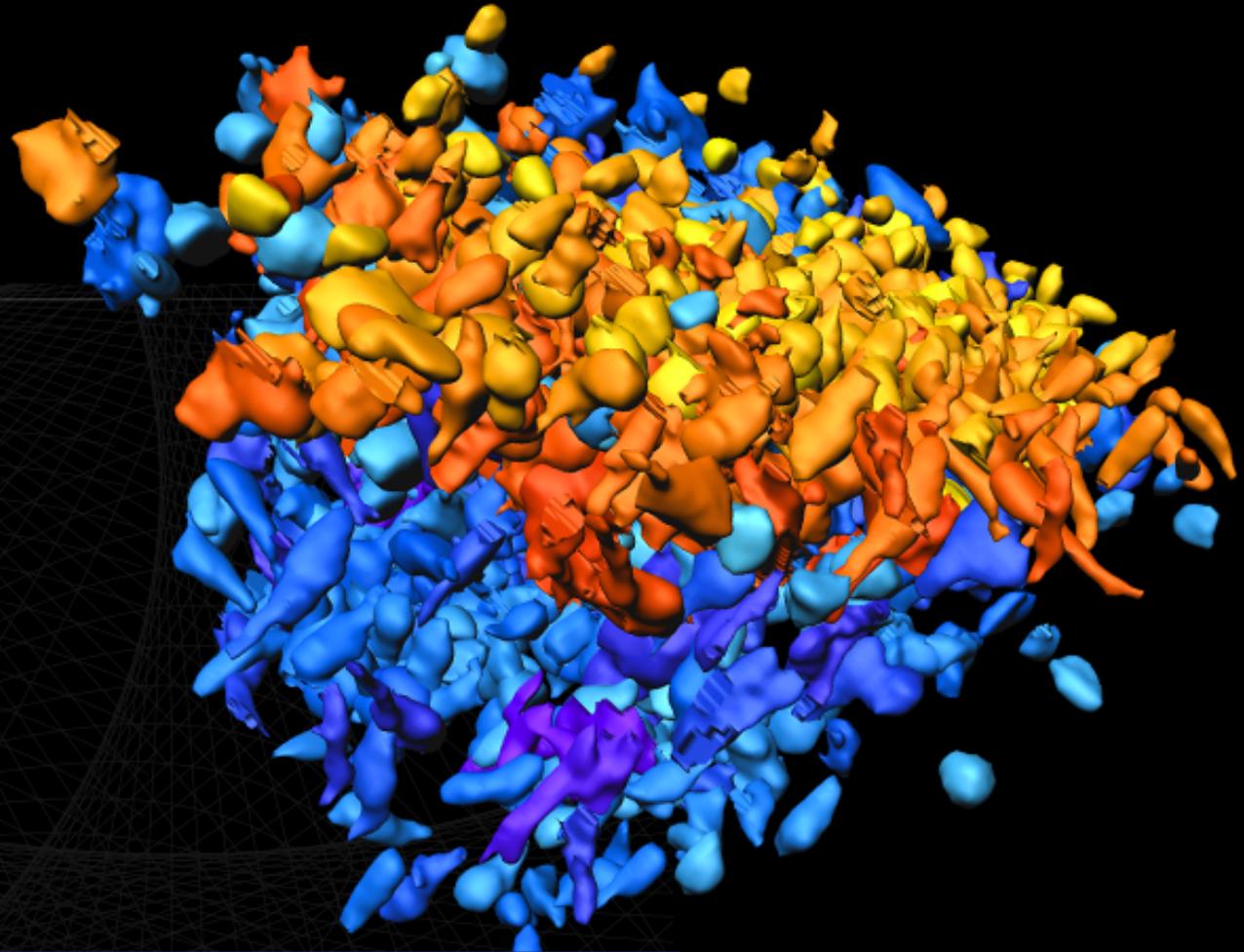


Introduction to Imaris



Imaris x64
9.0.2 [Oct 10 2017]
Build 44695 for x64
Copyright © 1993-2017 Bitplane AG
www.imaris.com
welcome@imaris.com

BITPLANE
an Oxford Instruments company

7th November 2017

Course Schedule

09:30-10:30	Session 1 – Understanding the Basics
10:45-12:30	Session 2 – Initial Scene Objects, Taking Snapshots and Making Movies
13:30-15:00	Session 3 – Adding New Objects to the Scene
15:30-17:00	Session 4 – Creating Cells

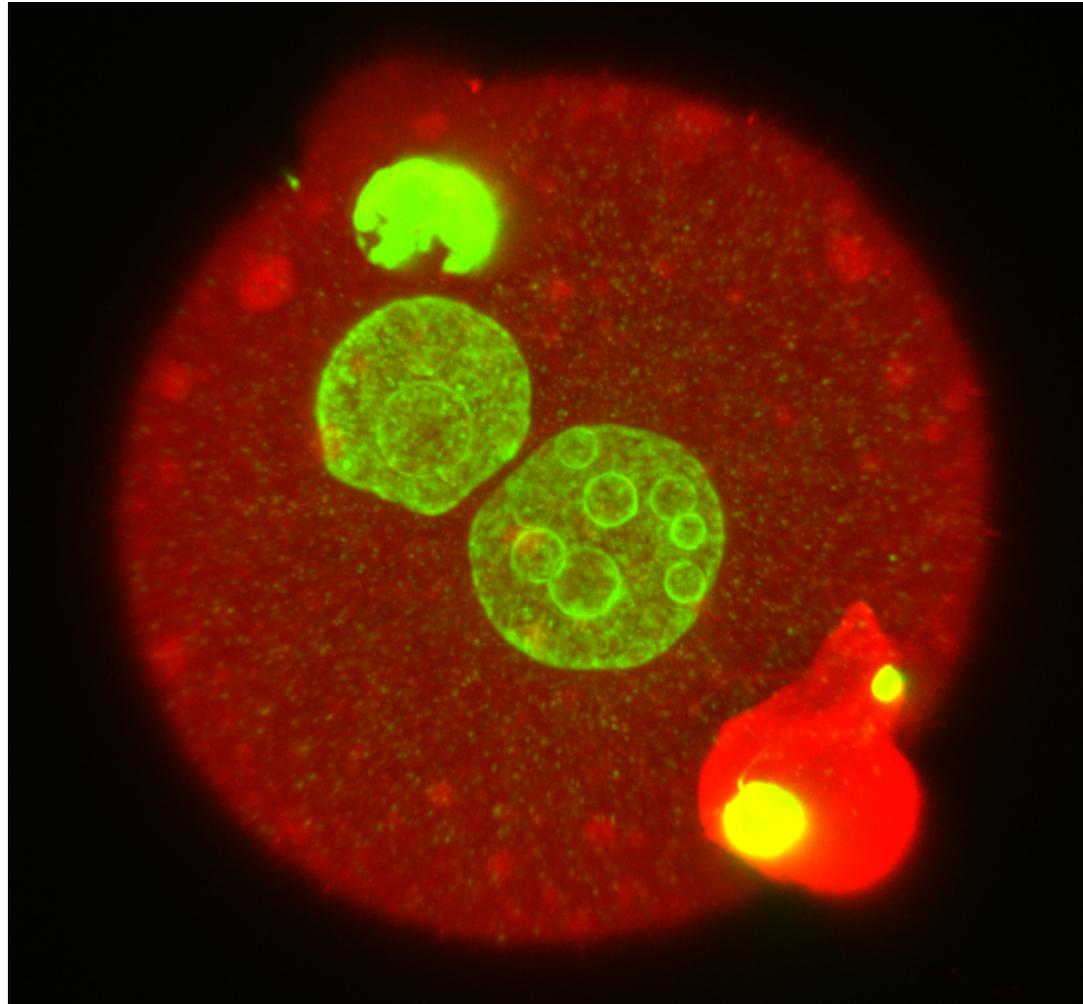
Session 3 – Adding New Objects to the Scene

1. Clipping plane
2. OrthoSlicer
3. Oblique Slicer
4. Spots
5. Surfaces



Adding New Objects to the Scene

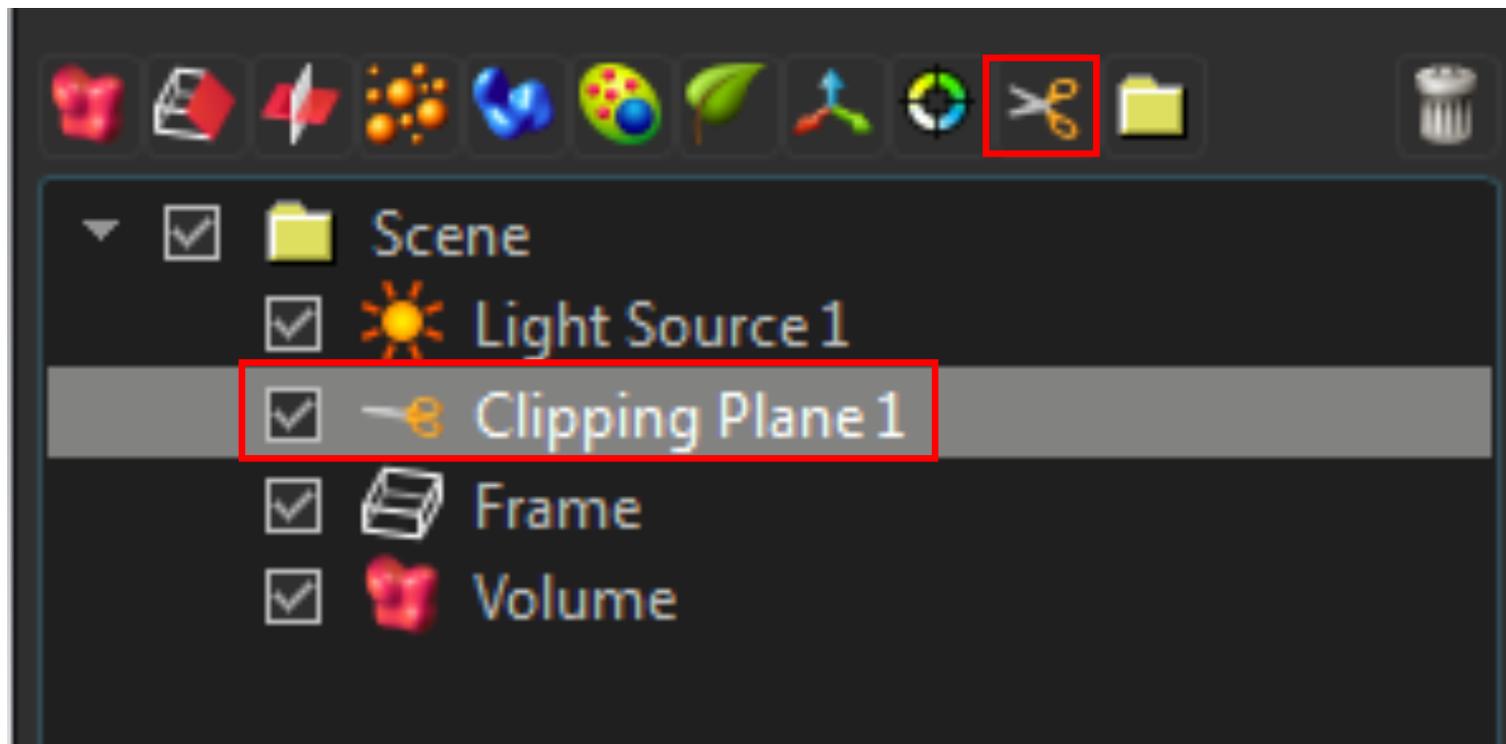
1. Add file Confocal Stack2 to Arena
2. View in Surpass
3. Remove Frame



Adding New Objects to the Scene

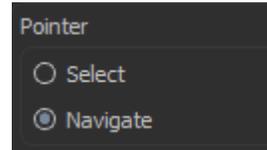
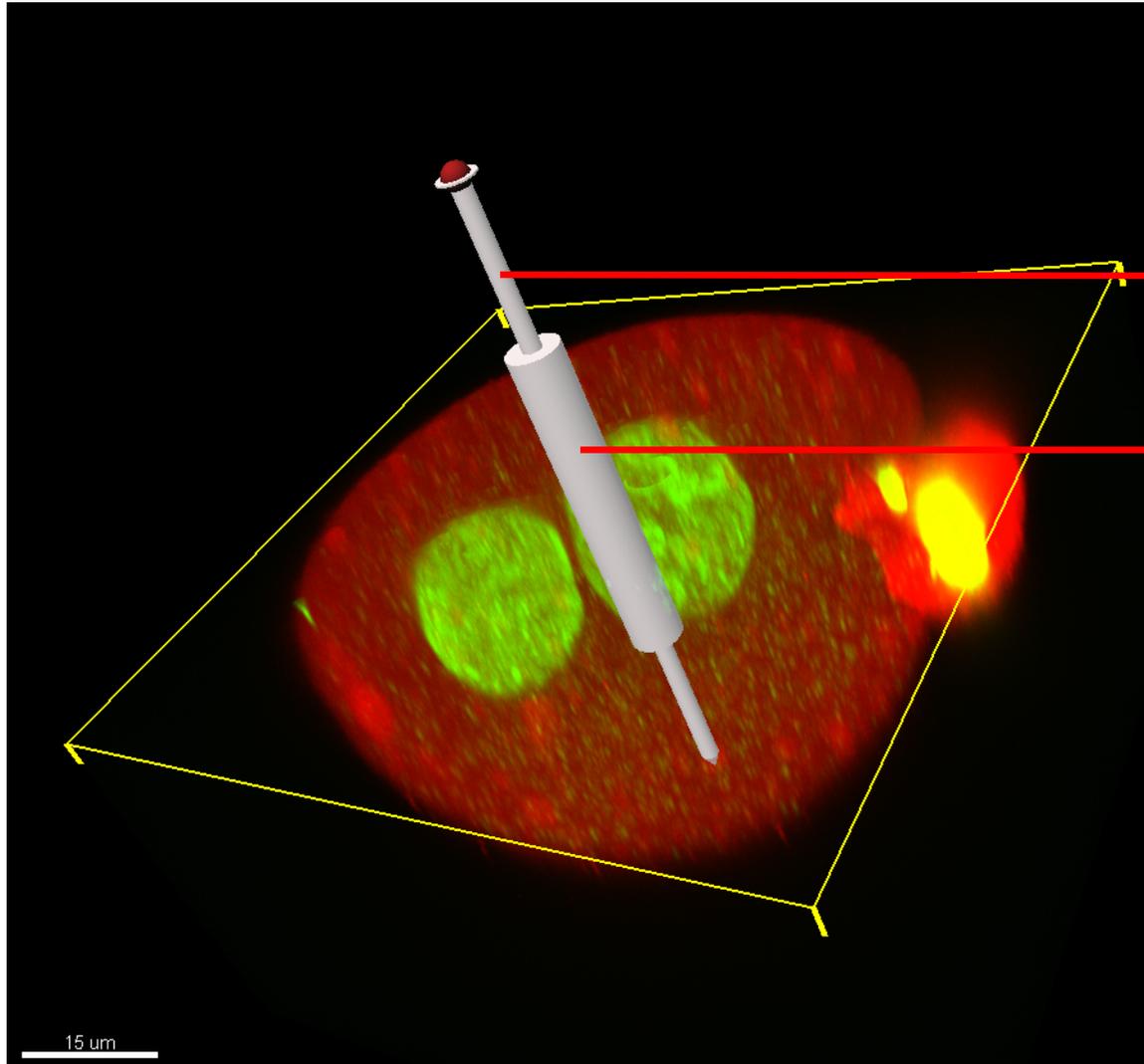
Clipping plane: Removes a section of your data to allow you to 'see inside'

