively.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Histogram

A histogram is a statistical data analysis, representing linear voxel within an image of the selected channel.

Change Channel Color

Click on the channel name to switch directly to the channel properties (Menu Edit - Image Properties - [Channels 1...n](#)).

See also:

Menu Edit - Image Properties... - [Channel 1...n](#)

Menu Edit - Show Display Adjustments - [Mouse & Keyboard PC](#)

Menu Edit - Show Display Adjustments - [Mouse & Keyboard Mac](#)

3.4.1 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Display Adjustments Window

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

See also:

Addendum - [Mouse & Keyboard PC](#)

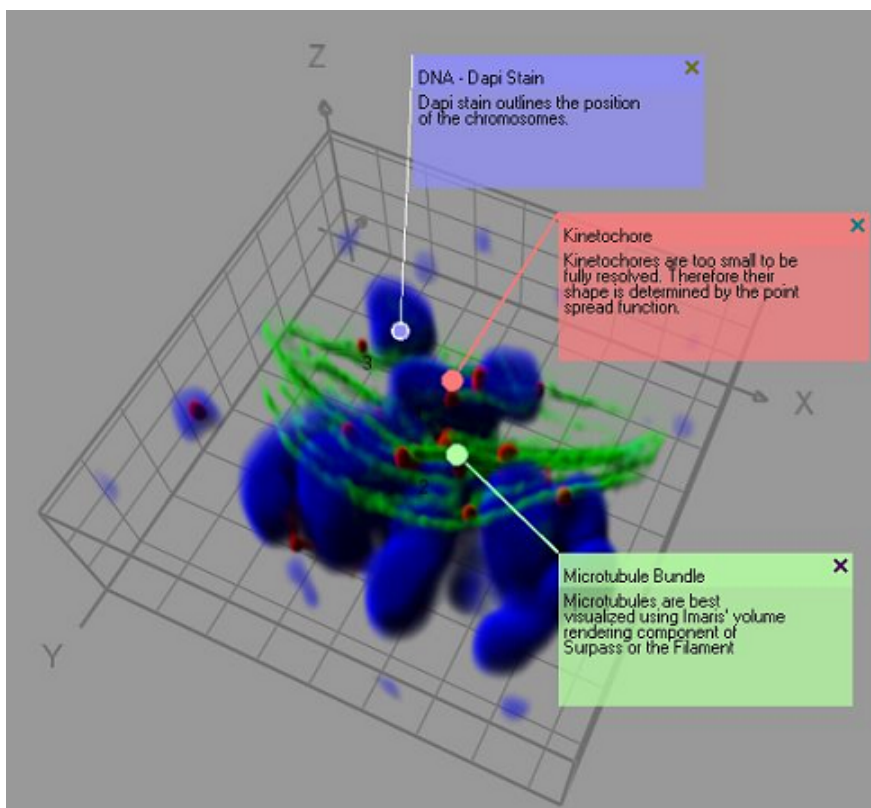
3.4.2 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Display Adjustments Window

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

See also:

3.5 InPress



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

3.5.1 Insert Figures and Annotations



To activate this module click on the Icon **InPress**, press Ctrl + Shift + P, or in the menu bar select menu File – InPress. To close Imaris InPress press the button **InPress**.

The Figures window appears on the right side of the screen.

The Figures window is open in all Imaris views and applies to all views.

For better handling it can also be un-docked by dragging it off its integrated position or clicking on the docking icon (top right, next to x).

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

Create a Figure

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

Add Annotations

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

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

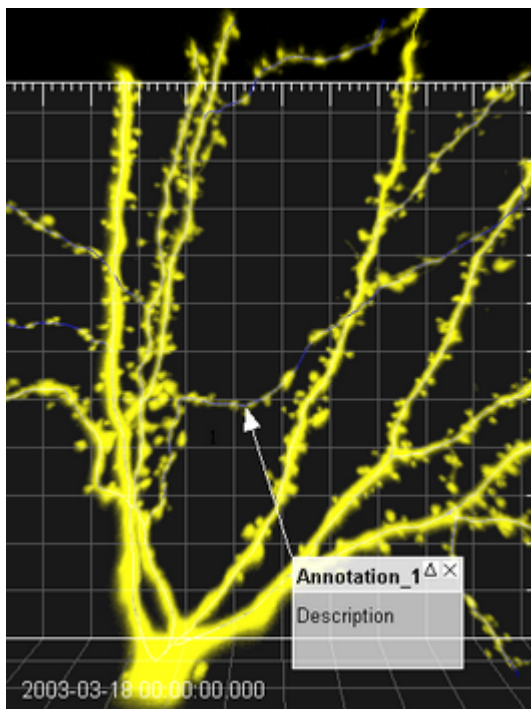
InPress Figure Tree

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

See also:

Menu File - [Export Scene...](#)

3.5.2 Position Box



Click in the image to set the 3D anchor point.

Move the mouse to elongate the line. With the second click you fix the top left corner of the text box

Move the mouse to adjust the text box size. With the third click you fix the text box size.


Move, Resize, and Hide the Annotation Box

The 3D anchor point can be moved by putting the mouse over the anchor point (until it changes to a cross) then click & drag the anchor point to a new location.

The annotation box can be moved by putting the mouse on the upper region of the box (until it changes to a cross) then click & drag the annotation box to a new location.

The annotation box can be resized by moving the mouse to an edge of the box (until the mouse changes to a resize icon) and then click & drag the edge.

The description area of the annotation can be closed (hidden) or expanded (shown) by clicking the triangle on the top right of any annotation box.

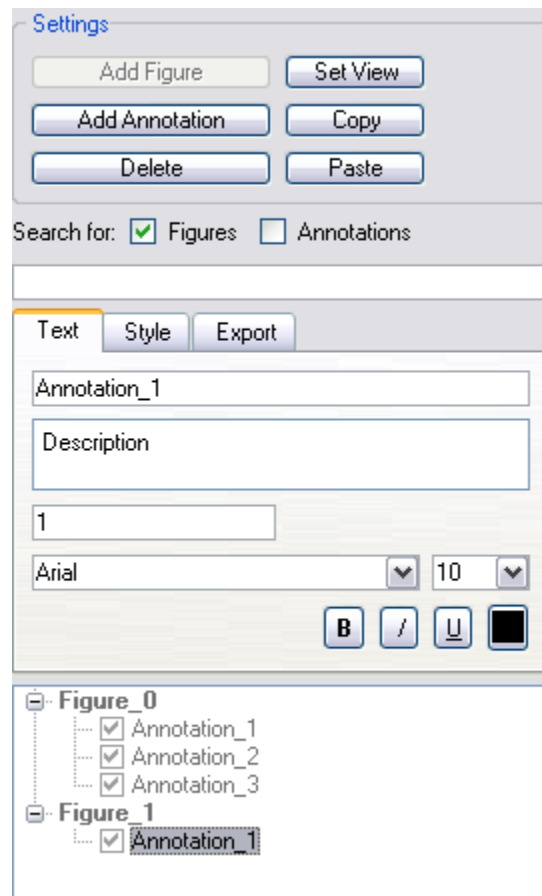
The annotation can be hidden by clicking the  on the top right corner of the box or hidden / show by (un) checking the box in front of the annotation in the InPress list view

The annotation number can be moved by putting the mouse over it (until it changes to a cross) then click & drag the annotation number to a new location.

Save Figures and Annotations in Scene File

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

3.5.3 Settings



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

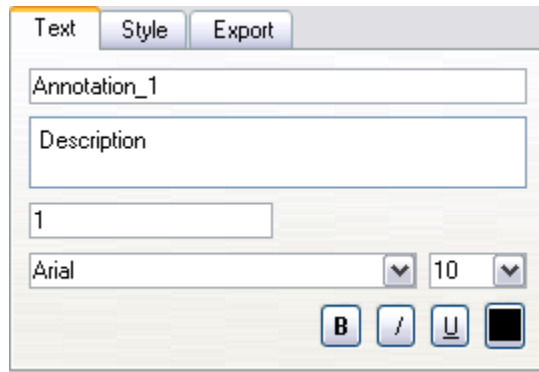
Search for Figures and Annotations

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

3.5.4 Tab Text - Style - Export

Tab Text

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



The top line controls the annotation title field.

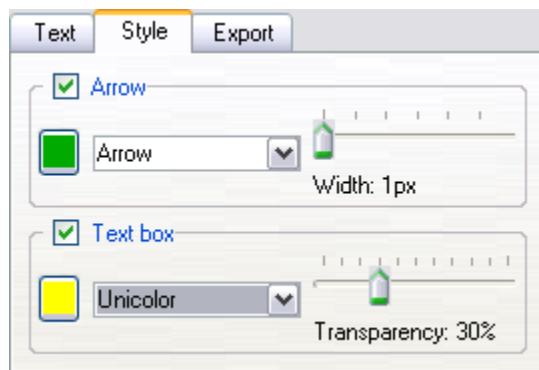
The second line controls the description field (text).

The third box by default contains the annotation running number (legend). To remove the number from the image, delete it from the text field

The font, font size, and color can be selected (from any installed system fonts). The settings for the different rows (title field, text field, and legend field) can be adjusted separately. To display the default settings click on the menu Edit - Preferences... and select [InPress](#).

Tab Style

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



The first check-box turns on or off the Arrow.

With a click on the color selection you can change the color of the graphical element. Click it to change the pointer to any desired color.

In the drop-down list you select the shape of the pointer. The pointer can have three possibilities; a sphere, an arrow, or a magnifier.

The slider controls the size of the line and the pointer.

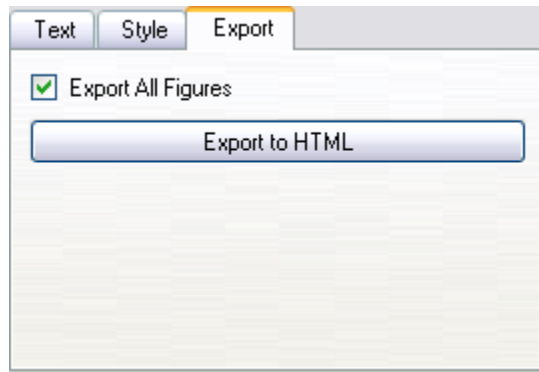
The second check-box turns on or off the Text box.

With a click on the color selection you can change the color of the text box.

The pointer can be colored in three ways; uni-color, black text area, or a black title area.

The slider controls transparency of the text box.

Tab Export



Check the check-box if you want to export all figures.

Highlight the figure in the InPress Figure Tree that you want to Export.

With a click on the button **Export to HTML** you open the Export Figure as HTML window.

Choose the desired file path, enter a file name to save the document.

A web page is created that contains the image name and location, each channel name, the number of time points, the image size, the number of slices, the voxel sizes, a snapshot of the view that is associated with the figure, and the figure name.

3.6 Add Time Points...

Additional time points can be added to the data set.

Select the required image and click **Open**.

The time points to be added must contain the same number of channels and be of the same format as the current data set. A warning message appears if the formatting does not match.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.7 Delete Time Points...

Superfluous time points can be deleted.

Parameters

Delete From, To

Specify the time points to be deleted and click **OK**. The selected time points are automatically deleted from the data set.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.8 Add Channels...

Adding channels is used to extend or complete the data set information if a microscope can only export one channel as a file at one time. All channels of a selected data set are added. The size of the data sets must be identical.

Window: Add Channels

Select the requested file and click **Open**.

All channels from this data set are added automatically. If the size of the data set does not match a warning message is issued.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.9 Delete Channels...

Deleting channels is used to erase unwanted information in a data set.

Select Channels to Delete

Channel 1...n

Select the channel to be deleted in the Delete Channels box and click **OK**. The effect is immediately visible on the image.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - Open - [Reader Configuration](#)

3.10 Add Slices...

Adding slices may become necessary if through manipulation or during file export or formatting the data set consists of less than the originally acquired number of slices.

Window: Add Slices

Select the requested file and click **Open**.

All slices from this data set are added automatically. The x-, and y-values of the two data sets must be identical and the same number of channels is required.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.11 Delete Slices...

Parameters

Slice number [1...n]

Specify the slice to be deleted and click **OK**. The slice is automatically deleted from the data set.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.12 Crop Time...

The total number of time points can be reduced at the beginning or end of the series of images.

Parameters

From, To

Enter the time points to be included in the data set and click **OK**. All other time points are deleted from the data set.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - Open - [Reader Configuration](#)

3.13 Resample Time...

The total number of time points can be reduced to display the images at a faster speed.

Parameters

New Number of Time Points

Specify the number of time points needed and click **OK**.

The process of resampling the time points takes a while. When the process is finished, the time bar displays the new number of time points.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - Open - [Reader Configuration](#)

3.14 Crop 3D...

Cropping the data set allows you to crop the images down to the region of interest. Cropping reduces the size of the data set and makes it easier and faster to handle the viewing and storing of the images.

Preview

Display of a sectional view of the actual image (current time point). A rectangle, representing the region of interest (ROI), is overlaid on all three views.

Select Crop Dimensions

X, Y, Z, From, To, Size

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

To move the ROI, click inside the rectangle, hold the mouse button down & drag the entire ROI around.

To shape the ROI, click on a handle, hold the mouse button it down and reshape the ROI. Side handles affect one direction, corner handles two directions.

The modifications apply to all slices and all time points of the image.

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

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - Open - [Reader Configuration](#)

3.15 Resample 3D...

Resampling reduces the voxel density in a data set to fasten its processing. Reducing the data size also deteriorates the resolution. Resampling reduces the number of voxels in a grid but keeps the original relationship between the voxels.

New Size

X, Y, Z

The fields display the current x-, y-, and z-values. The requested values can be directly entered in the fields.

Aspect Ratio

Fixed Ratio X/Y

Fixed Ratio X/Y/Z

The Aspect Ratio of the data set's dimensions can be kept by checking the respective Fixed Ratio options. Clicking the **OK** button resamples the data set to the entered values.

Use a Gaussian filter as low-pass before sampling down an image.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu File - Open - [Reader Configuration](#)

Menu Image Processing - Smoothing - [Gaussian Filter](#)

3.16 Change Data Type...

Type

From

Displays the current data set type.

To

Drop-down list to select the requested data set type from the supported types:

unsigned 8 bit for the range 0...255.

unsigned 16 bit (0...65535).

32 bit float.

Range Adjustment

Check field to determine how the data values are translated during the change.

None

Data values are imported in the new type.

Source Range to Target Range

Maximum data values are scaled to the new range (e.g., 0...255 to 0...65535).

Data Range to Target Range

Actual data range values are interpolated to the new range (e.g., 0...150 to 0...65535).

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

3.17 Preferences...

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

[System](#)
[Display](#)
[Loading](#)
[Calculation](#)
[Time](#)
[Surpass](#)
[Statistics](#)
[Licences](#)
[Update](#)
[Usage Data](#)
[3D Cursor](#)
[InPress](#)
[Advanced](#)

3.17.1 System

Displays the basic system parameters of your Windows computer.

Processor

Number of Processor
Processor Architecture
Processor Speed

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

Graphics

OpenGL Renderer
OpenGL Version
Pixel Shader
OpenGL Extensions

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

Operating System

OS
Version (Build)
Service Pack

Information about the operating system, the installed Version and the service pack.

Memory Status

Physical Memory installed
Physical Memory available

Information about the available amount of internal memory.

Window: Hardware Settings

Data Cache

Memory Limit

The memory limit defines how much RAM memory Imaris can use before caching on the disk starts. The value must stay below the total amount of installed RAM on the system to work properly. 32-bit systems can

not handle more than 2-3 GB per application.

Display

Texture Cache Limit

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

Open GL Test

The result of the open GL test displays on the right hand side.

Congratulations, your graphics board is able to display huge data.

Your graphics board is not capable of displaying huge data. Some features will be unavailable.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.2 Display

Select the viewing properties and the basic colors for the backgrounds and selection in the gallery.

Display

Interpolate

If checked, the images are automatically interpolated for a smoother display.

Texture Cache Limit (MB)

Before displaying any image data, Imaris converts the data into a configuration (called textures) that is optimized by the graphics hardware. The value of the Texture Cache Limit determines how many textures can be stored in RAM. Set the value to the memory of your graphics card.

Colors

Background Color

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

Button **Select...**

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

Background Color 2

Background marking the original position if an image is moved.

Button **Select...**

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

Checkered Background for Blending

When using the blending mode, a checkered background displays in Full 3D blend and in Surpass.

Tile Size

Allows definition of the tile size for a checkered background.

Linear Color Progress for Blending

The background displays a color gradient in blend progress projections and in Surpass.

Selection Color

Color of selection frame and drawing lines in contour surfaces.

Button **Select...**

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

Measurement Color

Color of measurements points and lines visible in the image (in the Slice view and the Surpass view).

Button **Select...**

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

Coordinate Axis/Scale Bar

Show Coordinates Axis

Show Date

Show Scale Bar

Show Time

Show relative Time

Select to display coordinate axis, date, scale bar or time on screen in Slider, Section, or Gallery view.

Off Screen Rendering (for saving Snapshots and Movies)

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

Show System Monitors

Check this box to display the system monitors in the Status Bar at the bottom of the screen. The first window displays the "reads per sec", the second the "writes per sec", the third the "write requests in queue" and the last the "percentage read cache hits". These are useful information especially if you work with huge data sets.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Menu File - [Snapshot](#)

3.17.3 Loading

Allows you to select the color assignment method used when loading data sets and to define the default colors. Images in Imaris format will display in the colors defined in the image file.

Take Colors from:

Default Colors

Use the default color selection to display the loaded data set. The parameter set Default Colors (see below) is available.

File Colors (color table or base color if available, otherwise default colors)

Use the original color definition of the loaded data set (usually stored in a lookup table). The parameter set Default Colors (see below) is not available.

Emission Wavelength (from file if available, otherwise default colors)

Use the color according to the emitted wavelength from the file (corresponds to the appearance under the microscope). The parameter set Default Colors (see below) is not available.

Please note that not all file formats support lookup tables and emission wavelength.

Default Colors

The parameter set Default Colors is available, if you select Default Colors in the parameter set Take Colors from (see above).

First Cannel

Second Channel

Third Channel

Other Channels

Display of the defined color.

Button **Select...**

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

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.4 Calculation

Calculation

Number of Processors

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

Image Processing History

Maximum Number of Commands in History

Defines the maximum number of image processing operations that can be undone. Each level of image processing requires an additional copy of the full image in memory. If your machine runs out of memory, set Maximum Number of Commands in History to 1.

Data Cache

Imaris uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

Memory Limit (MB)

The value of data cache limit controls the amount of data blocks Imaris will keep in memory at any time. Enter a value based on the following table.

PC

32 bit	Physical memory installed x 0,5; but not higher than 1.2 GB
64 bit	Physical memory installed x 0,5

Mac

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

Button **Apply**

Press this button to apply the changes.

Cache File Paths:

Display of the cache file paths.

Button **Add**

Button **Remove**

You can use the buttons to add or remove file paths in the list.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.5 Time

These are the default parameter settings for the Time Bar.

Play Back

Specify the play back mode for the Time Bar.

Play One Time

All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever

Once the play back has reached the last time point, it starts at the first time point again (never ending).

Swing Back and Forth Forever

When the last time point is reached, the time sequence is shown in reverse until the first time point is reached.

Frame Rate: ... Frames per Second

You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Toolbars - [Time Bar](#)

3.17.6 Surpass

Object Creation Buttons

Check the icons to be displayed on the Objects toolbar in the Surpass View.

Button **Move Up**

Button **Move Down**

Highlight an icon and click Move Up or Move Down to define the order of the icons in the Objects toolbar.

Key Frame Interpolation

Object Rotation Center (optimizing default user interaction)

This is the default parameter, select Object Rotation Center to create a rotated animation. The distance from the camera to the object rotation center is always the same.

Camera Rotation Center (optimizing fly through animation)

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

Key Frame Animation

Specify the play back mode for the Key Frame Animation.

Play One Time

All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever

Once the play back has reached the last time point, it starts at the first time point again (never ending).

Frame Rate ... Frames per Second

You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Surpass View - Overview - [Surpass Tree](#) (Objects toolbar)

3.17.7 Statistics

Show Statistic Values

The most desired set of statistics values can be specified (for display, export to MS Excel, or sorting). Check the values to be displayed when you open the tab Statistics in the Surpass View.

Show All

Check this box and all statistical values are selected. Un-check the box and all statistical values are un-selected.

For details please refer to the respective chapter:

Menu Edit - Preferences ... - Statistics - [Filament](#)

Menu Edit - Preferences ... - Statistics - [Measurement Points](#)

Menu Edit - Preferences ... - Statistics - [Spots](#)

Menu Edit - Preferences ... - Statistics - [Surfaces](#)

Menu Edit - Preferences ... - Statistics - [Volume](#)

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Surpass View - Overview - [Properties Area](#) (Tab Statistics)

Coloc - [Volume Statistics](#)

3.17.7.1 Filament

Statistic Values - Filament

In Figure 1, the presentation of the dendritic tree is simplified, by (•) representing a dendritic vertex, and (x) a spine vertex. Dendrite and Spine edges are represented with (—) or (---) respectively. Dendrite segments are noted with D and spine segments with S.

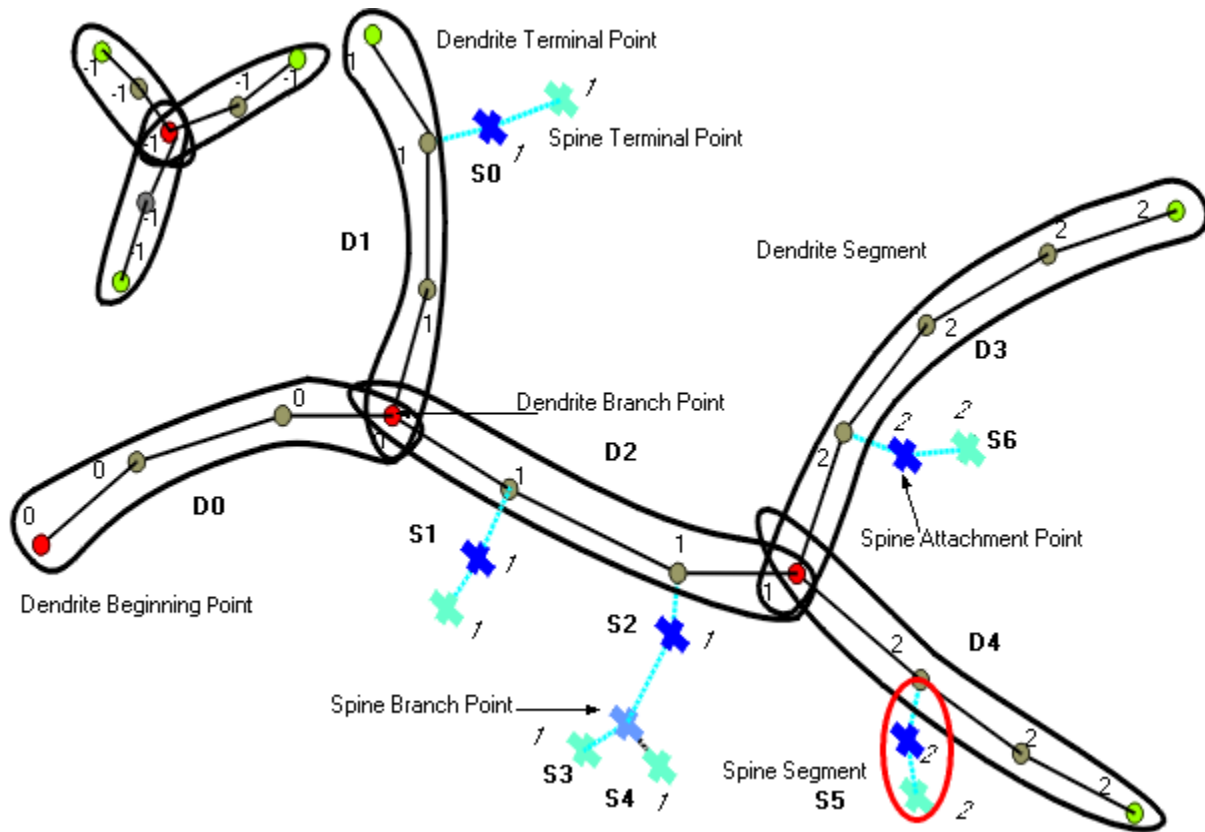


Figure 1

Filament - Dendrite Area

Defined as the sum of the areas of all the segment edges.

The area of an edge is defined as a surface area of a frustum (truncated cone).

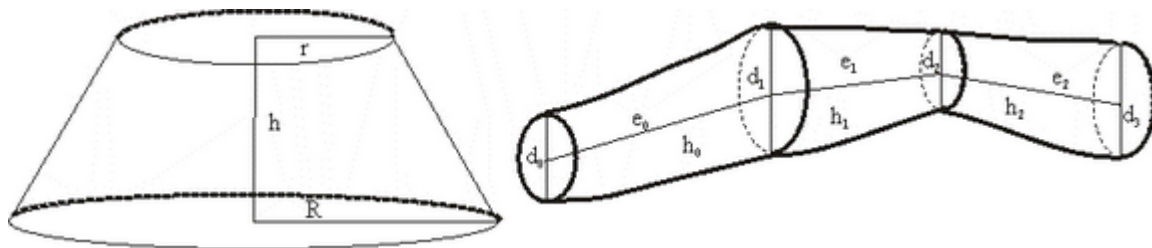


Figure 2

$$A(i) = (r(i) + r(i+1)) * \sqrt{((r(i) - r(i+1)))^2 + h(i)^2}$$

$$A = \sum A(i), \quad i = 0, \dots, n-1, n$$

n = number of edges of a segment

Filament - Dendrite Average Diameter

Defined as the mean value of the diameters measured at each vertex of the dendrite middle axis (in Figure 2 $(d_0 + d_1 + d_2 + d_3 + d_4)/5$.)

Filament - Dendrite Branch Depth

Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the dendritic graph.

Filament - Dendrite Branch Level

The Dendrite Branch Level is a numerical structure that starts unfolding from the filament beginning toward the terminal points, assigning Branch Level to dendrite segments at each branching point.

The Branch Level number is assessed according to the diameter calculations of the individual dendrite segments. The initial Branch Level at the beginning point is 1. At each branching point, the dendrite segment with smaller mean diameter sequentially increases Branch Level, while the dendrite segment with a greater diameter maintains the same Branch Level.

In the case of two dendrite segments with the same diameter, the segment with a smaller Branching Angle (refer to Figure 4) keeps the Branch Level, while the dendrite segment with a greater Branching Angle sequentially raises its Branch Level.

In the Figure 3 diameter d_1 is greater than d_2 ($d_1 > d_2$) and d_3 and d_4 are equal ($d_3 = d_4$). The Dendrite Branching Angle α_1 is smaller than α_2 ($\alpha_1 < \alpha_2$).

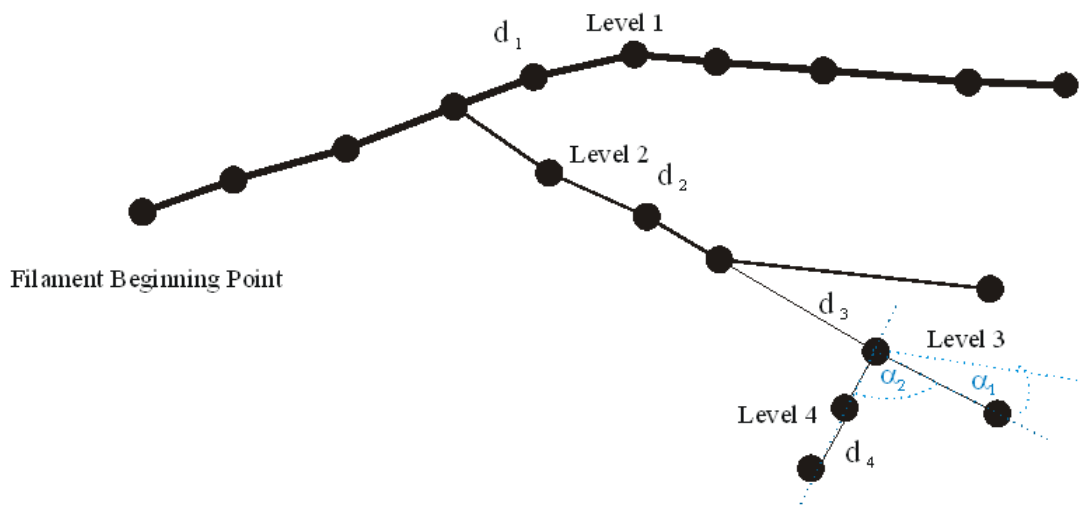


Figure 3

Filament - Dendrite Branching Angle

Defined as the angle between the extending lines from the branch point and its peripheral neighbor (Figure 4 angles marked with dashed blue arcs, α). Defined only if beginning point is determined.

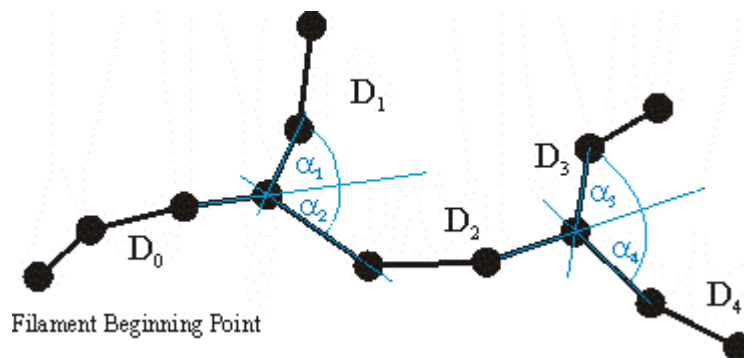


Figure 4

Filament - Dendrite Branching Angle B

The angle between the extending lines connecting the branch point with the neighboring branch points and the terminal points, respectively (Figure 5 angles marked with dashed green arcs, β). Defined only if beginning point is determined.

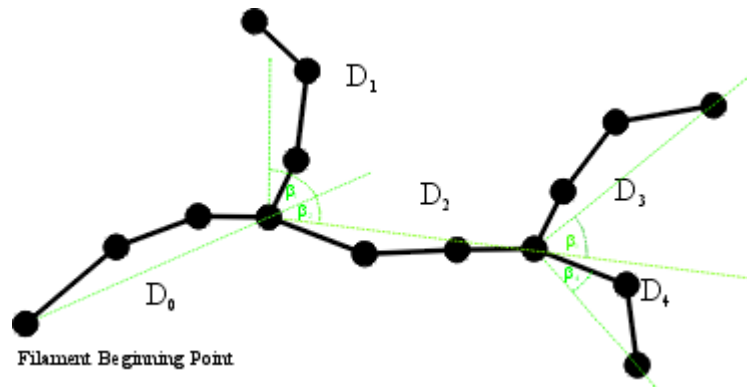


Figure 5

Filament -Dendrite Length

Defined as the sum of all edges between two branch points or between a branch point and a terminal point, respectively. (Figure 2 Dendrite Length $\rightarrow e_0 + e_1 + e_2$).

Filament -Dendrite Length (sum)

The sum of the length of all dendrites in the entire dendritic graph.

Filament -Dendrite Mean Diameter

The mean value of all dendrites diameters measured from the center to the dendrite surfaces using the lower threshold (automatic creation) value.

Filament -Dendrite No of Spines

The number of spines branching off a dendrite segment (in Figure 1 $D_0 \rightarrow 0$, $D_1 \rightarrow 1$, $D_2 \rightarrow 2$, $D_3 \rightarrow 1$, $D_4 \rightarrow 1$)

Filament - Dendrite Orientation Angle

The angle formed between extending line connecting distal vertices of the dendrite segment and X-axis of image within the XY plane (Figure 6 angles marked with a dashed orange arcs, γ).

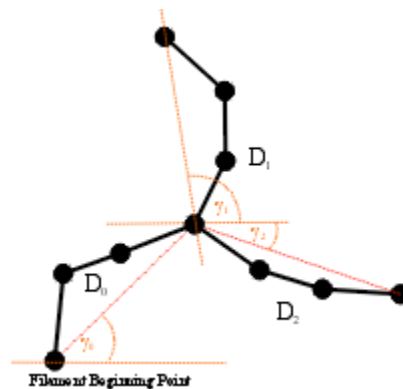


Figure 6

Filament - Dendrite Resistance

Measure of a dendrite ability to conduct an electric current. The dendrite resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account diameter variations. It has units of 1/mm and must be multiplied by specific material resistance in order to obtain a value of a resistor.

Filament - Dendrite Spine Density

Defined as the number of spines per unit length of dendrite.

Filament - Dendrite Straightness

Defined as the ratio between dendrite length and radial distance between two branch points (h) (Figure 7 value is always smaller than 1. If the Dendrite Straightness is 1 that means that the dendrite is completely straight).

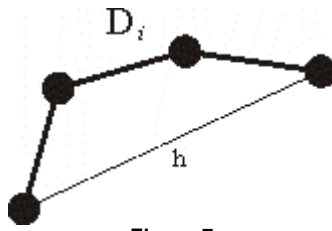


Figure 7

Filament - Dendrite Volume

Defined as the sum of all (frustum) volumes composing a dendrite (Figure 2).

$$V(i) = \left(\frac{\pi}{3} \right) * h(i) * (r(i)^2 + r(i) * r(i+1) + r(i+1)^2)$$

$$V = \sum V(i) \quad i = 0, \dots, n-1, n$$

n = number of edges of a segment

Filament - Full Branch Depth (sum)

Branch Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the dendritic graph. Full Branch Depth is the highest value of Branching Depth for the dendritic graph (in Figure 1→2). If no beginning point is defined, the value is set to "-1" (Figure 1).

