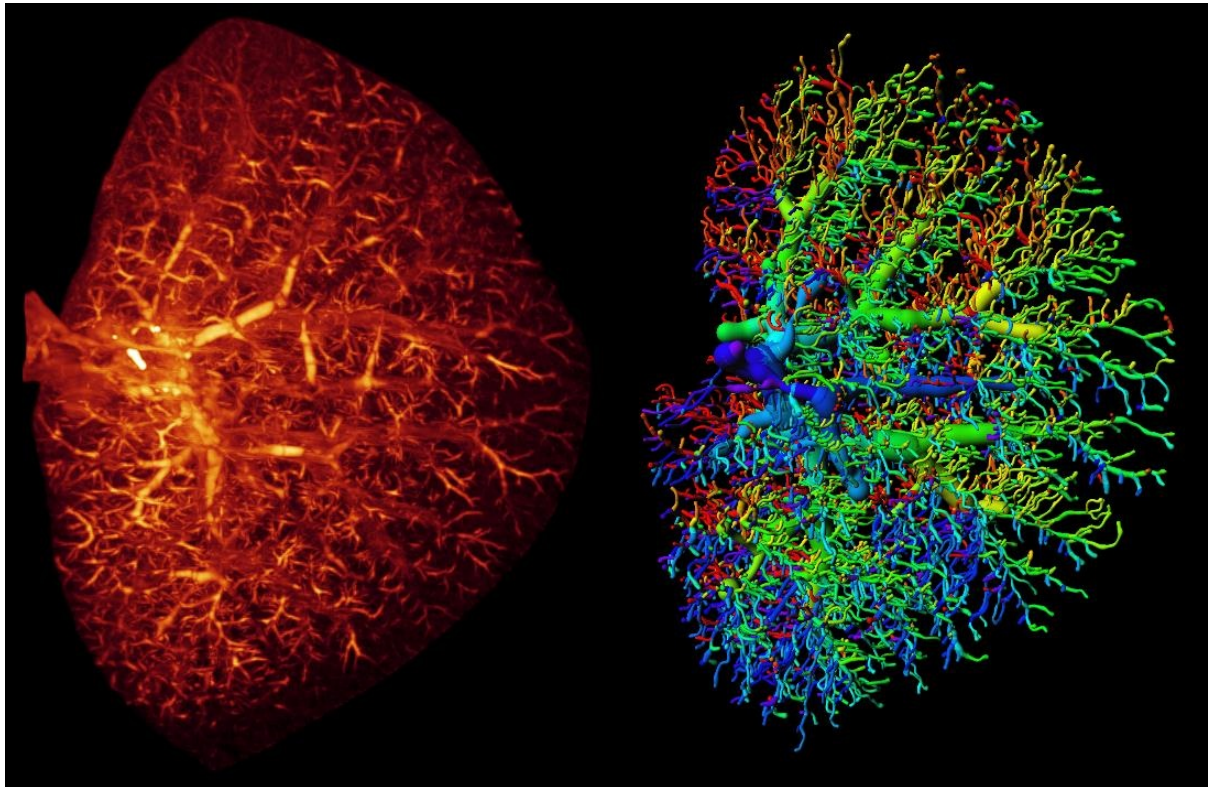


Imaris Basics Workshop Notes

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Notes Prepared by Dr Sarah Creed

Monash Micro Imaging at MHTP 1ST EDITION - June 2017



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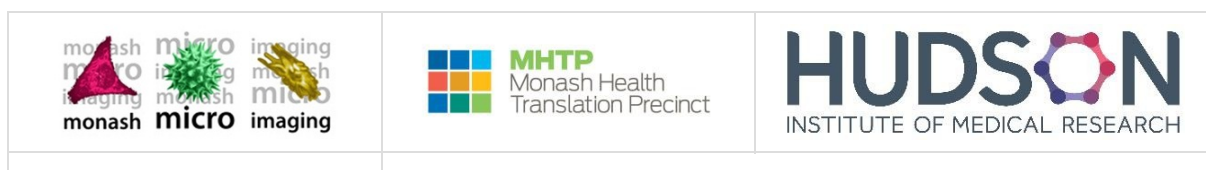


Table of Contents

Imaris Basics title page	1.1
PART 1: INTRODUCTION TO IMARIS AND LICENSE INFORMATION	1.2
PART 2: GETTING STARTED IN IMARIS	1.3
PART 3: VIEWING YOUR DATA	1.4
PART 4: IMAGE ADJUSTMENTS	1.5
PART 5: GETTING STARTED WITH SELECTION AND ANALYSIS TOOLS	1.6
Acknowledgements	1.7
Use and Distribution of Workshop Notes and Demonstration Images	1.8

PART 1: INTRODUCTION TO IMARIS AND LICENSE INFORMATION

Imaris 3D/4D Analysis Software

Imaris is a 3D/4D data visualisation and analysis software produced by Bitplane, an Oxford Instruments company. Imaris allows you to 3D capture snapshots and animations for accurate visualisation of your data, as well as creating 3D surface renders and selections for measurements and quantification.

Use and Licenses

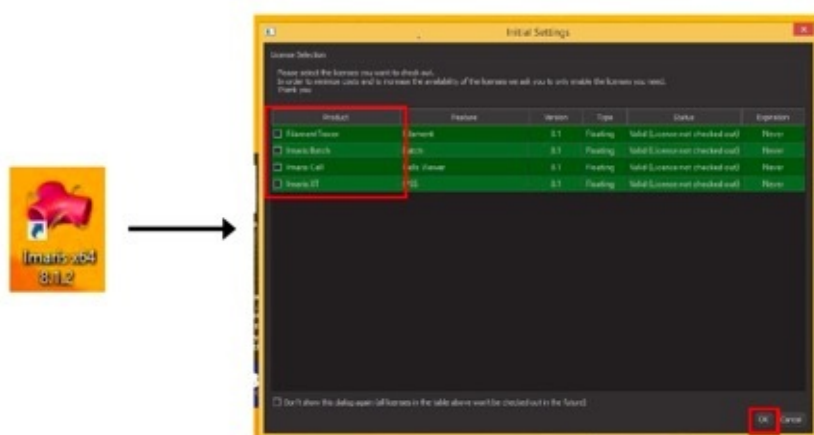
Imaris requires a license to operate. There are a number of institutional licenses available to run Imaris software on Monash Micro Imaging (MMI) computer workstations. These workstations, and the Imaris licenses, can be booked via the iLab booking system and are included as equipment available to all registered MMI users. Bookings on analysis workstations are counted towards your usage hours. To access these workstations, and the software licenses without registering with MMI, please contact MMI staff to discuss hourly rates.

You can access a free 10 day trial of Imaris at <http://www.bitplane.com/imaris/imaris>. Click on 'Download Imaris' and complete the form.

Information and quotes for purchasing your own Imaris license can also be accessed via the Bitplane website.

Imaris Versions

Imaris 9.2.0 was released in June 2018, and (at the time of writing) is the latest current version of Imaris. This is the version that should be used if licenses are available. Open Imaris from the desktop icon and select any special license(s) required from the selector by clicking the check box beside the licence and click OK. The standard license does not need to be selected, it will start automatically upon opening Imaris.



There are a limited number of licenses available for use which are shared with the facility on Monash campus. Please only select the license you need so other remain free for other people to use.

If Imaris is needed immediately but no licenses are available, Imaris 7.6.5 is available with a special license. Please see MMI-MHTP staff for assistance if you are not aware of how to access this.

This workshop and the accompanying notes will use Imaris 9 – but all notes are applicable to earlier version of Imaris, with the exception of “Arena Mode” which was an inclusion only in later versions of the software.

Information and Online Tutorials

You can find detailed technical information, helpful step-by-step guides, video tutorials and webinars for the Imaris software on the 'Bitplane Learning' website: <http://www.bitplane.com/learning>.

Using Imaris for the Workshop

If you are able to, please download and install the free Imaris trial to your laptop for use during the workshop. If you have already used the free trial previously or are unable to download this please inform MMI-MHTP staff so they can organise a training license for you. This is for temporary use only. It will be installed on your laptop before the workshop by MMI-MHTP staff and MUST be removed by staff at the conclusion of the workshop.

PART 2: GETTING STARTED IN IMARIS

Opening Images

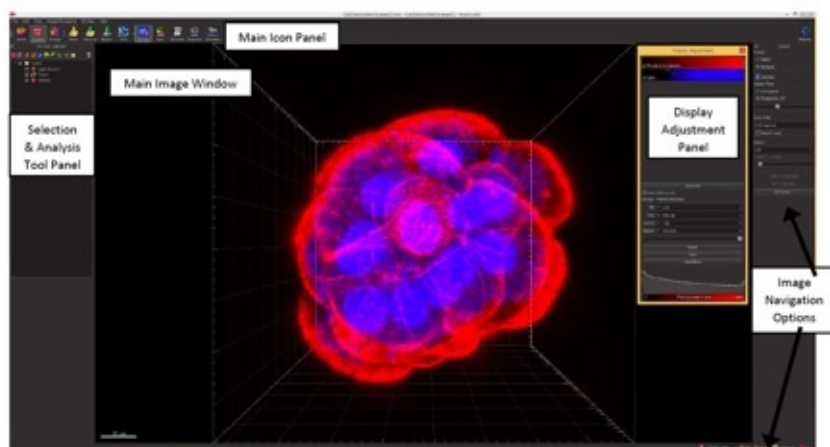
The Imaris software can read and open many different image formats, including standard formats such as .tif and .jpg as well as bio-formats from microscope/image capture software. It will automatically read the dimensions (channels, time, z-stacks) and display them appropriately in the software.

When using Imaris in Surpass mode you can open images by 'dragging and dropping' them into the Imaris window, or by going to **File -> Open** and selecting your file from the explorer window.

To add files to Imaris in Arena mode see the section on Arena below.

The Main Imaris Interface

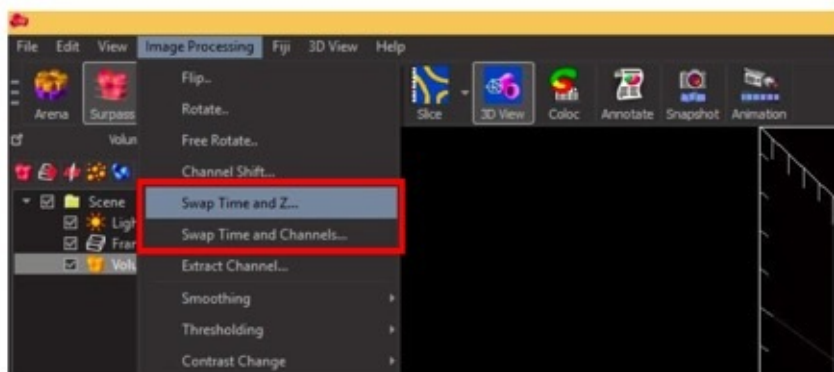
Imaris opens as a full window on your screen. In the main window there are a number of icon panels for different functions. These are outlined below.



Rearranging Image Dimensions

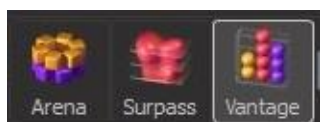
On rare occasions Imaris will read the dimensions of your image in the wrong order and display them incorrectly, for example displaying time points as 3D z-series or different channels as time points.

You can easily correct this by going to the **Image Processing** menu and selecting the dimensions you wish to swap; either **Swap Time and Z** or **Swap Time and Channels**.



Imaris Modes

There are 3 different ways to view your data in Imaris, which we will refer to here as “Operating modes”. These are Arena Mode, Surpass Viewing Mode and Vantage. You can switch between these modes by selecting the corresponding icon from the top left panel of the main Imaris window.



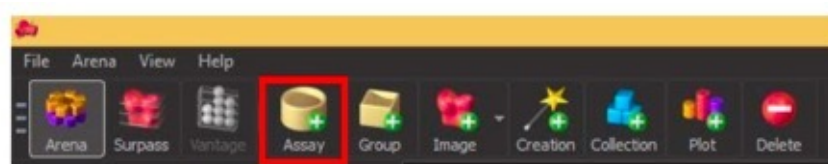
When you first open Imaris v8 it will start in **Arena Mode**. In the Arena you can pre-load all of the images you want to examine, and sort them into different experiments and groups. Arena is not present in earlier version of Imaris.

In **Surpass Viewing Mode** you can visualise the selected images in 2D, 3D or 4D (time series only), navigate around the image and carry out quantification.

In **Vantage** you can generate graphs and plots from your image data. This is rarely used, as most people export quantification data to other statistics programs such as Excel, SPSS or GraphPad Prism. As such the use of Vantage is not covered in the Imaris Basics workshop.

Using the Imaris Arena

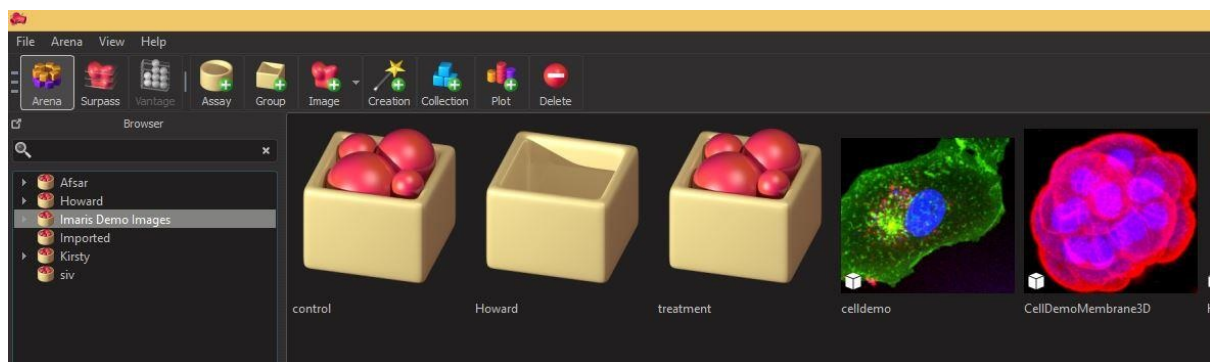
To use Arena, select your workspace or create a new one by clicking the **New Assay** icon.



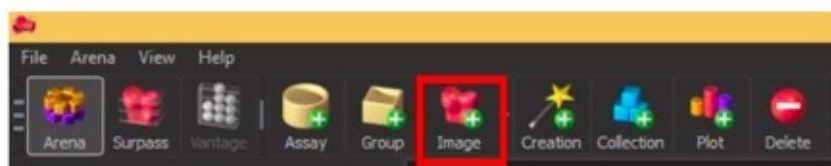
Give the “assay” or experiment folder a name and click **OK**.



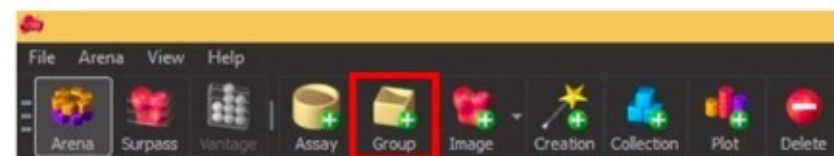
This will generate a new section in the Arena workspace where you can add images and groups.



You can now add images by dragging and dropping into the Arena workspace or clicking the **new Image** icon and selecting your file from the explorer window.



You can group your images together (ie: control vs treatment or by experiment date) by creating a new group within the workspace. Click on the **New Group** icon, name the group and click **OK**.



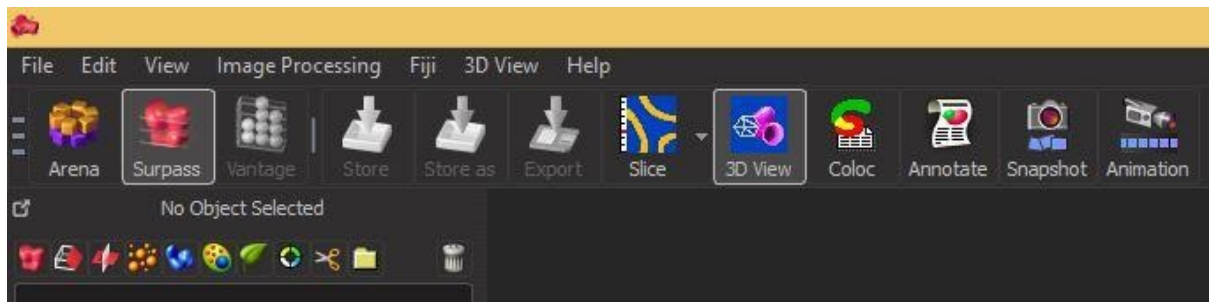
Drag and drop your images into the group icon in the workspace to move them.

To delete an image, group or assay from Arena select the image or icon and click the **Delete** button in the panel.

To open a specific image from Arena in the Surpass Viewing Mode, double click the image.

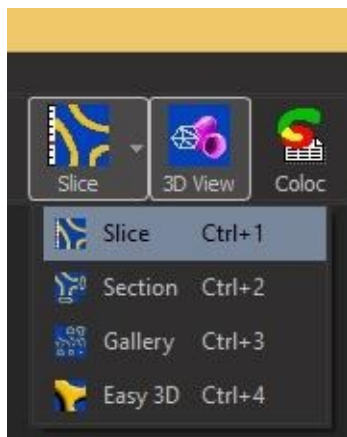
Options in Surpass Viewing Mode

In **Surpass Viewing Mode** your data will open as a single image in 2D, 3D or 4D, depending on the data type. This is called the **3D View**.



You can choose to work in this 3D View mode or you can select options from the **Slice/Easy 3D** icon. You can switch between the two easily using the respective icons in the top panel.

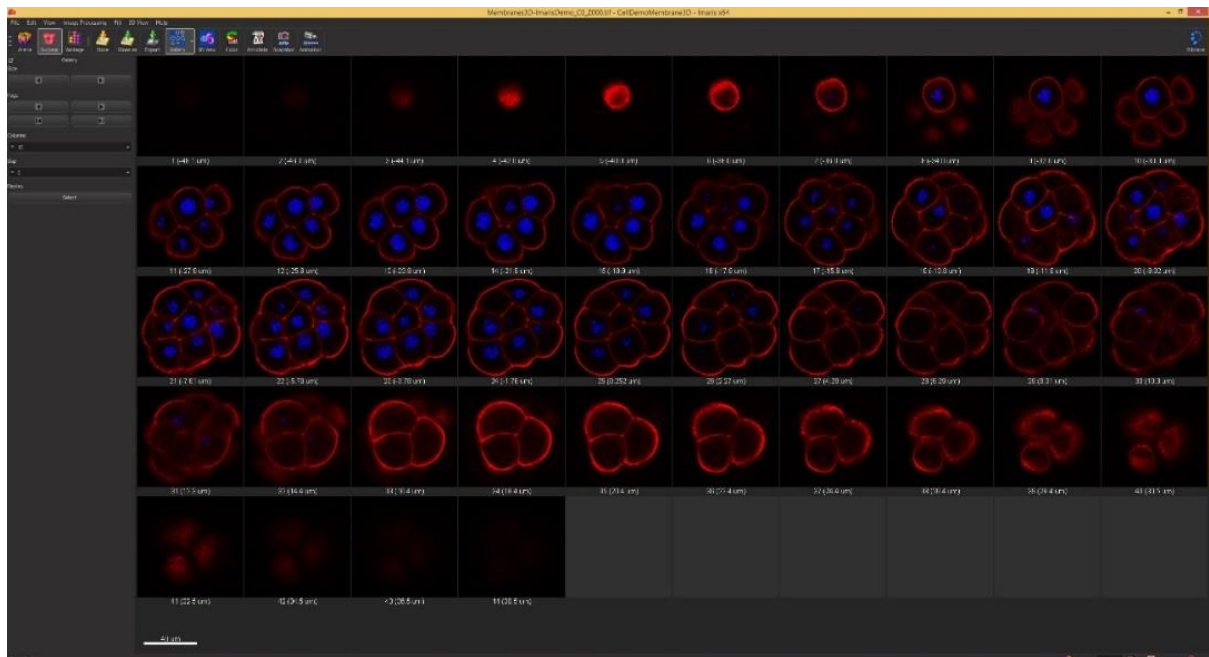
From the Slice/Easy 3D Menu you can select several different viewing options from the drop down menu, accessible by clicking on the small down arrow.



Slice will show each z-section of the 3D image as a single panel and allows you to scroll through the section using the slider on the left hand side of the window.



Another popular option is the **Gallery View**. This shows all slices from a 3D image in order in a gallery panel.