Click on scissor icon to add new Clipping Plane



Adding New Objects to the Scene

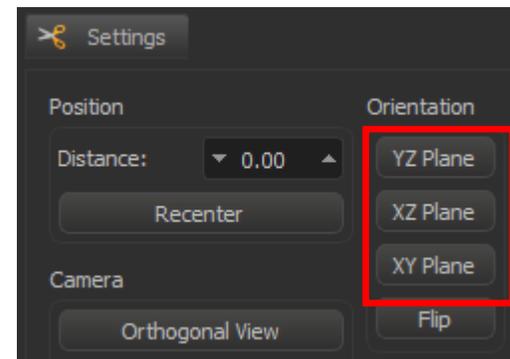
Clipping plane: Removes a section of your data to allow you to 'see inside'



Use Navigate to rotate image data
Use Select to interact with clipping tool

Upper rod rotates clipping plane

Lower rod moves position of clipping plane



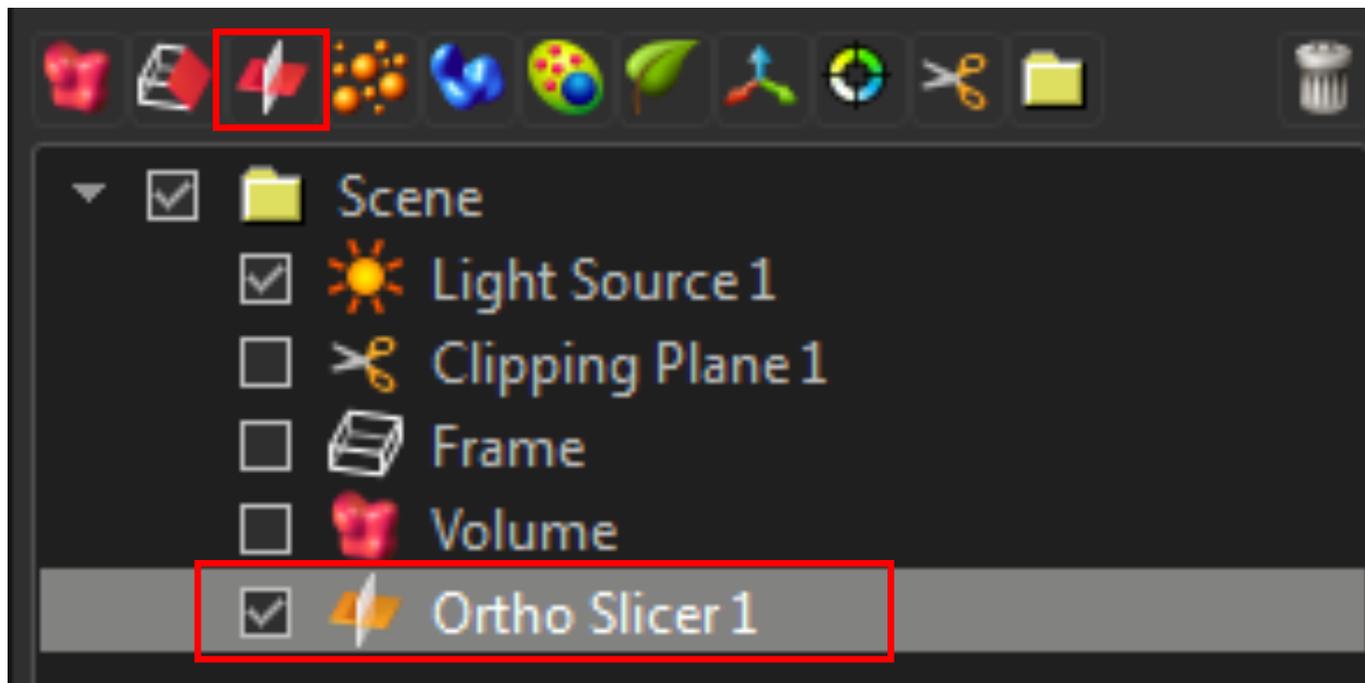
Use Orientation tools
to move clipping plane
onto different axis



Adding New Objects to the Scene

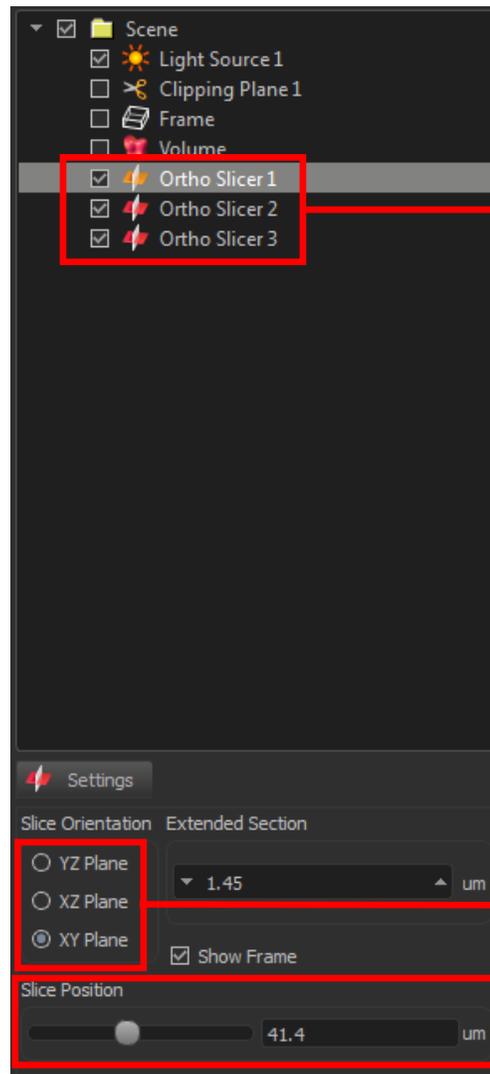
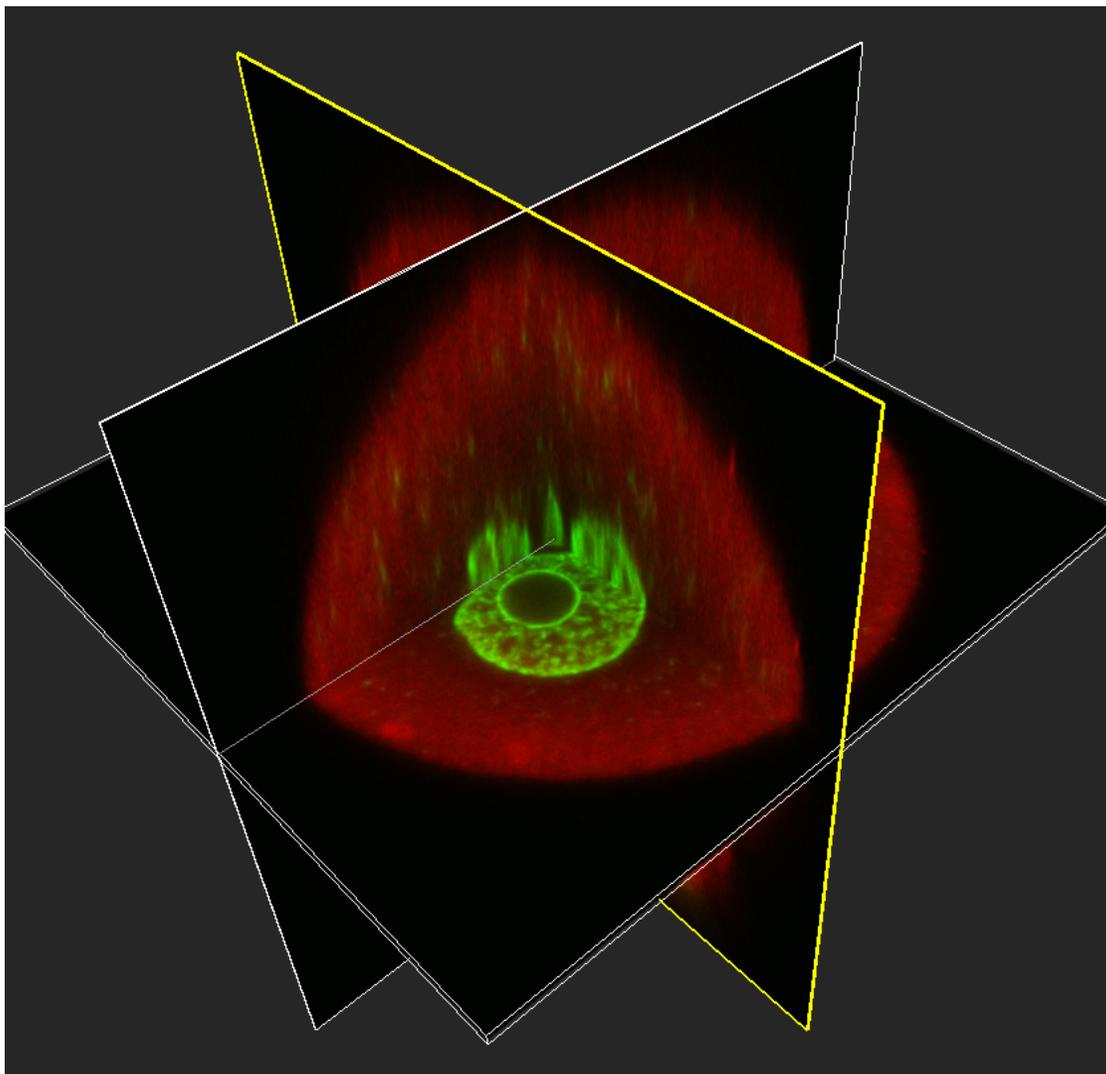
Ortho Slicer: 3D Orthogonal view in x, y, z

Click on cross-slice icon to add new Ortho Slicer



Adding New Objects to the Scene

Ortho Slicer: 3D Orthogonal view in x, y, z



Add multiple Ortho Slicers to view x,y,z planes (turn off Volume for Ortho view only)

Select plane of slicer

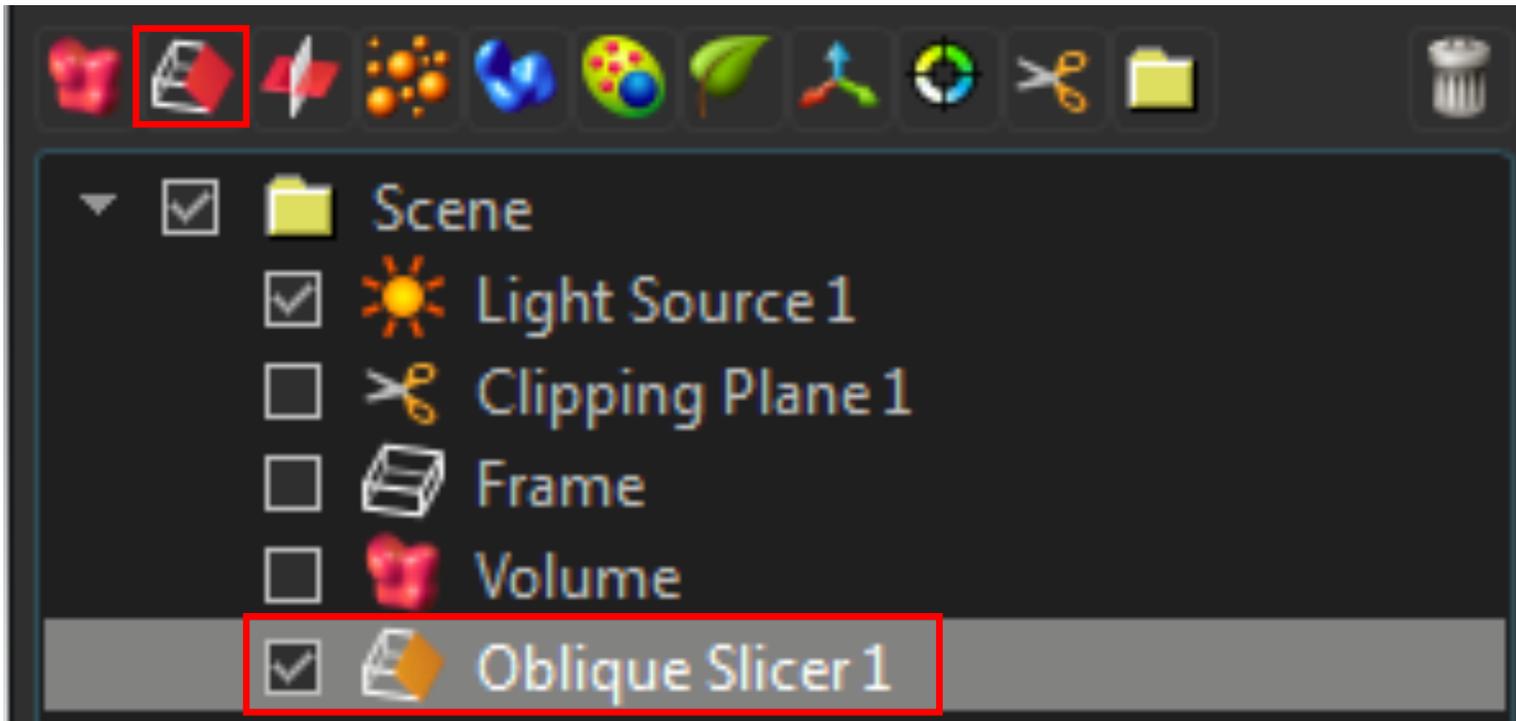
Move slice position to slide through planes



Adding New Objects to the Scene

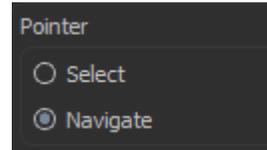
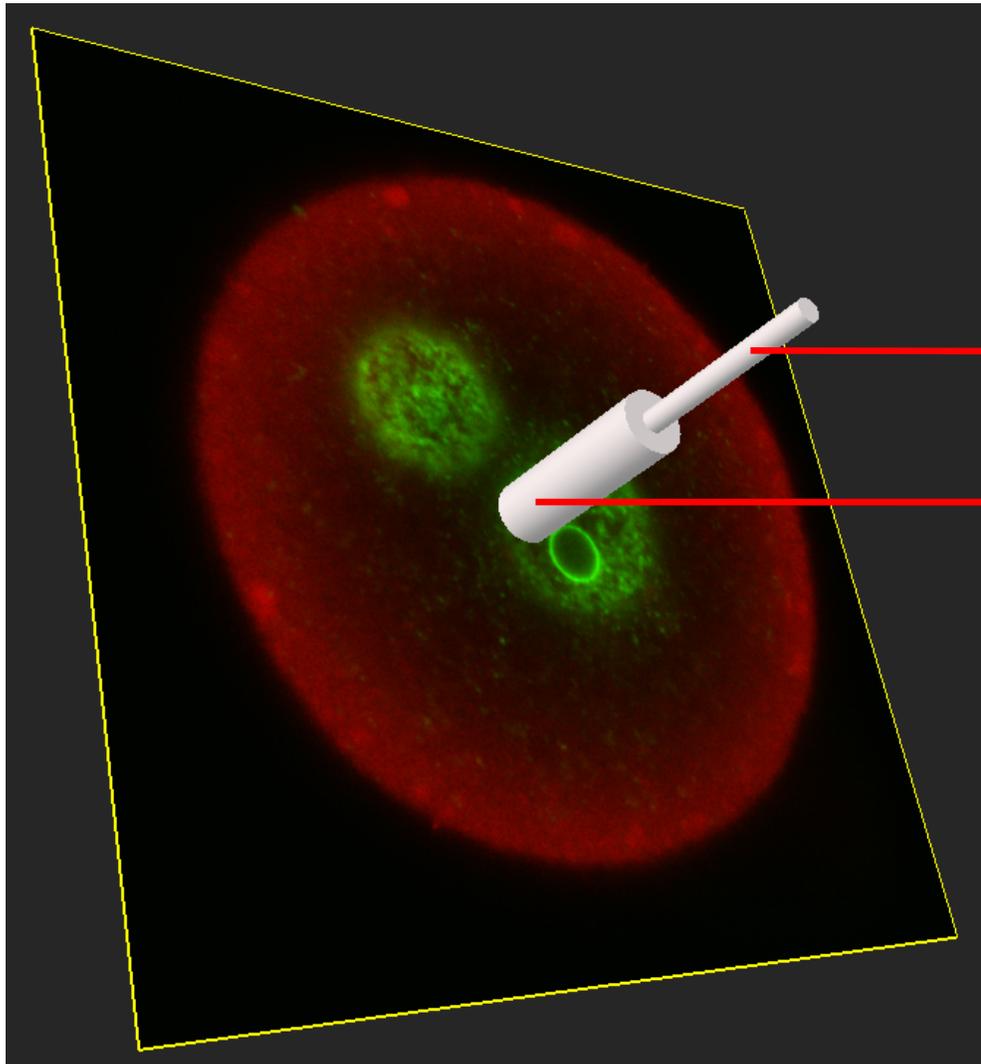
Oblique Slicer: Slice view through data in any orientation