Filament - Full Branch Level

Full Branch Level is the highest value of Branching Level for the dendritic graph (in Figure 3→4).

Filament - Length (Sum)

Defined as the sum of the lengths of all edges (both dendrites and spines) within the entire dendritic graph.

Filament - No. Dendrite Branch Points

The number of branching points in the entire dendritic graph (in Figure 1→3).

Filament - No. Dendrite Segments

The number of dendrite segments in the entire dendritic graph (Figure 1→8).

Filament - No. Dendrites Branches

The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments. In Figure 8 the Number of Dendrite Branches are indicated with Roman numerals, distance increments are represented with a green dashed line and the lower part illustrates the Number of Dendrite Branches in relation to distance from the beginning point.

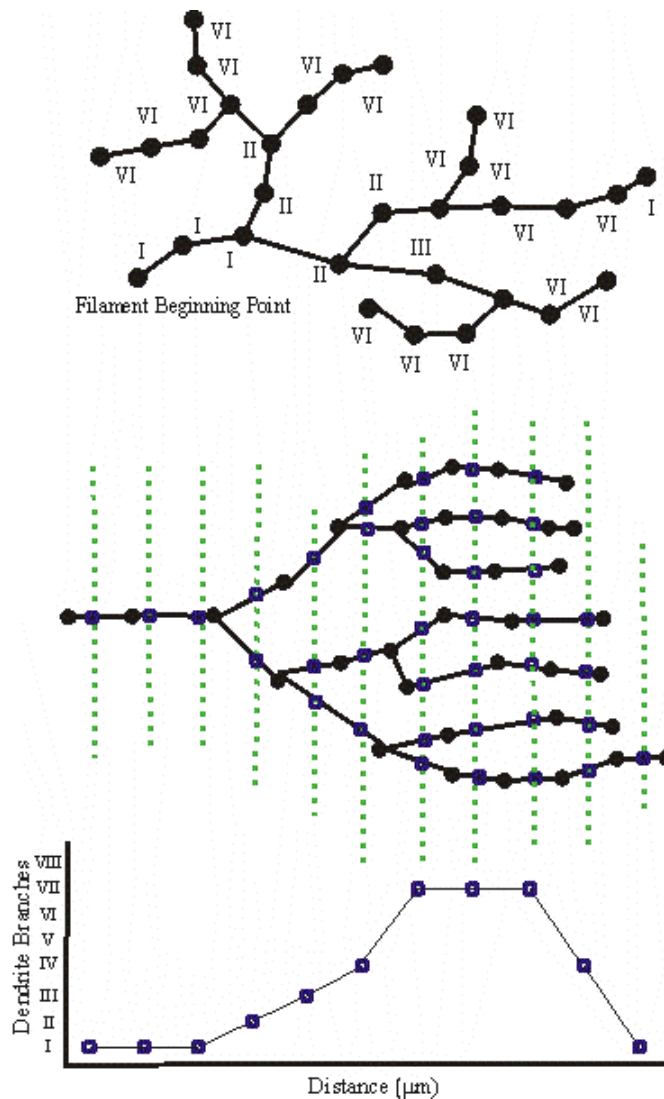


Figure 8

Filament - No. Dendrite Terminal Points

The number of dendrite terminal points (excluding the beginning point) in the entire dendritic graph (Figure 1 → 6).

Filament - No. Edges

The number of connections between vertices in the entire dendritic graph (Figure 1 → 34).

Filament - No. Spine Branching Points

The number of spine branch points in the entire dendritic graph (Figure 1 → 1). A spine branch point will only exist in a single spine that has more than one terminal point.

Filament - No. Spine Segments

The spine segment count in entire dendritic graph (Figure 1 → 7).

Filament - No. Spine Terminal Points

The number of spine terminal points in the entire dendritic graph (Figure 1 → 6).

Filament - No. Unconnected Parts

The number of unconnected graphs in the scene (Figure 1 → 2).

Filament - No. Vertices

The number of vertices in the entire graph (Figure 1 → 36).

Filament - Pt Branching Angle

The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 9 a angles are marked with δ). This value is defined only for graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the branching segments (in Figure 9 b angles marked with δ_2). The value is not calculated for branch points with more than three segments.

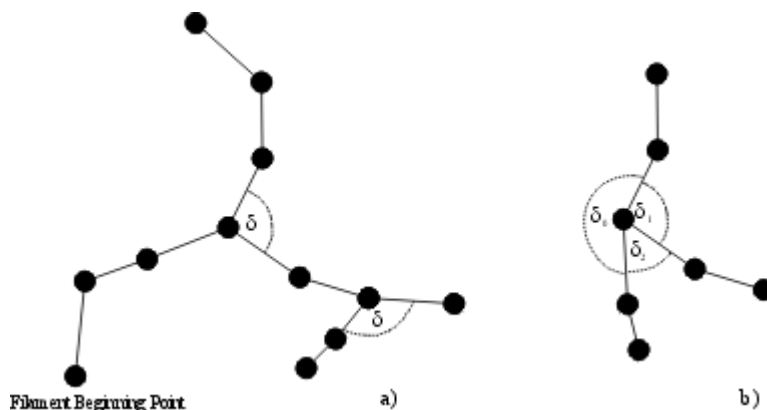


Figure 9

Filament - Pt Diameter

Defined as the value of the vertex's diameter (Figure 2).

Filament - Pt Distance

Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 8).

Filament - No. Scholl Intersections

Defined as the number of dendrite intersections (branches) on concentric spheres, defining dendrite spatial distribution as a function of distance from the beginning point. All spheres have a center at the beginning point (Figure 10). The 1.0 μm step resolution for the spheres can be changed under Preferences -> Advanced -> SurfaceAppFilament

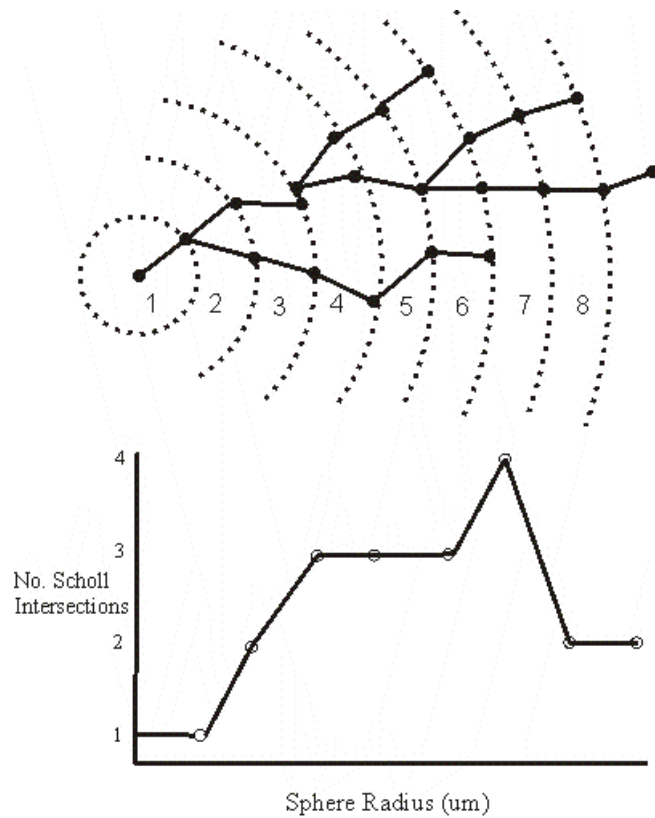


Figure 10

Filament - Spine Area

The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).

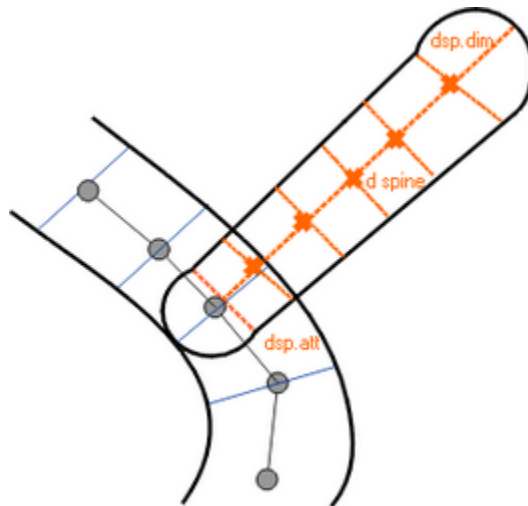


Figure 11

Filament - Spine Attachments Pt Diameter

Diameter of a vertex at the spine attachment point to the dendrite (Figure 11 dsp).

Filament - Spine Branch Depth

Spine Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the spine attachment point to a given point in the dendritic graph.

Filament - Spine Branch Level

A Spine inherits the Dendrite Branch Level from the dendrite segment that it is attached to.

Filament - Spine Avg Diameter

The mean value of spine diameters including attachment point diameter (Figure 11, average of dspine and all dsp.att).

Filament - Spine Branching

The number of spine branching points in a spine segment. The value is typically 0 or 1 (Figure 12 for S_0 0, S_1 1, S_2 0 and S_3 0).

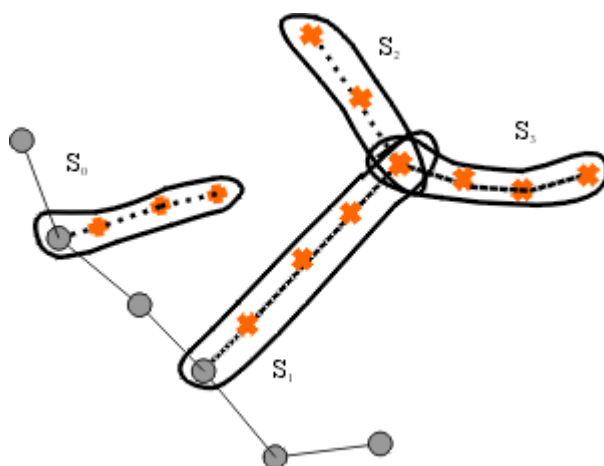


Figure 12

Filament - Spine Length

The Spine length is calculated by subtracting the sphere radius at spine the attachment point (Figure 11 d sp.att) from the sum of all spine segments lengths (esp) and radius of the spine terminal point (Figure 11).

Filament - Spine Length sum

The sum of the length of all spines in the entire dendritic graph.

Filament - Spine Orientation Angle

The angle formed between the extending line connecting distal spine vertices in the spine segment and X-axis of the image within the XY plane (defined in the same way as Dendrite Orientation Angle, refer to Figure 6).

Filament - Spine Resistance

Measure of a spine's ability to conduct an electric current. The spine's resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account the diameter variations. It has units of $1/\mu\text{m}$ and must be multiplied by specific material resistance in order to obtain a value of resistor (defined in the same way as Dendrite Resistance).

Filament - Spine Straightness

Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Dendrite Straightness, refer to Figure 7).

Filament - Spine Terminal Point Diameter

Diameter of a vertex at the spine terminal point (Figure 11 dsp.ter.).

Filament - Spine Volume

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

See also:

Surpass View - Filament - [Tab Statistics](#)

3.17.7.2 Measurement Points

Measurement Point statistics are automatically computed for each Measurement Point.

Show Statistic Values - Measurement Points

Measurement Points - Distance

Distance between the measurement points.

Measurement Points - Distance Sum

Sum of all measured distances.

Measurement Points - Number of Points

Number of measurement points.

Measurement Points - Point Intensity Center

Value of the point intensity center (average) in the different channels.

Measurement Points - Point Position X

Point position in x-axis.

Measurement Points - Point Position Y

Point position in y-axis.

Measurement Points - Point Position Z

Point position in z-axis.

See also:

Surpass View - Measurement Points - [Tab Statistics](#)

3.17.7.3 Spots

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

Show Statistic Values - Spots

Spots - Area

The sum of the triangle surfaces.

Spots - Diameter

The spots diameter.

Spots - Intensity Center

Intensity of the voxel in the Spot(s) center.

Spots - Intensity Max

Spots - Intensity Mean

Spots - Intensity Median

Spots - Intensity Min

Spots - Intensity StdDev

Spots - Intensity Sum

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

Spots - Number of Points per Time Point

Number of points per time point.

Spots - Number of Tracks

Number of tracks.

Spots - Number of Voxels

Number of the Spot(s) voxels.

Spots - Position X**Spots - Position Y****Spots - Position Z**

Spots position (x y z).

Spots - Speed

The spots speed is the instantaneous speed of the object (um/s).

$$S(t) = \frac{\sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}}{T(t) - T(t-1)}$$

S(t) = TrackSpeed

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

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

$T(t)$ = time in seconds at time point t

Spots - Time Index

The spots time index.

Spots - Total Number of Points

The total number of points.

Spots - Track AR1Mean

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Spots - Track AR1 X

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

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

AR1X = TrackAR1X

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

t_L = last time index of track

t_f = first time index of track

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

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

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

t_L = last time index of track

t_F = first time index of track

Spots - Track AR1 Y

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

Spots - Track AR1 Z

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

Spots - Track Area Mean

The mean track area.

Spots - Track Diameter Mean

The mean diameter of all spots along the track.

Spots - Track Displacement

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

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

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

D = TrackDisplacement

t_L = last time index of track

t_F = first time index of track

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

Spots - Track Duration

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

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

Duration = TrackDuration

$T(t)$ = time in seconds at time point t

t_L = last time index of track

t_F = first time index of track

Spots - Track Intensity Center Mean

The mean center intensity of all spots along the track.

Spots - Track Intensity Max

The max intensity of all spots along the track.

Spots - Track Intensity Mean

The mean intensity of all spots along the track.

Spots - Track Intensity Median

The median track intensity of all spots along the track.

Spots - Track Intensity Min

The min track intensity of all spots along the track.

Spots - Track Intensity StdDev

The standard deviation track intensity of all spots along the track.

Spots - Track Intensity Sum

The sum of the track intensity of all spots along the track.

Spots - Track Length

The Track Length is the total length of displacements within the track.

$$L = \sum_{t=t_f+1}^{t_L} \sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}$$

L = TrackLength

t_L = last time index of track

t_f = first time index of track

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

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

Spots - Track Number of Branches

The number of track branches.

Spots - Track Number of Fusions

The number of track fusions.

Spots - Track Number of Points

The number of track points.

Spots - Track Speed Max

The maximum value of the object's speed on the track.

Spots - Track Speed Mean

The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Spots - Track Speed Min

The minimum value of the object's speed on the track.

Spots - Track Speed Standard Deviation

The Standard Deviation value of the object's speed on the track.

Spots -Track Speed Variation

The parameter is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

σ - Standard Deviation

μ -Mean value

Spots - Track Straightness

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

Straightness = TrackStraightness
 Displacement = TrackDisplacement
 Length = TrackLength

Spots - Track Volume Mean

The mean track volume.

Spots - Volume

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

See also:

Addendum - [Terminology](#) (Standard Deviation)

3.17.7.4 Surfaces

Surfaces statistics are automatically computed for each Surface.

Show Statistic Values - Surfaces

Surfaces - Area

The sum of the triangle surfaces.

http://en.wikipedia.org/wiki/Moment_of_inertia#Principal_moments_of_inertia

Surfaces - Center of Homogeneous Mass X

Surfaces - Center of Homogeneous Mass Y

Surfaces - Center of Homogeneous Mass Z

Center of homogeneous Mass (voxel intensities are equal):

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

R : center of homogeneous mass (x y z)

r_i : center of a voxel (x_i y_i z_i)

n : number of i Surfaces

Surfaces - Center of Image Mass X

Surfaces - Center of Image Mass Y

Surfaces - Center of Image Mass Z

Center of Image Mass (the voxel intensities are not equal):

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

R : center of image mass (x y z)

m_i : voxel intensity

r_i : center of a voxel (x_i y_i z_i)

M : sum of voxel intensities

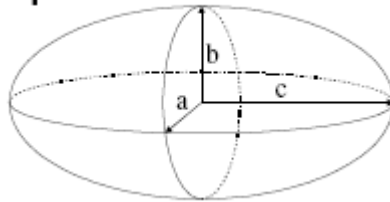
Ellipsoid Axis

In mathematics, an Ellipsoid is a type of quadric that is a higher dimensional analogue of an Ellipse. The equation of a standard Ellipsoid in an x-y-z Cartesian coordinate system is:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$

where a, b and c (the lengths of the three semi-axes) are fixed positive real numbers determining the shape of the Ellipsoid.

Ellipsoid



Surfaces - Ellipsoid Axis A X

Surfaces - Ellipsoid Axis A Y

Surfaces - Ellipsoid Axis A Z

These three parameters define the Vector of the Ellipsoid Axis a.

Surfaces - Ellipsoid Axis B X

Surfaces - Ellipsoid Axis B Y

Surfaces - Ellipsoid Axis B Z

These three parameters define the Vector of the Ellipsoid Axis b.

Surfaces - Ellipsoid Axis C X

Surfaces - Ellipsoid Axis C Y

Surfaces - Ellipsoid Axis C Z

These three parameters define the Vector of the Ellipsoid Axis c.

Surfaces - Ellipsoid Axis Length A

Surfaces - Ellipsoid Axis Length B

Surfaces - Ellipsoid Axis Length C

Surfaces - Ellipsoid (oblate)

Surfaces - Ellipsoid (prolate)

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

$a = 0$ it is an **Ellipse**

$a = b = c$ it is a **Sphere** (three equal sides)

$a \neq b \neq c$ it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

$a = b < c$ it is a prolate Spheroid (cigar-shaped)

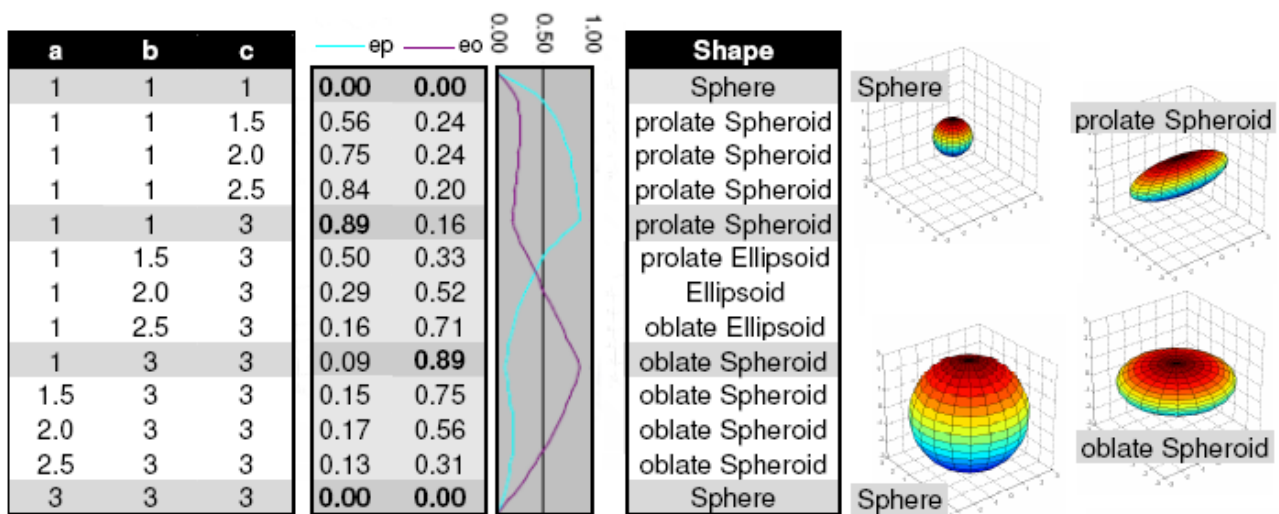
$a < b = c$ it is an oblate Spheroid (disk-shaped)

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

$e_{prolate}$ = prolate Ellipsoid

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

e_{oblate} = oblate Ellipsoid



Surfaces - Intensity Center

Surfaces - Intensity Max

Surfaces - Intensity Mean

Surfaces - Intensity Median

Surfaces - Intensity Min

Surfaces - Intensity StdDev

Surfaces - Intensity Sum

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

Surface- Number of Disconnected Components

The number of disconnected surfaces in the selected Surface object .

Surface- Number of Disconnected Components

Total number of created disconnected surfaces.

Surface- Number of Disconnected Components per time point

Total number of created disconnected surfaces in the selected time point.

Surfaces - Number of Surfaces per Time Point

Number of Surfaces per actual Time Point.

Surfaces - Number of Tracks

Number of tracks.

Surfaces - Number of Triangles

Number of the Surface object triangles.

Surfaces - Number of Vertices

Number of the Surface object vertices.

Surfaces - Number of Voxels

Number of the Surface object voxels.

Surface Object - Sphericity

Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, Ψ , of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle:

$$\Psi = \frac{\pi^{\frac{1}{3}} (6V_p)^{\frac{2}{3}}}{A_p}$$

V_p = volume of the particle

A_p = surface area of the particle

Surfaces - Time Index

Time index of the Surfaces.

Surfaces - Total Number of Surfaces

Total number of Surfaces.

Surfaces - Total Number of Triangles

Total number of Triangles.

Surfaces - Total Number of Voxels

Total number of Voxels.

Surfaces - Track AR1Mean

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Surfaces - Track AR1 X

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

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

$AR1X = TrackAR1X$

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

t_L = last time index of track

t_f = first time index of track

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

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

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

t_L = last time index of track

t_F = first time index of track

Surfaces - Track AR1 Y

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

Surfaces- Track AR1 Z

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

Surfaces - Track Area Mean

The mean sum of the triangle surfaces of a track.

Surfaces - Track Center of Homogeneous Mass X

Surfaces - Track Center of Homogeneous Mass Y

Surfaces - Track Center of Homogeneous Mass Z

Track center of homogeneous Mass (voxel intensities are equal):

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

R : center of homogeneous mass (x y z)

r_i : center of a voxel (x_i y_i z_i)

n : number of i Surfaces

Surfaces - Track Center of Image Mass X

Surfaces - Track Center of Image Mass Y

Surfaces - Track Center of Image Mass Z

Track center of Image Mass (the voxel intensities are not equal):

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

R : center of image mass (x y z)

m_i : voxel intensity

r_i : center of a voxel (x_i y_i z_i)

M : sum of voxel intensities

Surfaces - Track Displacement

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

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

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

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

Surfaces - Track - Track Displacement^2

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

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

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

Surfaces - Track Duration

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

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

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

Surfaces - Track Ellipsoid Axis AX Mean

Surfaces - Track Ellipsoid Axis AY Mean

Surfaces - Track Ellipsoid Axis AZ Mean

These three parameters define the Vector of the track Ellipsoid Axis a.

Surfaces - Track Ellipsoid Axis BX Mean

Surfaces - Track Ellipsoid Axis BY Mean

Surfaces - Track Ellipsoid Axis BZ Mean

These three parameters define the Vector of the track Ellipsoid Axis b.

Surfaces - Track Ellipsoid Axis CX Mean

Surfaces - Track Ellipsoid Axis CY Mean

Surfaces - Track Ellipsoid Axis CZ Mean

These three parameters define the Vector of the track Ellipsoid Axis c.

Surfaces - Track Ellipsoid Axis Length A Mean

Surfaces - Track Ellipsoid Axis Length B Mean

Surfaces - Track Ellipsoid Axis Length C Mean

These three parameters define the length of the Ellipsoid Axis a, b, and c.

Surfaces - Track Ellipsoid Oblate A Mean

This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Surfaces - Track Ellipsoid Prolate A Mean

This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Surfaces - Track Intensity Center Mean

This value describes the mean voxel intensity in the center of the Surfaces.

Surfaces - Track Intensity Max

This value describes the maximum voxel intensity of the Surfaces.

Surfaces - Track Intensity Mean

This value describes the mean voxel intensity of the Surfaces.

Surfaces - Track Intensity Median

This value describes the median voxel intensity of the Surfaces.

Surfaces - Track Intensity Min

This value describes the minimum voxel intensity of the Surfaces.

Surfaces - Track Intensity StdDev

This value describes the Standard Deviation voxel intensity of the Surfaces.

Surfaces - Track Intensity Sum

This value describes the sum of voxel intensity of the Surfaces.

Surfaces - Track Length

The Track Length is the total length of displacements within the Track.

$$L = \sum_{t=t_f+1}^{t_L} \sqrt{D_x(t, t-1)^2 + D_Y(t, t-1)^2 + D_Z(t, t-1)^2}$$

L = TrackLength

t_L = last time index of track

t_f = first time index of track

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

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

Surfaces - Track Number of Branches

A branch occurs when a Surface object is connected to several other Surface objects of a future time point.

Surfaces - Track Number of Fusions

A fusion occurs when a Surface object is connected to several Surface objects of a past time point.

Surfaces - Track Number of Surfaces

Number of Surfaces.

Surfaces - Track Number of Triangels

Number of Triangles.

Surfaces - Track Number of Voxels

Number of Voxels.

Surfaces - Track Speed

The Track Speed is the instantaneous speed of the object.

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

S(t) = TrackSpeed

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

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

$T(t)$ = time in seconds at time point t

Surfaces - Track Speed Avg.

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

Surfaces - Track Speed Variability

Standard Deviation of TrackSpeed.

Surfaces - Track Sphericity Mean

Describes the mean Track Sphericity.

Surfaces - Track Straightness

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

Straightness = TrackStraightness

Displacement = TrackDisplacement

Length = TrackLength

Surfaces - Track Volume Mean

Describes the mean Track Volume.

Surfaces - Volume

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

See also:

Surpass View - Surfaces - Tab Statistics

3.17.7.5 Volume

Volume statistics are automatically computed for each Volume object.

Show Statistic Values - Volume

Volume - Data Intensity Mean

Volume - Data Intensity StdDev

Volume - Data Intensity Sum

The voxel intensity statistics describe the voxels of the whole dataset (Mean, StdDev and Sum).

Volume - Data Volume

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

Volume - Number of Voxels

Number of the Volume voxels.

See also:

Surpass View - Volume - [Tab Statistics](#)

Addendum - [Terminology](#) (Standard Deviation)

3.17.8 Licenses

Here you can adjust the default license settings.

Available Licenses/Licenses Found

Feature Licenses

Display of all existing Imaris licenses on the system. If a particular license is currently not available (another computer is probably using it), it will be displayed grayed. You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

Check/Uncheck All

Check this box and all available Imaris licenses on the system are selected.

File Reader Licenses

Display of the actual available file reader licenses on the system.

Check/Uncheck All

Check this box and all available file reader licenses on the system are selected.

Information

Information text field how to configure the settings.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Menu Help - [License...](#)

3.17.9 Update

Here you can adjust the default update settings.

How often should be searched for updates?

Daily

Weekly

Never

Select either daily, weekly, or never.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.10 Usage Data

Here you can enable the capturing of data usage .

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.11 3D Cursor

Here you can adjust the default 3D Cursor settings.

3D Cursor

Box
Sphere
Cross
Torus

Here you can select the shape of the 3D Cursor.

Size
Color

Here you can select the size and color of the 3D Cursor.

Animation

This are the default animation parameters for the function InMotion.

Frequency

Adjust the animation frequency.

Amplitude X

Amplitude Y

Adjust the amplitude in x-, and y-direction.

Shear

Rotate

Select shear or rotate as animation mode.

Continuous

Discrete

Select continuous or discrete as animation sequence.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

[InMotion](#)

3.17.12 InPress

Here you can adjust the default InPress settings.

Format

Title Format

Text Format

Legend Format

Choose title format, text format or legend format and adjust the following parameter set.

Title, Text or Legend

Font

Font Size**Color****Format****Bold****Italic****Underlined**

The font, font size, color, and format can be selected (from any installed system font).

Button **Select...**

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

Style**Arrow Color****Arrow Width****Arrow Style****Text Box Color****Text Box Transparency****Text Box Style**

You can adjust the arrow color, width and style (Sphere, Arrow, Magnifier), text box color, transparency and style (Unicolor, Black Text, Black Title).

Button **Select...**

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

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.17.13 Advanced

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

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

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

3.18 File Type Associations...

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

File Extensions

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

Please note: The feature of the thumbnail extractor is not available for the Macintosh users.

.ims

Imaris Series, Imaris Classic

.imx

Imaris Scene

.ics .ids

Nikon ICS

.lsm

Zeiss LSM510

.zvi

Zeiss Axiovision

.lei

Leica LCS

.lif

Leica LIF

.pic

Biorad MRC 1024/600

.rbinf

TILLvisION

.stk

MetaMorph STK

.r3d .dv

Delta Vision

.oib .oif

Olympus OIB/OIF

.ipl

Scanalytics IPLab

.ome

Open Microscopy Environment XML

.inf .info

Leica Series

.tif .tiff

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

More

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

2D/3D Indicator

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

Show

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

2D Image (colored square).

2D Time Image (pink square and bars).

3D Image (green cube).

3D Time Image (blue cube and bars).

4 Menu View

Please refer to chapter:

Slice	Ctrl + 1	Slice View
Section	Ctrl + 2	Section View
Gallery	Ctrl + 3	Gallery View
Easy 3D	Ctrl + 4	Easy 3D View
Surpass	Ctrl + 5	Surpass View
Animation	Ctrl + 6	Animation
InMotion	Ctrl + 7	InMotion
Coloc	Ctrl + 8	Coloc

Fit	Ctrl + B
100%	Ctrl + A
Full Screen	F11
Navi	Ctrl + N

[Origin Bottom Left](#)
[Origin Top Left](#)
[Origin Bottom Right](#)
[Origin Top Right](#)

[Status Bar](#)

Toolbars - [Status Bar](#)

[Refresh](#) Ctrl + F

See also:

Addendum - [Mouse & Keyboard Mac](#)

4.1 Fit

Fits the entire scene into the view.

In the Status Bar you find the respective button **Fit**.

See also:

Toolbars - [Status Bar](#)

4.2 100%

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

In the Status Bar you find the respective button **100%**.

See also:

Toolbars - [Status Bar](#)

4.3 Full Screen

Displays the viewing area to full size of the monitor. To return to the standard window, click on the button **Full Screen** in the lower left corner.

In the Status Bar you find the respective button **Full Screen**.

See also:

Toolbars - [Status Bar](#)

4.4 Navi

The Navigation window displays an overview of the data set in the upper right corner of the viewing area. If you move the data set the overview updates in real-time. You can activate the Navigation window individually for each Imaris view.

In the Status Bar you find the respective button **Navi**.

Change Position

In the pointer mode Select you can click & drag the Navigation window to any desired corner.

Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.

Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

See also:

[Section View](#)

Toolbars - [Status Bar](#)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Select)

4.5 Origin

The location of the coordinate system origin can be set to any corner of all the views. It can be set to: Bottom Left (default), Top Left, Bottom Right, or Top Right. A selection is stored and used each time you start Imaris.

4.6 Refresh

Refresh the screen.

5 Menu Image Processing

The Image Processing menu provides a range of functions to change the image data. Frequently used functions are Baseline Subtraction, and Threshold Cutoff from the Image Thresholding submenu or the Gaussian Filter from the Image Smoothing submenu.

Button **Apply**

Button **OK**

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

[Flip...](#)

[Rotate...](#)

[Channel Shift...](#)

[Swap Time and Z](#)

[Swap Time and Channels](#)

[Smoothing](#)

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

[Thresholding](#)

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

[Contrast Range](#)

- Linear Stretch...
- Gamma Correction...
- Invert...
- Normalize Layers...

[AutoDeblur...](#)

5.1 Flip...

The channels can be flipped together or separately.

On the left hand side of the window Flip Channel(s) the actual channel(s) are displayed. Select the channels to be flipped by marking the check-box on the left.

All Channel(s)

Direction X, Y, Z

Check the requested Direction box.

Button **Apply**
Button **OK**
Button **Cancel**

Click the **Apply** button. The effect of the change can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

5.2 Rotate...

Rotate direction applies to all channels together. Single channel application is not possible.

All Channel(s)

Axis X, Y, Z

Enter the requested axis.

Orientation

Counter Clockwise, Clockwise

Select the respective orientation.

Button **Apply**
Button **OK**
Button **Cancel**

Click the **Apply** button. The effect result can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

5.3 Channel Shift...

The Channel Shift function allows to move channels relative to one another.

On the left hand side of the window Channel Shift the actual channel(s) are displayed. Select the channels that should be moved relative to the remaining channels.

Pixel Shift for All Selected Channel(s)

X, Y, Z

The translation is specified in terms of integer numbers of voxels to move along each of the x-, y-, and z-directions.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

5.4 Swap Time and Z

Exchange the t-axis and the z-axis and re-format the image in such a way that the time dimension is mapped onto the z-axis and the z-dimension is mapped onto the t-axis. The section viewer now shows x-t-, and y-t-views.

Start Function

Select in the menu Image Processing - Swap Time and Z.

The z-, and t-axes are exchanged. This allows you to play the images over z and t.

Play the Series

Click on Start (arrow to the right) in the Time Bar to play the series of images along the z-axis.

The Time Bar is visible in all display modes (Slice, Section, Gallery 3D Preview, and Surpass). It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Example:

Your data set has four Time Points, each Time Point has one Slice in z-dimension. Then you swap time and z. The result is a new data set with one Time Point and four Slices in z-dimension.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Toolbars - [Time Bar](#)

5.5 Swap Time and Channels

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

Start Function

Select in the menu Image Processing - Swap Time and Channels.

The t-axes and the channels are exchanged.