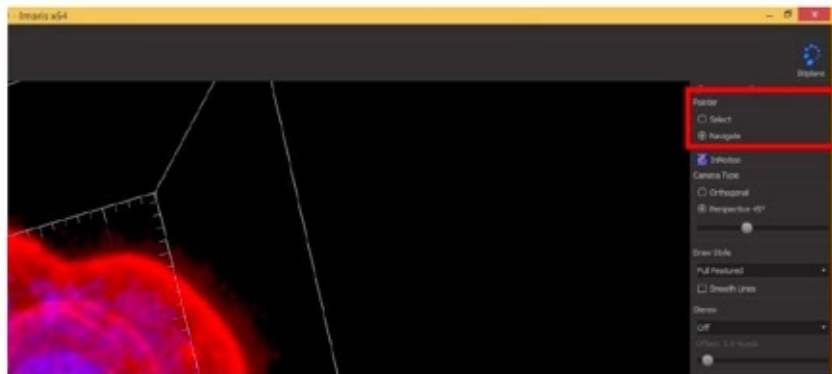


PART 3: VIEWING YOUR DATA

Navigating Within Your Images

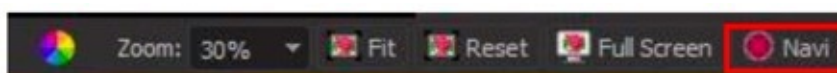
In Surpass Viewing Mode you can navigate around your 3D and 4D images (move and rotate) by clicking and dragging when the cursor is in navigate mode (it will look like two curved arrows when you hover over the image).

If the cursor is in section mode (normal single straight arrow when hovering over image) this will not work. You can switch between selection and navigation by hitting **Escape** on your keyboard, or using the **Pointer** selection buttons in the top right corner of the Imaris window.

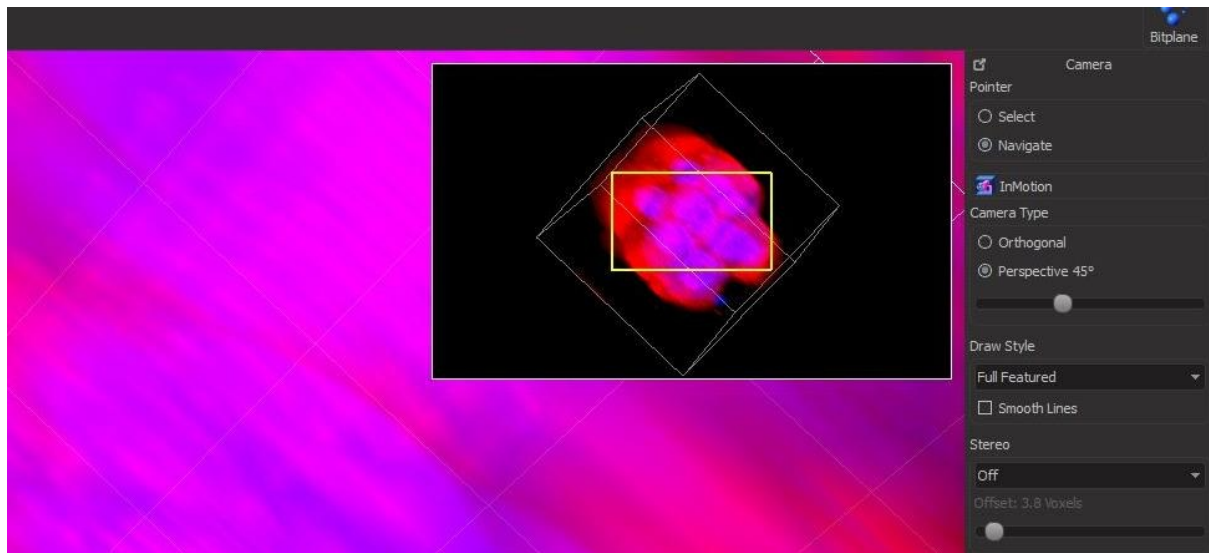


When navigating your image, if you stop dragging before you release the mouse button, the image will also stop moving. However, if you release the mouse button while continuing to drag the image it will continue to rotate automatically. Rotation will be on the angle and at the speed that you were dragging the image. To stop this continuous rotation, click on the image.

You can easily zoom in on your image using the scroll wheel on your mouse. Scroll up to zoom in and scroll down to zoom out. To visualise where you are in the original image while zoomed in, open the **Navigate** window by clicking the **Navi** icon on the bottom left of the Imaris window.



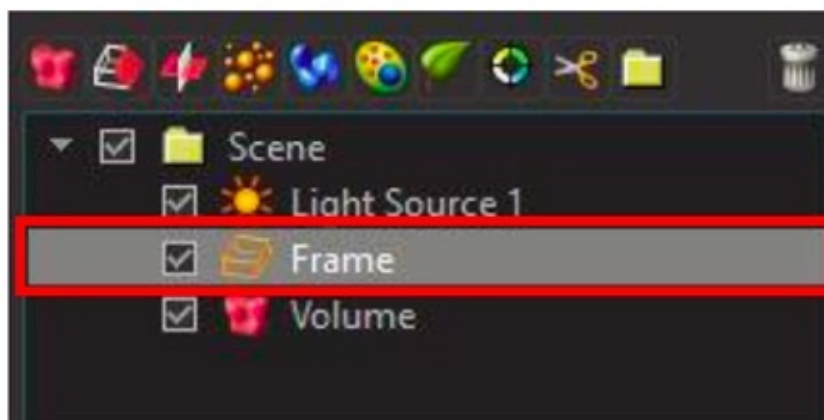
This will display a smaller version of the original image with a yellow outline demonstrating your current position in the image.



You can easily return the image to its original orientation and zoom at any time by clicking the **Fit** and **Reset** icons from the panel in the bottom left of the Imaris window.

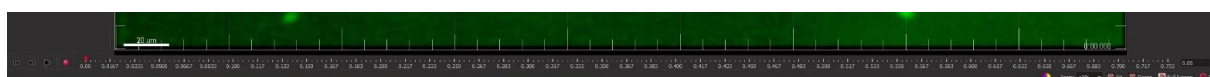


In Surpass 3D viewing mode images are displayed with a surrounding grid, or frame. You can turn this grid on and off by checking the box next to **Frame** in the right hand panel (analysis and selection tools) of the Imaris window, under Scene.



Viewing Time Data

When viewing 4D (time series) data you can navigate through time points using the slider at the bottom of the Imaris window.



To play continuously, click the small black arrow, or **play** button in the panel. To move one frame forward of backwards in the series use the **Skip** buttons. You can monitor your position through the series in the **Time Bar**.

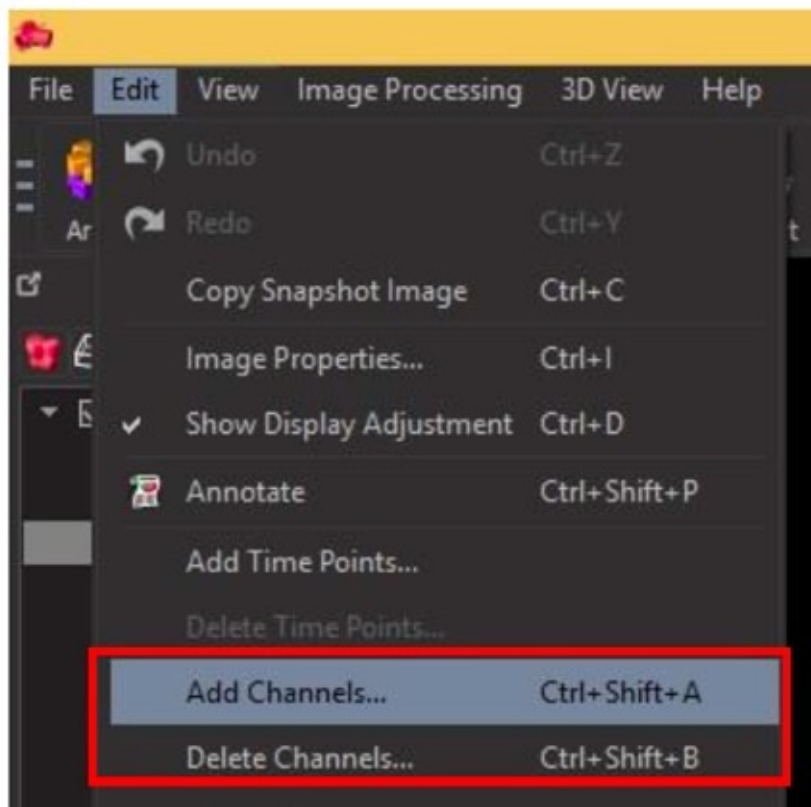


Adding and Deleting Channels

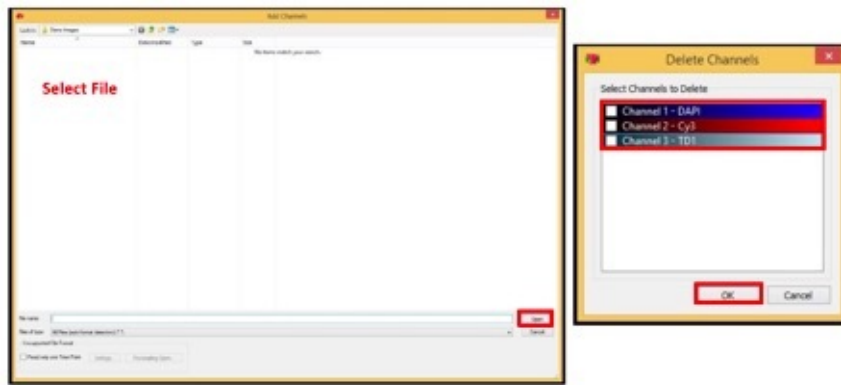
You can alter the channels in your images by using the **Add Channels** or **Delete Channels** tools.

This can be useful if your channels were captured as separate images or if you have an empty channel in a multi-channel file.

To add a channel to your image go to **Edit -> Add Channel...** (or use shortcut Ctrl + Shift + A). To remove channels go to **Edit -> Delete Channels...** (or use shortcut Ctrl + Shift + B).



To add a new file as a channel in your image, choose the file from the explorer window and click **OK**. When deleting channels, select the channel(s) you want to delete from the image and click **Open**.

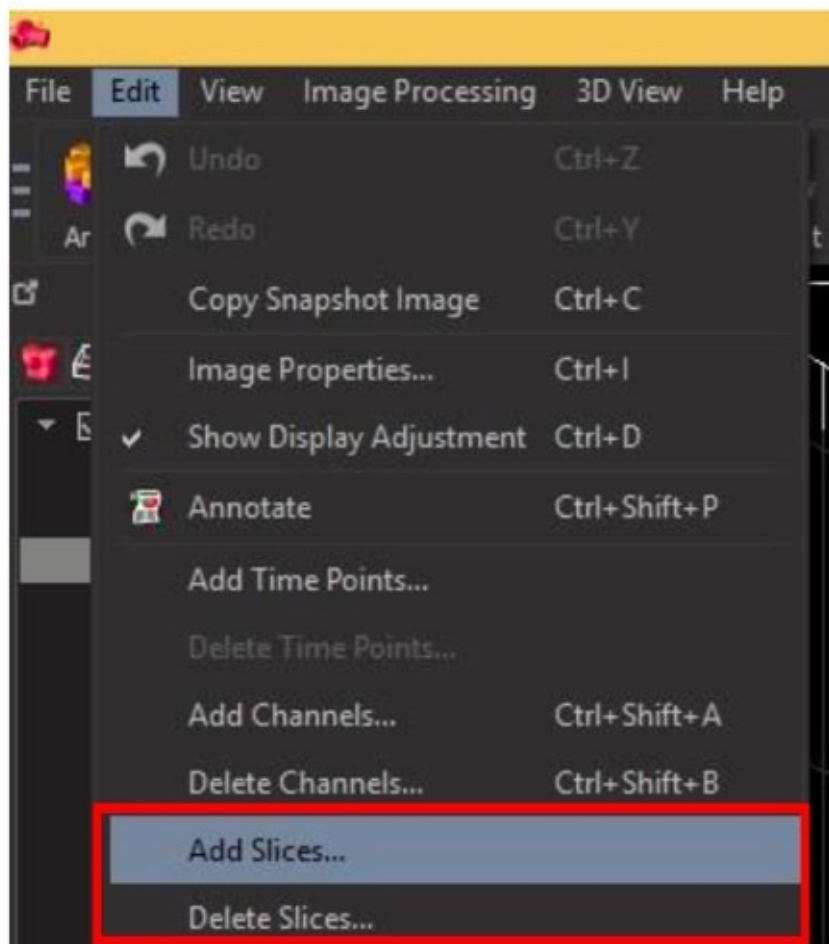


Adding or Deleting Slices

You can also add or delete slices from a z-stack or 3D image.

This can be useful if you have over sampled and have blank sections above or below your cells of interest in the z-stack.

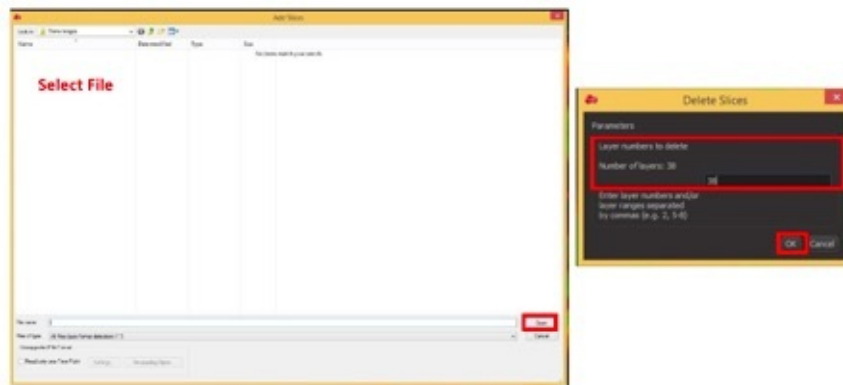
Go to the **Edit** menu and select the option you require; **Add Slices...** or **Delete Slices**.



This works in the same way as adding and deleting channels.

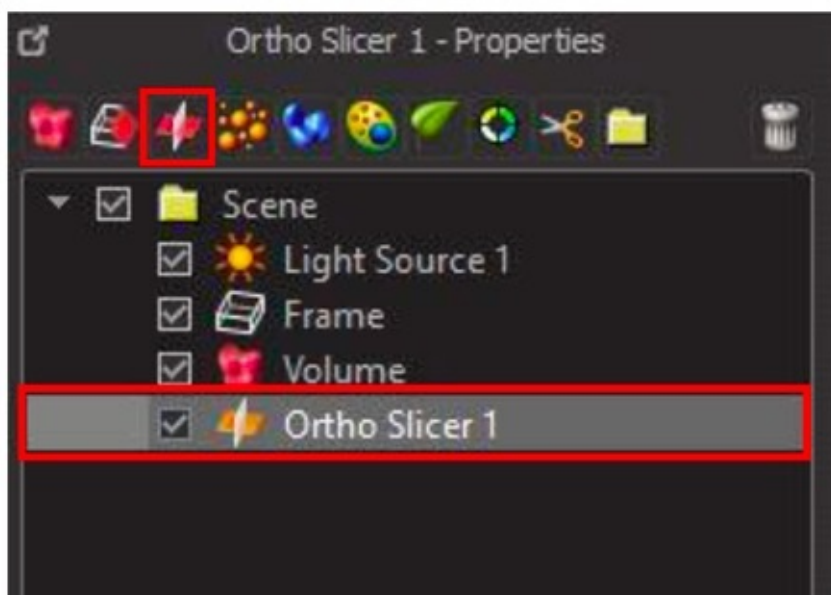
Slices can be adding by selecting a file containing the next set of z-slices, then click **Open**.

Delete by entering the number of the section(s) you wish to delete from the z-stack in the resulting window and click **OK**.



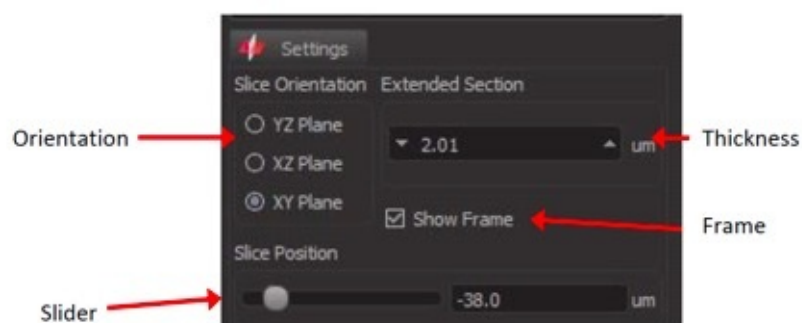
Orthoslicer

Orthoslicer is a tool found in the left hand side selection and analysis tools panel. Click on the **Orthoslicer** icon, it will appear as a layer in the scene, which you can turn on and off using the checkbox.



You can use the orthoslicer to navigate through the 3D image in xy, yz or xz. Select the orientation from the options, which will open in the bottom of the selection and analysis tools panel.

You can also change the thickness of the slicer and turn the orthoslicer frame on or off in the image.

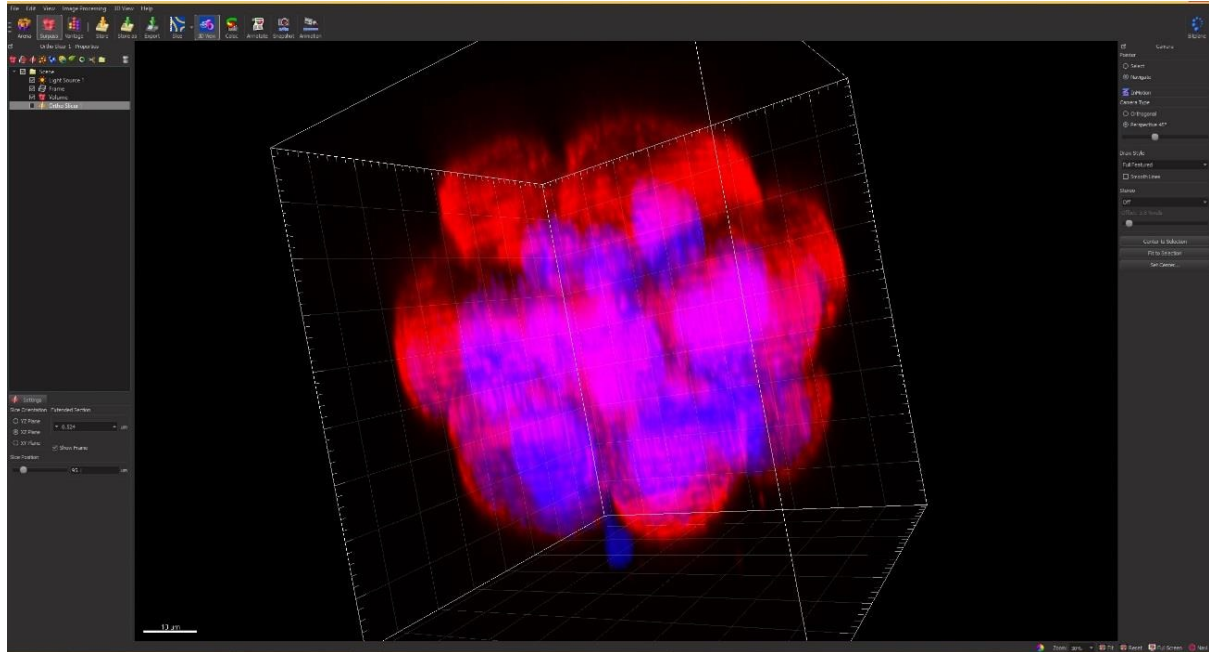


You can move the orthoslicer through the image using the slider for **Slice Position** at the bottom of the panel.

