PLUGIN for ***IMAGEJ***, that allows one to count the number of synapses of a neuron.

SCOPE OF THE WORK:

The plugin that will be presented below, is a joint work between the team "Structural Synaptic Plasticity"[1] of The Biomedical Research Centre of La Rioja (CIBIR) and "Programming and Symbolic Computation Team"[2] of University of La Rioja.

DESCRIPTION:

Synapses are the points of connection between neurons. The relevance of synapses comes from the fact that they are related to the computational capabilities of the brain. The possibility of changing the number of synapses may be an important asset in the treatment of some neurological diseases, such as Alzheimer.

This plugin provides a semi-automatic method for counting synapses.

This tool needs two images which are obtained using inmunostanning techniques. The images are obtained from the same neuron in culture using two antibody markers. In the example we use bassoon and synapsin, any two synaptic markers can work perfectly.

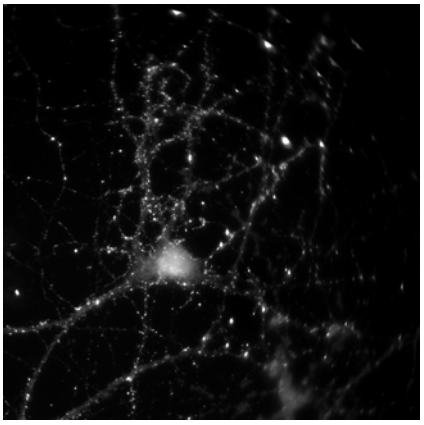


Image marked with bassoon

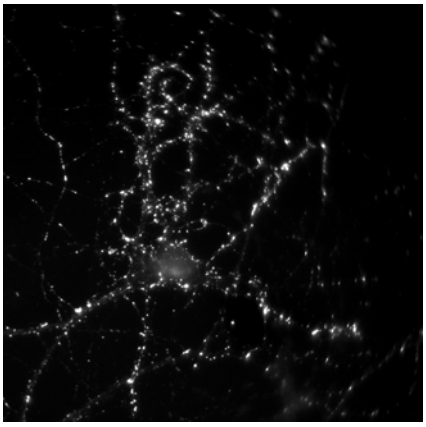


Image marked with synapsin

The program was designed to work with images from neurons in culture. In future version we will deal with the problems of in vivo stainning.

[1] http://www.cibir.es/cibir-investigacion/enfermedades-neurodegenerativas?start=1

[2] https://esus.unirioja.es/psycotrip/

INSTALLATION:

It's possible a installation on Windows (Xp, Vista and 7) GNU/Linux and Mac OS X.

To install the SynapCountJ plugin you should proceed as follows:

1. Install NeuronJ plugin:

   http://www.imagescience.org/meijering/software/neuronj/

2. Unrar the file synapcountj.rar.

   δ Copy the file *Macro_Make_Binary* into the macros where ImageJ is installed :

   - …/ImageJ/macros

   δ Copy the files SynapCountJ_.java, SynapCountJ_.clases and SynapCountJ_$BandPlot.class into the Plugins where ImageJ is installed:

   - …/ImageJ/Plugins

   You can create a folder or put them into a existing one.

3. Help -> Refresh Menu or start ImageJ (reboot if already open).

WORKING WITH THE PLUGIN:

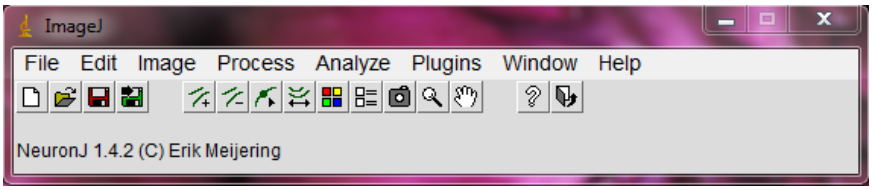
There are two steps:

1. Drawing the structure of the neuron.
2. Counting the number of synapses of a neuron.

Step 1: Drawing the structure of the neuron.