Example:

Your data set has four Time Points, each Time Point has one channel. Then you swap time and channel. The result is a new data set with one Time Point and four channels.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Menu Edit - Image Properties - [Channel 1 ... n](#)

Menu Edit - [Show Display Adjustment](#)

5.6 Extract Channel

The Extract Channels compare the color content of an image to reference color information. This function extracts and displays color data from the image referent point and use it as a representative color, for a new color channel creation. The new channel is generated based on the difference in the reference color point and the whole image color values.

The difference relates how closely the color information in the region matches the information represented by the color reference point, assigning the higher intensity for smaller difference. The result of the Extract Channel is a separate channel, which can be processed and viewed as any originally acquired channel in Imaris and used for easier segmentation of similarly colored multi-channel data sets.

- Click on the **Slice** icon in the Main toolbar.
- Change pointer mode from Navigate to **Select**.
- Select the referent color and click on it.
- Click on the Get value from the last measurement point Button displays associated RGB values in the numerical fields.
- Specify Color Difference by selecting the color space (either RGB or Perceptual).
- Select the Apply button. The effect of the change can be seen on the screen.
- When the results are satisfactory, click the OK button.
- The new channel is created and it's a part of the data set.
- To restore the initial image click Cancel.

The new extracted channel can be visualized like any other channel in Imaris by selecting the Edit menu and choosing the option [Display Adjustment Window](#). In the Display Adjustment Window un-check original channels, select the newly created channel and adjust its setting.

Channels

The channels can be selected by clicking on the check box.

Color of Interest

The Color of Interest option allows the reference color point to be present either as separate value for red green and blue or as the intensity value for a selected channel.

The reference color point is shown within the color wheel.

RGB

RGB triple value is displayed for the color reference point.

Intensity

Intensity of the selected channel is displayed for the color reference point.

Get value from the last measurement point Button

Clicking this button calculates the RGB or intensity values according to the selected color reference point.

Color difference

This option defines the color space of a newly created channel.

RGB

The RGB color space is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors.

Perceptual

Implements the color extraction using Lab color space. The Lab color model provides a perceptually uniform color space. A perceptually uniform color space indicates that a change in a color value produces a change of about the same visual importance. Lab color is designed to approximate human vision.

5.7 Smoothing

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

[Gaussian Filter](#)

[Median Filter](#)

5.7.1 Gaussian Filter

User Interface

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

Select the menu Image Processing - Image Smoothing - Gaussian Filter. The Gaussian Filter box is displayed.

Select the channels to be filtered by marking the check-box on the left of the channel.

Enter the requested filter width in the parameter field and click **Apply**. The effect of the change can be seen on the screen.

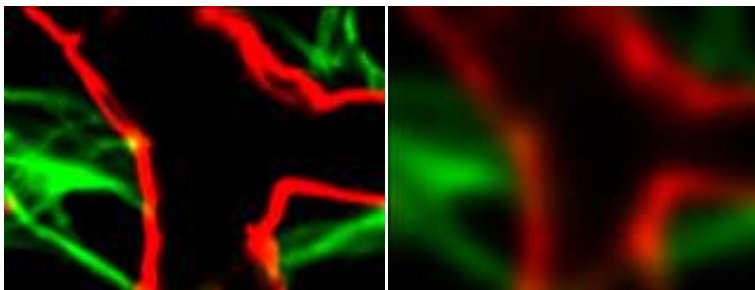
If necessary, readjust the value.

When the results are satisfactory, click the **OK** button.

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

Function

The Gaussian filter smoothes the image.



It has some nice properties:

Total image intensity is preserved.

Noise is suppressed homogeneously.

No artefacts are introduced.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Surpass View - Filament - [Data Input and Filtering](#)

5.7.2 Median Filter

User Interface

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

Select the menu Image Processing - Image Smoothing - Median Filter. The Median Filter box is displayed.

Select the channels to be filtered by marking the check-box.

Check the requested Filter Size and click the **Apply** button. The effect of the change can be seen in the image.

If necessary, readjust the value.

When the results are satisfactory, click the **OK** button.

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

Function

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

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

5.8 Thresholding

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

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

[Threshold Cutoff](#)

[Baseline Subtraction](#)

[Background Subtraction](#)

[Connective Baseline](#)

5.8.1 Threshold Cutoff

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

Select the menu Image Processing - Image Thresholding - Threshold Cutoff. The Threshold Cutoff box is displayed, showing a histogram of the voxels in the data set.

Select the channels to be cut by marking the check-box to the left of the channel bar.

Highlight a channel.

A colored bar in the histogram shows which channel has been selected.

Enter the requested values for that channel in the Threshold field or drag the bar in the histogram.

Repeat for each channel (if applicable).
Click **Apply**. The effect of the changes can be seen in the image.
If necessary, readjust the value.
When the results are satisfactory, click the **OK** button.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

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

5.8.2 Baseline Subtraction

Baseline Subtraction subtracts the baseline value from the intensity of every voxel in the image. If the result is positive, this is the new intensity value, if it is negative, the voxels intensity is set to zero. The Baseline Subtraction values for the channels may differ.

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

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

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

5.8.3 Background Subtraction

Background Subtraction applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background. The filter width is the only parameter of the background subtraction method.

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

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

5.8.4 Connective Baseline

The Connective Baseline works with two threshold values, a lower and an upper threshold. It applies these thresholds to divide the image into regions and then processes each region with a Baseline Subtraction using either the lower or the upper threshold. The three types of regions the method creates are the following:

Connected region of voxels where the intensity of every voxel within the region is below the lower threshold (Type A).

Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and no voxel within the region has an intensity greater than the upper threshold (Type B).

Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and at least one voxel within the region has an intensity greater than the upper threshold (Type C).

The intensities of voxels from regions of type A are set to zero, as are the intensities of voxels from regions of type B. Only voxels from regions of type C retain a nonzero intensity, namely the original intensity less the lower threshold value.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:

Surpass View - Filament - [Data Input](#)

5.9 Contrast Change

Linear Stretch...

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

Select the menu Image Processing - Contrast Change - Linear Stretch. The Linear Stretch box is displayed.

Select the channels to be stretched by marking the check-box.

Enter the requested values in the parameter fields New Maximum and New Minimum and click the **Apply** button. The effect of the change can be seen in the image.

If necessary, readjust the value.

When the results are satisfactory, click the **OK** button.

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

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Gamma Correction...

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

Select the menu Image Processing - Contrast Change - Gamma Correction. The Gamma Correction box is displayed with the default Gamma Value set to 1, therefore showing a straight line as a linear voxel representation.

Select the requested channels by clicking marking the check-box.

Enter the requested value in the Gamma value field or click directly in the gray Gamma Curve area to set the new gamma value point. The curve and the field value are set accordingly and the effect of the change can be seen on the channels (channels appear differently).

Click the **Apply** button

If necessary, readjust the value.

When the results are satisfactory, click the **OK** button.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

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

Invert...

The channels can be inverted together or separately.

Select the menu Image Processing - Contrast Change - Invert. The Invert box is displayed.

Select the channels to be inverted by marking the check-box on the left. The effect of the change can be seen in the image.

When the results are satisfactory, click the **OK** button.

The data set is modified.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

Normalize Layer(s)

This normalization adjusts brightness and contrast of individual z-slices to a uniform level. It is most useful for correcting illumination variations of scans from physical sections, and in some cases it can also reduce attenuation effects from z-stacks.

This image processing function first computes the mean and standard deviation values of the intensity for an entire image z-stack. Then it computes mean and Std.dev. for all individual z-slices. Finally, it adjusts the intensities of each z-slice with a linear transformation, to match the overall mean and Std.dev. value.

This procedure is applied for all z-slices of the dataset, for all time points and for each channel independently.

Voxels with zero intensity are excluded from this calculation - they are interpreted as background.

Select the menu Image Processing - Contrast Change - Normalize.

Select the channels to be inverted by marking the check-box on the left and click on the button **Apply**.

When the results are satisfactory, click the **OK** button.

The data set is modified.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

Histogram Equalization

Histogram equalization is a method of image processing by contrast adjustment using the image's histogram. The method is useful in images with close contrast values; in particular, this method can lead to better views of electron microscopy images.

Through this adjustment, the dynamic range and contrast of an image is altered. The resulting image features the highest possible contrast, uniformly distributed over the whole contrast range.

This method increases the global contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. In histogram equalization, each pixel is assigned a new intensity value based on its previous intensity level so that the output image contains a uniform distribution of intensities. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

Current minimum/maximum

These values define the limits of dataset intensities. Intensities below the Current minimum will be set to the new minimum and intensities above the Current maximum will be set to the new maximum.

New minimum/maximum

These values define the intensity range of the output dataset. By default, they are set to the

minimum/maximum achievable intensities of the data set.

To perform histogram equalization on the data set perform the following steps:

Select the menu Image Processing - Contrast Change - Histogram Equalization.

The Equalize Channel Histogram(s) window is displayed

Select the channels to be processed by marking the check-box

Enter the requested values in the parameter fields Current Minimum/Maximum, New Maximum/Minimum or accept the automatically optimized Min. and Max. limits.

Click the **Apply** button.

If necessary, readjust the value.

When the results are satisfactory, click the **OK** button.

Add to Batch Button

Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

5.10 Auto Deblur ...

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

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

Select the menu Image Processing - AutoDeblur

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

6 Menu Surpass

Please refer to chapter:

Clipping Plane	Surpass View - Clipping Plane
Contour Surface	Surpass View - Contour Surface
External Object	Surpass View - External Object
Filament	Surpass View - Filament
Frame	Surpass View - Frame
Group	Surpass View - Group
Light Source	Surpass View - Light Source
Measurement Point	Surpass View - Measurement Point
Oblique Slicer	Surpass View - Oblique Slicer
Ortho Slicer	Surpass View - Ortho Slicer
Spots	Surpass View - Spots
Surfaces	Surpass View - Surfaces
Volume	Surpass View - Volume

[Export Selected Objects...](#)

[Delete Selected Objects...](#)

Del

[Switch View Mode](#)

Esc

6.1 Export Selected Objects...

In the Surpass Tree highlight all Items to be exported. To export all Surfaces, Contours, and External objects, select theob.

Select menu Surpass - Export Selected Objects... .

The Save selection as External Objects box is displayed.

Select the directory and enter the name for the file to be saved and click **OK**. The object is saved as either as an Inventor file with the extension *.iv or as VRML2 file with extension *.vrmf. The saved object can be loaded as External objects to another data set.

See also:

Surpass View - Overview - [Scene File Concept](#)

Surpass View - [External Object](#)

6.2 Delete Selected Objects ...

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

Select menu Surpass - Delete Selected Objects.

The Delete selection window with a confirmation question is displayed.

See also:

Surpass View - Overview - Surpass Tree - Objects toolbar - [Button Delete ...](#)

Surpass View - Overview - [Scene File Concept](#)

6.3 Switch View Mode

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

Select

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

Navigate

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

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

See also:

Surpass View - Overview - [Camera Toolbar](#) (Pointer Select or Navigate)

7 Menu Help

[Quick Start Tutorials](#)

Ctrl + F1

[Keyboard and Mouse](#)

F1

[Reference Manual](#)

[Programming Interface](#)

[License...](#)

F2

[Check for Updates now...](#) F3

[Bitplane](#)
[About Imaris...](#)

See also:

Addendum - [Mouse & Keyboard Mac](#)

7.1 Quick Start Tutorials

The Quick Start Tutorials provide you with the basic information how-to-use Imaris but may also show yet unrecognized new features of the software to the advanced user.

The tutorials are designed to be followed sequentially, but if you are already familiar with Imaris the basic lessons may be skipped. The tutorials are cross-referenced by hyperlinks highlighted in blue underlined font. The Table of Contents and the Index provide further support for navigation in the tutorials.

Each tutorial is a hands-on seminar, therefore move Imaris to the left hand side of the screen and adjust the online tutorial on the right hand side of the screen and practice step-by-step.

7.2 Keyboard and Mouse

Click on this menu entry and the Reference Manual opens in the actual browser. The page for the global Mouse & Keyboard functions displays.

Global Mouse & Keyboard Functions

For PC User:

Addendum - [Mouse & Keyboard PC](#)

For Mac User:

Addendum - [Mouse & Keyboard Mac](#)

7.3 Reference Manual

The Reference Manual provides you with the basic information about Imaris: The menu(s), the different Imaris views and all adjustable parameters in the program.

To find a specific parameter use the "Search function" in the online version of the Reference Manual.

Context Sensitive Help

For PC User:

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

For Mac User:

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

7.4 Programming Interface

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

7.5 License...

Two different licensing schemes are available for the software, node-locked and floating. Node-locked licenses grant permission to run the licensed software on one computer (node) only. Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time.

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

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

Install and Upgrade Product Licenses for this Computer

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

Button **Online...**

Press this button and follow the instructions.

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

Button **Offline...**

Press this button and follow the instructions.

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

Button **Config...**

Press this button and follow the instructions.

Button **Next**

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

Window: Hardware Settings

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

Data Cache

Memory Limit

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

Display

Texture Cache Limit

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

Open GL Test

The result of the open GL test displays on the right hand side.

Congratulations, your graphics board is able to display huge data.

Your graphics board is not capable of displaying huge data. Some features will be unavailable.

Automatic Updates

Enable

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

Button **Set Default**

Click on this button to insert the calculated values (see above).

Licenses Running on this Computer

Name

Display of all existing Imaris products and their names.

Type

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

License Path

Display of the license path. Editable text field.

Button **Clear**

Click on this button to clear the license path.

Host ID

Display of the host ID. Editable text field.

See also:

Menu Edit - Preferences... - [Calculation](#)

Menu Edit - Preferences... - [Licenses](#)

Menu Edit - Preferences... - [Display](#)

7.6 Check for Updates now ...

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

7.7 Bitplane AG

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

7.8 About Imaris...

The Imaris start screen displays.

Imaris

Version number and release date.

Copyright 1993-2009 Bitplane AG
All rights reserved

European Patent Registry No. 05009677.7
Patent Pending.

Using XML parser expat: Copyright
1998, 1999, 2000. Thai Open Source
Software Center Ltd and Clark Cooper

www.bitplane.com
welcome@bitplane.com

8 Toolbars

In the standard Imaris view there are three different toolbars available. The [Main Toolbar](#), the [Status Bar](#) and the [Time Bar](#).

See also:
Surpass View - Overview - [Camera Toolbar](#)

8.1 Main Toolbar

Please refer to chapter:

Open	Menu File - Open
Save as	Menu File - Save as
Snapshot	Menu File - Snapshot
InPress	Menu File - InPress

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

Please refer to chapter:

Slice	Slice View
Section	Section View
Gallery	Gallery View
Easy 3D	Easy 3D View

Surpass	Surpass View
Animation	Animation
InMotion	InMotion

Coloc	Coloc
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Bitplane AG	Menu Help - Bitplane AG
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8.2 Status Bar

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

Zoom %

Imaris lets you enlarge or reduce the view of your data set with the Zoom. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, type a zoom value.

Mouse Clicks to Zoom

With a three-button mouse:

Click with the middle mouse button on the image, hold down the middle mouse button, and drag the mouse up or down.

Hold the Shift-key and click with the right mouse button on the image, and drag the mouse up or down.

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

Button **100%**

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

Button **Fit**

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button **Full Screen**

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window, click on the button Full Screen in the lower left corner.

Button **Navi**

Click on this button to toggle the display of the Navigation window of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. In the pointer mode Select you can click & drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner.

Change Position of the Navigation Window

In the pointer mode Select you can click & drag the Navigation window to any desired corner.

Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.

Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

See also:

Menu View - [Fit](#)

Menu View - [100%](#)

Menu View - [Full Screen](#)

Menu View - [Navi](#)

[Section View](#)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Navigate/Select)

8.3 Time Bar

If a time series is loaded, a Time Bar displays at the bottom of the main window. It contains a slider showing all Time Points in the series, a **Time Settings** button, **Play** and **Pause** button (arrow to the right), and a **Record** button (red dot).

The Time Bar is visible in the display modes Slice, Section, Gallery 3D Preview, and Surpass. It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Play and Record Time Series

Button  Time Settings...

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

Button 

Click on this button to play the time series. To pause the continuous display through all the Time Points in the time series re-click on the button.

Button 

To record the time series as movie click on the button **Record** (red dot). The Save As Movie window is displayed (see below).

Slider

To inspect any the Time Point in the time series manually navigate the slider as follows (be sure that the Pointer is in the Select mode):

Drag the slider handle to the left or right.

Select the slider handle with a left mouse click and then use the Arrow-keys to the left/to the right on the keyboard.

Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last Time Point.

The respective Time Point number is displayed on the right hand side of the slider in the number field.

Window: Save as Movie

File name

Enter a file name.

Save as type

Select the desired file type (QuickTimeMovie, TIFF series, or AVI).

Movie Settings

Compression Factor

Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate

Define how many frames are displayed per second.

Add to ImageAccess database

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

Play Movie with default Media Player when finished

Check the box to play the movie with the default media player when finished.

QTVR Settings

The QuickTimeVR Settings are not available.

See also:

Menu Edit - Preferences ... - [Time](#) (default parameter settings)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Select)

Surpass View - Overview - Camera Toolbar - [QuickTimeVR](#)

9 Slice View

How to Open the Slice View?

Click on the icon in the Main toolbar. If the icon Slice is not displayed click on the arrow in the group: Section, Gallery, Easy 3D, and select Slice.

Select menu View - Slice.

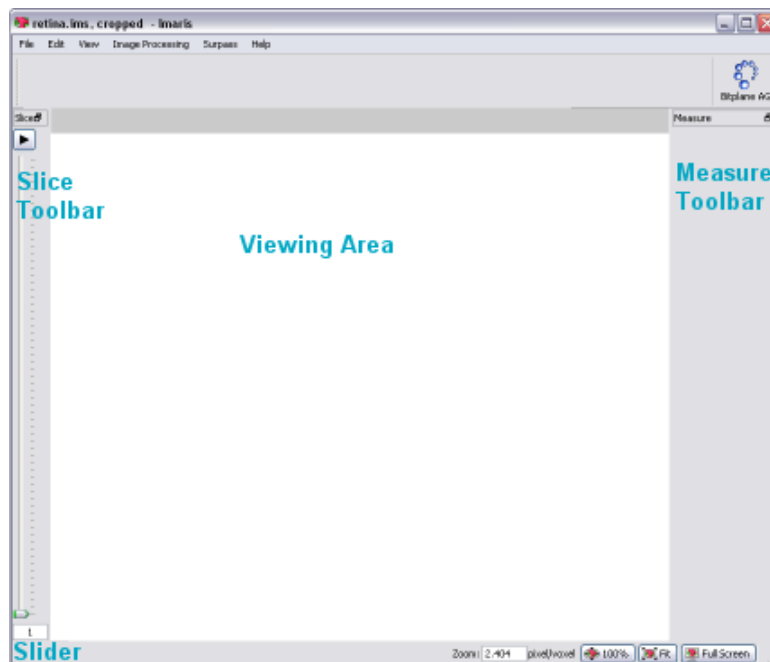
Press the key combination Ctrl + 1.

See also:

Slice View - [Mouse & Keyboard Mac](#)

9.1 Overview

The Slice view mode is the view in which an image is displayed when loading a data set into Imaris. The Slice representation shows a 2D view from the top in the z-axis. It shows all images in the data set along the z-axis, so you can inspect any slice along the z-axis for a general overview of the data set.



When moving the cursor over the image, the current voxel intensity and the 3D position in the data set (in brackets) is displayed in the lower left corner of the view.

Additional Information in the Status Bar

The Slice view also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)

The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:

Toolbars - [Status Bar](#)

9.2 Slice Toolbar

Slider

To inspect any slice in the data set along the z-axis you use the Slider. Navigate the slider as follows:

Drag the slider handle in the left control bar up and down.

Select the slider handle with a left mouse click and then use the Arrow-keys up/down on the keyboard.

Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last slice.

Select the slider handle with a left mouse click and then press the PageDown-key or the PageUp-key to display the first respectively last slice.

Enter the desired slice number in the number field at the bottom of the slider.

Button 

Click on the **Play/Pause** button to see a continuous display through all the slices in the stack. To pause re-click on the button.

Slice Number Field

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

9.3 Measure Toolbar

Type

Line

Set the two Measurement Points with two consecutive clicks. The result is displayed in the status field Distance.

Polygon

All Measurement Points are consecutively connected by lines and the displayed distance is the sum of the distances between the points.

Grid

Check this box to display a grid in the image.

Distance

The result of the measurement is displayed in the status field distance.

Button 

Click on **Delete** to clear the status field and start a new measurement.

9.4 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Slice View

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

See also:

Addendum - [Mouse & Keyboard PC](#)

9.5 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Slice View

Command + 1	Activates the Slice view
Click	Set a measurement point

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

10 Section View

How to Open the Section View?

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

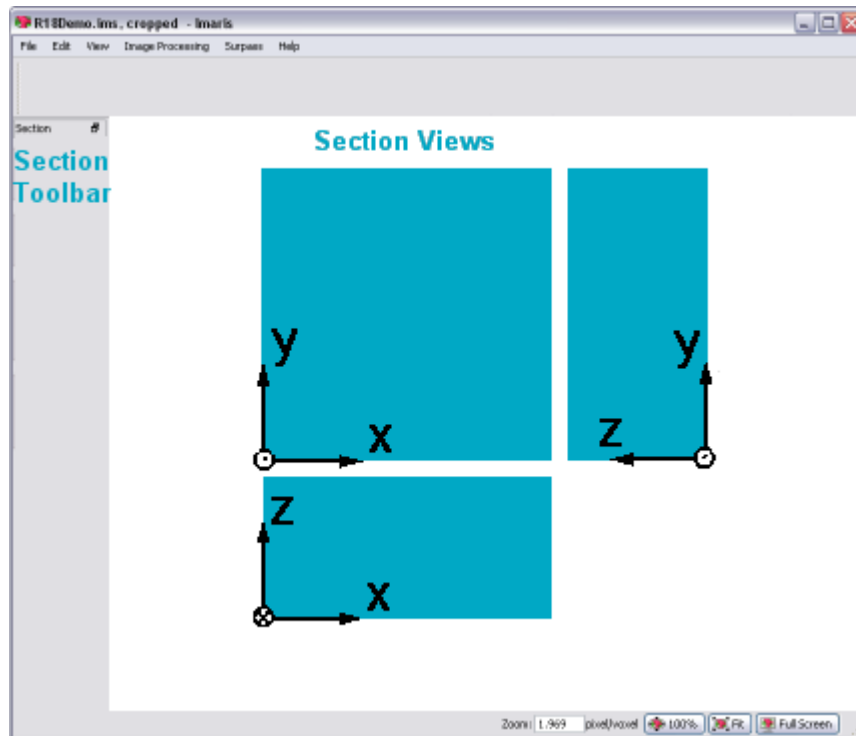
See also:

Section View - [Mouse & Keyboard Mac](#)

10.1 Overview

The Section view lets you inspect the environment of any given point within the data set by showing the cut through the point in the x-, y-, and z-axes or a defined portion of the data set (several slices together).

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



To select a point, click in any of the three views, hold the button down & drag the crosshairs to the requested point. The crosshairs immediately snap to the cursor when clicking anywhere in the image. The other views, connected by one coordinate, automatically follow the movements.

Additional Information in the Status Bar

The Section view also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)

The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:

Toolbars - [Status Bar](#)

10.2 Section Toolbar

View

Normal

Selects a single cut through the data set.

Extended

Additional colored lines are displayed in the viewing area. If Extended is checked the parameter sets Crosshair, Size and Mode are available.

Crosshair

The parameter set Crosshair is only available, if Extended is checked in the parameter set View (see above).

Normal

You may eliminate the Crosshair display on the image by un-checking the box. It is then only visible at the edges.

Extended

Select a portion of the data set in the x-, y-, and z-direction to be displayed. In the main screen, separate the two crosshairs. The planes between the crosshairs are rendered in the views showing the other axis. This allows you to see only certain interior structures without both the top and the bottom.

The image shows the white crosshair line together with colored focus range lines.

The focus range can be set in any of the three directions as follows: Move the cursor on a colored line. The cursor changes its form to a straight arrow with a bar.

Drag the line in the respective direction. The range is extended or reduced. Its depth is reflected in the x-, y-, and z-fields in the Size display. The Size values are indicated in the data set's dimensions, e.g., in m. It can also be adjusted directly by entering values in the Size fields (see below) or by means of their up and down arrows.

Un-check the Crosshair – Normal and/or Crosshair – Extended boxes to eliminate the crosshair and/or the focus range lines display on the image.

Size fields

The parameter set Size is only available, if Extended is checked in the parameter set View (see above). Display of the actual x-, y-, and z-position of the extended Crosshair. The positions can also be adjusted directly by entering values in the fields or by means of their up and down arrows.

Mode

The parameter set Mode is only available, if Extended is checked in the parameter set View (see above). Here you may choose the best display mode for the slice portion.

MIP

Calculated maximum over all slices in the range.

Mean

Calculated mean value over all slices in the range.

Blend

Calculated upon the transparency of the slices.

10.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Section View

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

See also:

Addendum - [Mouse & Keyboard PC](#)

10.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Section View

Command + 2	Activates the Section view
Click	Set the section center

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out
	Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

Open the Apple-menu, select System Preferences...

Click on the button Keyboard & Mouse.

Select the OS X mouse properties.

Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out
	Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

11 Gallery View

How to Open the Gallery View?

Click on the icon in the Main toolbar. If the icon Gallery is not displayed click on the arrow in the group: Section, Slice, Easy 3D, and select Gallery.

Select menu View - Gallery.

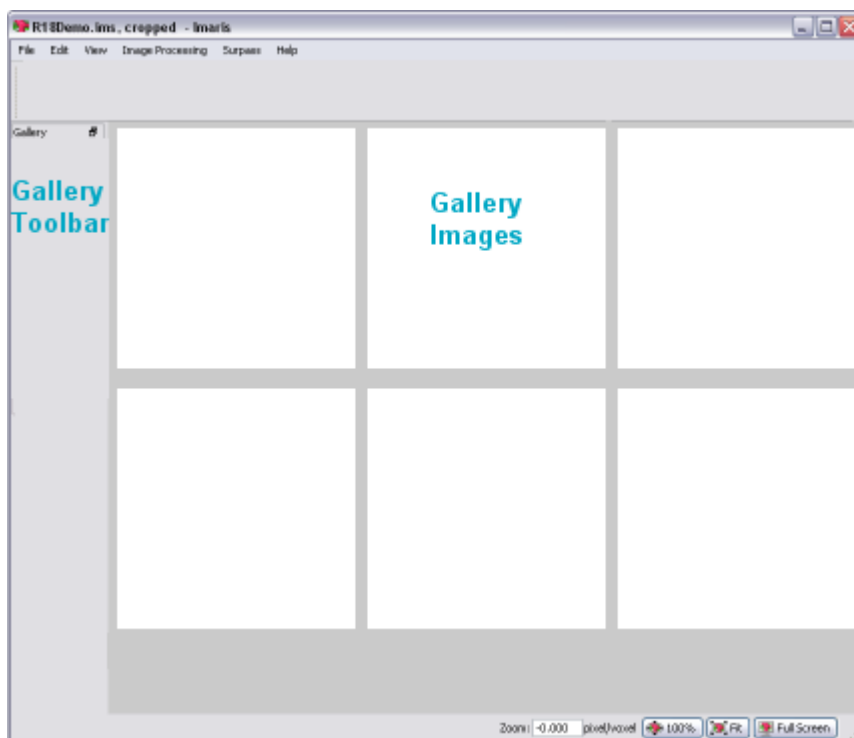
Press the key combination Ctrl + 3.

See also:

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

11.1 Overview

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



Additional Information in the Status Bar

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

See also:
Toolbars - [Status Bar](#)

11.2 Gallery Toolbar

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

Slice

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

Page

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

Columns

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

Skip

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

Display

Button **Select...**

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

Slice Number Display

The slice number is displayed beneath each slice.

11.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Gallery View

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

See also:

Addendum - [Mouse & Keyboard PC](#)

11.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Gallery View

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

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

Open the Apple-menu, select System Preferences... .

Click on the button Keyboard & Mouse.

Select the OS X mouse properties.

Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

12 Easy 3D View

How to Open the Easy 3D View?

Click on the icon in the Main toolbar. If the icon Easy 3D is not displayed click on the arrow in the group: Section, Gallery, Slice, and select Easy 3D.

Select menu View - Easy 3D.

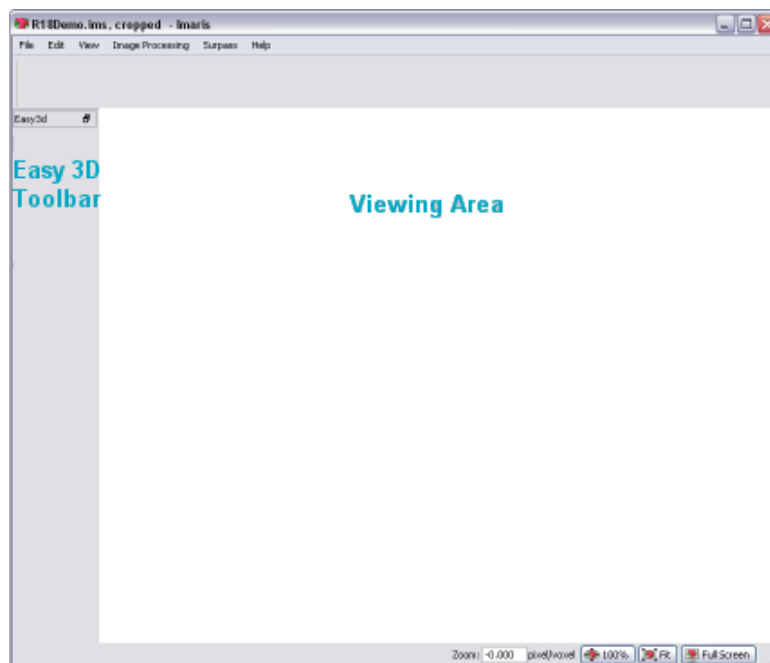
Press the key combination Ctrl + 4.

See also:

Easy 3D View - [Mouse & Keyboard Mac](#)

12.1 Overview

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



12.2 Easy 3D Toolbar

Rendering

MIP

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

Blend

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

Light

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

Light

Check the box to switch the light on and adjust the following parameters. There are two predefined light

setting parameters in Easy 3D Blend to choose from.

Left

Light from the left, shadow to the right.

Right

Light from the right, shadow to the left.

Button **Settings...**

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

Calculate

Auto

The image is calculated and displayed automatically.

Manual

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

Button **Calculate**

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

Window: Easy 3D Settings

Light

Directional Intensity

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

Ambient Intensity

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

Channel Opacity Combination

Allows you to control the opacity of the image.

Opaque

Translucent

Custom

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

12.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Easy 3D View

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

See also:

Addendum - [Mouse & Keyboard PC](#)

12.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Easy 3D View

Command + 4 Activates the Easy 3D view

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

13 Surpass View

The Surpass view provides extended functions for visualization, including:

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

How to Open the Surpass View?

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

Save and Load Surpass Configuration

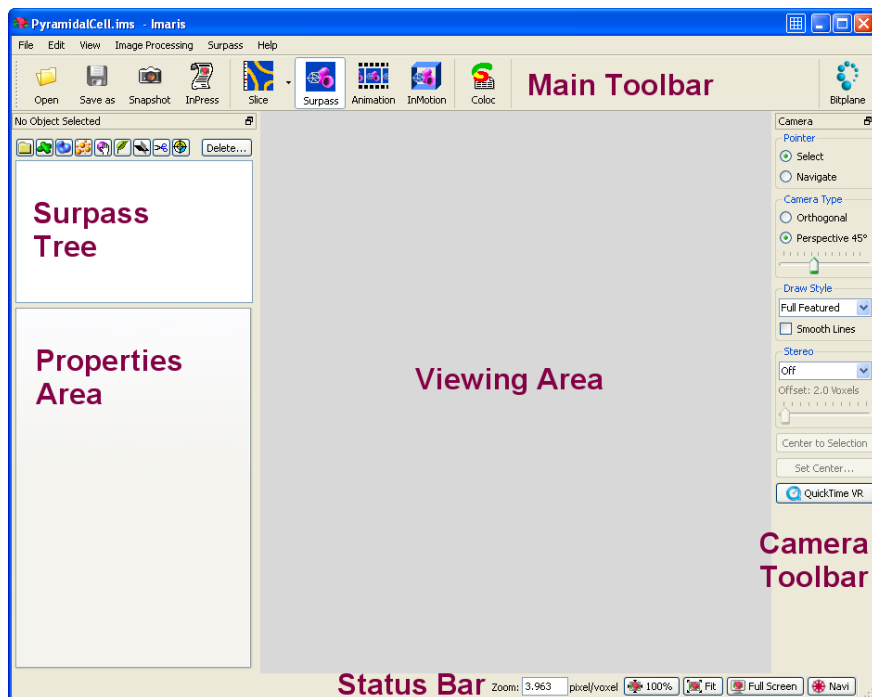
The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set. For details please refer to chapter Surpass View - Overview - [Scene File Concept](#).

See also:

- Surpass View - Overview - [Mouse & Keyboard Mac](#)
- Surpass View - [Ortho Slice](#)
- Surpass View - [Volume](#)
- Surpass View - [Surfaces](#)
- Surpass View - [Spots](#)

13.1 Overview

The Surpass main screen consists of six different areas:



On top you find the [Main Toolbar](#).

