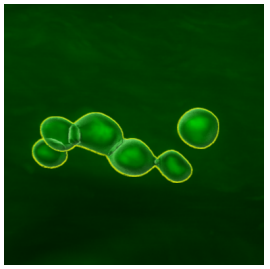
Jan 07, 2026

Quantification of neurons in cleared large ganglionated tissues



DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzjxn8lx1/v1

John-Paul Fuller-Jackson[1], Peregrine B Osborne[1], Janet R Keast[1]

[1]University of Melbourne

SPARC
Tech. support email: **info@neuinfo.org**

Janet R Keast
University of Melbourne

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzjxn8lx1/v1

Protocol Citation: John-Paul Fuller-Jackson, Peregrine B Osborne, Janet R Keast 2026. Quantification of neurons in cleared large ganglionated tissues. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzjxn8lx1/v1

Protocol status: Working
We use this protocol and it's working

Created: May 22, 2024

Last Modified: January 07, 2026

Protocol Integer ID: 100250

Keywords: imaris, segmentation, quantification, peripheral nervous system, autonomic ganglia, quantification of neuron, segmentation of neuron, large samples of ganglionate peripheral nerve tissue, cleared large ganglionated tissue, large ganglionated tissues this protocol, ganglionate peripheral nerve tissue, neuron distribution, 3d representation of the neuron distribution, segmentation, neuron, cleared large sample, quantification, accurate count, using imari

Funders Acknowledgements:
NIH SPARC
Grant ID: 3OT2OD023872

Abstract

This protocol is a semi-automated method using Imaris to quantify neurons in cleared large samples of ganglionate peripheral nerve tissue. The process involves the segmentation of neurons, which can then be filtered and manually curated. This not only provides accurate counts, but also a 3D representation of the neuron distribution.

Materials

Equipment

Ultramicroscope Blaze	NAME
Light sheet microscope	TYPE
Ultramicroscope	BRAND
140-006-739.04	SKU
https://www.miltenyibiotec.com/AU-en/products/ultramicroscope-blaze.html	LINK

Software

Imaris	NAME
Bitplane	DEVELOPER
http://www.bitplane.com/Default.aspx	REPOSITORY

Software

Imaris File Converter	NAME
Bitplane	DEVELOPER
https://viewer.imaris.com/download/ImarisFileConverter9_9_1w64.exe	SOURCE LINK

Software

NAME	DEVELOPER	SOURCE LINK
Imaris Stitcher	Bitplane	https://imaris.oxinst.com/products/imaris-stitcher

Large Volume Image Acquisition on Light Sheet Microscope

1 Mount sample on sample holder of light sheet microscope to optimize illumination of neuron-containing region.

2 Load sample into light sheet microscope, fully immersed in imaging medium.

3 At low magnification (1-2x), inspect the sample and identify the ganglionated region. If poorly illuminated, repeat Steps 1 and 2 until the region of interest is suitable illuminated by the light sheet.

4 At higher magnification (at least 8x), completely image the neuron-containing region using tile scanning if required. A z-step of 2 µm is suitable for neuron counting. Single-sided illumination is best due to differences in left and right illumination in asymmetrical samples. Ensure sufficient laser power and exposure for adequate illumination. Save files as OME-TIFF.

Image Preprocessing

5 Convert the image stack(s) from OME-TIFF to Imaris files (.IMS) using Imaris Converter.

Software

Imaris File Converter	NAME
Bitplane	DEVELOPER
https://viewer.imaris.com/download/ImarisFileConverter9_9_1w64.exe	SOURCE LINK

