

Neurite-J 1.1

ImageJ plug-in

User manual

October, 2014

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1. About Neurite-J 1.1

Neurite-J is an ImageJ (<http://rsbweb.nih.gov/ij/index.html>) plug-in that works as an adaption of the Sholl method providing a semi-automatic tool for neurite outgrowth quantification in organotypic cultures. Neurite-J gives a good description of neurite growth providing counts of neurite number and neurite area at different distances from the organotypic explant. Neurite-J 1.1 is the second available version of the Neurite-J plug-in for ImageJ software (<http://rsbweb.nih.gov/ij/index.html>). This version of the plug-in works under ImageJ 1.49 versions for Mac Os X, Linux and Windows systems (later versions have not been tested). Neurite-J is developed in the Neuroplasticity and Repair Group of the University Autonomous of Barcelona (UAB) by Dr. Abel Torres Espín.

The validation of the measures obtained by the plug-in are published in:

Torres-Espín A, Santos D, González-Pérez F, Del Valle J, Navarro X. Neurite-J: An Image-J plug-in for axonal growth analysis in organotypic cultures. J Neurosci Methods. 2014 Oct 30;236:26-39. doi: 10.1016/j.jneumeth.2014.08.005

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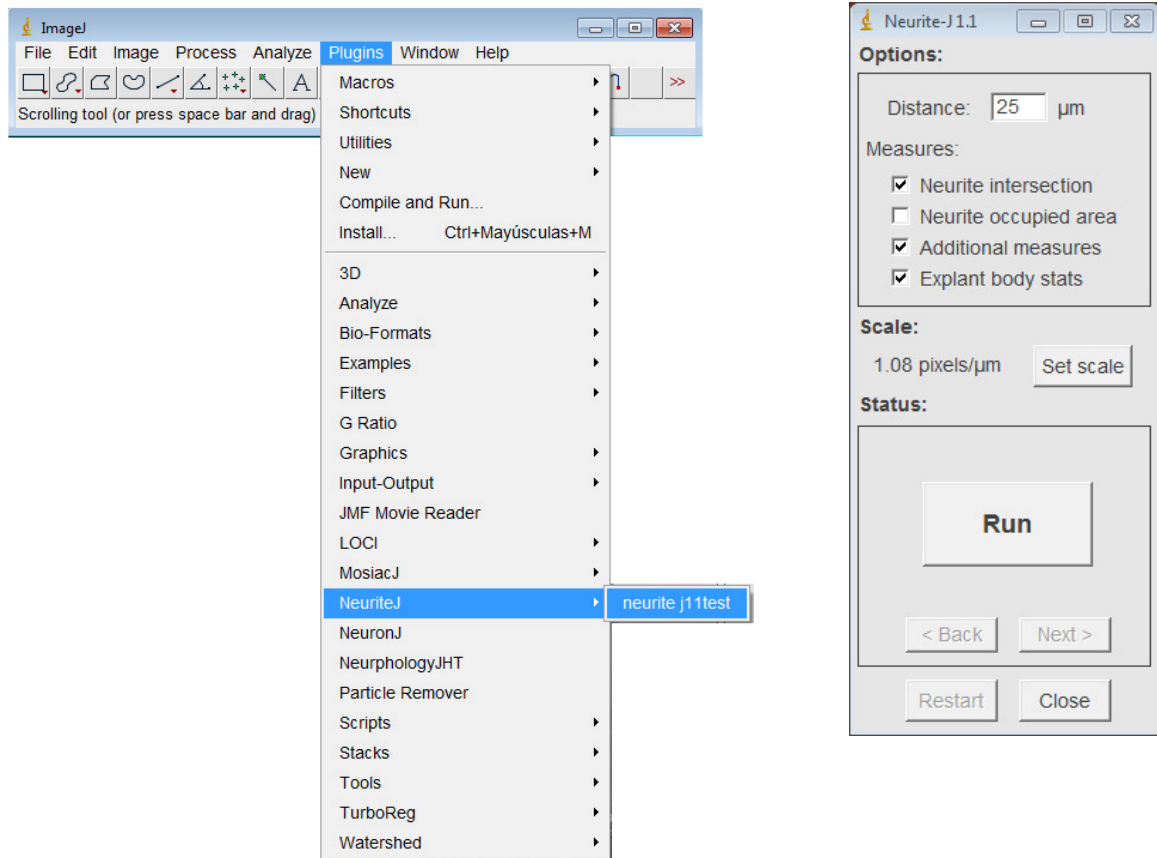
3. Neurite-J installation

Download the Neurite_J11.java file into `.../ImageJ/plugins/` folder. We recommend to create a NeuriteJ folder and save the file inside. To install Neurite-J use the *Plugins>Install...* or *Plugins>Complie and Run* command in ImageJ interface and select the Neurite_J11.java file.