On the left hand side you find the [Surpass Tree](#) and the [Properties Area](#). In the Properties Area you find all available parameters for the selected Surpass Tree Item. The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.

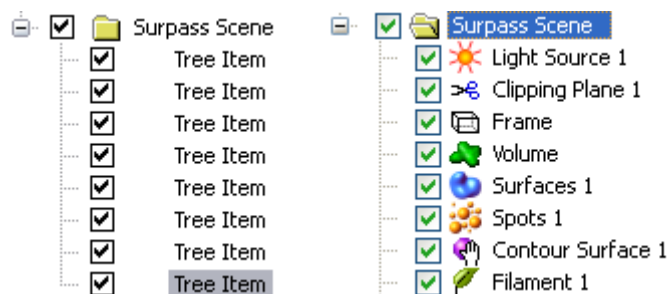
The [Viewing Area](#) displays all objects that are added to the Surpass Tree and are checked visible.

The [Camera Toolbar](#) includes a number of tools for data preparation, presentation and manipulation, most of which are displayed as buttons.

On the bottom you find the [Status Bar](#).

13.1.1 Surpass Tree

The Surpass Tree displays a tree list of all Surpass Tree Items that were added to the viewing area.



Structure

The tree list is automatically generated and updated when a Surpass Tree Item is added or deleted. The first added object generates a group (Surpass Scene). All following new objects are stored in this group. A name is generated automatically for each Surpass Tree Item. To change the name, double-click on the item and enter a new name. Move objects or groups from one group to another by dragging and dropping them with the left mouse button.

How to Add a new Surpass Tree Item?

All available Surpass Tree Items are available in the menu Surpass. To add new Surpass tree item click on the icon.



You find a list of all Items in the chapter Surpass View - Overview - [Properties Area](#).

Display

Each Surpass Tree Item includes a check-box. Check the box to make the object visible in the viewing area. Un-check the box to make the object invisible in the viewing area. The currently active object in the viewing area is highlighted in the Surpass Tree.

Groups

You can group objects into so-called component groups. Functions applied to the component group apply to all of its members. This facilitates the application of colors or the deletion of objects.

Please note: If a group folder is checked invisible, all Items in the folder are invisible.

Multiple Selection

You may select more than one listed Item at a time for an operation. The selection functions in Surpass correspond to the Windows functions:

Consecutive: Press and hold the Shift-key down and select the first, then the last entry to be selected from the list. All entries in between the two are also selected.

Selective: Press and hold the Ctrl-key down and select any required entries from the list.

All selected entries are highlighted and commands or operations apply to all of them.

Objects Toolbar

In the Objects toolbar you find a selection of Surpass Tree Items. To customize the Objects toolbar please refer to chapter Menu Edit - Preferences... - [Surpass](#) (Object Creation Buttons).

Button **Delete...**

To delete Surpass Tree Item, highlight the Item in the Surpass Tree and click the button **Delete...** . The Delete selection window with a confirmation question is displayed.

Naming Conventions

Objects are automatically named by Surpass as follows:

Clipping Plane	Clipping Plane n
Contour Surface	Contour n
External Object	External Object n
Filament	Filament n
Frame	Frame
Group	Group n
Surfaces	Surface n
Light Source	Light Source n
Measurement Point	Measurement Points n
Ortho Slicer	Ortho Slicer n
Spots	Spots n

[Volume](#)

Volume (only one volume can be created)

Surpass Tree Item Properties

Each Surpass Tree Item has its own set of adjustable parameters. They are displayed in the properties area.

Surpass Tree Item Tabs

Each Surpass Tree Item has its own set of adjustable parameters. They are grouped in different Tabs.

Save and Load Surpass Tree Configuration

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set. For details please refer to chapter Surpass View - Overview - [Scene File Concept](#).

See also:

Menu Edit - Preferences... - [Surpass](#) (Object Creation Buttons)

Menu Surpass - [Delete Selected Objects...](#)

Surpass View - Overview - [Properties Area](#)

Surpass View - [Group](#)

13.1.2 Scene File Concept

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

Export Scene File

The Scene File can be written as follows:

Select menu File - Export Scene in the Imaris menu bar.

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

Import Scene File

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

Select menu File - Import Scene..... in the Imaris menu bar.

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

See also:

Menu File - [Import Scene...](#)

Menu File - [Export Scene...](#)

Surpass View - Overview - [Surpass Tree](#)

Surpass View - [Group](#)

13.1.3 Properties Area

The Properties Area displays all available parameters for the selected Surpass Tree Item.

Surpass Tree Item - Properties

The name of the heading is a combination of the selected Surpass Tree Item, followed by "- Properties". If you select another Surpass Tree Item the heading changes accordingly.

Tab X

The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.

List of available Tabs:

<u>Surpass Tree Item</u>	<u>Parameter Tab</u>
Clipping Plane	Tab Settings
Contour Surface	Tab Settings Tab Color
External Object	Tab Settings Tab Color
Filament	Tab Create see Surpass View - Filament Creation Wizard Tab Settings Tab Creation Tab Draw Tab Edit Tab Statistics Tab Color
Fra me	Tab Settings Tab Color
Grou p	Tab Settings Tab Color Tab Statistics
Light Source	Tab Settings Tab Color
Measurement Point	Tab Settings Tab Edit Tab Intensity Tab Statistics Tab Color
Ortho Slicer	Tab Settings
Oblique Slicer	Tab Settings
Spots	Tab Create see Surpass View - Spots Creation Wizard Tab Settings Tab Creation Tab Edit Tab Statistics Tab Color
Surfaces	Tab Create see Surpass View - Surfaces - Creation Wizard Tab Settings Tab Creation Tab Statistics Tab Color

See also:

Surpass View - Overview - [Surpass Tree](#)

13.1.4 Creation Wizards

A Creation Wizard is a user interface element where you are led through a sequence of dialogs. In a Creation Wizard you are forced to perform a task in a specific sequence.

Examples for Creation Wizards in Imaris are the [Filament Creation Wizard](#), the [Spots Creation Wizard](#), and the [Surfaces Creation Wizard](#). Once you insert an new Item Filament, Spots, or Surfaces you are automatically on the Tab Create in the Properties Area.

See also:

Surpass View - Filament - [Creation Wizard](#)

Surpass View - Spots - [Creation Wizard](#)

Surpass View - Surfaces - [Creation Wizard](#)

13.1.5 Viewing Area

Pan

To move the image within the Surpass view (pan the object) choose the mouse pointer mode Navigate. Click and hold the right mouse button while dragging the mouse. Release right mouse button to place the image.

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

Right-click & drag	Pan image
--------------------	-----------

On a Mac with a one-button mouse:

Ctrl + click & drag	Pan image
---------------------	-----------

Rotate

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

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

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

Left-click & drag	Rotate image
-------------------	--------------

On a Mac with a one-button mouse:

Click & drag	Rotate image
--------------	--------------

How to Keep the Image Continuously Rotated?

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while you move the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Release the left mouse button while still dragging the mouse. The result is a continued rotation (speed of the rotation according to prior mouse motion). To stop the continued rotation re-click in the image area.

Zoom

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

Using the Mouse

Choose the mouse pointer mode Navigate. To zoom in on the image hold the middle mouse button and drag it towards you. To zoom out from the image hold the middle mouse button and drag it away from you.

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

Middle-click & drag	Move up: zoom out Move down: zoom in
---------------------	---

On a Mac with a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
-----------------------------	---

Using the Buttons in the Status Bar

Zoom %

Imaris lets you enlarge or reduce the view of your data set with the Zoom. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value.

Button **100%**

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

Button **Fit**

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button **Full Screen**

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window re-click on the button **Full Screen** in the lower right corner.

See also:

[Menu View](#)

Toolbars - [Status Bar](#)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Navigate)

13.1.6 Camera Toolbar

Pointer

Select

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

Navigate

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

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

Camera Type

Orthogonal

Orthogonal display using parallel lines.

Perspective X°

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

Slider X°

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

Draw Style

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

Full Feature

Shows all objects as they are.

Wireframe

Draws Surface objects, Surfaces, Spots, Filaments, and Measurement Points as red colored wireframe models.

Hidden Lines

Draws Surfaces as wireframe models and hides all background lines.

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

No Texture

Draws objects without textures.

Bounding Box

Shows only the boxes surrounding the objects.

Wireframe Overlay

Lays a red wireframe model over Surface objects, Surfaces, Spots, Filaments, and Measurement Points.

Low Resolution

Not yet implemented.

Points

Draws Surfaces as a point model.

Smooth Lines

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

Stereo

Off

No stereo display in the viewing area.

Red/Cyan Anaglyph

This display mode requires colored glasses.

Quad Buffer

This display mode requires shutter glasses.

Interleaved Rows

This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

Interleaved Columns

This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is

molded to have the form of dozens of tiny lenses or prisms per inch.

Offset

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

Slider

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

Button **Center to Selection**

Click on the button **Center to Selection** to move the selection in the center of the viewing area.

Button **Set Center...**

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

Please note: Rotation centers can be set on Surfaces, Contour Surface, Ortho Slicer and External objects

Button **Quick Time VR**

Please refer to the next chapter: Surpass View - Overview - [QuickTimeVR](#).

13.1.7 QuickTimeVR

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

Button **Quick Time VR**

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

Window: Save as Movie

File name

Enter a file name.

Save as type

The file type is QuickTimeVRMovie (*.mov).

Movie Settings

Compression Factor

Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate

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

Add to ImageAccess database

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

Play Movie with default Media Player when finished

Check the box to play the movie with the default media player when finished.

QTVR Settings

Here you define the degrees between two frames.

Total Angle Horizontally

Defines the angle of the horizontal movement, the default setting is 360.

Total Angle Vertically

Defines the angle of the vertical movement, the default setting is 180.

See also:

Toolbars - [Time Bar](#) (Play and Record Time Series)

Surpass View - [Overview](#)

Surpass View - Overview - [Camera Toolbar](#)

Animation - [Animation Window](#)

Animation - [Save Animation](#)

13.1.8 Mouse & Keyboard PC

General Mouse & Keyboard Functions in the Surpass View

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

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Select Mode

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

Mouse in Navigate Mode

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

See also:

Addendum - [Mouse & Keyboard PC](#)

13.1.9 Mouse & Keyboard Mac

General Mouse & Keyboard Functions in the Surpass View

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

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Select Mode

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Click	Select object
Command-click	Add object to selection/remove object from selection

Mouse in Navigate Mode

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

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
Open the Apple-menu, select System Preferences...
Click on the button Keyboard & Mouse.
Select the OS X mouse properties.
Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.
--

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Click & drag	Rotate image

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.2 Clipping Plane

The Clipping Plane cuts away objects on one side of the plane. It can be freely moved and rotated in the scene and allows you to look inside any object. The Clipping Plane consists of a colored frame with a white double rod in the middle.

To change the position and orientation of the Clipping Plane in the View Area the Pointer must be in Select mode.

How to Add a new Clipping Plane?

Select menu Surpass - Clipping Plane.

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

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

Change Position of Clipping Plane

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

Rotate Clipping Plane around Axes

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

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

13.2.1 Tab Settings

Position

Button **Recenter**

The Clipping Plane center is moved to the data set center.

Orientation

Button **YZ Plane**

Button **XZ Plane**

Button **XY Plane**

The Clipping Plane is oriented parallel to either the yz-plane, the xz-plane or the xy-plane.

Configuration

Button **Store**

Button **Recall**

After changing the position and/or orientation of the Clipping Plane, the new configuration can be stored by clicking on the **Store** button. The position previously stored can be recalled by selecting the **Recall** button.

Camera

Button **Orthogonal View**

The camera is set to a position which is perpendicular to the Clipping Plane.

Show Frame

To make the frame visible, check this box.

Perpendicular Movement

The pointer must be in the pointer mode Select. Place the mouse within the image. To change the orientation of the rod press the Ctrl button. The Clipping Plane is moved in the directions parallel to the plane.

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

13.2.2 Mouse & Keyboard PC

Clipping Plane Mouse & Keyboard Functions

Left-click on plane & drag

Move plane to desired position

Left-click on the bigger diameter rod & drag

Move plane to desired position

Left-click on the smaller diameter rod & drag

Rotate plane to desired position

See also:

Addendum - [Mouse & Keyboard PC](#)

13.2.3 Mouse & Keyboard Mac

Clipping Plane Mouse & Keyboard Functions

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

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.3 Contour Surface

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. You can employ this method whenever simple thresholding does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

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

How to Add a new Contour Surface?

Select menu Surpass - Contour Surface.

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

13.3.1 Tab Settings

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

Draw. Board/Size

YZ **X**
XZ **Y**
XY **Z**

Select the plane of the Contour Surface. Choose the yz-plane to draw in the x-direction. Choose the xz-plane to draw in the y-direction. Choose the xy-plane to draw in the z-direction. The size of the working channel is displayed in the corresponding field.

Drawing Mode

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

Visibility

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

None

The contour currently being drawn is made visible.

Next

The contour(s) next to the contour currently drawn is/are shown.

All

All contours are shown.

Slice Position

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

Slider

Slice (data field)

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

Mouse Interaction

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

Selection

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

Button **Copy**

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

Button **Paste**

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

Button **Calculate Surface**

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

Number of Triangles

The number of triangles is displayed.

Button **Reedit**

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

Button **Create Surfaces**

To create Surfaces out of the Contour object click on the button **Create Surfaces**. The Surfaces item appears in the Surpass Tree and displays in the viewing area.

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

Surpass View - Contour Surface - [Drawing Mode](#)

13.3.2 Drawing Mode

You can draw the contours in different draw styles.

Click

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

Time

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

Dist.

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

Edit Vertices

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

Move node

Hold down the Shift-key, click on the node and simultaneously drag the mouse.

Insert node

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

Delete node on PC

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

Delete node on Mac

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

Delete polygon on PC

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

Delete polygon on Mac

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

Size of Vertices and Lines

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

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

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

Surpass View - Contour Surface - [Mouse & Keyboard PC](#)

Surpass View - Contour Surface - [Mouse & Keyboard Mac](#)

13.3.3 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.3.4 Mouse & Keyboard PC

Contour Surface Mouse & Keyboard Functions

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

In the Drawing Mode Click

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

In the Drawing Mode Time and Distance

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

See also:

Surpass View - Contour Surface - [Tab Settings](#) (Drawing Mode)

Addendum - [Mouse & Keyboard PC](#)

13.3.5 Mouse & Keyboard Mac

Contour Surface Mouse & Keyboard Functions

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

In the Drawing Mode Click

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

In the Drawing Mode Time and Distance

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

See also:

Surpass View - Contour Surface - [Tab Settings](#) (Drawing Mode)

Addendum - [Mouse & Keyboard Mac](#)

13.4 External Object

Surpass provides the ability to import External objects (both, non-Imaris and Imaris objects) and display them concurrently with a Surpass object for comparison.

Only the External objects with the extension ***.iv** (Inventor file) or with extension ***.vrmf** (VRML2 files) can be imported.

The imported External objects can be rotated, scaled and translated.

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

How to Add a new External Object?

Select menu Surpass - External Object.

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

See also:

Menu Surpass - [Export Selected Objects ...](#)

13.4.1 Tab Settings

The object properties area displays a number of parameters for the positioning and display of the External object. The following display/handling options can be checked:

Box Handle

The object shows a surrounding box with handles. These handles allow to transform/distort the object in the x-, y-, and z-direction.

Trackball

The object shows surrounding circular lines. These lines allow rotation of the External object in any direction.

File Path Field

Display of the file path (to load the object, see below).

Button **Load...**

To select and load a data set as a External object click on the button **Load...** and the Load External Object File window is displayed. Select the file to be loaded as External object. The External object will be only loaded if the selected files have the extension ***.iv** (Inventor file) or ***.vrmf** (VRML2 files). Click on the button Open.

Button **Reset Size and Location**

Clicking this button sets the External object back to its original size and position before any translational, scaling or rotational operations have been performed.

Fix Ratio

If this option is checked and if scaling is performed on the External object by entering a value, the same scaling factor is applied to all three dimensions.

Position/Scaling

The following positional/scaling options are available:

Translation

Use these fields to move the External object in the x-, y-, or z-direction.

Rotation

Use this field to rotate the External object around the x-, y-, or z-axis.

Scaling

Use this field to scale the External object in one of the dimensions (or all together if Fix Ratio, see above, is checked). Fix Ratio is only valid with this method of scaling.

See also:

Menu Surpass - [Export Selected Objects ...](#)

13.4.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.4.3 Mouse & Keyboard PC

External Object Mouse & Keyboard Functions

Left-click white box & drag	Scale object from center
Left-click white box + Ctrl & drag	Scale object from opposite corner
Left-click trackball & drag	Rotate object
Shift + left-click trackball & drag	Set up a rotation axis

See also:

Addendum - [Mouse & Keyboard PC](#)

13.4.4 Mouse & Keyboard Mac

External Object Mouse & Keyboard Functions

Click white box & drag	Scale object from center
Click white box + Ctrl & drag	Scale object from opposite corner
Click trackball & drag	Rotate object
Shift + click trackball & drag	Set up a rotation axis

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.5 Filament

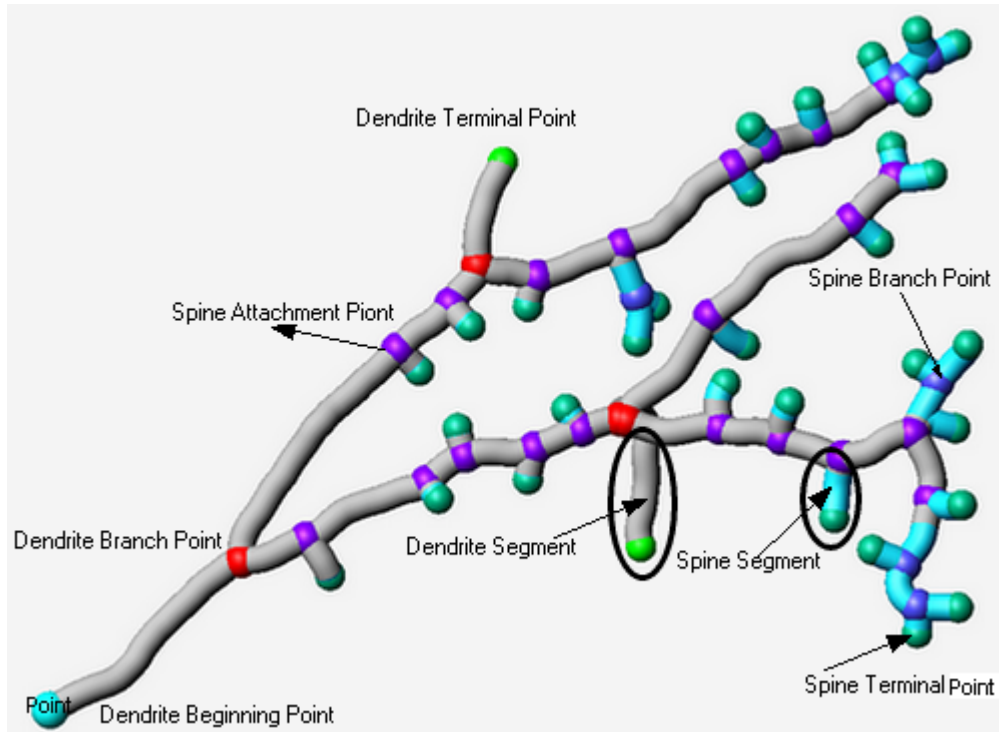
General

In IMARIS the dendritic trees are represented by a set of **vertices** and **edges**. The Vertex is drawn as a node or a dot and defines the center axis of the dendrites and an edge is presented as a line connecting two vertices. The entity of vertices and edges is referred to as graph.

Parts of the graph can be classified as spines either collectively, based on some statistical values, or individually by the user, by determined specifications.

Starting, ending and branching vertices are classified into **dendrite beginning point**, **dendrite branch point**, **dendrite terminal point**, **spine attachment point**, **spine branch point**, and **spine terminal point** depending on their position in the graph (refer to the picture below).

Segments are defined as the collection of edges between two starting, ending, or branching vertices. Segments are classified either as **dendrite segments** or **spine segments**. In a dendrite the edge is called a **dendrite edge**, in a spine it is called a **spine edge**.



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

After an automatic Filament extraction (see [Creation Wizard](#)) you can launch an editor operation to delete

Filaments, connect Filaments, or draw new Filaments. You may also skip the automatic Filament generation and process the data with semi-automatic or manual segmentation and Filament generation (see tab Draw).

Generated Filament objects will be visualized in the context of Surpass. This will allow multiple viewing combinations in which the Filaments are displayed individually or together with other cellular components that may have been imaged in other channels.

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

How to Add a new Filament?

Select menu Surpass - Filament.

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

See also:

Surpass View - Filament - [Creation Wizard](#) (for automatic Filament extraction)

Surpass View - Filament - [Tab Draw](#) (for interactive Filament tracing method)

13.5.1 Data Input

The FilamentTracer is designed to operate on 3D images, which provides sufficient resolution to resolve the Filaments to be studied in all three spatial directions. These images can be multi-channel (multiple colors) but only one channel will be traced at a time. The following input images are acceptable:

The ideal input is a fluorescently labeled confocal image. Such images generally have good contrast (selective labeling) and good resolution in all three spatial dimensions.

3D images can also be acquired using a standard microscope (non-confocal) equipped with a CCD camera and a stepper motor. Non-confocal optical arrangements do not provide resolution along the optical axis which is why every single plane consists of in-focus components and out-of-focus components. These images require deconvolution prior to tracing. The technique of deconvolution uses information about the imaging process (the point spread function) to remove the out-of-focus components from every section.

Finally there are 3D images taken in transmission light microscopy. Staining is usually less specific than in the fluorescent case and it will be more difficult to automatically segment the Filament. Deconvolution can also be applied to transmission images although - theoretically - its performance is weaker than in the fluorescent case.

13.5.2 Creation Wizard

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

Select the appropriate algorithm (or quit the automatic filament creation and draw the filaments interactively).

Define a Region of Interest

Select source channel and do some pre-processing.

Segmentation to specify filamentous and non filamentous parts.

Graph compilation and finishing.

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

See also:

Surpass View - Filament - Creation Wizard - [Algorithm](#)

Surpass View - Filament - Creation Wizard - [Preprocessing](#)

Surpass View - Filament - [Tab Draw](#) (for interactive filament tracing method)

13.5.2.1 Algorithm

In this first window of the Creation Wizard you decide, if you want to go for an fully automated filament creation using one of the two predefined algorithms (AutoPath-no loops or Threshold-loops, see below); or if you want to draw the filaments interactively.

If you do not want to use the automatic filament creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button

Terminate the creation wizard and activates the interactive filament tracing method.

Select Algorithm

The automatic filament creation provides two algorithms to choose from:

AutoPath (no loops)

Threshold (loops)

AutoPath (no loops)

The automated AutoPath Algorithm:

Produces a tree-like filament

Is based on local intensity contrast

Connects large start- and small end-points

If you select this algorithm please refer to chapter: Surpass View - Filament - Creation Wizard - [Autopath Algorithm \(no loops\)](#)

Threshold (loops)

The Threshold based Algorithm:

Produces a filament with loops

Is based on an absolute intensity threshold

Thinning to a skeleton

If you select this algorithm please refer to chapter Surpass View - Filament - Creation Wizard - [Threshold Algorithm \(loops\)](#)

Calculate Diameter of Filament from Image

Check this box and the Filament diameter is calculated based on the image data.

Un-check the box and the Filament diameter will taper smoothly from the starting point to the end points.

Region of Interest-ROI

Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Build Filament for all time Points

The option is available only if you work with a time data set. Check the box if you want to create a filament over all time points.

Filament Wizard Buttons

Button **Next**

Press **Next** to proceed with the next of the Creation Wizard.

Button **Finish**

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

Button **Cancel**

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

13.5.2.2 Autopath Algorithm (no loops)

The Autopath (no loops) algorithm consists of the following steps:

[Region of Interest](#)
[Points Diameter](#)
[Classify Dendrite Points](#)
[Dendrite Diameter](#)
[Classify Spine Points](#)
[Spine Diameter](#)
[Finish](#)

13.5.2.3 ROI

Region of Interest 1

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.

X:, Y:, Z:, Time

Min:, Max:, Size

Minimum, maximum and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields.

Alternatively the location and size of the ROI can be adjusted directly in the viewing area. Click on the arrows in the ROI preview rectangle (change to the pointer mode **Select**) and drag it to define new coordinates for X and Y. Location of the ROI can be adjusted by clicking inside the ROI preview rectangle and dragging (with mouse button held) to the chosen image location. The numbers in the value fields are updated automatically.

Memory Usage:

Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:

Display of the memory usage for the image size within the ROI.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired **Filament** and display the **Filament** in the viewing area.

Button **Cancel**

If you do not want to create Filament automatically click on **Cancel**.

13.5.2.4 Points Diameter

Select Source Channel

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

Starting Points

(largest Diameter: e.g. Dendrite Beginning)

Enter a value of the maximum (largest) diameter of dendrite to specify the Starting point diameter.

Seed Points

(Thinnest Diameter e.g. Dendrite Ending)

Enter a value of the minimum (smallest) diameter of dendrite to specify the Seed points diameter.

Spine

Detect Spines

Check this box to Optimize the Creation wizard for Spine Detection; creating the highly branched filament structure.

Un-check the box Detect Spines and the Spine channel selection and the Seed Points diameter of are disabled. Spines detection and creation process are not performed in the following creation procedure.

Select Source Channel

Select the respective source channel, from which Filament structure is to be computed. The default channel is the same as for the dendrite detection.

Seed Points

(Thinnest Diameter e.g. Small Spine Head)

Enter a value of the minimum (smallest) diameter of spine head to specify the Seed points diameter.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

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

Button **Cancel**

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

By reentering the Creation Wizard, either after selecting [Rebuilding Algorithm](#) or for [Processing additional ROI\(s\)](#) a new option exists for selecting a Starting Point.





See also:

Surpass View - Filament - [Tab Settings](#)
Surpass View - Filament - [Tab Draw](#) (for interactive filament tracing method)

13.5.2.5 Classify Dendrite Points




Thresholds are used to classify the detected dendrite's starting points and seed points.
The detected dendrite starting point(s) are visualized in the image by different colors. The respective histograms are displayed.
The threshold can be set manually or automatically.




Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Starting Points Threshold

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

Manual Starting Points addition/deletion in Creation Wizard

Manual addition of starting points in the filament structure is available by pressing Shift+ right-click. Shift+ right-click on an existing filament start point (created either automatically or manually added) and the selected starting point will be deleted.

Seed Points Threshold

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

Manual Seed Points Addition/Deletion in Creation Wizard

Manual Addition of Seed points in the filament structure is available by Shift+ left-click. Shift+ left-click on an existing point, (created either automatically or manually added) and the selected point will be deleted.

Remove Seed Points Around Starting Point(s)

Check the box and all seed points located within the radius of the sphere centered at the starting point are removed. The boundary of the sphere is defined by the value
Diameter of Sphere Region(s).

Diameter of Sphere Region(s)

Enter the diameter of the sphere centered at the beginning point. Default value set to twofold of the starting points diameter.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.





Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

13.5.2.6 Dendrite Diameter

The option is only available if in the step [Algorithm](#) the Calculate Diameter form the image is selected. The threshold value is based on the local contrast and the respective histogram is displayed. The defined threshold value is used for the dendrite diameter calculation.




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

Algorithm

The Algorithm provides two methods to calculate dendrite diameter.

Shortest distance from distance map

The dendrite diameter is calculated as the shortest distance between dendrite center point and dendrite surface.

Approximate circle of cross section area

The diameter of the approximate circle of cross-sectional area is determined as a value of the dendrite diameter.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

13.5.2.7 Classify Spine Points

The option is only available if in the step [Points Diameter](#) the Detect Spine option is selected.





Spine

Seed Points threshold

Thresholds is used to classify the spines seed points. The detected Spine seed points are visualized in the image. The respective histogram is displayed.

The threshold can be set manually or automatically.




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points are visualized in the viewing area.

The effect of changing the threshold value is directly visible in the viewing area.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.





Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

13.5.2.8 Spine Diameter

The option is only available if in the step [Points Diameter](#) the Detect Spine option is selected. The threshold value is based on the local contrast and the respective histogram is displayed. The defined threshold value is used for the Spine diameter calculation.




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

Algorithm

The Algorithm provides two methods to calculate Spine diameter.

Shortest distance from distance map

The Spine diameter is calculated as the shortest distance between dendrite center point and spine surface.

Approximate circle of cross section area

The diameter of the approximate circle of cross-sectional area is determined as a value of the spine diameter.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

13.5.2.9 Finish ROI Processing

The selected parameters will be used to complete the computation of a Filament structure in an image. By selecting one of six finishing options a Filament structure will be created in the following ways:

1. **Entire Image in This Time Point** - the entire image is processed with the same set of parameters used for the ROI processing and the filament structure is created for the entire image and the selected time point. Only available if you work with a time data set.
2. **Additional Region in This Time Point** - re-enters creation process (in step two-ROI) and uses the same the same set of parameters for a new ROI to create the filament structure only for the selected time point. Only available if you work with a time data set.
3. **Additional Regions in Other Time Point** - re-enters creation process (in step two-ROI) and uses the same set of parameters for a new ROI to create the filament structure for the new time point. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.
4. **Current Region in All Time Points** - re-enters creation process (in step two-ROI) and uses the same creation algorithm for a new ROI to creates the filament structure for all time points. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.
5. **Entire Image in All Time Points**- the entire image is processed with the same parameters used for the ROI processing and the filament structure is created for the entire image and all time points. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.
6. **All Done**- creates a filament structure only within an ROI.

Please refer to [Tab Creation](#). In step three of the Creation wizard – [Point Diameter](#), an option exists for selecting a Starting Point (Use existing filament or Detect a new starting point).

Note: The Use Existing Filament option requires that the new ROI overlaps with some portion of the existing filament.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

13.5.2.10 Threshold Algorithm (loops)

The threshold (loops) algorithm consists of the following steps:

[Preprocessing](#)
[Segmentation](#)
[Graph Compilation](#)
[Finish](#)

13.5.2.11 Preprocessing

Source Channel

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

Feature Preprocessing

Enable Feature Preprocessing

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

Appr. Filament Diameter

An approximate minimal Filament diameter has to be provided.

Preserve Edges

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

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

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

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

Button **Cancel**

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

See also:

Menu Edit - [Show Display Adjustment](#)

Menu Image Processing - Thresholding - [Connective Baseline](#)

Surpass View - Filament - [Tab Draw](#) (for interactive filament tracing method)

13.5.2.12 Segmentation

Segmentation

Fill Cavities (fully enclosed volumes)

Check this box to avoid hollow regions. These regions would be enclosed by a Filament mesh, which is not desired in most of the cases.

Connective Baseline

Type in two values for the lower threshold (colored line) and upper threshold (red line). You can also click into the histogram. Left mouse button for lower, right mouse button for upper threshold or you can click & drag the respective line.

Simple thresholding methods classify voxels purely based on a single intensity value. This approach often does not lead to good results because the staining, the illumination, and the detection operate within a

precision range. The connective thresholding scheme takes care of this and searches for object voxels using an intensity range rather than a single intensity.

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

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

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament click on **Cancel**.

See also:

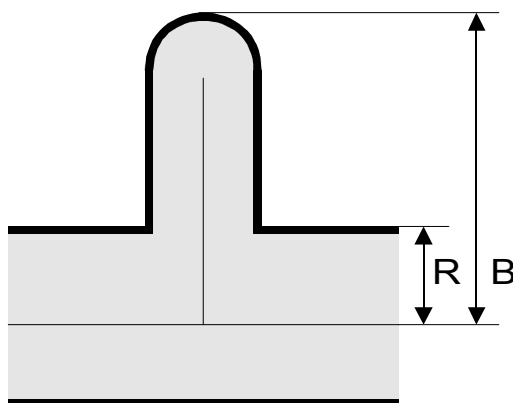
Menu Image Processing - Thresholding - [Connective Baseline](#)

13.5.2.13 Graph Compilation

Graph compilation

Minimal Ratio of Branch Length to Trunk Radius

Enter a minimal ratio of branch length to trunk radius (see figure below). Branches shorter than this value will be removed.



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

Find Dendrite Beginning Point

A dendrite beginning point has to be specified for specific statistic values (e.g. tree order). The point is selected from the trunk position with the biggest radius.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament click on **Cancel**.

13.5.2.14 Finish

Finish

To validate the automatically generated graph and the prior segmentation, you can inspect the result together with numerous Surpass modules: Ortho Slicer, Volume, Surfaces etc. You can optionally superimpose the segments with the original slices of the image stack using Imaris OrthoSlicers. OrthoSlicers are planes, which are perpendicular to any of the three spatial axes and extend throughout the entire image volume.

Build Filaments for all Time Points

Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters. They will be visible within the Surpass Tree. However the 3D Filament graph is only visible according to its time point.

Delete Working Channel

The Filament Working channel can be kept for other use. Principally it is not needed any more when finishing the Filament graph.

Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

This button is grayed in the last window of the Creation Wizard.

Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**

If you do not want to create Filaments click on **Cancel**.

13.5.3 Filament Properties

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

Tab Create

Only appears when you Add a new Filament.

Tab Settings

Change Filament appearance and inspect selection statistics.

Tab Creation

Re-enter the creation process.

Tab Draw

Manually draw filaments and connections.

Tab Edit

Select automatically filament parts and delete or smooth them.

Tab Statistics

Inspect all statistic values.

Tab Color

Change the color appearance (material) of the filament.

