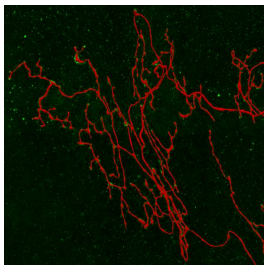
Dec 20, 2024

# Tracing complex nerve endings with Neurolucida 360



DOI
https://dx.doi.org/10.17504/protocols.io.x54v92294l3e/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**

John-Paul Fuller-Jackson
University of Melbourne



OPEN ACCESS

**DOI:** https://dx.doi.org/10.17504/protocols.io.x54v92294l3e/v1

**Protocol Citation:** John-Paul Fuller-Jackson, Peregrine B Osborne, Janet R Keast 2024. Tracing complex nerve endings with Neurolucida 360. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v92294l3e/v1

**Manuscript citation:**



**Protocol status:** Working

**Created:** June 07, 2024

**Last Modified:** December 20, 2024

**Protocol Integer ID:** 101370

**Keywords:** nerve ending, sensory neuroscience, segmentation, axon terminal, axon tracing, tracing of nerve ending, tracing complex nerve ending, complex nerve endings with neurolucida, complete visualisation of nerve ending, nerve ending, neurolucida, associated virus aav, immunohistochemistry, virus aav, signal of this tdtomato, sensory neuron, axon, tdtomato tag, entirety of the axon

**Funders Acknowledgements:**
NIH SPARC
Grant ID: 3OT2OD023872

## Abstract

This protocol describes the tracing of nerve endings labelled with an appropriate marker that fills the entirety of the axon and its terminal structure. In this example, a subset of rat sensory neurons was transfected with the adeno-associated virus AAV-PHP.S, which contained a TdTomato tag. The signal of this TdTomato was enhanced with immunohistochemistry to enable complete visualisation of nerve endings in tissue, which could then be traced in Neurolucida 360.

## Materials

Software

| | |
|---|---|
| Neurolucida 360 | NAME |
| MicroBrightField Bioscience | DEVELOPER |
| https://www.mbfbioscience.com/neurolucida360 | SOURCE LINK |

Software

| | |
|---|---|
| MicroFile+ (RRID: SCR_018724) | NAME |
| MBF Bioscience | DEVELOPER |

## Image acquisition

1 With a confocal microscope, image the nerve ending with at least 40x magnification and Nyquist sampling in XYZ. Use tile scans to completely capture the entire nerve ending. If tile scanning was used, stitch the image into a single image.

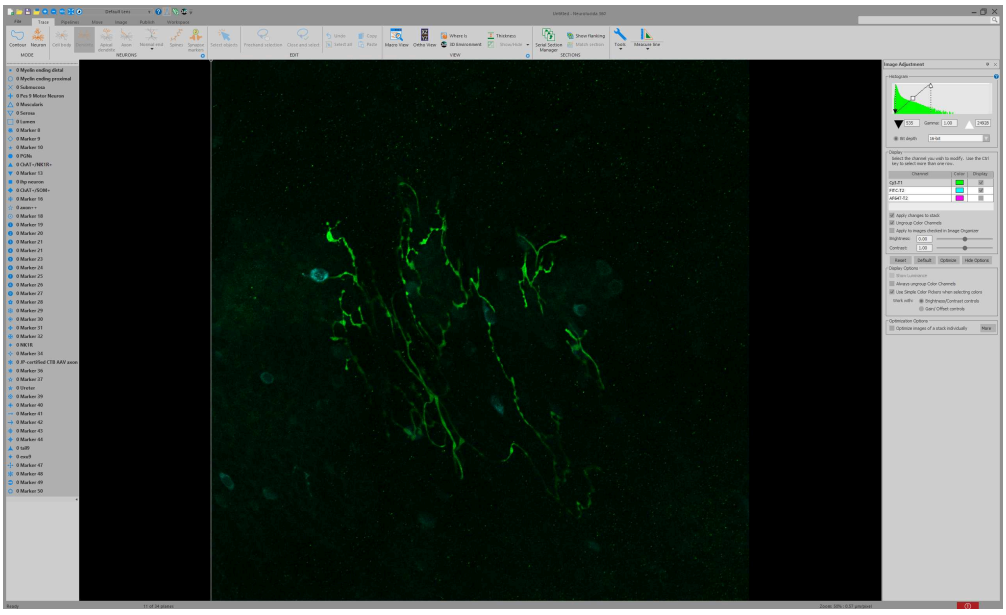
## Image pre-processing

2 Convert the image stack to JPEG2000 (JPX) using **Microfile+**.

| Software | |
|---|---|
| MicroFile+ (RRID: SCR_018724) | NAME |
| MBF Bioscience | DEVELOPER |

