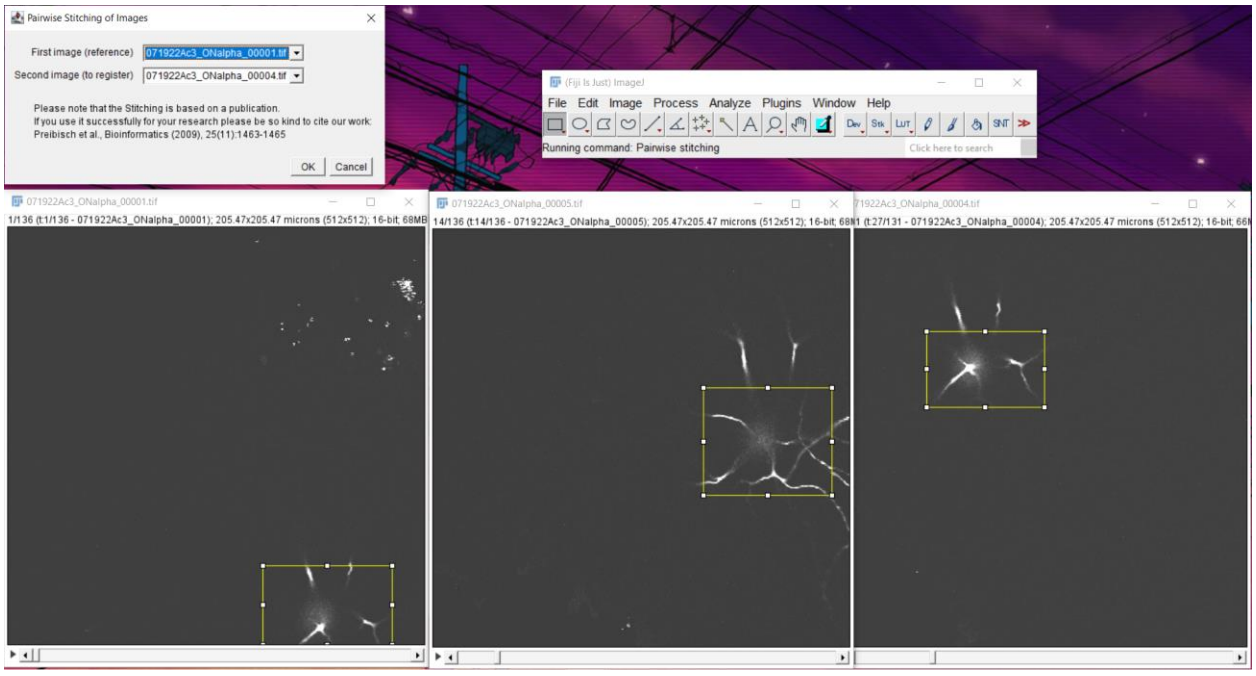
Initial Setup

Plugins that need to be with Fiji/ImageJ:

1. SNT (Simple Neurite Tracer)
2. DeInterleave
3. Pairwise Stitching

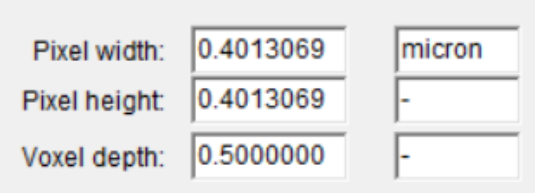
Loading Image into Fiji

1. Open the .tif file on Fiji (ImageJ). Your image likely has 3 channels joined together, which need to be separated.
2. Separate channels:
    a. On the toolbar, search for the DeInterleave plugin
    b. When asked “How many channels?”, input 3. (Note: You may close the windows of the channels not containing the neuron of interest.
3. If you have multiple images of the same neuron that needs to be stitched together, repeat the steps above until you have all the separate images ready.