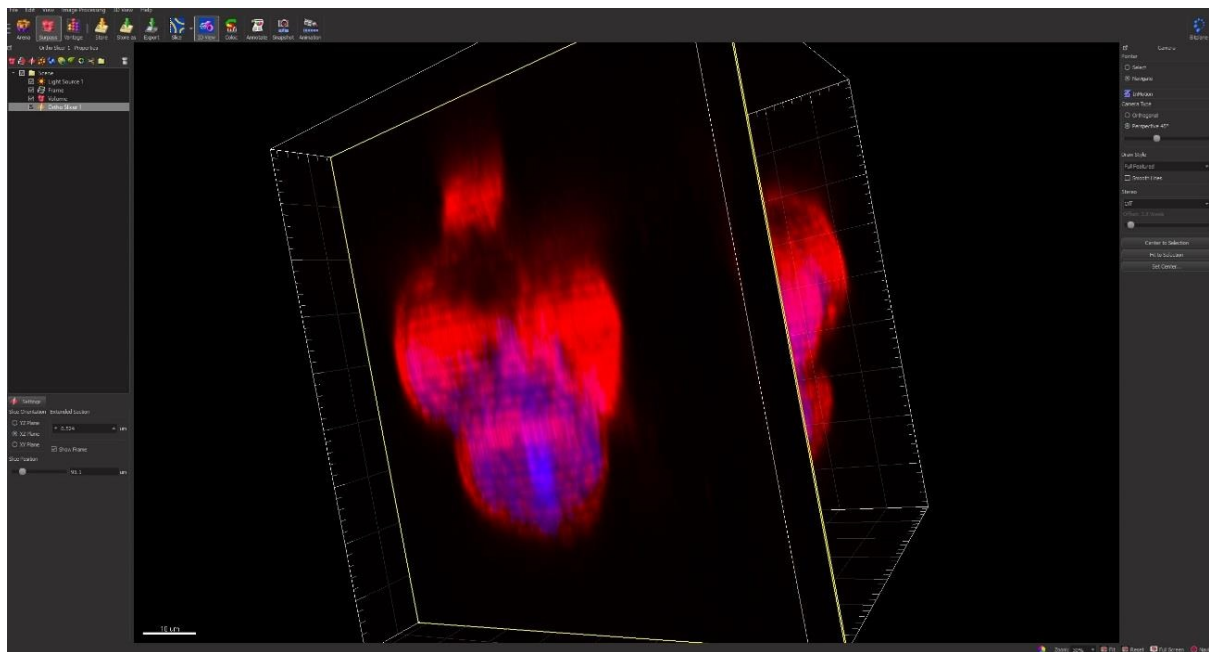
As you move through the image you will see only what is above the orthoslicer within the image.

Examples of a 3D image with and without the orthoslicer are shown below

Orthoslicer Off:

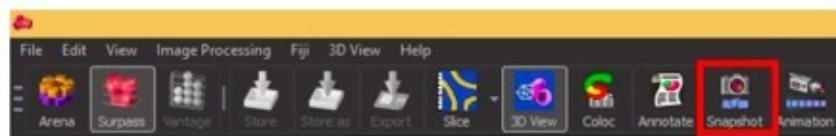


Orthoslicer On:



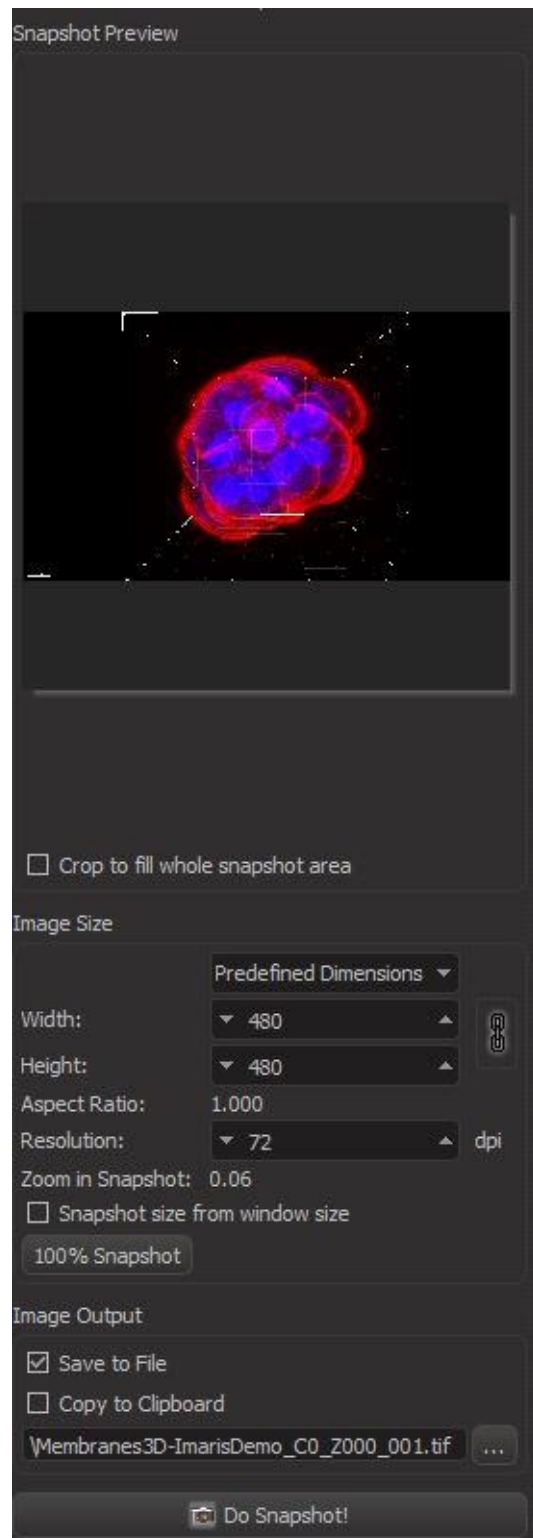
Snapshots

At any time you can capture a snapshot of the image on your screen using the **Snapshot** tool. Select **Snapshot** from the main menu panel.



You can adjust your original image in the main window to the orientation you need and make any adjustments.

You will see a preview of the final image in the Snapshot panel.



Check the box next to **Crop to fill whole snapshot area** to zoom the image to fill the entire space.

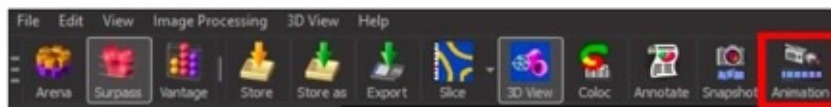
When you are happy with the image, input the resolution options for your final image into the **Image Size** section of the Snapshot panel.

Chose **Save to File** or **Copy to Clipboard**. If you chose to save to file, input your destination folder by clicking on the ... icon. Select your folder from the explorer window and name the image.

To capture the snapshot, click **Do Snapshot!**

Movies and Animations

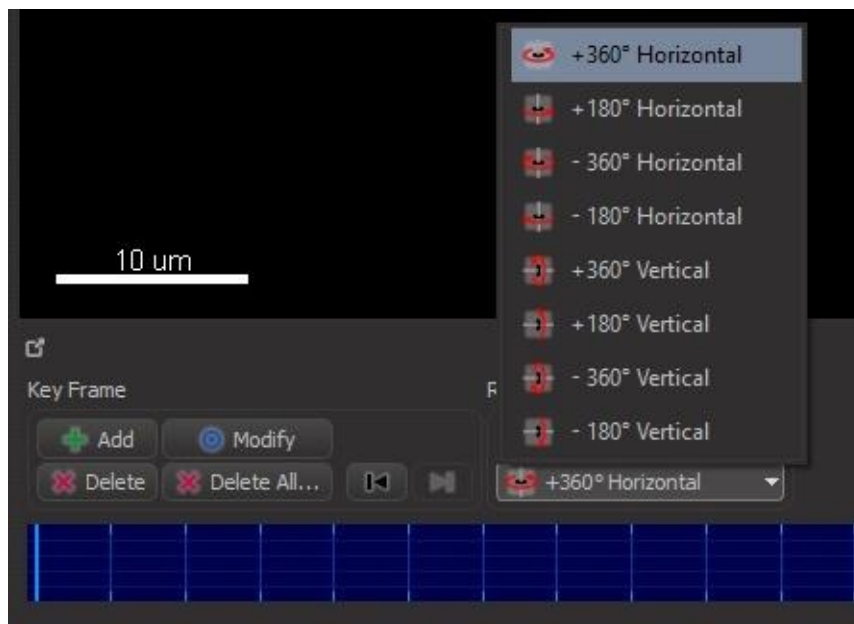
You can save movies, or animations, of your 3D images with a number of different actions. To start the animation capture select **Animation** from the main icon menu.



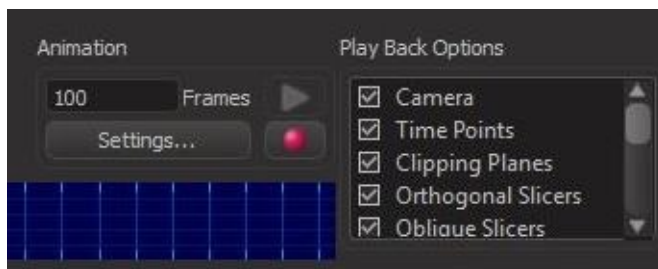
This will open the animation options and recording bar at the bottom of the Imaris window.



Here you can add animation frames for rotations from the pull up menu options. For other animations such as zooming, or using orthoslicer you will need to perform these during recording.



Set the number of frames you want to record and ensure any options you want included are selected under **Playback Options**.



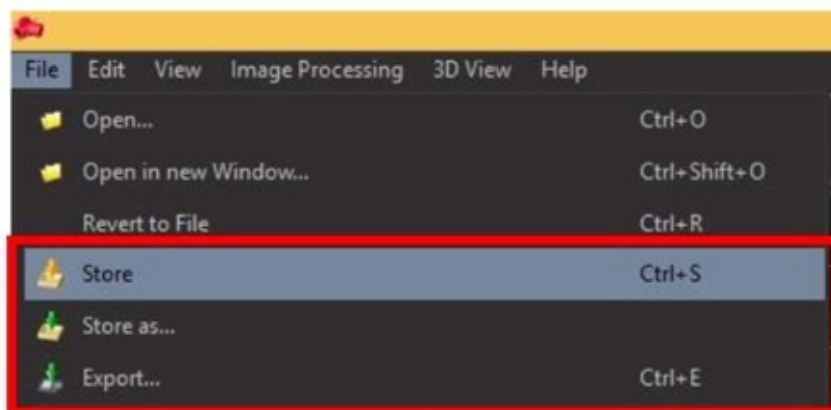
When you are ready to record your animation, click the **Record** button (red circle) on the animation bar. Select your location to save the movie file and click **OK**.

If you have added rotation options from the menu it will create this animation automatically. To record other movements, you must perform these yourself now as it records.

The recorded movie will be saved in your selected location and open and play automatically once it is complete.

Saving Options

You can save your changes to files in Arena by selecting **Store** (Shortcut Ctrl + S) from the **File** menu.



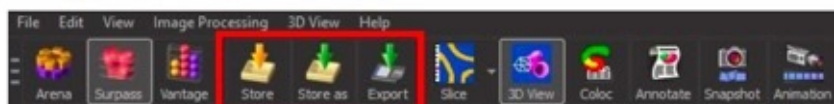
Store As will allow you to store changes to Arena under a new file name.

Use the **Export** option (Shortcut Ctrl + E) to save directly to a folder on the hard drive.

Note that Imaris likes to save in its own format (.ims). Selecting an Imaris file type from the options listed when saving will provide the most accurate version of your image. Imaris files can be opened in FIJI using bio-formats importer, configured for .ims files.

Selecting .tif will save each slice in your image as an individual file, although time series will give you the option to save all as a single file. Ensure the single file box is selected when saving time series as Tiffs.

You can also use the icons on the main panel for **Store** and **Export** options.

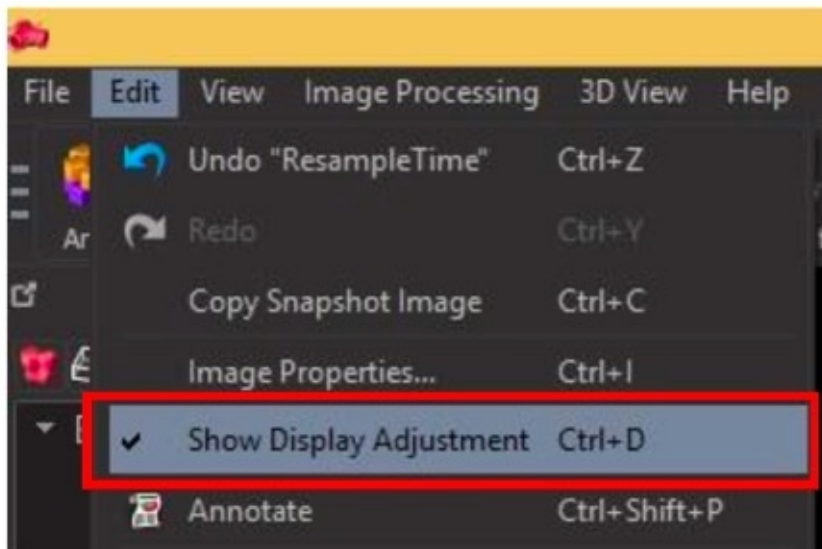


PART 4: IMAGE ADJUSTMENTS

Using the Display Adjustment Window

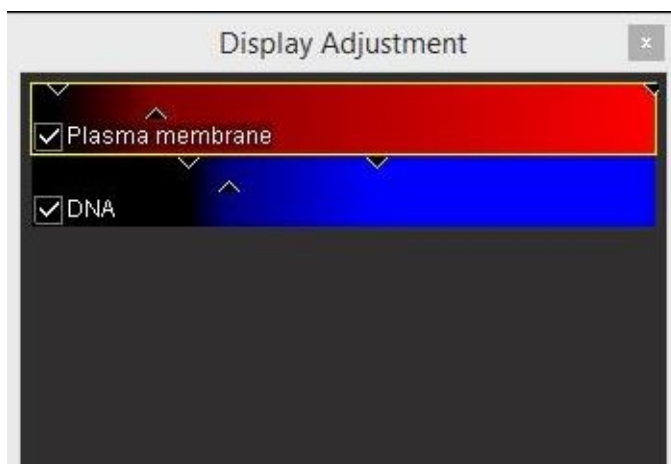
Many of the adjustment you will want to make to your images can be done within the **Display** Adjustment Window.

The **Display Adjustment** is a free floating window in the Imaris interface that you can move to any location. It usually opens by default when Imaris is launched. If the previous user has closed the window you may need to re-open it by going to **Edit -> Show Display Adjustment Window**.



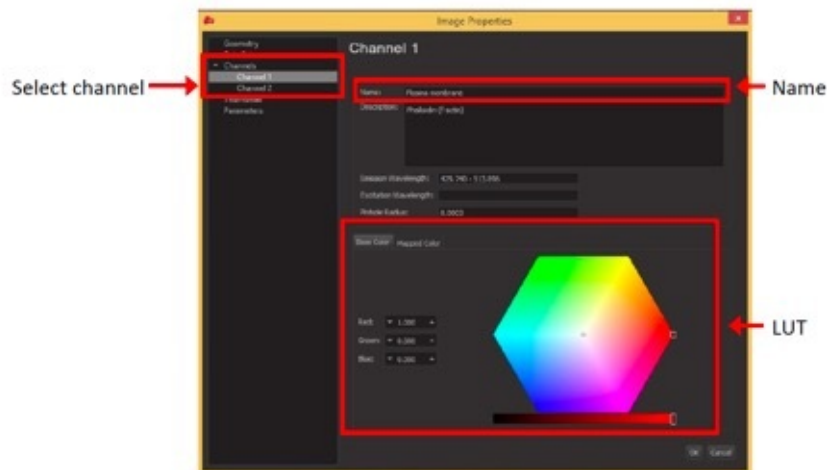
Click on a channel (anywhere in the coloured box) to make it the active channel in the window. The active channel will have a yellow border.

You can turn channels on or off in the image, without deleting them completely, using the check boxes next to the channels in the **Display Adjustment** window.



By double clicking on the channel name in the window you can open the **Image Properties**. You can also access this from **Edit -> Image Properties...** (or use shortcut Ctrl + I).

Under **Image Properties** you can select the different channels on the left hand panel and assign different LUTs to the channel, or assign a name (ie: from channel 1 to DAPI).

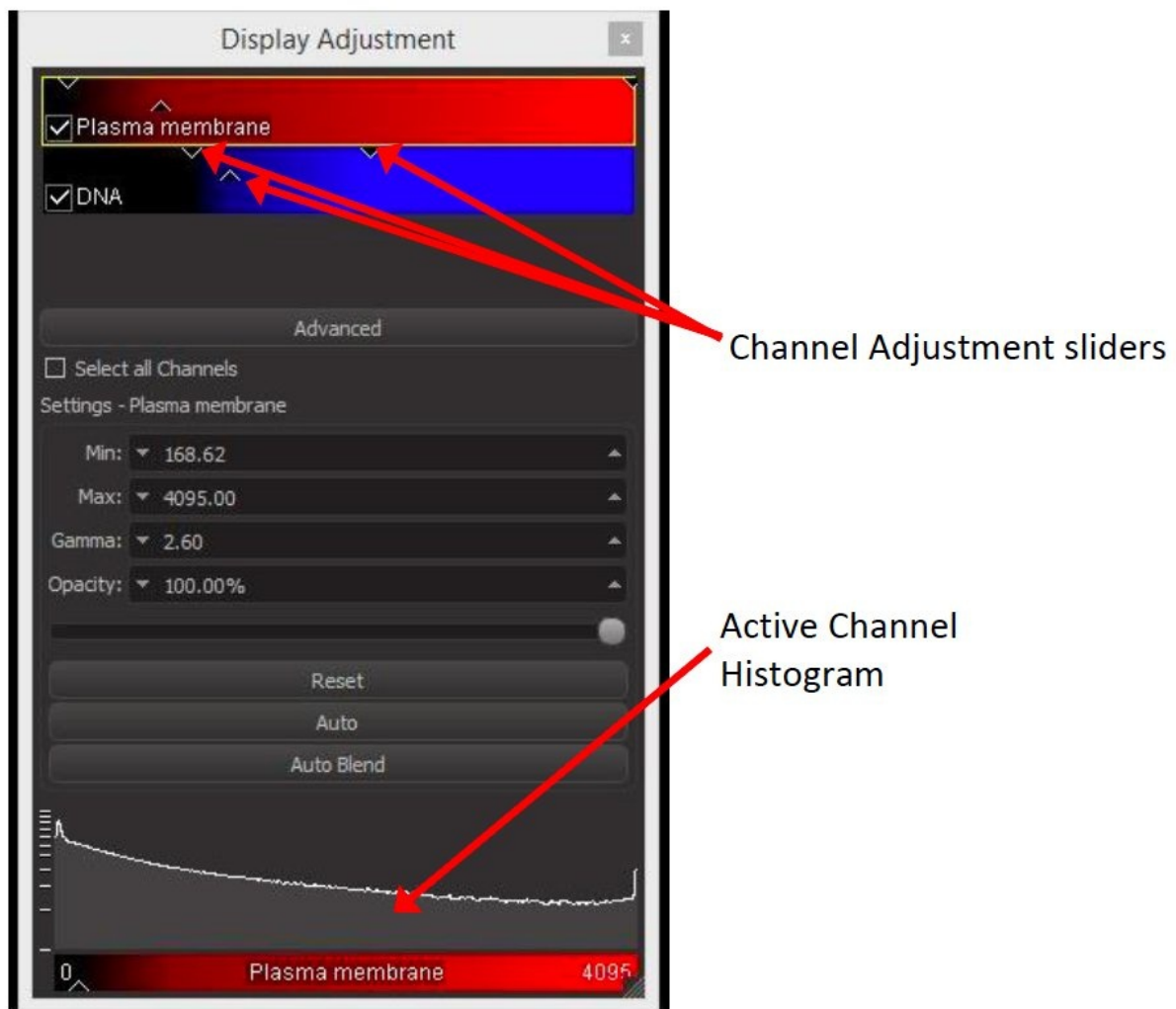


Under **Advanced** in the **Display Adjustment** panel you can find options for adjusting the brightness and contrast and the gamma. These are outlined in more details below.

Adjusting Brightness and Contrast

You can adjust the brightness and contrast on an image using the sliders in the channel boxes.

You can also adjust the brightness and contrast for the “active” channel or by adjusting the histogram under **Advanced** options in the **Display Adjustment** window.



You can use the **Reset** button at any time to return to the original brightness and contrast settings. Use the **Auto** button to allow the software to automatically adjust the brightness and contrast.

Gamma Adjustments

To change the **Gamma** on the active channel, use the up and down arrows in the **Gamma** box in **Advanced** options under the **Display Adjustment** window to increase or decrease the gamma value.

