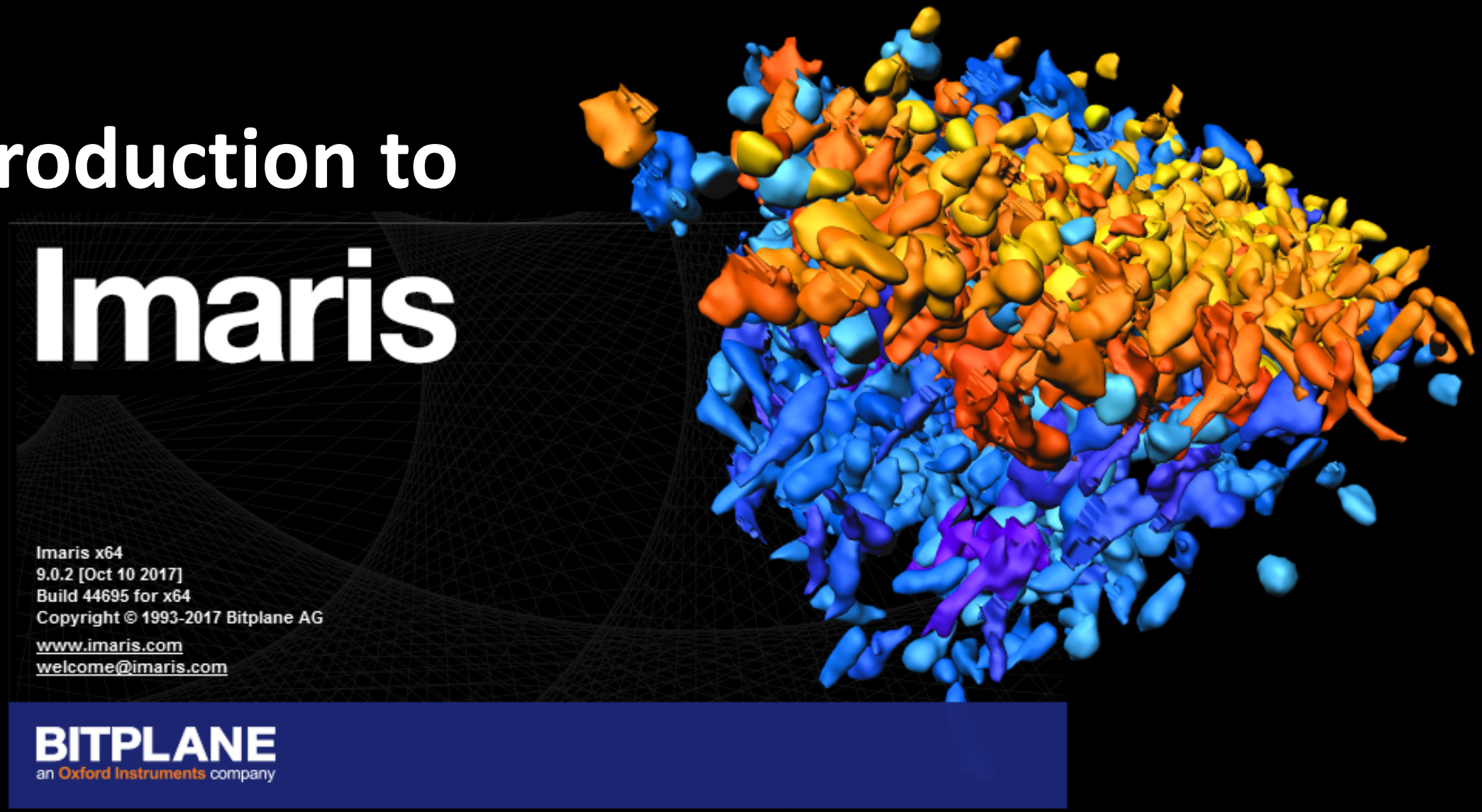
Introduction to



7th November 2017

Course Schedule

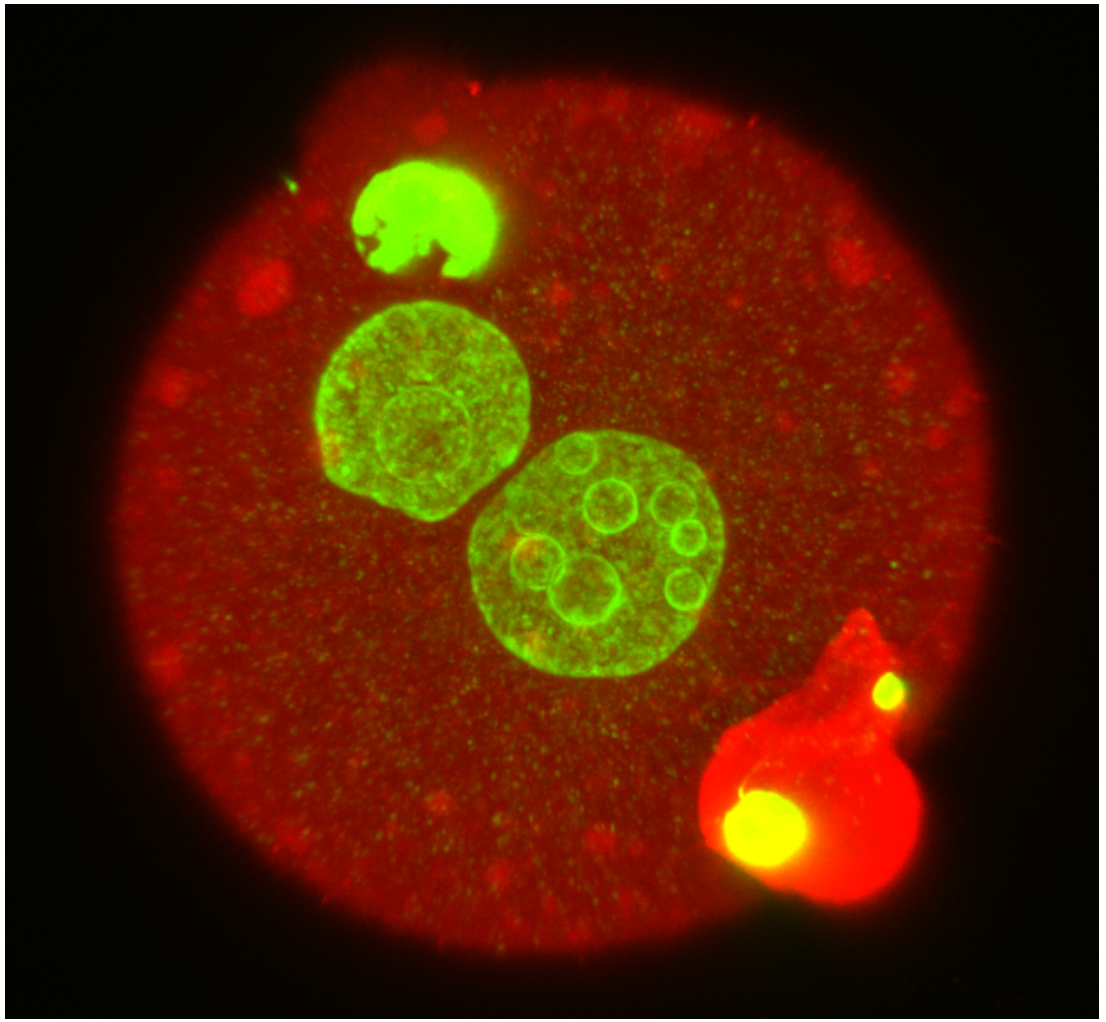
09:30-10:30	Session 1 – Understanding the Basics
10:45-12:30	Session 2 – Initial Scene Objects, Taking Snapshots and Making Movies
13:30-15:00	**Session 3 – Adding New Objects to the Scene**
15:30-17:00	Session 4 – Creating Cells

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

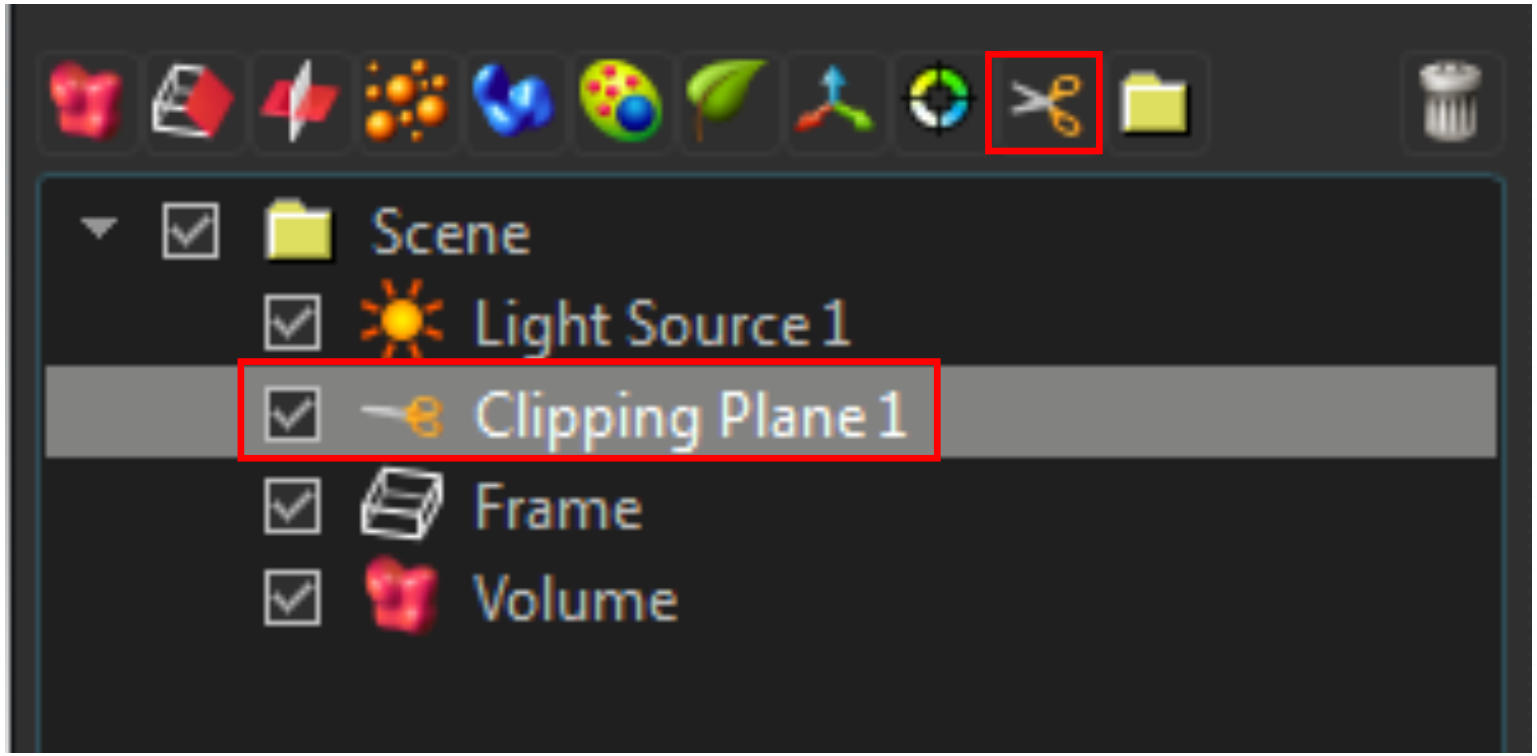
1. Add file Confocal Stack2 to Arena
2. View in Surpass
3. Remove Frame



Adding New Objects to the Scene

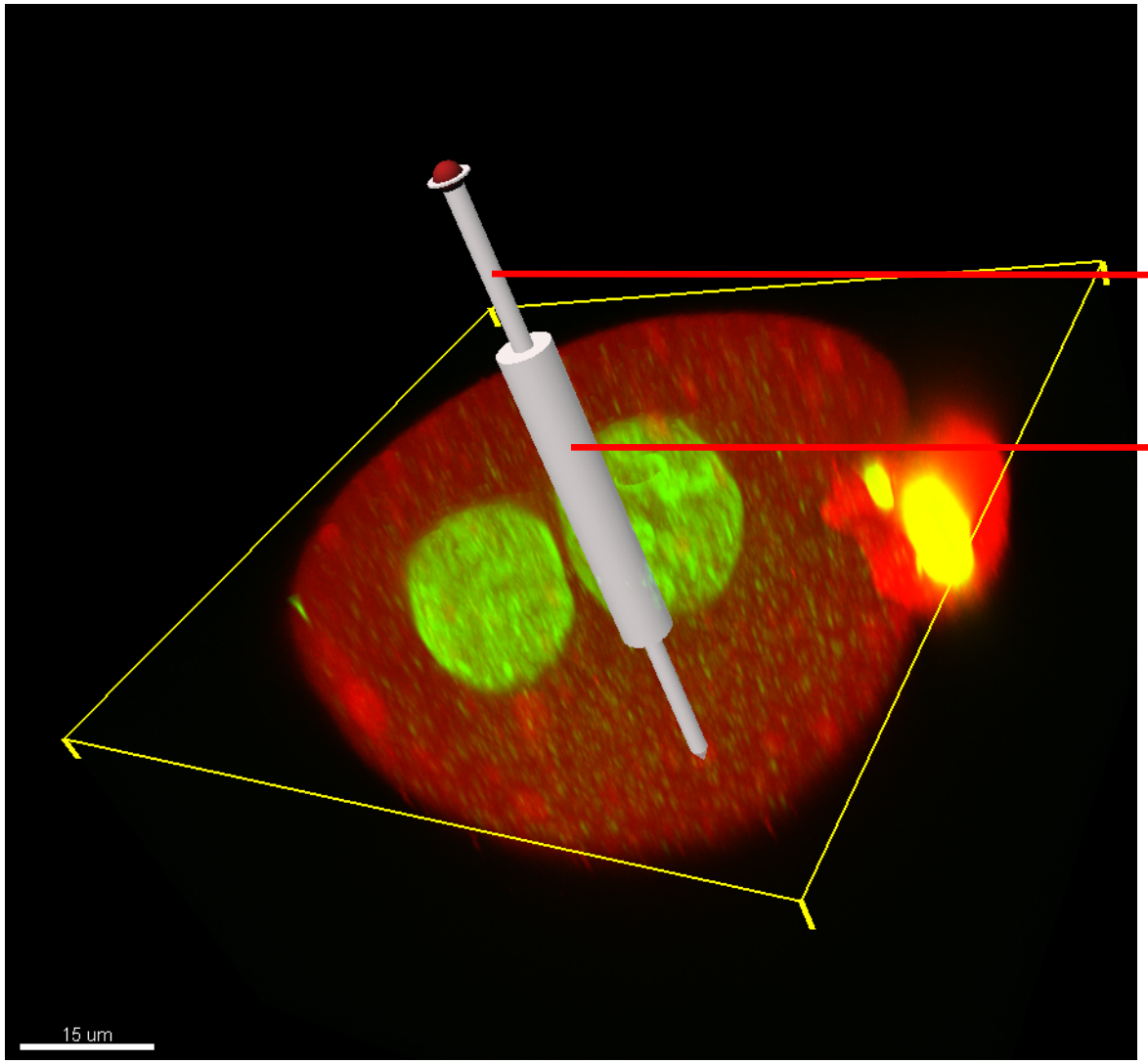
Clipping plane: Removes a section of your data to allow you to ‘see inside’

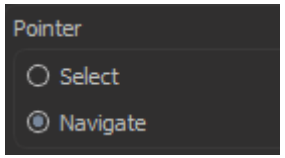
Click on scissor icon to add new Clipping Plane