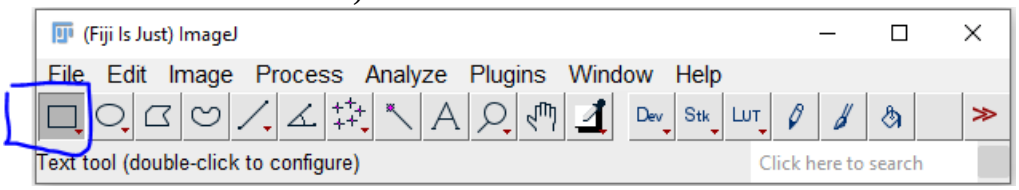


Stitching

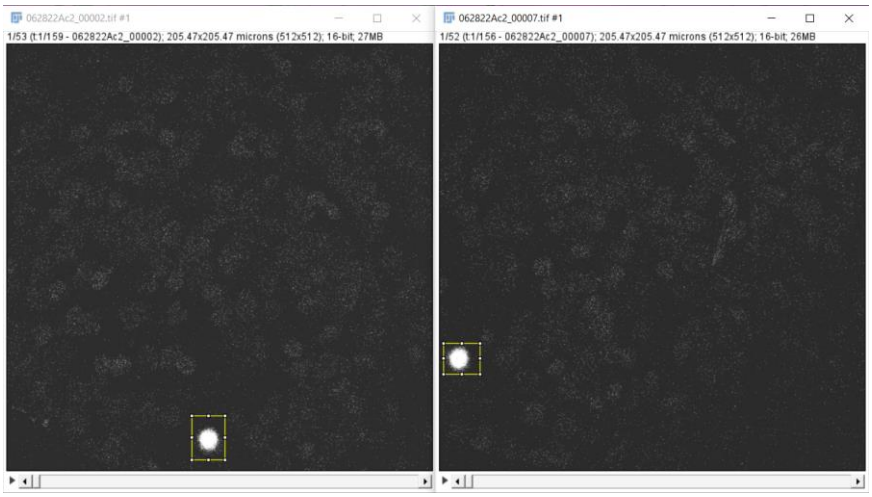
1. On the toolbar open Image -> Properties then copy the Pixel width (same as height) and change the Voxel depth to 0.5.



2. Stitching involves two images at a time. Remember to change the image properties to the same Pixel width, height, and Voxel depth every time.
3. Using the rectangle tool on the toolbar (selected by default), enclose the soma on both images at around the same layer. (NOTE: After you have stitched the image, Put the scaling back by looking Image -> Properties for the original pictures. Then go to the new stich image. Put pixel size correctly, set the unit correctly to , and voxel size 0.5. DO DIS BEFORE ANY TRACING)



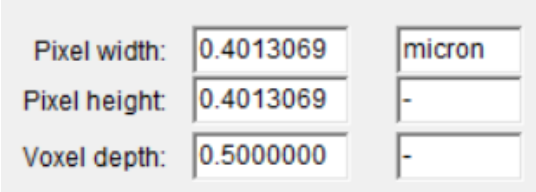
4. Search for the Pairwise stitching plugin (where it says ‘click here to search’) and select those two image files and click ok.
5. A new stitched image will be created, but you will need to input the correct dimensions again (the Pixel value and unit as micron). Stitch until you have a completed image.