Click on sliced object icon to add new Oblique Slicer



Adding New Objects to the Scene

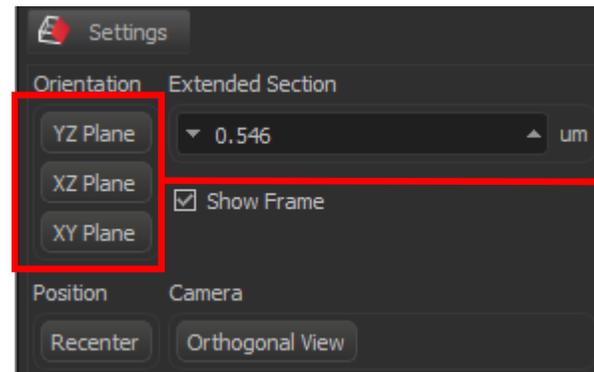
Oblique Slicer: Slice view through data in any orientation



Use Navigate to rotate image data
Use Select to interact with slicing tool

Upper rod rotates slice plane

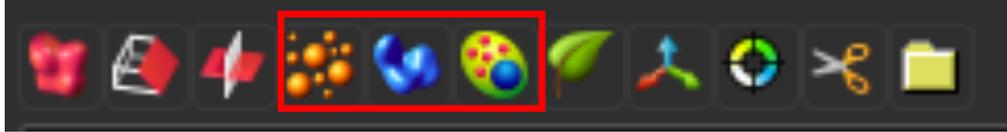
Lower rod moves position of slice plane



Use Orientation tools
to move slicing plane
onto different axis



Adding New Objects to the Scene

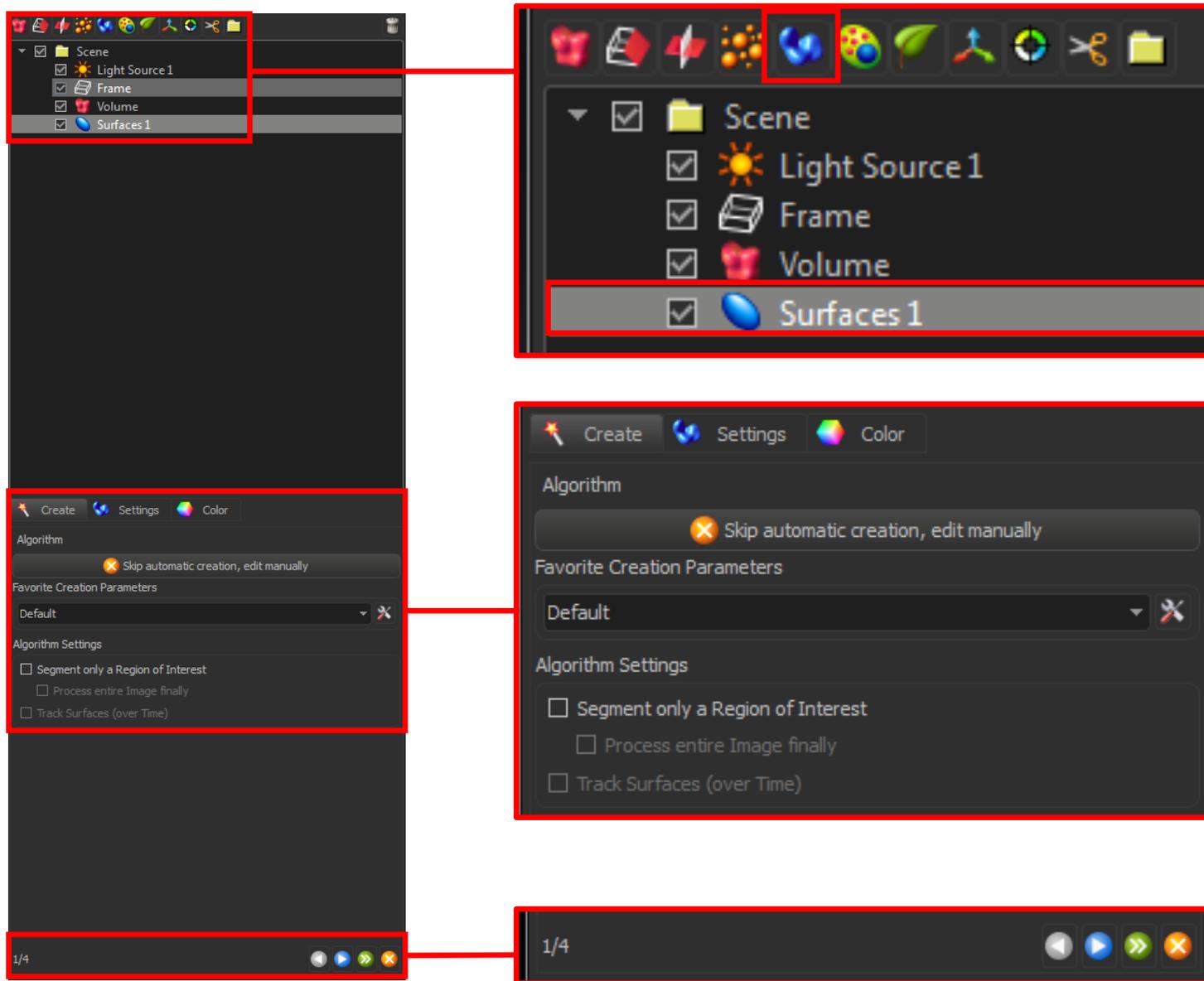


Use Spots, Surfaces and Cells to segment your data i.e. identify features of interest

- Use Spots to identify spherical objects e.g. vesicles
- Use Surfaces to identify irregular objects e.g. cell boundary
- Use Cells to link objects e.g. vesicles to cell
- Segmented objects can be surface-rendered and measured



Adding New Objects to the Scene

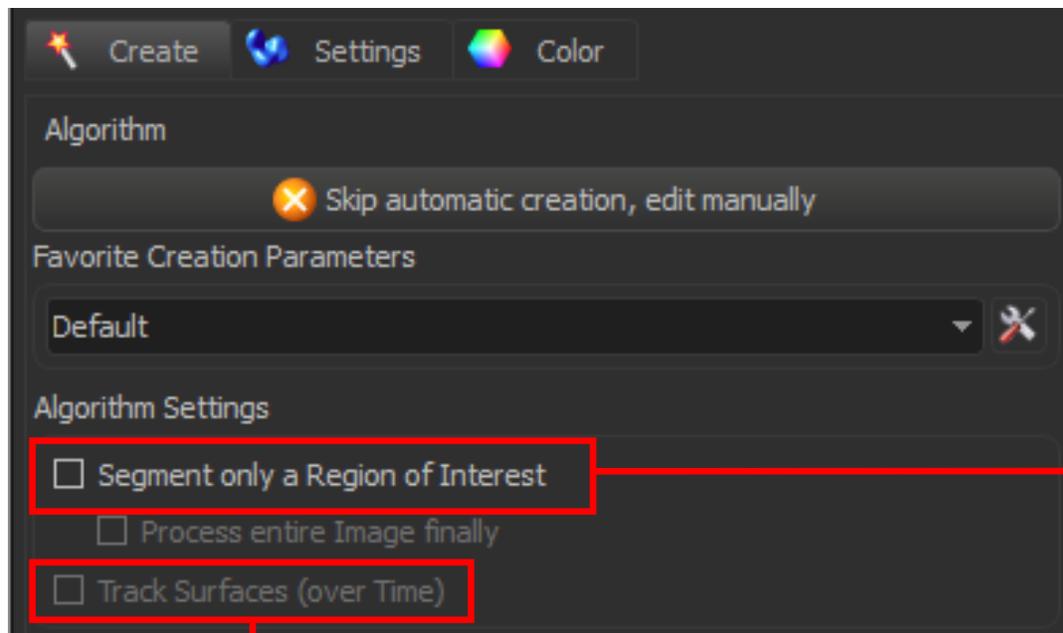


Click on the blue blobs icon to add new Surfaces

Creation parameters can be loaded from a pre-defined configuration, edited manually, or defined using a walk-through wizard

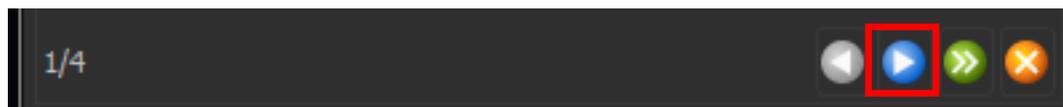


Adding New Objects to the Scene

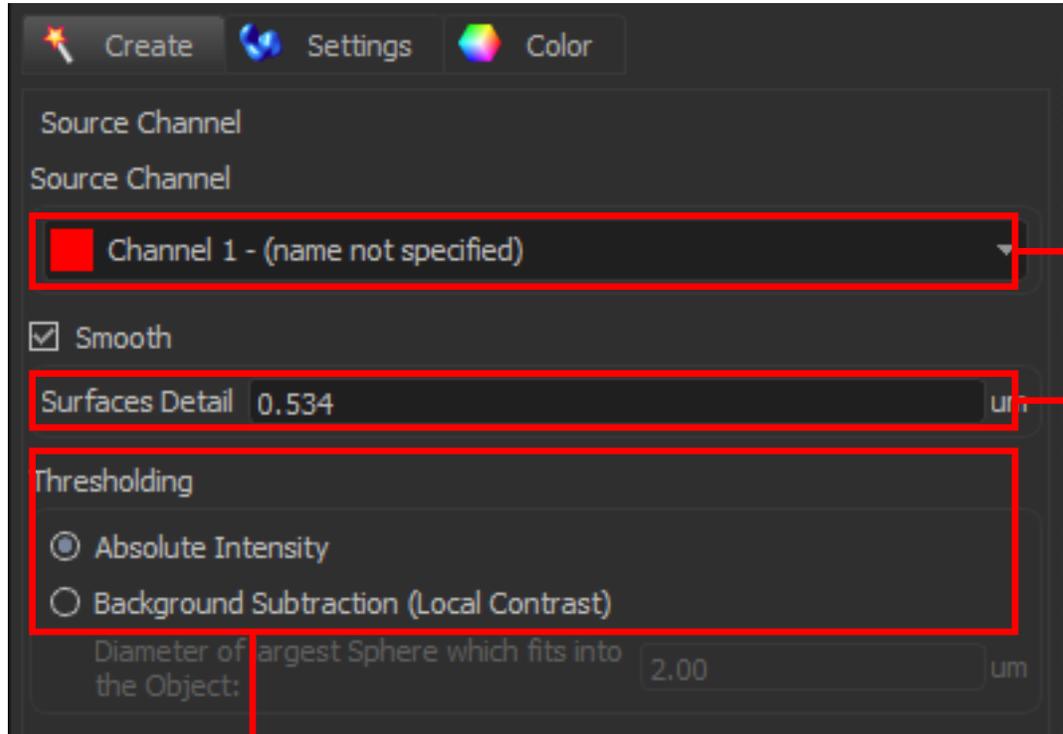


Useful for large datasets where defining the creation parameters can take a long time

Only relevant for live imaging data



Adding New Objects to the Scene

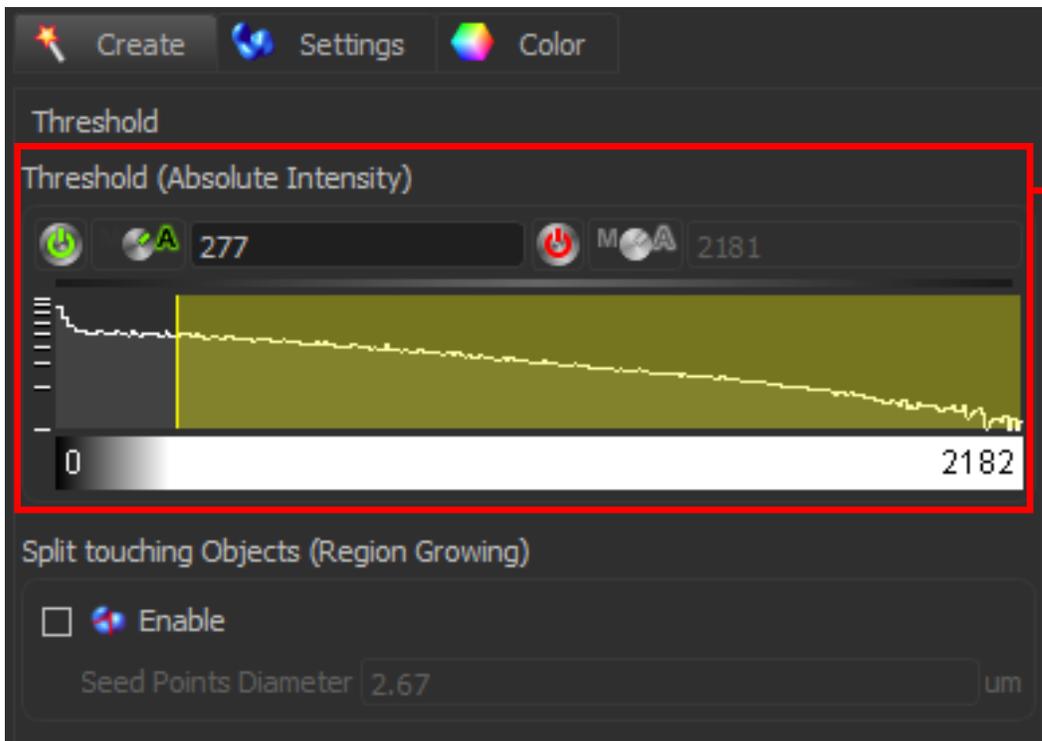


Specify channel to be used to create surfaces

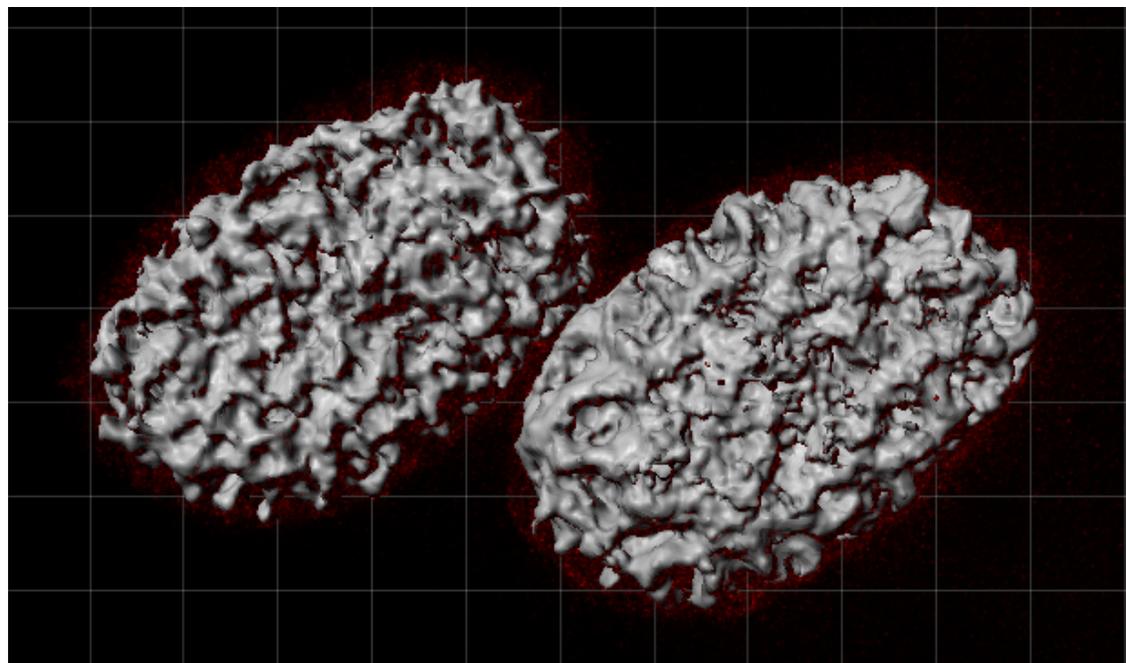
Determine surface level detail; smaller values will give more detail.