Adding New Objects to the Scene

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

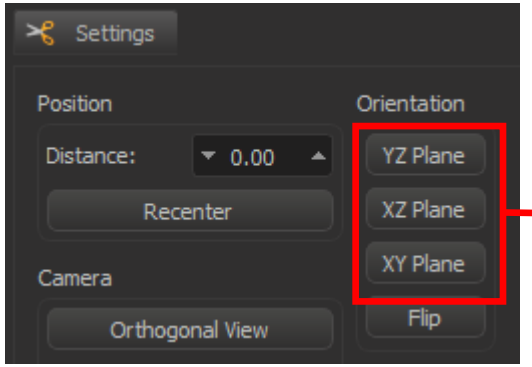




Use Navigate to rotate image data
Use Select to interact with clipping tool

Upper rod rotates clipping plane

Lower rod moves position of clipping plane

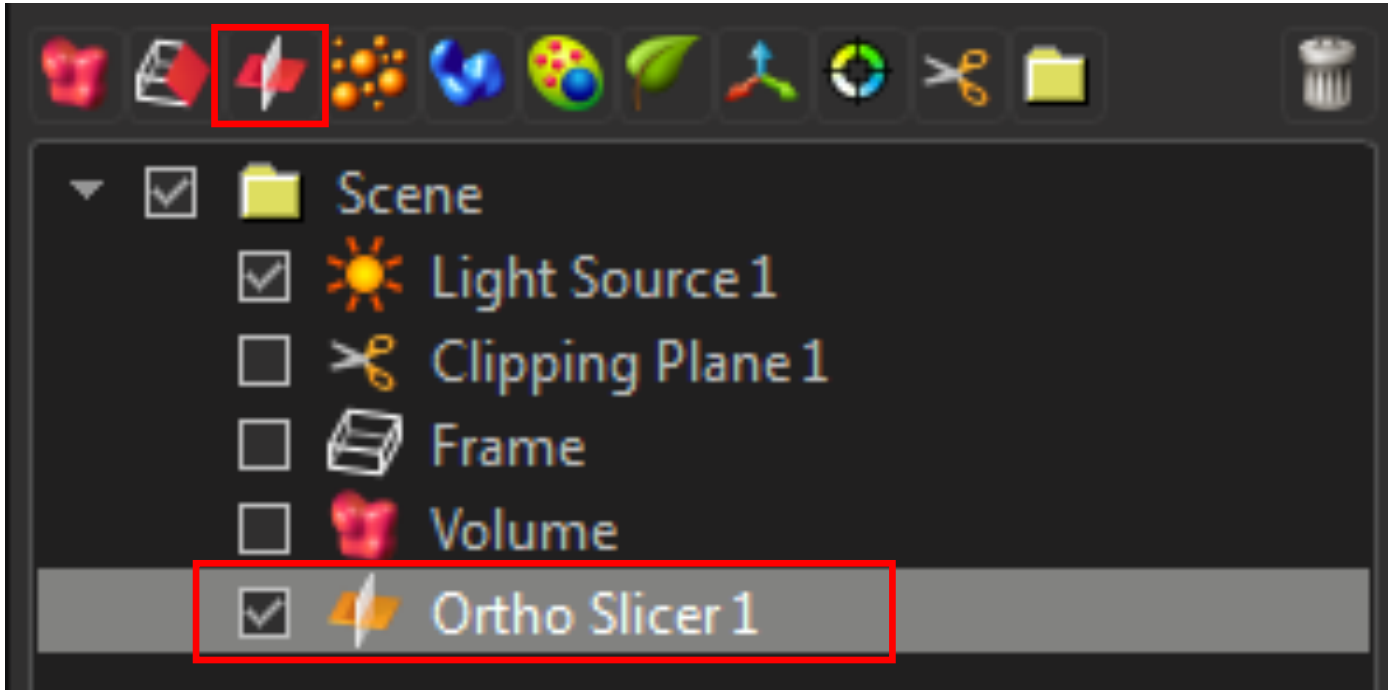


Use Orientation tools to move clipping plane onto different axis

Adding New Objects to the Scene

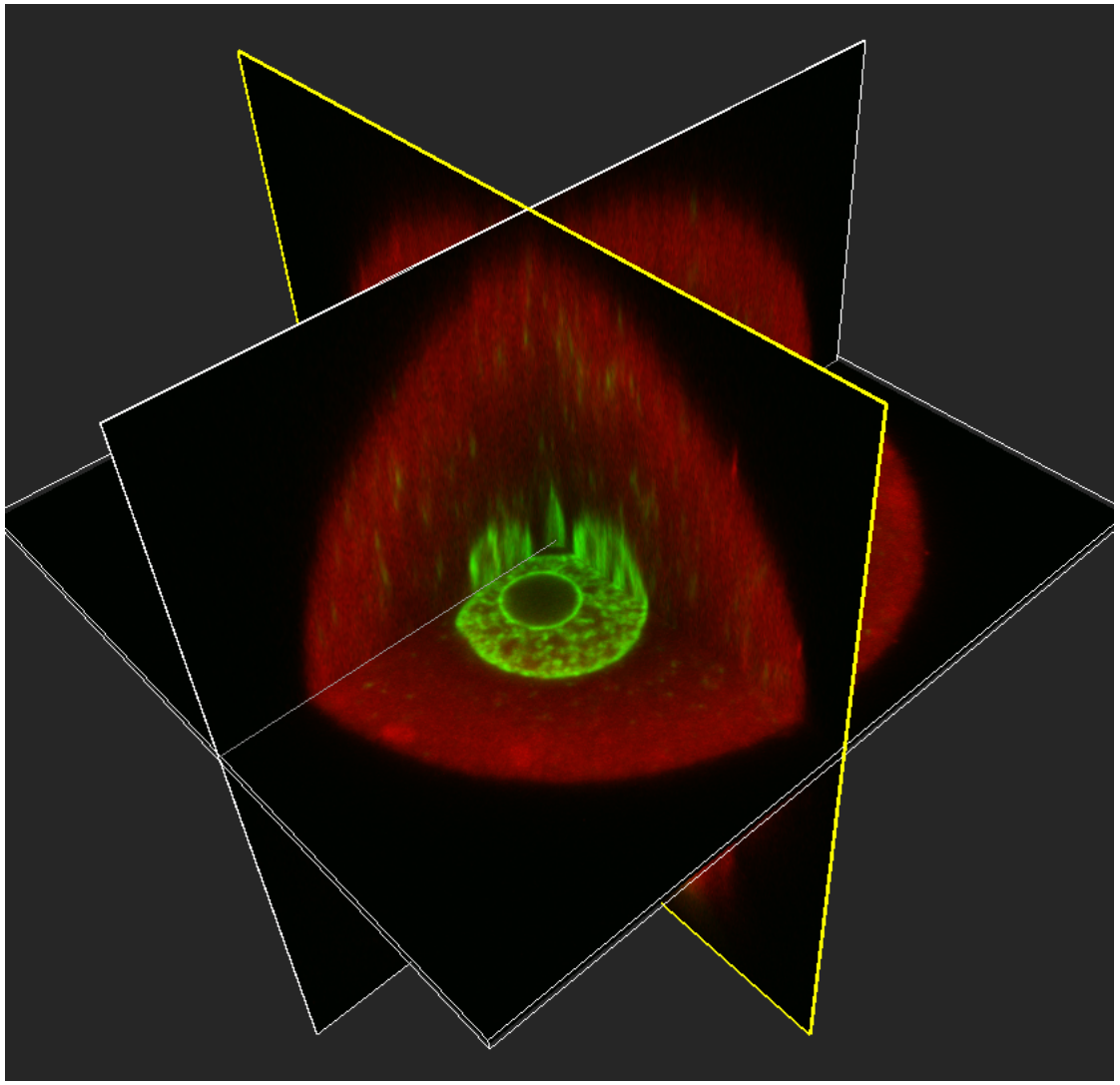
Ortho Slicer: 3D Orthoganol view in x, y, z

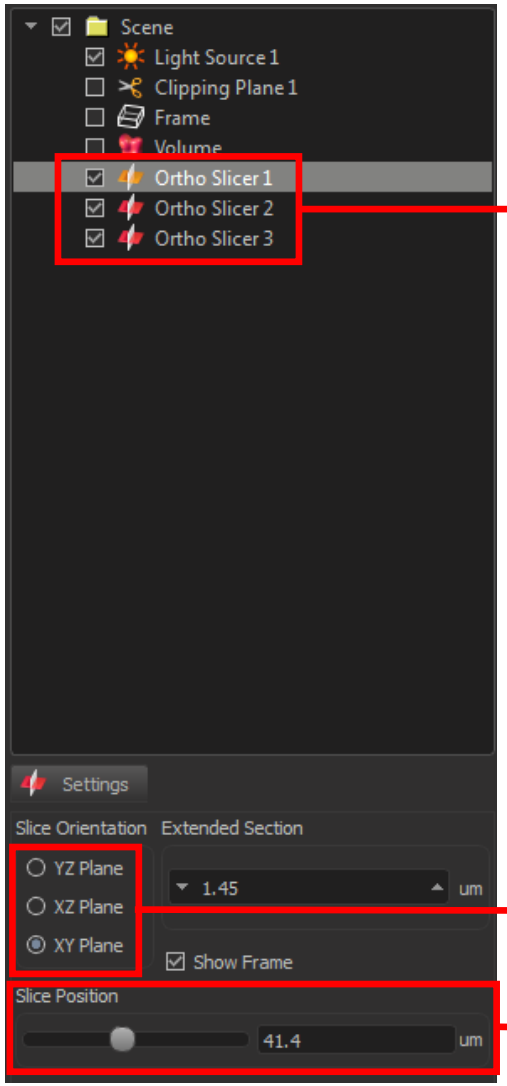
Click on cross-slice icon to add new Ortho Slicer



Adding New Objects to the Scene

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





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

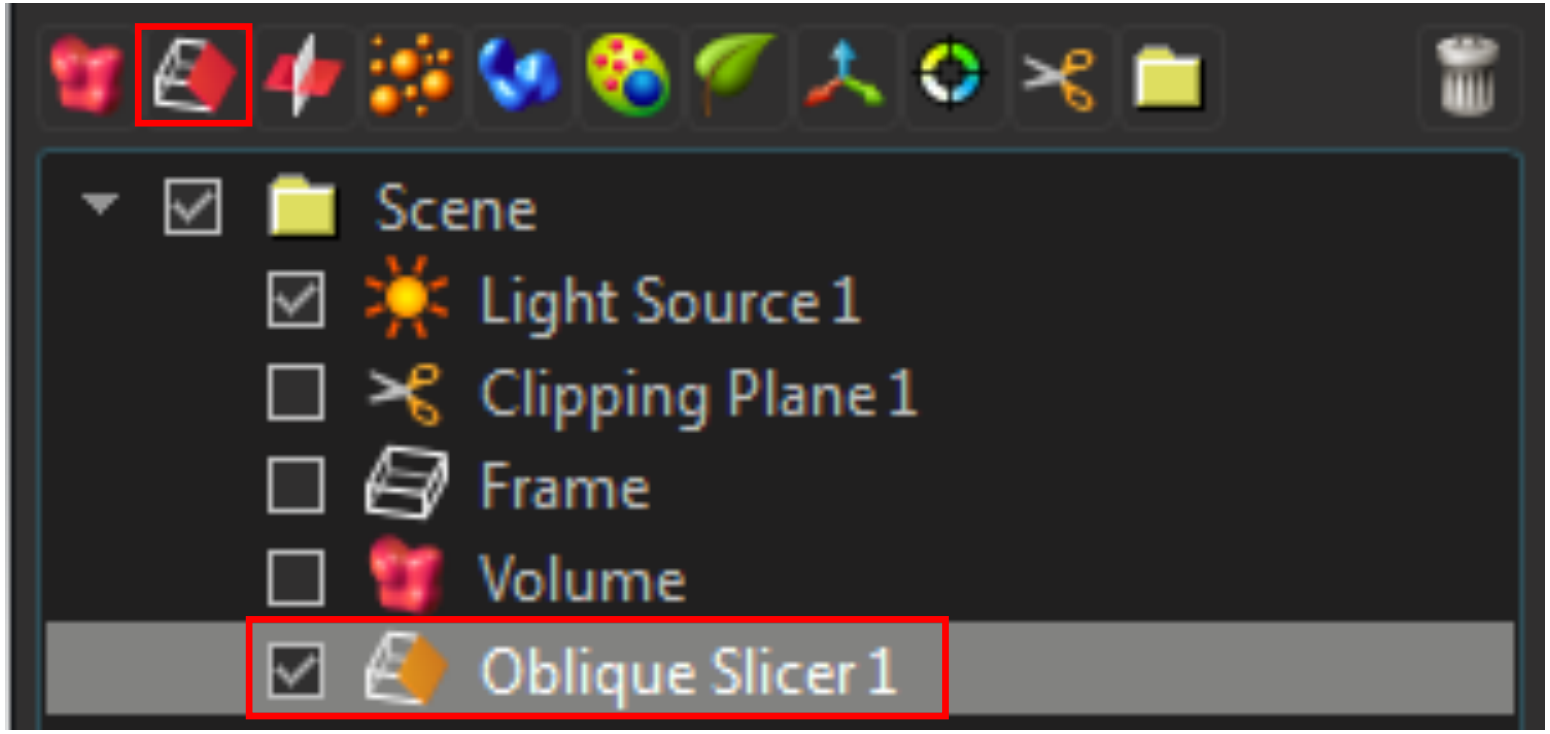
Select plane of slicer

Move slice position to slide through planes

Adding New Objects to the Scene

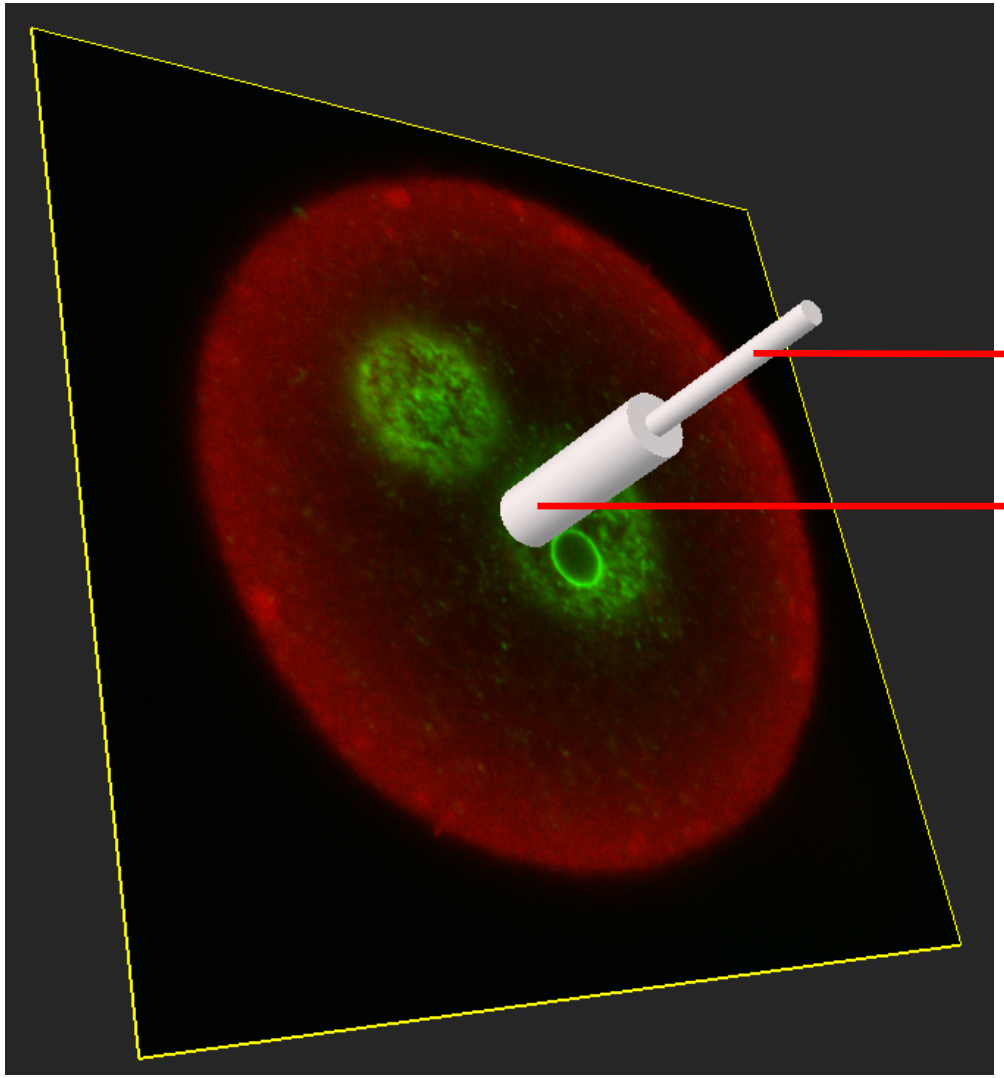
Oblique Slicer: Slice view through data in any orientation