4. How to use Neurite-J

4.1 User interface

Open Neurite-J using *Plugins>NeuriteJ>Neurite J11* command. The user interface will appear:



Options Box:

Distance Textbox: the distance in the specified units that plug-in will use to radial increase of Sholl rings.

Measures Checkbox: the plug-in provide two possible measures, the number of intersections at each distance from the body and the occupied area by the neurites for each ring. The plug-in can return additional information from intersection profiles, such as the Nmax, the critical value and the Dmax (Additional measures) or the area and the circularity of the explant body (Explant body stats) (see Torres-Espín et al., 2014).

Scale Label: show the current scale ratio specified by the user. If no scale ratio is provided, the ImageJ take the original scale of the current picture.

Set Scale Button: use this button to open the set scale ImageJ dialog windows and specify the pixel to real unit conversion. The conversion ratio will appear in the Scale label inside Neurite-J interface (see above).

Status Box: show the messages guiding the users during the image analysis process. By default appears the **Run Button** that starts the image processing.

Back Button: return to previous step in the image analysis.

Next Button: goes to the next step in the image analysis.

Restart Button: return to original image and restart the plug-in if it is pressed in any moment during image processing.

Close Button: close the plug-in. Notice that also it is possible exit to Neurite-J using the close button in the window title bar of the plug-in.

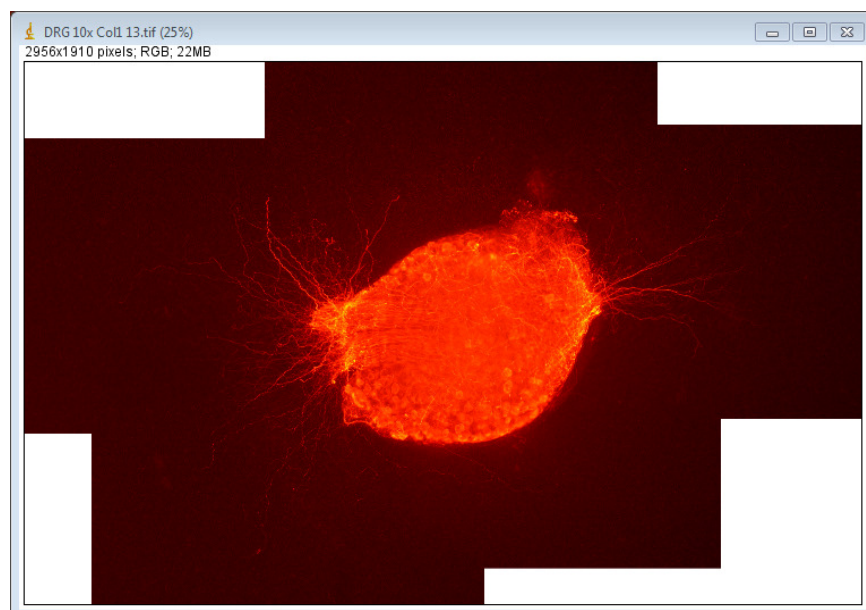
4.2 Before start

Neurite-J can work with any type of image file that ImageJ can open. Neurite-J is developed to work with pictures obtained by microscopy. Typically, to take all the axonal outgrowth from an organotypic culture some pictures are needed. In this case, all the pictures should merge into a mosaic prior to be analyzed with Neurite-J. Don't worry if some parts of the mosaic image are white, the Neurite-J will remove during image processing.