## Opening file in Neurolucida 360

3 Open the JPEG2000 file in **Neurolucida 360**.

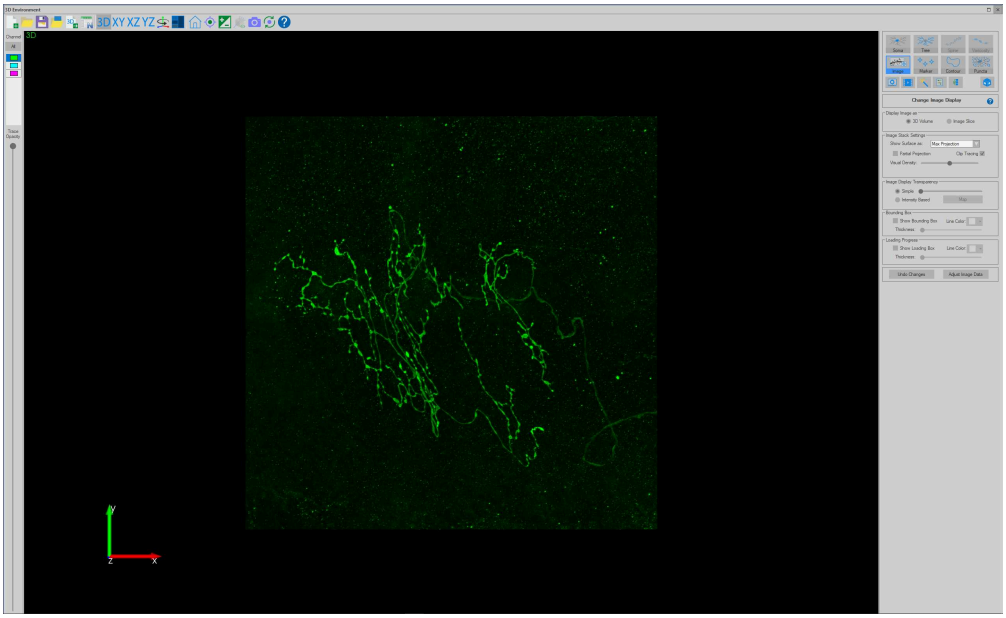


| Software | |
|---|---|
| Neurolucida 360 | NAME |
| MicroBrightField Bioscience | DEVELOPER |
| https://www.mbfbioscience.com/neurolucida360 | SOURCE LINK |

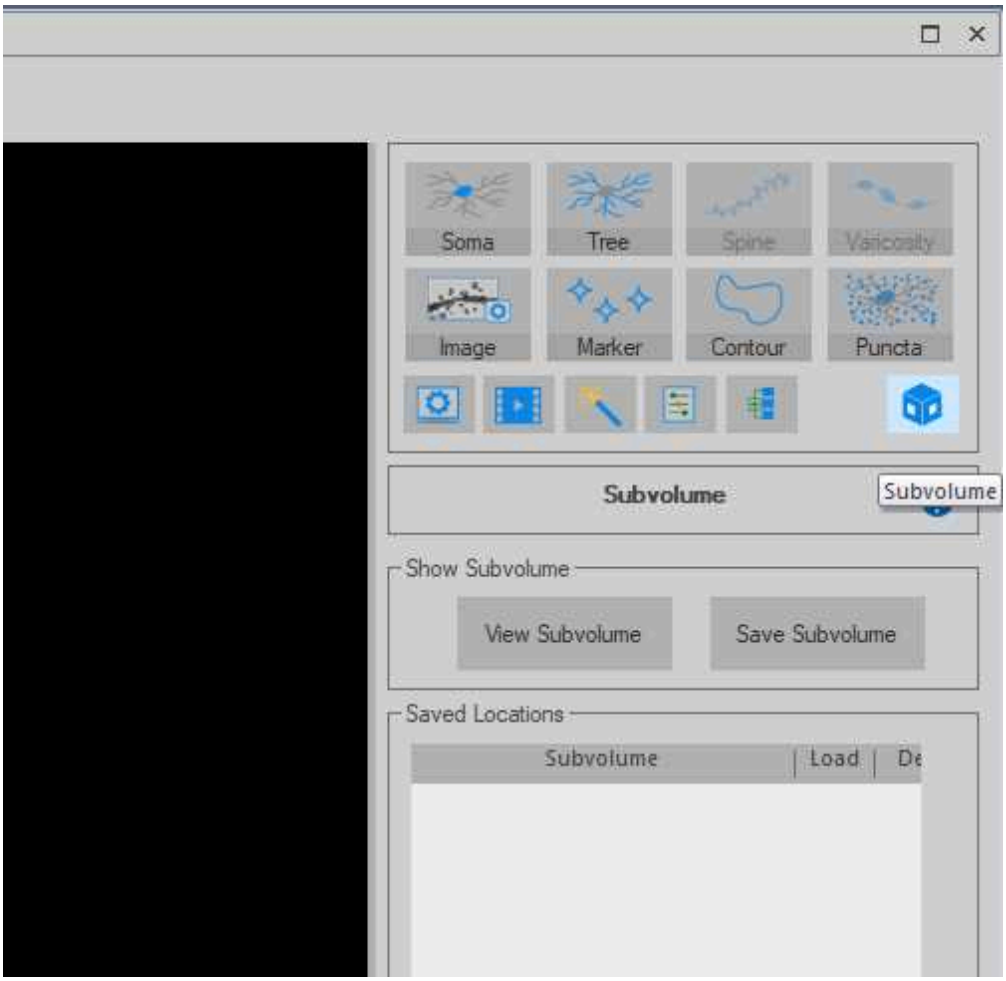
4 Thoughout all the subsequent Steps, save progress. This will prompt a saving of the JPEG2000 (if display settings were altered) and the saving of a data file. Choose .XML as the file type for saving the data file. Keep the .XML in the same file location as the associated JPEG2000.