See also:

Surpass View - [Filament](#) (Add a new Filament)

Surpass View - Filament - [Tab Settings](#)

Surpass View - Filament - [Tab Creation](#)

Surpass View - Filament - [Tab Draw](#)

Surpass View - Filament - [Tab Edit](#)

Surpass View - Filament - [Tab Statistics](#)

Surpass View - Filament - [Tab Color](#)

13.5.4 Tab Settings

Style

Line**Cylinder****Cone**

The visualization of the graph is done as lines with a specified thickness (pixel), as cylinders (with a defined diameter), or cones (using the measured graph diameter).

Show Dendrites

Check the box in the heading to highlight dendrite points in the filament. The special points are highlighted with color-coded spheres.

Beginning Point

Check this box to highlight the dendrite beginning points.

Branch Points

Check this box to highlight the dendrite branch points.

Terminal Points

Check this box to highlight the dendrite terminal points.

Show Spines

Check the box in the heading to highlight spine points in the filament. The special points are highlighted with color-coded spheres.

Attachment Points

Check this box to highlight the spine attachment points.

Branch Points

Check this box to highlight the spine branch points.

Terminal Points

Check this box to highlight the spine terminal points.

Render Quality

With the slider you can adjust the render quality of the filament.

13.5.5 Tab Creation

Rebuild

Select from the list Algorithm to re-enter the creation process at the beginning of the [Filament Creation Wizard](#), but with all the prior parameter values recalled for re-use or editing.

For details please refer to chapter:

Menu Surpass - Filament - [Creation Wizard](#).

Button **Rebuild Objects**

Click on this button to start the rebuild.

Click on drop down window and two choices are available for re-entering the Filament Creation Wizard, depending on whether the existing Filament graph should be **discarded**, or **re-used** in combination with additional Filament detection. In the Filament Creation Wizard the same basic steps are executed and the filament structure is recreated according to the parameters in the selected Rebuild algorithm

Rebuild algorithm

1. Algorithm (delete data)
2. Algorithm (keep data)

1. Algorithm (delete data)

Re-enters the Filament Creation Wizard for creating a new filament. Previously created filament structure will be discarded.

2. Algorithm (keep data)

Re-enters the Filament Creation Wizard for creating a newly added filament that is merged with the existing one.

In step three of the Creation wizard – [Point Diameter](#), a new option exists for selecting a Starting Point.

Use existing filament

Detect a new starting point

Use existing filament- the previously determined starting point will be used in the new filament structure. Therefore, in the next creation step (Starting point threshold) an option for Manual Start point editing in (shift+right click) will not be available.

Detect a new starting point -a new starting point of the filament will be determined, based on the value given for the largest diameter.

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

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Filament computation can be repeated for the same region, a different region, or the entire image. Creation Parameters values are listed.

13.5.6 Tab Draw

Method

When it is not possible to use Imaris automatic filament creation algorithms, either semi-automatic or manual creation will allow you to create filament objects by manually marking the object location in using method AutoPath, AutoDepth or Manual.

AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available. If you select this method please refer to chapter [AutoPath](#) (Surpass View - Filament - Draw - AutoPath).

AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed). If you select this method please refer to chapter [AutoDepth](#) (Surpass View - Filament - Draw - AutoDepth).

Manual

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

Diameter

Here you adjust the filament diameter.

Type

Dendrite

Spine

Select as draw type Dendrite or Spine.

Button **Set Selection as Starting Point(s)**

If you select as Method **AutoPath** (see above, selection of the drawing Method) the button **Set Selection as Starting Point(s)** is available.

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

Slice

If you select as Method **Manual** (see above, selection of the drawing Method) the Slice parameter settings are available.

YZ Plane

XZ Plane

XY Plane

Select the plane orientation of the drawing board: yz, xz, xy.

Automatic Placement

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

x ... um

Display of the Slice the position.

Slider

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

See also:

Menu Edit - [Show Display Adjustment](#)

Surpass View - Overview - Camera Toolbar - [Pointer](#)

Surpass View - Filament - Draw - [AutoPath](#)

Surpass View - Filament - Draw - [AutoDepth](#)

Surpass View - Filament - Draw - [Manual](#)

13.5.6.1 AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available.

Drawing in AutoPath Mode on a PC:

Select AutoPath and Shift + right-click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.

Place the mouse cursor at the ends of the structure. The computed path is displayed.

Shift + click and the displayed path is definitely drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

PC Mouse & Keyboard Function Overview in the AutoPath Mode

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

Drawing in AutoPath Mode on a Mac:

Select AutoPath and Shift + Ctrl + click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.

Place the mouse cursor at the ends of the structure. The computed path is displayed.

Shift + click and the displayed path is definitely drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

Mac Mouse & Keyboard Function Overview in the AutoPath Mode

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

13.5.6.2 AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed).

Drawing in AutoDepth Mode on a PC:

Select AutoDepth, press the Shift-key and hold down the left mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. A pre-filtering would be then necessary.

PC Mouse & Keyboard Function Overview in the AutoDepth Mode

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

Drawing in AutoDepth Mode on a Mac:

Select AutoDepth, press the Shift-key and hold down the mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. A pre-filtering would be then necessary.

Mac Mouse & Keyboard Function Overview in the AutoDepth Mode

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

13.5.6.3 Manual

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

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

If you select Manual the Slice parameter settings are available.

Drawing in Manual Mode on a PC:

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

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

PC Mouse & Keyboard Function Overview in the Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ

Z Set the plane orientation to XY

Drawing in Manual Mode on a Mac:

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

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

Mac Mouse & Keyboard Function Overview in the Manual Mode

Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Ctrl + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

13.5.7 Tab Edit

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

Mouse Selects

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

Point

Point selects only the position near to the mouse pointer.

Segment

Segment selects the Filament between two vertices that are branch points, beginning point, or ending points.

Branch

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

Select Parts from Filament

There are various methods to make automatic selections on the whole Filament graph.

Button **Rel. Branch...**

Rel. Branch selects short parts of the Filament defined by the ratio of the branch length to trunk radius B/R (see figure above).

Button **Loops...**

Loops finds circular closed Filaments limited by a maximum length.

Button **Invert**

Invert selects all unselected parts of the graph and de-selects all selected parts.

Button **Abs. Branch**

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

Button **Path**

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

Button **Clear**

Clear removes the selection.

Process Selection

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

Button **Assign as Dendrite**

Click on this button to assign the selection as dendrite.

Button **Assign as Spine**

Click on this button to assign the selection as spine.

Button **Assign as Dendrite Beginning Point**

Click on this button to assign the selection as dendrite beginning point.

Button **Smooth**

Removes the roughness of the current selection. It affects the position and the diameter of all segments except the branch points and end points.

Button **Center**

Click on this button to re-center the filament. The position of the center axis is refined according to the peak intensity in the vicinity of the initial position.

Button **Diameter...**

Click on this button to recalculate the filament diameter. The window Calculate Diameter (see below) opens.

Button **Duplicate**

Click on this button to copy the selection into a new Filament item. The new Filament item appears in the Surpass Tree.

Button **Join**

Click on this button to join two unconnected filament points or segments. Select the objects to be joined in the image by Ctrl + left-click (Mac: Command+ left-click). The selected point or segment will be highlighted, then click Join. A new filament structure will be created structurally joining previously unconnected points or segments.

Button **Delete**

Click on this button to delete the current selection.

Window: Calculate Diameter

Small Diameter

Type-in the smallest Filament diameter in the selected region.

Large Diameter

Type-in the largest Filament diameter in the selected region.

Contrast Threshold

Type-in the contrast threshold in the selected region.

Process Filament

The following buttons apply to the entire Filament.

Button **Smooth**

Smooth removes roughness from the Filament path. It affects the position and the diameter of all segments except the branch points and end points.

Button **Center**

Click on this button to re-center the filament. The position of the center axis is refined according to the peak intensity in the vicinity of the initial position.

Button **Diameter...**

Click on this button to recalculate the Filament diameter. The window Calculate Diameter (see above) opens.

Button **Split**

Unconnected parts of the Filament can be split into separate Filament objects. The new objects will appear as new Items in the Surpass Tree. This allows individual coloring and individual statistics.

Button **Merge...**

In Imaris it is possible to merge two or several Filament objects into one Filament object. This button is only available if you have two or more Filament items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Filament X" displays (see below).

Button **Export**

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

Window: Merge selected Objects to "Filament X"

Check the boxes in front of the desired Filaments or use the button **Select All**.

Button **Clear All**

Use this button to clear the Filaments selection.

Button **OK**

If you want to merge the Filaments click on **OK**.

Button **Cancel**

If you do not want to merge the Filaments click on **Cancel**.

Connect & Translate selected Edges (Vertices)

Check this box to connect and translate selected edges.

13.5.8 Tab Filter

Please note: First of all you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Dendrite) is displayed at the bottom of the threshold histogram.

Duplicate Selection to New Filament Button

Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:

13.5.9 Tab Statistics

Filament statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - [Filament](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall

b) Detailed

c) Selection

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.


Tab Overall

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

Tab Detailed

A pull down list offers three types of **Detailed** statistical calculations:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable. Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values

If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Tab Selection

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers three types of statistical calculations from a Selection:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Filament](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

Highlights the object in the viewing area

Highlights the object on the tab Detailed


Displays of the values on the tab Selection

Button **Filament** - Duplicate Selection to new Filament.

Click on this button and all selected elements are copied to a new Filament object. The new Filament appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Filament](#)

13.5.10 Tab Color

Tab Color edit, manage and assign colors material to the Filament object.

Object Type

Choice of Object type is presented in drop down window. Select either Dendrite or Spine and depending on the chosen object type selection of the variables is listed.

The Two color types are available:

[Base](#)

[Statistics Coded](#)

13.5.10.1 Color Type-Base

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.5.10.2 Color Type-Statistics Coded

Statistics Coded

Depending on the object type selection Statistical variable selection is listed in drop down window. For example, if Object type is chosen to be Dendrite, available Statistical type will be Dendrite linked variable.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area.

Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

Show Title

Check this box to see the title in the statistics colorbar.

Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**

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

Button **Load**...

Click on the button **Load** to display the color Table File box. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for colorbar.

Min - lower color limit

Max - upper color limit

Button **Auto**

Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

13.5.11 Mouse & Keyboard PC

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

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

AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:

Addendum - [Mouse & Keyboard PC](#)

13.5.12 Mouse & Keyboard Mac

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

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

AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Ctrl + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)

X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.6 Frame

The Frame serves to outline the object in the viewing area. In the Surpass Tree check the check-box Frame to display the frame in the viewing area. Un-check the box to make the frame invisible.

How to Add a new Frame?

Select menu Surpass - Frame.

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

13.6.1 Tab Settings

Highlight Frame in the Surpass Tree and click on the tab Settings. The Frame Settings display in the properties area.

Box and Time Settings

Box

Check the parameter to display a box around the Frame.

Line Width

Adjust the box line width with the respective slider.

Shading

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

Shadow

The entire Surpass scene can produce shadows on the three planes of the Frame object. Check this box to display the shadows. Use the Shading slider (see above) to adjust the contrast. The rendering is done in real-time, and it requires a good performance of the graphics board (depending on the complexity of the scene).

Time Colorbar

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

Grid and Tickmarks Settings

Grid

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

Tickmarks

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

Fix to Data Set

Check Fix to Data Set to fix the Frame to the same side when navigating the object.

Spacing

Specify the grid and tickmark spacing.

Labels Settings

Axis Labels

Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked the parameter Label Color and Label Font is available (see below).

Label Color

Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Color Editor window to change the color of the label on the coordinate axes.

Label Font

Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Font Editor window to change the font to label the coordinate axes.

13.6.2 Tab Color

Tab Color edit, manage and assign colors material to the Frame.

13.7 Group

Groups are folders in the Surpass Tree that contain other Surpass objects. They are visible in the objects area and allow you to create a treelike hierarchy of Surpass objects. When the first Surpass object is created, it is automatically placed in a Group called Surpass Scene. Additional Groups can be created in order to sort objects of the scene.

How to Add a new Group?

Select menu Surpass - Group.

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

How to Add Items in a new Group?

Click on an Item in the Surpass Tree list and drag it onto the Group object. Once released, the Item is added to the Group.

Like all other objects, Groups have a visibility check-box. This box affects the visibility of all objects in the Group.

See also:

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Overview - [Scene File Concept](#)

13.7.1 Tab Settings

Number of Objects

Displays the number of objects in the Group.

Button **Show All**

If you click this button the check-box(es) of the Item(s) in the Group are checked.

Button **Hide All**

If you click this button the check-box(es) of the Item(s) in the Group are un-checked.

13.7.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.7.3 Tab Statistics

The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations. To perform further analysis of large amounts of statistical data, use the Group Level Statistic. The Statistics tab collects and displays the Group's statistical values, which are lists of the statistical value summaries of all created objects contained in the specified Group.

For further analysis select the Export button and the statistic values of all objects are exported as a single file.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall

b) Detailed

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

Tab Overall

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Tab Detailed

A pull down list offers three types of Detailed statistical calculations:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Settings Button -Configure a List of visible statistics values

Click on the button Settings and switch to the window Preferences

Search Button - Search by object ID

Type in the object ID in the data field. Click on the button Search.

Highlights the object in the viewing area

Highlights the object on the tab Detailed

Displays of the values on the tab Selection

Export Button - Export Statistics on Tab Display to File

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically.

Export Button - Export All Statistics to File

All of the statistics (that are enabled in the statistics settings) will be exported.

With a click on the button Export the window Export Statistics opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

13.8 Light Source

A Light Source serves to illuminate objects in the viewing area. A light source node serves as the location and orientation of a light source.

How to Add a new Light Source?

Select menu Surpass - Light Source.

The Surpass Tree list is automatically updated and in the Light Source - Properties the Settings parameter are displayed. Multiple light sources can be added in the Surpass Tree.

Move Light Source

To change the Light Source position and direction, choose the pointer mode Select and drag the cone or line.

Light Intensity

Click on the cylinder above the cone and move it closer or further away from the object to change the light intensity.

See also:

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

13.8.1 Tab Settings

Fix to Data Set

Check the box to fix the Light Source to the same side when navigating the object. When the box is un-checked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

13.8.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.8.3 Mouse & Keyboard PC

Light Source Mouse & Keyboard Functions

Left-click cone (or line) & drag	Change direction
Left-click cylinder & drag	Change intensity

See also:

Addendum - [Mouse & Keyboard PC](#)

13.8.4 Mouse & Keyboard Mac

Light Source Mouse & Keyboard Functions

Click cone (or line) & drag	Change direction
Click cylinder & drag	Change intensity

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.9 Measurement Point

How to Add a new Measurement Point?

Select menu Surpass - Measurement Point.

The Surpass Tree list is automatically updated and in the Measurement Point - Properties the Edit parameters are displayed.

13.9.1 Tab Settings

Point Shape

Tetrahedron

Cube

Sphere

Here you can choose from three different shapes for the Measurement Points (tetrahedron, cube, or sphere).

Filled

Check the box and the point shape is solid.

Point Diameter

Here you can define the Measurement Point diameter.

Point Labels

Name

Check the check-box to display the name of a Measurement Point in the viewing area.

Intensity

Check the check-box to display the intensity of a Measurement Point in the viewing area. The intensity of the channel is displayed in brackets.

Line Mode

Pairs (AB, CD, ...)

Any two consecutive points are connected by a line.

Polygon (ABCD ...)

All points in the Group are connected consecutively (P1-P2-P3-P4, etc.).

Line Width

Here you define the line width.

Line Labels

Distance

The measured distance between two points is displayed next to the line connecting the two Measurement Points. Check the check-box to display the distances in the viewing area.

Velocity

Only accessible if you have a time data set. Check the box to display the speediness between two Measurement Points.

Tip: If you do not have the module ImarisTrack you can easily use the velocity function to track single objects over several time points and measure the velocity.

Button **Color**

Button **Font**

Label Color and Font

To change the color or font of the label (display of the line length) click on the respective icon.

Tip: You can find the parameters to change the color of the Measurement Points and connecting lines on the tab Colors.

Visible for all Time Points

Only accessible if you have a time data set. Check the box to display the inserted Measurement Points, lines, and annotations at all time points.

See also:

Surpass View - Measurement Point - [Tab Color](#)

13.9.2 Tab Edit

Autodepth Position

In the mouse pointer mode Select:

Shift-click with the left mouse button to add a new point. Left-click to select an existing point. Shift-left-click to reposition a selected point.

Intersect with

The first two parameters are relevant if you want to insert Measurement Points in a volume rendered data set. For the rest the last parameter is crucial.

all visible Channel

In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this parameter and all visible channel are relevant for the placement of the Measurement Points.

specific Channels

In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this option and choose the channel directly from the drop down list.

The selected channel is relevant for the placement of the Measurement Points.

solid Objects

Check this box and the Measurement Points are placed on the surface of an object. Un-check the box and you can also place Measurement Points in an object.

Button **Delete All Points**

Click on this button to delete all Measurement Points. Alternatively click on the Delete-key on the keyboard.

Selected Point

Click to select an existing point. If no point is selected the following parameters are grayed. Re-click on a

selected point to un-select the point.

Button **Delete**

Click on this button to delete the selected Measurement Point.

Name

Text field to edit the Measurement Point name.

Position

X

Y

Z

T

Data field of the x-, y-, z- and t-coordinates.

See also:

Menu Edit - [Show Display Adjustment](#)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Select)

13.9.3 Tab Intensity

Imaris reads out the intensity profile of each voxel against the distance. Here you select the channels that should be displayed in the histogram and you choose the desired connecting lines between the Measurement Points.

Histogram

The histogram displays the intensity profile along the lines. The labeled lines represent the Measurement Points. The x-axis represents the length of the profile. On the left hand side of the histogram you find the display of the channel(s) intensity range. The channel selection is located below the histogram. Here you can switch channels in the intensity profile on or off. Below the channel selection you find the profile selection. In the drop-down list select the desired line and the histogram updates accordingly.

Button **Snapshot**

Click on this button to do a Snapshot of the histogram. The window Save Snapshot as... opens.

See also:

Menu File - Save as... [Snapshot](#)

13.9.4 Tab Statistics

Measurement Point statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - [Measurement Points](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall

b) Detailed

c) Selection

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.






Tab Overall

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

Tab Detailed

A pull down list offers three types of **Detailed** statistical calculations:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values

If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Tab Selection

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers three types of statistical calculations from a Selection:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Buttons at the bottom of the Statistics tab

Button **Settings** - Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Measurement Points](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

Highlights the object in the viewing area

Highlights the object on the tab Detailed


Displays of the values on the tab Selection

Button **Measurement Points** - Duplicate Selection to new Measurement Points.

Click on this button and all selected elements are copied to a new object. The new Measurement Points appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

Buttons Overview

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click

See also:

Menu Edit - Preferences... - Statistics - [Measurement Points](#)

13.9.5 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.9.6 Mouse & Keyboard PC

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + left-click	Add a new measurement point
Shift + left-click	Reposition selected measurement point
Left-click on existing point	Select measurement point
Mouse wheel in Add mode	Turn wheel up: Increase size of next measurement point
	Turn wheel down: Decrease size of next measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of the shape around the mouse pointer changes accordingly. This helps a lot in handling 3D data sets.

See also:

Menu Edit - Preferences ... - [3D Cursor](#) (adjust 3D Cursor Shape)

Addendum - [Mouse & Keyboard PC](#)

13.9.7 Mouse & Keyboard Mac

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + click	Add a new measurement point
Shift + click	Reposition selected measurement point
Click on existing point	Select measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of shape around the mouse pointer changes accordingly. This helps a lot in handling 3D data sets.

See also:

Menu Edit - Preferences ... - [3D Cursor](#) (adjust 3D Cursor Shape)

Addendum - [Mouse & Keyboard Mac](#)

13.10 Oblique Slicer

The Oblique Slicer represents a plane that can be freely moved and rotated within the data set and allows you to look inside any object. The Oblique Slicer consists of a colored frame with a white double rod in the middle.

How to Add a new Ortho Slicer?

Select menu Surpass - Oblique Slicer.

The Surpass Tree list is automatically updated and in the Oblique Slicer - Properties the Settings parameter are displayed. The Oblique Slicer is displayed in the viewing area (depending on the default parameter on the tab Settings).

Change Position of the Oblique Slicer

You can change the position of the Oblique Slicer. Choose the pointer mode Select, highlight the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Oblique Slicer to the required position.

Rotate Oblique Slicer around Axes

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

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

Surpass View - Ortho Slicer - [Tab Settings](#)

13.10.1 Tab Settings

Orientation

Button **YZ Plane**

Button **XZ Plane**

Button **XY Plane**

Change the Slicer direction, if required, by selecting the adequate plane.

Position

Button **Recenter**

Click on this button to re-center the Slicer.

Extended Section

The section is a single cut through the data set, the colored lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame

Check the box to display a colored frame around the Slicer.

Camera

Button **Orthogonal View**

The camera is set to a position which is perpendicular to the Slicer.

13.10.2 Mouse & Keyboard PC

Oblique Slicer Mouse & Keyboard Functions

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

See also:

Addendum - [Mouse & Keyboard PC](#)

13.10.3 Mouse & Keyboard Mac

Oblique Slicer Mouse & Keyboard Functions

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

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.11 Ortho Slicer

Ortho Slicer represent an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. When adding slices, Surpass suggests per default the directions z, x, and y as sequence for the first three and all subsequent slices.

How to Add a new Ortho Slicer?

Select menu Surpass - Ortho Slicer.

The Surpass Tree list is automatically updated and in the Ortho Slicer - Properties the Settings parameter are displayed. The first Slice is displayed in the viewing area (depending on the default parameter on the tab Settings).

See also:

Surpass View - Ortho Slicer - [Tab Settings](#)

13.11.1 Tab Settings

Slice Orientation

YZ Plane

XZ Plane

XY Plane

Change the slice direction if required by selecting the adequate plane.

Extended Section

The section is a single cut through the data set, the colored lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame

Check the box to display a colored frame around the active slice.

Slice Position

Slider

Choose the relative position of the Slice in the data set by means of the slider or a direct entry of the slice position given as the offset distance from the data set origin (equals the absolute coordinates).

13.11.2 Mouse & Keyboard PC

Ortho Slicer Mouse & Keyboard Functions

Left-click plane & drag	Change slice position
Right-click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section
Arrow up	Change slice position up
Arrow down	Change slice position down

See also:

Addendum - [Mouse & Keyboard PC](#)

13.11.3 Mouse & Keyboard Mac

Ortho Slicer Mouse & Keyboard Functions

Click plane & drag	Change slice position
Ctrl + click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.12 Spots

The Spots object is available to model point-like structures in the data. It provides a procedure to automatically detect point-like structures, an editor to manually correct detection errors, a viewer to visualize the point-like structures as spheres, and statistics output.

How to Add new Spots?

Select menu Surpass - Spots.

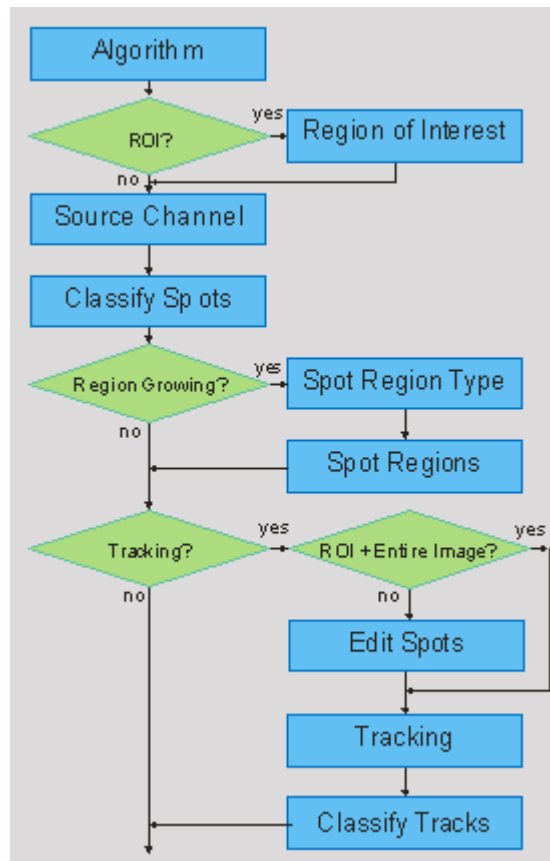
The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Spots - Properties.

See also:

Suprass View - Spots - [Creation Wizard](#)

13.12.1 Creation Wizard

This is the flow diagram of the Spots Creation Wizard:



In the first window of the Creation Wizard you find the algorithm settings. Here you have to decide, if you want to segment only a **Region of Interest (ROI)**, if you want to use the **Region Growing** for the Spot detection, and if you want to create **Tracks** after the **Spot** detection.

For further details please refer to chapter [Algorithm](#), [Region of Interest](#), [Source Channel](#), [Classify Spots](#), [Spot Region Type](#), [Spot Regions](#), [Edit Spots](#), [Tracking](#), and [Classify Tracks](#).

13.12.1.1 Algorithm

Algorithm Settings

If you do not want to use the automatic Spot creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button

Terminate the creation wizard and activates the manual Spots creation.

Segment only a Region of Interest

Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and

analyzes. Defining and processing ROI allows other parts of an image to be unchanged. Check this box if you want to segment only a [Region of Interest](#). If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally

Process entire Image finally this option appears only, if Segment only a Region of Interest is checked. Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters ([Complete ROI](#)). Un-check this box and only the region of interest is processed.

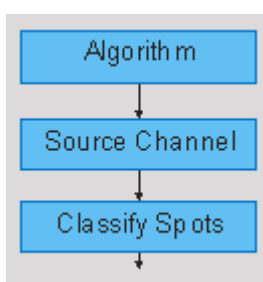
Different Spot Sizes (Region Growing)

Check this box if you want to apply the region growing, starting on the tab [Spot Region Type](#).

Track Spots (over Time)

Check this box if you want to track Spots over time, for details please refer to tab [Tracking](#).

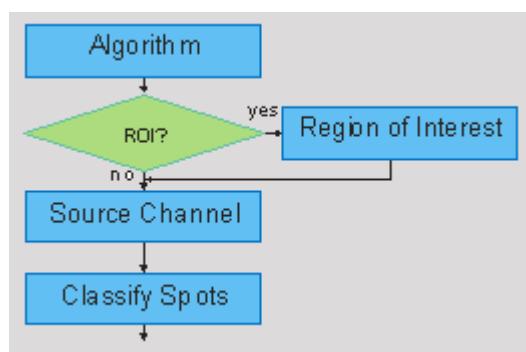
Some Examples



nothing checked

Structure of the Creation Wizard is:

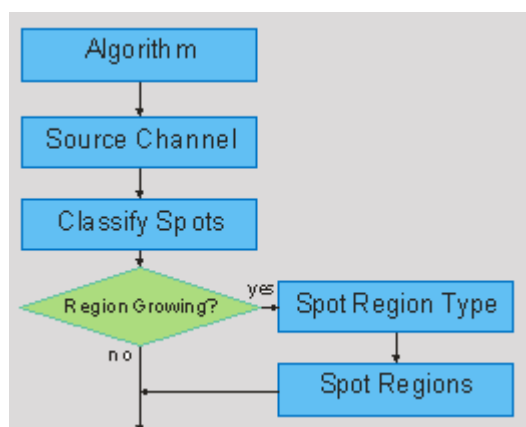
- 1/3 [Algorithm](#)
- 2/3 [Source Channel](#)
- 3/3 [Classify Spots](#)



ROI

Structure of the Creation Wizard is:

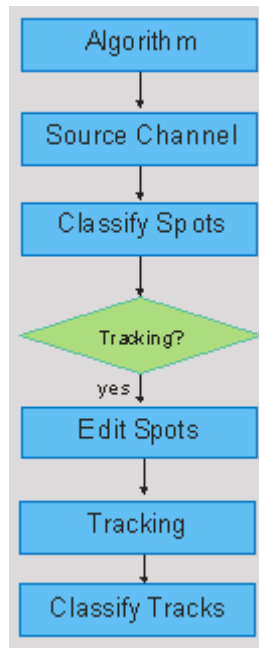
- 1/4 [Algorithm](#)
- 2/4 [Region of Interest](#)
- 3/4 [Source Channel](#)
- 4/4 [Classify Spots](#)



Region Growing

Structure of the Creation Wizard is:

- 1/5 [Algorithm](#)
- 2/5 [Source Channel](#)
- 3/5 [Classify Spots](#)
- 4/5 [Spot Region Type](#)
- 5/5 [Spot Regions](#)

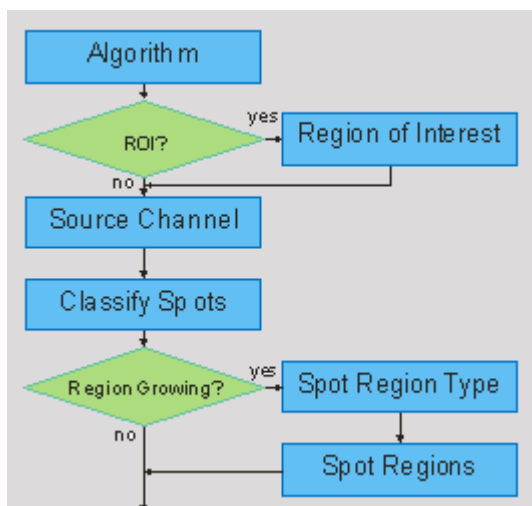


Tracking

Structure of the Creation Wizard is:

- 1/6 [Algorithm](#)
- 2/6 [Source Channel](#)
- 3/6 [Classify Spots](#)
- 4/6 [Edit Spots](#)
- 5/6 [Tracking](#)
- 6/6 [Classify Tracks](#)

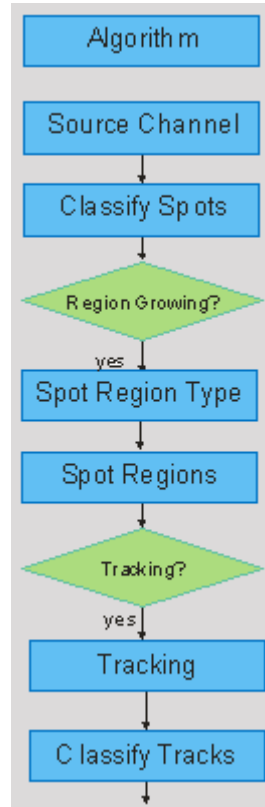
Combinations



ROI and Region Growing

Structure of the Creation Wizard is:

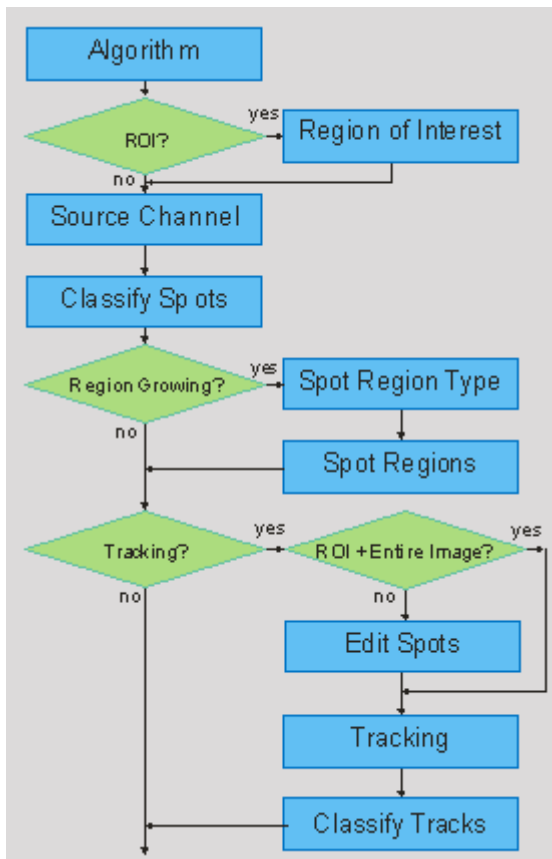
- 1/6 [Algorithm](#)
- 2/6 [Region of Interest](#)
- 3/6 [Source Channel](#)
- 4/6 [Classify Spots](#)
- 5/6 [Spot Region Type](#)
- 6/6 [Spot Regions](#)



Growing and Tracking

Structure of the Creation Wizard is:

- 1/7 [Algorithm](#)
- 2/7 [Source Channel](#)
- 3/7 [Classify Spots](#)
- 4/7 [Spot Region Type](#)
- 5/7 [Spot Regions](#)
- 6/7 [Tracking](#)
- 7/7 [Classify Tracks](#)



ROI, Region Growing and Tracking

not Entire Image

Structure of the Creation Wizard is:

- 1/9 [Algorithm](#)
- 2/9 [Region of Interest](#)
- 3/9 [Source Channel](#)
- 4/9 [Classify Spots](#)
- 5/9 [Spot Region Type](#)
- 6/9 [Spot Regions](#)
- 7/9 [Edit Spots](#)
- 8/9 [Tracking](#)
- 9/9 [Classify Tracks](#)

ROI, Region Growing and Tracking

plus Entire Image

Structure of the Creation Wizard is:

- 1/9 [Algorithm](#)
- 2/9 [Region of Interest](#)
- 3/9 [Source Channel](#)
- 4/9 [Classify Spots](#)
- 5/9 [Spot Region Type](#)
- 6/9 [Spot Regions](#)
- 7/9 [Tracking](#)
- 8/9 [Classify Tracks](#)
- 9/9 [Complete ROI](#)

Spots Wizard Buttons

Button **Back**

This button is grayed in the first window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots click on **Cancel**.