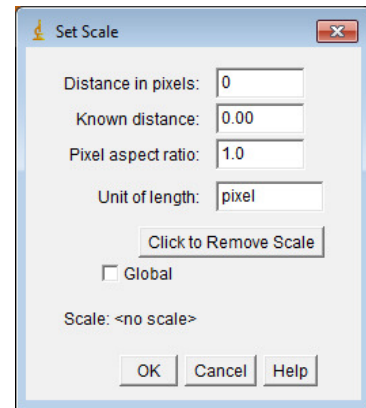
4.3 Image analysis with Neurite-J

To ensure the correct analysis of the pictures, the Neurite-J works sequentially. All the steps that need user interaction will be notified in the status box (see above).

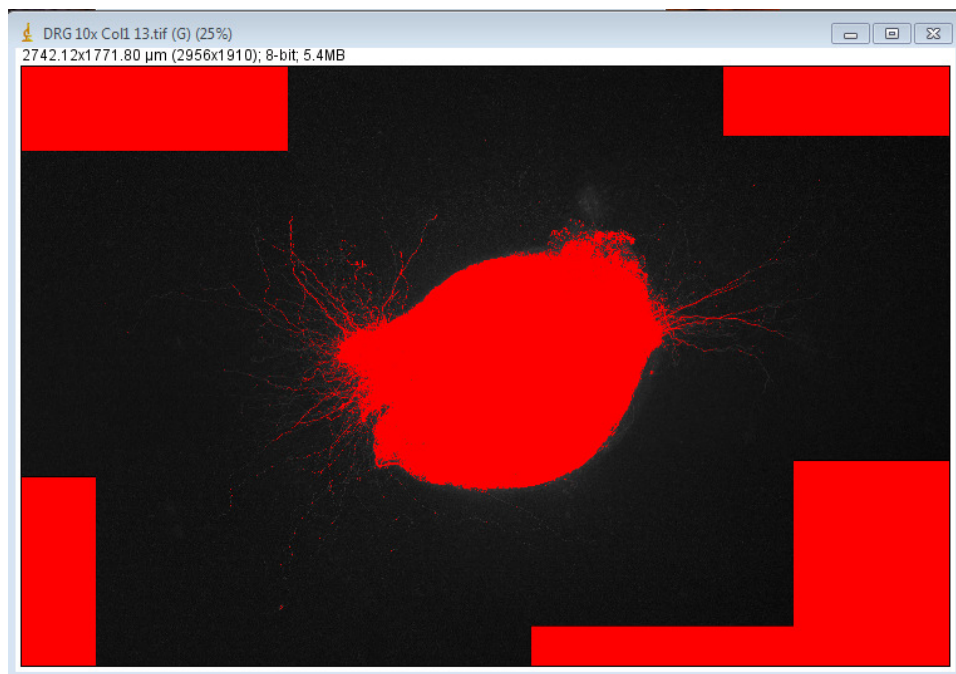
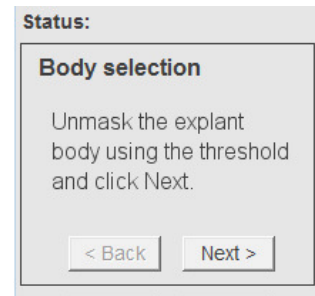
Step 1. Opening the image.