## Subvolume 3D view of nerve endings

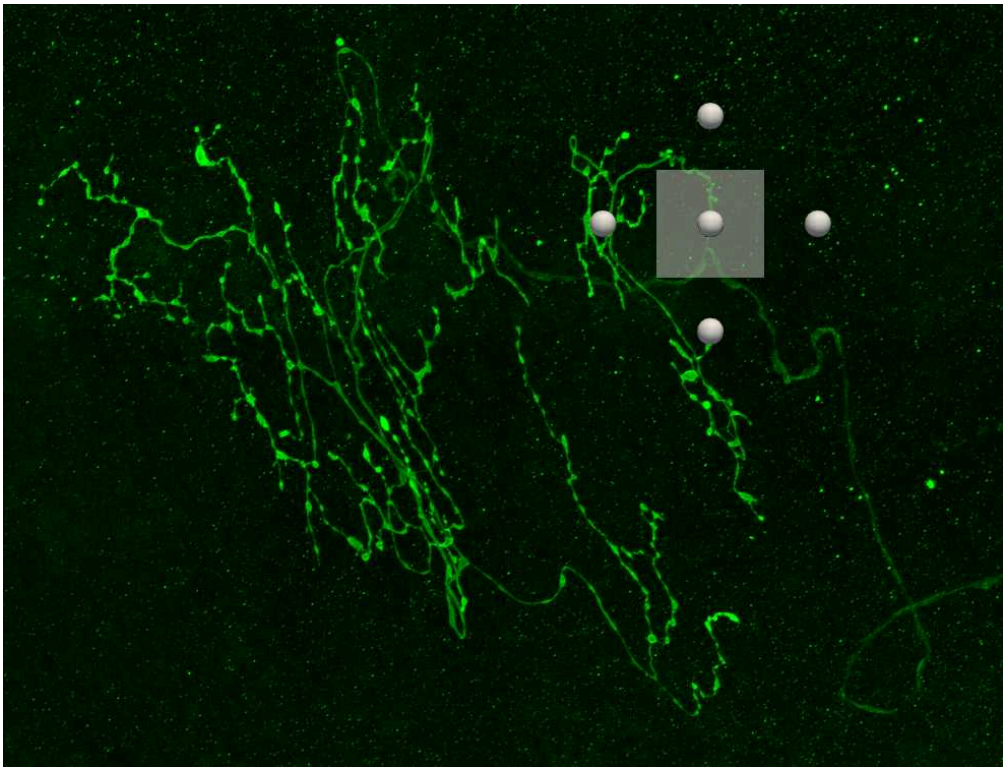
5 Open the **3D Environment** to view the dataset in 3D.