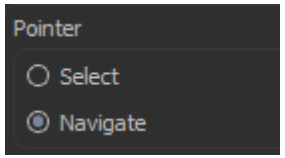
Click on sliced object icon to add new Oblique Slicer



Adding New Objects to the Scene

Oblique Slicer: Slice view through data in any orientation

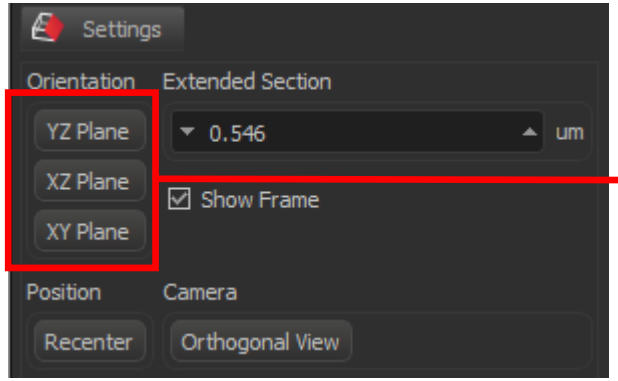




Use Navigate to rotate image data
Use Select to interact with slicing tool

Upper rod rotates slice plane

Lower rod moves position of slice plane



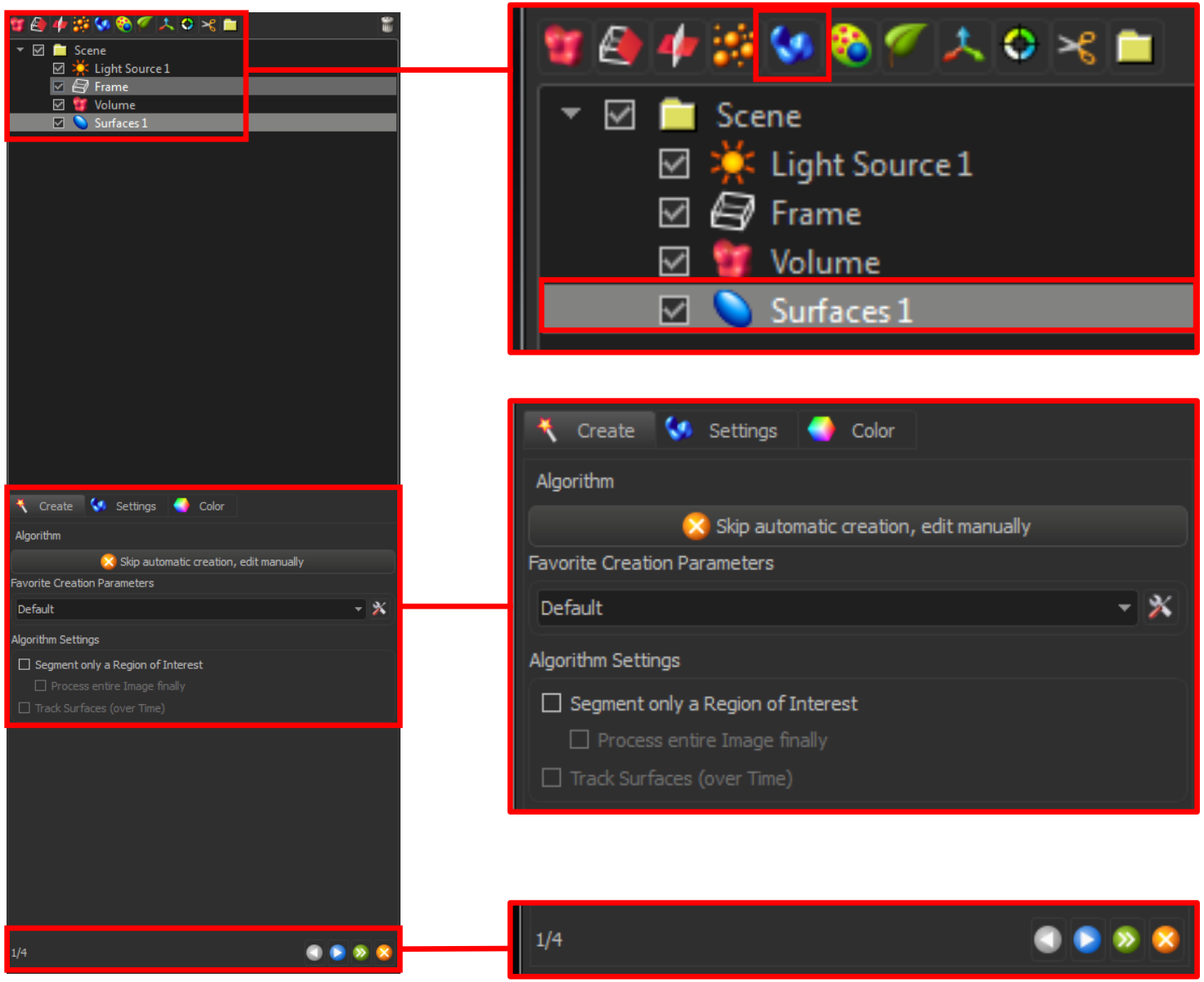
Use Orientation tools to move slicing plane onto different axis

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

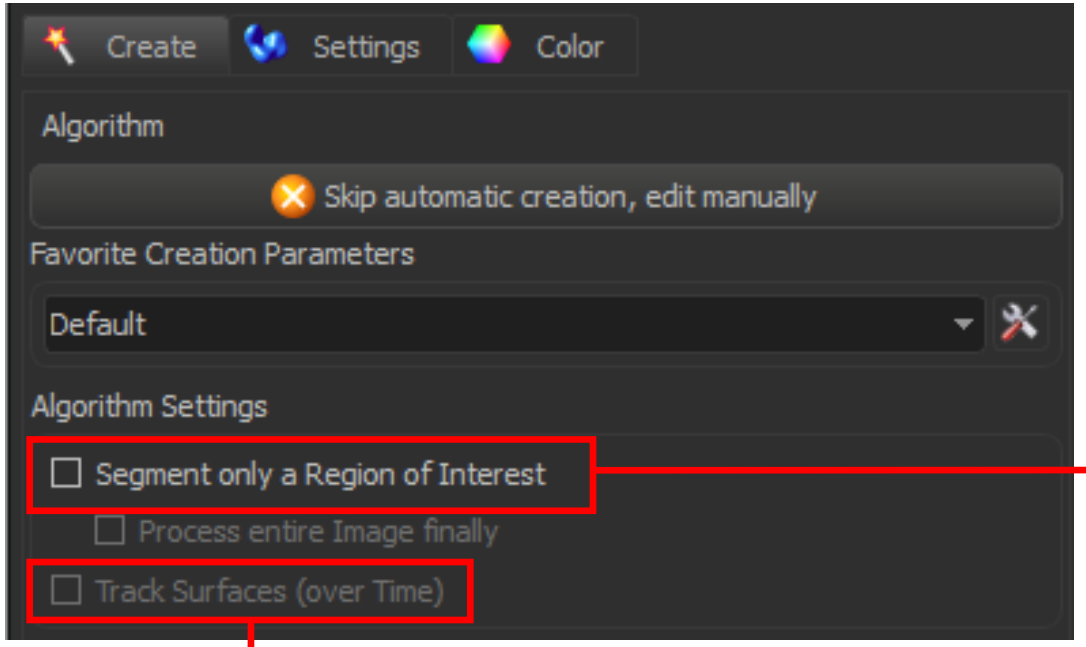
Adding New Objects to the Scene



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

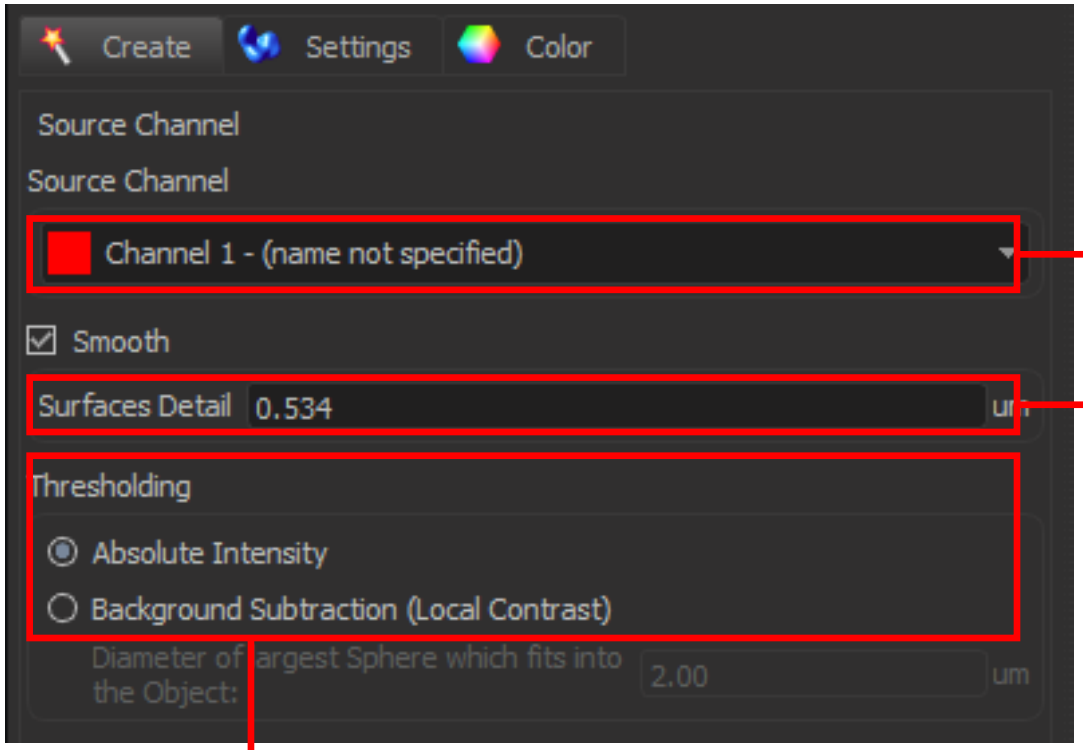
Adding New Objects to the Scene



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

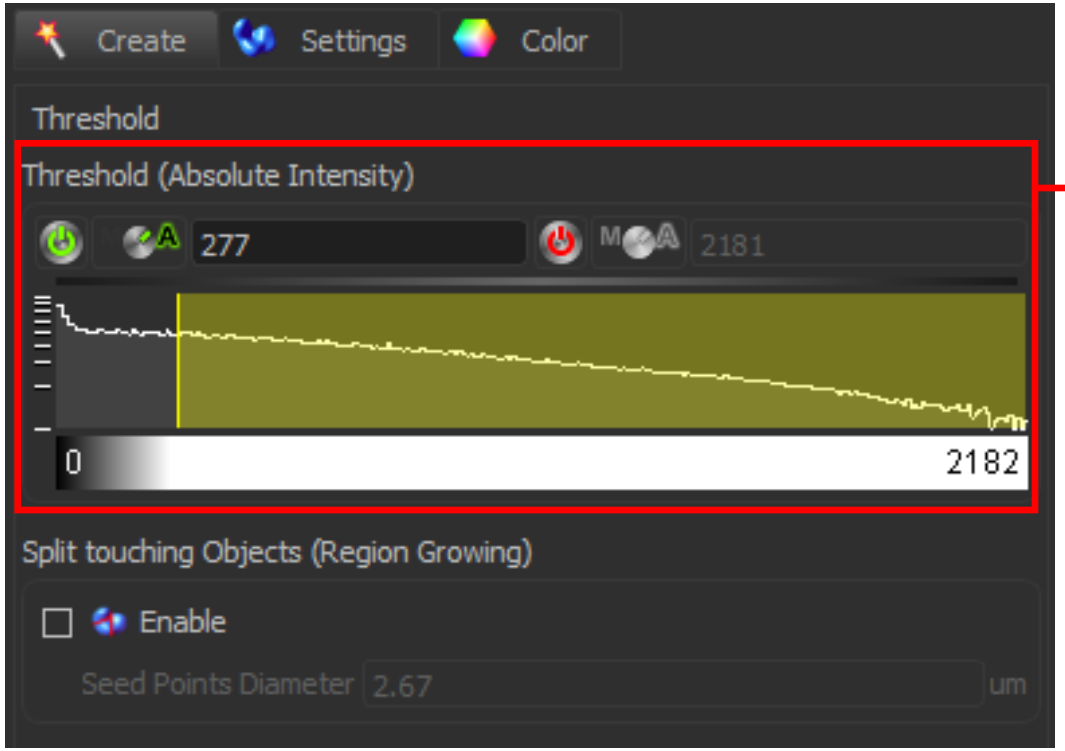


Specify channel to be used to create surfaces

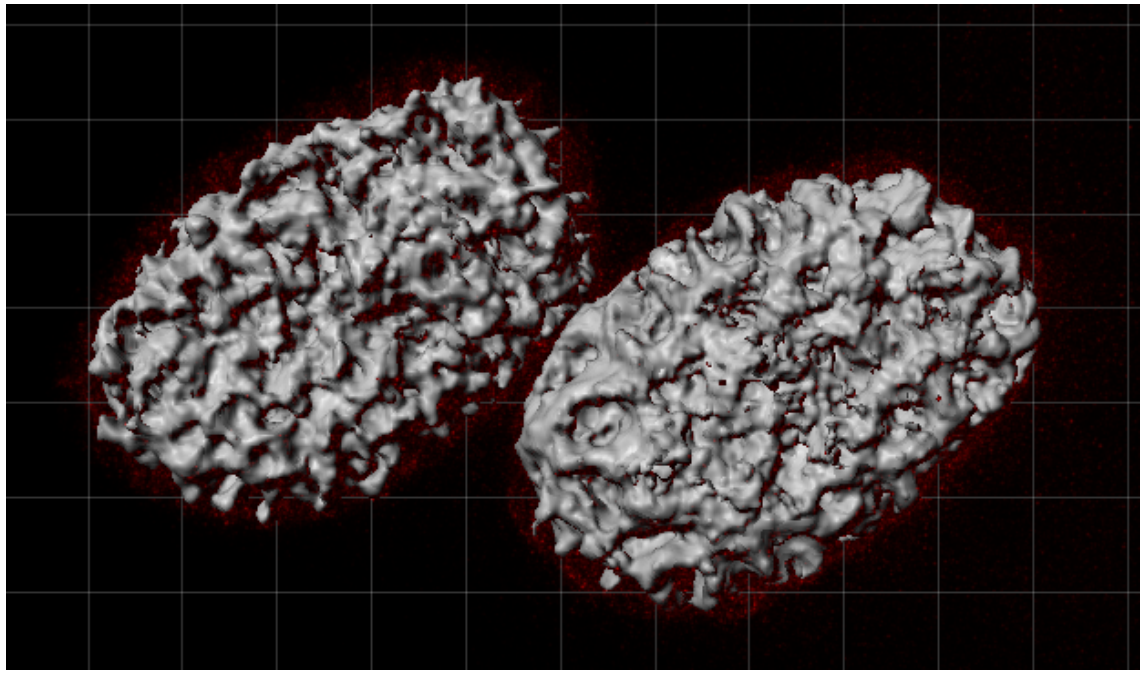
Determine surface level detail; smaller values will give more detail.