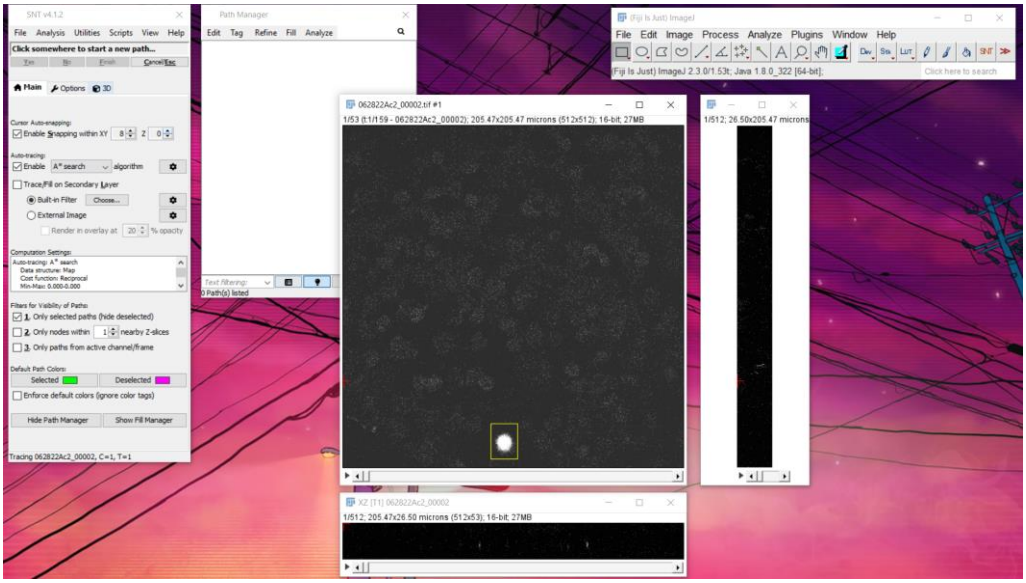
6. Spilt the stitched image into components. Save the images into filenamecellname_cell.tif and filenamecellname_chat.tif

Tracing

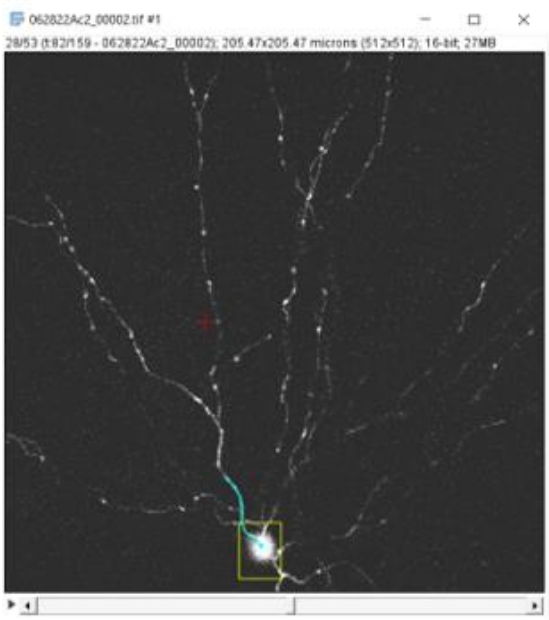
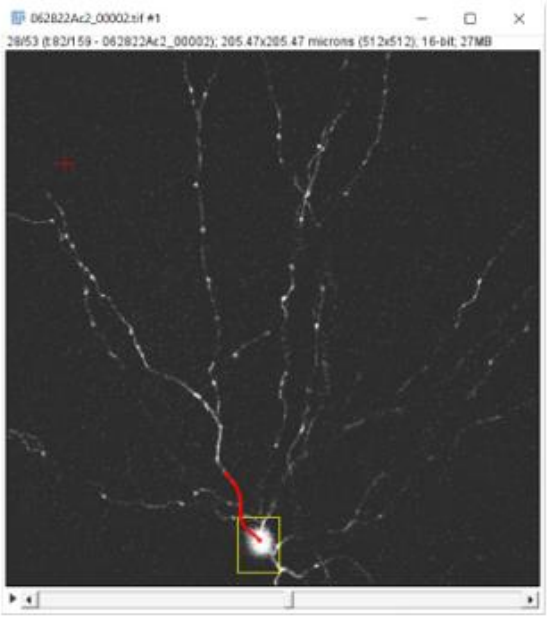
1. If you did not need to stitch multiple images, ensure that your voxel depth is 0.5.
    a. On the toolbar open Image -> Properties then copy the Pixel width (same as height) and change the Voxel depth to 0.5.