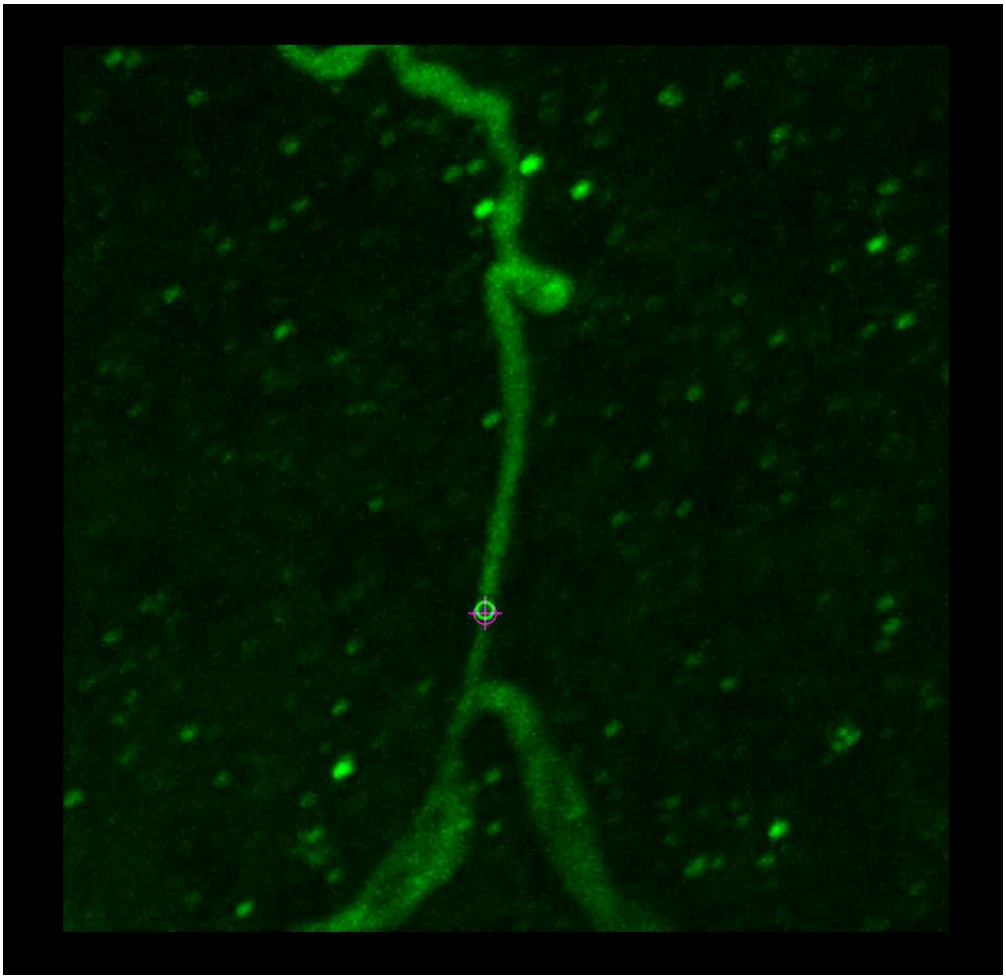
6 In the top right-hand menu, click on **Subvolume**.