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

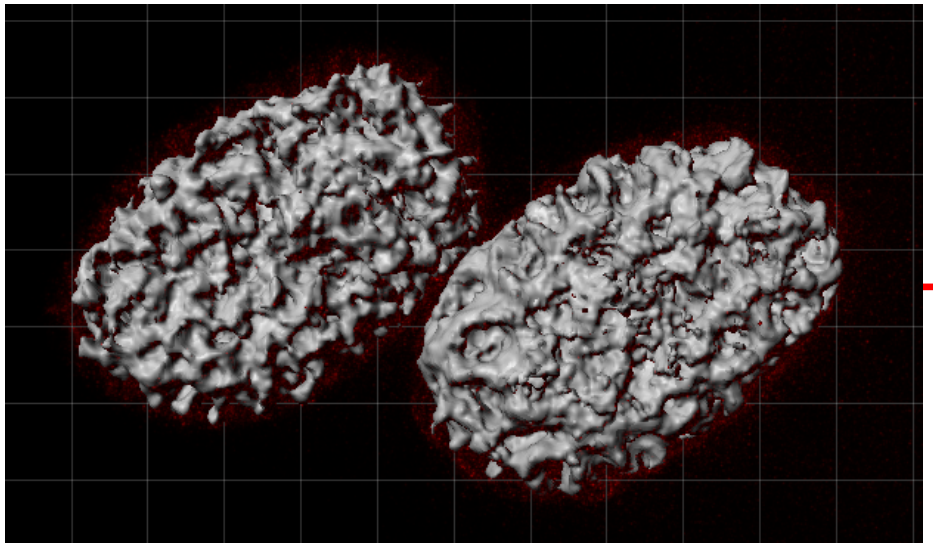
Adding New Objects to the Scene



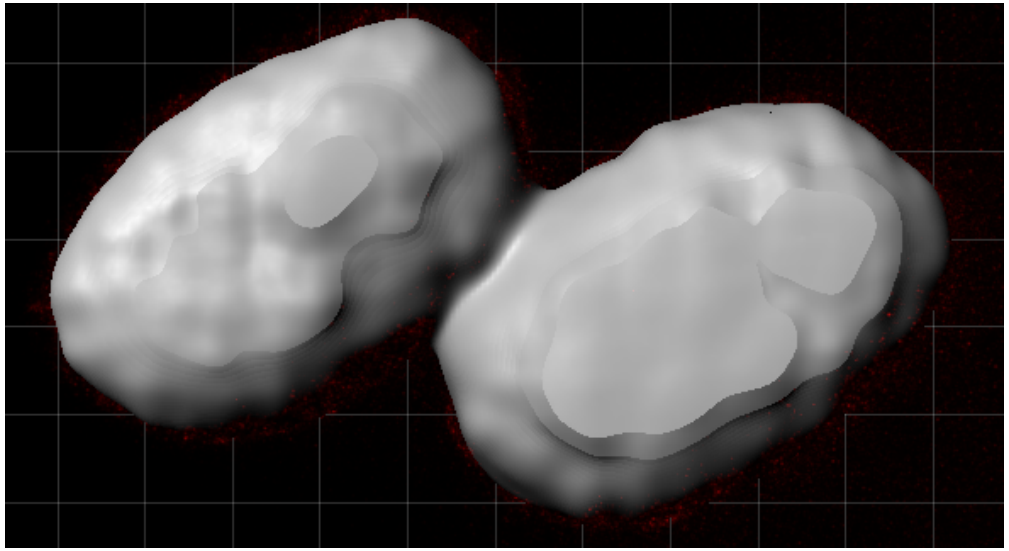
Thresholding (setting the values to determine what is object and what is background) can be done manually or automatically



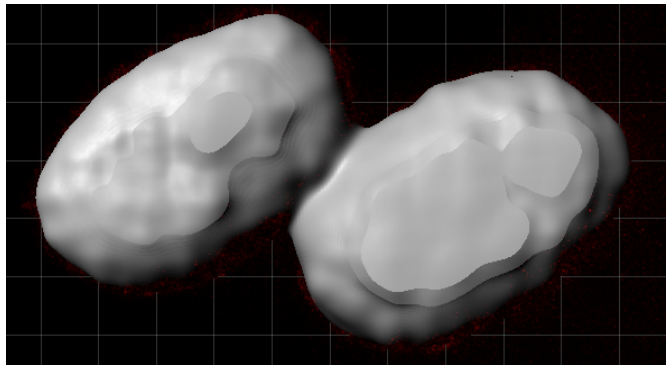
Adding New Objects to the Scene



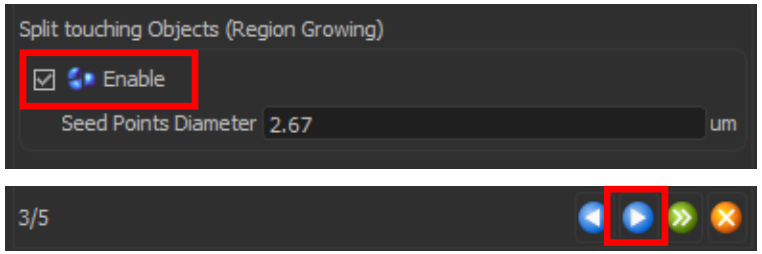
Object surface is too detailed. Return to previous step, increase Surface Detail to e.g. 2 µm

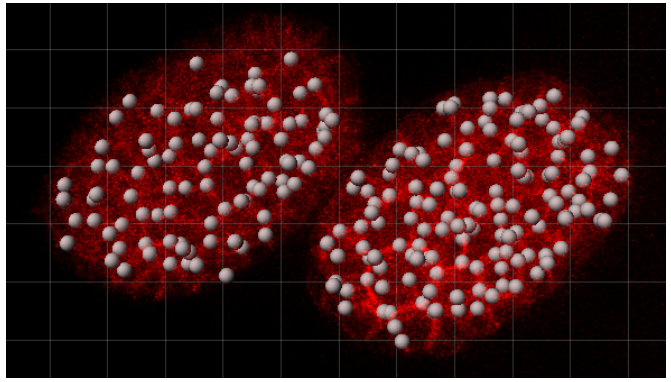


Adding New Objects to the Scene

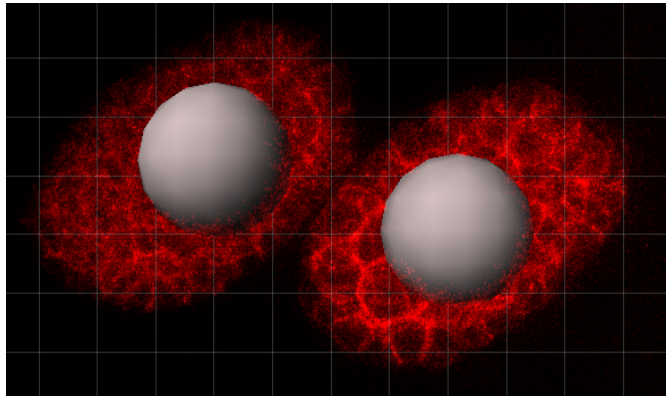


Objects are joined. Tick Split touching Objects (Region Growing) option to separate into two.



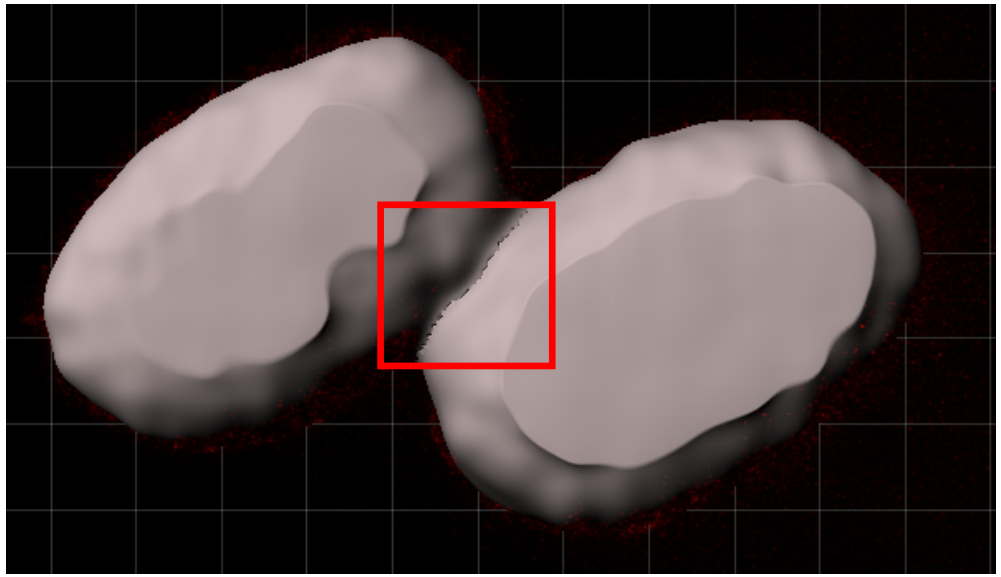


Seed points are too small. Return to previous step and increase Seed Points Diameter to e.g. 25 µm



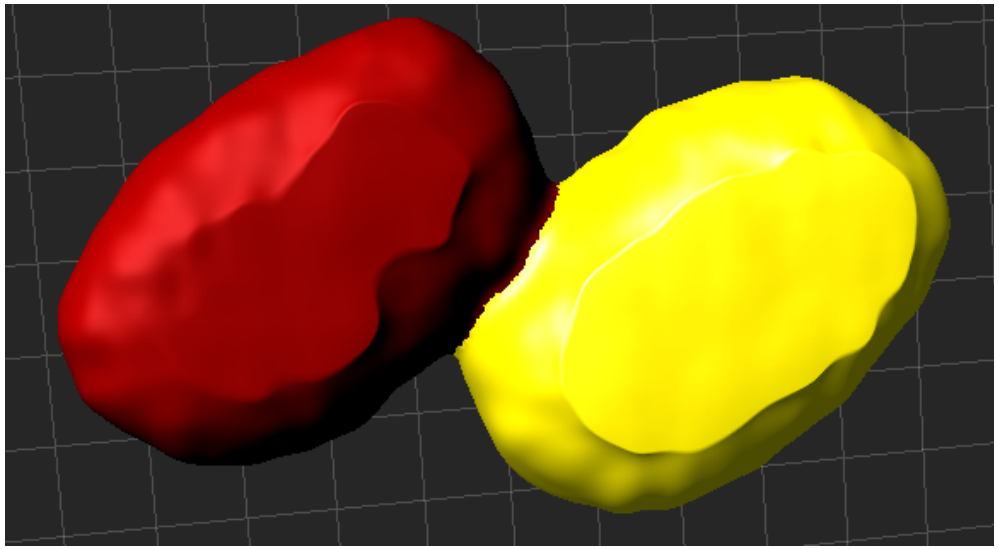
Two seed points – should split object as required

Adding New Objects to the Scene

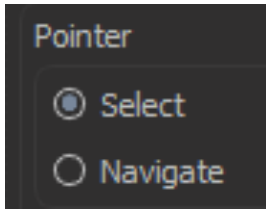


Threshold now correct and objects are separated.

Click green double-chevron button to complete surface build.



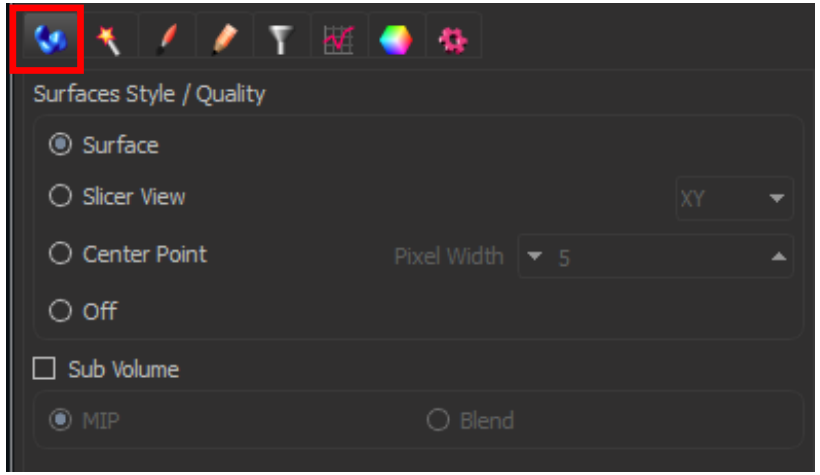
Switch pointer to Select mode and click on objects to check segmentation. Turn off Volume in the Scene to see rendered Surface alone.