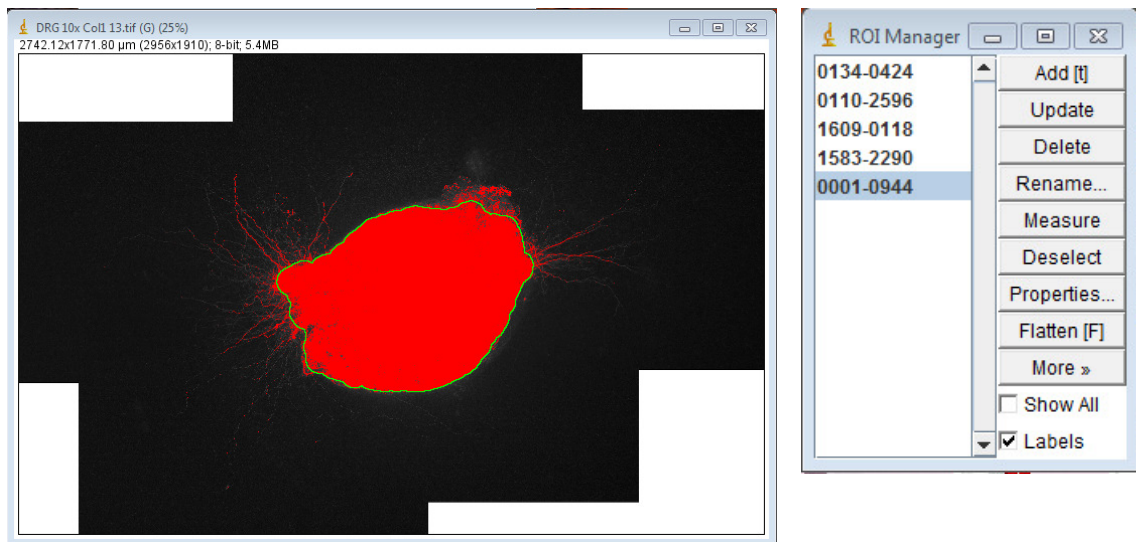


Step 2. Introduce and ensure the correct image scale. You can press the set scale button or access to scale set up windows using the *Analyze/Set scale...* command. Internet provides some tutorials about scale and spatial calibration of an image. Notice that if you select the global option in the Set Scale dialog, you can skip this step in a new analysis.

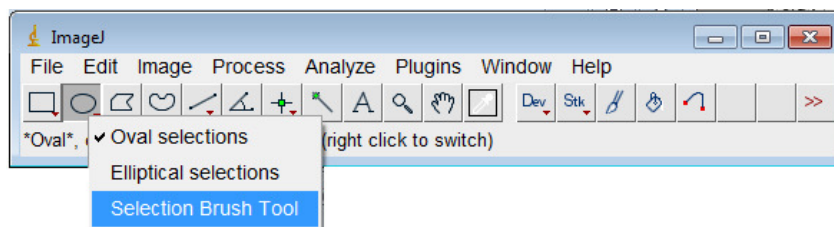
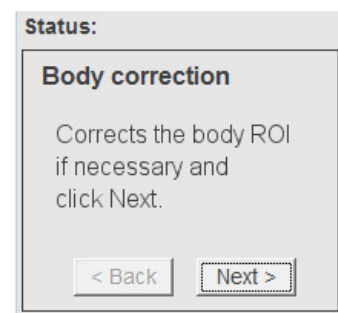


Step 3. Press Run button. Neurite-J will start the image processing. After some image conversion, the plug-in will prompt the user to select the organotypic body and the Threshold dialog will open. Using the threshold scroll bar, the organotypic body should be selected like in the picture. Don't worry if some neurites are selected, the plug-in will obviate. Ensure that all the culture body is thresholded. At the end, press Next button. A ROI including the body selection will be created and added to the ROI manager dialog.

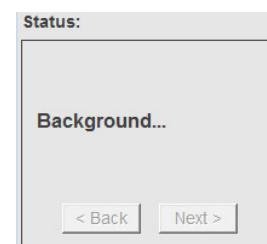




Step 4. Correction of the body selection. Sometimes the automatic selection of the organotypic body may include undesirable parts. If it is the case, you can correct the body selection prior to continue using the Selection Brush Tool (see the picture below, automatically selected by the plug-in) or another selection tool (<http://rsb.info.nih.gov/ij/docs/tools.html>). Once it is corrected, press Next button.