13.12.1.2 Region of Interest

Region of Interest 1

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.

X:, Y:, Z:, Time

Min:, Max:, Size

Minimum, maximum and Size of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields.

Alternatively the location and size of the ROI can be adjusted directly in the viewing area. Click on the arrows in the ROI preview rectangle (change to the pointer mode **Select**) and drag it to define new coordinates for X and Y. Location of the ROI can be adjusted by clicking inside the ROI preview rectangle and dragging (with mouse button held) to the chosen image location. The numbers in the value fields are updated automatically.

Memory Usage:

Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:

Display of the memory usage for the image size within the ROI.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots automatically click on **Cancel**.

See also:

Surpass View - Overview - [Camera Toolbar](#) (change to pointer mode Select)

13.12.1.3 Source Channel

Source Channel

Select the respective source channel, from which Spots are to be computed.

Spot Detection

Estimated Diameter

Spots with diameter smaller than this are not detected. This is also the diameter of the inserted Spots.

Background Object Subtraction

If checked, the background will be removed prior to spot detection. Technically background subtraction smoothes the image. The intensity is the one of a Gaussian filtered channel (gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of spot radius.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots click on **Cancel**.

13.12.1.4 Classify Spots

The Spots filtering is incorporated into the creation process.

A filtering allows removal of objects that would interfere with interpretation of results.

Quality: The 'Quality' is the intensity at the center of the spot in the channel the spot was detected.

If 'Background Subtraction' was not checked this is the intensity of the channel Gaussian filtered by 3/4 of spot radius.

If 'Background Subtraction' was active the intensity is the one of a Gaussian filtered channel from above minus the intensity of the original channel Gaussian filtered by 8/9 of spot radius.

The Filter type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Spots) is displayed at the bottom of the threshold histogram.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots click on **Cancel**.

13.12.1.5 Spot Region Type

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked ([Algorithm Settings](#)).

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

Spot Regions from

Absolute Intensity

Local Contrast

You can set the border using the Absolute Intensity or the Local Contrast as criteria. In both cases as next step a working channel is displayed in the viewing area.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots click on **Cancel**.





13.12.1.6 Spot Region

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked ([Algorithm Settings](#)).

Spot Regions

The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local Contrast.




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Absolute Intensity

Automatic

In the working channel a copy of the intensity histogram of the source channel is displayed. Regions above the threshold are displayed in white. Now you adjust the threshold of the working channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

Local Contrast

Automatic

In the working channel the regions with a local contrast brighter than the background are displayed in white. Now you adjust the threshold value of the local contrast channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

Diameter from

Region Volume

The Spot(s) radius is calculated from the region volume (the region volume and the Spot volume are equal).

Region Border

The radius of the calculated Spot(s) is the smallest distance from each Spot center to the region border.

Create Color Coded Region Channel

Additional to the Spots the Region Channel is displayed. Each region has a different color.

Preview

Here you select the preview settings.

Ortho Slicer

Check this box if you want to see the ortho slicer in the preview.

Volume Rendering

Check this box if you want to see a volume rendering in the preview.

Slicer Plane

XY, XZ, YZ

If you check the box Ortho Slicer (see above) these radio buttons are available to select the slicer plane.

Show Spots

Check this box to see the spots in the preview.

Button **Change Regions Color**

Click on this button to change the regions color.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Spots click on **Cancel**.

13.12.1.7 Edit Spots

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

Autodepth Draw

Intersect with

The auto depth function will automatically calculate the Spot depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spot.

all visible Channel

specific Channel - selected channel will be used for Spots depth computation

solid Objects

Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Process Selection

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click

Delete Spot	Left-click to select, then click on the the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

Button **Duplicate**

Click this button and all selected Spots appear in a new group in the Surpass Tree (Spots Selection).

Button **Recenter**

Click on the Recenter button results in change of the selected Spots position. The new center is placed in position of the local intensity maximum in the vicinity of the initial spot center.

Button **Delete**

Click on this button to delete the selected Spot(s).

Button **Clear**

Click this button to clear the Spot selection.

No Spot Selected/Selected Spot

Position X: Y: Z: [um]

Display of the Spot position.

Diameter [um]

The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index

Display of the Spot position.

Spots

In Imaris it is possible to merge two or several Spots items into one Spots item.

Button **Merge ...**

This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

Window: Merge selected Objects to "Spots X"

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **Clear All**

Use this button to clear the Spots selection.

Button **OK**

If you want to merge the Spots click on **OK**.

Button **Cancel**

If you do not want to merge the Spots click on **Cancel**.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Tracks click on **Cancel**.

See also:

Surpass View - Overview - [Camera Toolbar](#) (Pointer Navigate/Select)

13.12.1.8 Tracking

This tab only appears, if **Track Spots (over Time)** is checked ([Algorithm Settings](#)).

Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions the best matches to candidate Spots can be determined. All of the algorithms except for Connected Components use a Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernable pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert

This algorithm models the motion of each Spot as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components

For this algorithm, Spots in adjacent time-points are considered connected if the Spot spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). This is the only algorithm that automatically handles lineage (spots that split or merge). It will only work with images that have excellent time resolution, because the Spot cannot ever jump a distance larger than its own size.

Parameters

Max Distance

The parameter Maximum Distance disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.

The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

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

Intensity Weight

Based on the information obtained from the measured Spots intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

$$\text{TotalCost} = \sum(\text{all connections}) \text{DistanceCost} + \text{IntensityWeight} * \text{IntensityCost}$$

The parameter is only available if you select Autoregressive Motion Expert (see above).

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Tracks automatically click on **Cancel**.

13.12.1.9 Classify Tracks

The Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Spots (over Time)** is checked ([Algorithm Settings](#)).

A filtering allows removal of objects that would interfere with interpretation of results.

The Filter type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of the selected objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Tracks) is displayed at the bottom of the threshold histogram.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**

If you do not want to create Tracks click on **Cancel**.

13.12.1.1 Complete ROI

This tab only appears, if **Process entire Image finally** is checked ([Algorithm Settings](#)).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Spots Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

This button is grayed in the last window of the Creation Wizard.

Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**

If you do not want to create Spots/Tracks click on **Cancel**.

13.12.2 Tab Settings

On the tab Settings you can adjust the rendering quality to produce faster performance of the display when the number of Spots is very large. The size of the Spots can be changed via the Radius Scale box.

Spots Style/Quality

Off

Select this radio button and the Spots are turned off. They are not visible any more in the viewing area.

Sphere

The Spots display as spheres.

Center Point

The Spots display as center points.

Please note: The computation of Spheres is time consuming. If you have many Spots in your data set Imaris automatically switches to the display mode Center Points. If you would like to switch to the Spheres mode again select Sphere and confirm the changes.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Off

Select this radio button and the Tracks are turned off. They are not visible any more in the viewing area.

Line

The connections are shown as lines.

Cylinder

The connections are shown as cylinders.

Width

Diameter

Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality

When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Tracks Path

Color Editor

Select a desired color for the Track display.

Displacement

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale

Edit field for the displacement vector radius.

Color Editor

Select a desired color for the displacement arrow.

Dragon Tail

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider

Adjust the number of time points using the slider.

13.12.3 Tab Creation

Rebuild

Select from the list on which step of the [Spots Creation Wizard](#) you want to re-enter the creation process.

For details please refer to chapter:

Menu Surpass - Spots - Creation Wizard - [Algorithm](#)

Menu Surpass - Spots - Creation Wizard - [Region Growing](#)

Menu Surpass - Spots - Creation Wizard - [Tracking](#)

Button **Rebuild Objects**

Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Spots computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator with all adjusted parameter values.

13.12.4 Tab Edit

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

Autodepth Draw

Intersect with

The auto depth function will automatically calculate the Spot depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spot.

all visible Channel

specific Channel - selected channel will be used for Spots computation

solid Objects

Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Selection

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click
Delete Spot	Left-click to select, then click on the the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

Duplicate Button

Click this button and all selected Spots appear in a new group in the Surpass Tree (Spots Selection).

Recenter Button

Clicking on the Recenter button changes the position of the selected Spots. The new center is placed in the position of the local intensity maximum, which is in the vicinity of the initial spot center.

Connect Track Button

Click this button to connect two existing tracks. Select two Spots in two different tracks at the subsequent time points (just one Spot per time point) and click on the button Connect Track. The connection is automatically created between all selected objects.

Disconnect Track Button

Select a Spot and click on the button Disconnect Track. The connection from the selected Spot to the next Spot in sequence is deleted (the connection after the Spot).

Correct Drift Button

If you have an object drift (e.g. a cell drift) during image acquisition you can use this button to apply a drift correction for the whole data set. Select a reference Track. A click on the button Correct Drift will translate the entire image in such a way that the selected object remains stationary in the corrected image. The result is directly visible in the viewing area.

Edit Parameters

Crop largest common region after drift

For 3D visualization a compact image stack is usually preferred. This parameter leaves only the gray values in the common region, which is defined in all layers. The rest is set to zero and cut away.

Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction.

Please note: The drift correction changes the data set.

Button **Delete**

Click on this button to delete the selected Spot(s).

Button **Clear**

Click this button to clear the Spot selection.

No Spot Selected/Selected Spot

Position X: Y: Z: [um]

Display of the Spot position.

Diameter [um]

The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index

Display of the Spot position.

Spots

In Imaris it is possible to merge two or several Spots items into one Spots item.

Create Track from Selection... Button

Clicking this button creates a track for the selected Spot(s).

- Set the **Mouse selects** option to **Spots**.
- Add new Spot with Shift+ left click or select the already created one.
- Clicking on the **Create Track from Selection** button brings up the Create track from Selection Window

Create track from Selection Window

1. Select the Source channel

2. Spot Detection

Estimated Diameter

This is the diameter of the inserted Spots. Spots with a diameter smaller than this are not detected. .
There are two options for a Surface creation Threshold:

Background Subtraction

If checked, the background will be removed prior to spot detection.

3. Tracking algorithm

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

There are two algorithms to choose from:

Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernable pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

4. Max Distance

The parameter Maximum Distance disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.

5. Max Gap Size

It can happen that the object segmentation fails for some time points. This can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Track if it reappears. The number defines the maximum consecutive time points until the object re-appears.

6. End Time Point

The parameter specifies the last time point for the tracking algorithm implementation.

OK Button

Cancel Button

Button Merge ...

This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

Window: Merge selected Objects to "Spots X"

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **Clear All**

Use this button to clear the Spots selection.

Button **OK**

If you want to merge the Spots click on **OK**.

Button **Cancel**

If you do not want to merge the Spots click on **Cancel**.

See also:

Surpass View - Overview - [Camera Toolbar](#) (Pointer Navigate/Select)

13.12.5 Tab Filter

Please note: First of all you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are

considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of the selected objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Spots) is displayed at the bottom of the threshold histogram.

Button **Duplicate Selection to New Spots**

Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:

Surpass View - Overview - [Surpass Tree](#)

13.12.6 Tab Statistics

Spots statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - [Spots](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall

b) Detailed

c) Selection

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.






Tab Overall

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of

statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

Tab Detailed

A pull down list offers three types of **Detailed** statistical calculations:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Statistical variable classification:

1. Statistical variables associated with objects (Spots, Surface-volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables Average values are displayed per time point. Changing time point, statistical variable value and image would change consequently.

2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement, track intensity). Track statistical values are not time dependent, since connecting moving object traces in image time series creates Tracks.

All values

If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Tab Selection

A pull down list offers three types of statistical calculations from a Selection:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Buttons Overview

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Spots](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

Highlights the object in the viewing area

Highlights the object on the tab Detailed


Displays of the values on the tab Selection

Button Spots - Duplicate Selection to new Spots.

Click on this button and all selected elements are copied to a new Spots object. The new Spots appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xml). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Spots](#)

13.12.7 Tab Color

Tab Color edit, manage and assign colors material to the Spots or Tracks.

Object Type

Choice of Object type is presented in drop down window. Select either Point (for Spots) or Tracks and depending on the chosen object type selection of the variables is listed.

For **Object Type Points** two color types are available:

[Base](#)
[Statistics Coded](#)

For **Object Type Tracks** three color types are available:

[Base](#)
[Statistics Coded](#)
[Time Mapped](#)

13.12.7.1 Color Type-Base

Base

Assign the Spots/Track color via the Color Editor. Tab Color edit, manage and assign material colors of a Spots/Track.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.12.7.2 Color Type -Statistics Coded

Statistics Coded

Depending on the chosen object type (either Point or Tracks) Statistical variable selection is listed in drop down window. For example, if Point is chosen as Object type, available Statistical data will be Point linked variable. Similarly, variable for Tracks will be listed, if object type is selected to be Tracks.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is colorbar range for Points and lower one is colorbar for the Tracks.

Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

Show Title

Check this box to see the title in the statistics colorbar.

Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**

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

Button **Load** ...

Click on the button **Load** to display Table File box is displayed. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for colorbar.

Min - lower color limit

Max - upper color limit

Button **Auto**

Click the button **Auto** the system detects the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

13.12.7.3 ColorType -Time Mapped

Time Mapped

This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage

from 0% (complete opacity) to 100% (complete image transparency).

13.12.8 Mouse & Keyboard PC

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Left-click
Select multiple Spots	Ctrl + left-click
Add new Spot	Shift + left-click
Delete Spot	Left-click to select, then click on the the button Delete
Change Spot diameter	Mouse wheel

See also:

Addendum - [Mouse & Keyboard PC](#)

13.12.9 Mouse & Keyboard Mac

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Click
Select multiple Spots	Command + click
Add new Spot	Shift + click
Delete Spot	Click to select, then click on the the button Delete
Change Spot diameter	Mouse wheel

See also:

Addendum - [Mouse & Keyboard Mac](#)

13.13 Surfaces

The Surfaces visualization is a computer-generated representation of a specified gray value range in the data set. It creates an artificial solid object in order to visualize the range of interest of a volume object.

How to Add a new item Surfaces?

Select menu Surpass - Surfaces.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Surfaces - Properties.

See also:

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Surfaces - [Creation Wizard](#)

13.13.1 Creation Wizard

In the first window of the Creation Wizard you find the algorithm settings. Here you have to decide, if you want to segment only a **Region of Interest (ROI)**, use the **Seeded Region Growing** for the Surfaces detection, create **Tracks** after the **Surfaces** detection or Skip automatic creation, and go to [Contour](#) drawing .

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button

Terminate the creation wizard and activates the manual Surface creation.

For further details please refer to chapter [Algorithm](#), [Region of Interest](#), [Source Channel](#), [Threshold](#), [Split](#)

[touching Objects \(Region Growing\)](#), [Classify Seed Points](#), [Classify Surfaces](#), [Tracking](#), [Edit Surfaces](#), [Classify Tracks](#), and [Complete ROI](#).

13.13.1.1 Algorithm

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button

Terminate the creation wizard and activates the manual Surface creation.

Algorithm

Segment only a Region of Interest

Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Check this box if you want to segment only a [Region of Interest](#). If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally

Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.

Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters ([Complete ROI](#)). Un-check this box and only the region of interest is processed.

Track Surfaces (over Time)

Check this box if you want to track the resulting Surfaces over time, for details please refer to tab [Tracking](#).

Some Examples

Intensity Threshold	Structure of the Creation Wizard is:
	1/4 Algorithm
	2/4 Source Channel
	3/4 Threshold
	4/4 Classify Surfaces
Intensity Threshold + ROI	Structure of the Creation Wizard is:
	1/5 Algorithm
	2/5 Region of Interest
	3/5 Source Channel
	4/5 Threshold
	5/5 Classify Surfaces
Seeded Growing	Region Structure of the Creation Wizard is:
	1/7 Algorithm
	2/7 Source Channel
	3/7 Threshold
	4/7 Split touching Objects (Region Growing)
	5/7 Region Seed Points
	6/7 Classify Seed Points
	7/7 Classify Surfaces

Intensity Threshold	Structure of the Creation Wizard is:
+	1/7 Algorithm
Tracking	2/7 Source Channel
	3/7 Threshold
	4/7 Classify Surfaces
	5/7 Edit Surfaces
	6/7 Tacking
	7/7 Classify Tracks

ROI	Structure of the Creation Wizard is:
+	1/11 Algorithm
Seeded Region	2/11 Region of Interest
Growing	3/11 Source Channel
	4/11 Threshold
	5/11 Split touching Objects (Region Growing)
	6/11 Region Seed Points
	7/11 Classify Seed Points
	8/11 Classify Surfaces
	9/11 Edit Surfaces
	10/11 Tacking
	11/11 Classify Tracks

Seeded Region	Structure of the Creation Wizard is:
Growing	1/10 Algorithm
+	2/10 Source Channel
Tracking	3/10 Threshold
	4/10 Classify Surfaces
	5/10 Split touching Objects (Region Growing)
	6/10 Region Seed Points
	6/10 Classify Seed Points
	7/10 Edit Surfaces
	8/10 Tacking
	9/10 Classify Tracks

Surfaces Wizard Buttons

Button **Back**
 This button is grayed in the first window of the Creation Wizard.

Button **Next**
 Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
 Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

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

13.13.1.2 Region of Interest

Region of Interest 1

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.

X:, Y:, Z:, Time

Min:, Max:, Size

Minimum, maximum and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields.

Alternatively the location and size of the ROI can be adjusted directly in the viewing area. Click on the arrows in the ROI preview rectangle (change to the pointer mode **Select**) and drag it to define new coordinates for X and Y. Location of the ROI can be adjusted by clicking inside the ROI preview rectangle and dragging (with mouse button held) to the chosen image location. The numbers in the value fields are updated automatically.

Memory Usage:

Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button Add

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button Delete

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:

Display of the memory usage for the image size within the ROI.

Button Cancel

If you do not want to create Filament automatically click on **Cancel**.

Surfaces Wizard Buttons**Button Back**

With **Back** you return to the previous window of the Creation Wizard.

Button Next

Press **Next** to proceed with the next window of the Creation Wizard.

Button Finish

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button Cancel

If you do not want to create Surfaces click on **Cancel**.

See also:

Surpass View - Overview - [Camera Toolbar](#) (change to pointer mode Select)

13.13.1.3 Source Channel**Source Channel**

Select the respective source channel, from which Surfaces are to be computed.

Smooth

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

Threshold

There are two options for Surface creation Threshold:

Absolute Intensity

Background Subtraction (Local Contrast)

If option **Background Subtraction** is selected **Diameter of largest Sphere which fits into the Object** should be determined.

Either use the automatically computed value or type in a new value in the data field.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**





Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.4 Threshold




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

General

Automatic

The initial threshold value is automatically computed using an algorithm based on [T.W. Ridler and S. Calvard, "Picture thresholding using an iterative selection method", IEEE Trans. System, Man and Cybernetics, SMC-8 (1978) 630-632].

Manual

Define the requested threshold by clicking into the histogram or by entering the value directly in the data field.

Seeded Region Growing

If you want to apply the seeded region growing for the Surfaces creation check the respective option on the first window of the Surfaces Creation Wizard, tab [Algorithm Settings](#). During the region growing process around each seed point the region grows until a border stops the growing. The threshold you set above is the border of the region growing.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.5 Split touching Objects (Region Growing)

This tab only appears, if **Split touching Objects (Region Growing)** is checked ([Algorithm Settings](#)).

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

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.6 Region Seed Points

Seed Detection

Estimated Diameter

Type in the estimated diameter of a seed point.

13.13.1.7 Classify Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if **Split touching Objects (Region Growing)** is checked ([Algorithm Settings](#)).

A filtering allows removal of objects that would interfere with interpretation of results.

The Filter type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold.

The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.8 Classify Surfaces

The Surfaces filtering is incorporated into the creation process.

A filtering allows removal of objects that would interfere with interpretation of results.

The Filter type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.9 Tracking

This tab only appears, if **Track Surfaces (over Time)** is checked ([Algorithm Settings](#)).

Algorithm

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

Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernable pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion

This algorithm models the motion of each Surface as an autoregressive AR1 process. This model is appropriate if your Surface perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Surface will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which the Surface is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter,

and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert

This algorithm models the motion of each Surface as an autoregressive process. This model is appropriate if your Surfaces perform any kind of continuous motion. In the expert mode the tracks are predicted by the additionally established algorithm called Intensity Weight (see below).

Connected Components

This algorithm models the motion of continuous connected components.

Parameters

Max Distance

The parameter Maximum Distance disallows connections between a Surface and a candidate match if the distance between the predicted future position of the Surface and the candidate position exceeds the maximum distance.

The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

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

Intensity Weight

Tracks are computed by minimization of a cost function that combines distance based and intensity based costs. "Intensity Weight" is the factor by which IntensityCost is taken into account:

$$\text{TotalCost} = \sum_{\text{all connections}} \text{DistanceCost} + \text{IntensityWeight} * \text{IntensityCost}$$

The parameter is only available if you select Autoregressive Motion Expert (see above).

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.1 Edit Surfaces

Here you find the manual editing tools for the Surfaces.

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

Edit Mode

Add/Delete

This activates a semi-automatic surface segmentation (see below). Single objects can be added by one mouse click. If you select **Add/Delete** as edit mode, the Add/Delete Properties are available (see below).

Cut

This activates the Surface Scissors function (see below). You can manually split two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available (see below).

Autodepth Draw

The auto depth function will automatically calculate the Surface depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:
all visible Channels
specific Channel
solid Objects

Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Add/Delete Properties

The Add/Delete Properties are only available, if you select **Add/Delete** as edit mode (see above).

There are two different techniques available:

Marching Cubes

Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

Position the autodepth cursor to the center of the object.

Adjust the size of the box to the approximate size of the object (use the mouse wheel).

Shift + click to create the new object.

Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

Position the autodepth cursor to the border of the object (the size of the box does not influence the result).

Shift + click to create the new object.

Process Selection

Button **Clear**

Click this button to clear the Surfaces selection.

Button **Duplicate**

Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

Duplicate to all Timepoints

If you check this box the selection is not only active for the current time point but for all time points in which the object appears.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

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

Change to the pointer mode Select.

Select as Edit Mode the mode Cut (see above).

Shift + click on the Surface object.

A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular)

Click on the button **Cut Surface** (see below) and the object splits into two individual Surface objects with two different colors.

Button **Cut**

Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear**

Click on this button to clear the selection.

Close Borders

Check this box to add a surface along the cutting line and close the surface.

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**

Button **Mask Selection ...**

Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Surfaces

In Imaris it is possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**

This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask

When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map

Constant Inside/Outside

Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:

The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:

If you leave the box un-checked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**

If you want to apply the mask click on **OK**.

Button **Cancel**

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

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button **Select All**.

Button **Clear All**

Use this button to clear the Surfaces selection.

Button **OK**

If you want to merge the Surfaces click on **OK**.

Button **Cancel**

If you do not want to merge the Surfaces click on **Cancel**.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.1 Classify Tracks

The Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Surfaces (over Time)** is checked ([Algorithm Settings](#)).

A filtering allows removal of objects that would interfere with interpretation of results.

The Filter type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either

type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Tracks) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.1.1: Complete ROI

This tab only appears, if **Process entire Image finally** is checked ([Algorithm Settings](#)).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Surfaces Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

This button is grayed in the last window of the Creation Wizard.

Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

13.13.2 Tab Settings

Surfaces Style/Quality

Surface

Select this option button and the created Surfaces is displayed in the viewing area.

Center Point

Select this option button and the Surfaces displayed as center points.

Off

Select this option button and the Surfaces are turned off. They are not visible any more in the viewing area.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Off

Select this radio button and the Tracks are turned off. They are not visible any more in the viewing area.

Line

The connections are drawn as lines.

Cylinder

The connections are drawn as cylinders.

Width**Diameter**

Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality

When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Tracks Path

Displacement

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Color Editor

Select a desired color for the displacement arrow.

Time Color

Selecting Time Color draws lines, cylinders or truncated cones in colors with a range from blue to white and dark to bright corresponding to the range of time points. Alternatively it is possible to assign a single color via the Color Editor. Deselect Time Color and click on the Color symbol to open the Color Editor.

Color Editor

Select a desired color for the Track display.

Radius Scale

Edit field for the displacement vector radius.

Dragon Tail

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider

Adjust the number of time points using the slider.

See also:

Surpass View - Surfaces - [Creation Wizard](#)

13.13.3 Tab Creation

Rebuild

Select from the list on which step of the [Surfaces Creation Wizard](#) you want to re-enter the creation process.

For details please refer to chapter:

Menu Surpass - Surfaces - [Algorithm](#)

Menu Surpass - Surfaces - [Tracking](#)

Button **Rebuild Objects**

Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Surfaces computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator with all adjusted parameter values.

13.13.4 Tab Draw

The Draw tab allows the user to semi-automatically create further Surface objects.

The Draw tab has two sub tabs:

[Add/Delete](#)

[Contour](#)

13.13.4.1 Add/Delete

Add/Delete

This activates a semi-automatic surface segmentation.

Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:

all visible Channels

specific Channel

solid Objects

Select as reference for the Surface depth the first visible channel, all visible channels, or other solid objects.

Add Surface using:

There are two different techniques available for adding the Surface Objects:

Marching Cubes

Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

Position the cursor to the center of the object.

Adjust the size of the box to the approximate size of the object (use the mouse wheel).

Shift + click to create the new object.

Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

Position the cursor to the border of the object (the size of the box does not influence the result).

Shift + click to create the new object.

13.13.4.2 Contour

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. You can employ this method whenever simple threshold does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

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

The Contour Tab has three sub tabs:

[Board](#)

[Mode](#)

[Autofit](#)

13.13.4.2.1 Board

A drawing plane is displayed in the viewing area.

Orientation

This option defines the orientation of the drawing plane. Once orientation of the drawing plane is selected, the drawing planes of all contours are set in the same position. By default the orientation is set to be in the xy plane-z-direction.

Choose the yz-plane to draw in the x-direction. Choose the xz-plane to draw in the y-direction. Choose the xy-plane to draw in the z-direction.

Visibility

This option defines the Visibility of previously drawn contour lines.

None

The contour currently being drawn is made visible.

Next

The contour(s) next to the contour currently drawn is/are shown.

All

All contours are shown.

Resolution

This option controls the smoothness and complexity of the surface created by the contour lines. It can be used to reduce the number of triangles in a surface object according to a user-defined value. There are two options:

Automatic

The Automatic resolution depends on the contour line vertex spacing.

Manual

The manual resolution is controlled with the slider.

Setting the Resolution to 100% corresponds to the data set resolution. Setting the resolution to 1% reduces the voxel resolution to 1% of original data set.

Preserve feature

If the box Preserve feature is selected, a Surface calculation is then performed using anisotropic Gaussian filtering. This feature aims to reduce image noise without removing significant parts of the image content, ensuring that thin and sharp features are not absent from the created surface. The filter size is calculated adaptively and separately for each plane. It produces a family of contour lines, and each resulting line is a

combination between the original image and a filter that depends on the local content of the original image. As a consequence, the created Surface the features elongated along the structure and narrow across are preserved, while the noise is removed.

If the Preserve feature is not selected an isotropic Gaussian filter is used for surface creation. The resulting Surface has consistently smooth, shape since the smoothing is made along the structures giving.

Selection

The following button actions apply to the previously created contour lines.

Button Cut

To cut a contour line in a plane click on the button Copy.

Button Copy

To copy a contour line in a plane click on the button Copy.

Button Paste

To paste a contour line in click on the button Paste.

Button Delete

To delete a contour line click on the button Delete

Button Smooth

To smooth a contour line edge click on the button Smooth.

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slider

Button <

Click on this button to switch to the previous slice with a contour line.

Button >

Click on this button to switch to the next slice with a contour line.

Slice (data field)

The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the chapter [Shortcuts Overview](#) in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Import Button

This option allows you to import the old manual contour surfaces and incorporate them in the new Contour surface segmentation.

Open a scene file containing manual surface objects created in Imaris 6.3 or lower versions. Click on the Import button

Click on the Import button to bring up the window **Merge the selected objects to 'Surface No.'** Select the

manual surface objects you want to import and click on the OK button. The selected surfaces are now imported.

Window Merge selected objects to 'Surface No.'.

Select All button

Selects all Contour surfaces from the list

Clear All button

Clears the selection

OK

Confirm the selection

Cancel

Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

13.13.4.2.2 Mode

Drawing Modes

In the contour line creations there are two different drawing modes: **Active** and **Inactive**.

Active

The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines. **Inactive**

In the inactive drawing mode the existing contour lines can be modified or deleted.

You can select the respective mode by using the **ctrl+space key** on the keyboard.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Please refer to the section 'Shortcuts Overview' for a listing of the respective shortcuts.

Several segmentation tools are provided allowing you to select areas in the current slice for contour line drawing.

Select one of the shape object tools in the Drawing Mode. Left clicking on a tool icon activates a drawing tool.

Once a drawing style tool is selected you may adjust the specific parameters for that drawing mode.

Once a segmentation tool is activated and adjusted, activate the drawing mode by changing the pointer mode to Select (press the ESC-key) and then press the **ctrl+space key**. The drawing mode is activated.

By default, contour lines are drawn in a transparent gray color. The color of the contour lines can be modified using a dialog under the Color Tab.

Click

Clicking within the image inserts a point, a so-called vertex. You can now define subsequent vertices by clicking with the left mouse button. Successive points will be connected with straight-line segments.

Time

Click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms), specified in the corresponding field. The interval can be changed by manually specifying the required value. This drawing style allows you to draw a structure that contains fine details.

Insert a vertex after ms

The number specifies the time interval (in ms) between two inserted vertices.

Distance

Click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (um), which is specified in the corresponding field. To change the interval, specify the required value in the object properties area. This drawing style is recommended if the structure is smooth.

Vertex spacing um

The number specifies the distance interval (in um) between inserting two inserted vertices.

Circle

The Circle drawing tool inserts a circular contour line. The Circle tool always draws a contour line from the center; that is, the place you initially click will be the center point of the contour line. To change the circle radius specify the required value in the object properties area. In the parameters you can also specify the number of contour vertices to be inserted. To create the contour line, move the mouse pointer over the orthogonal slicer plane. A preview outline of the circle is then displayed. To create a contour line, click with the left mouse button on the desired position.

Radius um

Defines the radius of the circle in um.

Scrolling the mouse wheel can also change the radius of circle.

Number of vertices

Defines the number of vertices in the circle line.

Isoline

The Isoline drawing tool generates contours at a fixed iso-value so the geometrical features of the contour line takes into account the image's intensity values. Connecting the points with a constant intensity value forms the contour line.

The Isoline drawing mode selects the maximum intensity iso-value under the mouse pointer and creates a contour line by tracing points of uniform intensity. Clicking with the left mouse button on the orthogonal slicer plane selects the largest connected area that contains the all voxel with the same intensity values. The current iso-value under the mouse pointer is displayed in the parameter field Intensity and is updated automatically as you move the mouse. To create a contour line, click with the left mouse button on the desired position.

Reduce density to numerical field %

Defines the density of inserted vertex in relation to the data set.

A density of 100% inserts a contour vertex at each pixel of the data set.

A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Intensity

Displays the value of the voxel intensity under the mouse pointer.

Magic wand

The Magic wand tool creates a contour line by selecting the largest connected area that contains the maximum intensity voxels and all voxels within a user-defined tolerance range. The Tolerance range defines the tool's sensitivity; the higher value you set, than wider image area that is selected. The Tolerance range can be specified in a box in the Parameter panel.

Move the mouse pointer over the orthogonal slicer plane and a preview of the contour line is displayed. The Intensity value in the Parameter panel is updated as the mouse pointer is moved across over the orthogonal slicer plane. To create a contour line, click with the left mouse button on the desired position.

The Intensity with the corresponding Tolerance value is stored in the parameter section of the label field. This makes it possible to easily correct a selection at a later time.

It is possible to modify the range even after the seed voxel has been selected.

Reduces the density to numerical field %

Defines the density of the inserted vertex in relation to the data set.

A density of 100% inserts contour vertex at each pixel of the data set.

A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Tolerance

Defines the range of intensity limits in percentages.

The tolerance 0% selects pixels with exactly the same intensity value as the point under the mouse pointer.

The tolerance value of 100% selects all intensities within the intensity range of the data set. Values in between are interpolated linearly. The tolerance can also be changed incrementally by scrolling the mouse wheel.

Intensity

Displays the value of the voxel intensity under the mouse pointer and the tolerance range.

Detects holes box

This option enables you to create a contour line that contains holes of selected areas. If the Detects holes

box is selected the line is generated around holes, outlining the hole. If the box is un-checked, the outer line of the selected region is drawn and the holes are not visible.

Channel Selection

All Visible Channel

Specific Channel

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

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slider

Button <

Click on this button to switch to the previous slice with a contour line.

Button >

Click on this button to switch to the next slice with a contour line.

Slice (data field)

The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the chapter Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Import Button

This option allows you to import the old manual contour surfaces and incorporate them in the new Contour surface segmentation.

Open a scene file containing manual surface objects created in Imaris 6.3 or lower versions. Click on the Import button

Click on the Import button to bring up the window **Merge the selected objects to 'Surface No.'** Select the manual surface objects you want to import and click on the OK button. The selected surfaces are now imported.

Window Merge selected objects to 'Surface No.'.

Select All button

Selects all Contour surfaces from the list

Clear All button

Clears the selection

OK

Confirm the selection

Cancel

Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is

displayed in the viewing area.

13.13.4.2.3 Autofit

Autofit is a method that can help the user automate a manual segmentation.