Specify the thresholding method. Background subtraction will be better at isolating small features, Absolute Intensity will be better at covering larger features

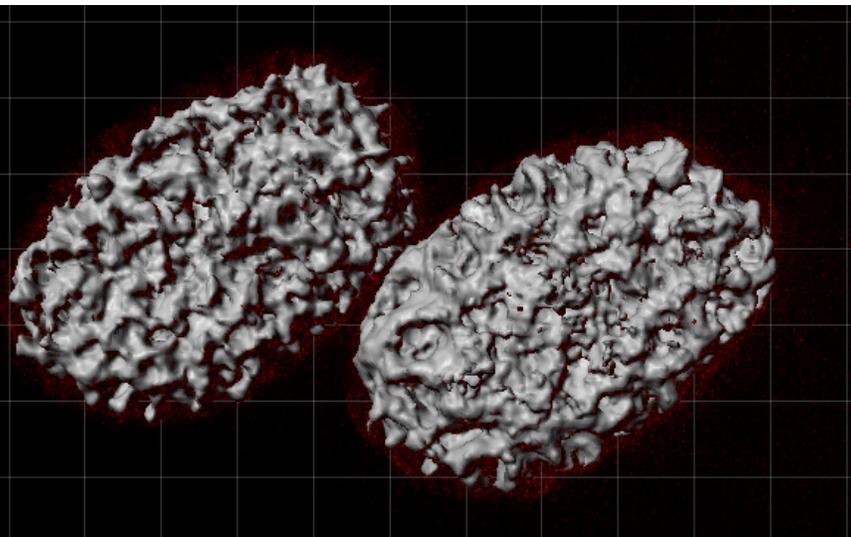
Adding New Objects to the Scene



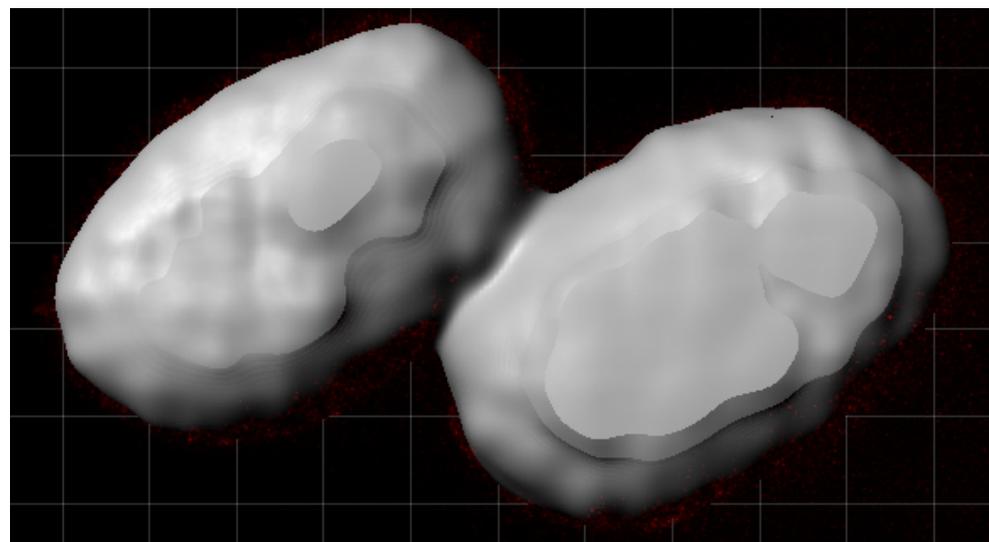
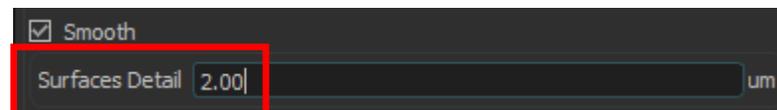
Thresholding (setting the values to determine what is object and what is background) can be done manually or automatically



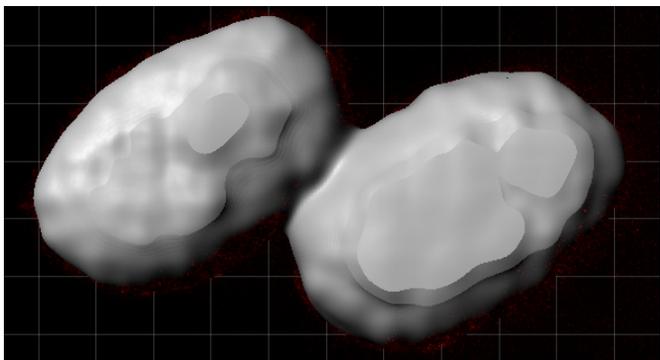
Adding New Objects to the Scene



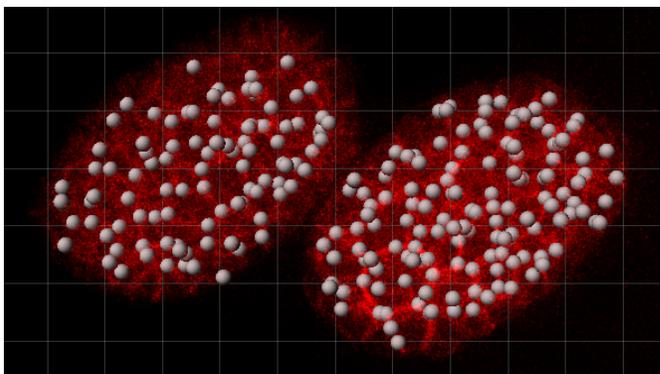
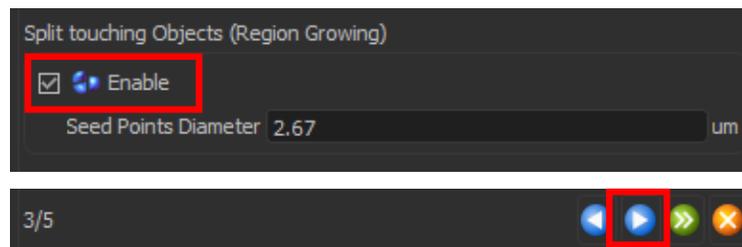
Object surface is too detailed. Return to previous step, increase Surface Detail to e.g. 2 μm



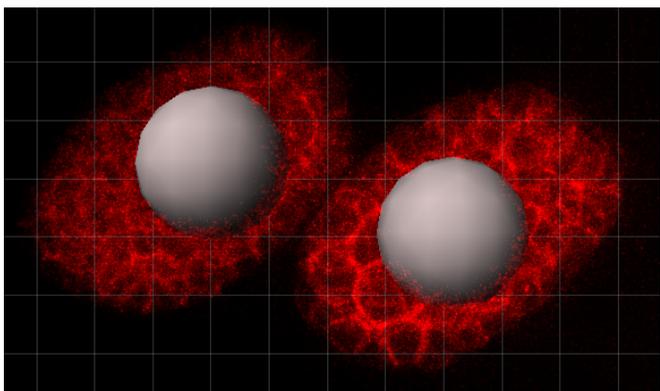
Adding New Objects to the Scene



Objects are joined. Tick Split touching Objects (Region Growing) option to separate into two.



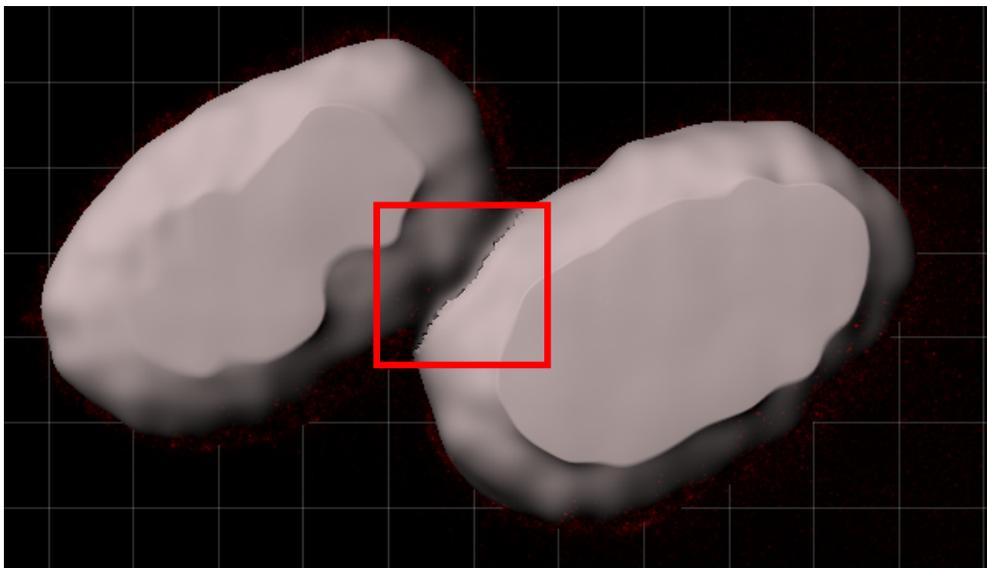
Seed points are too small. Return to previous step and increase Seed Points Diameter to e.g. 25 μm



Two seed points – should split object as required



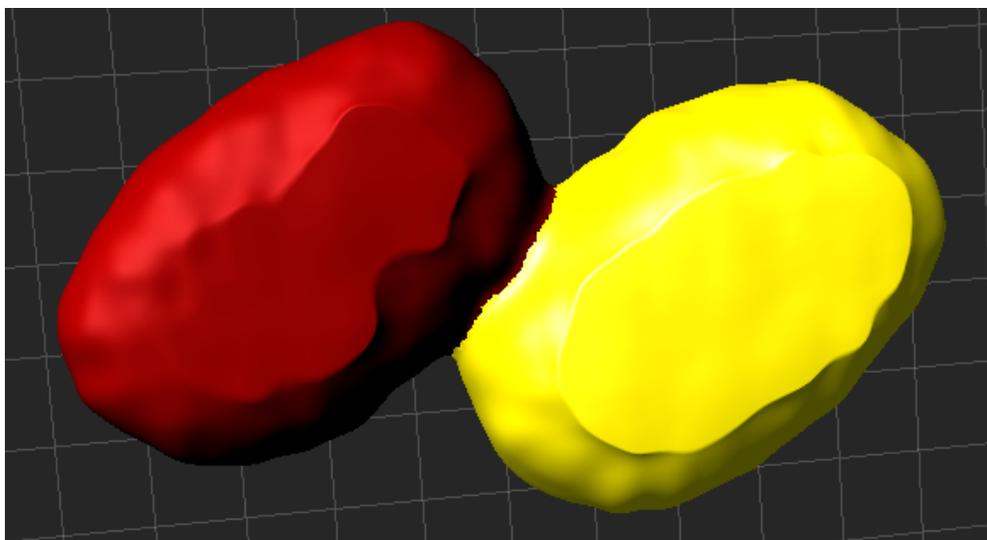
Adding New Objects to the Scene



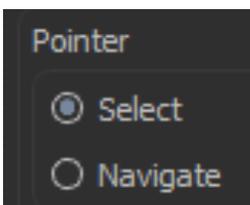
Threshold now correct and objects are separated.



Click green double-chevron button to complete surface build.



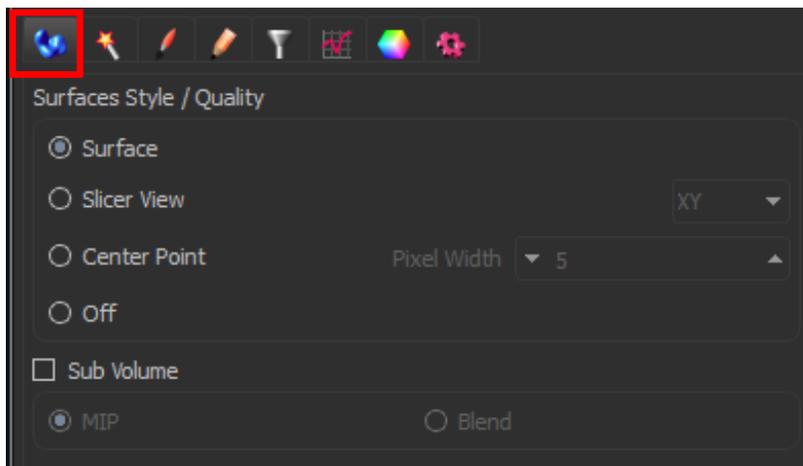
Switch pointer to Select mode and click on objects to check segmentation. Turn off Volume in the Scene to see rendered Surface alone.