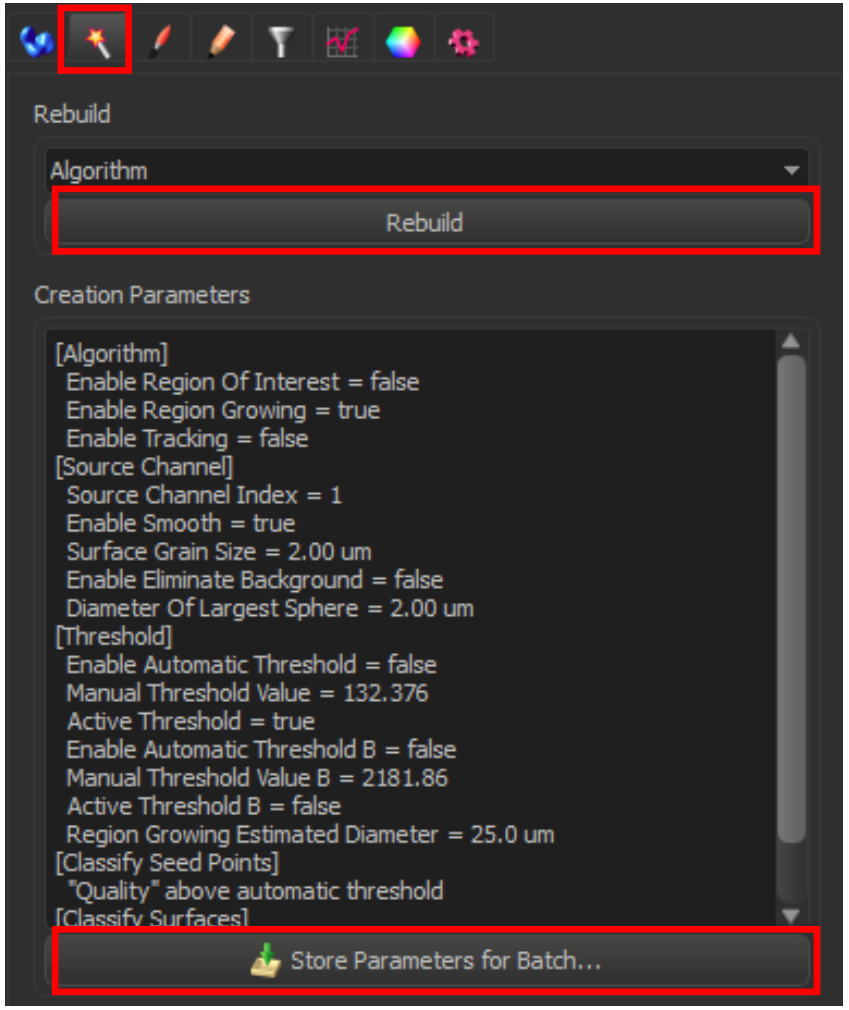
Adding New Objects to the Scene

Surface Object Options

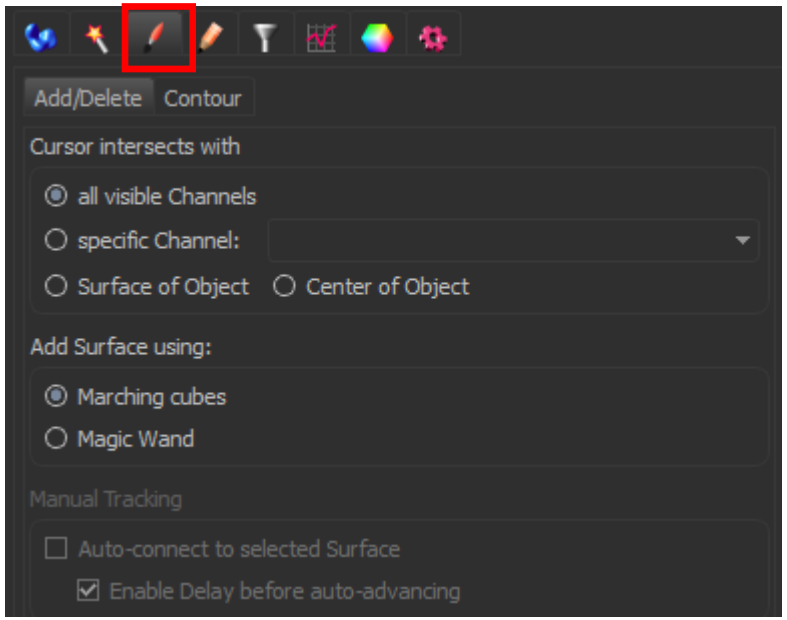
1. View



2. Rebuild



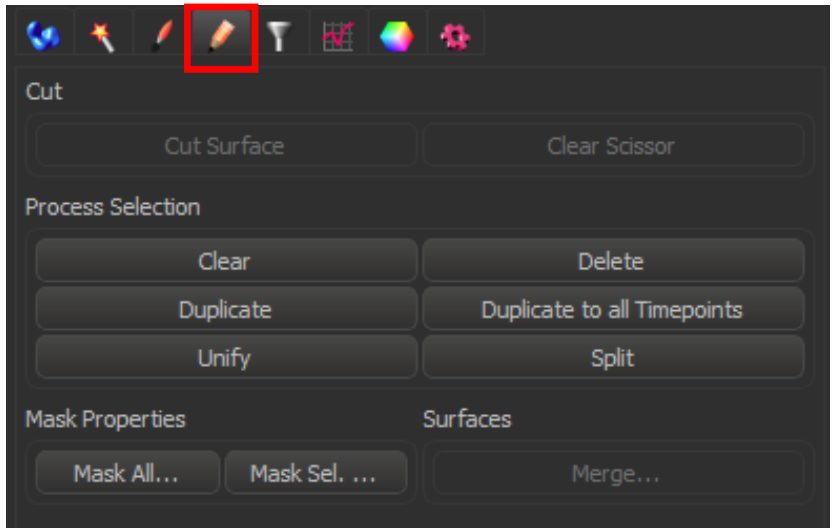
3. Edit



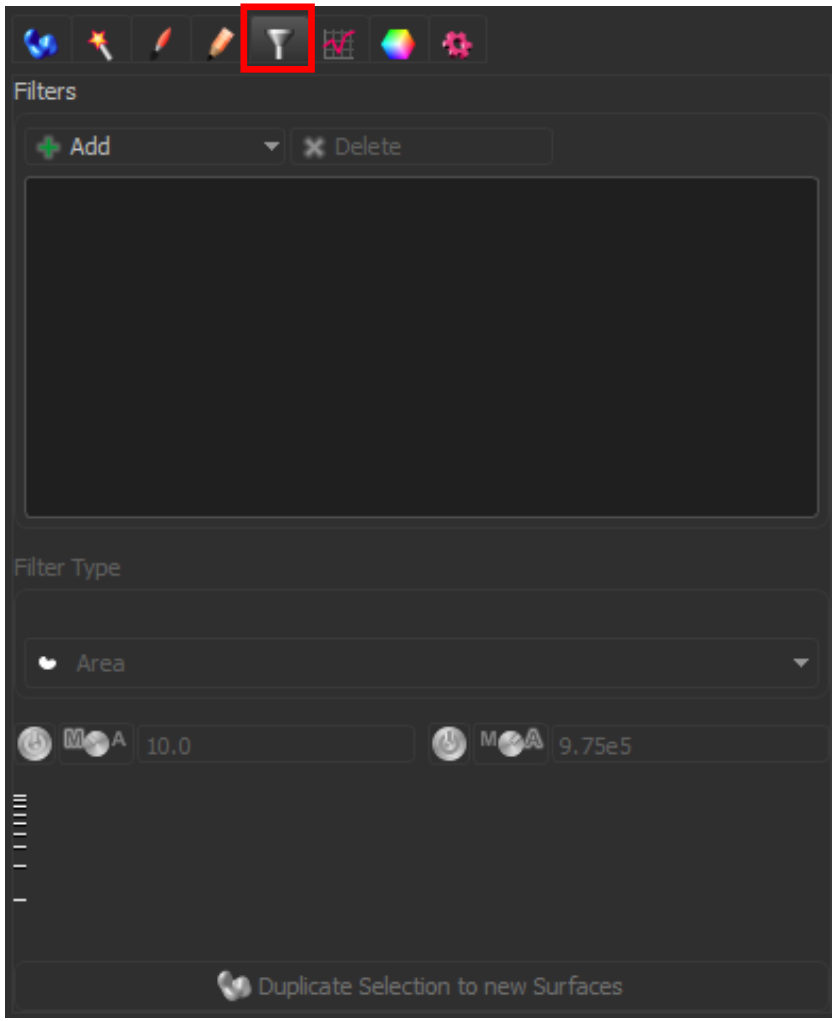
Adding New Objects to the Scene

Surface Object Options

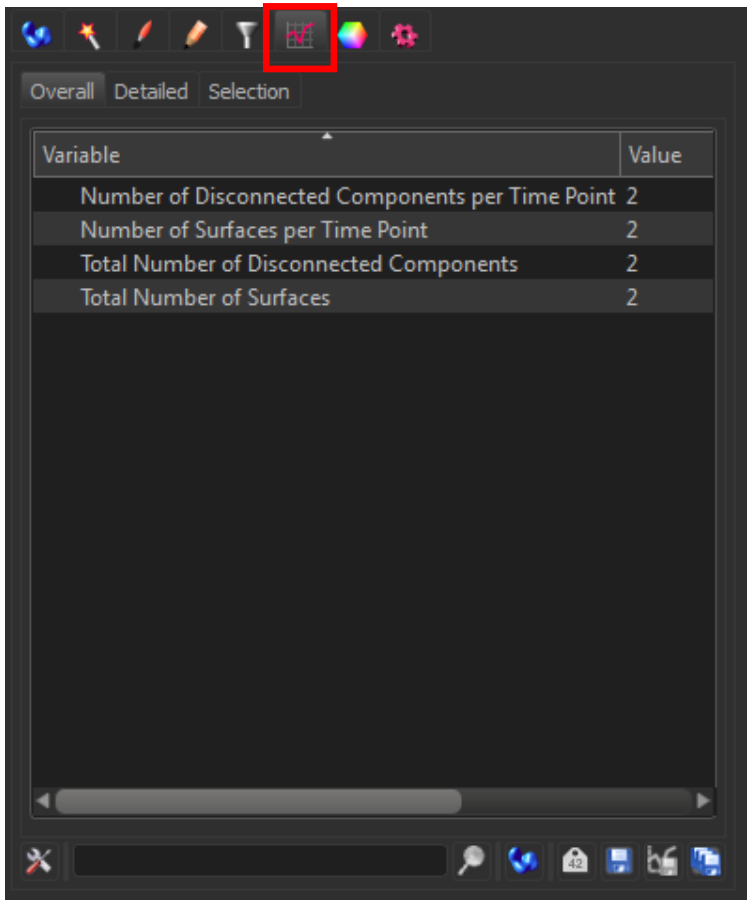
4. Cut



5. Filter



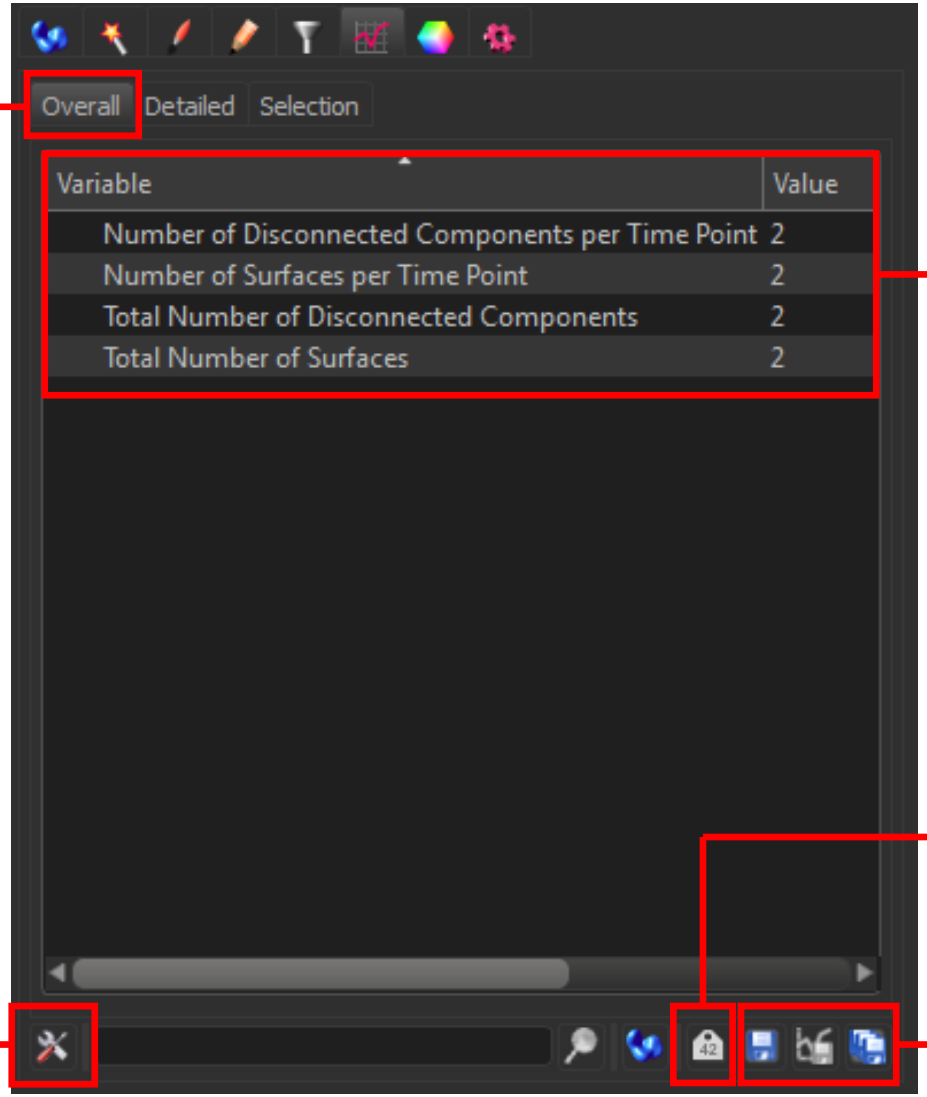
6. Stats



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)

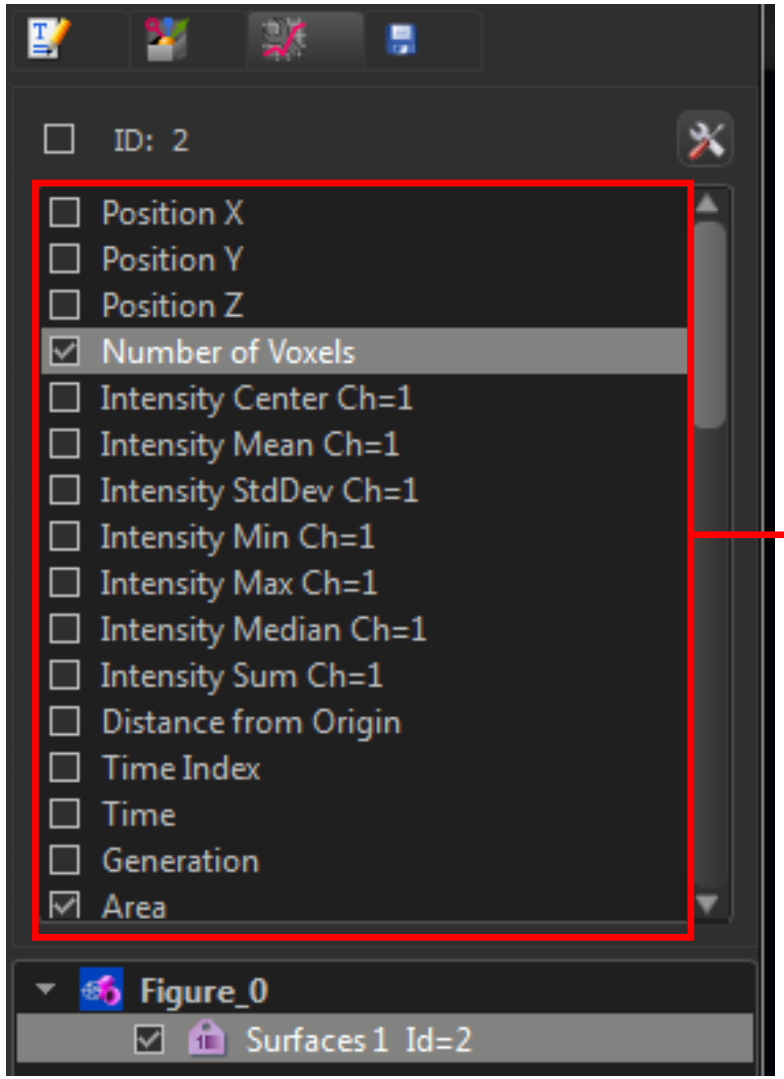
Annotations (need to select object first)