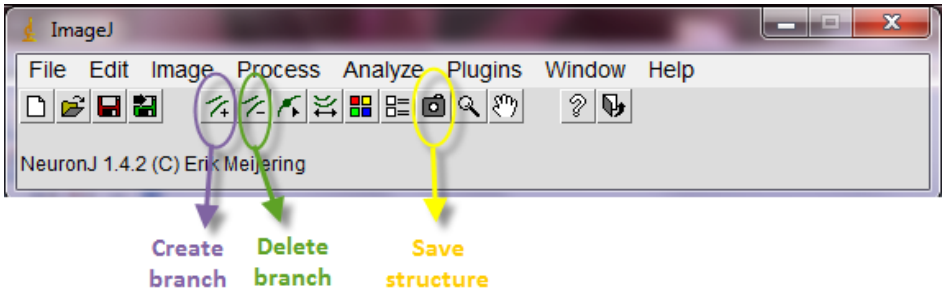
We are going to draw the neuron morphology from one of those pictures, an inmuno from a structural marker such MAP2B should work as well.

First of all, we must draw the structure of the neuron. From ImageJ, we load the NeuronJ plugin (Plugins → NeuronJ). Afterwards we can see this toolbar:

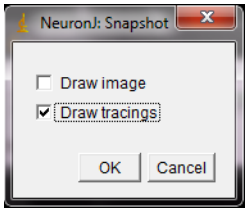


Then, we open one of the images explained at the beginning of this maunal (bassoon or synapsin) clicking on this button  .

Now, we have to draw the structure:



The tracing of dendrites is initiated by moving the mouse to the beginning of a dendrite of interest and clicking the (left) mouse button. The NeuronJ plugin shows the path from the current mouse position in the image to the clicked point. Move the mouse roughly along the dendrite until the path suggested by NeuronJ starts to deviate too much from what is considered the correct tracing. Clicking the mouse button again causes the program to fix the displayed path and to start the computation of paths from the newly clicked point. This procedure can be repeated until the end of the dendrite is reached, which is indicated by double-clicking the mouse button.



When we have drawn the structure, we extract the tracing from the image. Click on the button  and choose the option “Draw tracings”.

We save the image with extension ‘.Tiff’ (File → Save As → .Tiff).

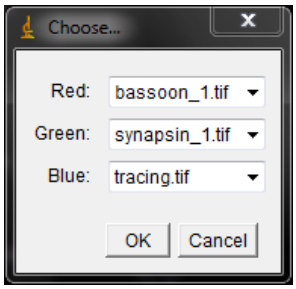
Finally, we close the NeuronJ plugin  .

Step 2: The quantification of synapses.

From ImageJ, open the two images with the bassoon and synapsin antibody markers and the image with the structure of the neuron.

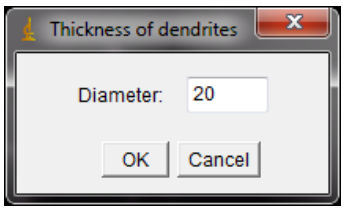
Start the plugin SynapCountJ (Plugins → SynapCountJ).

The following dialog is open:



Select the image with the bassoon marker for the red channel, the image with the synapsin marker for the green channel, and the tracing for the blue channel.

Introduce the thickness of the dendrite (in pixels):



