NeuroTracker User Guides

Version 1.0 (20210118)

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Contents

Changelog

- V1.0 (20210118); Copied contents of neurotracker guide from 'UndergradRemoteProjects' document, and limited to neurotracker-specific segments only.

NeuroTracker User Guide

Warning: If you are running NeuroTracker to analyze neural imaging data (or any other of our analysis tools) and you haven't been trained on the system(s) yet, please ask for help first. Also, if anything happens during analyses that you are unsure of, please ask for assistance to ensure data integrity. Add the 'ProcessPixels' plugin to the plugins folder of Fiji to allow running the neurotracker software.

- Select Plugins –> Tracking –> NeuroTracker
  - Run your neural imaging analyses

Running NeuroTracker

- **Read the NeuroTracker troubleshooting section below when you are finished with this section.**
- Run NeuroTracker in Fiji per the instructions above.

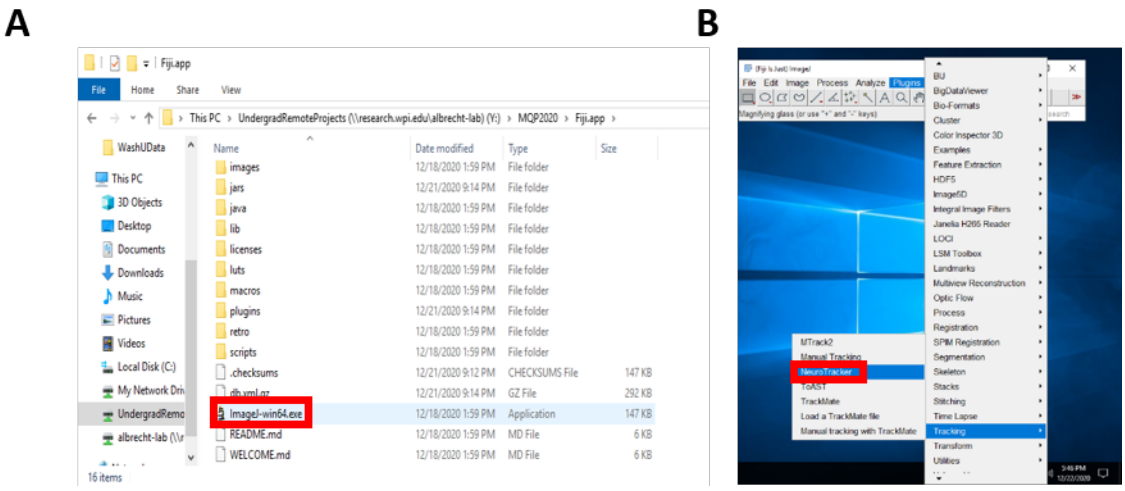

Figure 1: (A) Start Fiji using the 'ImageJ*.exe' executable present in the Fiji folder. (B) Choosing NeuroTracker to begin neural analysis.

- The first page that appears is generally one that doesn't need editing for running neural analysis on 5x objective wide-field data. Thus, if this is your predominant dataset, then simply click 'Ok' at the bottom. Otherwise, modify the parameters shown and then click 'Ok' to continue. Unless you have moving animals, leave the 'Velocity Predict' radio button *unselected* at the bottom to speed up neural analysis.
- Select the folder you want to run the neural analysis on. This folder should contain a single/series of TIFF image files taken using our epifluorescent microscopes.
- Select the range of files to analyze in this run of neurotracker. Note that this setting will run over the total number of TIFF files in the folder whatever their names happen to be. Thus, if your files have names $[0, 1, 2, 3, 4, 5].tiff$, then entering a range of $3 - 6$ will cause NeuroTracker to analyze files $[2, 3, 4, 5].tiff$. Enter the range in the format of the default, filled-in example. Select 'Ok' to continue. To analyze only one file, enter the range in the following format: $4 - 4$.