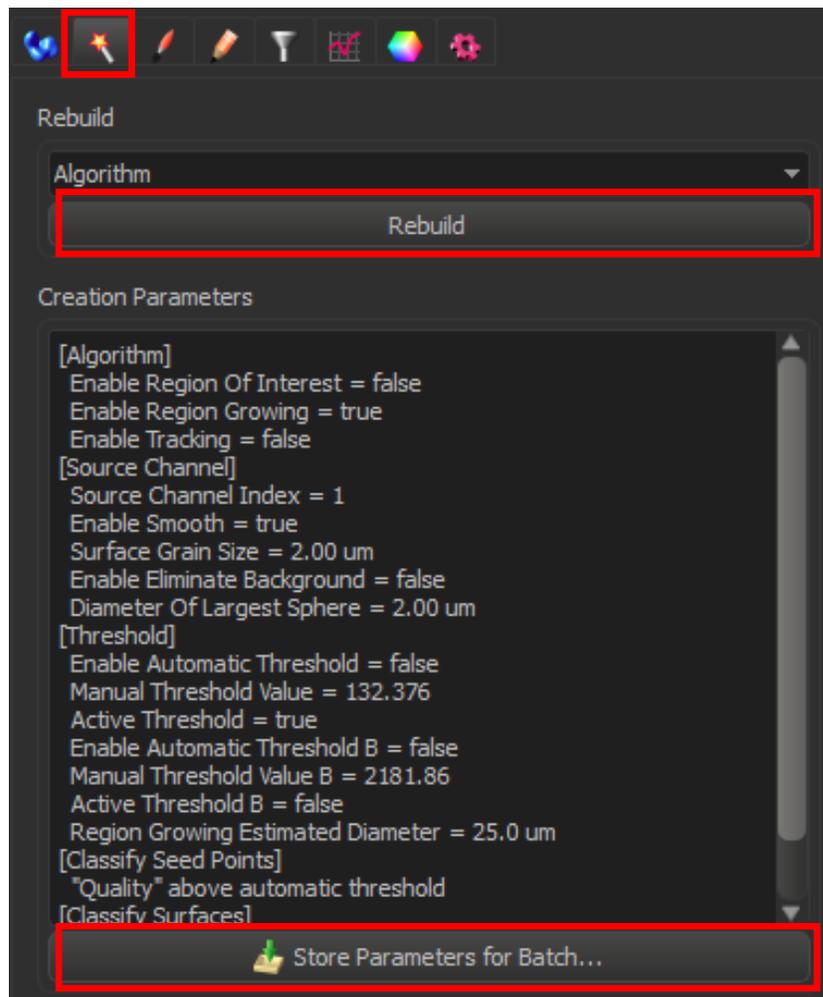
Adding New Objects to the Scene

Surface Object Options

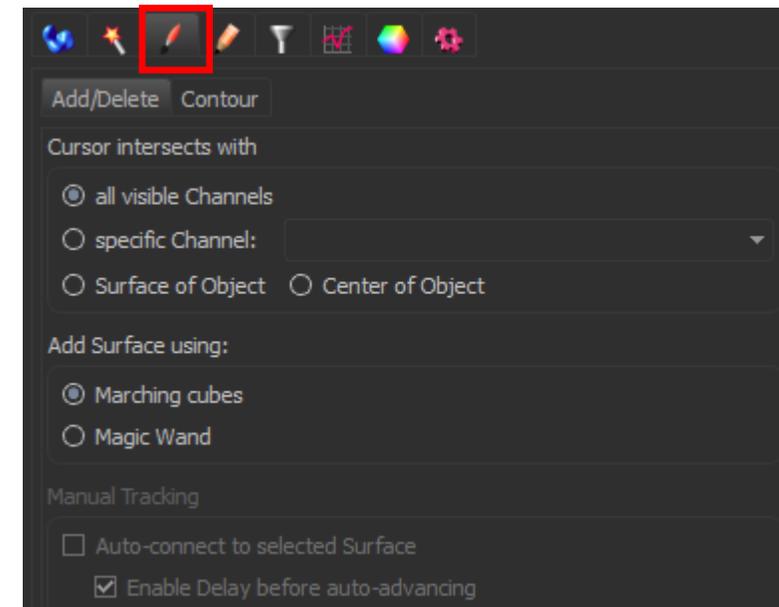
1. View



2. Rebuild



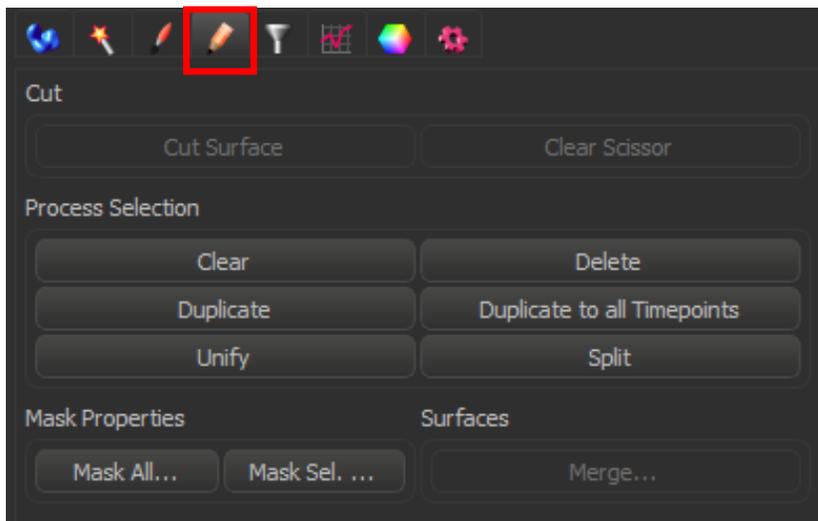
3. Edit



Adding New Objects to the Scene

Surface Object Options

4. Cut



Cut

Cut Surface Clear Scissor

Process Selection

Clear Delete

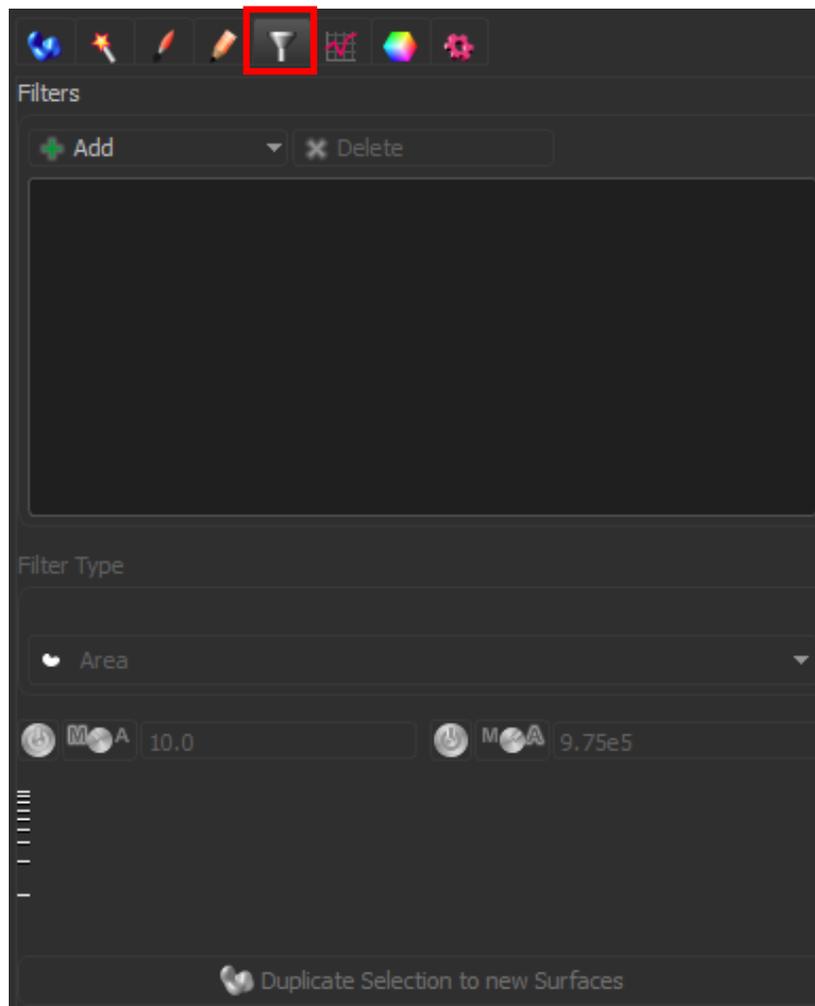
Duplicate Duplicate to all Timepoints

Unify Split

Mask Properties Surfaces

Mask All... Mask Sel. ... Merge...

5. Filter



Filters

Add Delete

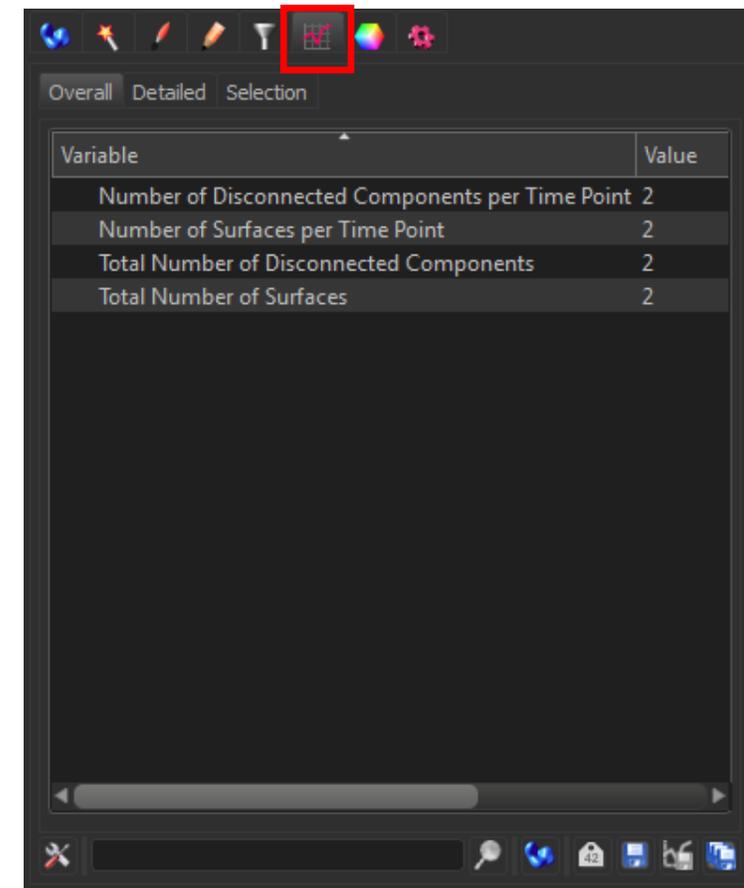
Filter Type

Area

M A 10.0 M A 9.75e5

Duplicate Selection to new Surfaces

6. Stats



Overall Detailed Selection

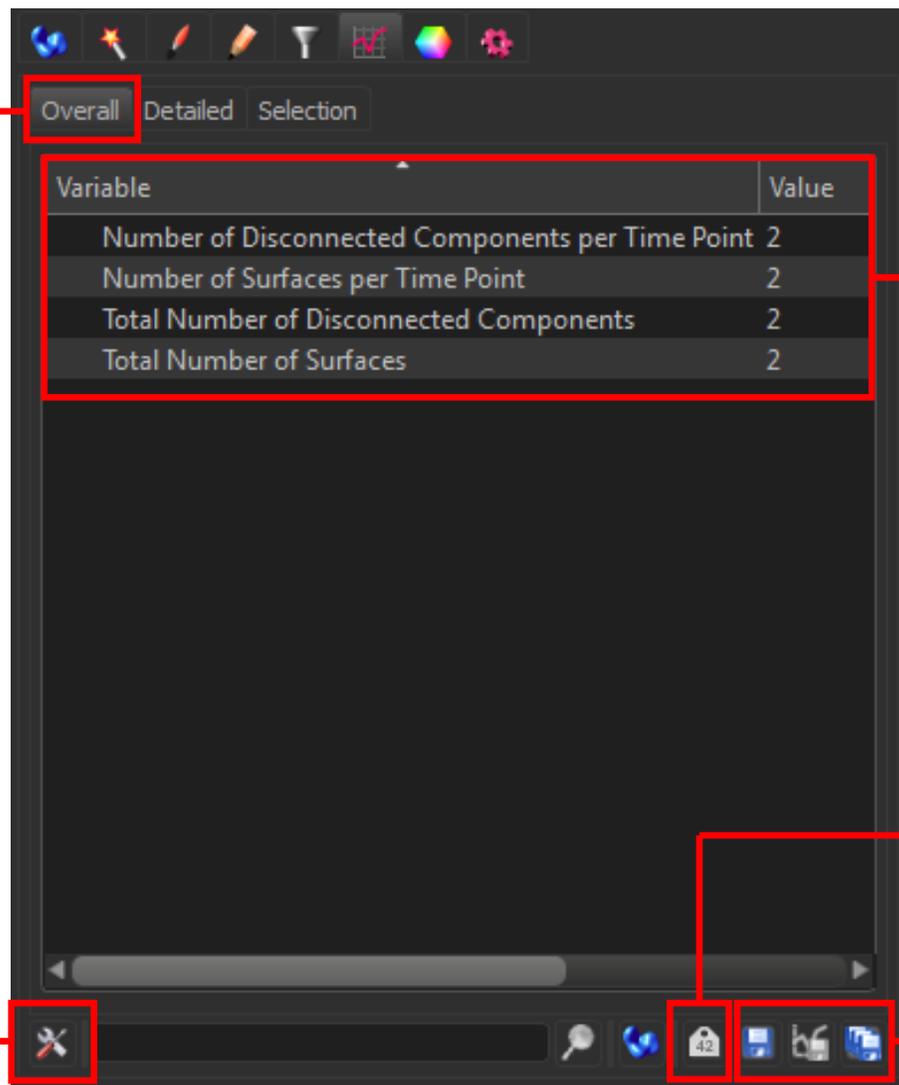
Variable	Value
Number of Disconnected Components per Time Point	2
Number of Surfaces per Time Point	2
Total Number of Disconnected Components	2
Total Number of Surfaces	2



Adding New Objects to the Scene

Surface Object Options: Stats tab

Specify whether displayed stats relate to the total population or specific objects



Stats (window may need to be expanded to the right to see all information, or use lower slider)

Annotations (need to select object first)

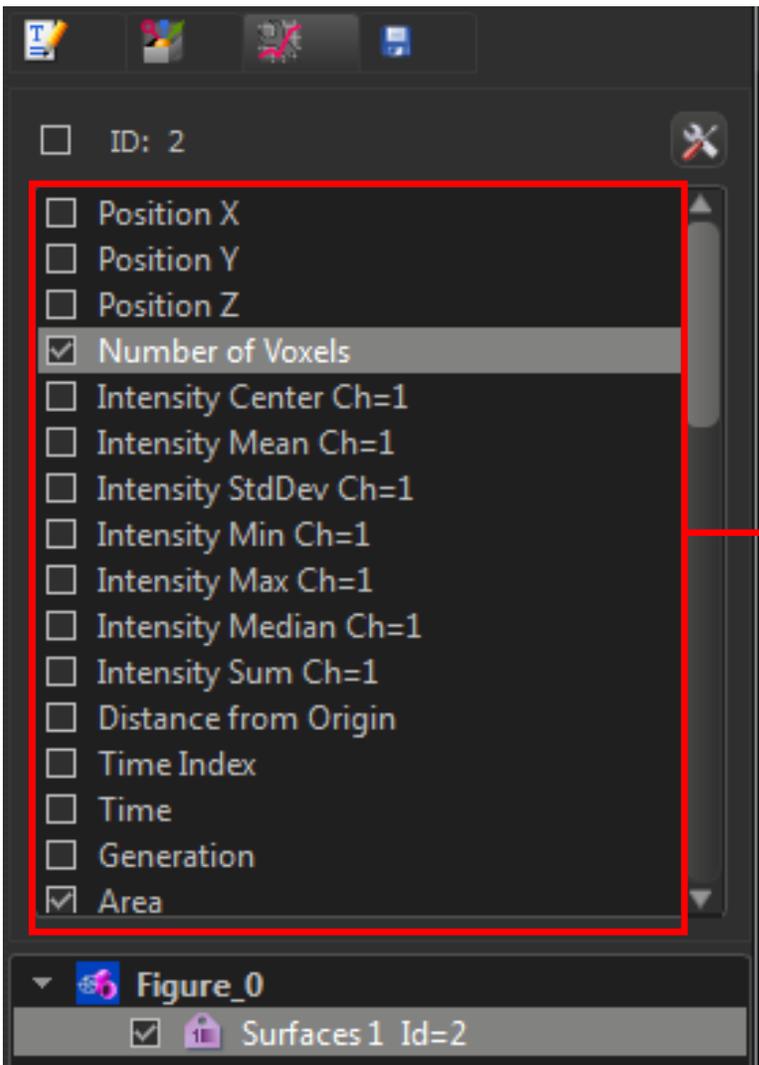
Specify what measurements are to be made

Export options



Adding New Objects to the Scene

Surface Object Options: Stats tab (Annotations)