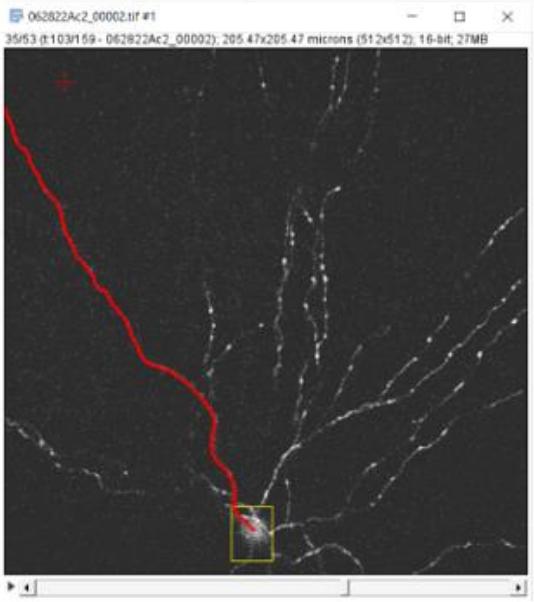
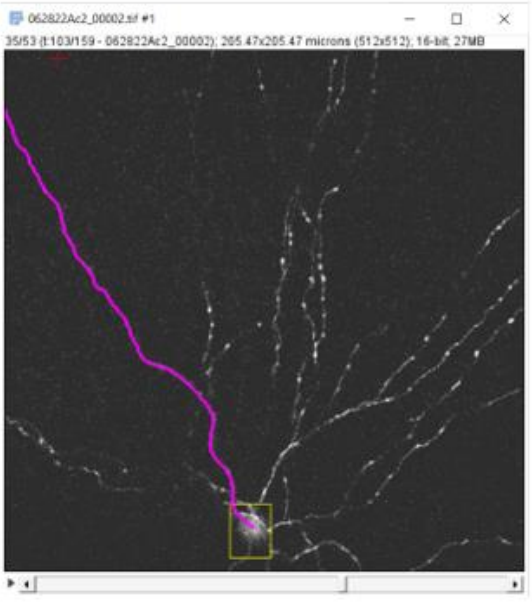
2. Select the window of the .tif you want to trace and search for the SNT plugin.
    a. An SNT menu will open, click SNT
    b. Click OK
3. You'll have a window setup that looks similar to the image below.



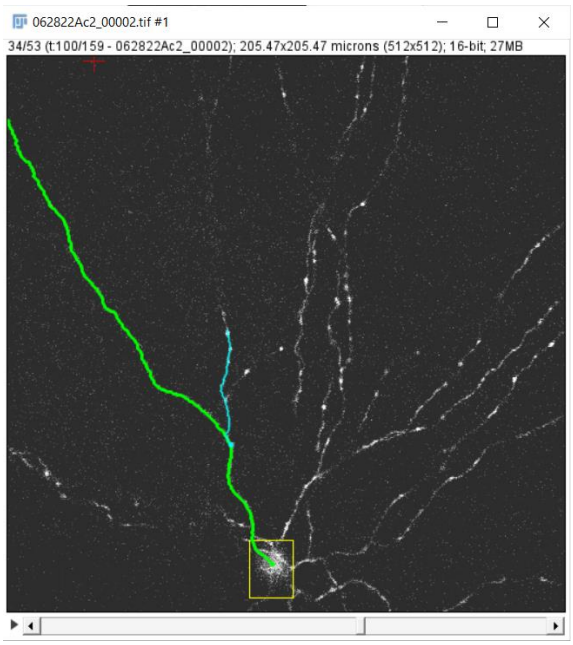
4. You can now begin tracing your image.
5. You want to trace from the soma outwards, so your first left click will be approximately in the middle of the soma. (NOTE: You want all paths to originate from ONE point, otherwise you will end up with multiple .swf files. You should only have ONE .swf file when you are saving your trace).
6. From the first point, click on a dendrite (you may need to scroll through the layers). A path will automatically trace between the first and second points. Then press "y" to confirm that's the right path. It should turn red.