6 For tile scan acquisitions, stitch the separate .IMS files together using Imaris Stitcher.

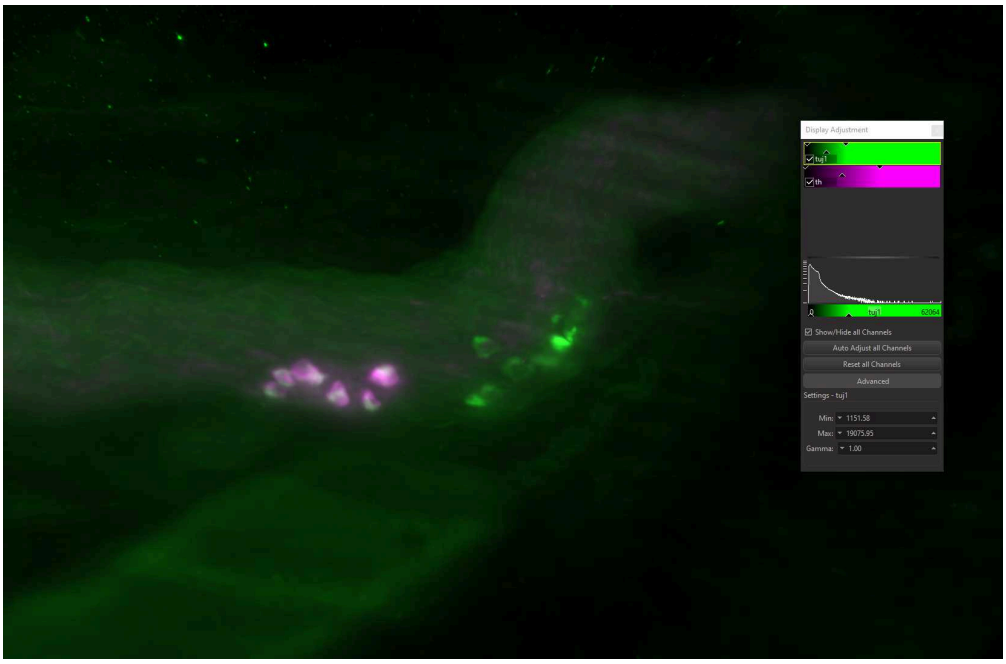
Software	
Imaris Stitcher	NAME
Bitplane	DEVELOPER
https://imaris.oxinst.com/products/imaris-stitcher	SOURCE LINK

Neuron Visualization in Imaris

7 Open the stitched .IMS file in Imaris.

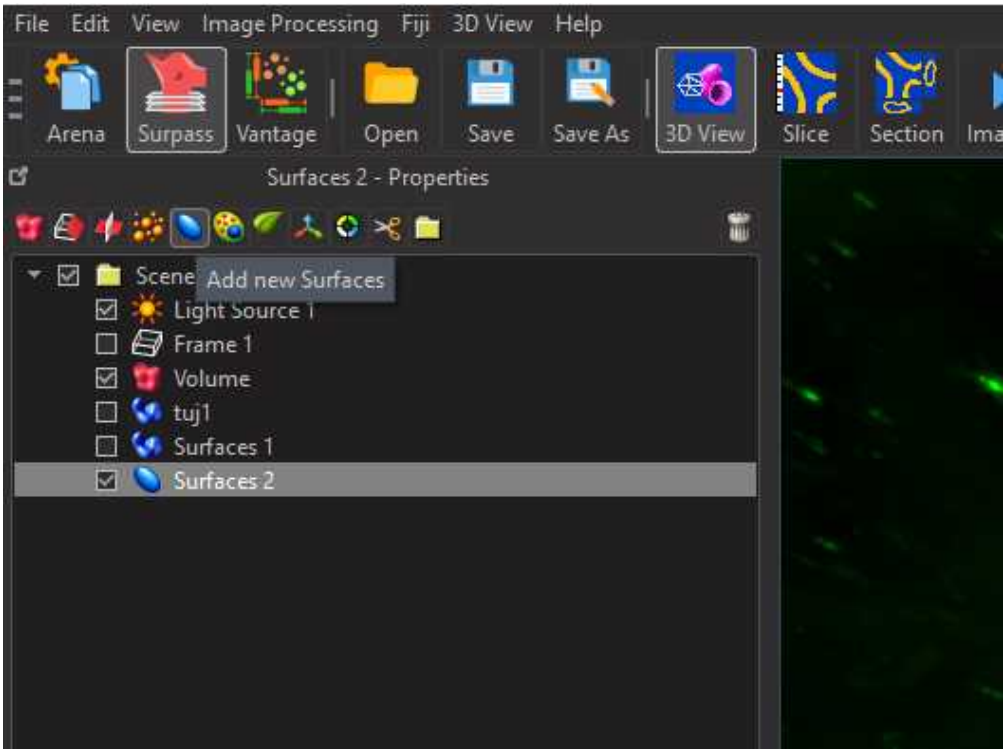
Software	
Imaris	NAME
Bitplane	DEVELOPER
https://imaris.oxinst.com/big-data	SOURCE LINK