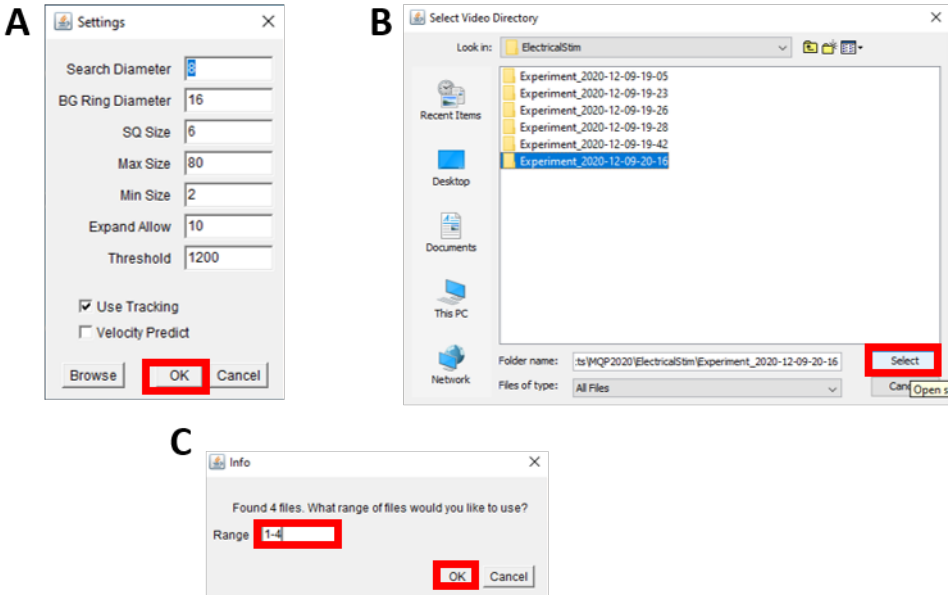

Figure 2: (A) Click through this menu for typical analysis of neural data (5x objective). (B) Select the folder with relevant .tiff files to analyze. (C) Set your range using the format shown.

- Open two Fiji windows to allow changing various settings for the analysis:
  - Image –> Adjust –> Brightness/Contrast
  - Image –> Adjust –> Threshold
- Select the radio button at the bottom of the thresholding window that says 'Don't reset range' to prevent the brightness/contrast changing when the threshold is adjusted. This is important to do every time you start NeuroTracker for ease of use.
- In the window labeled B&C, move the 'minimum' slider all the way to the left, and then adjust the 'maximum' slider to the left until the neurons of interest are clearly visible in the fluorescent image that Fiji opened. Sliding the 'maximum' slider too far left will wash out the image, and so find a setting that allows clear identification of the neurons, without extra white background.

- **Warning: The following step must be completed with the TIFF file showing the first frame of the image stack (shown at the top of the image window as '$1/n$'. For example:** $1/300$**). If the first frame is not showing, scrub the slider all the way to the left at the bottom of the window, or move your mouse wheel to move the slider accordingly.** Now work in the 'Threshold' window. If the 'Default' or 'Red' options do not appear in the window, choose these options in the multiselect menus. Select 'Dark Background' if not already selected. Move the lower slider all the way to the right, and then adjust the upper slider towards the right until the red overlay that appears on the screen just covers the first neuron that you are interested in selecting. Once the area of thresholding is correct, and a minimum of the particular animals gut is also showing, left click in the middle of the thresholded area of the neuron of interest. You will notice a thick yellow box appear around the thresholded neuron, and a number appear. The number is the 'animal number' designation that is used by NeuroTracker and MATLAB later to individually identify the animal and its associated neural imaging data that is being tracked in this run of the analysis software. *It sometimes helps to scrub the video stack forward before selecting a neuron to see if the animal moves a lot or its neuron becomes covered by something like another animal. Moving animals or animals with weird events that happen to them during one of the videos should be excluded, and you should just move on to choosing another animal.* The Log window that appears when a properly thresholded neuron is clicked indicates