Export options

Specify what measurements are to be made

Adding New Objects to the Scene

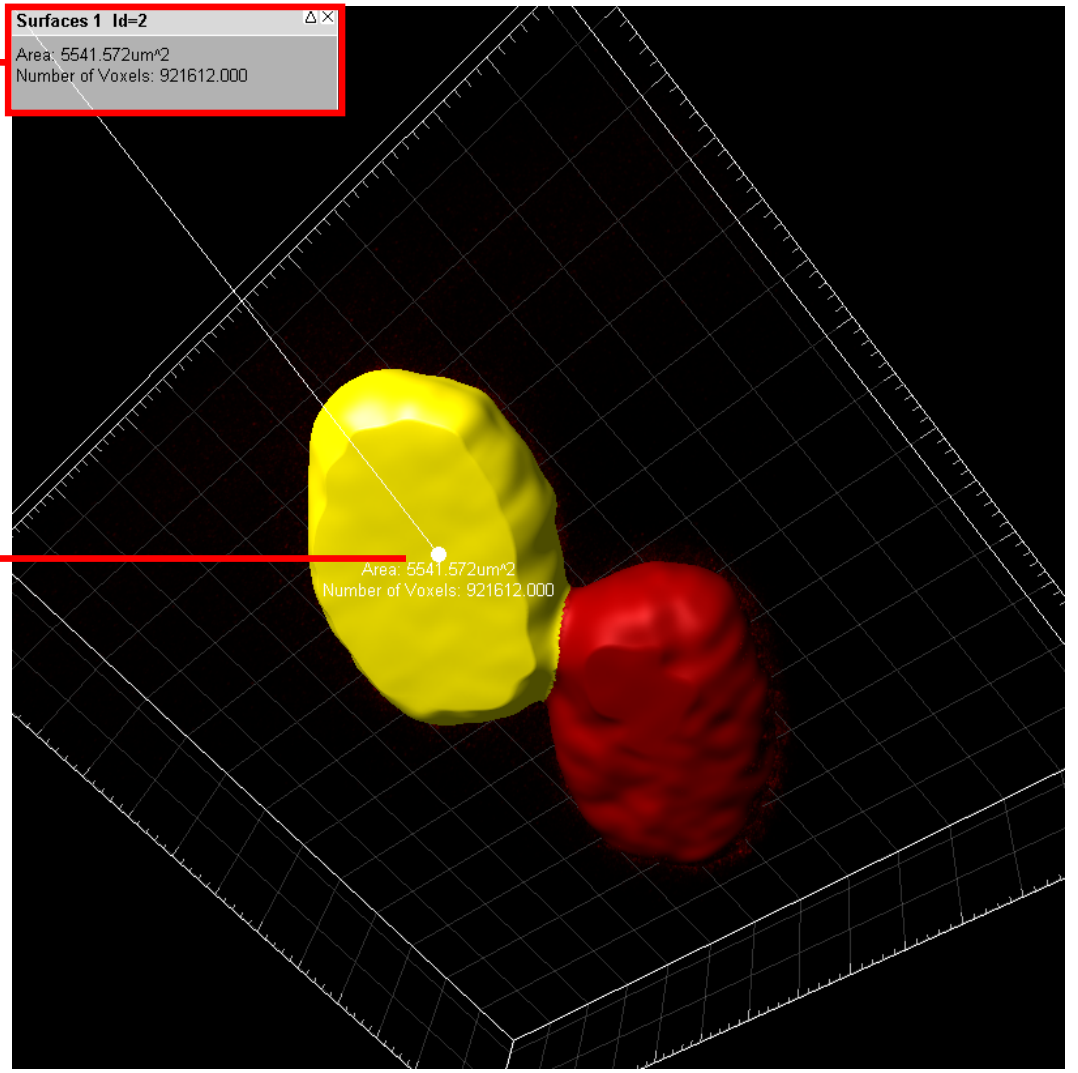
Surface Object Options: Stats tab (Annotations)