8 In the Display Properties (Ctrl+D), rename each channel according to the immunolabel. Adjust each channel's Display Settings to best visualize the neurons in the image. A gamma correction of 1.2-1.6 is typical, to correct for differences in illumination across the sample.

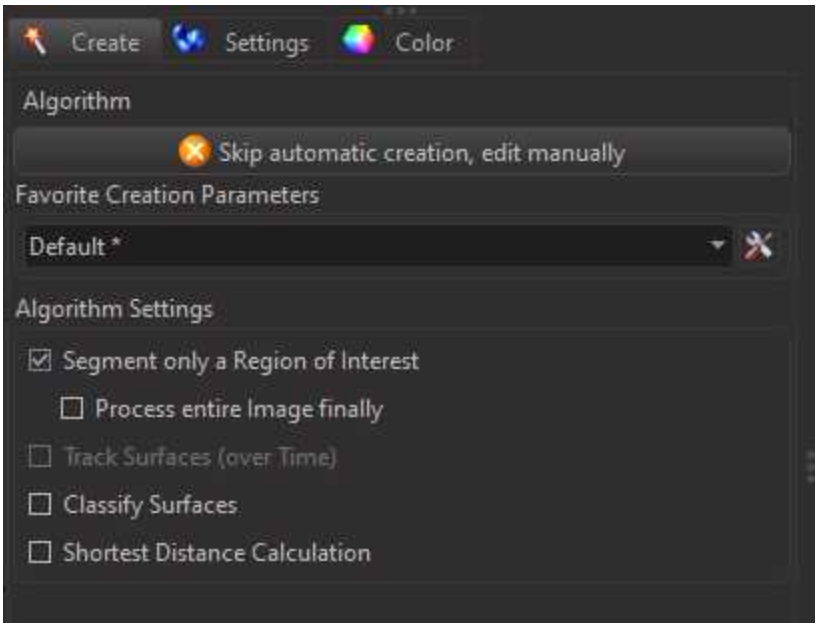


Semi-automatic segmentation of neurons with Surfaces

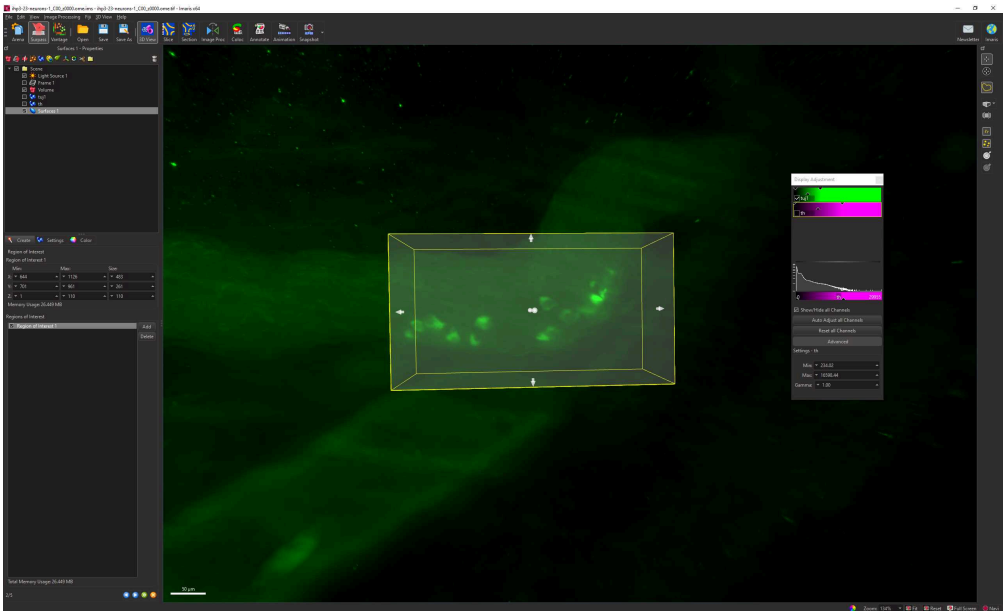
9 In Object Panel, click on Surfaces.