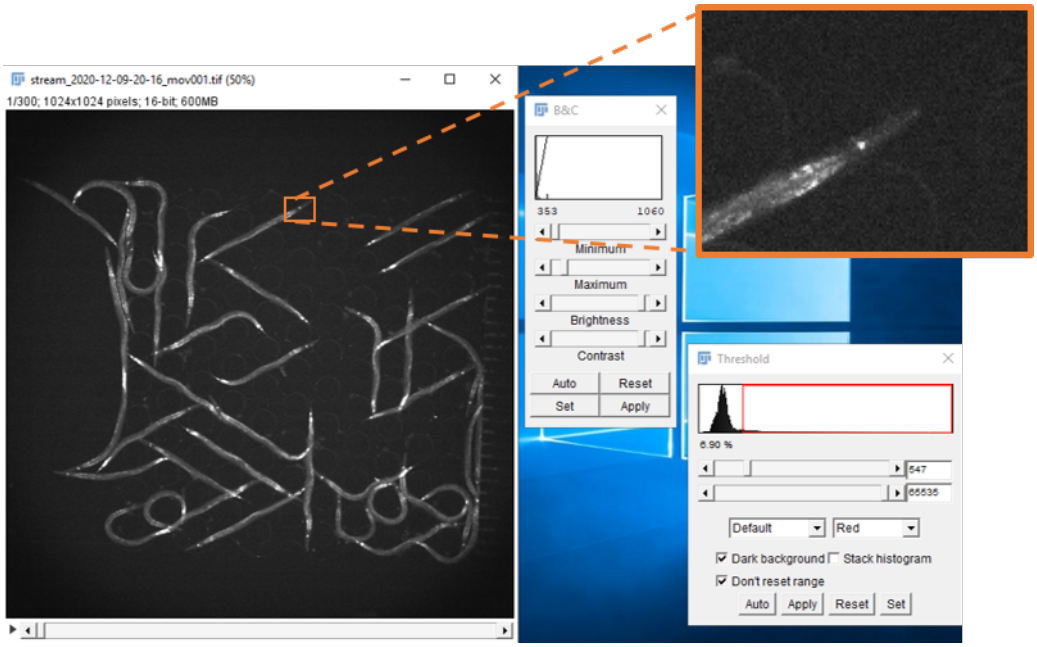

Figure 3: (A) An example of well adjusted brightness using Fiji. Note that the neuron in the head we are interested in is clearly visible and distinct from other body features. Inset image shows enlarged version of neuron properly adjusted for brightness.

the x and y position of the neuron in pixels that was selected, along with the threshold selected for that particular animal. Thresholds should be adjusted for each animal you select. If you mis-select a neuron, close the image window, click through any warning windows that appear, and start from the beginning.

- Repeat the last step for as many neurons as you wish to run in this iteration of NeuroTracker.
- When you've selected all neurons of interest, press the 'space bar' on the keyboard to begin the analysis. You must have the image window selected for this to work. Avoid clicking the inside the image window with any moust clicks as this will edit the list of positions that NeuroTracker will run on, and create false and annoying data that must be cleaned out later. NeuroTracker will go through each of the positions you've selected and automatically calculate the important parameters that define the fluorescent activity of the neuron. Once the analysis of each frame for a particular animal is finished, a graph of the background fluorescence (red) and associated neural activity (black) appears for one-half of a second, so you can confirm the shape matches what you expect. Additonally, notice that a positions file becomes present in the folder where the image stack files are present. This allows continuing the analysis if it had to be stopped at some point, though the correct starting range for the continuation of analysis will need to be appropriately chosen (see above). To continue the analysis, select 'yes' when prompted by "Positions file detected.....".

- When NeuroTracker has fully finished the analysis of the files you indicated to run at the beginning, an error window appears saying "NeuroTracker has finished/completed....". Simply select 'Ok' to exist the program. Note the creation of data text files in the folder where the video/tiff stack originally resided.