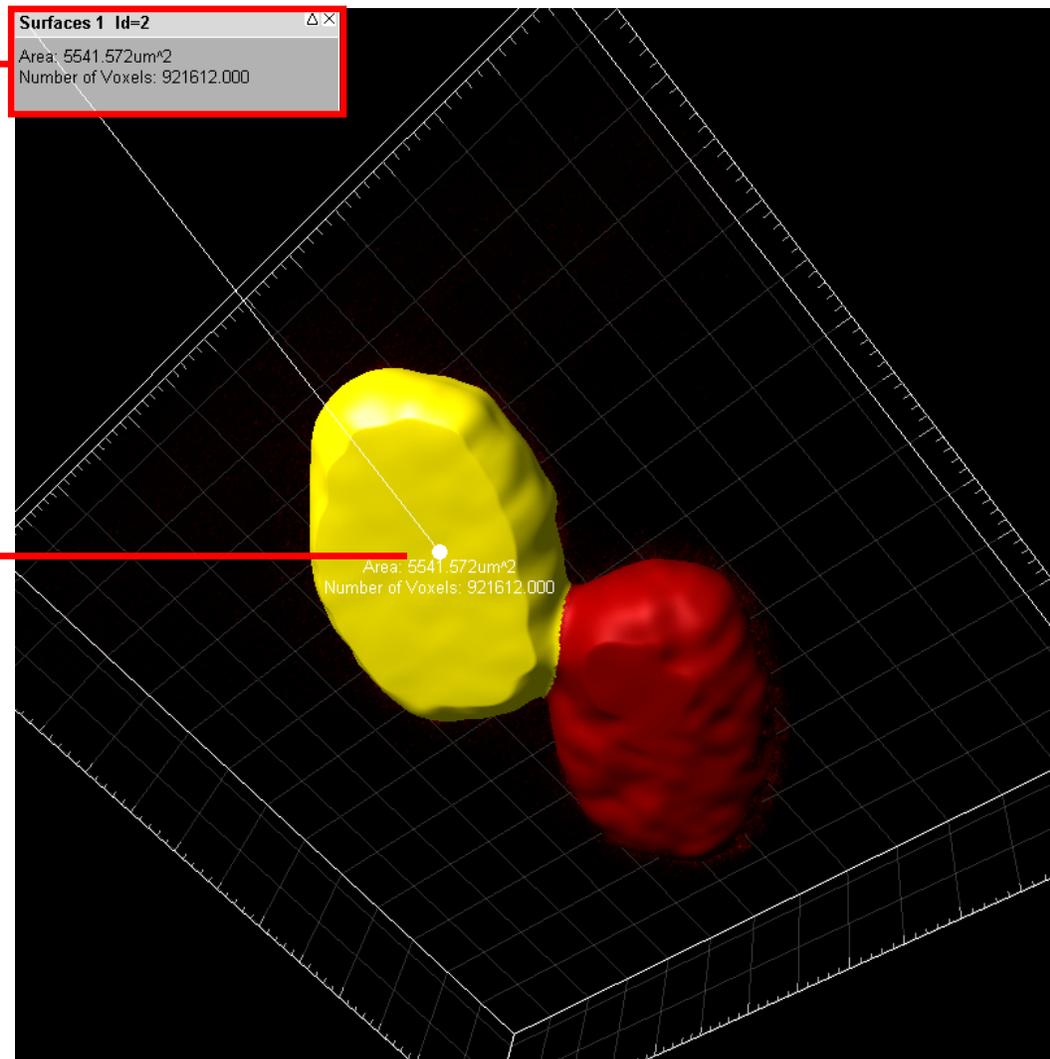


Text box option

Select stats to be displayed

Arrow locked to centre of object

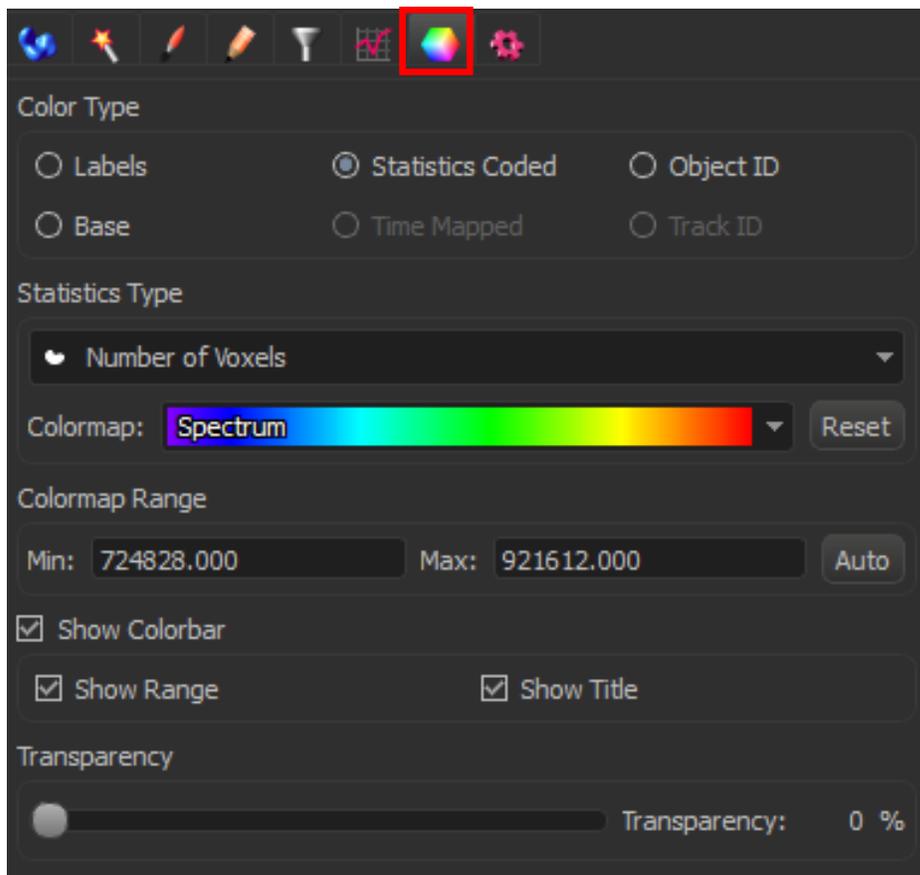
Surfaces 1 Id=2
Area: 5541.572um²
Number of Voxels: 921612.000



Adding New Objects to the Scene

Surface Object Options

7. Colour



Color Type

Labels Statistics Coded Object ID

Base Time Mapped Track ID

Statistics Type

Number of Voxels

Colormap: Spectrum

Colormap Range

Min: 724828.000 Max: 921612.000

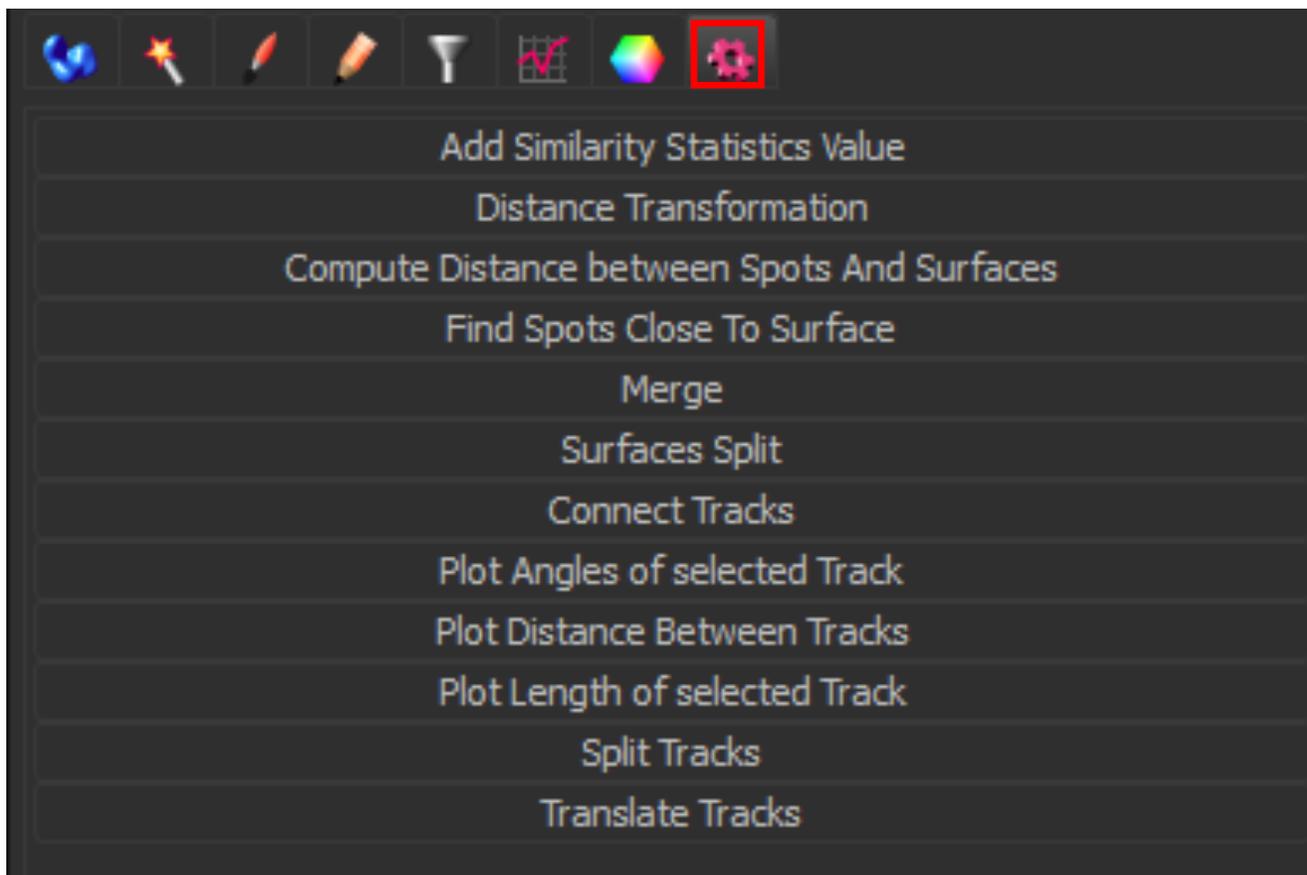
Show Colorbar

Show Range Show Title

Transparency

Transparency: 0 %

8. XT Functions



Add Similarity Statistics Value

Distance Transformation

Compute Distance between Spots And Surfaces

Find Spots Close To Surface

Merge

Surfaces Split

Connect Tracks

Plot Angles of selected Track

Plot Distance Between Tracks

Plot Length of selected Track

Split Tracks

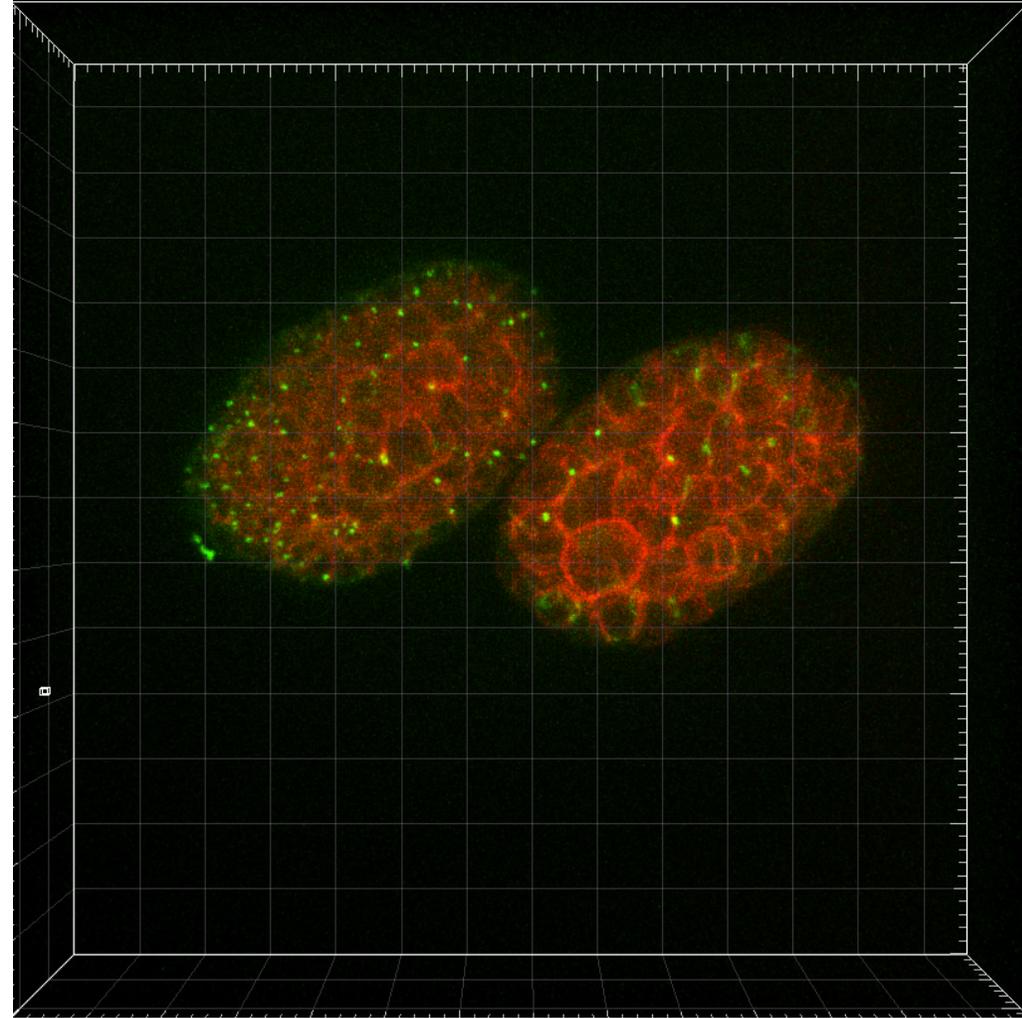
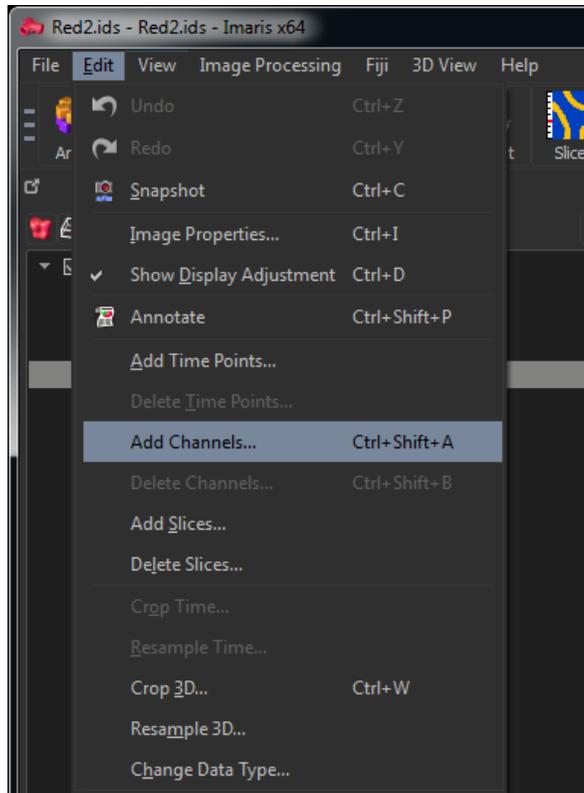
Translate Tracks