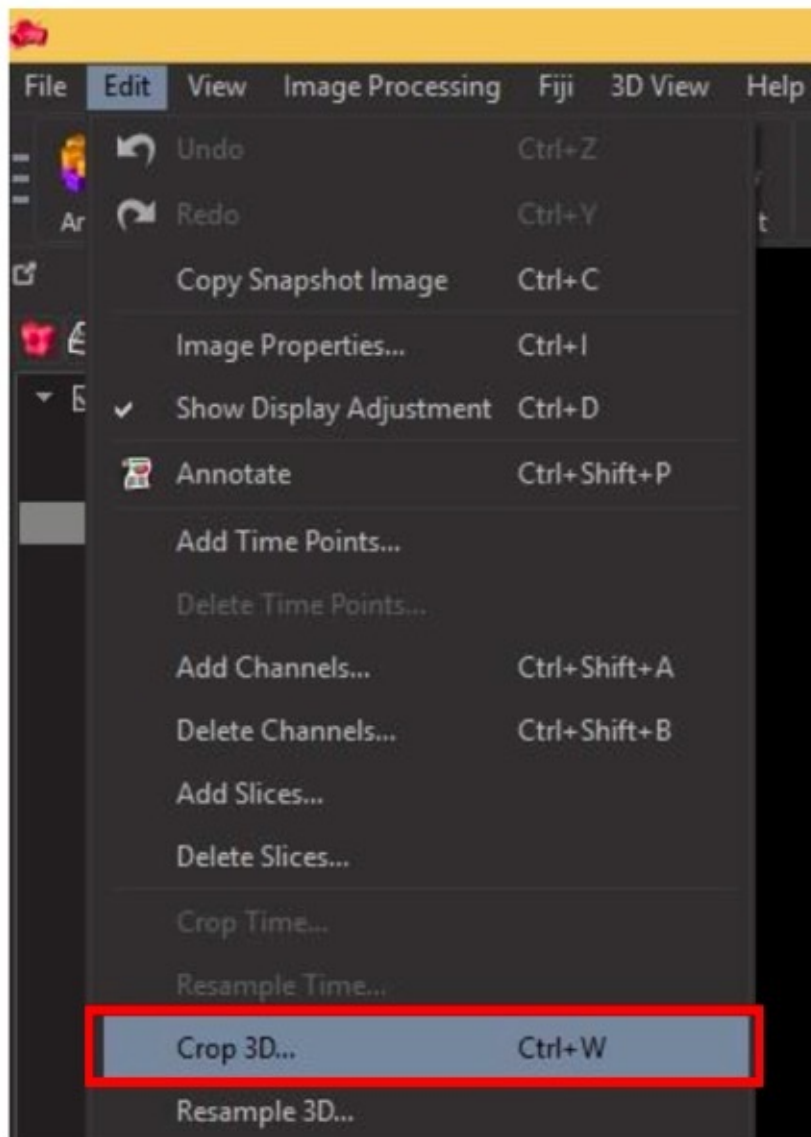


You can also type values directly into the gamma box.

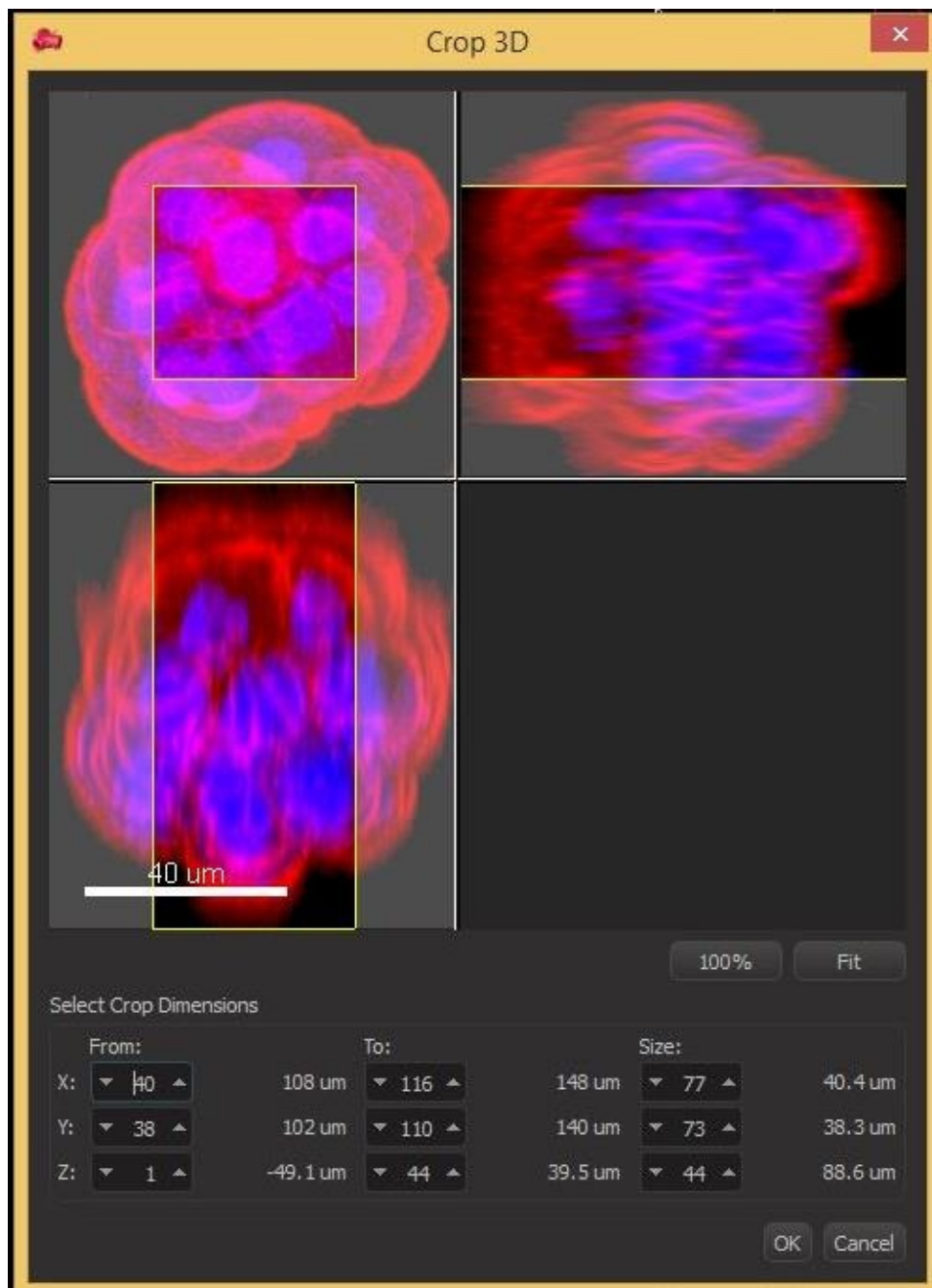
Use gamma adjustments with caution as this is a non-linear adjustment to your image. Gamma adjustments can skew quantification and any non-linear adjustments to images are not permitted in published journal articles.

Cropping Images

To crop images in Imaris go to **Edit -> Crop 3D...** (or use shortcut Ctrl + W).



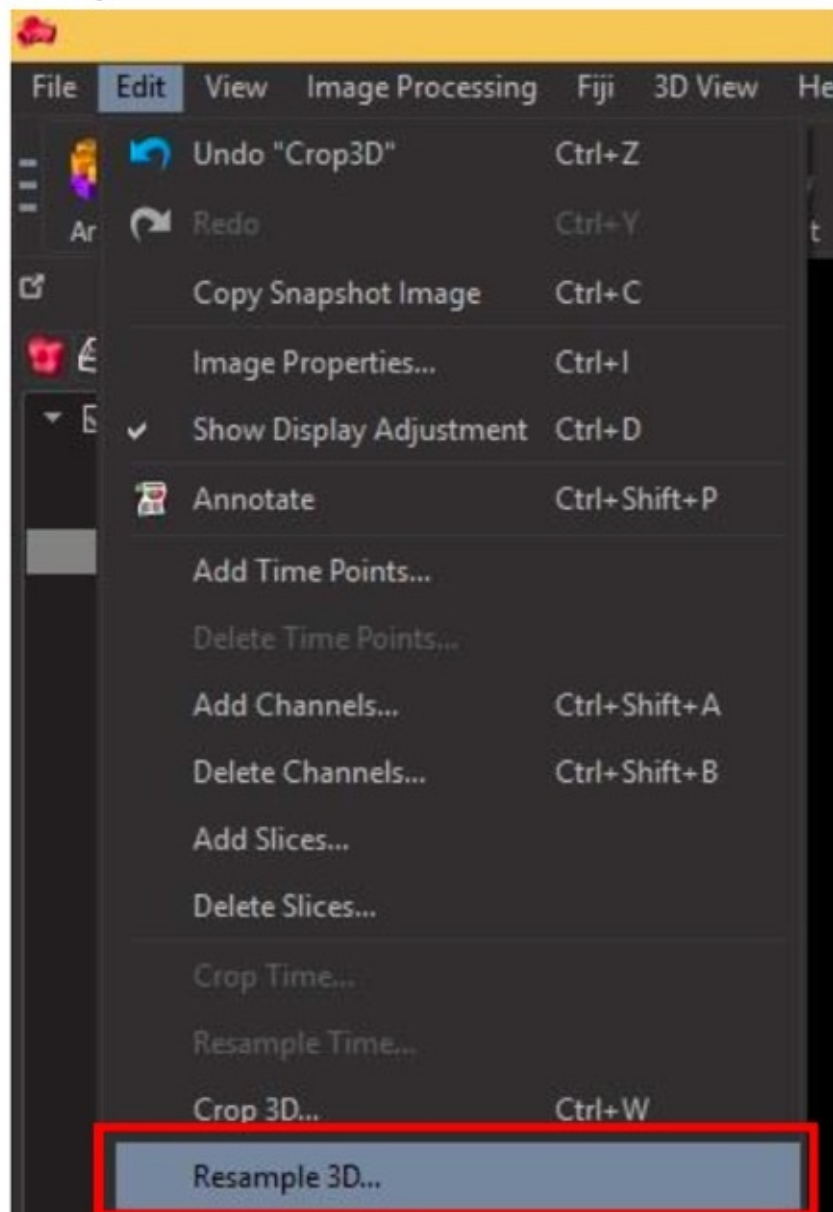
The crop window displays your image in xy, yz and xz. Adjust the yellow bounding boxes in each image, or type values directly into the **Crop Dimensions** boxes to crop in all 3 dimensions. Select **OK** to apply the changes.



To crop a 2D image in Imaris, use the same **Crop 3D...** tool. Only a single xy image will open in the crop window. Adjust the bounding box and apply changes in the same manner as a 3D image.

Resizing and Resampling

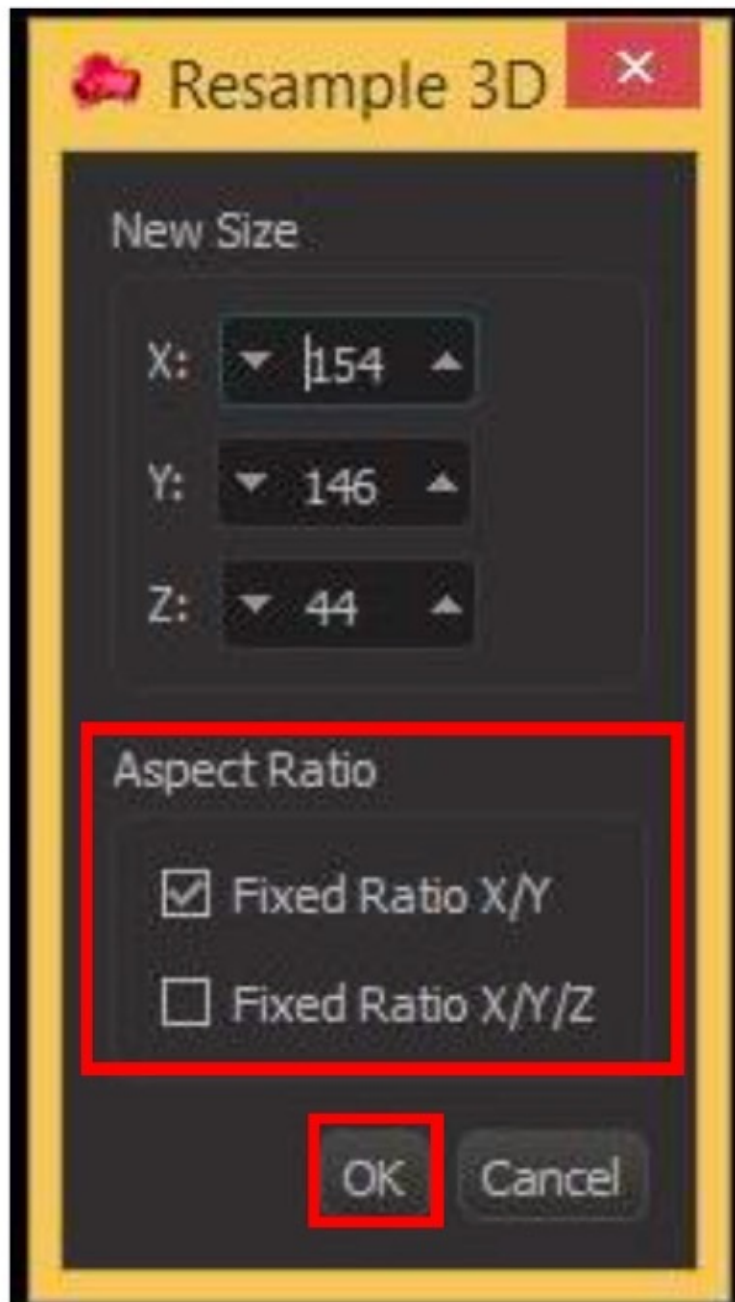
Images in Imaris can be resized by “resampling” the pixel information within the image. To resample your image go to **Edit -> Resample 3D...**



In the **Resample 3D** window use the up and down arrows to adjust the pixel size for the x, y and z dimensions. You can also type values directly into the boxes.

The x and y dimensions will usually come up as fixed ratio by default. To maintain the ratio with the z dimension as well check the box next to **Fixed Ratio X/Y/Z**.

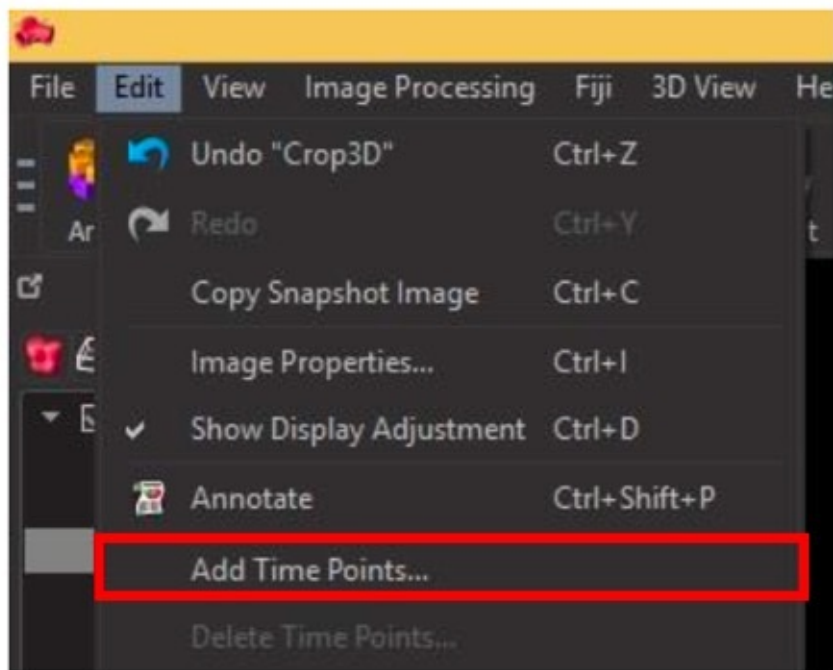
When one of these boxes is checked, changing the value for one dimension will adjust the corresponding dimension(s) accordingly.



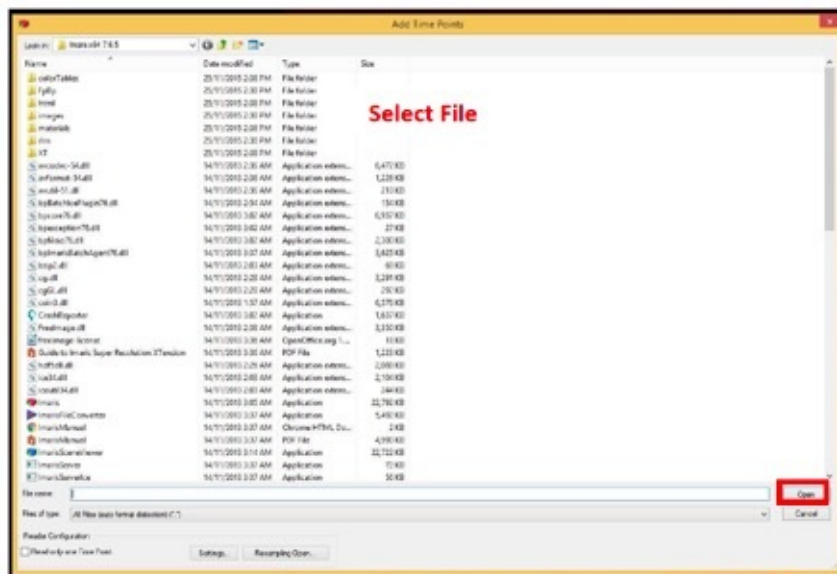
Click **OK** to apply the changes to your image.

Editing Time Series

You can add multiple time series files together using the **Add Time Points...** function. To do this go to **Edit -> Add Time Points....**

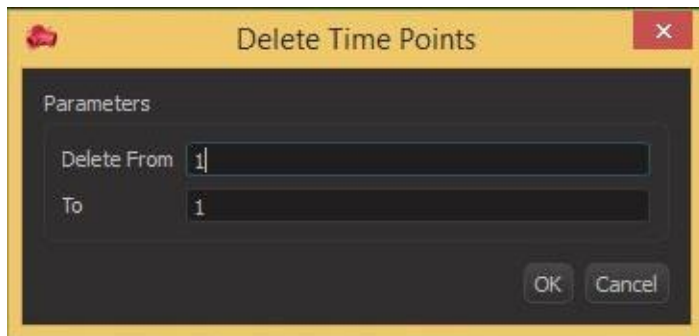


The same as adding channels or z-sections, from the explorer window select the file you want to add on to your time series and click **Open**.

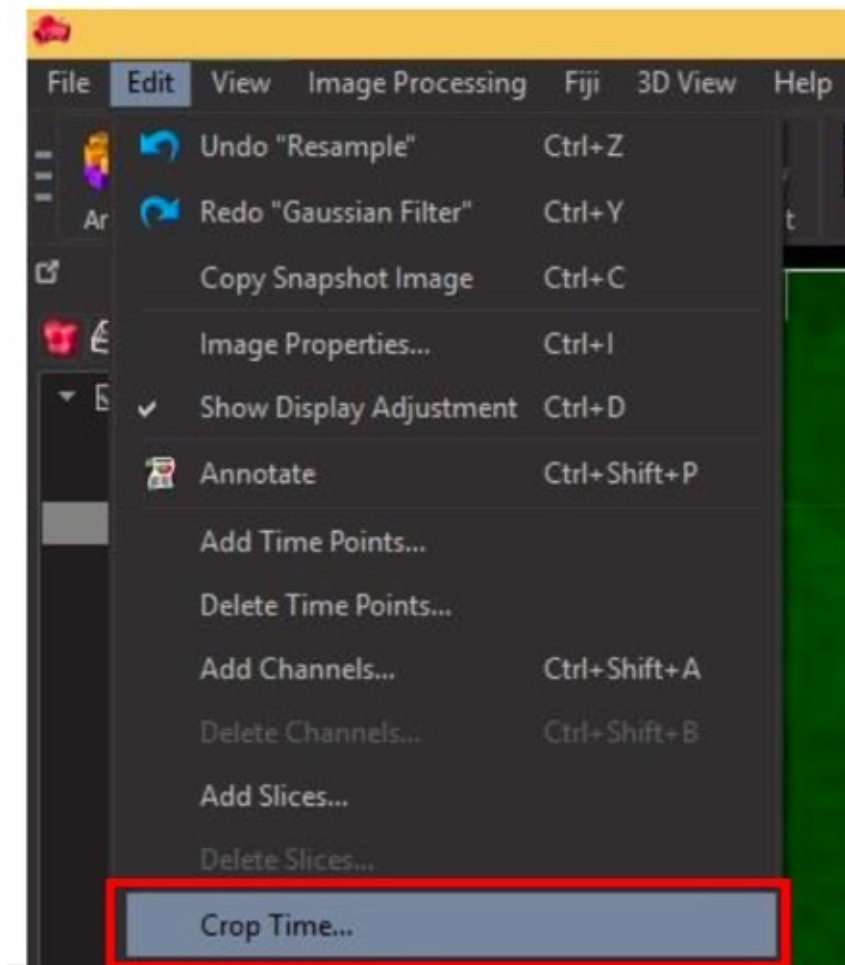


Repeat this for any additional files you want to combine, ensure you add them in the correct time order.