Text box option

Select stats to be displayed

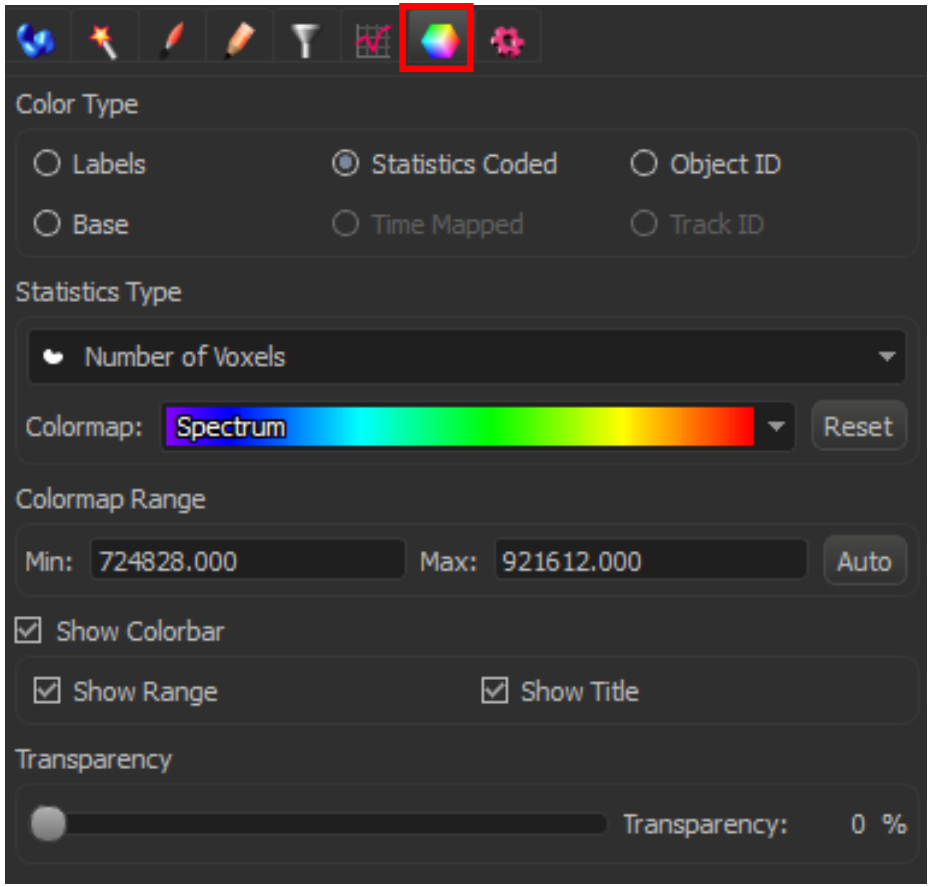
Arrow locked to centre of object



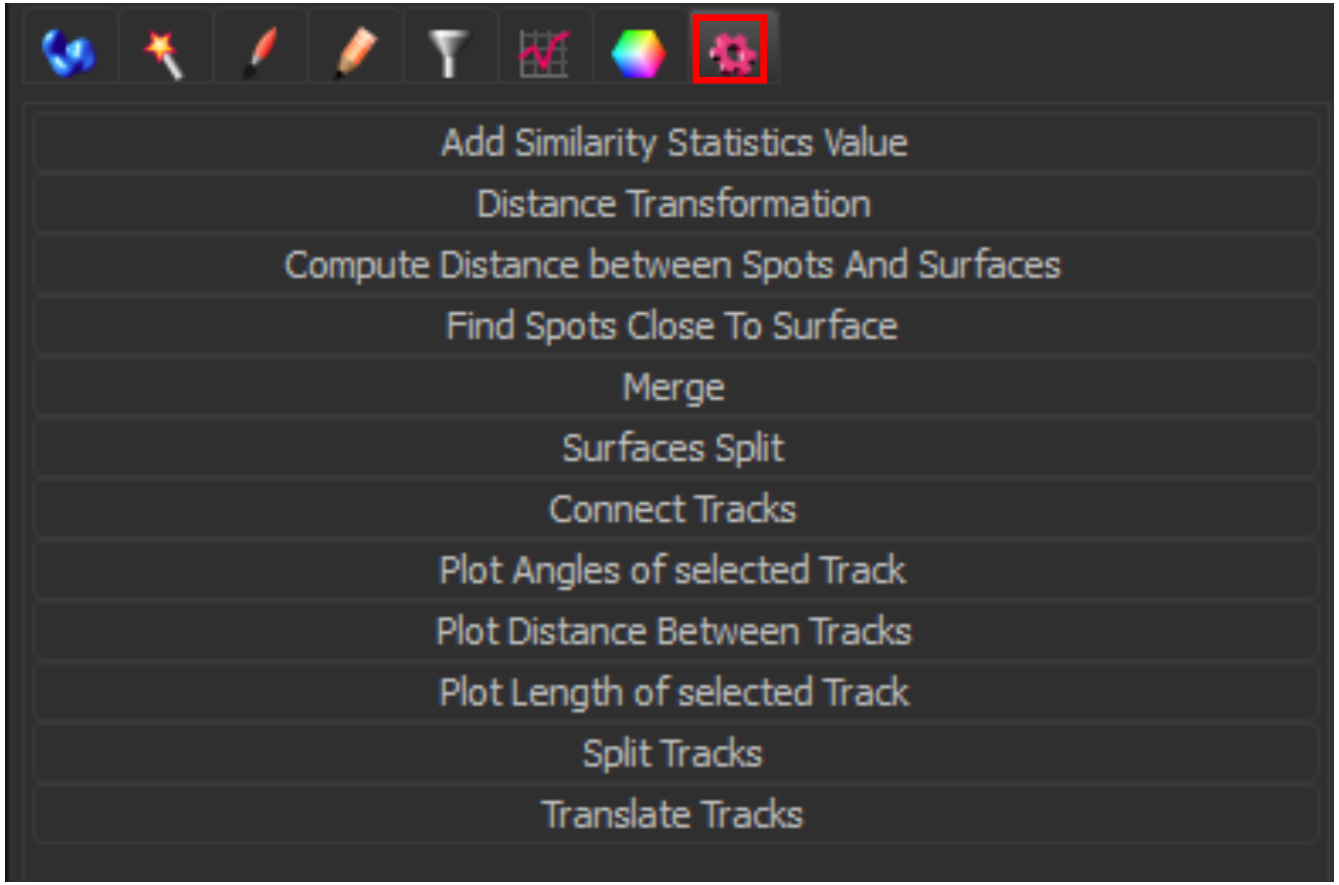
Adding New Objects to the Scene

Surface Object Options

7. Colour



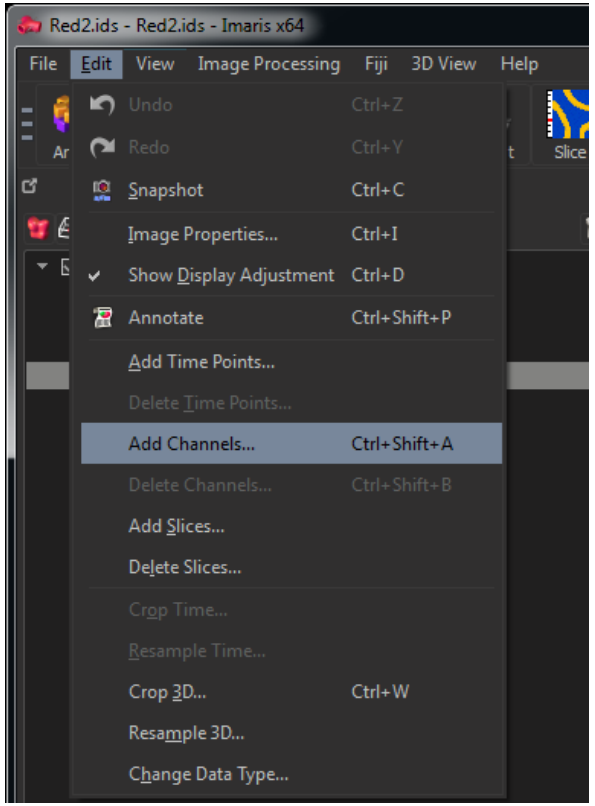
8. XT Functions

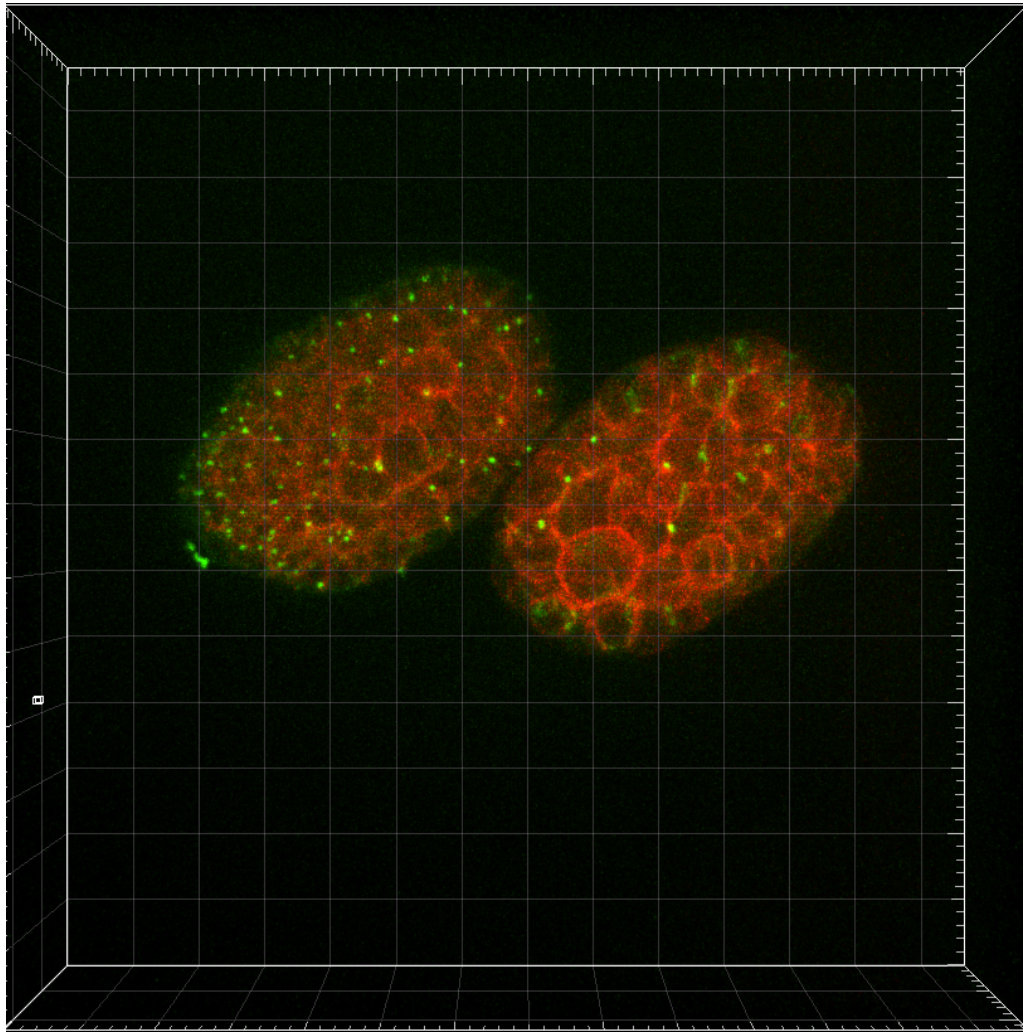


Adding New Objects to the Scene

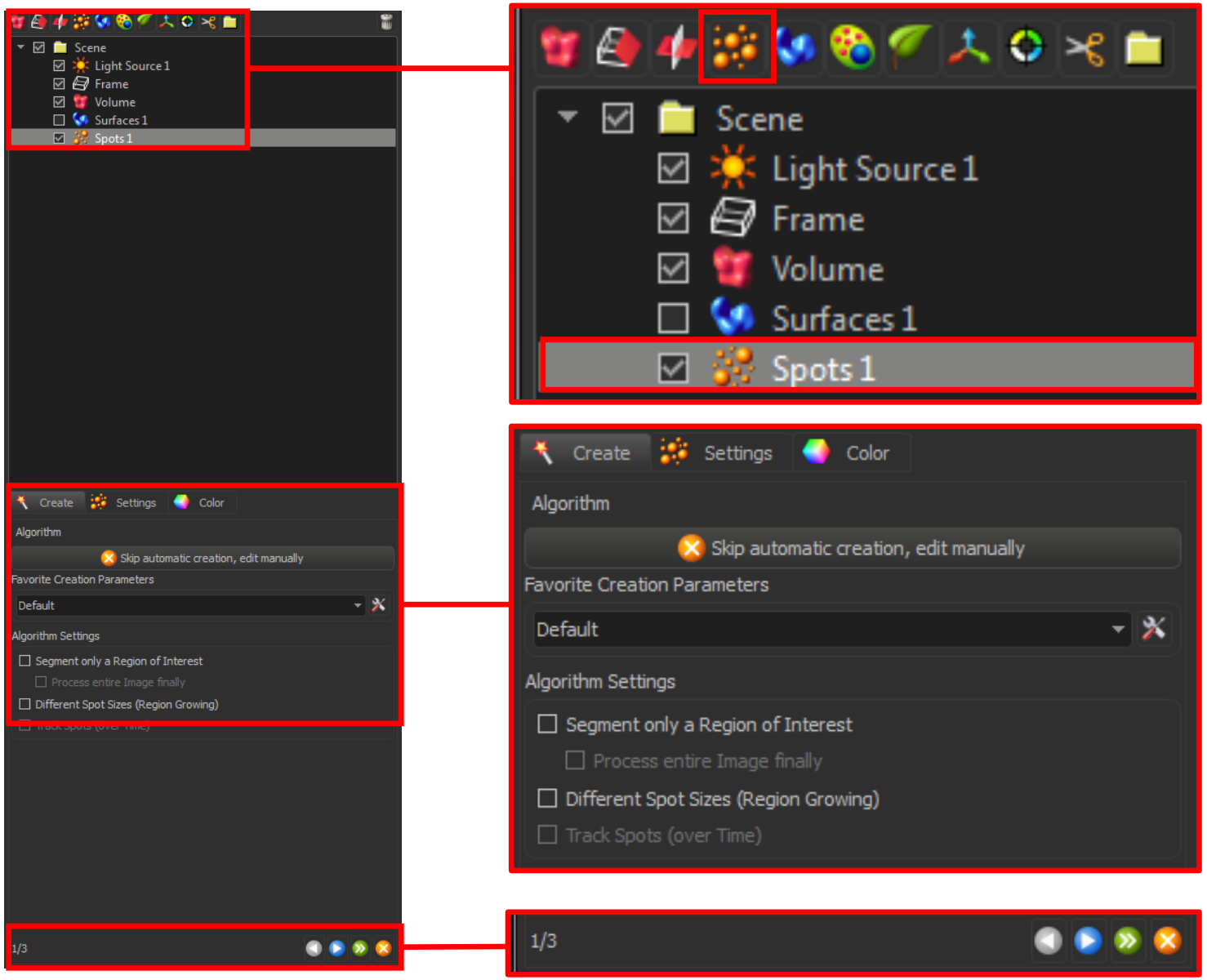
Add new channel to existing dataset

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





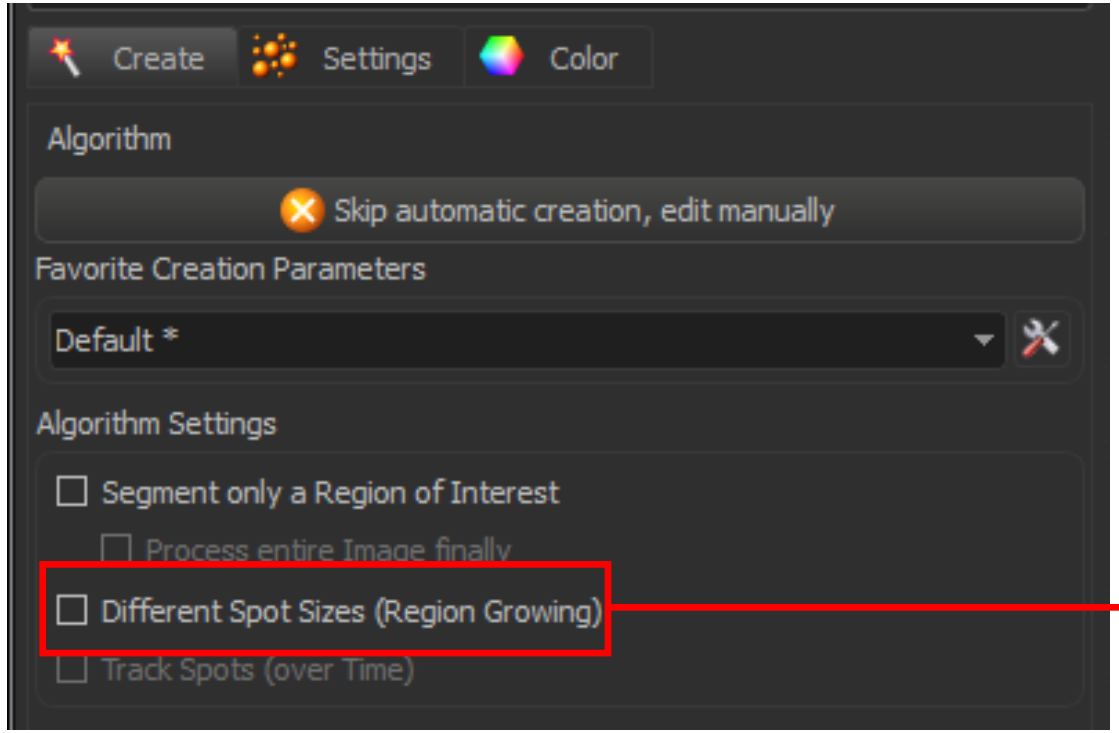
Adding New Objects to the Scene



Click on the orange blobs icon to add new Spots

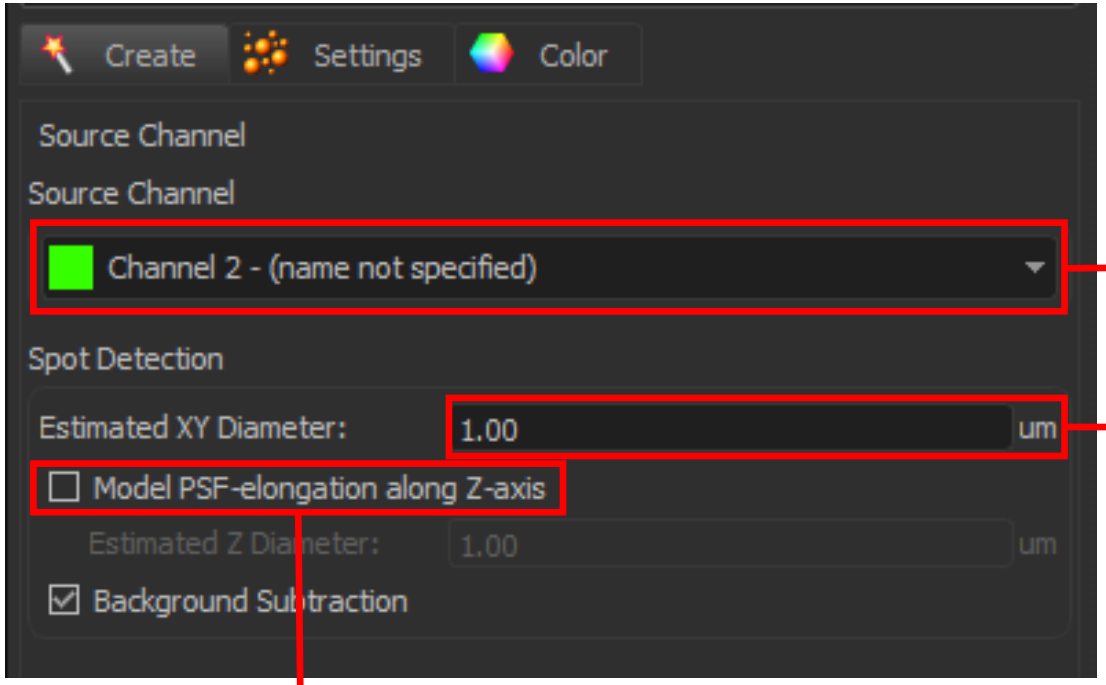
As with Surfaces, creation parameters can be loaded from a pre-defined configuration, edited manually, or defined using a walk-through wizard

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

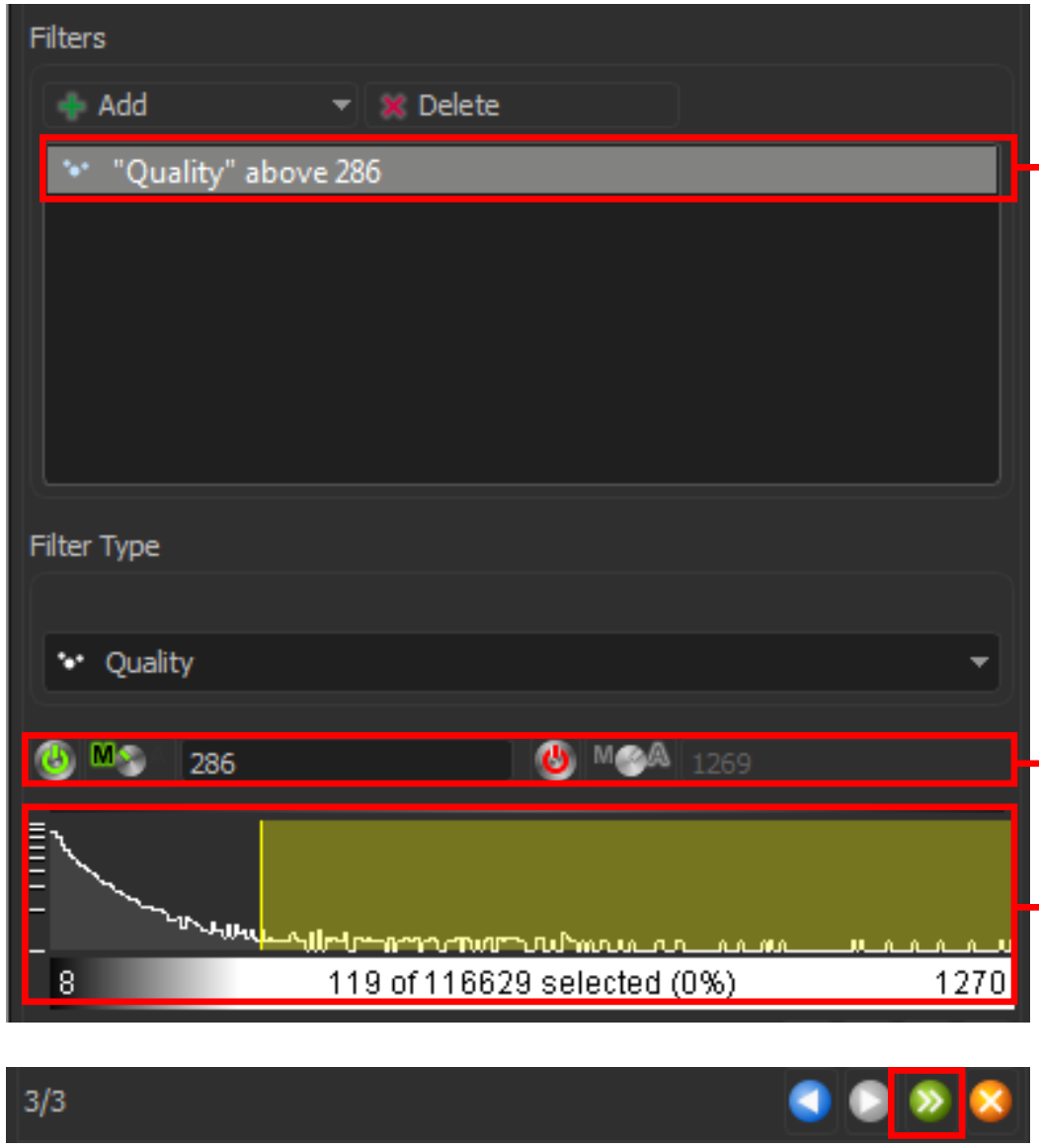


Select correct channel

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



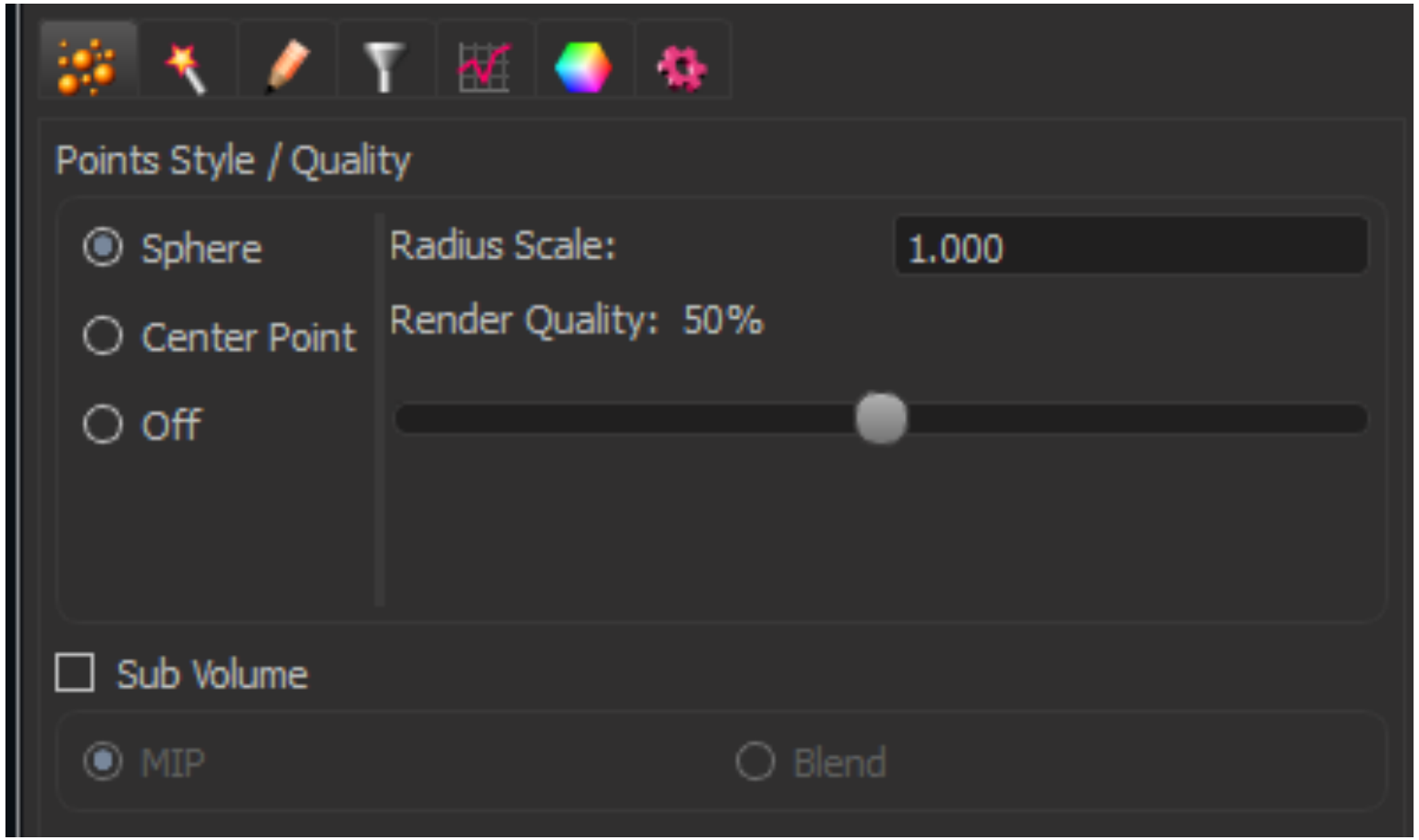
Use filters to limit number of displayed spots;
The 'Quality' is the intensity at the centre of the spot in the channel the Spots was detected.