7 Move the **Subvolume** to the desired start region of the nerve ending.

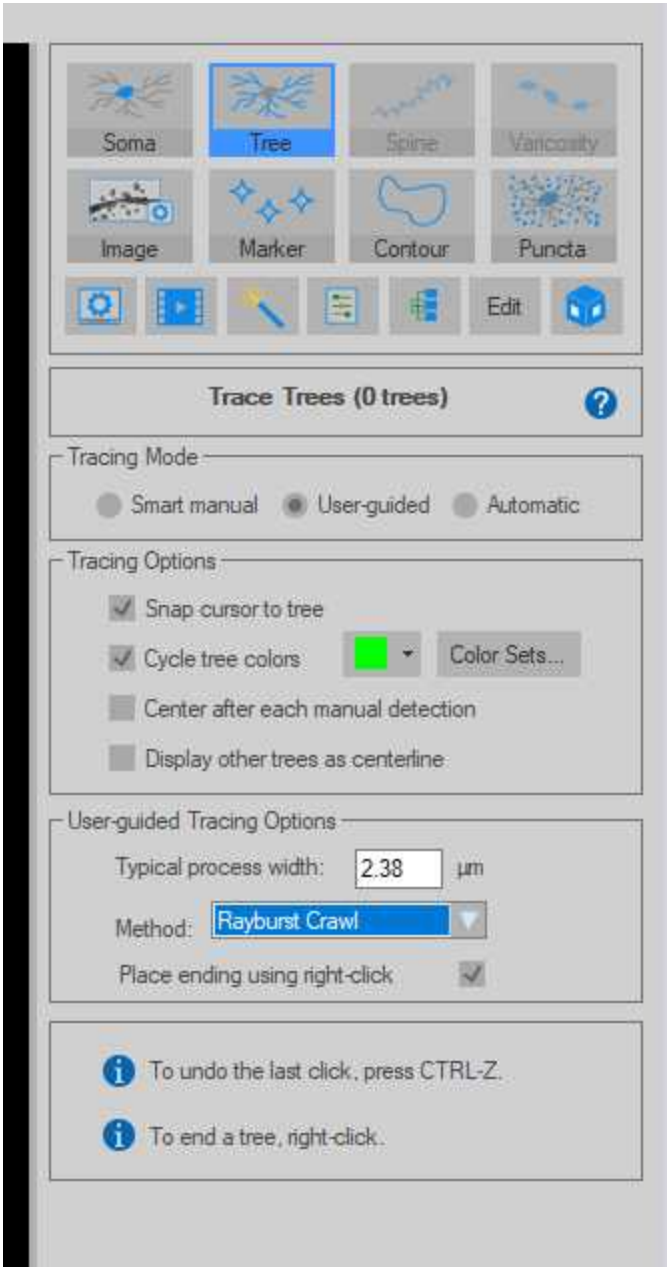


8 Click **View Subvolume** to view only the region selected.



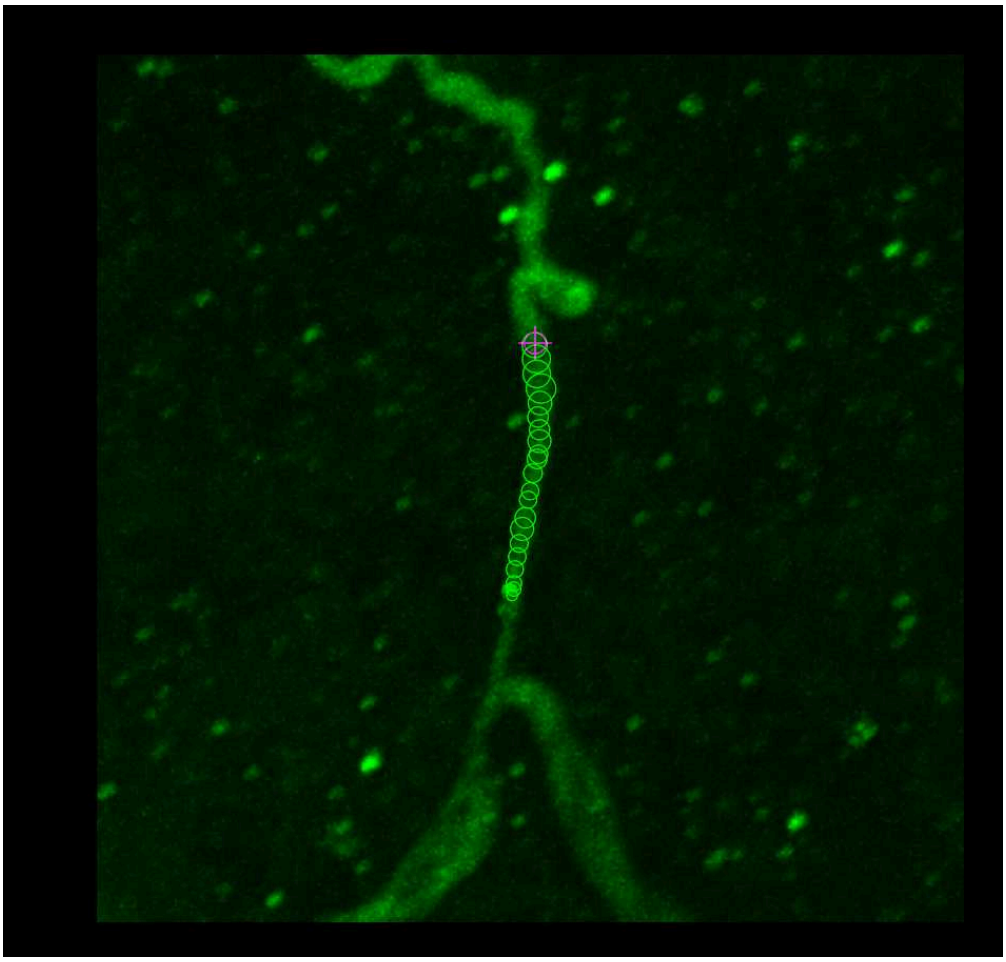
## 3D tracing of nerve ending

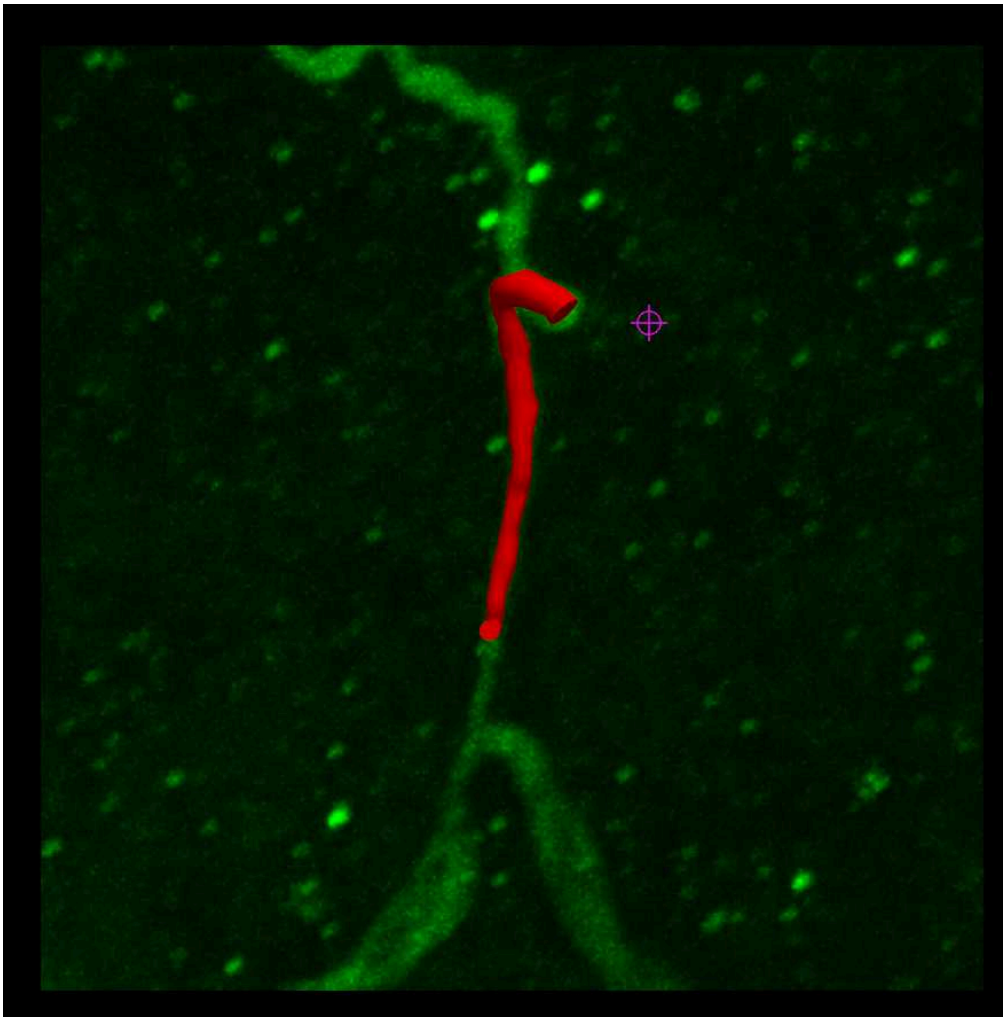
9 Using the channel in which the AAV-PHP.S fluorescence was acquired, start the tracing by clicking on **Tree** in the right-hand side panel. Select **User-guided** and under the **User-guided Tracing Options** select **Rayburst Crawl** as the **Method**.