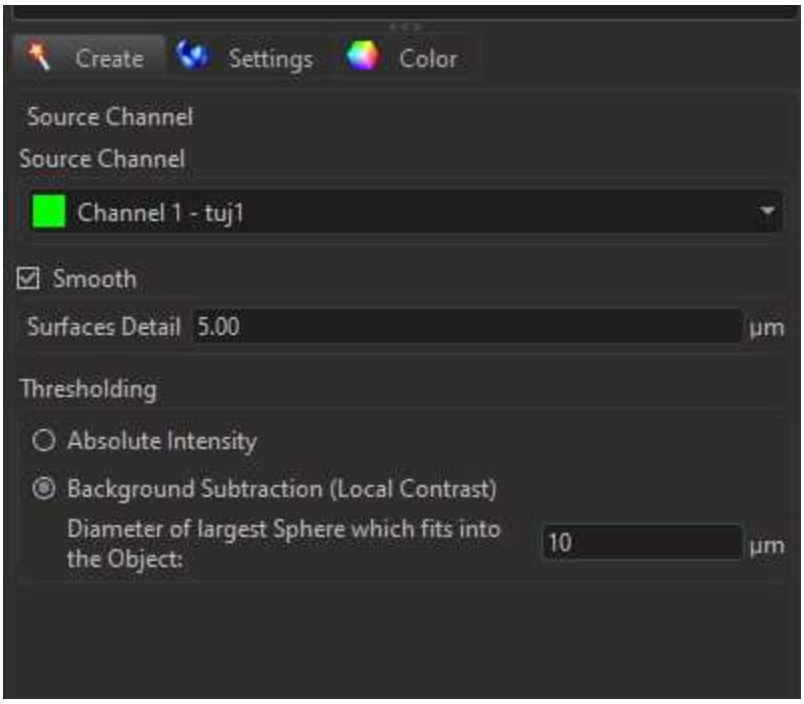
10 In the first part of the Surface Creation Wizard, tick *Segment only a Region of Interest*. Leave all other options unticked. Click next (blue arrow icon at the bottom of panel).



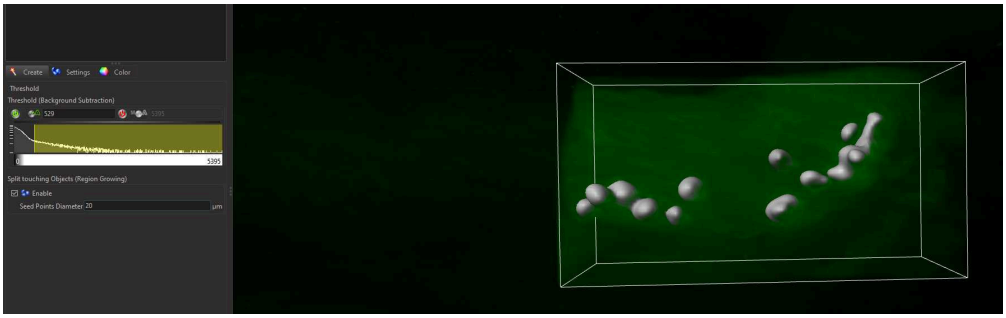
11 In the second part of the Surface Creation Wizard, manually adjust the region of interest to encompass the neurons to be segmented (and thus counted). Click next.