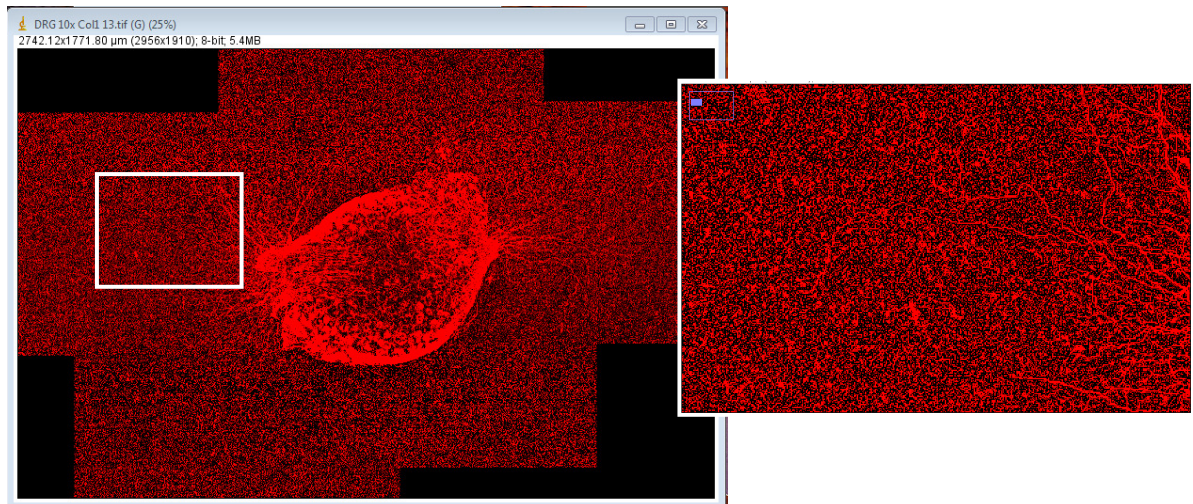


Step 5. Background correction. This is an automatic step where plug-in will make some image enhancements.

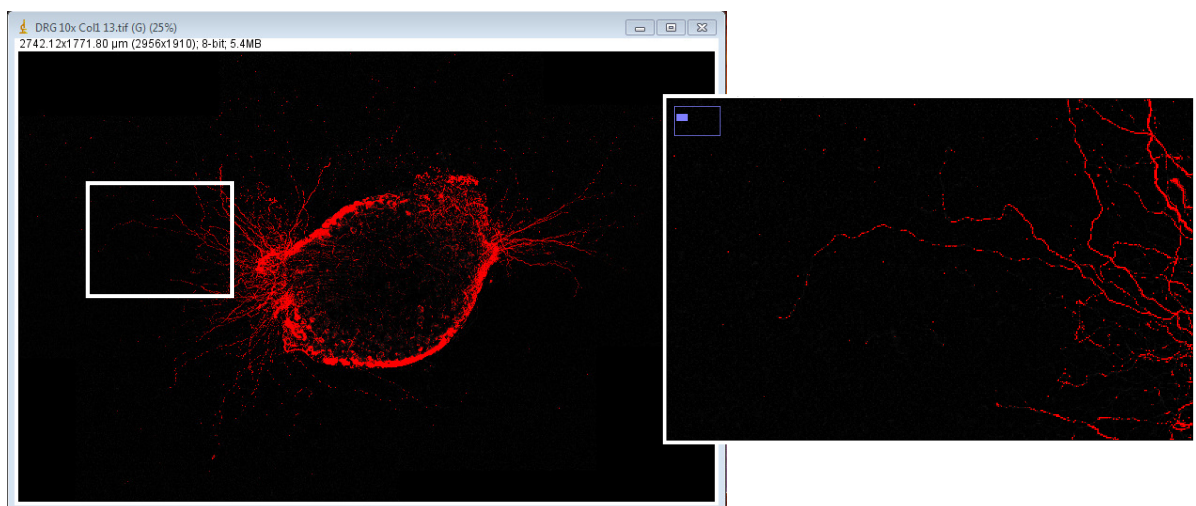


Step 6. Neurite thresholding. Probably is the most important step. It is in this point when the user can introduce some variability in their pictures analysis. Then, it is important to select the neurites property. Too much noise selection could overrate and deform the neurites, too low selection could eliminate neurites to the analysis. The next pictures could be a guide. After correct neurite thresholding press Ok button.