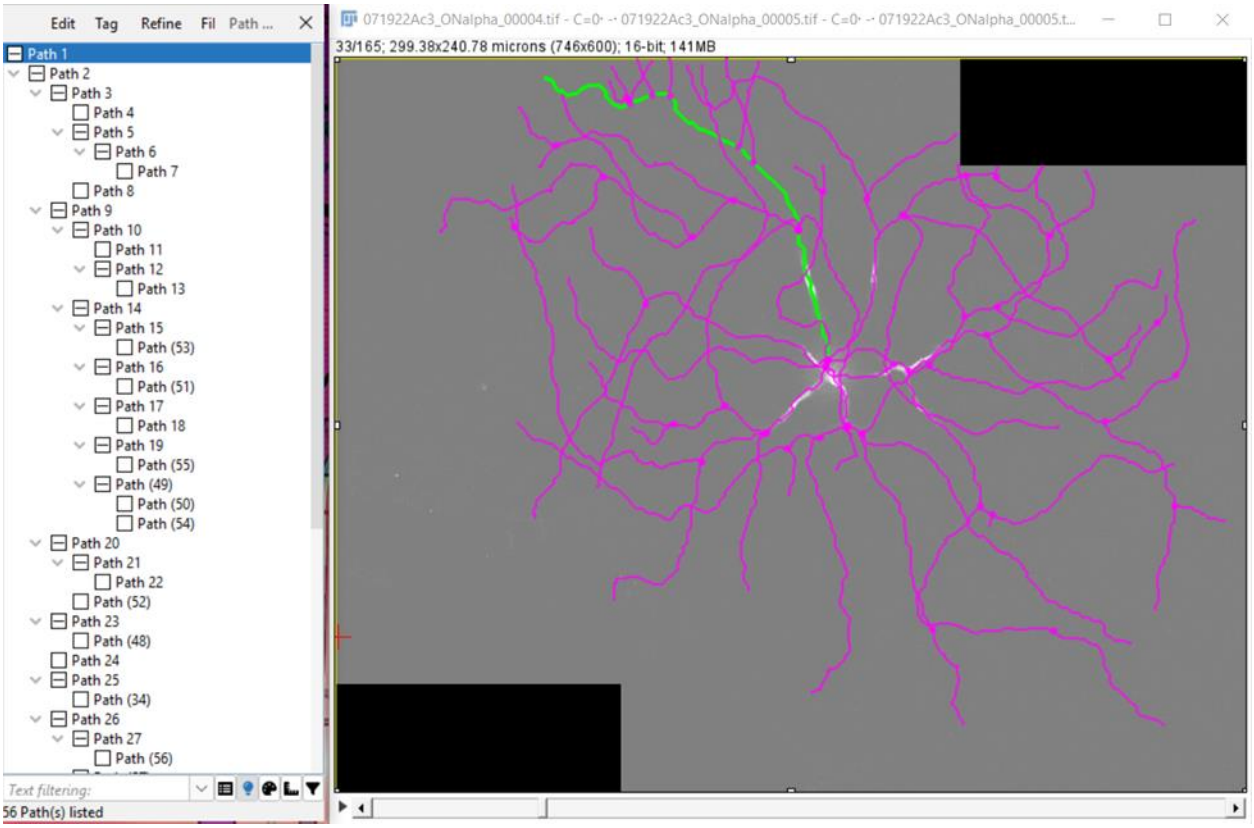
7. Keep clicking outwards, further down the dendrite, pressing “y” after each selection.
8. After tracing a branch to the end, click “f” to finalize that selection. It should turn purple.

9. Now that you’ve finalized the branch, you can fork branches off of it. You can select the branch closest to your cursor by pressing “g”. The selected branch should turn green.
10. Hold alt and click at the start of a fork to begin appending a new branch.



11. You should keep forking off a branch, never starting an entirely new path. At the end of tracing, you should see all the branches stemming from each other. You can check the Path Manager and see that all the paths are under Path 1.