- **Read or re-read the NeuroTracker troubleshooting section below.**

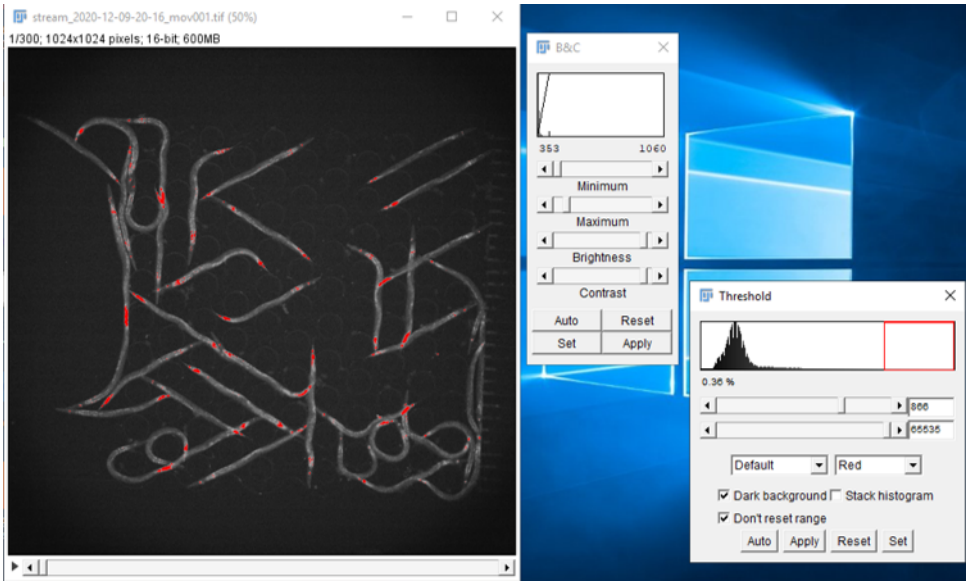

Figure 4: Threshold settings are changed to limit the areas of high fluoresence indicated by Fiji.

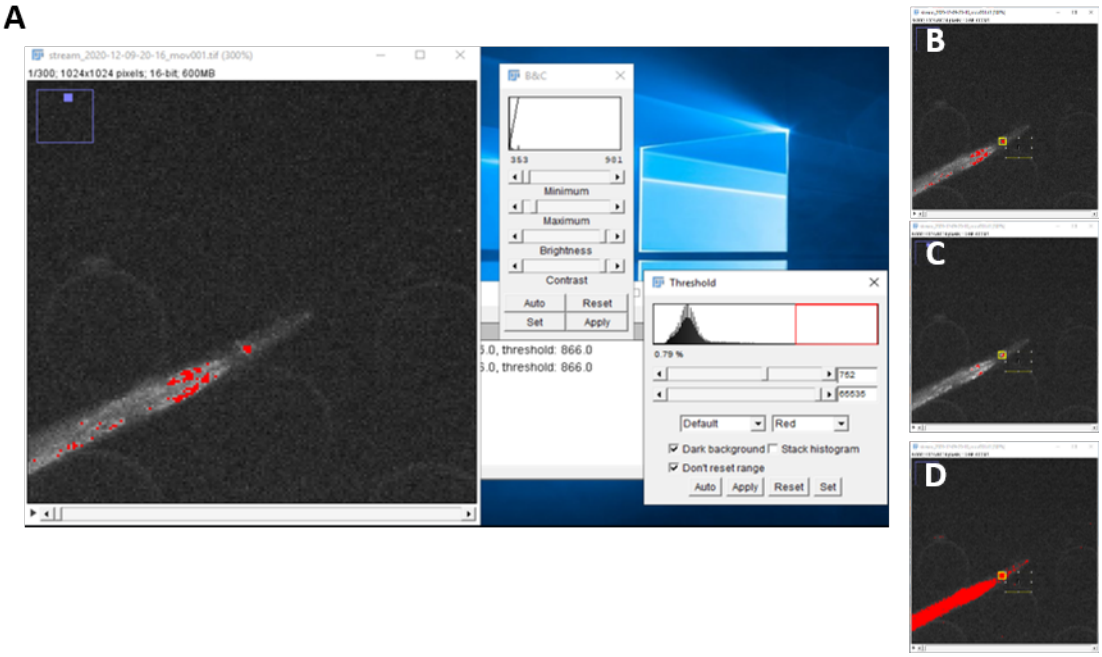

Figure 5: ((A) Enlarged image of thresholded neuron with a dense color blob over the center of the neuron of interest and no suprathreshold areas on the dendritic process leading to head. Note the separation of the thresholded neuron red area from suprathreshold regions in the gut and other areas. (B) Properly thresholded neuron. (C) Over thresholded neuron, reduce the threshold value to achieve the region characteristics of A/B. (D) Under thresholded neuron, increase the threshold to achieve region characteristics of A/B.

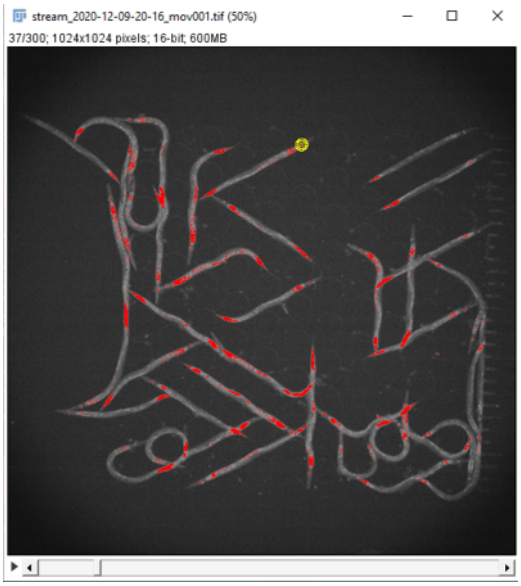

Figure 6: View of NeuroTracker when running properly.