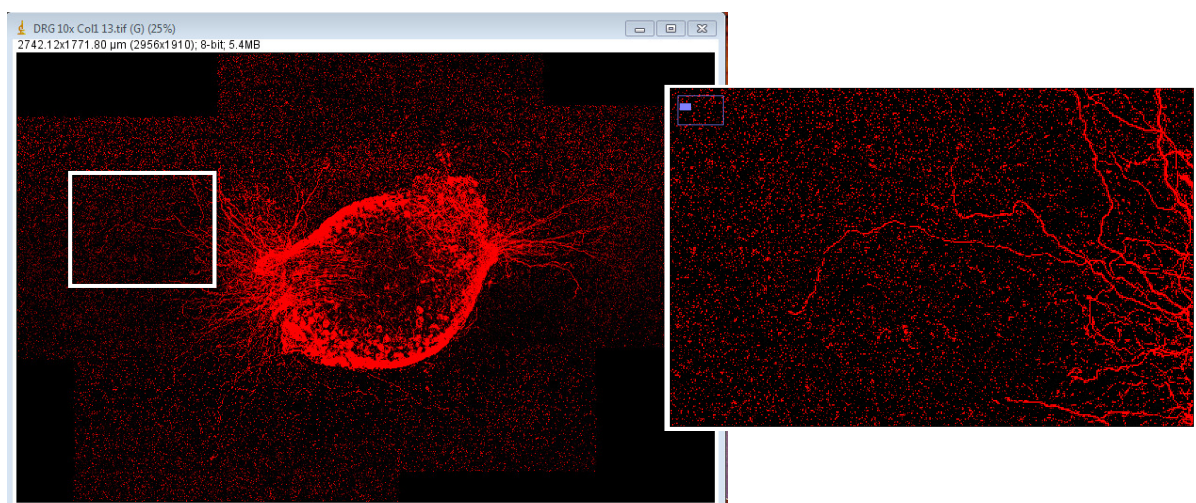
Incorrect thresholding. Too much noise selection:



Incorrect thresholding. Low neurite selection:

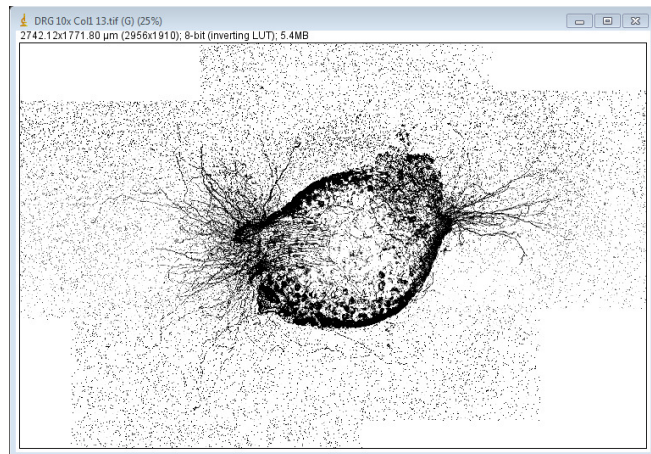
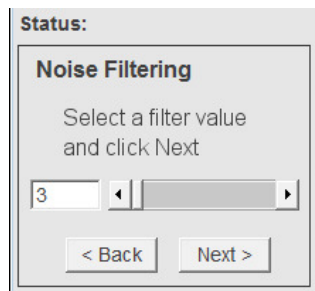


Correct thresholding. Notice that no so much noise is selected while all the neurites are thresholded.

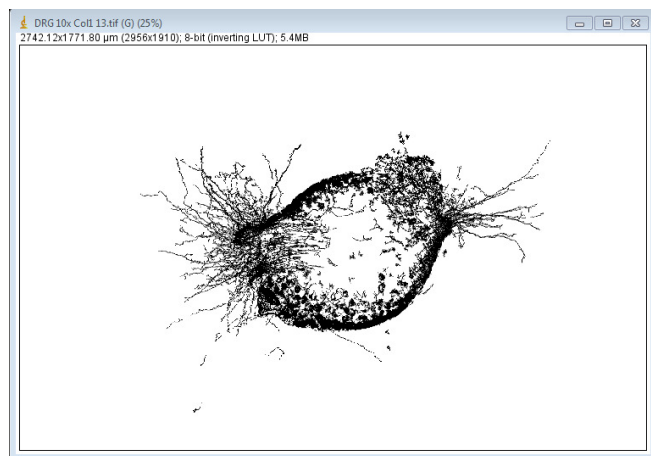
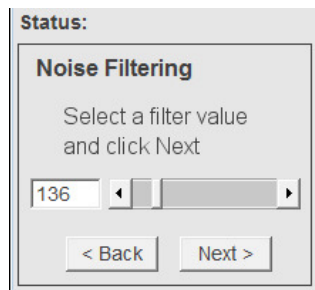


Step 7. Noise filter selection. The automatic noise filtering clears the background and generates a black and white (B/W) picture (see below), but some particles and extra noise could remain after filtering. In this step the user can select the decided filter value. We recommend to select the value that allow to see the maximal number of neurites without introducing to much noise (see below). Once the noise filter is selected press Next button.