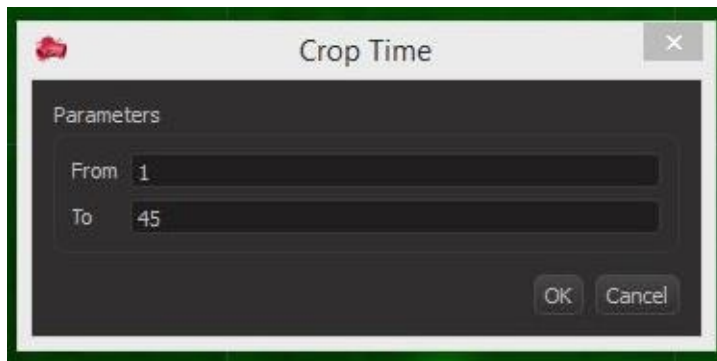
You can also delete time points from a series by going to **Edit -> Delete Time Points....** (as above) In the resulting window, enter the time points you want to delete from the series and click **OK**.



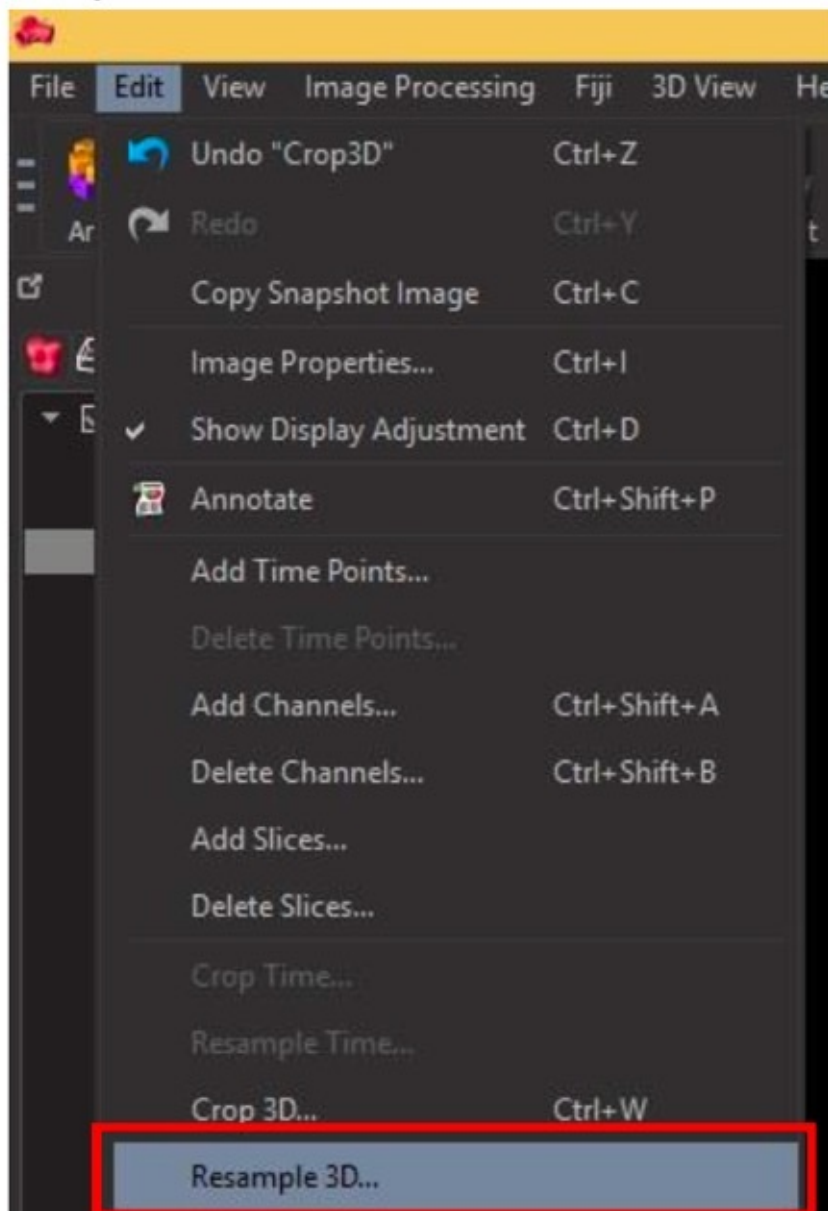
Similar to this is the **Crop Time** function. This is also found under the **Edit** menu.



Under the **Crop Time** window enter in the first and last time points you want to keep and click **OK**. Imaris will discard any time points before and after the numbers you have entered.



The last way of editing your time series is using **Resample Time**. This allows you to reduce the total number of time points by removing frames at set intervals. Find this under the **Edit** menu as well.



In the **Resample Time** window enter new number of frames you want to have in your time series. This number should be divisible by the total number of frames to ensure even resampling. Click **OK** to apply the change to your time series.

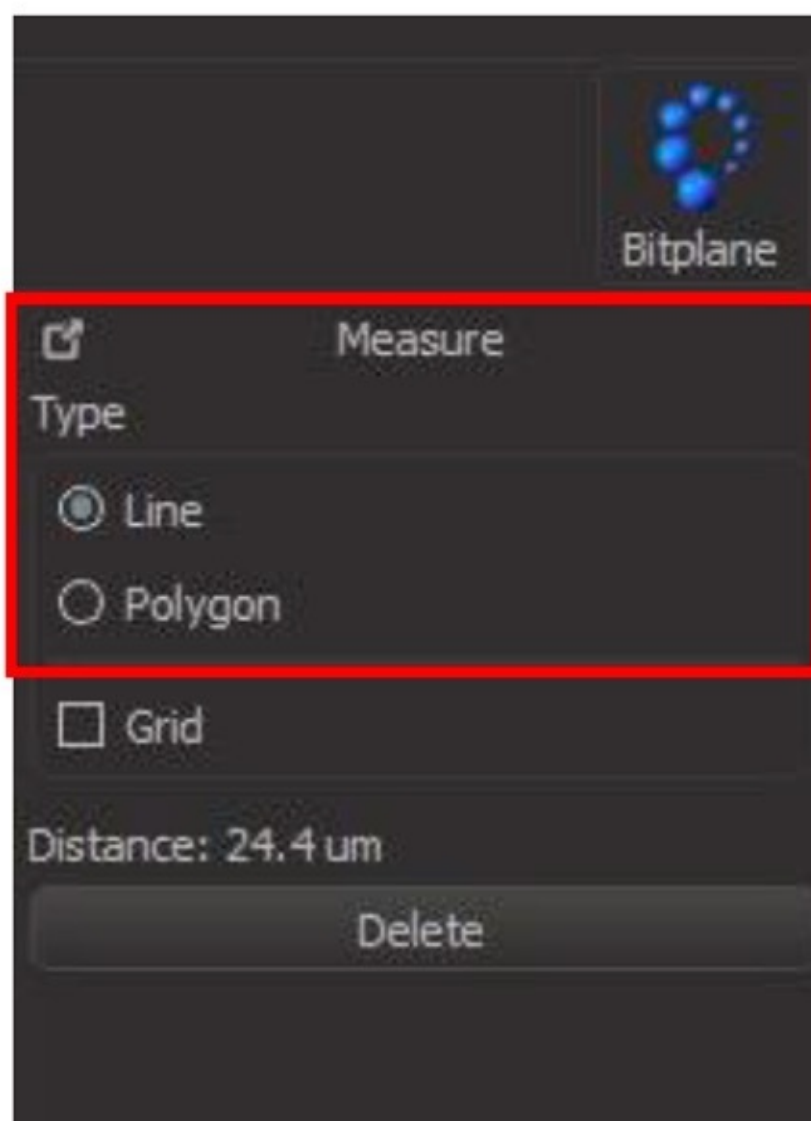


PART 5: GETTING STARTED WITH SELECTION AND ANALYSIS TOOLS

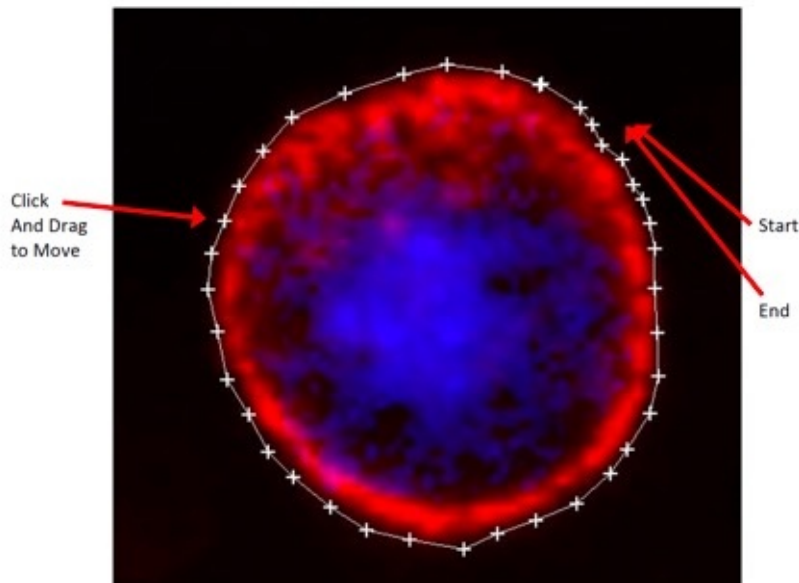
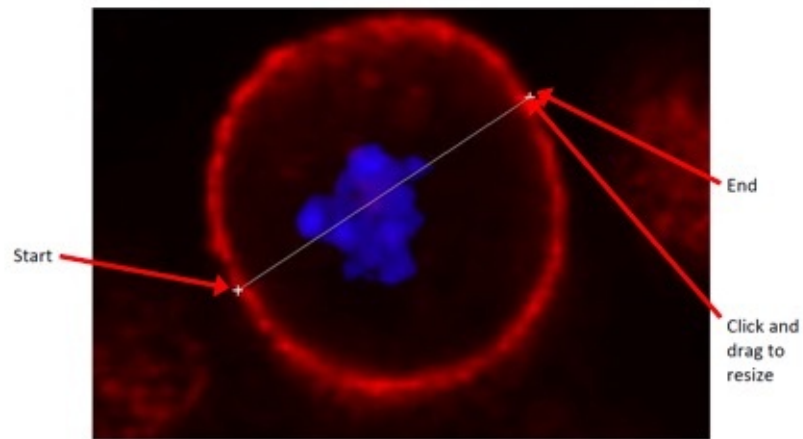
Basic Distance Measurements

A lot of the quantitation in Imaris requires you to select certain features in the image using the selection tools. However you can quickly and easily perform a 2D measurement for a distance between two points in your image, or a circumference, without the need for selection.

Switch your image into **Slice** view and scroll to the plane you want to measure. From the right hand side panel, select line (for distance) or polygon (for circumference) under tool type.

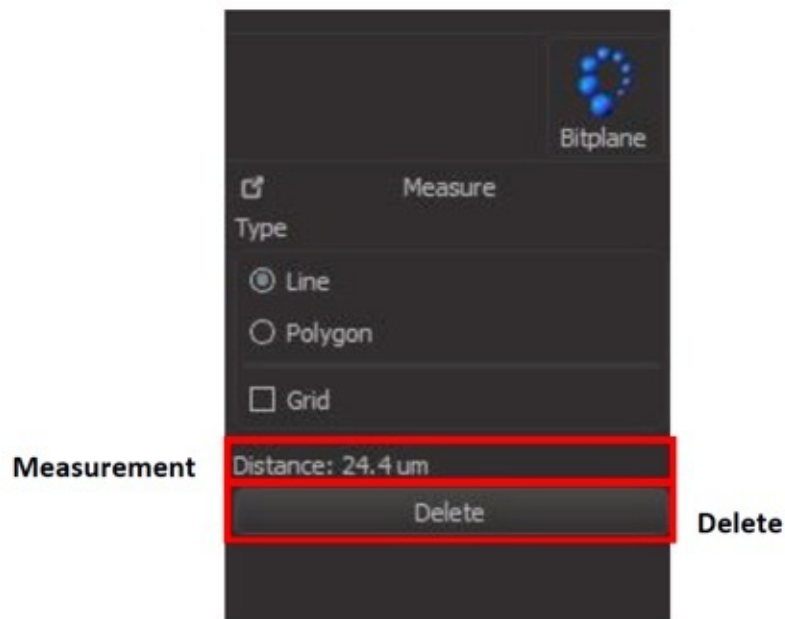


Click on the image at your starting point, then your secondary point for a line, or all points around the object to create a polygon. You can move points by clicking inside the box and dragging.



The distance of the line or polygon you have drawn will be displayed in the right hand side panel.

You can immediately draw another line on the image by clicking another location as your start point. Polygons will add to the existing line and therefore old lines must be deleted before drawing a new polygon. Delete a line or polygon with the **Delete** button on the panel.

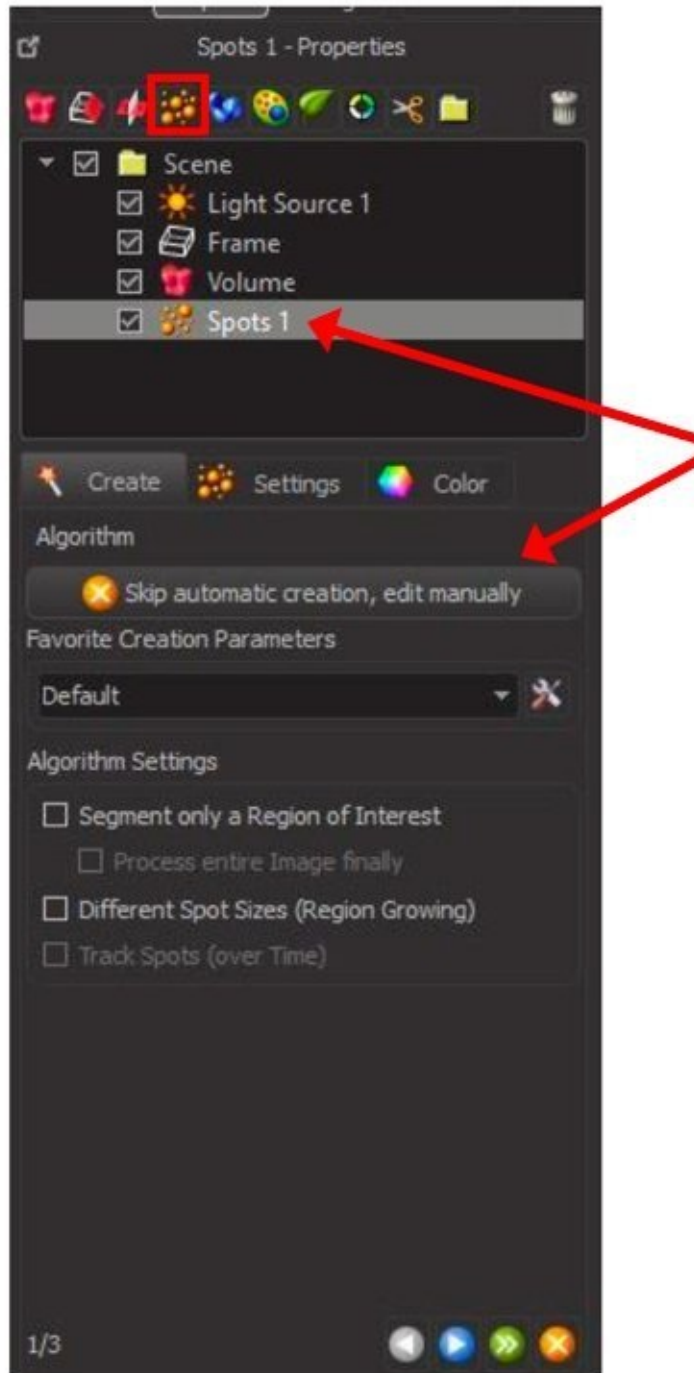


This can be a quite laborious process so it is only recommended for a small number of measurements.

Spots Selection

To select objects in your images for counting and analysis you can use the **Spots** selection tool.

To begin selection click the **Spots** icon in the Selection and Analysis Tools panel.



This will add a layer to your Scene called “Spots 1” and open the Spots Creation Wizard at the bottom of the panel. The layer can be turned on and off in the scene by checking or unchecking the box beside it here.

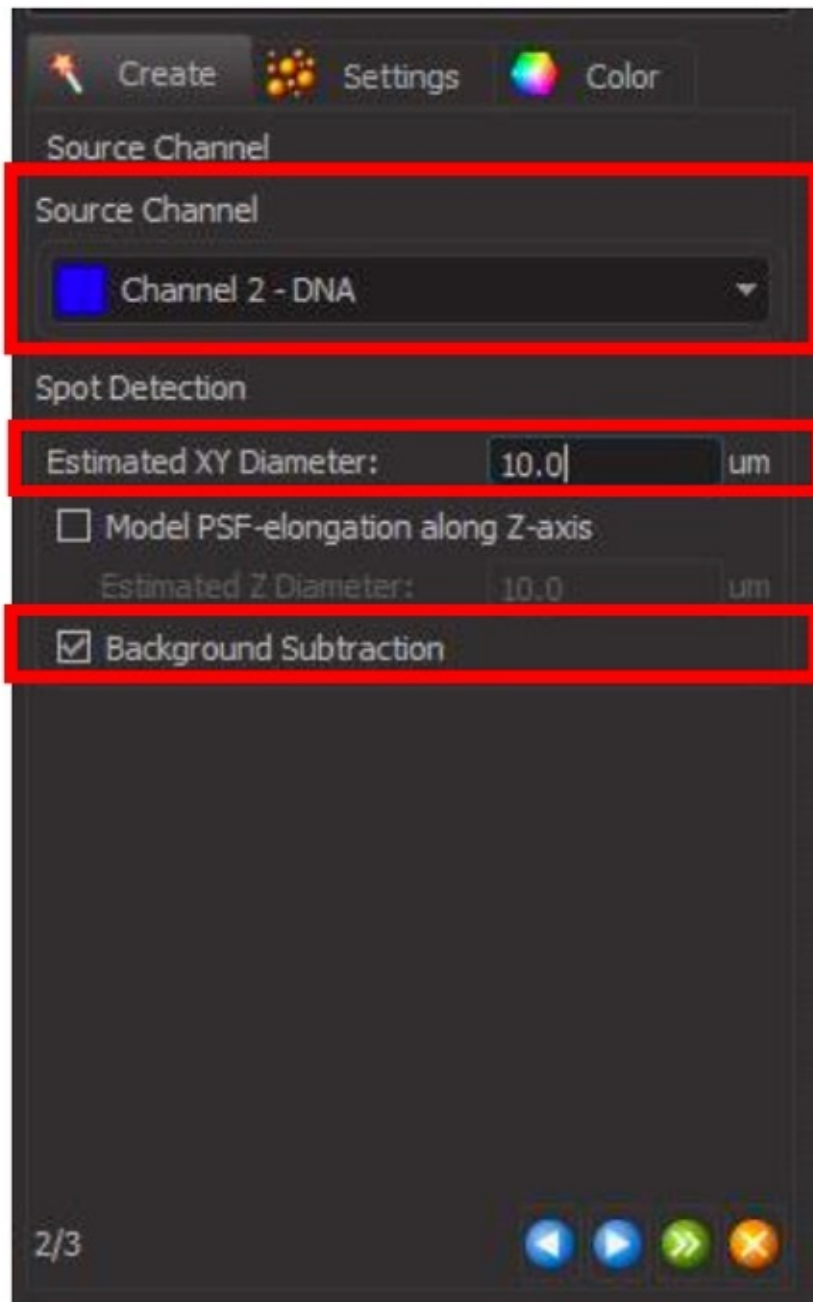
In the first step of the Spots Creation Wizard, leave the creation parameters as default.

For particularly large images you can check the box to **Segment only a Region of Interest** in order to keep processing times down. Select the option to **Process entire image finally** to apply the selection to the whole image after selection.

Click on the **blue arrow** to proceed to the next step.

Use the **green arrow** to skip to the end and use automatic creation with the default parameters unchanged.

Use the **orange cross** to cancel the selection wizard at any point during creation.