12 In the third part of the Surface Creation Wizard, under *Source Channel* select the channel containing the neurons of interest. Tick *Smooth* and set *Surfaces Detail* to 5 µm. Under *Thresholding* select *Background Subtraction (Local Contrast)*. Set the *Diameter of the largest Sphere which fits into the Object* to 10 µm. Based on subsequent neuron detection success, these values can be modified.

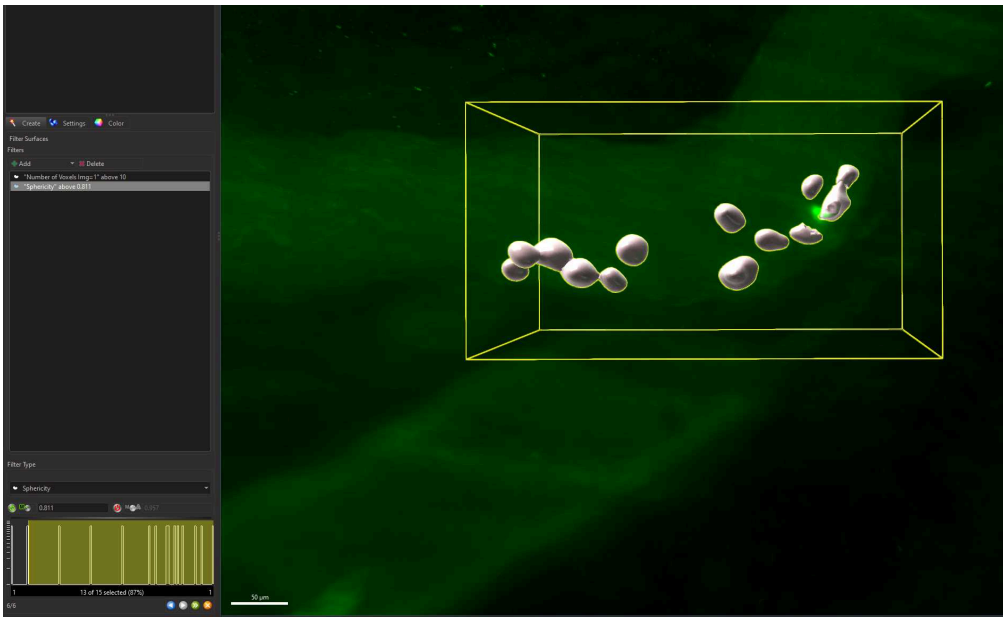


13 In the fourth part of the Surface Creation Wizard, a preview of the Surfaces is shown on the image. Under *Threshold*, the Surface can be adjusted based on background subtraction. Adjust the thresholding until the previewed Surface just obscures the signal it is detecting. Tick *Seed Points* and set *Seed Point Diameter* to 20 µm (this value can be modified to optimize selection of neurons and splitting neurons that are closely positioned).
For samples in which illumination of neurons varies drastically across the sample, it may be better to break down the Surface creation into separate Regions of Interest, to adjust each accordingly.