Saving Traces

1. When you are done tracing out the whole neuron: Save Traces As -> .traces. Example: 120222Ac3.traces
2. Go to Path Manager -> Analyze -> Skeletonize
3. Save skeleton under filenamecellname_skel.tif. Example: 120222Ac3_skel.tif

Filling

1. Open raw image and traces in SNT
2. Select all paths
3. Click *Fill → Fill Out* on the Path Manager window
    a. If an error arises, create and delete a random path, then try again
4. Click "Start" and scroll through image in z for about 5 seconds
5. Click "Stop", "Store", and "Reload"
6. Select the stored fills
7. Click "Export As", then "Binary Mask"
8. On the top menu, click *Process → Image Calculator*
9. Multiply the traced image and binary mask together
    a. Click the "32-bit float" box
    b. Click "Yes" when asked to process all images
10. Convert image to 8-bit
    a. Image → Type → 8-bit
11. On the main Fiji window, click *Stk → Z-Project*
    a. Use either Max Intensity or Average Intensity
        i. If using Average Intensity, go to *Adjust → Brightness/Contrast* and click "Auto"
    b. Save as a .tif file. (Specifically, save this as your max projection and add maxProj at the end of the file name. Example: 120222Ac3_maxProj.tif

Chat Surface Finder

1. Select all paths. Save as filenamecellname.swc
2. Call CHAT_analyzer_GUI from MATLAB Command Windows.
3. Load Chat image -> Choose the CHAT image.
4. Load dendrite image -> Choose the dendrite image (the one color image).
5. Leave 'boxes per line' alone or adjust the number, depending on your image. Change "peak shift in adjacent boxed" to 18.
6. Auto detect peaks -> Compute surface. Find the two CHAT bands in the 3D surface.
7. Select box. Click on the graph to define the CHAT bands. Get the two smaller peaks. The higher peak is the soma.
8. Save surfaces. Save as chat_surfaces.mat. (Note: Make sure this file is saved under the folder of the cell you are working on).

RGC Morphology Analyzer

1. Run RGCMorphologyAnalyzer from MATLAB Command Window.
2. File -> Load skeleton image (skell.tif file).
3. Load surfaces and Load your .swc file for that cell
4. Draw surface on skeleton.
5. Select Lower surfcae to CHAT On, Upper surface to chat_off, choose Unwarp. Once it's done, it redraws as unwrap.
6. Analyze -> Stratification profile and Arbor properties.
7. Checked Bistratified for cells that are bistratified so it does analyses for upper and lower layers.
8. When you're done, click 'File' -> Save analyzed data file as arborData.mat. (Note: Make sure this file is saved under the folder of the cell you are working on).

Cell Folder

Your cell folder should look like this (have all these files), if all the above steps are performed properly.

Name	Modified	Modified By	File size	Sharing	Activity
062623Ac3.swc	July 9	Gregory William Schwartz	90.2 KB	Shared	
062623Ac3.traces	July 9	Gregory William Schwartz	29.9 KB	Shared	
062623Ac3_cell.tif	July 9	Gregory William Schwartz	68.7 MB	Shared	
062623Ac3_chat.tif	July 9	Gregory William Schwartz	68.7 MB	Shared	
062623Ac3_maxProj.tif	July 10	Gregory William Schwartz	799 KB	Shared	
062623Ac3_skel.tif	July 9	Gregory William Schwartz	68.7 MB	Shared	
062623Ac3_stitched.tif	July 9	Gregory William Schwartz	137 MB	Shared	
0626Ac3_dAC_OFFOS_00001.tif	July 9	Gregory William Schwartz	128 MB	Shared	
0626Ac3_dAC_OFFOS_00002.tif	July 9	Gregory William Schwartz	117 MB	Shared	
arborData.mat	July 9	Gregory William Schwartz	87.5 KB	Shared	
chat_surfaces.mat	July 9	Gregory William Schwartz	11.4 MB	Shared	
morph_summary.png	July 14	Gregory William Schwartz	56.6 KB	Shared	
part1.tif	July 9	Gregory William Schwartz	42.5 MB	Shared	
part2.tif	July 9	Gregory William Schwartz	39.0 MB	Shared	