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

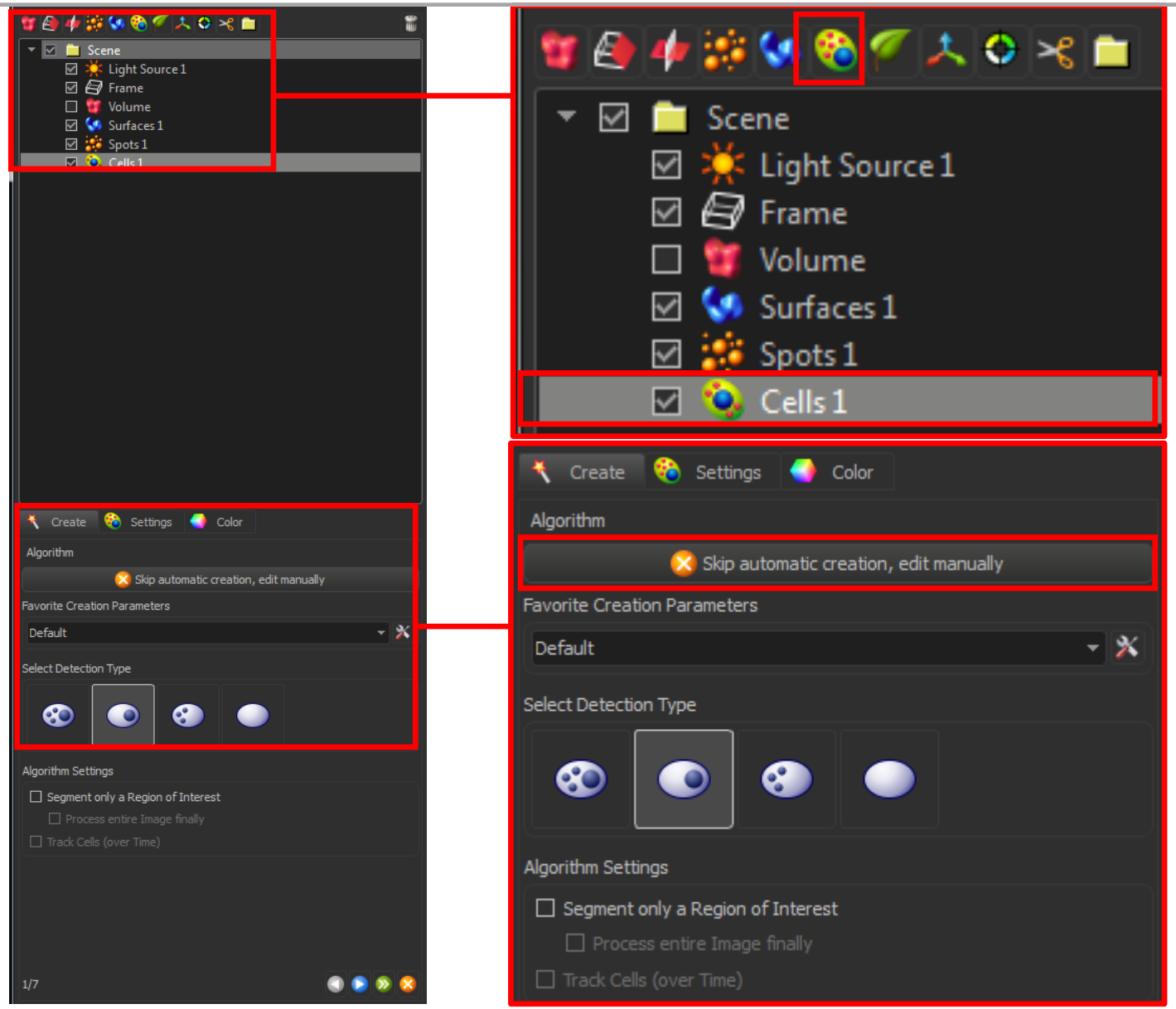
Adding New Objects to the Scene

Spot Object Options



Options essentially the same as for Surfaces

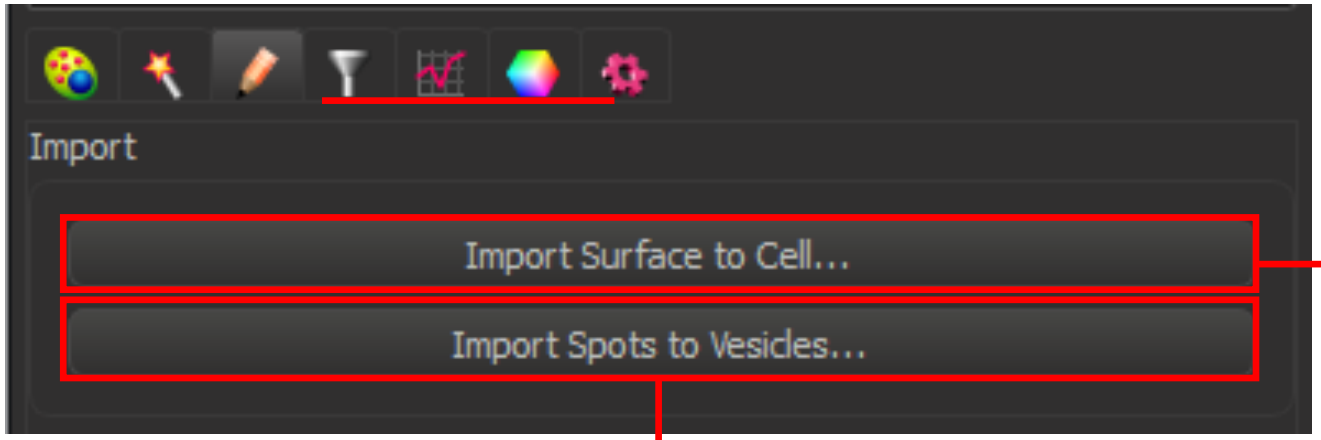
Adding New Objects to the Scene



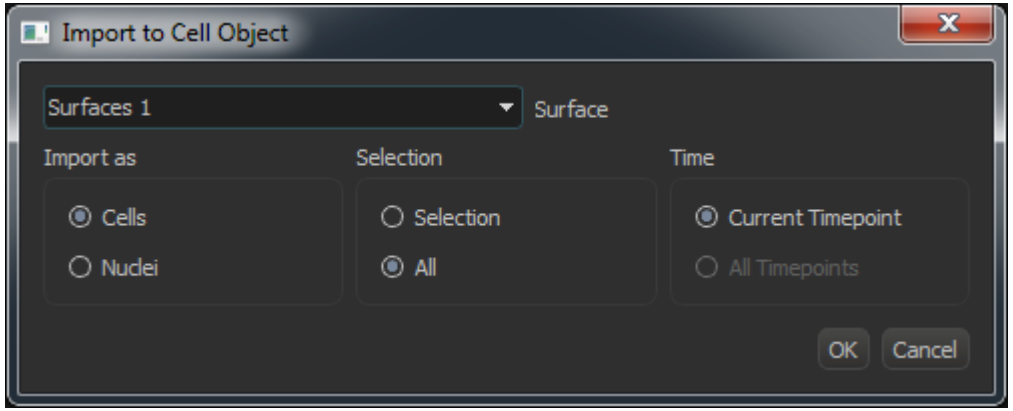
Click on the orange blobs icon to add new Spots

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

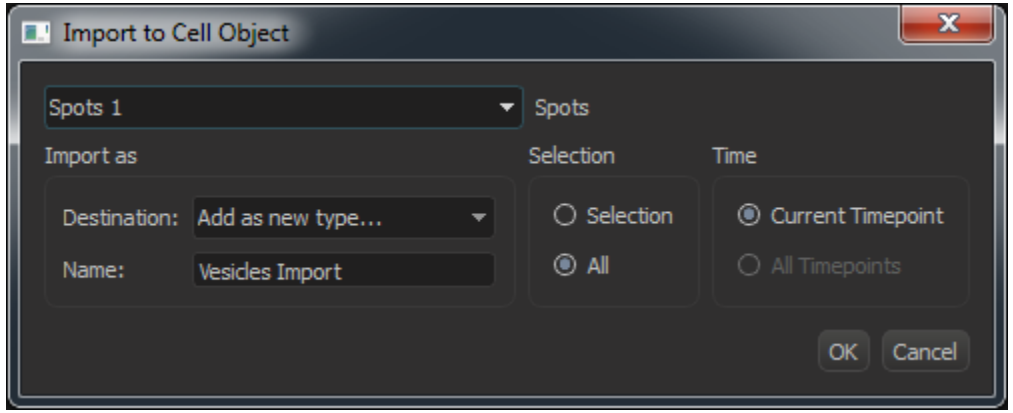
Adding New Objects to the Scene



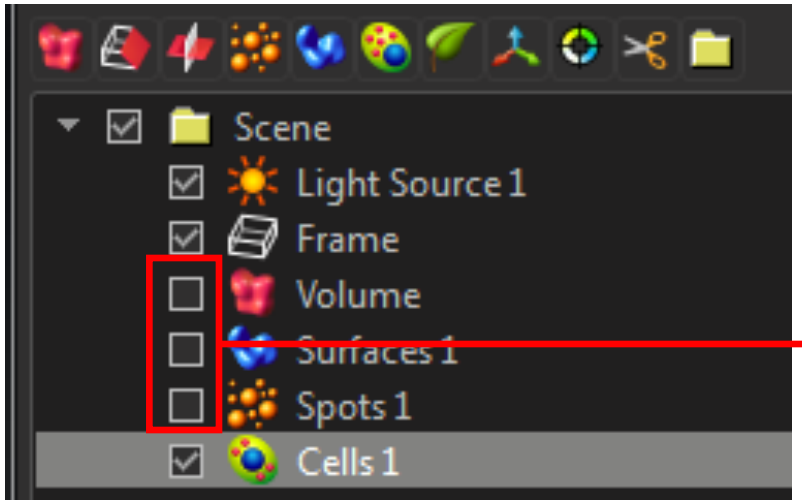
Assign Surfaces objects to Cells



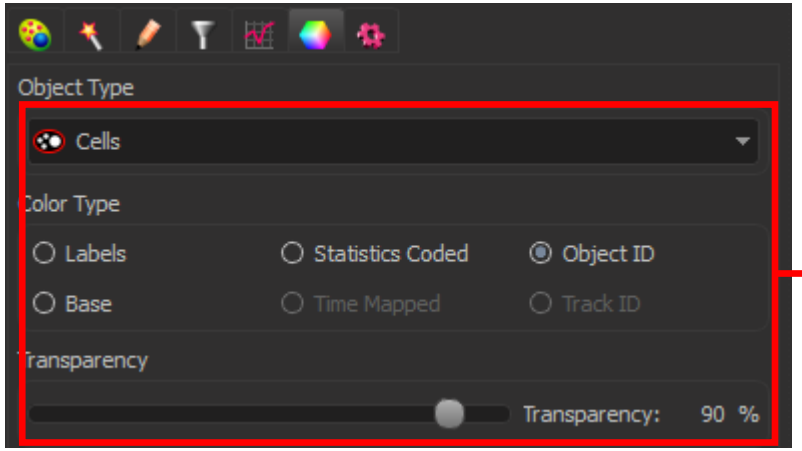
Assign Spots objects to Cells



Adding New Objects to the Scene

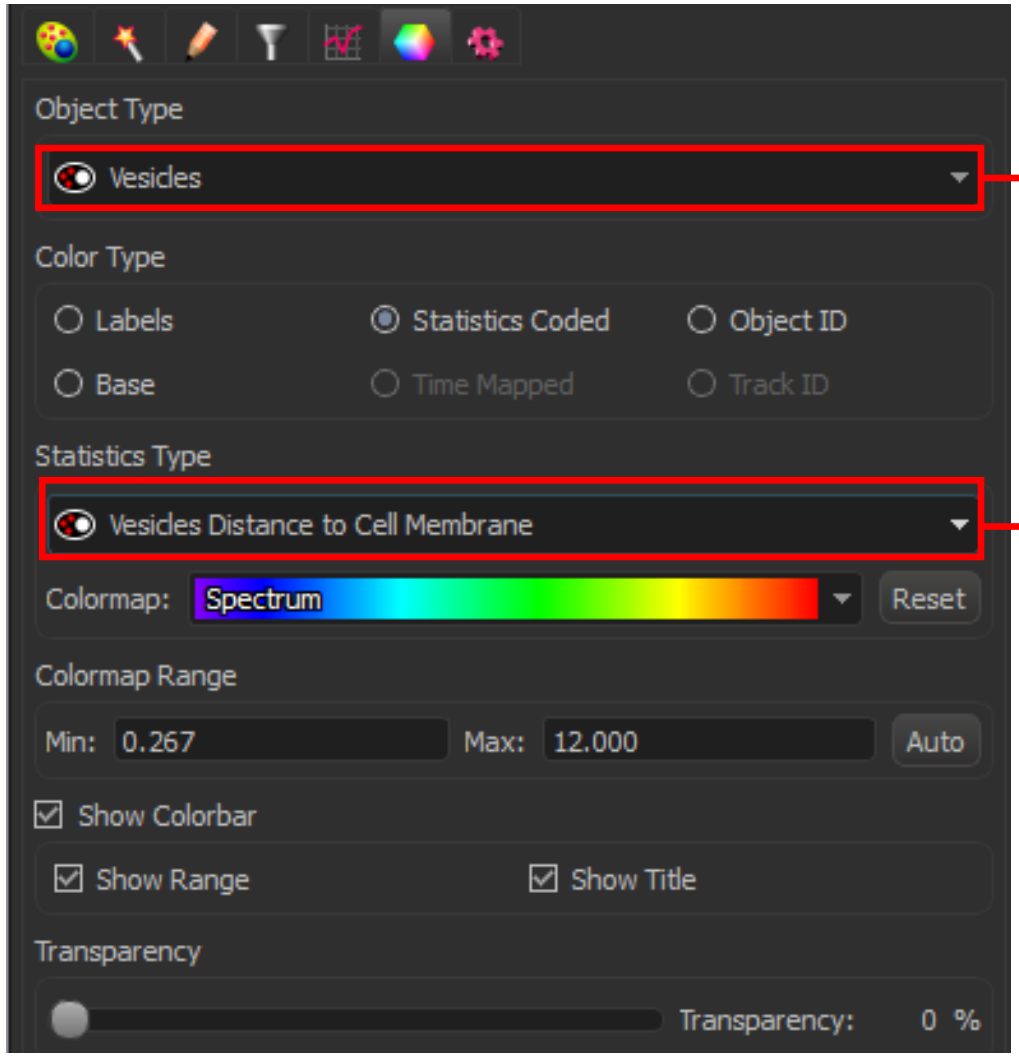


Turn off Volume, Surfaces & Spots



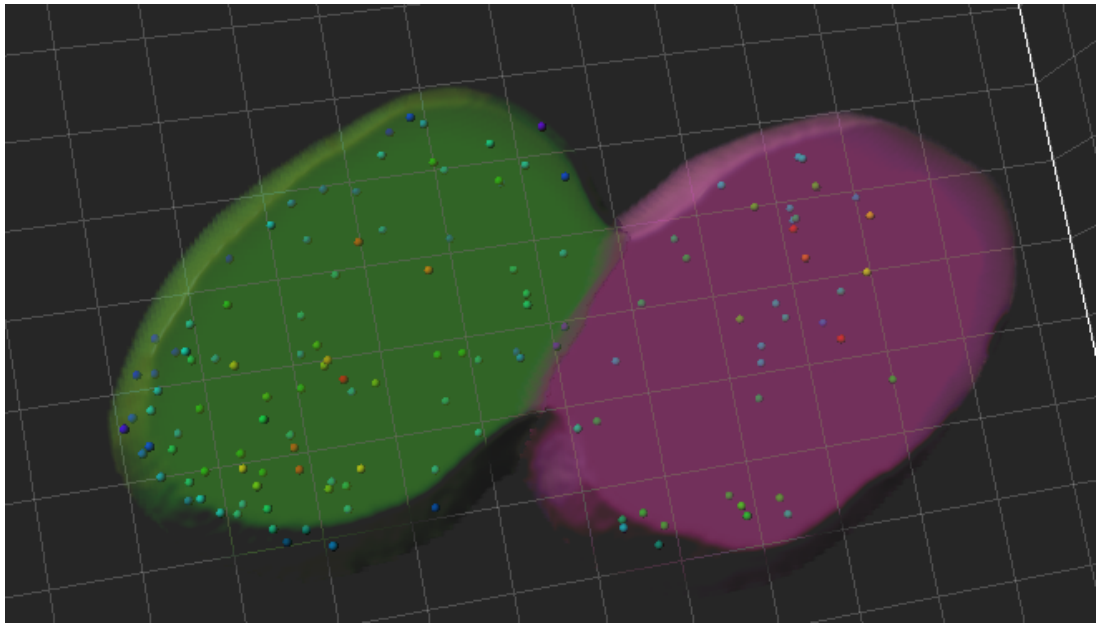
Adjust Transparency on Cells

Adding New Objects to the Scene



Select vesicles

Select Stats



Making Movies (Animation)

Group Exercise 4

Re-create Cells, using Cells Wizard