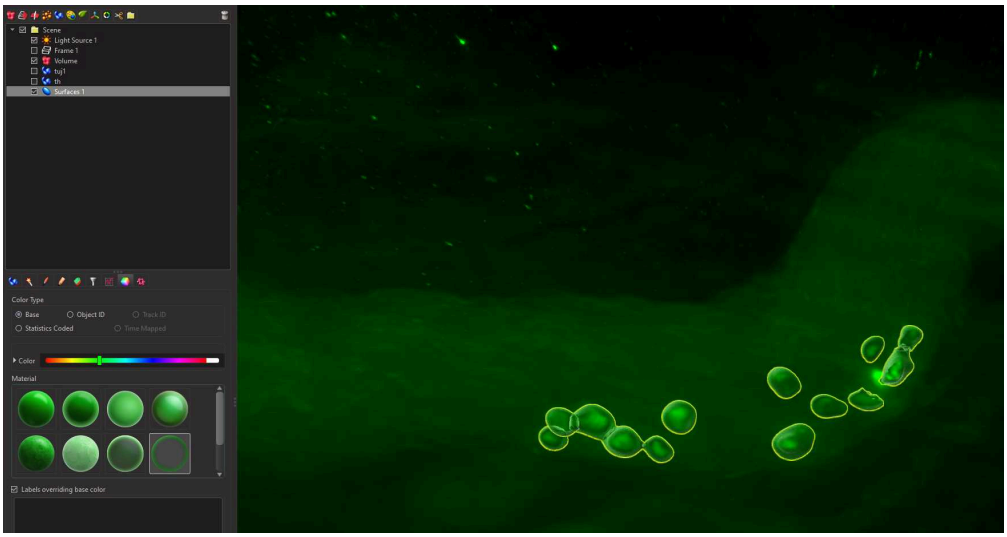


14 In the fifth part of the Surface Creation Wizard which is only added when *Seed Points* are enabled, the suggested Seed Points on the image can be filtered according to many metrics - in this example *Quality* was chosen and the selection thresholded to the point when all neurons were identified by Seed Points.

15 In the sixth and final step of the Surface Creation Wizard, the final Surfaces are shown, and can be filtered again based on many criteria. *Number of Voxels* filters Surfaces based on size, which is useful for quickly removing very large and very small things that are not neurons but were included (for example, axon tracts immunolabelled by the same marker). Another useful metric to filter by is *Sphericity*, which helps remove Surfaces based on non-spherical objects (again, axon tracts).
Click on the Green arrow icon to finish the creation of the Surface.

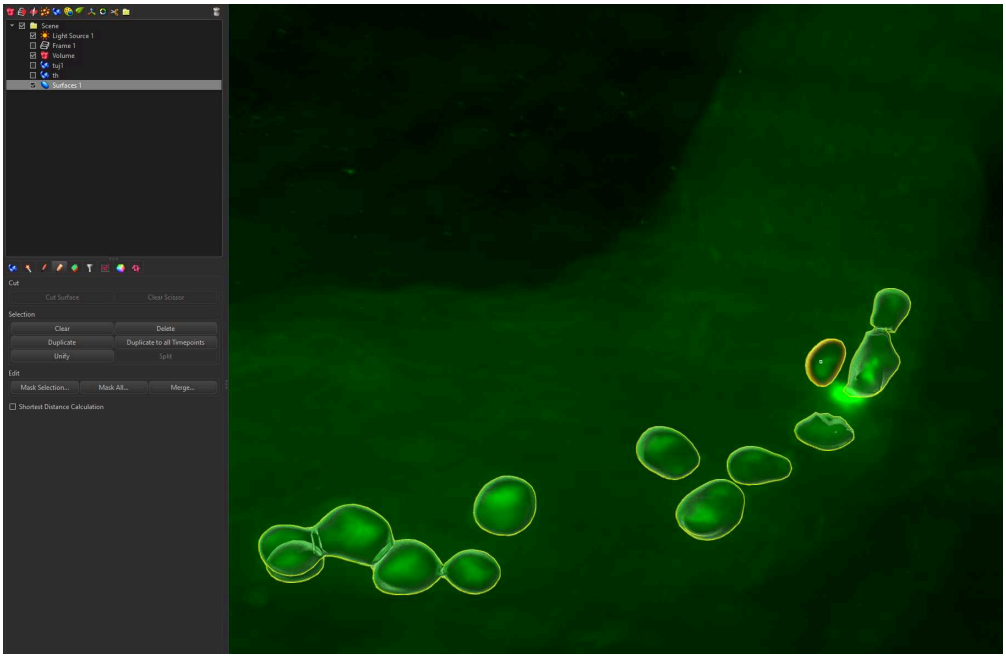


16 With the Surface segmentation of the neurons complete, manual editing can still be performed for anything that was not filtered out during the Surface Creation Wizard. With the Surface selected, click on the colourful cube icon to bring up display options for the Surface. Under *Material* a range of surface textures can be selected, with the semi-transparent most useful for identifying which Surfaces correctly represent neurons.