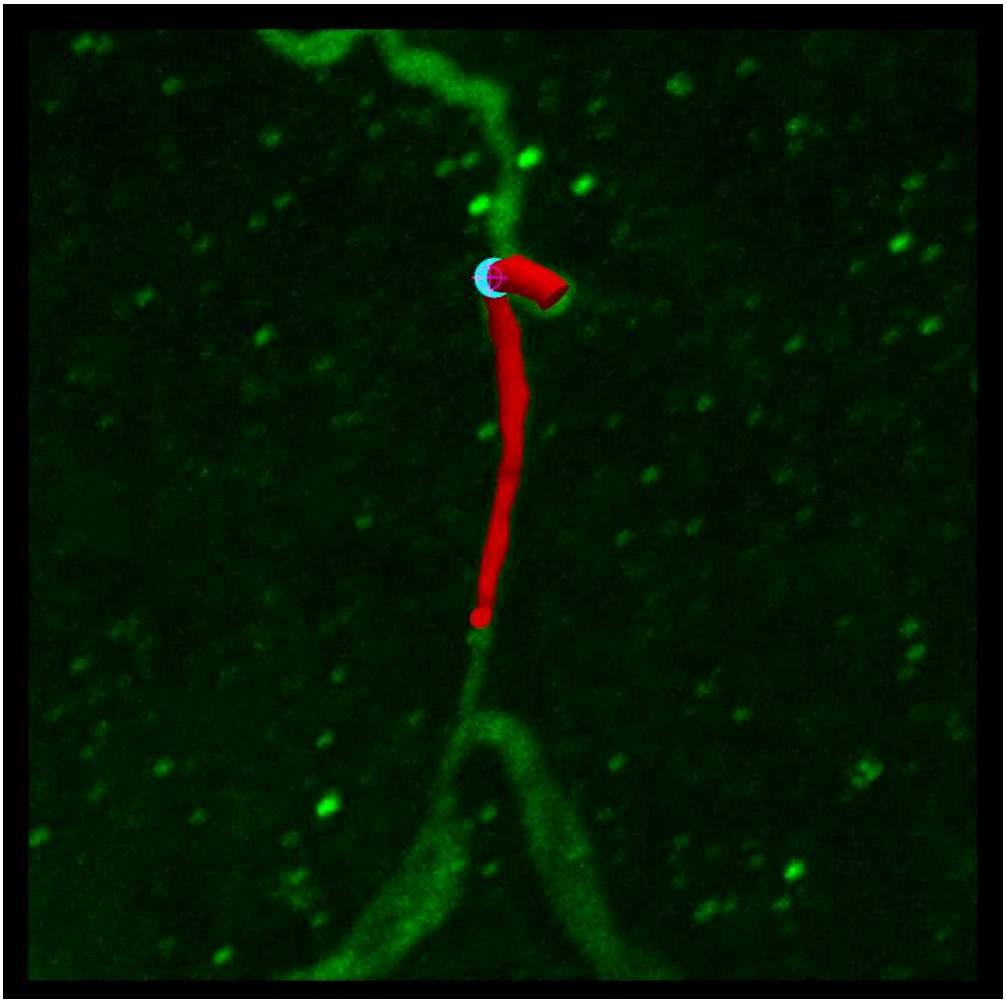

10 Move the cursor onto the desired axon to be traced in the **Subvolume** image stack, a small circle should appear on the axon of approximately the same diameter as the nerve ending. This is the starting point of the tracing. **Left click** to start tracing.

11 Move the cursor along the nerve ending. A string of circles of the axon diameter should follow the cursor, these are individual points of the nerve ending tracing. Go as far as possible without the points deviating from the axon. **Left click** to confirm points, current position will become the new start position. **Right click** ends the tracing after positions are established.