Autofit is a deformable contour model used to create and optimize the contour line segmentation, so that the localization and accuracy of the segmented object is improved.

It is a semi-automatic, user interactive detection, robust against noise in the image. The contour line creation procedure consists of two steps: initial user segmentation and refinement by the Autofit algorithm.

In the first step the model requires the user input, an initial curve (rough outline) closed to the boundaries of the object to be segmented.

The next step is to perform an Autofit algorithm. The algorithm then tries to deform this initial shape so that it fits onto the contours of the image. The Autofit algorithm performs an iterative optimization process where the sum of external and internal energies along the contour line approach a local minimum.

The external energy is computed from the original image intensity gradients and it should be minimal when the contour line is located at an object boundary.

The internal energy is based on the shape of the contour line. The Autofit algorithm simulates the contour line as an elastic rubber band and the internal energy is defined by the physical constraints that describe the contour. It should be minimal when it meets some regularity and smoothness constraints regarding the shape of the line (curvature is minimized and contour vertices are encouraged to be equidistant).

The outcome of the Autofit algorithm is an optimized line, a good fit between the external forces, which aims at the object boundaries, and deformable internal forces that targets regularity and smoothness.

The method, which is applied by Autofit, is known under the name of active contour model or snakes. Kass, Witkin and Terzopoulos published the seminal work on the method.

M. Kass and A. P. Witkin and D. Terzopoulos (1988), Snakes: Active Contour Models, International Journal of Computer Vision, 1(4): 321-331

The Autofit algorithm in Imaris is based on the following publication:

Donna J. Williams and Mubarak Shah (1992), A Fast Algorithm for Active Contours and Curvature Estimation, CVGIP: Image. Understanding, 55, (1), 14-26.

Please Note

The Autofit algorithm does not solve the entire problem of finding contours in images, but rather, depends on mechanisms like interaction with a user, interaction with some higher level image understanding process and information from image data.

The accuracy and computation time depends on the convergence criteria used in the Autofit.

The model is sensitive to the initial contour line estimate and algorithm parameters.

The automatically determined parameters provide the optimal values for the Autofit algorithm.

Perform Autofit button

Clicking on this button performs an Autofit calculation.

Presets

Shape Smooth slider Accurate

This setting affects the shape of the resulting contour line. If the Shape slider is set to be more on one side the resulting contour line can be encouraged either to be **smooth** or more **accurate** with original image intensity gradients.

Impact Weak slider Strong

The impact signifies how strongly the algorithm will affect the contour line.

If the impact is set to be **Weak**, the algorithm performs only a few iterations and does not move the vertex very far from the initial contour line.

If the impact is set to be **Strong**, the number of iterations will increase and the vertexes can be moved to find edges that are more distant from the initial contour line.

Advanced

This option activates only if the **Presets** option is not selected.

Continuity

The Continuity value affects the vertices space regularity and tends to keep the distances between each pair of adjacent vertices equal. Setting a high continuity value enforces the contour vertices to be equally spaced

on the line.

Curvature

The Curvature value constrains the shape of the contour line. Setting a high value for Curvature, results in a circularly shaped smooth contour line.

Image Energy

The Image Energy value constrains the intensity gradients on the contour. Setting a high value places the contour line at the intensity high gradients.

Reach Distance um

This parameter controls the maximal distance the contour vertex can move away from the original position during a single iteration.

Setting a high value enables the algorithm to find edges at more distant locations from the initial contour line and therefore decreases the risk of falling into local minima. However, the algorithm then has a tendency of detecting edges different from the desired object.

Maxi iteration

This parameter defines the Maximum number of iterations to be performed. The Autofit algorithm terminates if the specified maximum iterations have been performed.

SSC iterations

Scale space continuation is a method for preventing the Autofit algorithm to place a contour line at the local minima created by noise. First a large Gauss filter is used and then the Autofit algorithm iterates while slowly reducing the size of the Gauss filter. SSC iterations define the number of Autofit algorithm iterations

For example, if the maximum number of iterations is set to 30 and the number of SSC iterations is set to 3, the Autofit algorithm is run three times. The first time with a Gauss filter with three times the specified standard deviation, then for the second time with two times the standard deviation and finally, for the third time with the specified standard deviation. 10 iterations are performed maximally each time.

Gauss numerical field um

The standard deviation of the Gauss filter applied prior to the algorithm.

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slider

Button <

Click on this button to switch to the previous slice with a contour line.

Button >

Click on this button to switch to the next slice with a contour line.

Slice (data field)

The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the chapter Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Import Button

This option allows you to import the old manual contour surfaces and incorporate them in the new Contour surface segmentation.

Open a scene file containing manual surface objects created in Imaris 6.3 or lower versions. Click on the Import button

Click on the Import button to bring up the window **Merge the selected objects to 'Surface No.'** Select the manual surface objects you want to import and click on the OK button. The selected surfaces are now imported.

Window Merge selected objects to 'Surface No.'.

Select All button

Selects all Contour surfaces from the list

Clear All button

Clears the selection

OK

Confirm the selection

Cancel

Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

13.13.4.2.4 Shortcuts Overview

You can select the drawing mode by using the **ctrl+space** key. 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.

Spots Mouse & Keyboard Functions

up or down arrow keys	Move up / down one slice
left/right arrow keys	Move up / down to next slice which contains contour lines
C key	Copy contour lines from current slice
X key	Cut contour lines on current slice
V key	Paste contour lines to current slice
Del key	Delete all contour lines on current slice
O key	Smooth all contour lines on current slice
A key	Perform Autofit Algorithm on current slice
+ key	Increase contour line width
- key	Decrease contour line width
Mouse wheel	Change circle radius or magic wand tolerance

Drawing mode **Active**

Backspace key	Abort drawing of currently opened contour line
Enter key	Stop drawing of currently opened contour line and leave it open
U key	Change to contour line union mode Union indicated by a "+" sign next to the mouse cross cursor
S key	Change to contour line subtraction mode Subtraction indicated by a "-" sign next to the mouse cross cursor

Drawing mode **Inactive**

L key	Toggle lock/unlock orthogonal slicer
T key + Left-click on line (vertex)	Translate contour line or vertex
R key + Left-click on line (vertex)	Rotate contour line
S key + Left-click on line (vertex)	Scale contour line
D key + Left-click on line (vertex)	Delete contour line or vertex

Union mode

The result of Union mode is a single contour line whose area is the minimum superset of the overlapping input contour line areas.

Subtraction mode

The result of subtraction mode is a single contour line whose area is the area of the original line minus the operator line area.

13.13.5 Tab Edit

Here you find the manual editing tools for the Surfaces.

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

Edit Mode

Cut

This activates the Surface Scissors function for manually spiting two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available.

Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

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

Change to the pointer mode Select.

Select as Edit Mode the mode Cut.

Shift + click on the Surface object.

A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular)

Click on the button **Cut Surface** and the object splits into two individual Surface objects with two different colors.

Button **Cut Surface**

Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear Scissor**

Click on this button to clear the selection.

Close Borders

Check this box to add a surface along the cutting line and close the surface.

Mouse selects

Specify what object is select by a single mouse click.

**Surface
Track**

Process Selection

Button **Clear**

Click this button to clear the Surfaces selection.

Button **Duplicate**

Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

Button **Duplicate to all Timepoints**

Click this button and the selection is not only active for the current time point but for all time points in which the object appears.

Button **Unify**

Click this button and the selected Surfaces are unify into one Surface object.

Button **Split**

Click this button and the selected Surfaces are separated into two Surface objects.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**

Button **Mask Selection ...**

Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Surfaces

In Imaris it is possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**

This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask

When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map

Constant Inside/Outside

Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:

The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:

If you leave the box un-checked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**

If you want to apply the mask click on **OK**.

Button **Cancel**

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

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button **Select All**.

Button **Clear All**

Use this button to clear the Surfaces selection.

Button **OK**

If you want to merge the Surfaces click on **OK**.

Button **Cancel**

If you do not want to merge the Surfaces click on **Cancel**.

13.13.6 Tab Filter

Please note: First of all you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest and, from that selection, include or exclude objects based on a range of criteria. In Imaris you can set two threshold values, Lower and Higher Threshold. The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be outside the interval defined by the two threshold values.

In the viewing area only those objects meeting all filter criteria are shown interactively and instantly highlighted.

Filters

Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.






Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.





Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Filter Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).




Compact Lower and Upper Threshold


Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.



In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on

the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold. Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of selected objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surface) is displayed at the bottom of the threshold histogram.

Duplicate Selection to New Surfaces Button

Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:

Surpass View - Overview - [Surpass Tree](#)

13.13.7 Tab Statistics

Surfaces statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - [Surfaces](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall

b) Detailed

c) Selection

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.


Tab Overall

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are five Categories: Surface , Spots , Tracks , Dendrites  and Spines .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

Tab Detailed

A pull down list offers three types of **Detailed** statistical calculations:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all values within a set of the selected variable. Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Statistical variable classification:

1. Statistical variables associated with objects (Spots, Surface-volume, area, intensity values, position,

speed, displacement) have values at every time point. Therefore for these variables Average values are displayed per time point. By changing the time point, statistical variable value and image are consequently changed.

2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement, track intensity). Track statistical values are not time dependent, since connecting moving object traces in image time series creates Tracks.

All values

If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Tab Selection

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers three types of statistical calculations from a Selection:

Specific Values

Average Values

All Values

Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values

If the option All values is selected, all statistical values will be displayed for the selected image element.

Buttons Overview

	PC:	Mac:
Select Surface	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Surfaces](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

Highlights the object in the viewing area

Highlights the object on the tab Detailed


Displays of the values on the tab Selection

Button **Surfaces** - Duplicate Selection to new Surfaces.

Click on this button and all selected elements are copied to a new Surface. The new Surface appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Surfaces](#)

13.13.8 Tab Color

Tab Color edit, manage and assign colors material to the Surfaces or Tracks.

Object Type

Choice of Object type is presented in drop down window. Select either Surfaces or Tracks and depending on the chosen object type selection of the variables is listed.

For **Object Type Surfaces** two color types are available:

[Base](#)

[Statistics Coded](#)

For **Object Type Tracks** three color types are available:

[Base](#)

[Statistics Coded](#)

[Time Mapped](#)

13.13.8.1 Color Type- Base

Tab Color edit, manage and assign material colors of a Surfaces/Track.

RGB

The **RGB** color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuse reflection of a matte, uneven material.

Specular- reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from material defines self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.

In Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and could be used with other Imaris objects.

13.13.8.2 Color Type- Statistics Coded

Statistics Coded

Depending on the object type selection (either Surfaces or Tracks) Statistical variable selection is listed in drop down window. For example, if Object type is chosen to be Surfaces, available Statistical type will be Surface linked variable. Similarly, Statistical variable for Tracks will be listed, if Object type is selected to be Tracks.

Statistics Colorbar Properties

Statistics colorbars are displayed in the viewing area. The upper one is colorbar range for Surfaces and lower one is colorbar for the Tracks.

Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

Show Title

Check this box to see the title in the statistics colorbar.

Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**

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

Button **Load** ...

Click on the button **Load** to display the color Table File box. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for colorbar.

Min - lower color limit

Max - upper color limit

Button **Auto**

Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

13.13.8.3 Color Type - Time Mapped

Time Mapped

This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

13.14 Volume

How to Add a new Volume?

Select menu Surpass - Volume.

The Surpass Tree list is automatically updated and in the Volume - Properties the Settings parameter are displayed. A data set can have only one volume.

13.14.1 Display Adjustments

To set the correct Display Adjustments for the Volume rendering of your data set please refer to chapter Menu Edit - [Show Display Adjustment](#).

13.14.2 Tab Settings

Mode

MIP (max)

In the Maximum Intensity Projection mode only the brightest point (the point with maximum intensity) of all layers along the viewing direction displays. If your data set has more then one channel the combination color displays. A layer in the data set with high red and high green intensity displays yellow.

Normal Shading

The result of the normal shading is a depth effect on the screen. The surfaces turned away from the light source appear darker.

MIP (min)

A Maximum Intensity Projection with minimum intensity of all layers along the viewing direction.

Blend

The result of the bland mode is a depth effect on the screen. One effect of the blend mode is, that the borders appear darker. In the blend mode all values along the viewing direction including their transparency are used for the calculation.

Shadow Projection

The result of the shadow projection is a depth effect on the screen. An object between light source and the surface you look at projects a shadow.

If Shadow Projection is selected, the parameter Fix Light(s) to Data Set is available.

Fix Light(s) to Data Set

Check the box to fix the x-, y-, and z-axes to the same side when navigating the object. When the box is un-checked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

This check-box is only available, if Shadow Projection is selected.

Rendering Quality

If the rendering quality is set to 1.000, then 1pixel represents 1 voxel. If you decrease the rendering quality the image becomes coarse-grained.

View Aligned Planes

Check this box to see the display planes. Examine the planes while rotating the data set in the viewing area.

See also:

Preface - [Terminology](#)

13.14.3 Tab Draw

Volume Painter

On the tab Draw you can find the settings for the Volume Painter. Use this Volume Painter to adjust the voxel intensities before you do a segmentation based on Surfaces, Spots, or Filament. (If an automatic algorithm is not able to separate two objects, the intensity between these two objects can be "erased" in advance.) You can draw disks, spheres, or hallow spheres into the selected image channel.

First select the desired channel and painter shape.

Rotate the view to execute a vertical cut.

Then customize the painter settings.

Change to the pointer mode Select.

Move the cursor to the desired position.

Hold down the Shift-key and click to add the shape.

In the viewing area you see a preview of the painter tool.

To re-edit a shape use the point selection.

Set intensity to 0 to erase a region, or set the intensity to 255 to fill a region.

To fix the shape click on the button **Set Intensity**.

Please note: The button Set Intensity changes the data set.

Selected Channel

Select the respective source channel.

Painter Shape

Here you select the painter shape.

Disk

The painter is disk shaped. In the settings selection adjust the diameter, width and intensity of the disk (see below).

Sphere

The painter is a sphere. In the settings selection adjust the diameter and intensity of the sphere (see below).

Hollow Sphere

The painter is a hollow sphere. In the settings selection adjust the diameter and intensity of the hollow sphere (see below).

Selection

Diameter

Here you select the diameter of a disk, sphere, and hollow sphere. If your mouse has a wheel, you can use the wheel to adjust the diameter.

Width

Here you select the width of a disk.

Intensity

Here you select the painter shape intensity. The shape preview in the viewing area changes accordingly. If you want to cut parts out of the channel select 0 as intensity value.

Button **Set Intensity**

In the last step you click on this button to fix the shapes. The button **Set Intensity** changes the data set and it is not possible to re-edit the shapes again.

Button **Delete All**

Click on this button to delete all inserted shapes in the preview.

Selected Point

You can use the following data fields and the button **Delete** to re-edit or delete a selected point.

Position [um] X, Y, Z

To select a point change to the pointer mode Select and click on a point. The color of the selected point changes. The point position displays in the x, y, and z data field. To move the point click on the new position in the viewing area or edit the x, y, or z data field.

Button **Delete**

Click on this button to delete the selected point.

13.14.4 Tab Statistics

Volume statistics are automatically computed. For the statistical variable values please refer to the chapter Menu Edit - Preferences... - Statistics - [Volume](#). Statistic tab collects and displays image statistical values in Imaris. Statistic tab enable us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enabling convenient organization and analysis of heterogeneous statistical data. Statistical View is configured as follows:

a) Overall

b) Detailed

c) Selection

In statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort columns click on the arrow in column heading. Click again to reverse the sort.

Tab Overall

Output of the **Overall** statistics provides an overview of the image single determining variables.

Buttons at the bottom of the Statistics tab

Button **Settings** - Configure List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Volume](#).

Button **Search** - Search by object ID.


Type in the object ID in the data field. Click on the button **Search**.

Highlight the object in viewing area
Highlight the object on the tab Detailed
Display of the values on the tab Selection

Button **Surfaces** - Duplicate Selection to new Surfaces.

Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

Button **Export** - Export Statistics on Tab Display to File.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

With a click on the button **Export** you open the Window Export Statistics. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Volume](#)

14 Animation

The Key Frame Animation function defines several subsequent views (key frames) of your image, which will be used to create an animation. Imaris generates smooth transitions between the selected views by interpolation.

How to Open the Key Frame Animation Window?

Click on the icon in the Main toolbar.
Select menu View - Animation.
Press the key combination Ctrl + 6.

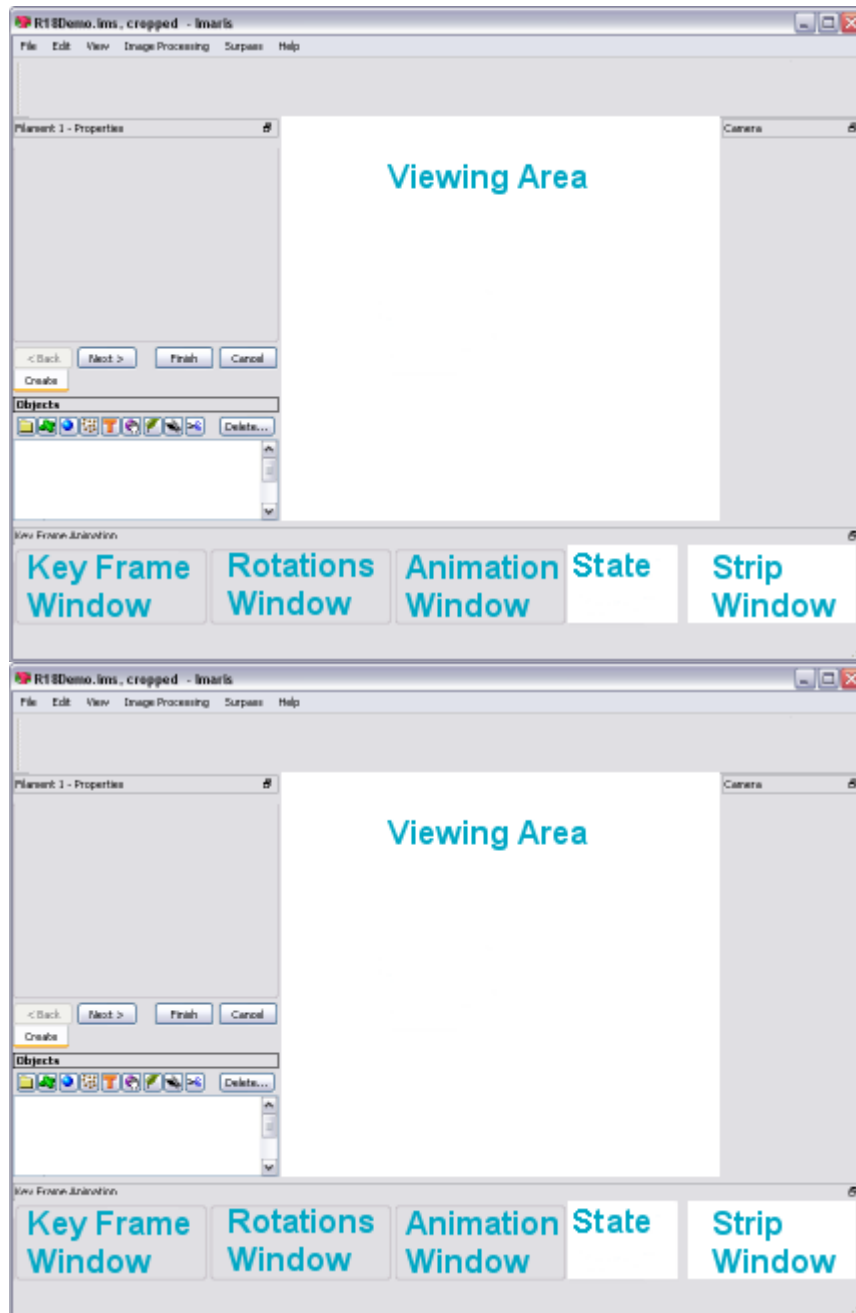
See also:

Menu Edit - Preferences... - [Surpass](#)

Animation - [Mouse & Keyboard Mac](#)

14.1 Overview

The Key Frame Animation consists of different areas:



At the bottom of the screen the following windows are displayed: the [Key Frame Window](#), the [Rotations Window](#), the [Animation Window](#), the [State](#) selection, and the [Strip Window](#).

14.2 Key Frame Window

In the Key Frame window you define and manage the key frames of your movie.

Button **Add**

Use this button to insert user-defined key frames in the strip sequence.

Button **Modify**

Use this button to modify user-defined key frames in the strip sequence.

Button **Delete**

Use this button to delete user-defined key frames in the strip sequence.

Button **Delete All**

Click on this button to delete all user-defined key frames in the Strip window.

Large spaces between the user-defined key frames (thick blue lines) will cause that section of the movie to play more slowly. Placing the lines close together will cause that section of the movie to play more quickly. Use this button to set a constant speed throughout the entire movie.

Button **II<**

Button **II>**

With the arrow buttons you step to the previous (arrow to the left) or next (arrow to the right) user-defined key frame.

Buttons Overview

Add	Add new user-defined key frame
Mod.	Modify active, user-defined key frame
Del	Delete active, user-defined key frame
Del. All	Delete all key frames
II<	Go to Previous user-defined key frame
II>	Go to Next user-defined key frame

For the complex use of these buttons please refer to chapter Animation - [Strip Window](#).

See also:

Animation - [Create Animation](#)

14.3 Rotations Window

Use the templates to quickly define standard camera motions for the key frame animation system.

Templates

Select one of the predefined templates. The key frames are inserted automatically. Click on the button **Play** to see the animation.

Button **Custom**

Click on this button to display the Custom Rotation window to freely define the rotation (see below).

Space

Screen

The rotation axis is associated to the x-, y-, and z-axis of the screen.

World

The rotation axis is associated to the x-, y-, and z-axis of the data set.

Axis

X

Y

Z

Turn along the x-, y-, or z-axis.

Direction

+

Turn to the left.

-
Turn to the right.

Angle

Select or type in the desired angle.

Button 

Click on this button to accept the changes.

Button 

Click on this button to cancel the changes.

14.4 Animation Window

Frames

The number in the field **Frames** indicates the number of thin blue lines within the Strip Panel. This number defines the duration of animations.

Button 

To open and adjust the Key Frame settings click on this button. For details please refer to chapter Menu Edit - Preferences... - [Surpass](#).

Button 

Click on this button (arrow to the right - **Play/Pause**) to start the movie preview. To pause the movie preview re-click on this button.

Button 

To record the movie click on this button (red dot - **Record**). Selecting this button the Save As Movie window is displayed (see below).

Window: Save as Movie Window

File name

Enter a file name.

Save as type

Select the desired file type (QuickTimeMovie, TIFF series, or AVI).

Movie Settings

Compression Factor

Select a compression factor between 0 (high quality) and 100 (low quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate

Define how many frames are displayed per second.

QTVR Settings

The QuickTimeVR Settings are not available.

Add to ImageAccess database

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

Play Movie with default Media Player when finished

Check the box to play the movie with the default media player when finished.

Menu Edit - Preferences... - [Surpass](#) (default Key Frame settings)
Surpass View - Overview - [QuickTimeVR](#)

14.5 Play Back State

In the State selection you can adjust the play back settings.

Camera

Check: Play back the previously recorded Camera positions for each key frame.

Un-check: Do not play back the recorded Camera positions. The Camera stays in the same position. You can change the Camera position manually while playing the animation.

Time Points

Check: Play back the previously recorded Time Points for each key frame.

Un-check: Do not play back the recorded Time Points. The Time Points do not automatically change during the animation. You can change the Time Points manually while playing the animation.

Colors

Check: Play back the previously recorded Colors for each key frame.

Un-check: Do not play back the recorded Colors. The Colors do not automatically change during the animation. You can change the Colors manually while playing the animation.

Display Adjustments

Check: Play back the previously recorded Display Adjustments for each key frame.

Un-check: Do not play back the recorded Display Adjustments. The Display Adjustments do not automatically change during the animation. You can change the Display Adjustments manually while playing the animation.

Clipping Plane (Position)

Check: Play back the previously recorded Clipping Plane positions for each key frame.

Un-check: Do not play back the recorded Clipping Plane positions. The Clipping Plane positions do not automatically change during the animation. You can change the Clipping Plane positions manually while playing the animation.

Orthogonal Slicer (Position)

Check: Play back the previously recorded Orthogonal Slicer positions for each key frame.

Un-check: Do not play back the recorded Orthogonal Slicer positions. The Orthogonal Slicer positions do not automatically change during the animation. You can change the Orthogonal Slicer positions manually while playing the animation.

Oblique Slicer (Position)

Check: Play back the previously recorded Oblique Slicer positions for each key frame.

Un-check: Do not play back the recorded Oblique Slicer positions. The Oblique Slicer positions do not automatically change during the animation. You can change the Oblique Slicer positions manually while playing the animation.

Objects Show/Hide (Object Visibility)

Check: Play back the previously recorded objects for each key frame.

Un-check: The visibility of the objects in the viewing area can be changed manually during the animation. Check or un-check Surpass Tree Items during the animation and the effect is directly visible in the viewing area.

See also:
Animation - [Create Animation](#)

14.6 Strip Window

The Strip window provides the working area. To display the views of the different key frames left-click on a line to display the corresponding image in the viewing area.

Lines in the Strip Window

Thick Blue Line

A thick blue line indicates a user-defined key frame.

White Line

A white line represents the active key frame.

Thin Blue Line

A thin blue line stands for an interpolated frame.

Actions in the Strip Window

Add user-defined Key Frame

Click on a thin blue line (interpolated key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add** to insert a user-defined key frame in the strip sequence.

Click on a thick blue line (user-defined key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add**. Each time you click on the button Add you insert a new user-defined key frame right to the last active key frame.

Please remember: If you select a thin blue line, you define a new user-defined key frame exactly on this location. If you select an already user-defined key frame the new key frame is halfway between the selected and the next user-defined key frame on the right hand side.

Delete user-defined Key Frame

Click on a key frame and then on the button **Delete**.

Move user-defined Key Frame

To move a key frame in the Strip window drag the key frame line while holding down the mouse button. The first and last key frames can not be moved.

Modify user-defined Key Frame

Select the key frame in the Strip window. Turn the image to a new position and click on the button **Modify**. The new position for the selected key frame is saved.

Copy user-defined Key Frame

To copy a key frame in the Strip window select the initial key frame with a click. The scene in the viewing area changes accordingly. Right-click on the key frame in the Strip window at the desired copy position. The scene in the viewing area does not change. Click on the button **Add** to insert the user-defined key frame.

Please remember: Click on a key frame to change the camera position in the viewing area accordingly. Right-click (if you use a three button mouse) on a key frame and the view does not change.

Strip Window Overview

Click on key frame	Select key frame and change view accordingly
Right-click on key frame	Select key frame, view does not change
Button Add	Add new user-defined key frame
Button Mod.	Modify selected, user-defined key frame
Button Del	Delete selected, user-defined key frame

Menu Edit - Preferences... - [Surpass](#) (default interpolated frame settings)

14.7 Create Animation

The Key Frame Animation feature in Surpass allows you to create an animation from views (key frames). It interpolates frames between the user-defined views to create a smooth movie.

1. Adjust Pre-Settings

Create an object (Surfaces, Volume, etc) from which to create the animation.

In the Main toolbar click on the icon **Animation**. The Key Frame Animation window displays on the bottom of the screen and is divided in the Key Frame window, Rotations window, Animation window, State window, and Strip window.

In the Animation window you find the data field Frames. Enter the number of frames to be included in the movie.

Click the **Settings** button and enter the number of frames per second.

2. Add Key Frames

Turn the image view to the first position.

Capture the first view with a click on the button **Add** in the Key Frame window (records position). The key frame appears in the Strip window.

Move the image to the next viewing position.

Click on the button **Add** in the Key Frame window to set the next key frame position (records new position). The new key frame appears in the Strip window.

Repeat as desired, until all user-defined key frames are added.

3. Select Play Back Settings

In the State selection you can adjust the play back settings. Please refer to chapter Animation - [Play Back State](#) for details.

4. Play the Animation

In the Animation window click on the button **Play** to start the animation.

Example: Handle Camera

Use 360 Template

Use the templates to quickly define standard camera motions for the key frame animation system.

In the Rotations window select +360 Horizontal. Five key frames are inserted automatically. The first and the last key frames are identical.

In the State window check Camera.

In the Animation window click on the button **Play** to see the animation. The camera rotates 360 horizontally.

Capture Individual Camera Positions

With the pointer in Navigate mode, position the image to create the view for the first key frame.

In the Key Frame window click the button **Add**. With the first click on **Add** you insert the first and last key frames, they are identical. The key frames appears in the Strip window.

Position the image to the desired view for the next key frame and click the **Add** button to place the next key frame.

Repeat as desired, until all user-defined key frames are added.

In the State window check Camera.

To preview the movie, click in the Animation window on the button **Play**. The camera position changes automatically.

Move the Camera Position Manually During the Play Back

Create an animation.

In the State window un-check Camera.

Play the animation and change the Camera position during the play back manually.

Example: Handle the Orthogonal Slicer

Individual Orthogonal Slicer Positions

Add the Surpass Tree Item Orthogonal Slicer and check the box in the Surpass Tree.

Adjust the Orthogonal Slicer for the first key frame and click on the button **Add**.

Move the Orthogonal Slicer before you add the next key frame and so on.

In the State window check Orthogonal Slicer.

Play the animation. The Orthogonal Slicer position changes automatically.

Change Orthogonal Slicer Position Manually During the Play Back

Create an animation.

In the State window un-check Orthogonal Slicer.

Play the animation and change the Orthogonal Slicer position during the play back manually.

See also:

Animation - [Overview](#)

Animation - [Key Frame Window](#)

Animation - [Rotations Window](#)

Animation - [Animation Window](#)

Animation - [Play Back State](#)

Animation - [Strip Window](#)

Animation - [Save Animation](#)

14.8 Save Animation

When you are satisfied with the movie, there are two possibilities to save the animation. Either as an Imaris Scene File or as a movie-file (*.avi).

In Imaris as Scene File

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

Save Different Animations in Different Scene Files

Saving different animations of the same data set requires saving of different Scene Files. After generating a new movie by the Key Frame Animation, rename each time the Scene File, e.g. add "Movie" at the end of the Scene File name. Open the menu File and select Export Scene... . In the dialog window type in the new Scene File name (e.g. PyramidalCellSceneAMovie.imx) and click on **Save**.

Record the Animation as AVI Movie

To save the animation as AVI movie click in the Animation window on the button **Record** (red dot). The Save As Movie dialog window is displayed on screen. As File name type in the corresponding Scene File name, use the default Movie Settings and click on **Save**. The file extension for the AVI movie is *.avi.

See also:

Menu File - [Export Scene...](#)

Surpass View - Overview - [Scene File Concept](#)

Surpass View - Overview - [Quick TimeVR](#)

Animation - [Animation Window](#) (Button Record)

14.9 Mouse & Keyboard PC

Animation Mouse & Keyboard Functions

Ctrl + 6 Activates the Key Frame Animation

In the Strip Window

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

14.10 Mouse & Keyboard Mac

Animation Mouse & Keyboard Functions

Command + 6 Activates the Key Frame Animation

In the Strip Window

With a one-button mouse:

Click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Ctrl + click on key frame	Select key frame, view does not change

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

15 InMotion

The function InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view. This helps to get an understanding of the image depth, while manipulating with Surpass objects: Spots, Filaments, Measurement Points.

How to Start the InMotion Mode?

- Click on the icon in the Main toolbar.
- Select menu View - InMotion.
- Press the key combination Ctrl + 8.

To switch the mode off click on the icon Surpass in the Main toolbar.

Adjust 3D Cursor Settings

To adjust the 3D cursor settings click on menu Edit - Preferences... - [3D Cursor](#).

See also:

Toolbars - [Main Toolbar](#)

InMotion - [Mouse & Keyboard Mac](#)

15.1 Mouse & Keyboard PC

InMotion Mouse & Keyboard Functions

Ctrl + 8	Activates the InMotion function
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15.2 Mouse & Keyboard Mac

InMotion Mouse & Keyboard Functions

Command + 8	Activates the InMotion function
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16 Coloc

In fluorescent microscopy, colocalization describes the presence of two fluorochromes at the same physical location. ImarisColoc provides extended functionality for the analysis and visualization of colocalization in multi-channel data sets. It provides an automated selection of colocalization based on a method introduced by Costes & Lockett at the NIH, NCI/SAIC.

Coloc processes 2D, 3D, and 4D data sets. It operates on two channels simultaneously and measures the degree of overlap of the two channels.

With Coloc you can determine and display locations within the data set with common information from all channels, e.g., to illustrate where all types of proteins can be found simultaneously or to detect low-density tissues in all channels.

The desired range for signal analysis can be limited by defining intensity thresholds or sets of intensity values for each of the involved channels. All voxels falling inside every of these channel-specific intensity limits represent a colocalization. They are emphasized in the view and statistics are calculated and displayed. When the selection is changed, the numeric and visual feedback happens in real-time.

The result of the colocalization as a whole can be saved as a separate channel, which can then be processed and viewed as any originally acquired channel in Imaris. Multiple colocalization images are easily combined into a single view in ImarisSurpass.

Coloc is an optional module within the Imaris program and is not available as a standalone program.

How to Display the Coloc Main Screen?

