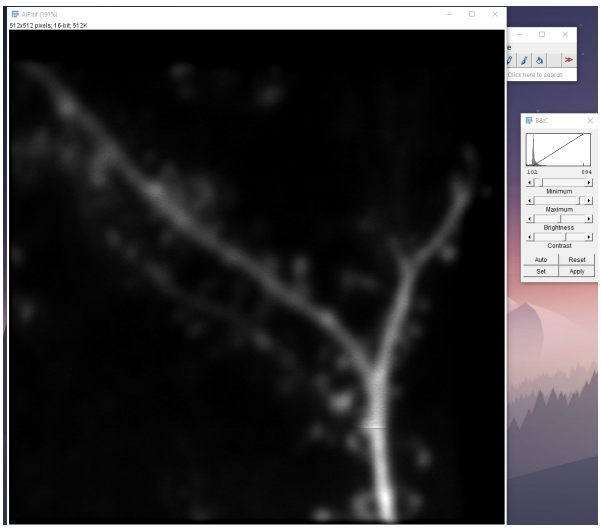
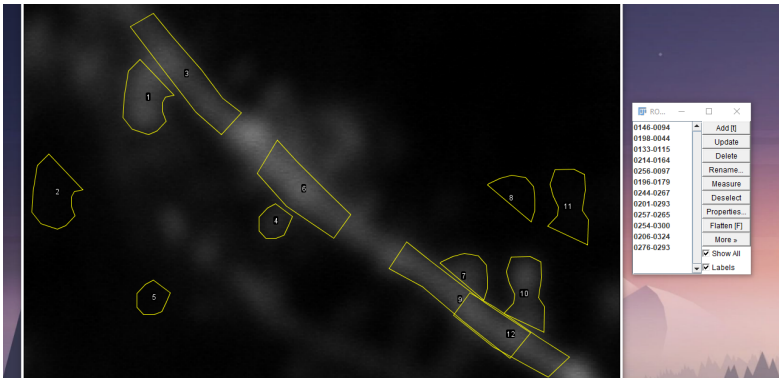
Exceprt from: **Spine Analysis Protocol. Kyle Jenks, Sur lab, MIT 2022-06-07**

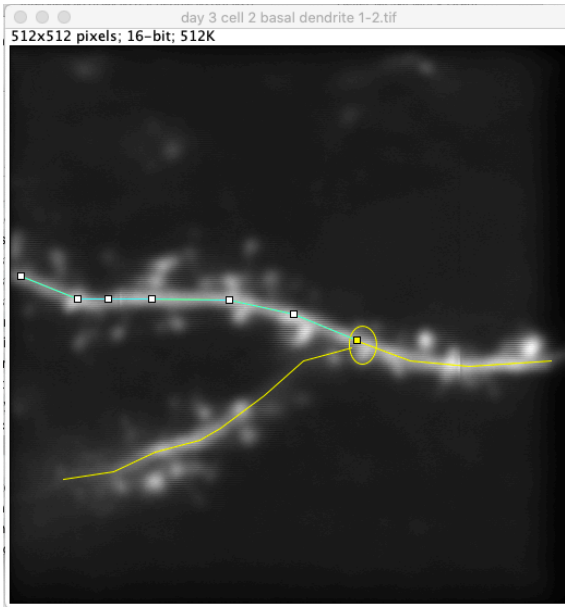
ROI **Selection** – **Spines**



1. save the average intensity projection as a ".tif " file
2. Open in Fiji or imageJ
3. Image->adjust->Brightness/Contrast and play around with the settings until you feel you can clearly make out the spine borders. Try not to overcorrect and lose dim spines. If the image quality is poor and you cannot differentiate spines from background, or if the image seems blurry, make a note and move on to the next segment.
4. Use ctrl+ or the magnify icon tool to zoom in on the left most side of the dendrite.
5. Use the "polygon selections" tool to draw a border around the first spine
6. When you complete the polygon, press "t"
7. This will add the selected (ROI) to the ROI manager
8. Drag the polygon to a nearby empty region of background
9. Press t to add the background
10. Check "show all" and "labels" in the ROI manager
11. Draw a rectangular shape on the dendritic shaft below the spine, and approximately 2-3 spine widths either side of the spine center. Draw around bright regions of the shaft, or shift the shaft ROI over. if the spine is near a branch point, please make sure the entire dendritic ROI is on the branch the spine belongs to.

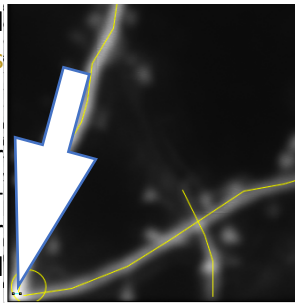


12. Press t
13. Repeat steps 12-18 for the remaining spines. Spines should be 1, 4, 7 etc. Background 2, 5, 8 etc and shafts 3, 6, 9 etc. If you lost the pattern, select the last ROI in the ROI manager and delete until you are back to where the mistake occurred.
14. When you have selected all spines you trust, go to ROI manager, More -> save to save ROIs
15. clear the ROI manager. (Select all and DELETE)
16. use the segmented line tool (right click line -> segmented line) to trace dendrite branch(es) in the recording. A drawn segment should not pass thorough any branch points. If you reach a branch point, edge of the frame or end of the visible dendrite end the segmented line ROI by ctrl clicking. (See image)



17. Press t to add to the ROI manager

18. Then annotate the next branch until all dendritic branches have been traced by segmented lines. (some recordings may have only a single branch). Be sure to annotate all branches for which you drew “dendritic ROIs” in step 11.



19. Use the oval tool to circle the branch points if they exist. This doesn’t have to be super precise. Each branch point circle should have the endpoints to 3 segment ROIs inside it - even if one of the connected segments doesn’t have any spines, or is very short or near the edge of the screen, please annotate it with a tiny line. (See arrow in the attached image).
20. Check that you have covered the dendrite and all branch points.
21. Save theses ROIs, but Append “_dend” to the filename before saving. This prevents overwriting the spine ROIs and helps identify the right file for clustering analysis. be careful not to overwrite your spine ROIs! (ROI manager, More -> save to save ROIs).
    i. “0280-0296_dend.roi"
    ii. or “RoiSet_dend.zip”
22. Close the ROI manager, results window, and -Ch2.tif (don’t save the movie)
23. Repeat for remaining segments

Notes: Do not take spines if you are unsure of their borders. Spines do not always contact the dendritic shaft, the neck may not be visible. In this case, just select the visible portion. Spines should not overlap with shaft ROIs, but shaft ROIs can overlap with one another.