Troubleshooting

- If the selection box for each frame of the analysis (square box) doesn't track the neuron or the analysis stops automatically ("NeuroTracker has lost track of the neuron. . . . . "), do the following. Click the 'space bar' once to stop the analysis at the current point (unless NeuroTracker does this automatically), scrub backwards (***backwards only***) in the video file using the slider at the bottom of the image window, and reset B&C/Threshold as per the section above. Left click on the neuron (the yellow selection areas will disappear and only a single red square will remain). If the yellow square/circles are obscuring your view of the thresholded neuron and you do not see the crosshairs, press the up, down, left or right arrow keys to move the yellow squares off of the neuron of interest. Once the neuron is re-thresholded is selected, click the 'space bar' on the keyboard to continue.

- If for any reason you do not wish to include an animal for any reason, you can remove this animal from the final manipulated dataset in MATLAB by pressing the 'Q' key on the keyboard once. You will notice the analysis stop for around a quarter second as the selection is registered, and then the analysis will continue. Note that this will not remove the animal from being analysed in subsequent videos, but sets a value in the output text files which is recognized by the MATLAB data import script.
- If a tracking error occurs at the end of tracking the frames for a particular animal, but you wish to preserve its neural data, restart the analysis for that particular video, and overwrite the data for the other neurons until you get to the animal that caused the error. Adjust B&C/Threshold until the animal's neuron is correctly analyzed, and then let NeuroTracker continue the analysis as normal.
- If errors occur during analysis, it is always better to restart analysis on the video that caused the error (changing B&C/Threshold) than try to fight with the analysis at whatever point the error is occurring.
- Do not select animals that may move out of frame, are moving considerably, have two distinct neurons showing up when thresholded, or have another animal that moves overtop of them or causes them to move. All of these situations lead to data corruption and errors in the final analysis.
- Do not select animals that when thresholded present two side-by-side neural spots that are suprathreshold. This will cause needless noise in the data as the computer attempts to stick with the highest activity region.

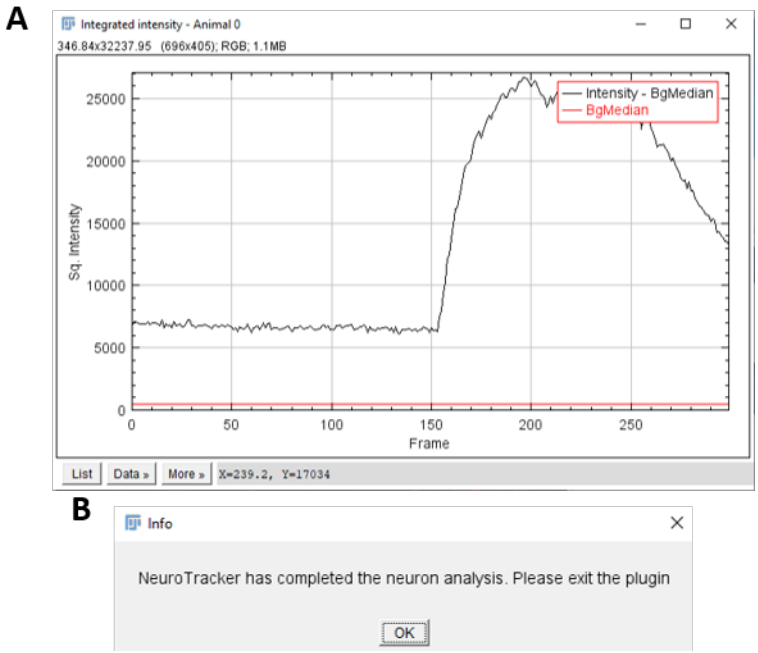

Figure 7: Graph of neural activity as analyzed for an individual animal by NeuroTracker. This view appears briefly before the program continues with the analysis. Note the smooth nature of the curve from the data recorded. Rapid spiking is not characteristic of calcium activity, and indicates movement errors.