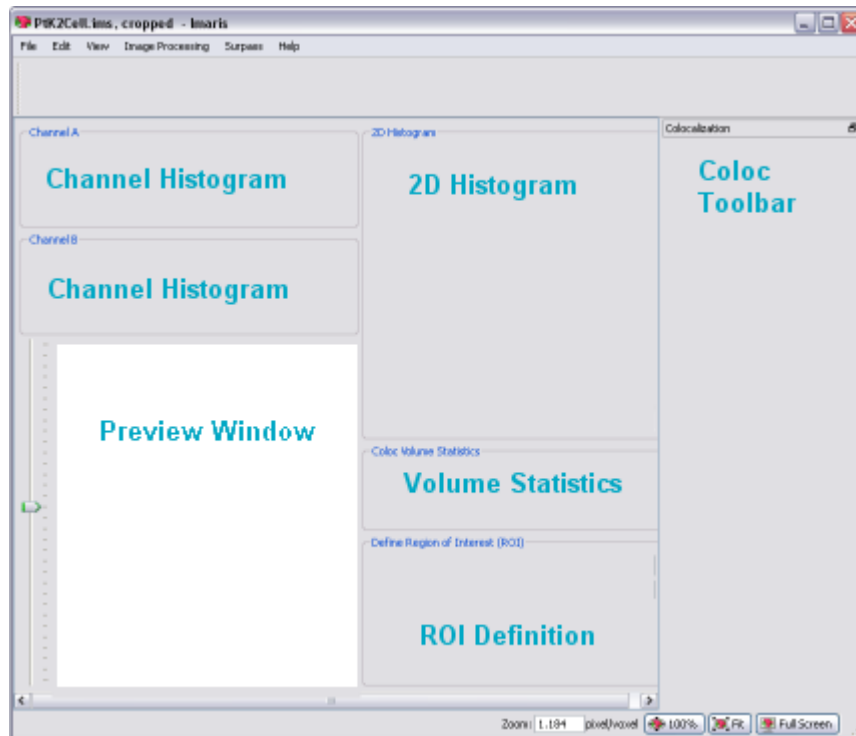
- Click on the icon in the Main toolbar.
 - Select menu View - Coloc.
 - Press the key combination Ctrl + 7.
-

See also:

Coloc - [Mouse & Keyboard Mac](#)

16.1 Overview

The Coloc main screen consists of different areas:



[Channel Histogram](#) (channel A, channel B). This area allows the selection of the two channels for the colocalization analysis.

[Preview Window](#). This area visualizes the data set together with a preview of the colocalized region.

[2D Histogram](#). This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.

[Volume Statistics](#). This area displays statistics about the resulting colocalized volume. The display adapts instantly to all modifications in the selection.

[ROI Definition](#). This feature allows you to define a region of interest (ROI) for the entire analysis.

[Coloc Toolbar](#). The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

16.2 Channel Histogram

This area allows the selection of the two channels for the colocalization analysis. The intensity histograms are displayed for the currently selected channels.

Select Channel A and Channel B

The two channels for colocalization detection must be selected first.

Select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Threshold

The threshold for each channel can then be defined by clicking in the histogram, dragging the colored line directly, or by entering the value in the threshold field.

Adjust Histogram Settings

If necessary, you can adjust in the Coloc toolbar the histogram mode and histogram options.

See also:

Coloc - [Coloc Toolbar](#)

Coloc - [Mouse & Keyboard PC](#)

Coloc - [Mouse & Keyboard Mac](#)

16.3 Preview Window

This area visualizes the data set together with a preview of the colocalized region. It displays a Slice representation of the data set, i.e. a 2D view from the top in the z-axis direction. On the left hand side, a sliding bar with a handle allows inspecting and displaying the single slices in the data set along the z-axis. The Preview window updates in real-time the display of the colocalized region based on the actual settings of the colocalization parameters. The original channels can be switched on or off in the Display Adjustments window.

Time Settings

There are no time-dependent parameters for the colocalization analysis. The Preview window always displays the currently selected time point of the data set. The values in the Statistics window always refer to the volume of the current time point. However, when the **Build Coloc Channel** button is pressed, the Coloc channel and statistical values for all time points are calculated.

See also:

Menu Edit - [Show Display Adjustment](#)

Coloc - [Mouse & Keyboard PC](#)

Coloc - [Mouse & Keyboard Mac](#)

16.4 2D Histogram

This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.

Adjust Channel Thresholds

Effects of the modification are instantly visible in the channel histogram A and B, and in the Preview window.

Selection Mode Threshold

The channel thresholds are visible in form of a rectangle.

Point with the cursor on the 2D histogram.

Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.

Click on the edge and drag it to any another position in the histogram.

Selection Mode Polygon

The channel thresholds are visible as an intensity area in form of a free configurable polygon.

You draw the polygon on the 2D histogram. This method is especially useful for eliminating channel bleed through regions along the border of the lower intensity bins. After checking the Polygon mode, the overlaid threshold rectangle in the 2D histogram is converted into a polygon with four corner points. You can now start to modify this polygon or to draw a completely new one. The initial point of the polygon is visible as white filled.

Set Point

Press and hold the Shift-key. The cursor turns into a cross with a little sign. Click on the 2D histogram with the left mouse button to place a point (vertex). Any additional point connects with lines to the initial point and the last point inserted.

Insert Point in a Line

Press and hold the Shift-key. The cursor turns into a cross with a little " + " sign. Point on the line between two vertices and click on the line with the left mouse button to add a point.

Automatic Point Setting

Press and hold the Ctrl-key. The cursor turns into a cross with a little " + " sign and a stack symbol. Drag the cursor around. New vertices are added automatically at each change of cursor position.

Move Point

Point on one of the vertices, click & drag the vertex around to the desired location. The polygon changes its shape.

Move Line

Point on a line between two vertices, click & drag the line around to the desired location. The polygon changes its shape.

Move Polygon

Point somewhere beside of the polygon edge, click & drag the polygon around to the desired location.

Delete Point

Shift + click on a point to delete the point.

See also:

Coloc - [Coloc Toolbar](#) (selection Mode Threshold or Polygon)

16.5 Volume Statistics

The values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. The values are calculated by the following formulas.

Please note: The calculated Coloc Volume Statistics are not automatically saved in the data set. To save or export the statistics you have to build a coloc channel (Coloc - Coloc Toolbar - [Build Coloc Channel](#)).

Definitions

Regions:

Data set	All data set voxels
Coloc	Colocalized voxels
Object A	Voxels with channel A intensity above threshold A
Object B	Voxels with channel B intensity above threshold B
ROI	Region of interest

Indicators:

N	Number of voxels
SA _i	Channel A intensity of voxel
SB _i	Channel B intensity of voxel
SA _{avg} , Coloc	Average channel A intensity in the Coloc region

Volume A	Number of voxel channel A x voxel volume
Volume B	Number of voxel channel B x voxel volume
Material A	Channel A voxel signal intensity
Material B	Channel B voxel signal intensity

Number of colocalized voxels

Total count of colocalized voxels N_{coloc}.

% of data set colocalized

Percentage of total data set voxels colocalized.

$$\frac{N_{coloc}}{N_{dataset}} \cdot 100\%$$

% of volume A above threshold colocalized

Percentage of channel A voxels above threshold A colocalized.

$$\frac{N_{coloc}}{N_{objectA}} \cdot 100\%$$

% of volume B above threshold colocalized

Percentage of channel B voxels above threshold B colocalized.

$$\frac{N_{coloc}}{N_{objectB}} \cdot 100\%$$

% of material A above threshold colocalized

Percentage of channel A material above threshold A that is colocalized.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in objectA} SA_i} \cdot 100\%$$

% of material B above threshold colocalized

Percentage of channel B material above threshold B that is colocalized.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in objectB} SB_i} \cdot 100\%$$

% of ROI colocalized

Percentage colocalization of channel A and channel B volume inside the region of interest.

$$\frac{N_{coloc}}{N_{mask}} \cdot 100\%$$

% of ROI material A colocalized

Percentage colocalization of ROI channel A material.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in mask} SA_i} \cdot 100\%$$

% of ROI material B colocalized

Percentage colocalization of ROI channel B material.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in mask} SB_i} \cdot 100\%$$

Pearson's coefficient in colocalized volume

Pearson correlation of channel A and channel B inside the colocalized region.

Please note: The correlation value ranges between 1 and -1. A value of 1 represents perfect correlation, 0 no correlation, and -1 perfect inverse correlation.

$$R_{AB,coloc} = \frac{\sum_{i \in coloc} (SA_i - SA_{avg,coloc})(SB_i - SB_{avg,coloc})}{\sqrt{\sum_{i \in coloc} (SA_i - SA_{avg,coloc})^2 \sum_{i \in coloc} (SB_i - SB_{avg,coloc})^2}}$$

Pearson's coefficient in data set volume

Pearson correlation of channel A and channel B inside the entire data set volume.

$$R_{AB,dataset} = \frac{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})(SB_i - SB_{avg,dataset})}{\sqrt{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})^2 \sum_{i \in coloc} (SB_i - SB_{avg,dataset})^2}}$$

Pearson's coefficient in ROI volume

Pearson correlation of channel A and channel B inside the region of interest.

$$R_{AB,mask} = \frac{\sum_{i \in mask} (SA_i - SA_{avg,mask})(SB_i - SB_{avg,mask})}{\sqrt{\sum_{i \in mask} (SA_i - SA_{avg,mask})^2 \sum_{i \in mask} (SB_i - SB_{avg,mask})^2}}$$

Original Mander's coefficient A

Original Mander's coefficient A = $\text{Sum}(\text{SAiSBcoloc}) / \text{Sum}(\text{SAi})$
SBcoloc=1 if SBi>0, otherwise SBcoloc=0

Original Mander's coefficient B

Original Mander's coefficient B = $\text{Sum}(\text{SBiSAcoloc}) / \text{Sum}(\text{SBi})$
SAcoloc=1 if SAi>0, otherwise SAcoloc=0

Mander's coefficient A (thresholded)

Thresholded Mander's coefficient A = $\text{Sum}(\text{SAiSBcoloc}) / \text{Sum}(\text{SAi})$
SBcoloc=1 if SBi>thresholdB, otherwise SBcoloc=0

Mander's coefficient B (thresholded)

Thresholded Mander's coefficient B = $\text{Sum}(\text{SBiSAcoloc}) / \text{Sum}(\text{SBi})$
SAcoloc=1 if SAi>thresholdA, otherwise SAcoloc=0

See also:

Menu Edit - Image Properties... - Channel 1...n - [Tab Coloc Statistics](#) (to export the statistics)

Coloc - Coloc Toolbar - [Build Coloc Channel](#) (to build the coloc channel)

16.6 ROI Definition

Define Region of Interest (ROI)

This feature allows you to define a region of interest (ROI) for the entire analysis. A third channel can be selected as a masking area for the entire analysis. A masking channel is e.g. a third microscope channel acquired with settings that allows defining a region of interest by simple intensity thresholding. All voxels outside of the region of interest defined by the mask channel are ignored for the colocalization analysis. The volume excluded by the mask appears hatched in the Preview window. The mask channel is used in conjunction with the Automatic Threshold function. For this function, the selection of an appropriate region of interest is essential.

Mask Dataset

Check this box to activate the function.

X% of Data selected

Displays the percentage of selected data.

Channel Selection

Select the channel to be used from the drop-down list.

Channel Histogram

Point with the cursor into the histogram and click to set the threshold. A bolded line marks the threshold in the histogram and the corresponding value is indicated in the threshold field. The masked volume of the data set appears hatched in the Preview window. Drag the cursor in the histogram to modify the threshold. Effects of the modification are instantly visible in the threshold field as well as in the Preview window.

Threshold Value

Editable threshold value field.

Mask Channel and Automatic Threshold

When the mask is set as required, click the button **Automatic Thresholding** to calculate the thresholds. Build the colocalization channel by clicking the button **Build Coloc Channel**.

See also:

Coloc - Coloc Toolbar - [Automatic Thresholding](#)
Coloc - Coloc Toolbar - [Build Coloc Channel](#)
Coloc - [Mouse & Keyboard PC](#)
Coloc - [Mouse & Keyboard Mac](#)

16.7 Coloc Toolbar

The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

Selection Mode

You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bolded line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

Threshold

To adjust the threshold value using the mouse (see above 1. and 3.), select this mode.

Polygon

To draw a polygon line in the 2D histogram (see above 4.) select this selection mode.

Automatic Thresholding

Please refer to chapter Coloc - Coloc Toolbar - [Automatic Thresholding](#).

Histogram Mode

The histogram mode options are used to define the calculation basis for the colocalization within the data set.

Single Slice

The histograms are calculated and displayed for the current slice only.

Single Time Point

The histograms are calculated and shown for the entire 3D stack of the current time point. This mode allows for faster browsing through the data set along the z-axis.

All Time Points

The histograms are calculated and shown for an entire time data set, i.e. for all time points within the 4D data set.

Histogram Options

The histogram mode options are used to define the display of the 1D and 2D histograms.

Ignore Border Bins

A border bin is always the first and last value in a histogram. Often a lot of voxels fall into the bin with the lowest intensity. In a frequency plot this causes a massive and unnecessary downscale of all other intensity bins which are of more interest. The border bins can therefore be ignored when scaling the display of the histogram. Ignore is selected as default. With most data sets, checking the Ignore Border Bin and Logarithmic options allows a more representative histogram display.

Logarithmic

The frequency plot is shown in a logarithmic scaling instead of a linear scaling.

Color Coded

Applies a false color look-up table to the histogram to improve the visibility of intensity differences. The high frequencies (bins in the 2D histogram into which a lot of voxels accumulate) are shown in yellow-to-white, bins with little voxels are shown in blue-to-black.

Coloc Intensities

The Coloc intensities option defines the brightness of the calculated colocalization displayed in the Preview window.

Source Channels

Uses the intensities of the two channels for displaying the intensity of a colocalized voxel as the square root of the product of the intensities of the original channels.

Constant Value

Displays the intensity of a colocalized voxel defined by the specified value. No distinction is made whether high-intensity or low-intensity voxels colocalize as long as they are within the selection.

Coloc Color

Coloc color allows you to specify personal color settings for the colocalization display in the Preview window.

Button **Edit**

Clicking on the button **Edit** opens the Color window. You can either select one of the predefined colors to apply on the colocalization areas or define, add, and apply your own color definitions.

Button **Build Coloc Channel**

Please refer to chapter Coloc - Coloc Toolbar - [Build Coloc Channel](#).

Button **Build Time Dep. Coloc**

Please refer to chapter Coloc - Coloc Toolbar - [Build Coloc Channel](#).

Button **Channel Statistics**

Please refer to chapter Menu Edit - Image Properties... - Channel 1...n - [Tab Coloc Statistics](#).

See also:

Coloc - [Channel Histogram](#)

Coloc - [Preview Window](#)

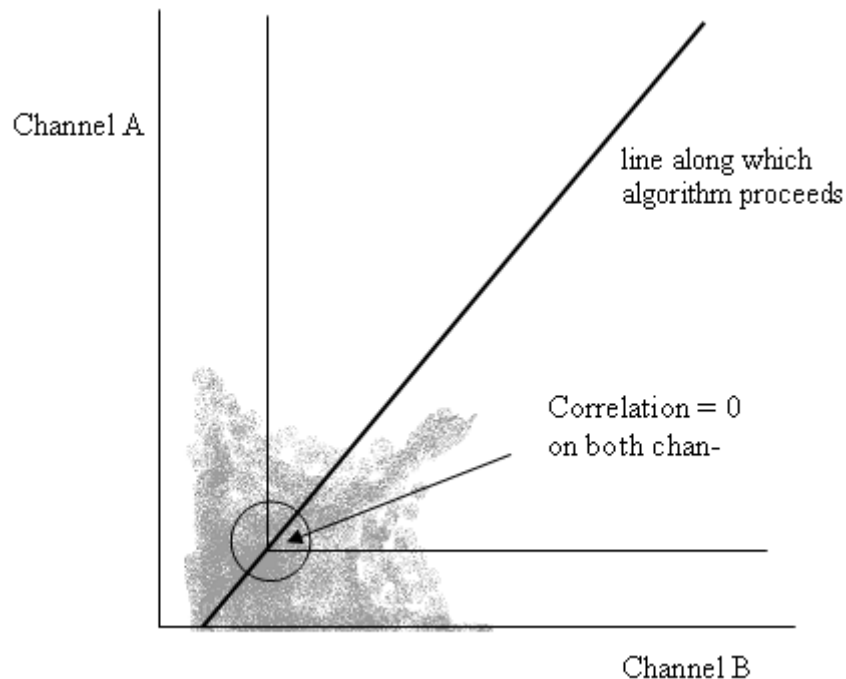
Coloc - [2D Histogram](#)

16.7.1 Automatic Thresholding

Button **Calculate Thresholds**

This button performs an automatic threshold run on both channels based on an algorithm developed by Costes and Lockett at the National Institute of Health, NCI/SAIC, which is based on the exclusion of intensity pairs that exhibit no correlation (Pearson's correlation below zero).

The automatic threshold search is done using the 2D histogram. Starting with the highest intensity value, the algorithm reduces the threshold value step by step along a line (see figure below) and computes the correlation coefficient of the image using only voxels with intensities below the threshold. The algorithm continues reducing the thresholds until the correlation reaches 0, thus defining the automatic threshold.



P-Value

PSF Width

As the size of the Point Spread Function please enter the length along the Z Axis. This can be a calculated theoretical length.

Links to calculators on the WWW you can find e.g. on the Bitplane homepage.

The default in Imaris is not actually related to the PSF but only to the diagonal of the voxel size.

For more information about the P-Value and PSF Width please refer to the following publication:

Automatic and Quantitative Measurement of Protein-Protein Colocalization in Live Cells

Sylvain V. Costes,*y Dirk Daelemans,z Edward H. Cho,* Zachary Dobbin,* George Pavlakis,z and Stephen Lockett*

* Image Analysis Laboratory, National Cancer Institute, Frederick, Maryland;

y National Cancer Institute/Science Applications International Corporation, Frederick, Maryland; and

z Human Retrovirus Section, National Cancer Institute, Frederick, Maryland

Biophysical Journal Volume 86 June 2004 3993-4003

16.7.2 Build Coloc Channel

Button **Build Coloc Channel**

Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set. The channel can then be saved and is part of the data set.

Button **Build Time Dep. Coloc**

Clicking this button calculates the colocalization channel according to the selected settings (calculating an automatic threshold individually for each time point) and adds it to the data set. The channel can then be saved and is part of the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File – Save As or click the **Save as** button in the toolbar. The Save As box is displayed. Select the directory and enter the

name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

Button **Channel Statistics** (for the new Coloc channel)

After building the Coloc channel, its statistics become available in the Image Properties. The button **Channel Statistics** displays directly the Image Properties window with the statistics for the calculated channel. The name of the channel, the description of the source channels and the selection method are indicated and displayed in the Image Properties on the Tab Coloc Statistics.

Export Statistics

The statistics can be exported.

Button **Export**

In the Image Properties window click the **Export** button. The Export Coloc Statistics window is displayed. Select the directory and enter the name for the file to be saved. The statistics are saved as .csv Excel file and can be opened directly with Microsoft Excel.

See also:

Coloc - [Volume Statistics](#)

16.8 Basic Operation

The basic use of Coloc in the daily work flow comprises the following steps:

- Select the channels.
- Adjust selection mode and histogram mode.
- Set thresholds.
- Check the statistics.
- Build and save the Coloc channel.

Select Channel A and Channel B

In the 1D histograms select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Selection Mode and Histogram Mode

Before you set the thresholds, please check the appropriate parameters in the Coloc toolbar on the right hand side of the screen:

- Check as Selection Mode Threshold.
- Decide, which Histogram Mode you need. Choose Single Slice for calculating the histogram for the current slice only. Or choose Single Time Point to calculate the histogram for the entire 3D time point. Or choose All Time Points for the entire 4D image.

Set Thresholds

Colocalization occurs where the voxels are above threshold in channel A and channel B. You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bolded line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

A. On the 1D Channel Histogram

Point with the cursor on the histogram. The cursor turns into a cross.

Click on the histogram to determine a threshold. The bolded left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.

Click on the line and drag it to another position in the histogram. Effects of the modification are instantly visible in the other indications as well as in the Preview window.

Perform for both channels until the settings are as desired.

B. By Entering a Value

Click in the Threshold field of one of the channels and enter a value, then press Enter. The bolded left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot. Effects of the modification are instantly visible in the other indications as well as in the Preview display.

Perform for both channels until the settings are as desired.

C. On the 2D Histogram

Point with the cursor on the 2D histogram. The cursor turns into a cross.

Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.

Click on the edge and drag it to any another position in the histogram.

D. On the Preview Window

Click with the cursor on the image in the Preview window. An active contour is displayed, outlining image elements brighter than the location clicked.

Drag the line on the image to define the threshold of channel A. The selection works best when starting with brighter spots and dragging towards darker areas.

Press and hold the Shift-key, click with the cursor on the image, then drag the line to determine the threshold of channel B.

Check the Statistics

The statistical values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. For details please refer to chapter Coloc - [Volume Statistics](#).

Build Coloc Channel

Button **Build Coloc Channel**

Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File - Save As or click the **Save as** button in the toolbar. The Save As box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

16.8.1 Advanced Examples

The colocalization channel can be visualized like any other channel in Imaris. In the following examples, a Coloc channel was built on the retina data set.

Visualize the Coloc Channel in Easy 3D

Example 1: Visualize and Adjust the Coloc Channel in Imaris Easy 3D

After building the Coloc channel, click on the **Easy 3D** button in the Imaris menu bar.

Check the desired Rendering mode parameters and adjust the Settings if necessary, then click the **Calculate** button. The 3D image of channel is calculated and displayed.

As the colocalized region always lays inside the initial channels, making the initial channels invisible or more transparent allows a better view of the colocalized region.

In the Imaris menu bar select Edit – Show Display Adjustment.

In the Display Adjustments window un-check one of the two original channels, then click the button **Calculate** again. The 3D image of the original and the Coloc channel is calculated and displayed.

In the Imaris menu bar select Edit – Show Display Adjustment.

In the Display Adjustment window select the Coloc channel and adjust its settings.

In the Display Adjustment window select the remaining original channel and adjust its settings. In this example the channel is set as partly opaque.

After each modification, click the **Calculate** button again to calculate the result.

Visualize the Coloc Channel in Surpass

The colocalization channel can be visualized like any other channel in Surpass.

Example 2: Create Surfaces of the Coloc Channel and Add the Two Initial Channels as Volume Rendering

After building the Coloc channel, click on the **Surpass** button in the Imaris menu bar.

In the Objects toolbar click the **Add New Surfaces** button.

In the Surfaces Properties window check and select the Coloc channel.

Select the desired parameters. In this example, we set the threshold just below the lowest intensity values in the histogram.

In the menu Surpass select Volume.

In the Imaris menu bar select Edit – Show Display Adjustment and un-check the Coloc channel. The Surpass Volume object displays only the two initial channels. Then select the two initial channels and adjust their settings, e.g. decrease the blend opacity.

16.9 Mouse & Keyboard PC

Coloc Mouse & Keyboard Functions

Ctrl + 7 Activates the Coloc view

Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Left-click on histogram Set new threshold
Left-click on line & drag Move threshold

2D Histogram

Left-click Set channel A and channel B thresholds
Left-click on line & drag Move channel A and channel B thresholds

Preview Window

Left-click & drag Set threshold channel A
Shift + left-click & drag Set threshold channel B

Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + left-click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Ctrl + left-click	Add multiple new points
Left-click & drag (on point or line)	Move point or line
Left-click & drag (outside polygon)	Move polygon
Delete	Delete single point

16.10 Mouse & Keyboard Mac

Coloc Mouse & Keyboard Functions

Command + 7	Activates the Coloc view
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Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Click on histogram	Set new threshold
Click on line & drag	Move threshold

2D Histogram

Click	Set channel A and channel B thresholds
Click on line & drag	Move channel A and channel B thresholds

Preview Window

Click & drag	Set threshold channel A
Shift + click & drag	Set threshold channel B

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences...
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.

Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Command + click	Add multiple new points
Click & drag (on point or line)	Move point or line
Click & drag (outside polygon)	Move polygon
Delete	Delete single point

17 Addendum

In this addendum you find the Imaris global [Mouse & Keyboard PC](#) and [Mouse & Keyboard Mac](#) functions, tips and tricks how to customize your [Imaris Interface](#) in daily routine, and a [Terminology](#) table with frequently used technical terms and specific image processing terms.

17.1 Mouse & Keyboard PC

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

Ctrl + 1	Activates the Slice view
Ctrl + 2	Activates the Section view
Ctrl + 3	Activates the Gallery view
Ctrl + 4	Activates the Easy 3D view
Ctrl + 5	Activates the Surpass view
Ctrl + 6	Activates the Key Frame Animation
Ctrl + 7	Activates the Coloc view
Ctrl + 8	Activates InMotion
Ctrl + A	Sets zoom factor to 1 pixel per voxel and centers the image to the window
Ctrl + B	Adjusts zoom factor and pan position to best fit the image into the window
Ctrl + C	Copies current view to the clipboard
Ctrl + D	Shows the Display Adjustment window
Ctrl + E	Saves Surpass scene file
Ctrl + F	Refreshes the textures of all views
Ctrl + I	Allows to inspect and edit the image properties
Ctrl + L	Opens Surpass scene file
Ctrl + N	Toggles display of Navigation window
Ctrl + O	Opens existing file
Ctrl + P	Allows to edit the settings of Imaris
Ctrl + Q	Exits the application

Ctrl + R	Discards all changes and opens the current data set again
Ctrl + S	Saves the current data set
Ctrl + T	Saves the current view as a *.tif file
Ctrl + W	Crops X, Y, or Z
Ctrl + Z	Revokes the last action
Ctrl + Shift + A	Adds channels of another image
Ctrl + Shift + B	Deletes selected channels
Ctrl + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
F11	Maximizes the viewing area to the full size of your monitor
Ctrl + F1	Opens Quick Start Tutorials
Alt + F4	Exits the application
Del	Deletes selected objects
Right-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Left-click & drag	Rotate image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective chapter in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - Mouse & Keyboard PC
Slice View -	Mouse & Keyboard PC
Section View -	Mouse & Keyboard PC
Gallery View -	Mouse & Keyboard PC
Easy 3D View -	Mouse & Keyboard PC
Surpass View -	Overview - Mouse & Keyboard PC
	Clipping Plane - Mouse & Keyboard PC
	Contour Surface - Mouse & Keyboard PC
	External Object - Mouse & Keyboard PC
	Filament - Mouse & Keyboard PC
	Light Source - Mouse & Keyboard PC
	Measurement Point - Mouse & Keyboard PC
	Oblique Slicer - Mouse & Keyboard PC
	Ortho Slicer - Mouse & Keyboard PC
	Surface- Shortcuts Overview
	Spots - Mouse & Keyboard PC
Animation -	Mouse & Keyboard PC
InMotion -	Mouse & Keyboard PC
Coloc -	Mouse & Keyboard PC

Context Sensitive Help

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

17.2 Mouse & Keyboard Mac

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

Command + 1	Activates the Slice view
Command + 2	Activates the Section view
Command + 3	Activates the Gallery view
Command + 4	Activates the Easy 3D view
Command + 5	Activates the Surpass view
Command + 6	Activates the Key Frame Animation
Command + 7	Activates the Coloc view
Command + 8	Activates InMotion
Command + A	Sets zoom factor to 1 pixel per voxel and centers the image to the window
Command + B	Adjusts zoom factor and pan position to best fit the image into the window
Command + C	Copies current view to the clipboard
Command + D	Shows the Display Adjustment window
Command + E	Saves Surpass scene file
Command + F	Refreshes the textures of all views
Command + I	Allows to inspect and edit the image properties
Command + L	Opens Surpass scene file
Command + N	Toggles display of Navigation window
Command + O	Opens existing file
Command + ,	Allows to edit the settings of Imaris
Command + Q	Exits the application
Command + R	Discards all changes and opens the current data set again
Command + S	Saves the current data set
Command + T	Saves the current view as a *.tif file
Command + W	Crops X, Y, or Z
Command + Z	Revokes the last action
Command + Shift + A	Adds channels of another image
Command + Shift + B	Deletes selected channels
Command + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
Command + F11	Maximizes the viewing area to the full size of your monitor
Command + F1	Opens Quick Start Tutorials
Del	Deletes selected objects
Ctrl-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

Open the Apple-menu, select System Preferences... .

Click on the button Keyboard & Mouse.
Select the OS X mouse properties.
Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective chapter in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - Mouse & Keyboard Mac
Slice View -	Mouse & Keyboard Mac
Section View -	Mouse & Keyboard Mac
Gallery View -	Mouse & Keyboard Mac
Easy 3D View -	Mouse & Keyboard Mac
Surpass View -	Overview - Mouse & Keyboard Mac
	Clipping Plane - Mouse & Keyboard Mac
	Contour Surface - Mouse & Keyboard Mac
	External Object - Mouse & Keyboard Mac
	Filament - Mouse & Keyboard Mac
	Light Source - Mouse & Keyboard Mac
	Measurement Point - Mouse & Keyboard Mac
	Oblique Slicer - Mouse & Keyboard Mac
	Ortho Slicer - Mouse & Keyboard Mac
	Surface- Shortcuts Overview
	Spots - Mouse & Keyboard Mac
Animation -	Mouse & Keyboard Mac
InMotion -	Mouse & Keyboard Mac
Coloc -	Mouse & Keyboard Mac

Context Sensitive Help

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

17.3 Customize Interface

In this chapter you find a collection of tips and tricks to customize the Imaris interface for your daily routine.

Histogram

Logarithmic or linear histogram display.

On the left hand side of a histogram there are horizontal lines representing a linear or logarithmic histogram display.

Click on these lines to change the display from logarithmic to linear and vice versa.

The default setting is logarithmic.

Windows

You can plug windows in Imaris in or out.

Example: Key Frame Animation window

Plug out:

Click on the header of the Key Frame Animation window and drag the window to any free space on the screen.

On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged out, you can drag it on any free space on the screen.

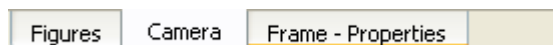
Plug in:

On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged in on its original place.

Tabbed Toolbars

Try to un-dock the toolbars and dock them all at the same side. Especially in combination with InPress, this saves a lot of screen area.

Example: In the Section view you can bring the toolbar Figures (InPress), Camera, and Properties to the left side at the bottom.



Objects Toolbar

You can adjust the Objects toolbar to your personal preferences.

Open the menu Edit - Preferences - Surpass - Object Creation Buttons to adjust your personal Objects toolbar.

Display Mode Blend and Bland Opacity

You can select the Display Mode Blend in the Surpass view for a Volume object, in the Section view (Extended) and in the Easy 3D view. Any change in the Display Adjustment window in the parameter Blend Opacity is only visible, if a Blend Mode is selected.

17.4 Terminology

Term	Description
Channel	A channel is a particular piece of information that has been recorded for all voxels. Most of the time in light microscopy, the different channels are intensities that have been measured at different wavelengths. For instance, a two-channel data set contains two intensities for each voxel, one intensity measured in channel 1 (e.g., green light) and one intensity in channel 2 (e.g., red light). Ideally, the intensity values present in different channels are independent of one another.
Coordinates	Any position in the data set can be described either in the voxel grid (e.g., voxel no. 5 in x-direction) or by a coordinate, which corresponds to a m). The distance relative to a defined origin in the real world (e.g. 2 latter) requires the correct calibration of the data set, i.e. that the voxel size is defined appropriately. Generally, in Imaris the origin of the data set is in the left bottom corner. If the data set was cropped from a larger data set, the bottom left corner may have minimum coordinates that are not equal to (0,0,0).
Image stack	A 3D data set is sometimes called an image stack consisting of 2D images stacked on each other.

MIP	A maximum intensity projection (MIP) is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.
Original Data Set	A 3D data set that cannot be derived from any other data set using Bitplane software. Usually "Original data sets" have been acquired using an image sensor and are loaded into Imaris.
Rendering	A technique that visualizes a multi-dimensional data set as a two-dimensional image that can be displayed on a computer screen or printed on a laser printer.
Resultant Image	An image that has been computed using Imaris.
Standard Deviation	<p>In probability and statistics, the standard deviation of a probability distribution, random variable, or population or multiset of values is a measure of the spread of its values. It is defined as the square root of the variance.</p> <p>The standard deviation is the root mean square (RMS) deviation of values from their arithmetic mean. For example, in the population {4, 8}, the mean is 6 and the standard deviation is 2. This may be written: {4, 8} 6. In this case 100% of the values in the population are at one standard deviation of the mean.</p> <p>The standard deviation is the most common measure of statistical dispersion, measuring how widely spread the values in a data set are. If the data points are close to the mean, then the standard deviation is small. Conversely, if many data points are far from the mean, then the standard deviation is large. If all the data values are equal, then the standard deviation is zero.</p> <p>The standard deviation (s) of a population can be estimated by a modified standard deviation (s) of a sample.</p>
Threshold	A threshold in Imaris is a gray value, which acts as a limit. All gray values above (or below) are assigned a particular functionality.
Time Point	A time point is a single 3D image containing one or many channels. The term is used regardless of what data is actually stored in the channels. In particular, the channels could contain data taken at different points in time with a changing object.
Volume Rendering	A rendering technique which operates directly on the voxel data. The input image consisting of a collection of voxels arranged in a regular grid is converted directly into the 2D output image.
Voxels	A volume data set is composed of volume elements called voxels. The voxels are the smallest units within the image about which we have distinct information in the form of a measured intensity. The dimensions of an individual voxel are specified in most image file formats supported by Imaris.