Adding New Objects to the Scene

Add new channel to existing dataset

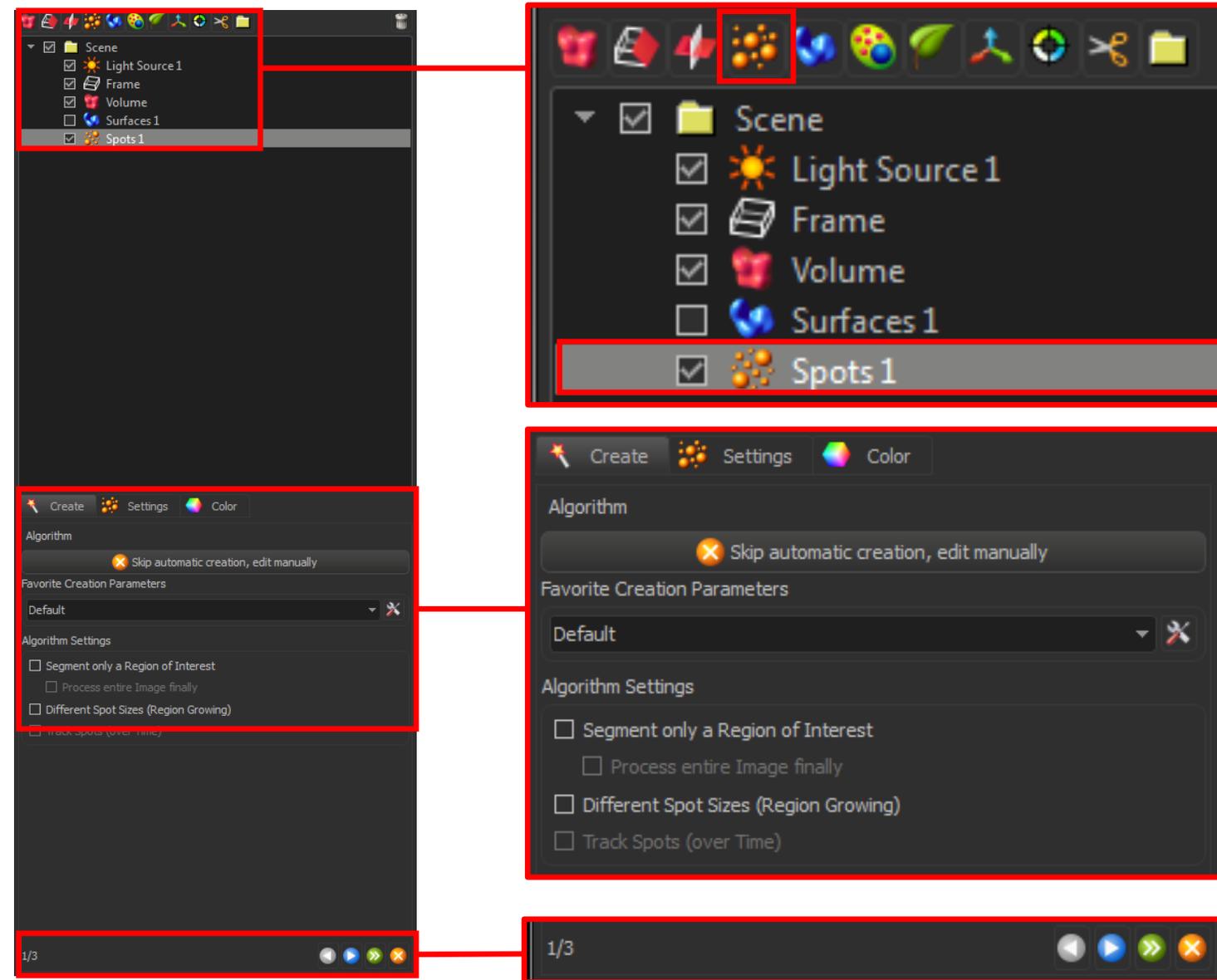
1. Edit>Add Channels... (Ctrl+Shift+A)
2. Select Green2.ids
3. Adjust Display settings



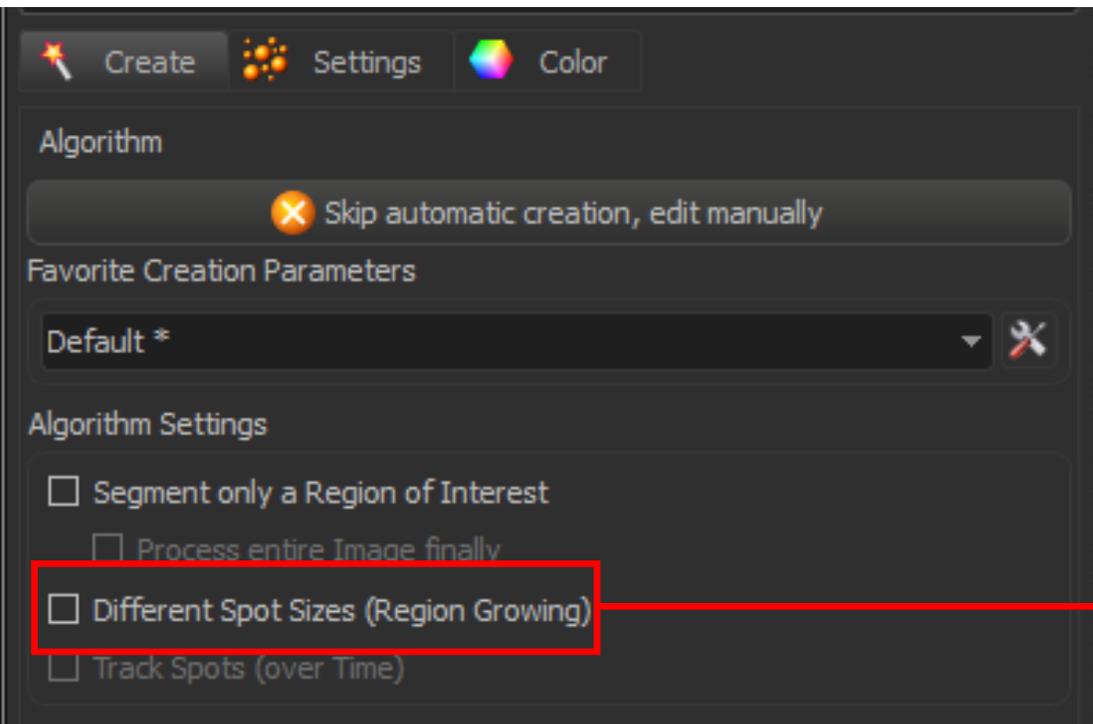
Adding New Objects to the Scene

Click on the orange blobs icon to add new Spots

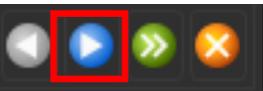
As with Surfaces, creation parameters can be loaded from a pre-defined configuration, edited manually, or defined using a walk-through wizard



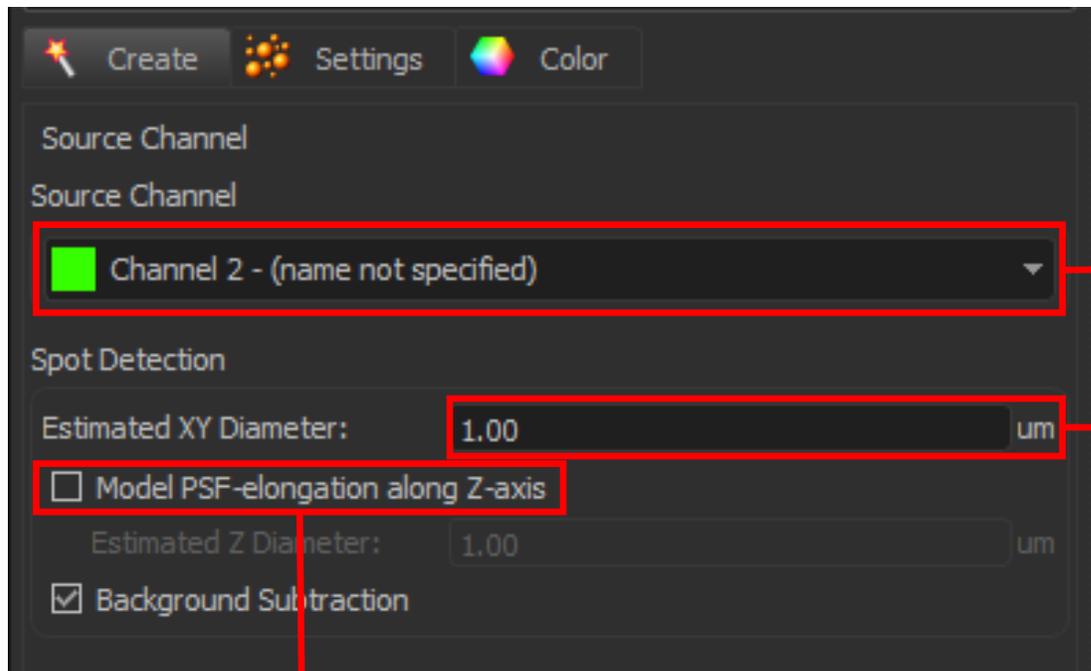
Adding New Objects to the Scene



Tick this option if the spots are of different sizes. This adds additional steps to the creation wizard.



Adding New Objects to the Scene



Select correct channel

Use Slice view to get an estimate of XY diameter

Selecting this option gives better spot identification along the z-axis

Adding New Objects to the Scene

The screenshot displays the 'Filters' panel in the Imaris software. At the top, there are 'Add' and 'Delete' buttons. A filter is applied: '"Quality" above 286'. Below this, the 'Filter Type' is set to 'Quality'. A control bar shows a green power icon with 'M' and a value of '286', and a red power icon with 'M' and 'A' and a value of '1269'. Below the control bar is a histogram showing a signal distribution. The x-axis of the histogram ranges from 8 to 1270, with a vertical line at 286. The text below the histogram reads '119 of 116629 selected (0%)'. At the bottom of the interface, there are navigation buttons: a blue left arrow, a grey right arrow, a green double right arrow, and an orange close button. The page number '3/3' is visible in the bottom left corner.

Use filters to limit number of displayed spots;
The 'Quality' is the intensity at the centre of the spot in the channel the Spots was detected.

Thresholding can be set automatically or manually

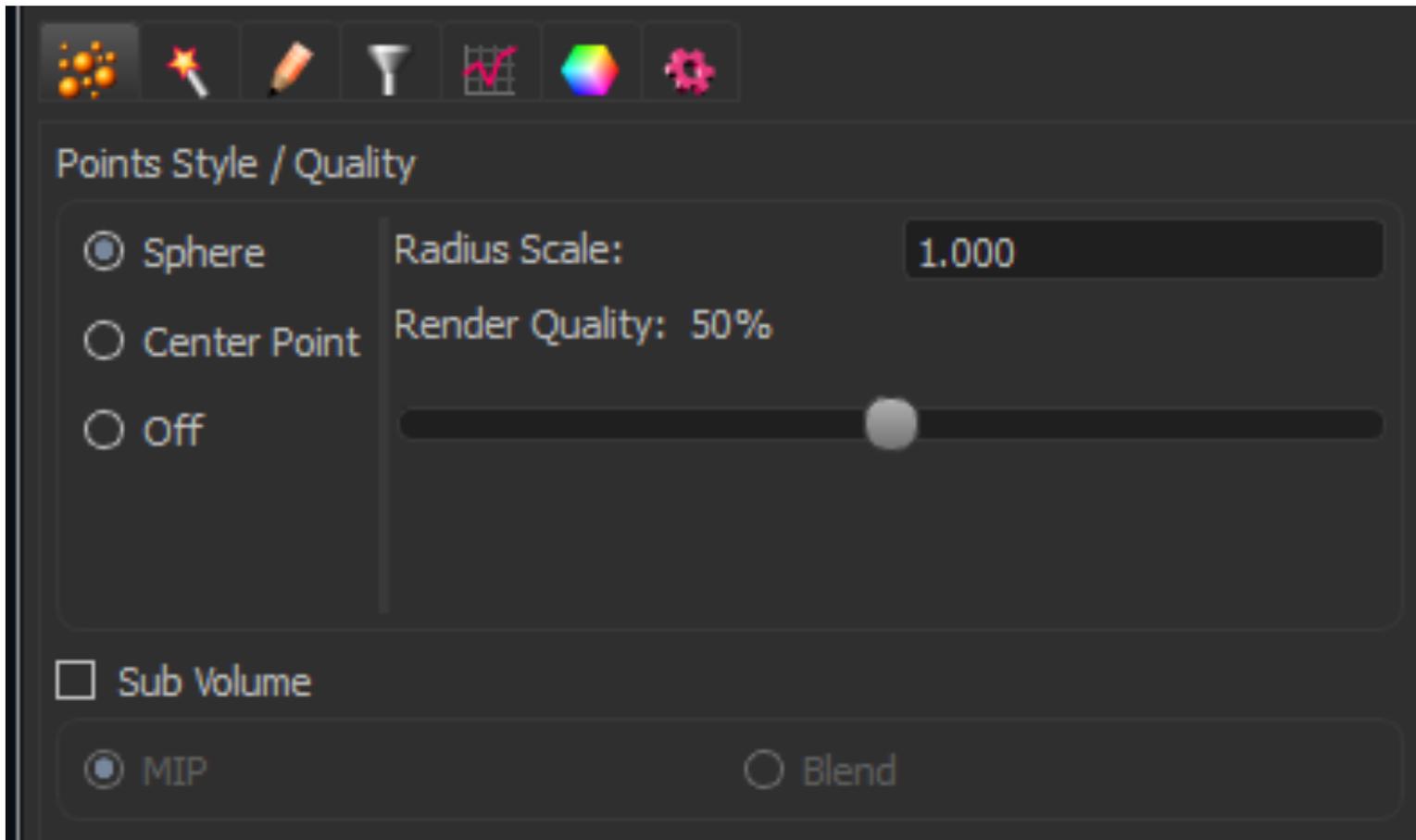
Thresholds can be defined using the histogram.

