



Quantification of Neurite lengths How To

1. - *Obtain a sample image sequence.* Download and decompress the “Phase Contrast Image Sequence” sample from the Validation Data, in the NEURITE web page.
2. - *Start ImageJ and open image sequence.* Start ImageJ and open the sequence by doing a import sequence with function "File > Import > Image sequence" and select one file of the sequence.
3. - *Run Neurite Tracing plug-in.* Open "Neurite tracing" plugin selecting the option “Plugins > Neurite > Neurite Tracing”
4. - Select the minimum and maximum neurite width in the sequence. In this case select 3 as a minimum width and 4 as maximum width. These values represent the average minimum and maximum neurites thick in pixels.
5. - *Select a reference frame and perform the segmentation in this frame.* Select the first frame in the sequence as a reference frame. Next press the “Segmentation” button ( Segment) in the “Neurite Tracing” *plug-in* window to do the segmentation process. This operation perform an eigenanalysis in the image, this is necessarily to trace the neurites in the reference frame.
6. - *Trace the desired neurites in the reference frame.* Select the “draw neurite” tool ( Draw Neurite) from the *plug-in* window, later do a mouse click in the beginning of the primary neurite near to the neuron stump (figure 1a), follow the neurite shape with the mouse and click in the final of the neurite right in the growth cone (figure 1b). In the same way, trace the one of the two second neurites; first click in the bifurcation with the primary, follow the shape with the mouse and do a double click in the end of the neurite (figure 1c) to set free the trace. Finally trace the last neurite (figure 1d).

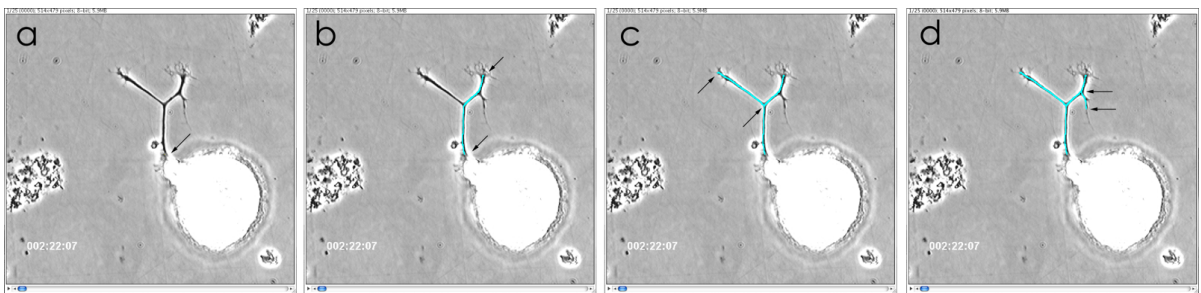


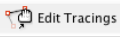


Figure 1. Tracing neurites. A) The beginning of the primary neurite. B) The primary neurite traced showing the beginning and ending point. C) One of the secondary neurites traced showing the beginning and ending points. D) The other the secondary neurites traced showing the beginning and ending points.

7. - *Edit neurite information.* Do a double click in one row of the result window; a small popup window must be appearing there you can edit the neurite information (name, type and color). When you edit the data with the adequate information press the “Accept” button.

8. - *Perform an automatic trace frame by frame in the following images.* Select the reference frame in the sequence (the three traces must be appear over the neurites), latter select the three rows corresponding to the traced neurites in the “Result Window” and finally press the “Next Single Image” button () in the *plug-in* widow.

The *plug-in* trace automatically the neurites in the next frame in the sequence. You should note that three other rows appear in the “results window” and these are selected. Also the three neurites are traced over in the frame two. To automatic trace the neurites in the next frame you only need to press the “Next Single Image” button () again, because the current traced neurites are already selected in the “Results Window”. In order to follow this tutorial repeat this process five more times until the frame number eight in the sequence.