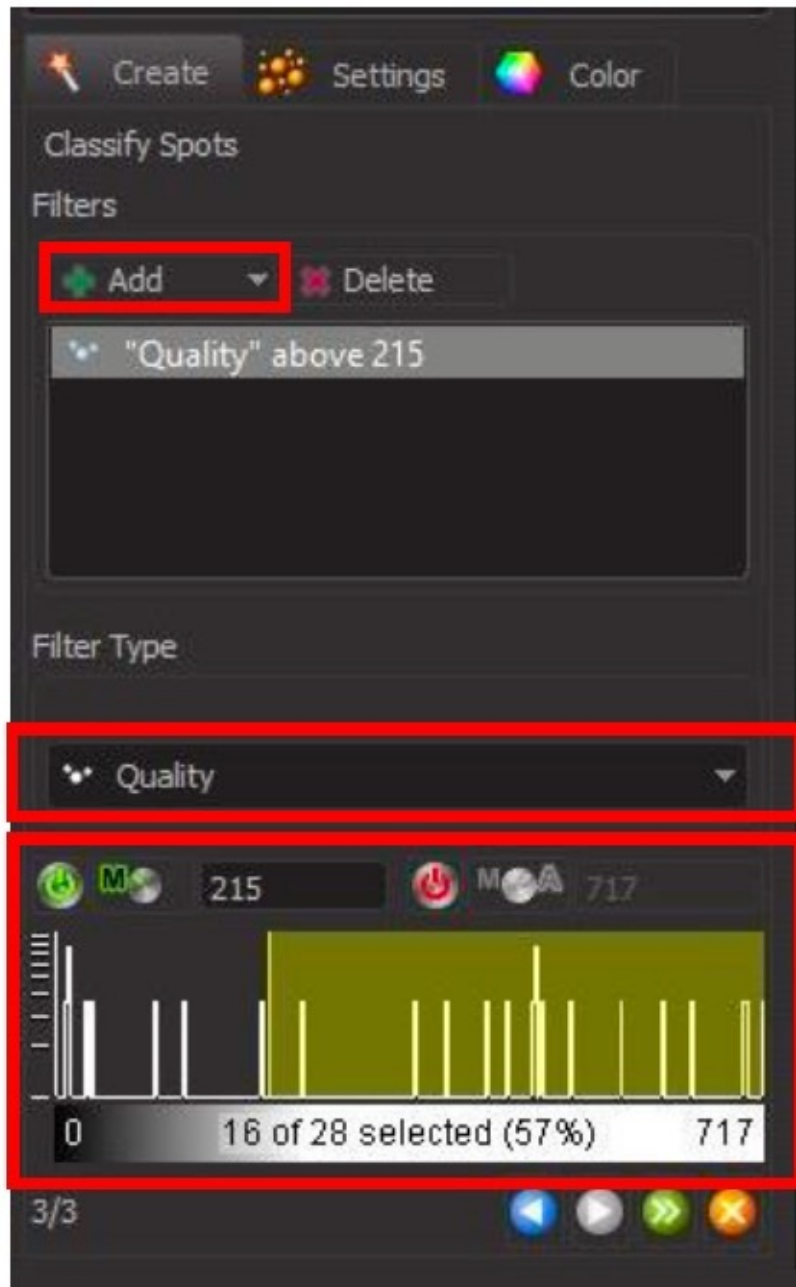


In the next step of the Spots Creation Wizard, Select the channel you want to segment from the drop down list under **Source Channel**. It can often be helpful to turn off other channels in the image to see your channel of interest more clearly.

Change the **Estimated XY Diameter** to fit the objects you are selecting. You can usually get an idea of the size by comparing to the scale bar in the image.

For most Spot Selection you will leave PSF-elongation modelling unchecked, but select **Background Subtraction**.

Click on the **blue arrow** to proceed to the next step, or the **green arrow** to complete the selection without changing any other parameters.



In the third step of the Spots Creation Wizard use filters to remove any non-specific selections.

Choose the filter from the drop down menu and adjust the level with the **Histogram**.

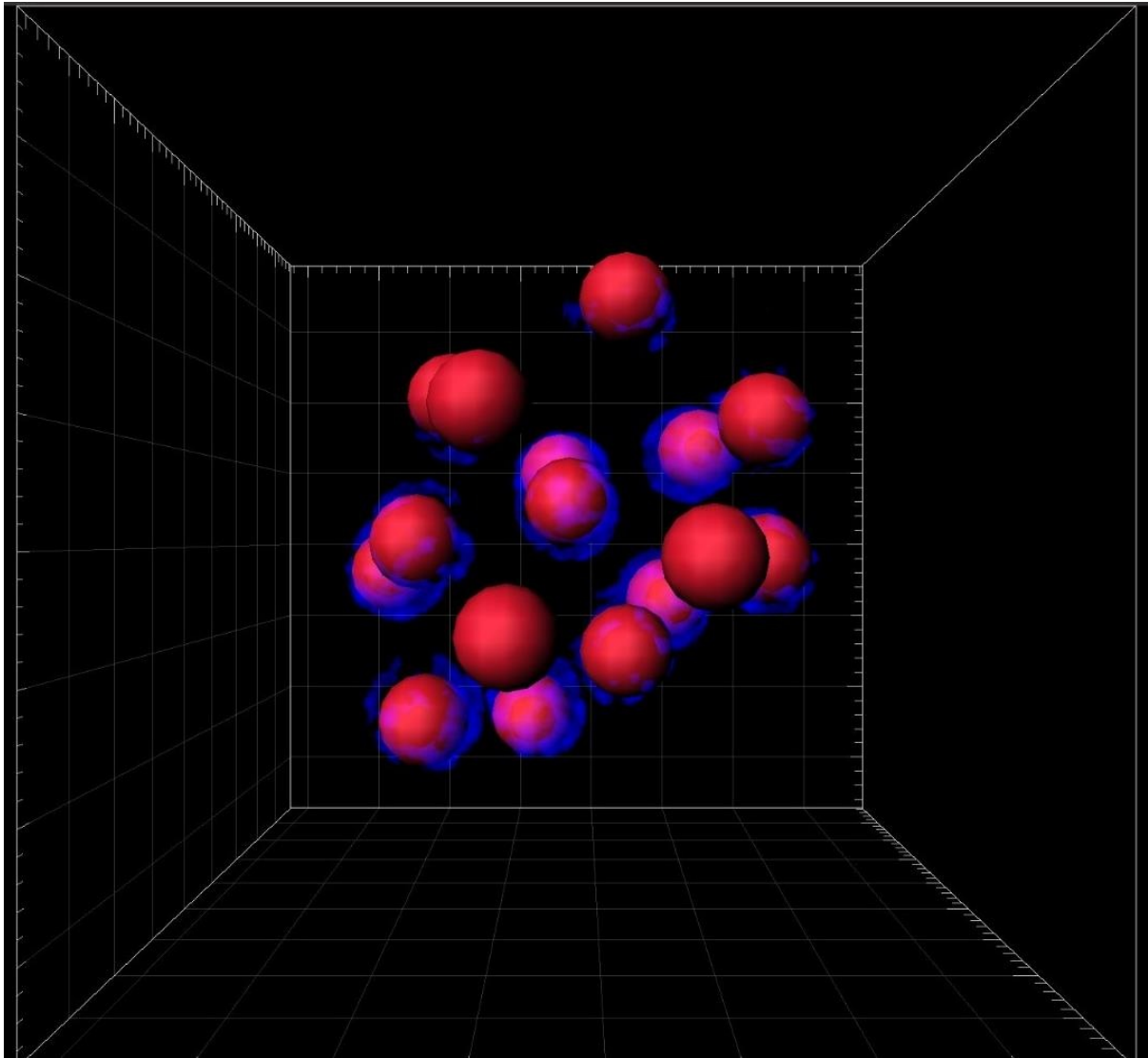
You can move and rotate the image to check the selection throughout the entire 3D environment before applying the filter and completing selection.

To use more than one filter, adjust your first, then click **+ Add**

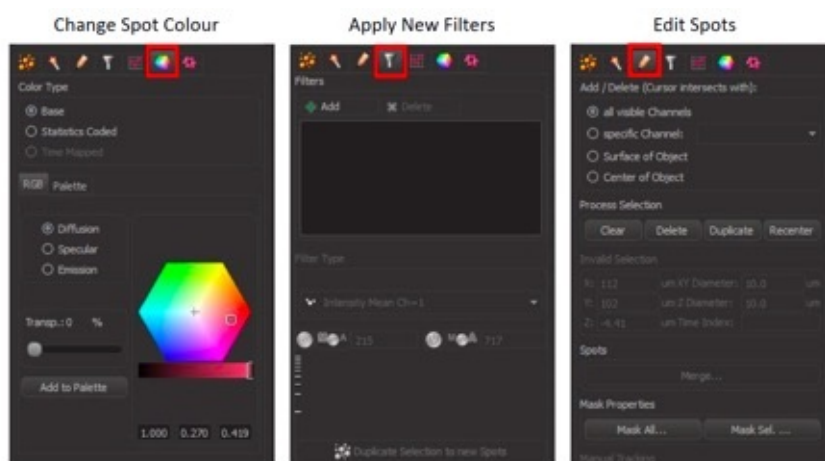
and choose and adjust your second filter.

Click the **green arrow** to complete the selection.

The spots selection is now complete and all selected objects will be 'marked' by a sphere overlayed in the image.

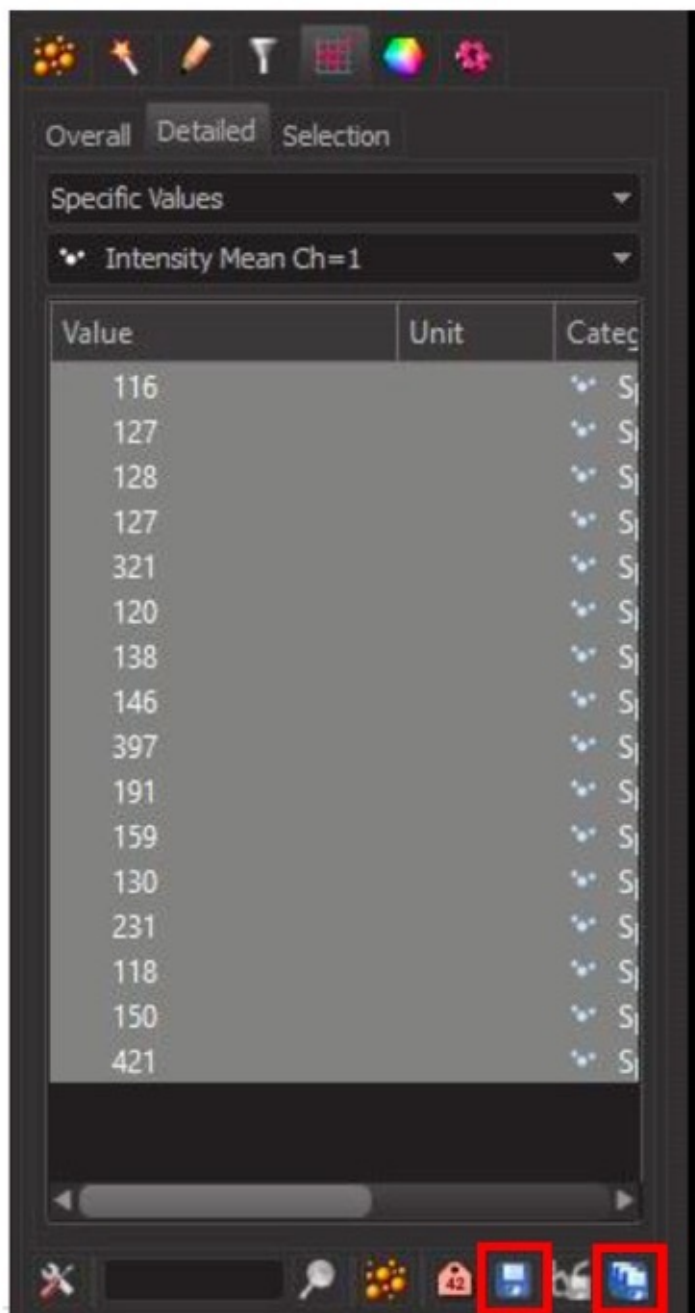


The Spots Selection Wizard in the bottom left hand corner will now be replaced with a series of tabs which allow you to further edit the spots.



In the “Edit” tab you can select Spots within the image by switching the cursor to the selector and clicking on the spot. You can then recenter, duplicate or delete the spot.

Selecting the "Wand" tab will allow you to go back and rebuild the creation algorithm from the start if you are unhappy with the final results and cannot correct it using editing.



The "Graph" tab contains measurements for the selected spots.

From the drop down menu you can choose specific values or averages. Under specific values, you can further select a measurement of interest.

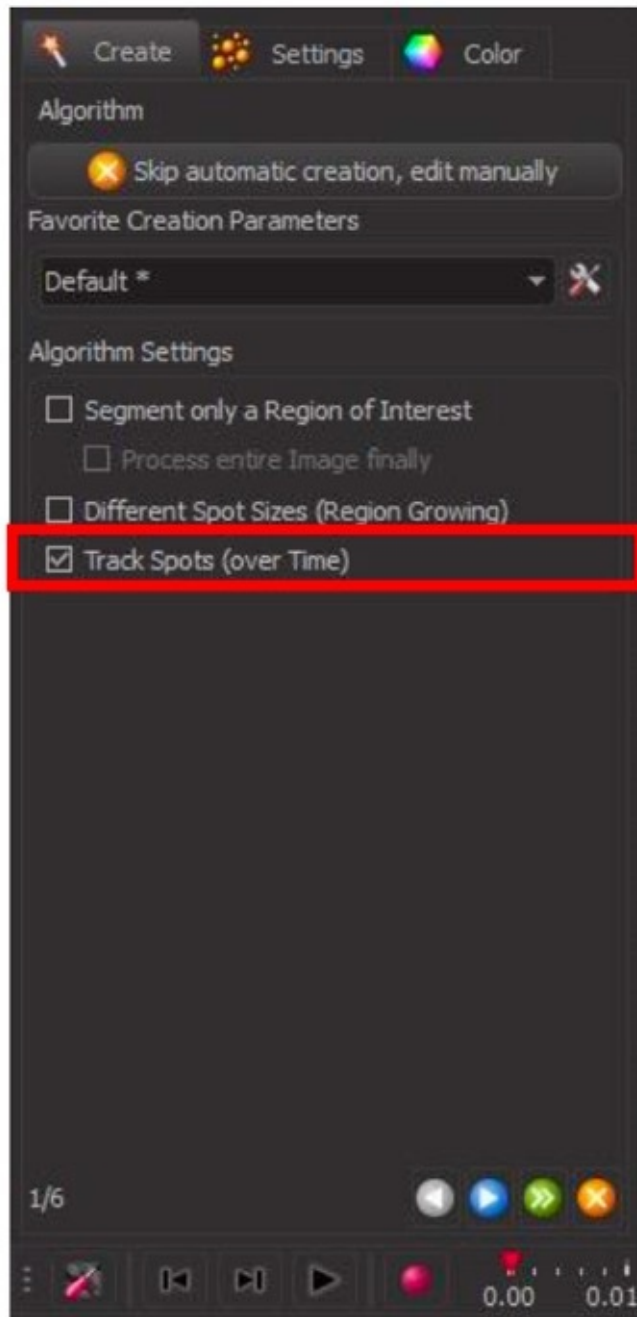
Specific values will show you the value for each individual spot in the image, averages will show you all values averaged over the entire selection, as well as count.

You can extract these measurement values to an Excel spreadsheet. Use the **Single Save** button at the bottom to save only the currently displayed values. Or use the **Multiple Save** button to save all values. In the resulting explorer window select the location and name the file. The Excel sheet will open after saving.

Basic Tracking

Spots Creation can also be used on time series to track objects through time.

Begin the process as you did above, by selecting **Spots** from the Selection and Analysis Tools panel.



This time, the Spots Creation Wizard has recognised you are working on a time series. You may notice there are now 6 steps in the wizard instead of 3.

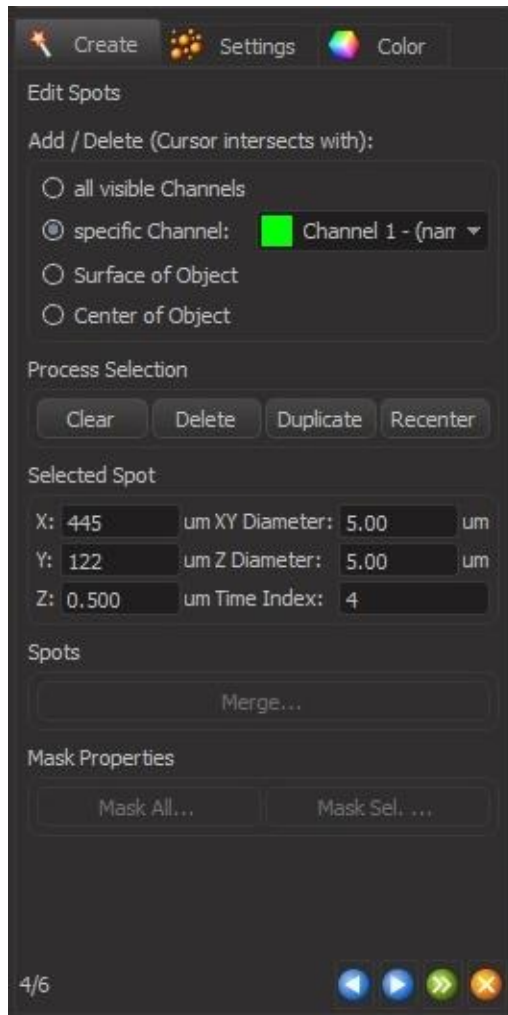
In the first step, **Track Spots (over Time)** will now show. Ensure this box is checked and set all other parameters as before.

As before, use the **blue arrow** to proceed to the next step, the **green arrow** to skip to the end and use automatic creation with the default parameters unchanged or the **orange cross** to cancel the selection wizard at any point during creation.

Steps 2 and 3 are used for initial detection of the spots and are identical to the previous spots selection, using channel, diameter and filters to select the objects in your image.

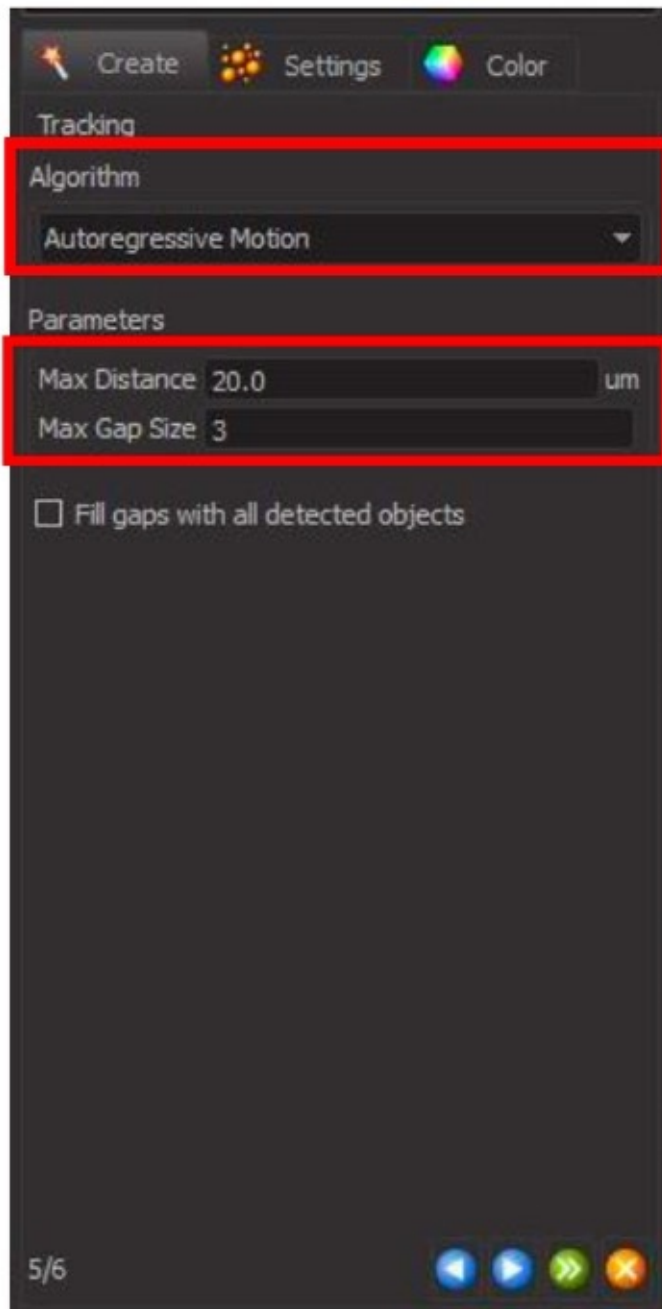
As with single images you can scroll through your time series and rotate and move in 3D at any point during selection to check your accuracy in space and time.

Once your objects have been selected, you can apply filters to the tracking over time.



In step 4 you can manually select spots (switch cursor to selection mode and click on the specific spot) to delete, duplicate or recenter, as previously in the post-selection editing.