Turn Spots on and off in the Object List to see the underlying signal



Adding New Objects to the Scene

Spot Object Options



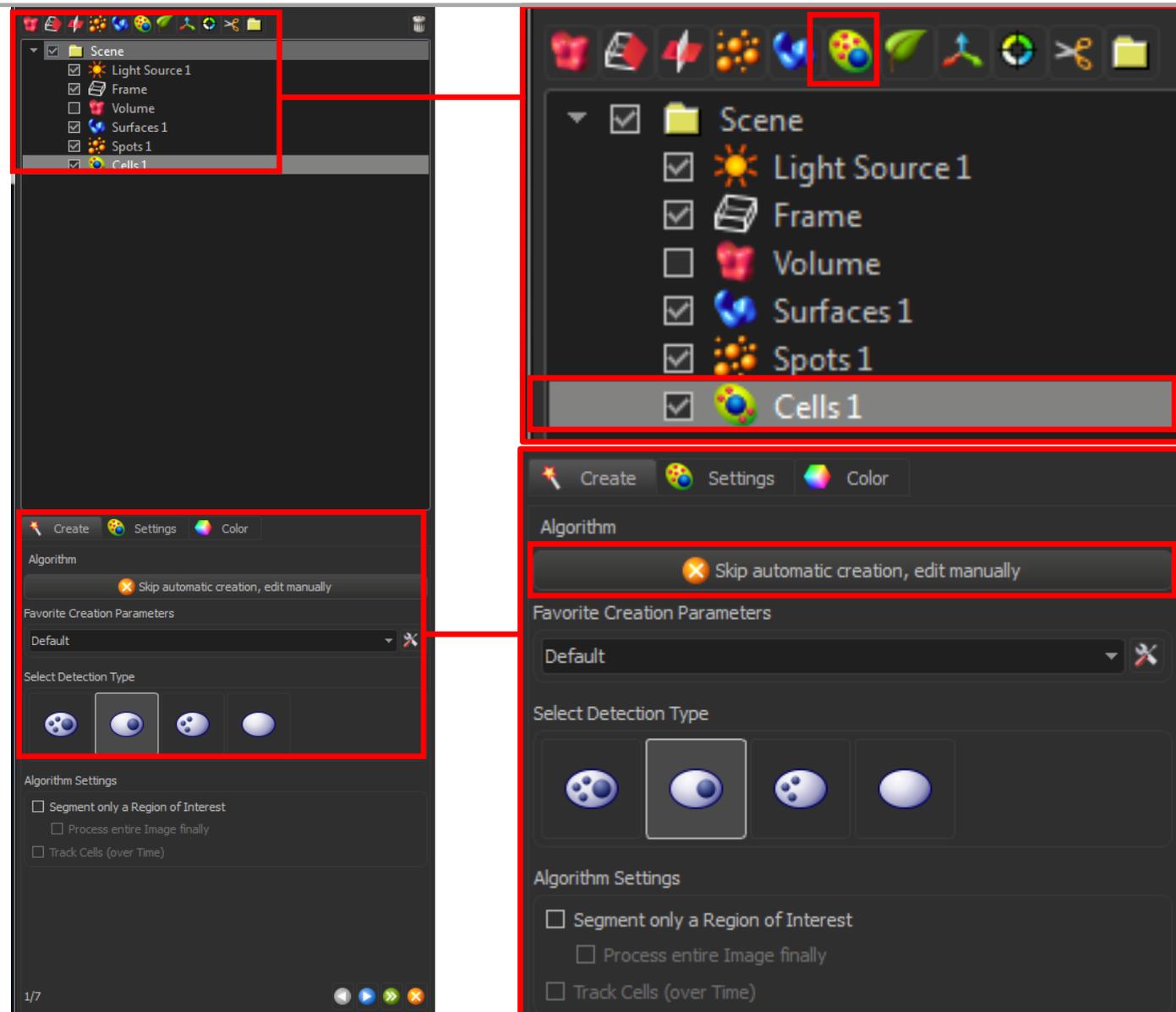
Options essentially the same as for Surfaces



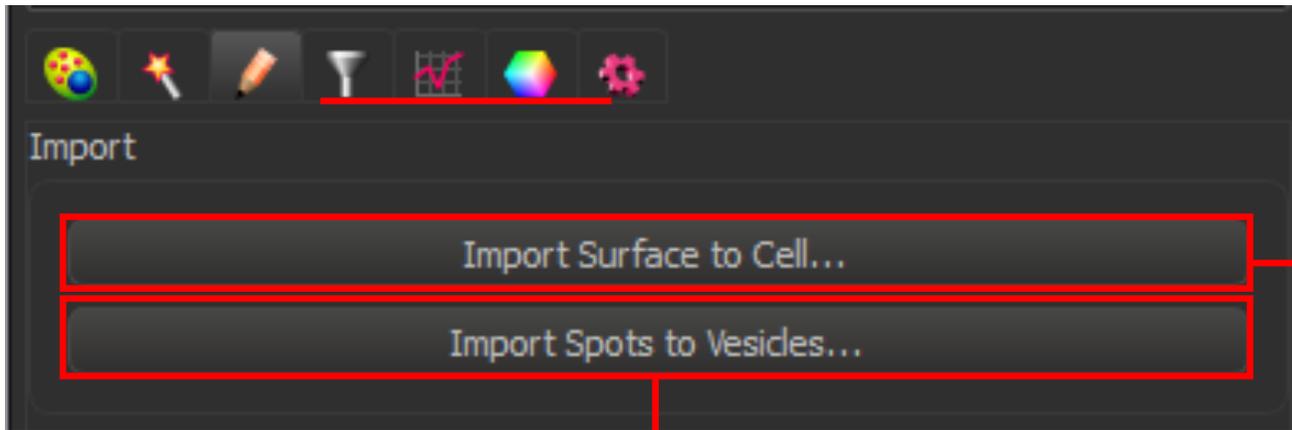
Adding New Objects to the Scene

Click on the orange blobs icon to add new Spots

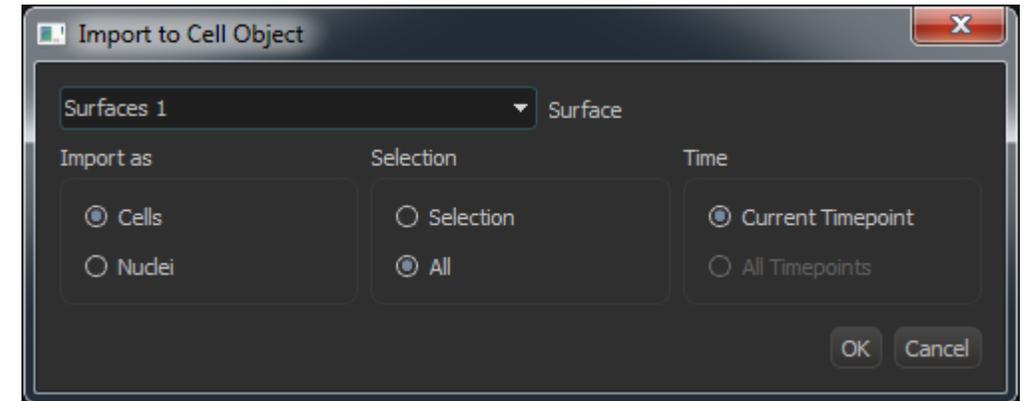
As with Surfaces & Spots, creation parameters can be loaded from a pre-defined configuration, edited manually, or defined using a walk-through wizard



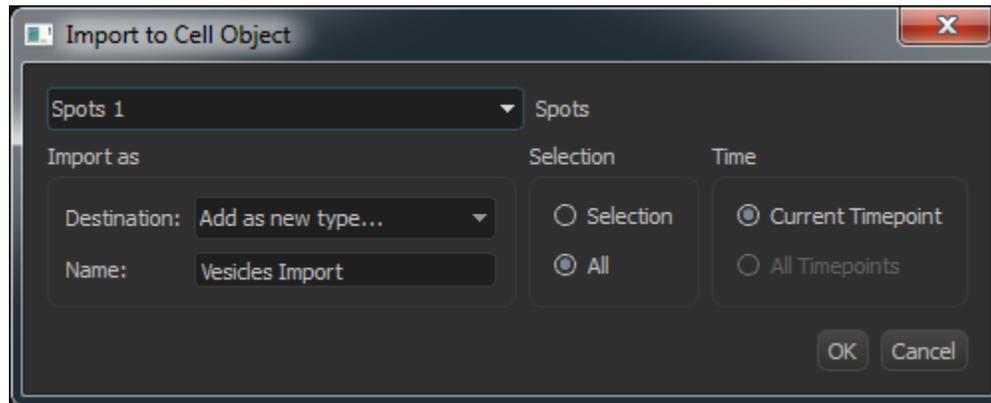
Adding New Objects to the Scene



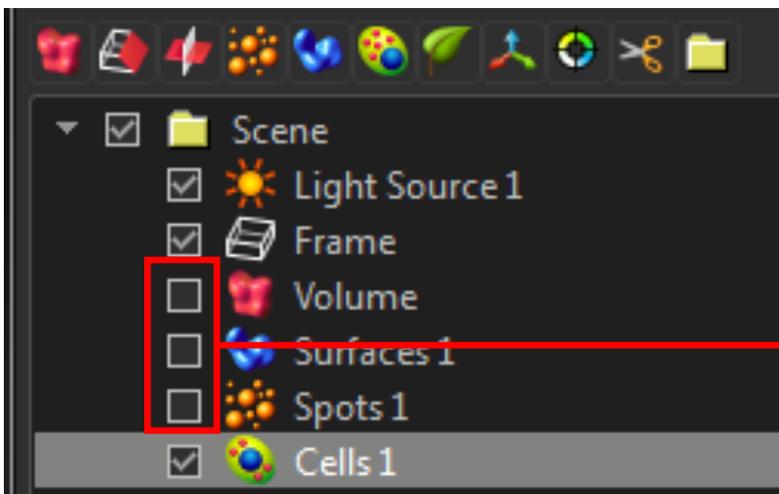
Assign Surfaces objects to Cells



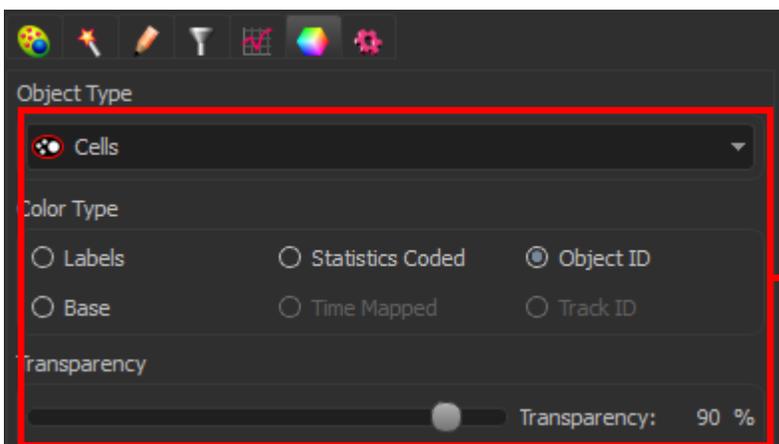
Assign Spots objects to Cells



Adding New Objects to the Scene



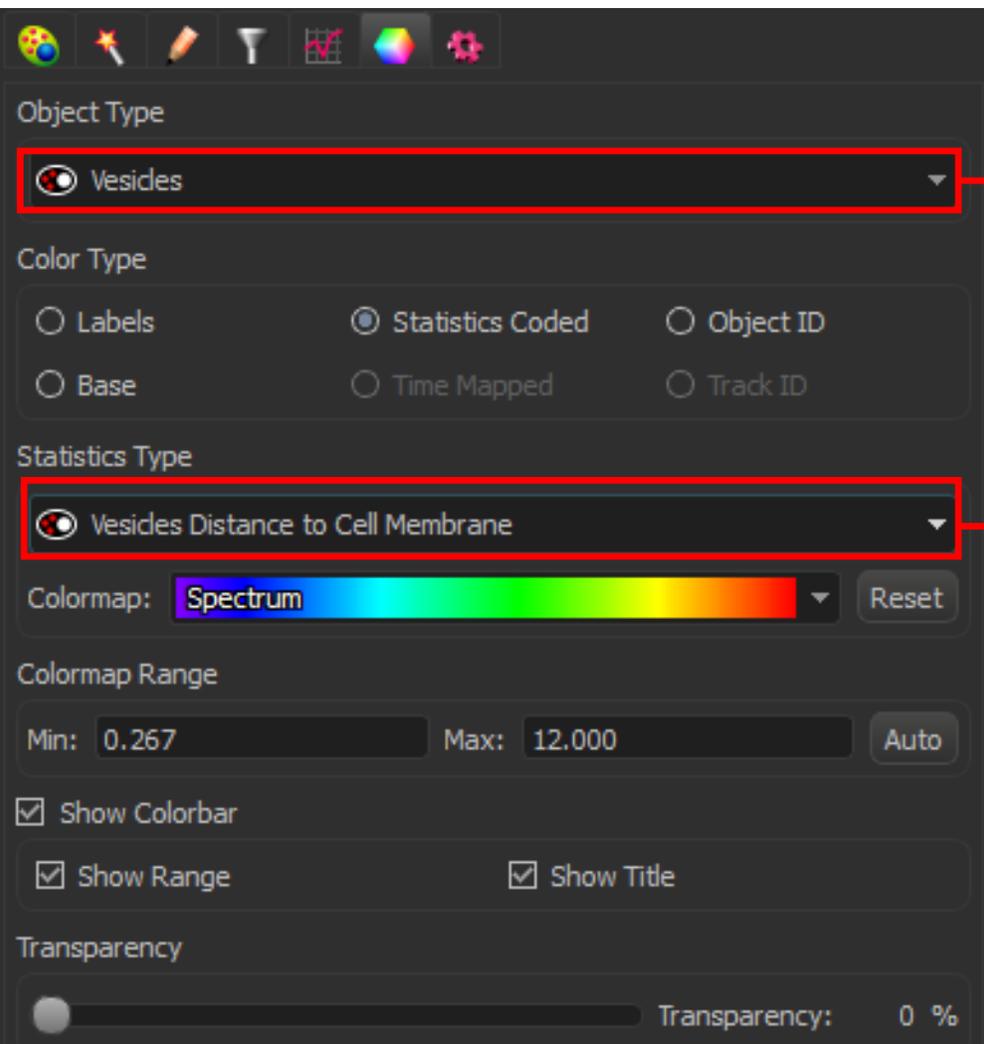
Turn off Volume, Surfaces & Spots



Adjust Transparency on Cells



Adding New Objects to the Scene

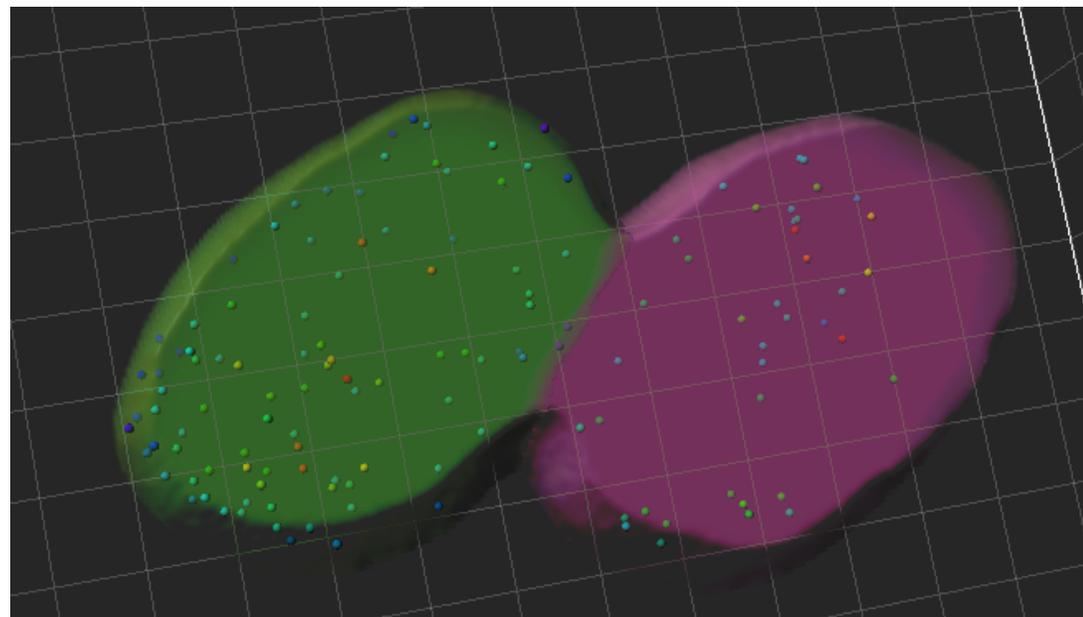


The screenshot shows the Imaparis software interface with the following settings:

- Object Type:** Vesicles (highlighted with a red box)
- Color Type:** Statistics Coded (selected)
- Statistics Type:** Vesicles Distance to Cell Membrane (highlighted with a red box)
- Colormap:** Spectrum (with a color bar ranging from blue to red)
- Colormap Range:** Min: 0.267, Max: 12.000 (with an Auto button)
- Show Colorbar:** Checked
- Show Range:** Checked
- Show Title:** Checked
- Transparency:** 0 %

Select vesicles

Select Stats



Making Movies (Animation)

Group Exercise 4

Re-create Cells, using Cells Wizard