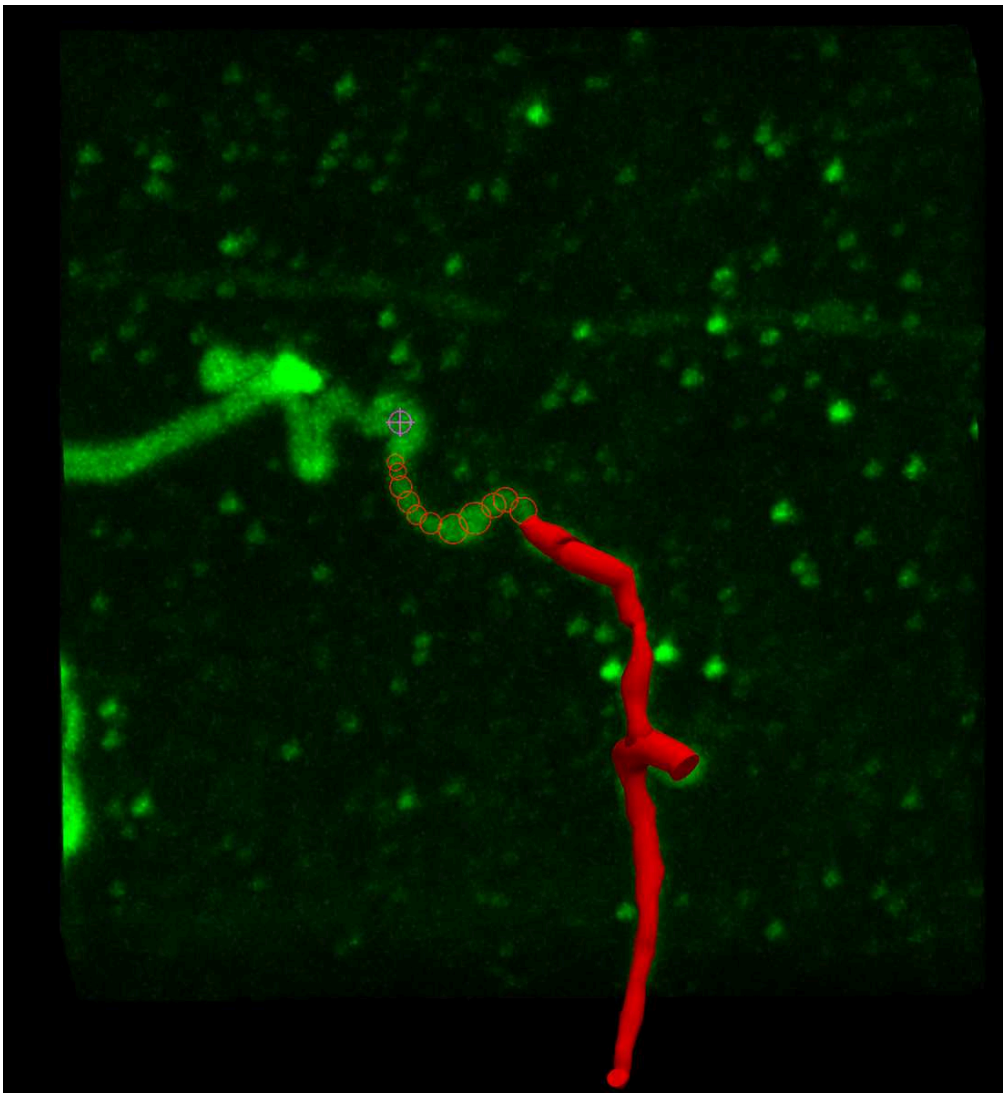




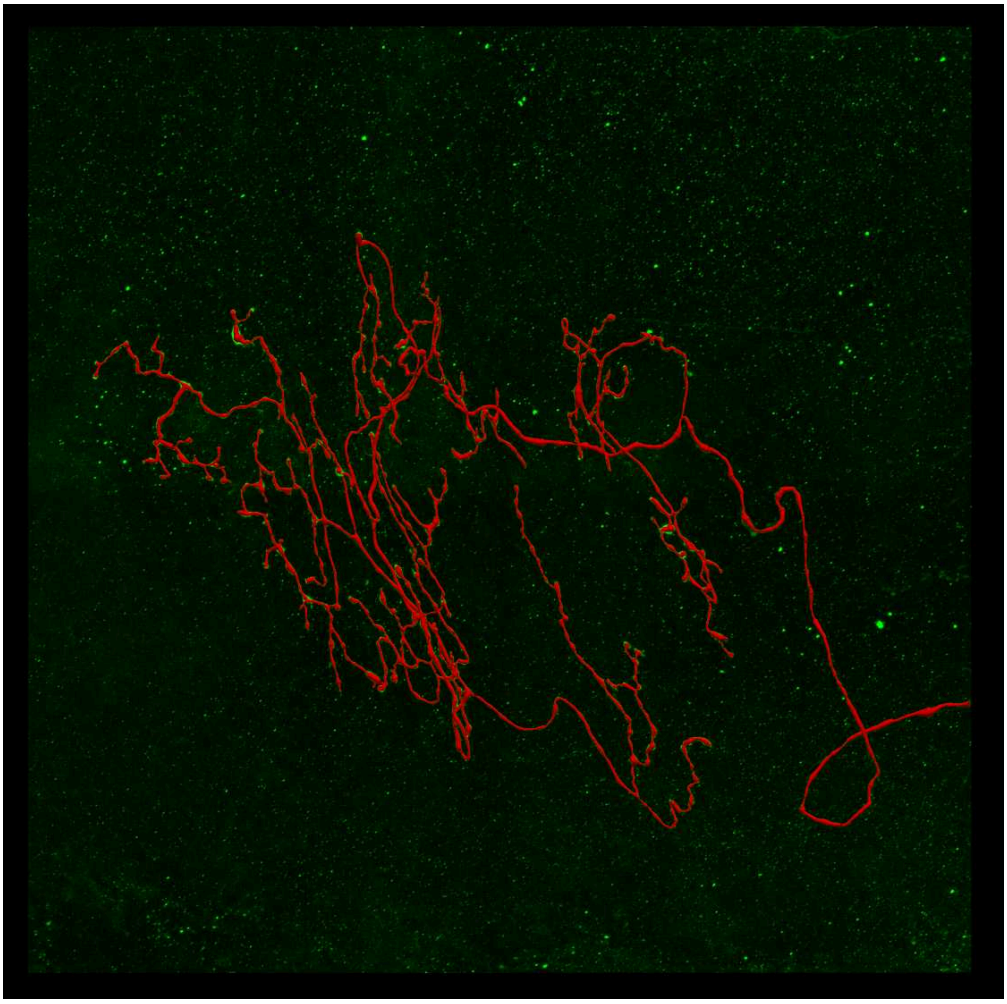
12 For branches, hover the cursor over the branch point of the currently traced nerve ending until a 3D point is highlighted. This can only be done after ending a tracing with a **Right Click**.



13 **Left click** on the branch point to start a connected branch, and continue tracing like in Step 11.



14 When the tracing approaches the edge of the **Subvolume** region, the **Subvolume** region will automatically shift in the direction of the tracing to allow for seamless tracing of nerve endings without the need to view the entire image stack. Continue tracing until the end of all the nerve endings, adding branches where necessary.



## Protocol references

Wiedmann, N.M., Fuller-Jackson, J.-P., Osborne, P.B., Keast, J.R., 2024. An adeno-associated viral labeling approach to visualize the meso- and microanatomy of mechanosensory afferents and autonomic innervation of the rat urinary bladder. The FASEB Journal 38, e23380. https://doi.org/10.1096/fj.202301113R