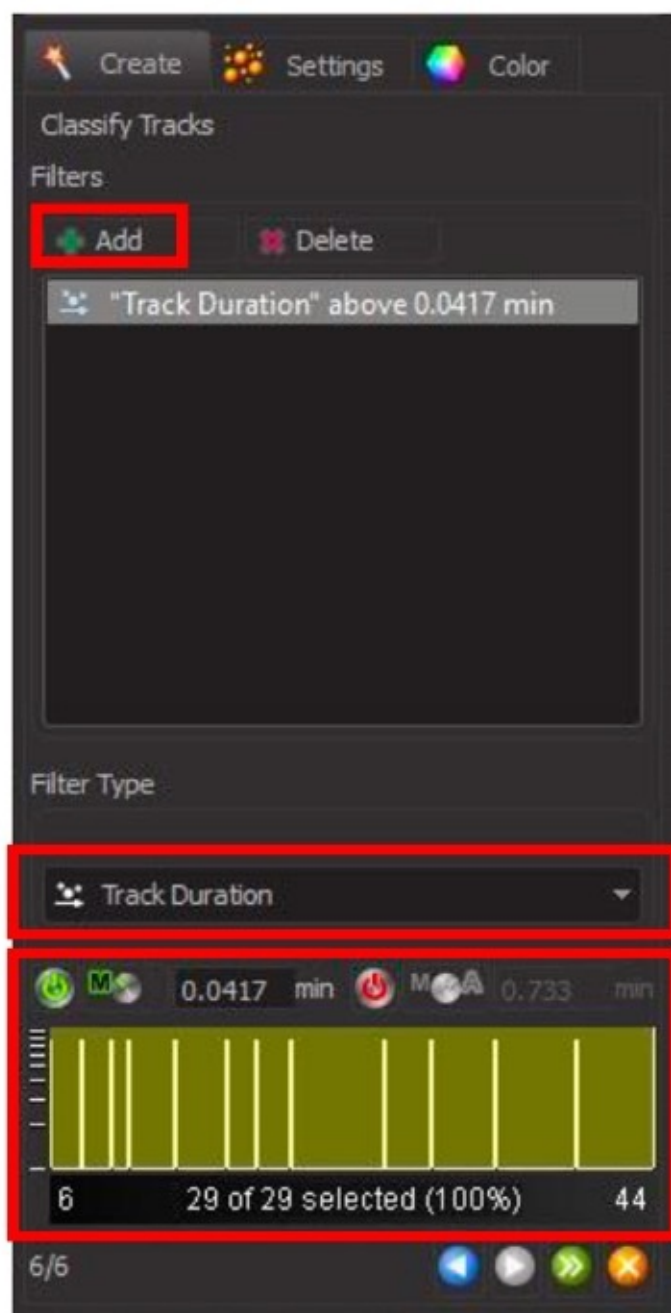
This allows you to remove any detected object that you don't want tracked (ie: cells that leave the field of view during tracking, etc).



In step 5 of the wizard, you can select the type of **Tracking Algorithm** to apply to the time series from the drop down menu. For most tracking **Autoregressive Motion** will work well to track movement.

You can also adjust the **Tracking Parameters**, including the **Maximum Distance** to track an object and the **Maximum Gap Size** (ie: how many frames to between an object disappearing and reappearing to allow linking the tracks together).

Proceed to the next step with the **blue arrow** or complete the selection without changing any other parameters with the **green arrow**.



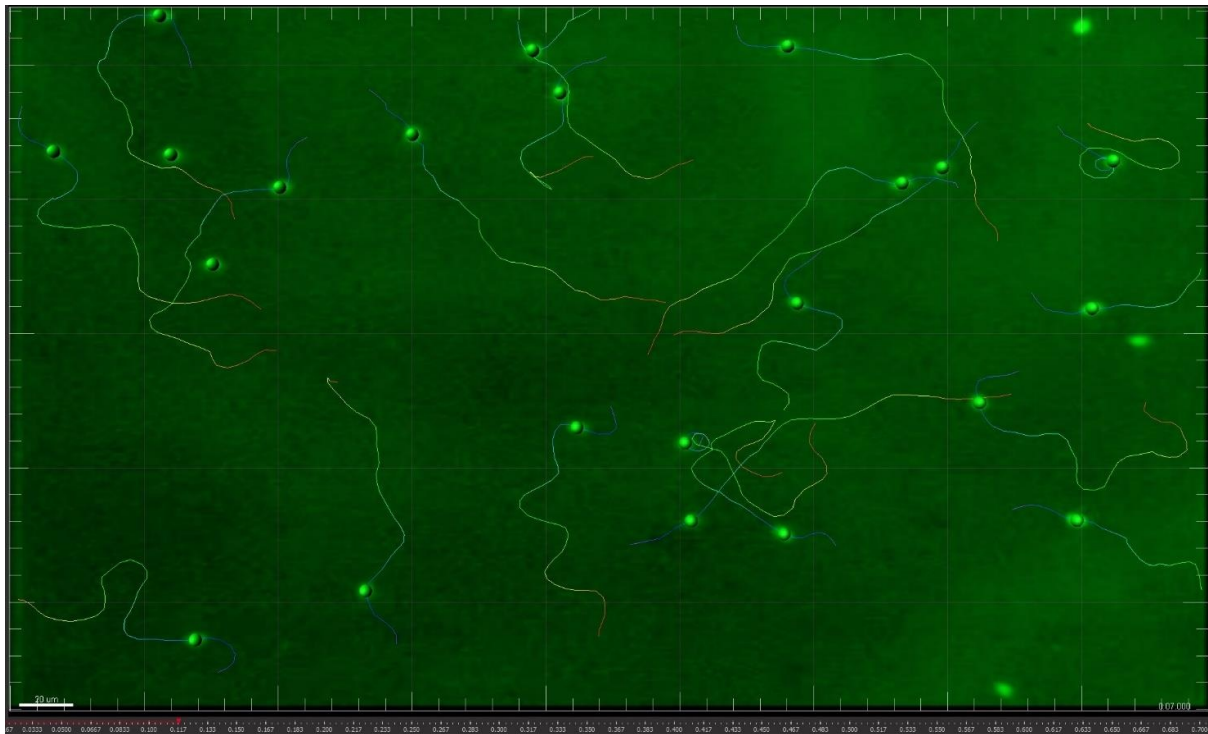
In step 6 of the Spots Tracking Wizard, you can apply filters similar to step 3 of Spots Selection.

Here you can filter on properties such as Track Duration.

Select a filter from the drop down menu and use the **Histogram** to adjust. Add a new filter using the **+ Add** button.

Complete the Spots Selection and Tracking using the **green arrow**.

Following selection and filtering, all detected objects will be overlayed in the image with Spots and a path of the track will be displayed, colour coded for time.



As with the basic Spots Selection tool, the panel in the bottom left hand corner will now be replaced with a series of tabs which allow you to further edit the spots and tracks. Spot Colour and Filters tabs are similar to Spot Selection.



The “Edit” tab now has the option to select Spots or Tracks for editing.

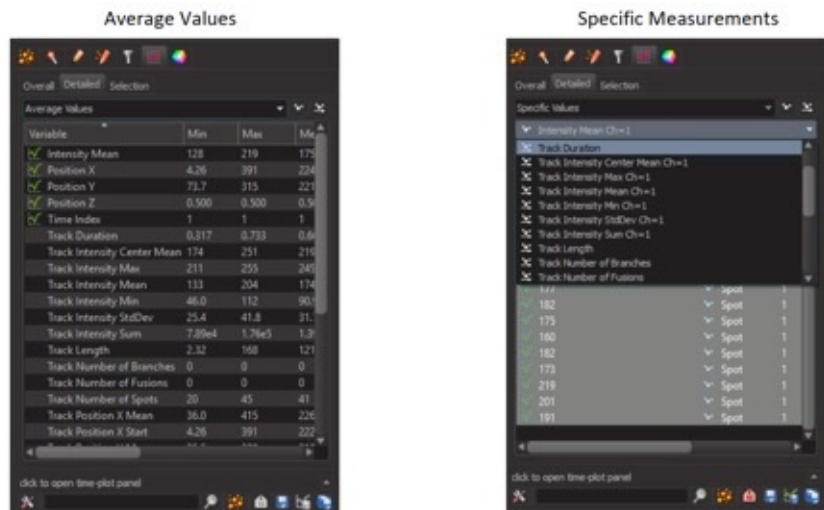
You can select a specific spot or track in the image or select a track in the editing tab.

Tracks can’t be deleted here, but you can select two shorter tracks that you have recognised as being from the same object and connect them, by selecting both and clicking **Connect**.

You can also spit a track that the wizard has detected as a single object, if it was in fact from 2 separate objects. Select the track and click **Disconnect**. Enter the point at which you want the track separated.

Under the “Graph” tab you will now find measurements for the tracks over time.

As with Spots, you can select **Specific Values** or **Averages**. Under Specific Values you can select measurements such as track length track duration.

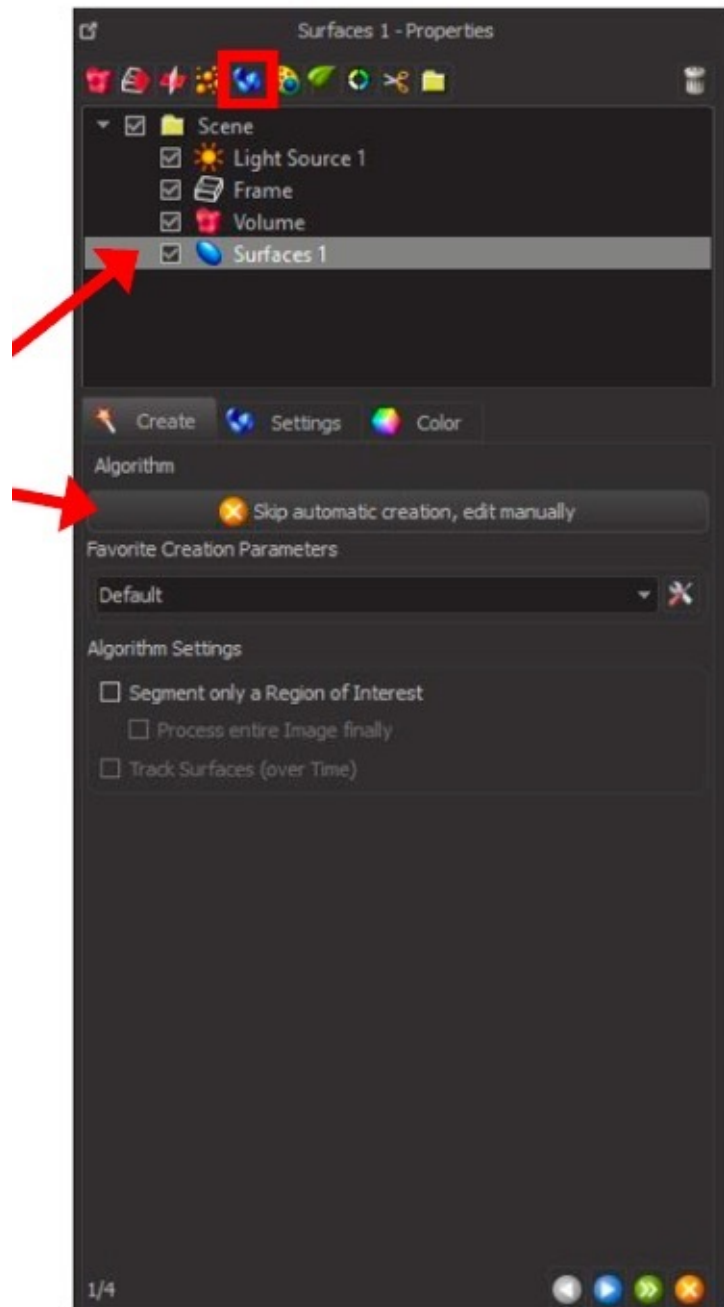


You can save these measurements to Excel files as before.

Creating Surfaces

To select connected objects, surfaces or volumes in your images use the **Surfaces** selection tool.

To begin, choose **Surfaces** from the Selection and Analysis Tools panel.

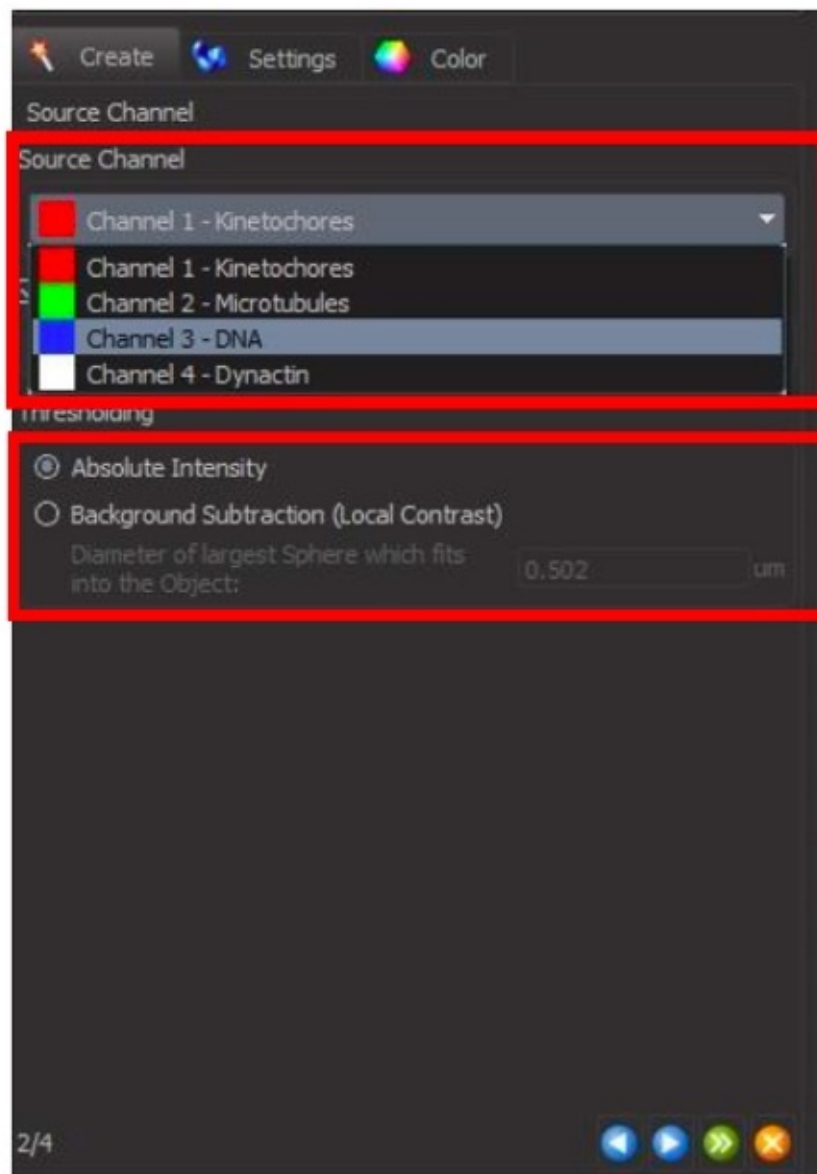


As for spots, this will add a layer to your Scene called

“Surfaces 1” and open the Surfaces Creation Wizard at the bottom of the panel.

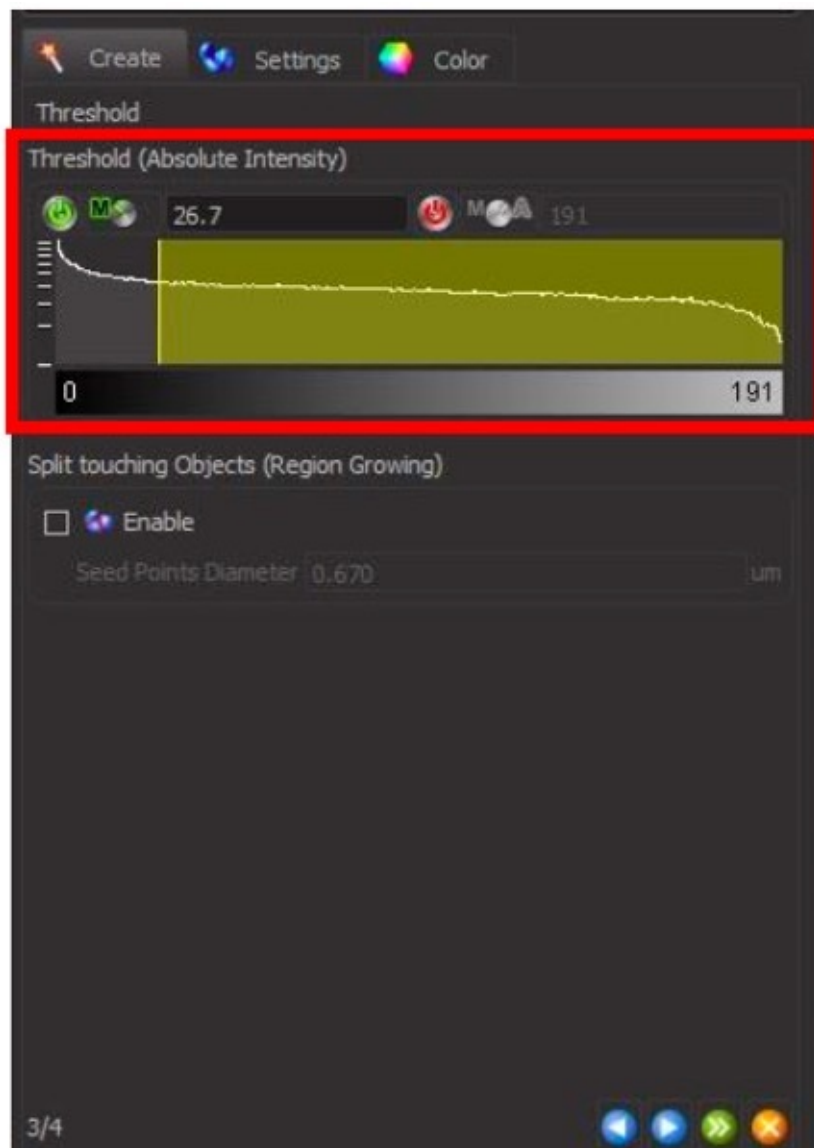
Step 1 of the Surfaces Creation Wizard is similar to Spots. Leave the creation parameters as default and check the box to **Segment only a Region of Interest** and **Process entire image finally** for large images

Click on the **blue arrow** to proceed to the next step or the **green arrow** to skip to the end and use automatic creation with the default parameters unchanged. Use the **orange cross** to cancel the selection wizard at any point during creation.



In step 2, Select your channel from the drop down menu and choose either **Absolute Intensity** or **Subtract Background** under **Thresholding**.

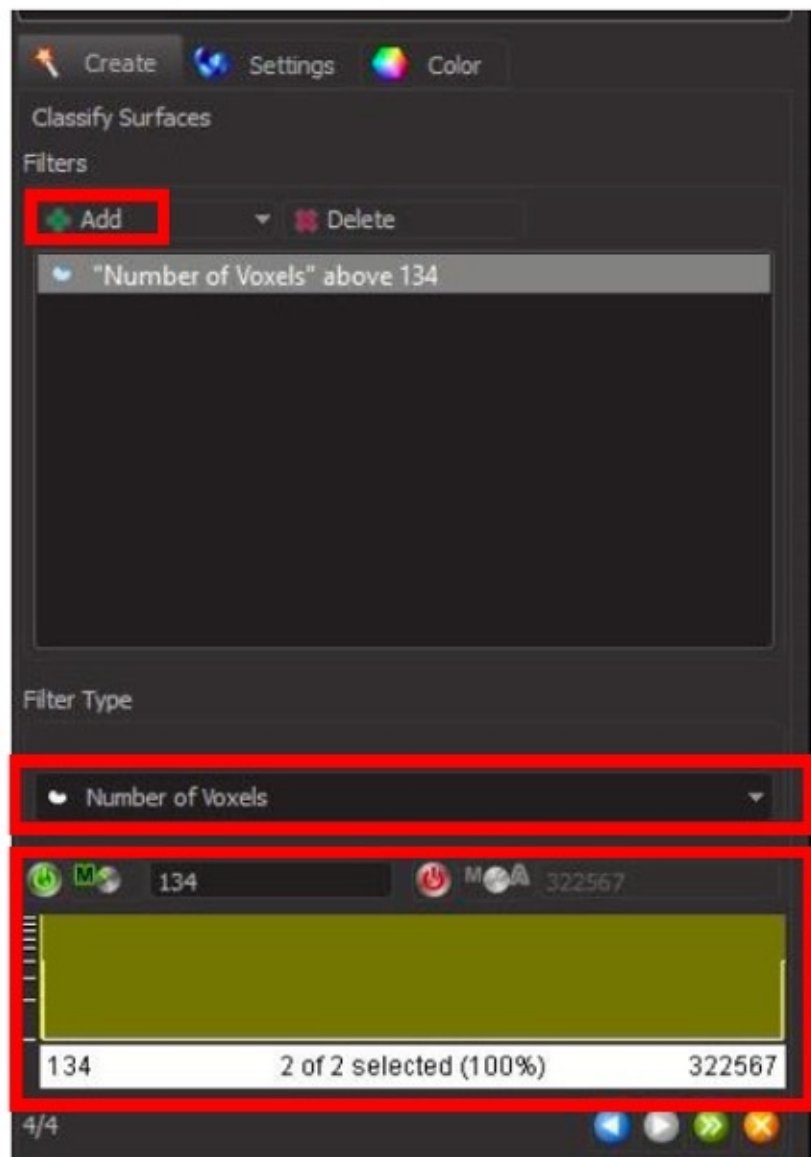
Click the **blue arrow** to proceed to the next step.