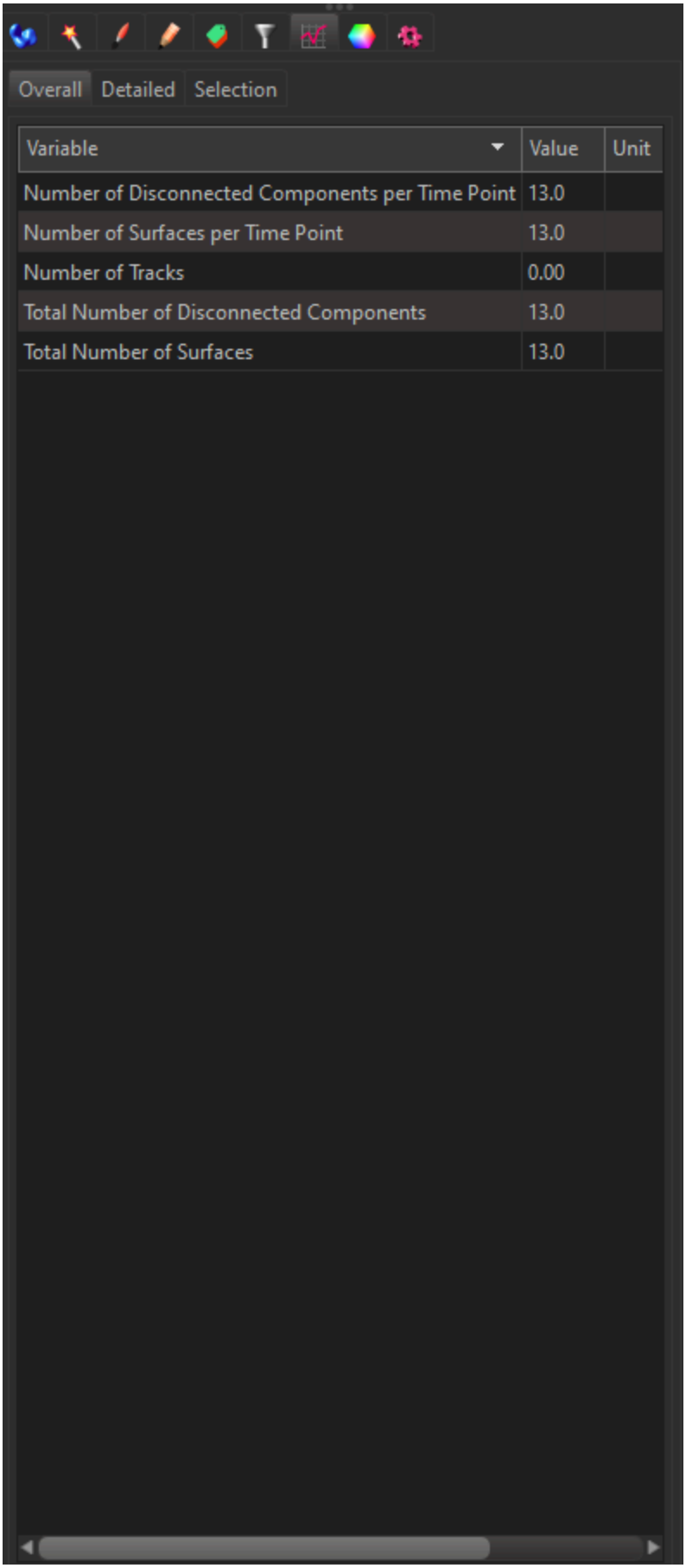


17 With the Surface selected, change to the Edit tab (pencil icon) to then select individual Surfaces and modify/delete them. Selected Surfaces will be highlighted with a more

intense yellow hue.



18 To view the total number of neurons in any given Surface, click on the Measurement tab (icon of a line graph). Under Overall, the *Total Number of Surfaces* will be displayed. The Save icon below allows for this to be exported as a .CSV.


Overall
Detailed
Selection
Variable
Value
Unit
Number of Disconnected Components per Time Point
13.0
Number of Surfaces per Time Point
13.0
Number of Tracks
0.00
Total Number of Disconnected Components
13.0
Total Number of Surfaces
13.0