9. - *Edit a neurite tracing.* At this point you should be in the frame eight in the sequence, only two neurites are traced (because one disappears) and the frame eight corresponding rows selected in the “Results Window”. The primary neurite change the direction and a new neurite appear (figure 2a). To follow the primary neurite you must be edit this neurite trace, this can be done by selecting the “Edit tracing” tool () in the *plug-in* window. Once you select the tool, place the cursor next to the end of the trace corresponding to the primary neurite, when the trace change the to yellow color do a click (figure 2b), now you can edit the trace; move the mouse to the desired end of the neurite and double click with the mouse to set free the trace (figure 2c).

The traces can be edited selecting the final point (same as you do before) or selecting the beginning point, this fix the final point and you can edit the trace.

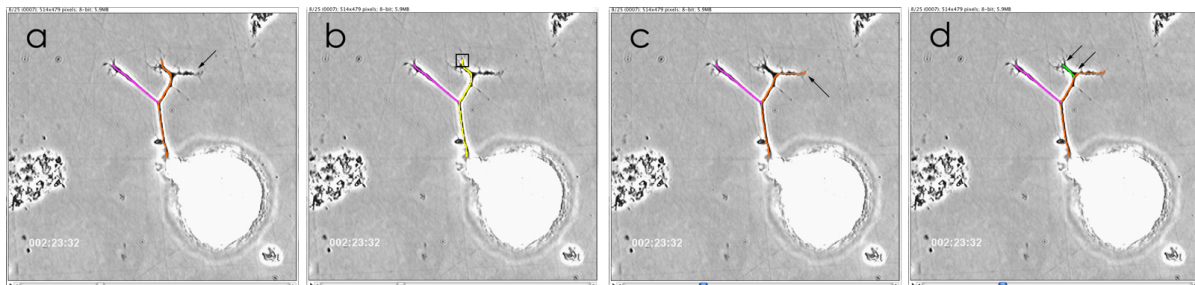
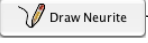






Figure 2. Edit and add neurites. A) Erroneous tracing of the primary neurite, arrow shows the right direction. B) The primary neurite in editing condition the black square indicate the specified area to make the edit click. C) The edited primary neurite, the arrow show the new position of the ending point. D) The new secondary neurite traced, arrows show the beginning and ending points.

10. - *Add a neurite in the middle of quantification.* After editing the primary neurite (step 9) you can add a new secondary neurite to the sequence quantification, you need to select the “draw neurite” tool () and trace the new secondary neurite doing a click in the beginning of the neurite right in the bifurcation, follow the neurite shape and finally double click in the end of the neurite (figure 2d).

The new neurite are traced, and one new row appear in the “results window”, the information in the row can be edited follow the directions of the step 7.

11. - *Perform an automatic trace in a section of the sequence.* In this point you should be in the frame eight in the sequence, with three traced neurites. Go to the end of the “Results Window” and select the three rows corresponding to the number eight frame if they are not. Select the number of frames to trace the neurites in the spinner () next to the “next section images” button () , in this case put 17 frames. Finally press the “next section images” button () to start the automatic tracing.

If in one moment of the automatic tracing process the traces made by NEURITE are erroneous or you are not satisfied them, you could stop the process at any time pressing the “stop process” button () , and later edit the traces follow the directions of the step 9.

12. - *Save Tracings.* Finally you can save the traces for a later inspection, adjustments or edit traces. In order to save the neurite traces go to the File menu in the *plug-in* window and select the “Save Traces As” option, a file chooser window appear, in there you should select the folder and the name of the file and press the save button. A .ntf file is created with the assigned name.

13. - *Export neurite lengths.* To analyze the neurite lengths you can export the neurite lengths to a Excel file. To export the tracings you should select the “Export Lengths to Excel” option in the “File” menu of the *plug-in* window a file chooser window appear, in there you should select the folder and the name of the file and press the save button. A .xls file is created with the assigned name.