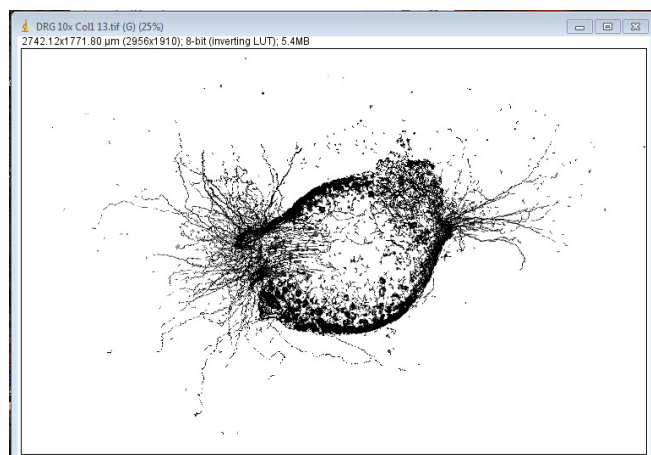
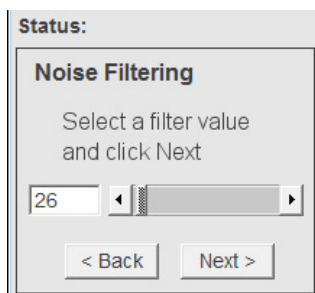
Low filter: _____