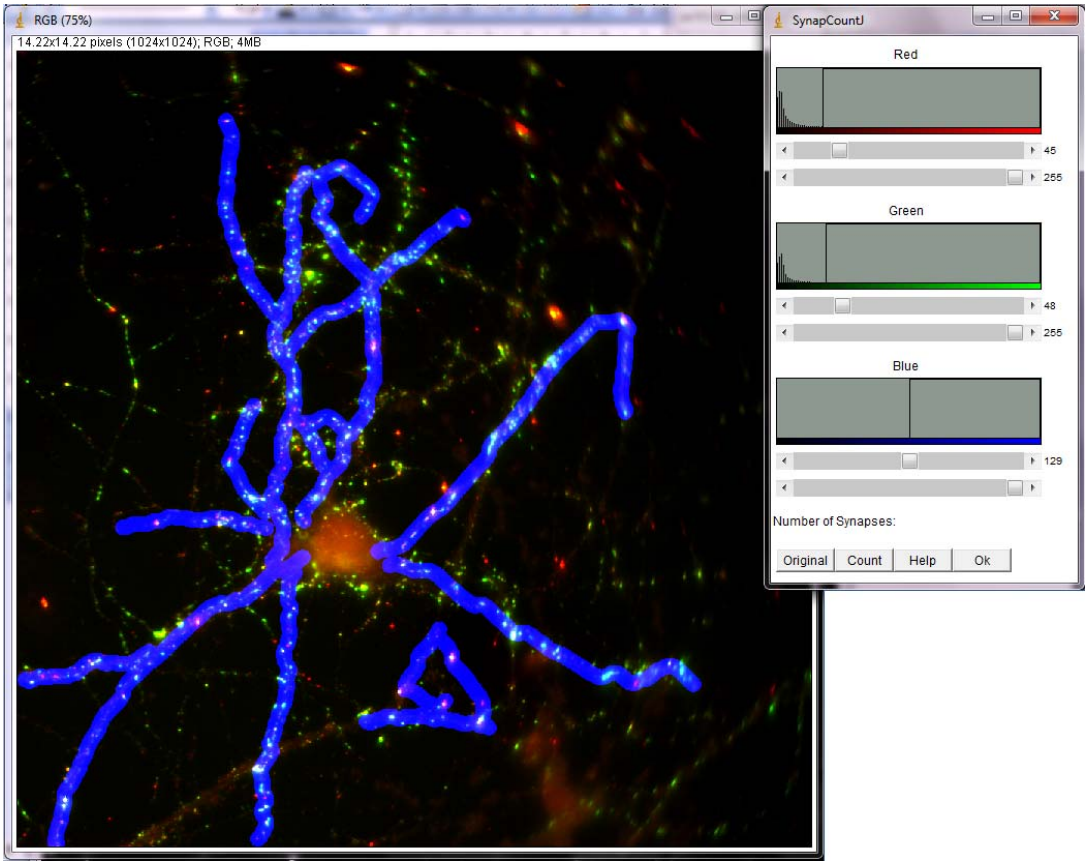
The plugin show the images with the two markers and the one with the structure overlapped, thus:

i. The red channel is for the image with **bassoon**.

ii. The green channel is for the image with **synapsin**.

iii. The blue channel is for the image with the **structure** of the neuron.

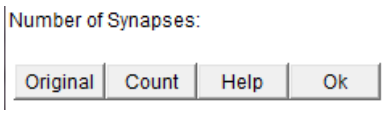
Then, the following screen is shown:



To find the synapses, we are looking for the points where the three channels match up, these points are white. However, it is worth noting that the synapses are not only the fully white points, but also the ones whose color is close enough to white.
So, we select a range of white values in which we estimate there are synapses. Notice that only the synapses located on the dendritic tree are selected, in such a way we reduce the false positives and confine the dendrites to analyze

When we choose a range of white values, the areas in that range are highlighted to provide a better estimation of the synapses of the neuron.

The buttons which appear in the dialog box mean:



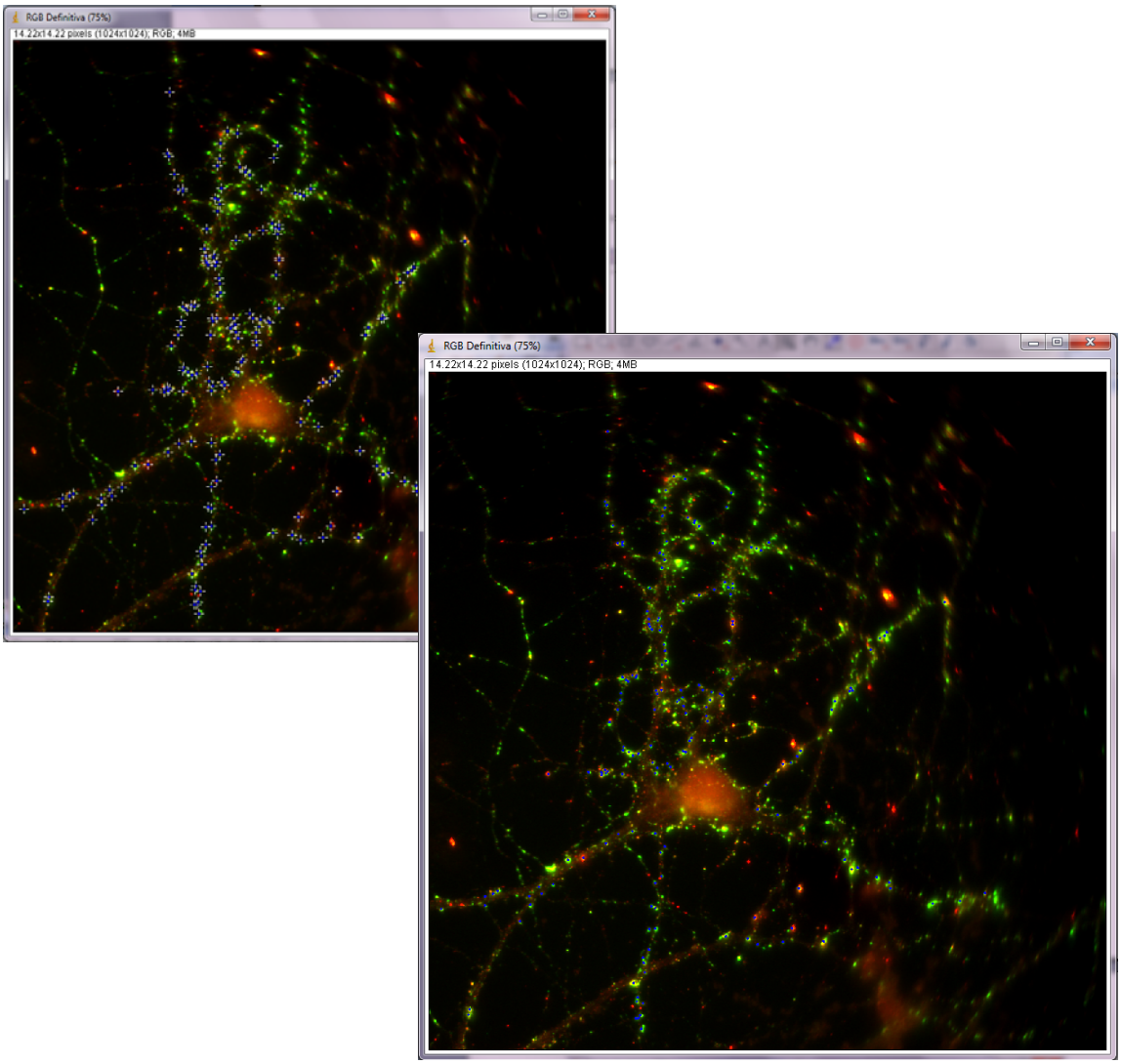
Original: back to the original image.

Count: show the number of synapses which are found for a concrete range of values next to the label “Number of Synapses”.

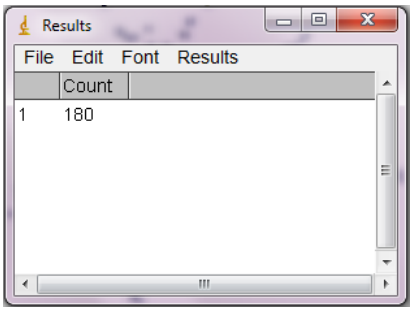
Help: information and help about this dialog.

Ok: Do The quantification of synapses, and return the followings results:

ϕ The image named “Final RGB” shows the synapses which are marked with blue points.



ϕ In the results table appears the number of synapses.



ϕ In the last image, named “RGB”, we find the image with the three channels overlapped.