In step 3, use the **Histogram** to adjust the detection threshold until you have the optimal fit for your data.

The surface overlay should cover as much of your signal as possible with minimal excess noise detection.

Proceed to the next step with the **blue arrow** or complete the selection with the **green arrow**.



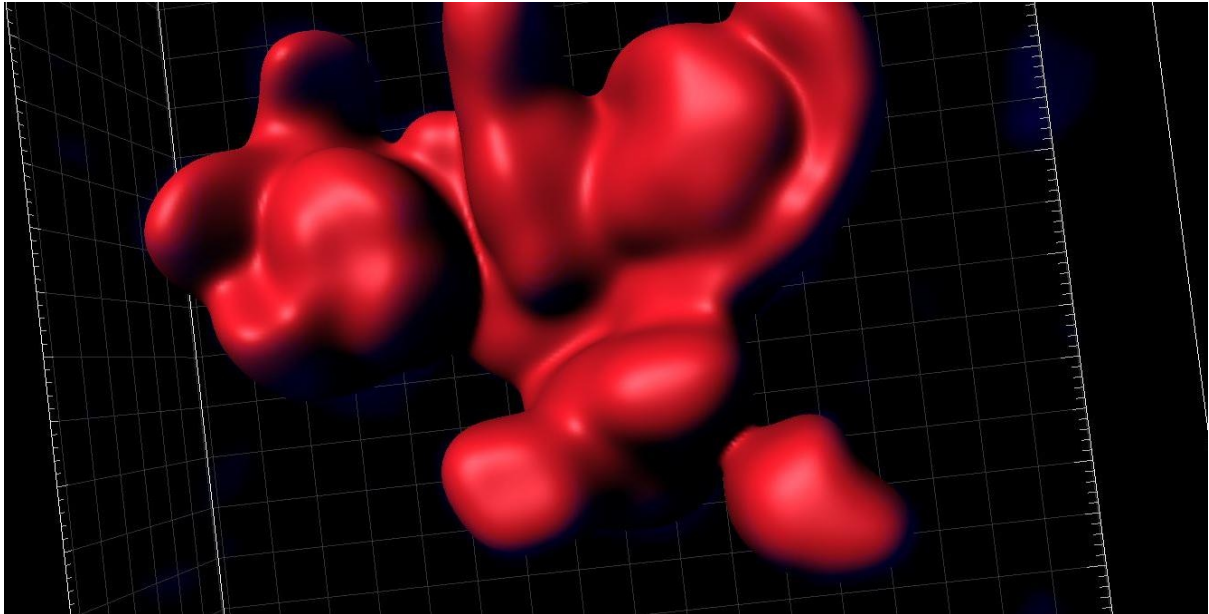
Finally, use filters to remove any non-specific selections.

Select the filter from the drop down menu and adjust the parameters in the **Histogram**.

Add new filters using the **+ Add** button.

Complete the selection process by clicking on the **green arrow**.

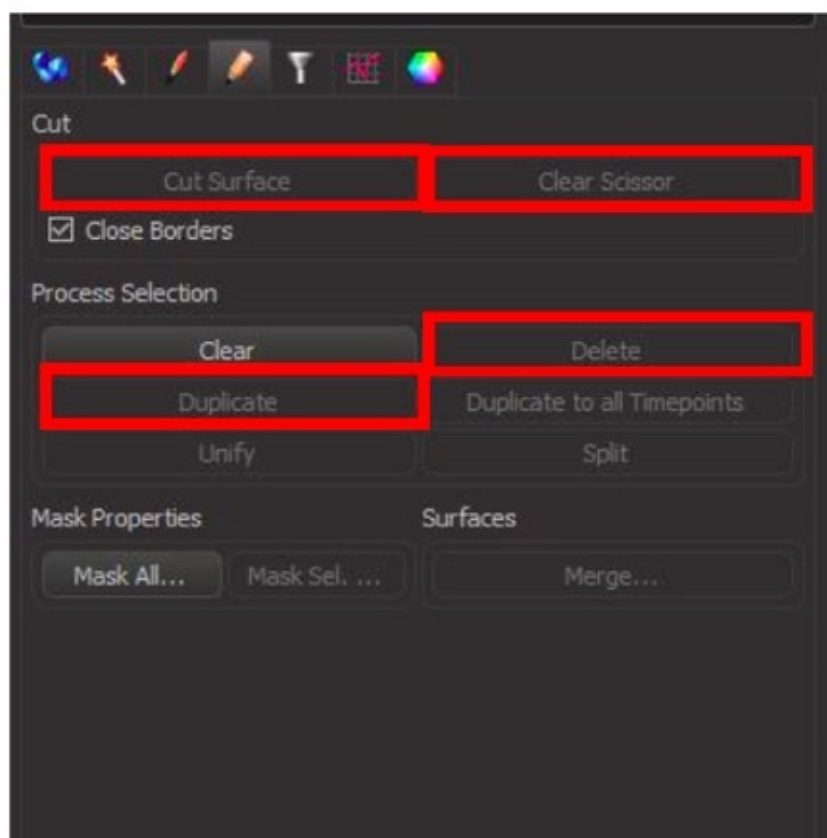
You should now see a surface render overlaying the signal in your image.



You can again change the colour of your Surfaces or apply new filters using the tabs in the bottom left hand panel.

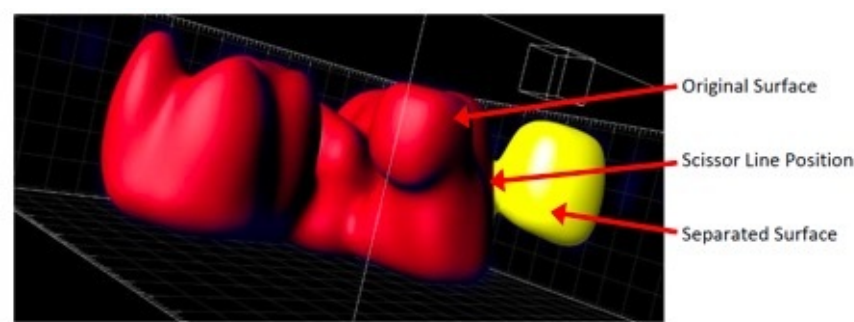
Under the “Edit” tab you now have options to cut surfaces, along with duplicating and deleting, as for spots.

To edit a surface, switch the cursor to selection mode and click on the surface you want to edit, followed by clicking your desired function in the tab.



To use the “scissors” to cut a surface, first navigate the image to the orientation that best shows the join you want to cut. Switch the cursor to section and hold shift then click on the join. A line will appear across the surface. If you are unhappy with the orientation or position, click on **Clear Scissor** and adjust the image orientation and try again.

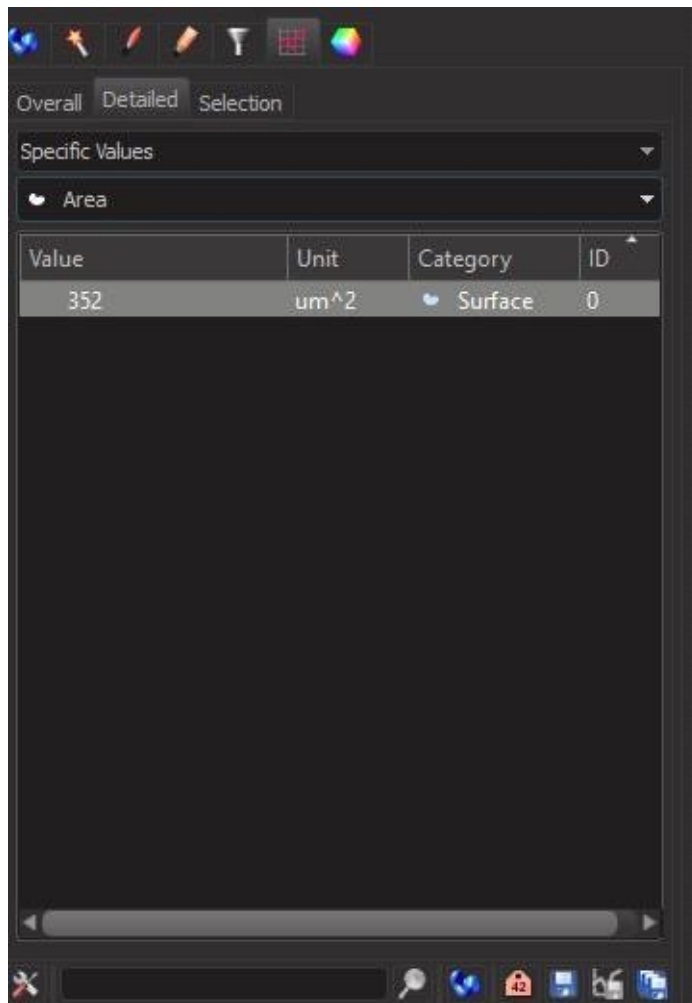
When you are happy with the line position, click on **Cut Surface** to separate the two parts of the surface object.



You can find measurements for the selected surfaces under the “Graph” tab. As with Spots, you can select **Specific Values** or **Averages**. Under Specific Values you can select measurements such as area, intensity and volume of the surfaces.

A screenshot of a software interface's 'Graph' tab. It features a table titled 'Average Values' with four columns: 'Variable', 'Min', 'Max', and 'Me'. The table lists various statistical and geometric measurements for a selected surface. The interface includes a toolbar at the top with icons for selection, manipulation, and visualization, and a bottom toolbar with icons for zooming and navigation. The 'Detailed' tab is selected, and the 'Selection' sub-tab is active.

Variable	Min	Max	Me
Intensity Median	5.00	5.00	5.00
Intensity Median	81.0	81.0	81.0
Intensity Median	7.00	7.00	7.00
Intensity Min	0.00	0.00	0.00
Intensity Min	0.00	0.00	0.00
Intensity Min	0.00	0.00	0.00
Intensity Min	0.00	0.00	0.00
Intensity StdDev	13.3	13.3	13.3
Intensity StdDev	10.3	10.3	10.3
Intensity StdDev	51.4	51.4	51.4
Intensity StdDev	8.35	8.35	8.35
Intensity Sum	3.53e6	3.53e6	3.53e6
Intensity Sum	2.54e6	2.54e6	2.54e6
Intensity Sum	2.83e7	2.83e7	2.83e7
Intensity Sum	2.59e6	2.59e6	2.59e6
Number of Disconnected ...	1	1	1
Number of Vertices	62688	62688	62688
Position X	9.36	9.36	9.36
Position Y	7.05	7.05	7.05
Position Z	2.74	2.74	2.74

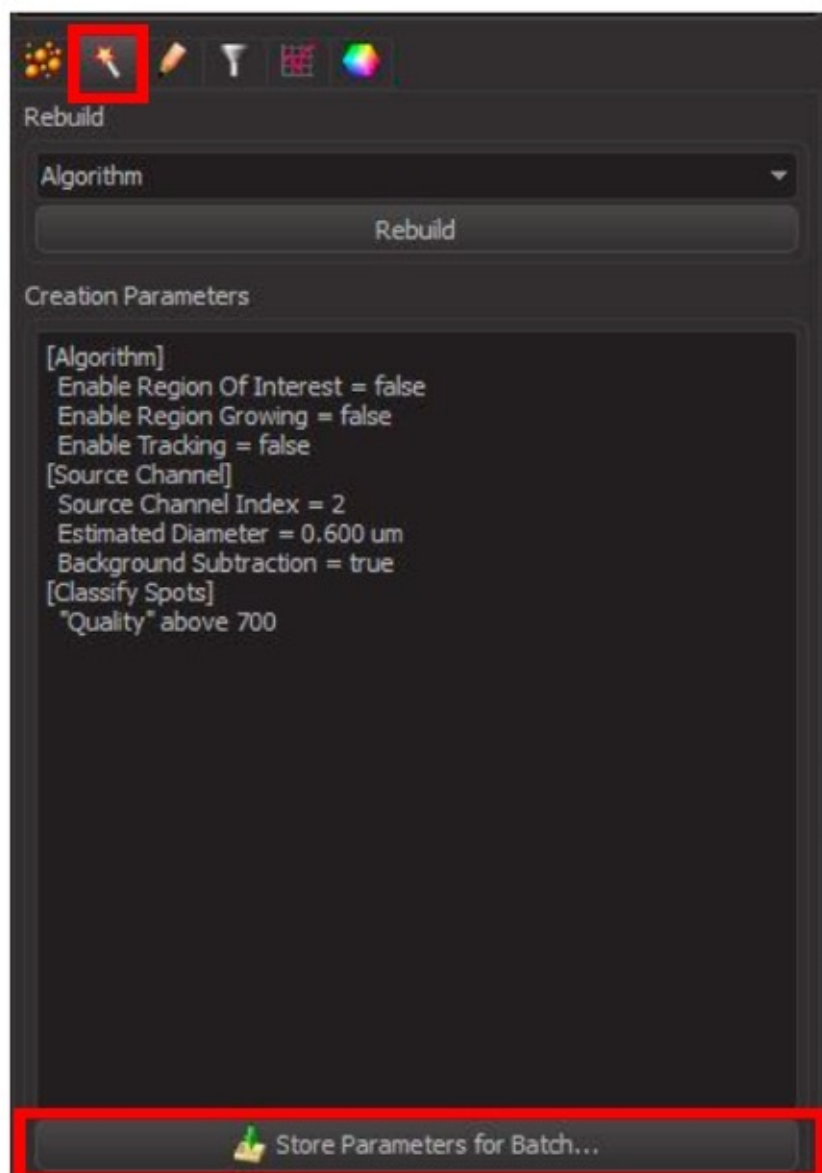


Again you can save these measurements to Excel files using the same methods as Spots and Tracking.

Saving and Reusing Selection Criteria

Often we need to use the same selection criteria throughout our analysis to maintain consistency and prevent bias in our results. This can easily be done by saving and reusing our initial selection algorithms.

To save your selection algorithm generate your selection with optimal parameters. After finishing selection go to the “Wand” or rebuilding tab and click on **Store Parameters for Batch**.

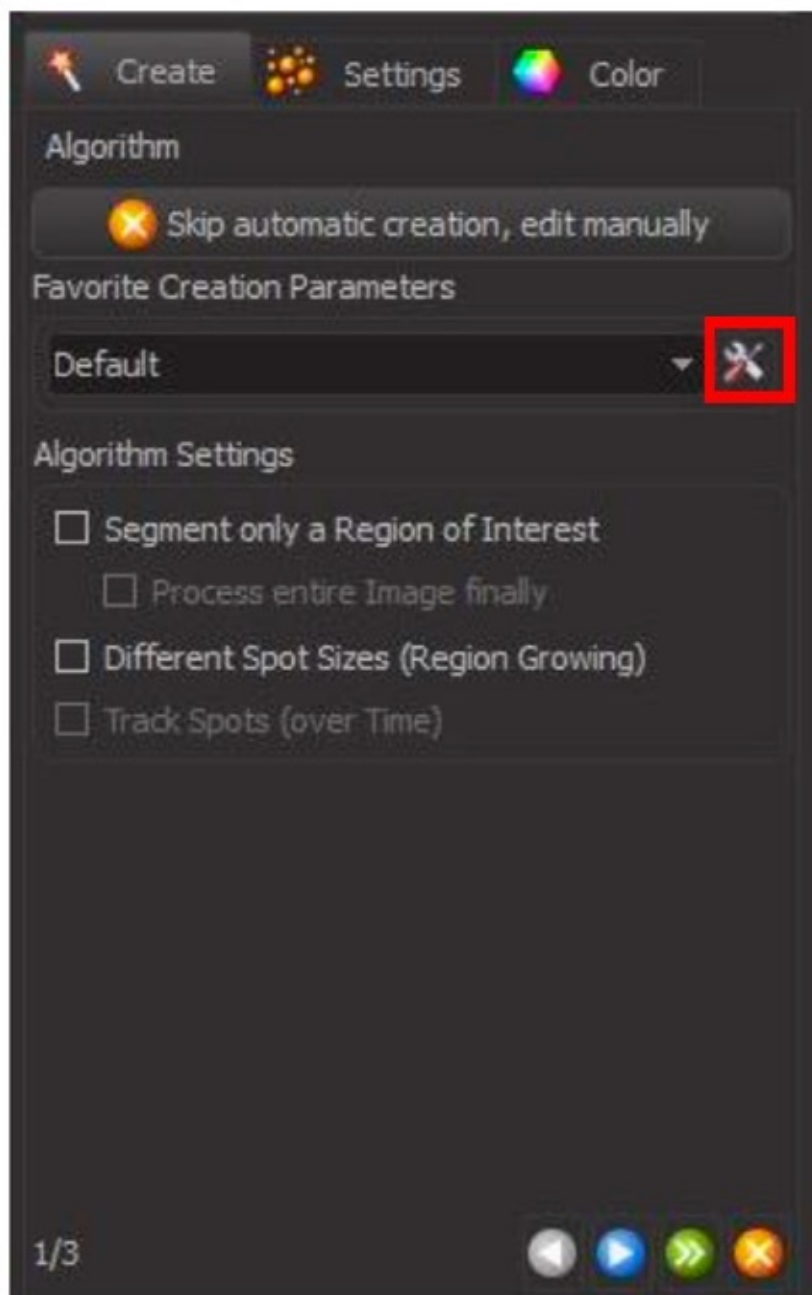


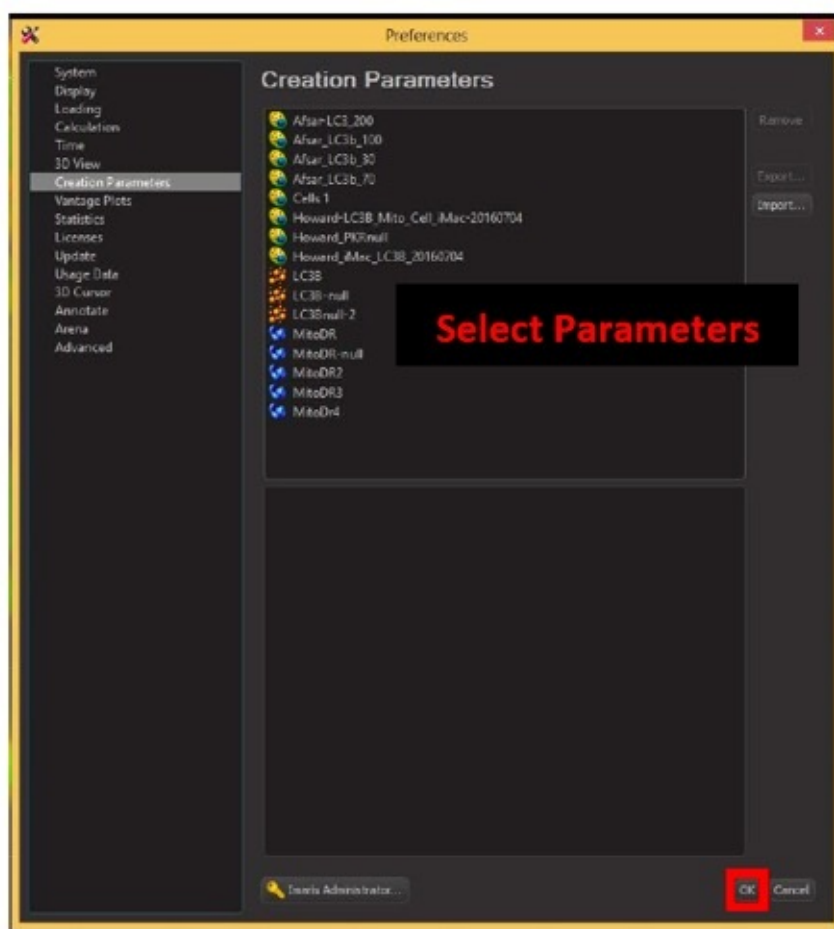
In the popup window, give your parameters a name and select the storage location in either your Arena workspace or under the general use Favourite Creation Parameters. Click **OK** to save.



To reuse saved parameters, select the **tools** button under **Favourite Creation Parameters** in step 1 of any creation wizard.

Select the stored parameters that you want to use and click **OK**.





If you stored your parameters under Favourite Creation Parameters when saving, they may also be found in the **Favourite Creation Parameters** drop down menu.

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