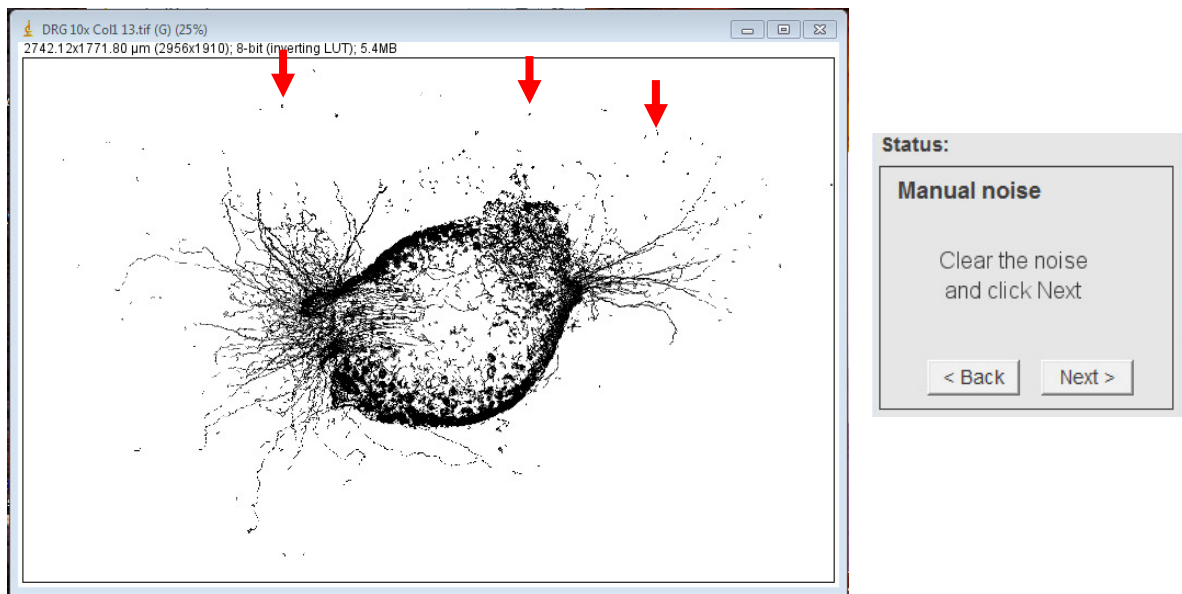
High filter: _____



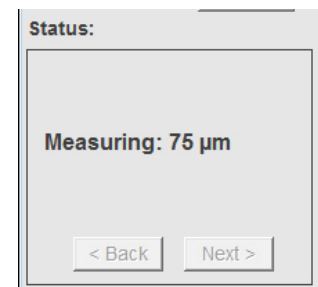
Correct filter: _____



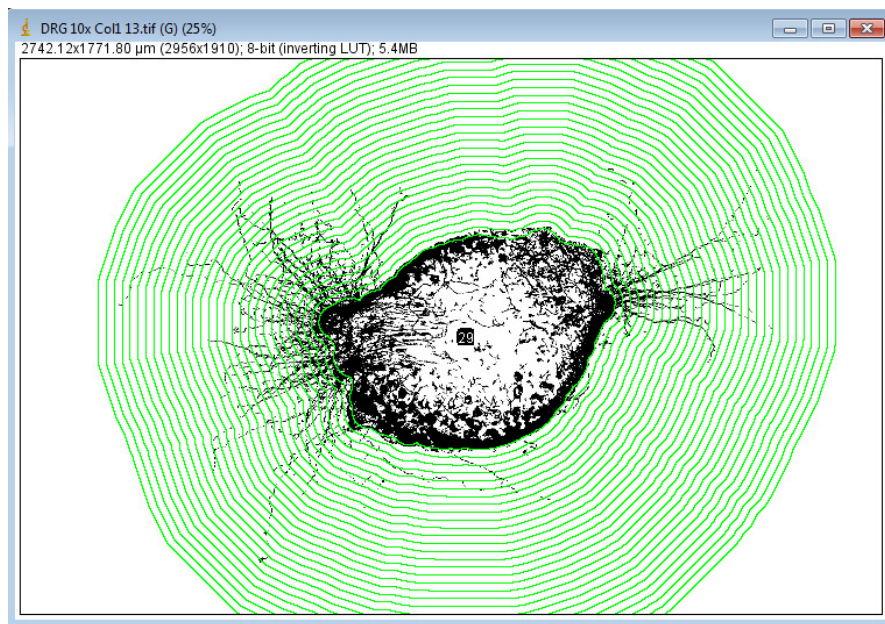
Step 8. Manual noise elimination. In this step of the process it is important to remove all the black pixels that user did not want to be analyzed.



To manual eliminate the undesirable noise, the Paintbrush tool with white color is automatically selected by the plug-in. The user can draw over the black pixels to erase it. It is possible to modify the brush size using the options of the Paintbrush tool (right or double click in the tool icon). You can also make a selection with any selection tool and erase the selected area with the SUPR key. After the manual cleaning, the resulting image is the measurable ones that will be analyzed. To continue press Next and the measuring process will start.



Result. After measuring process, the picture will redraw with the concentric rings and the Results window will open (see below). To analyze another image, close the current picture and start again with step 1. At this point, the Results window and ROI manager dialog will be opened and full. Both windows will be cleared by the Neurite-J plug-in to allow new analysis. Remember that if you select the global scale option in the set scale step (step 2), you do not have to do it for the new picture.



Results						
File	Edit	Font	Results			
	Distance (μm)	Intersections	Math	Value	Body Stat	Body Value
1	0	91	Nmax	99	Area(μm ²)	466714.971
2	25	90	RC(μm)	75	Circularity	0.784
3	50	90	Dmax(μm)	625		
4	75	99				
5	100	91				
6	125	85				
7	150	72				
8	175	65				
9	200	50				
10	225	55				
11	250	43				
12	275	33				
13	300	27				
14	325	16				
15	350	18				
16	375	16				
17	400	10				
18	425	10				
19	450	11				
20	475	3				
21	500	6				
22	525	4				
23	550	1				
24	575	3				
25	600	2				

5. Out of Memory. It is possible that too big pictures cause Out of Memory problems during image processing. To solve this issue, you can increase the amount of memory that ImageJ use in *Edit/Options/Memory & Threads* command. The memory changes will produced after ImageJ restart.

6. Contact us

For any suggestions, problems or errors communication, don't forget to send an e-mail to atpspin@gmail.com with NeuriteJ as a subject.