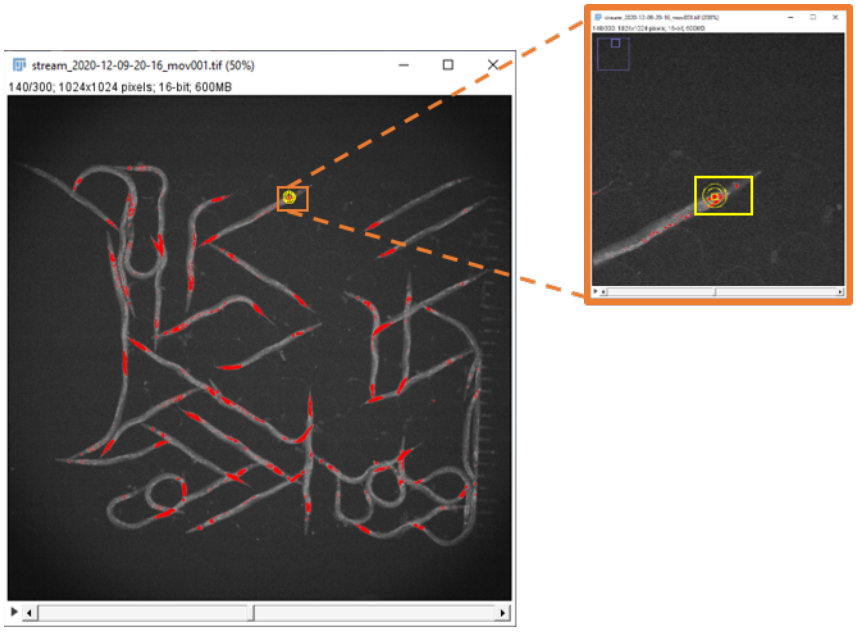

Figure 8: Example of automated ROI that is no longer tracing the neuron correctly. This ROI must be reset to be over the neuron for data to be accurate and correct. Inset image shows enlarges view.

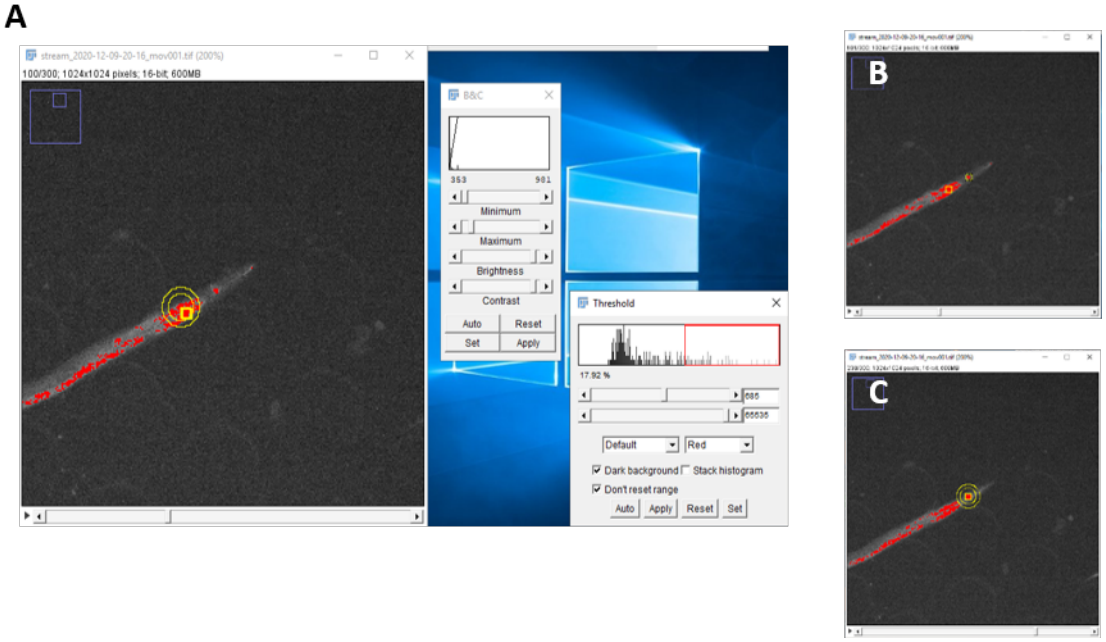

Figure 9: (A) Enlarged view of mistracked neuron upon re-thresholding the image. (B) The center of the suprathreshold region of the neuron of interest in re-selected in this view with a single left mouse click. (C) Resumption of correct neural analysis after computer ROI error.