

SynapCountJ

PLUGIN for IMAGEJ,
that allows you to count
the number and calculate the density
of synapses of a neuron



UNIVERSIDAD
DE LA RIOJA

Gadea Mata Martínez

gadea.mata@gmail.com

gmata.ext@riojasalud.es



Centro de Investigación
Biomédica de La Rioja

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 Center or 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 immunostaining techniques. In the example, these images are obtained from the same neuron in culture using two antibody markers; bassoon (a presynaptic scaffolding protein) and synapsin (a synaptic vesicular protein). Any set of two synaptic markers can work perfectly.

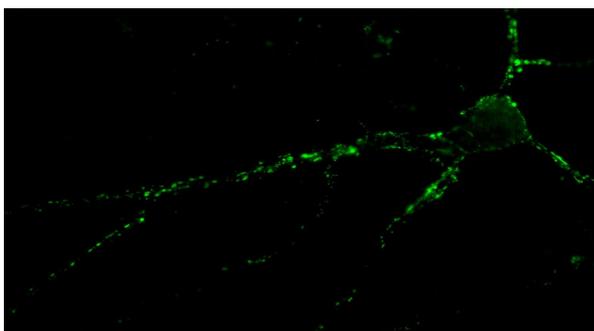


Image marked with bassoon

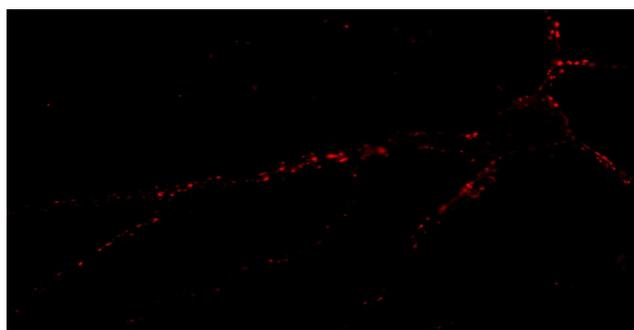


Image marked with synapsin

The program was designed to work with images from neurons in culture. However, in future versions we will deal with the problems of brain slices.

INSTALLATION:

SynapCountJ can work 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. Install *Bio-Formats* plugin: <http://www.loci.wisc.edu/software/bio-formats>
3. Unrar the file *synapcountj.rar*
 - φ Copy the file *Macro_Make_Binary* into the macros where *ImageJ* is installed:
 - .../ImageJ/macros

1 <http://www.cibir.es/cibir-investigacion/enfermedades-neurodegenerativas?start=1>

2 <https://esus.unirioja.es/psycotrip/>

- φ Copy the files *SynapCountJ.jar* into the Plugins where *ImageJ* is installed:
 - .../ImageJ/Plugins

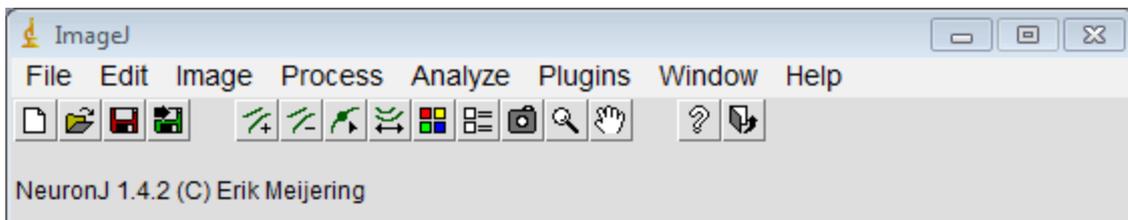
You can create a folder or put them into an existing one.

- φ Help → 'Refresh Menu' or start *ImageJ* (reboot if already open).

WORKING WITH NEURONJ PLUGIN:

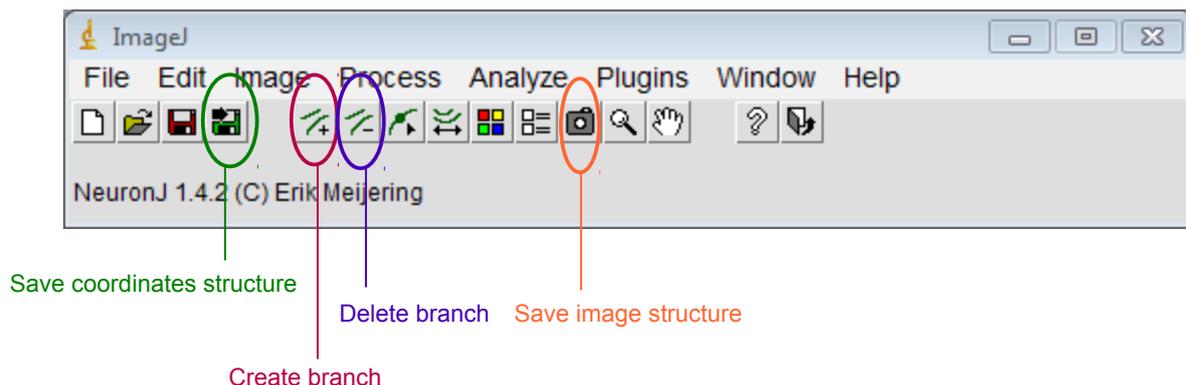
First, we are going to draw the dendritic neuronal morphology from one of those pictures, an immunocytochemistry from a dendritic structural marker such *MAP2B* should work as well.

First of all, we must draw the structure of the neuron. To this end we have to 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 manual (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 trace.

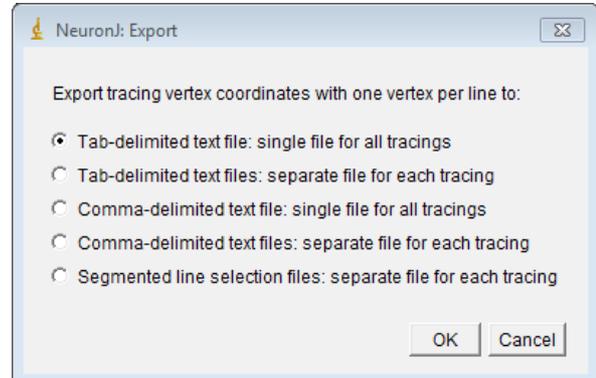
Clicking the mouse button again makes the program fix the displayed path and start the computation of paths from the newly clicked point. This procedure can be repeated till the end of the dendrite has been reached, which is indicated by double-clicking the mouse button.

When the structure is draw, we should extract the trace from the image.

Click on the button  and choose the first option "Tab-delimited text file: single file for all tracings".

The plugin save a file with the extension '.txt'. This file contains the coordinates of all the dendrites selected and all the traces under the name of tracingN1, tracingN2 and so on.

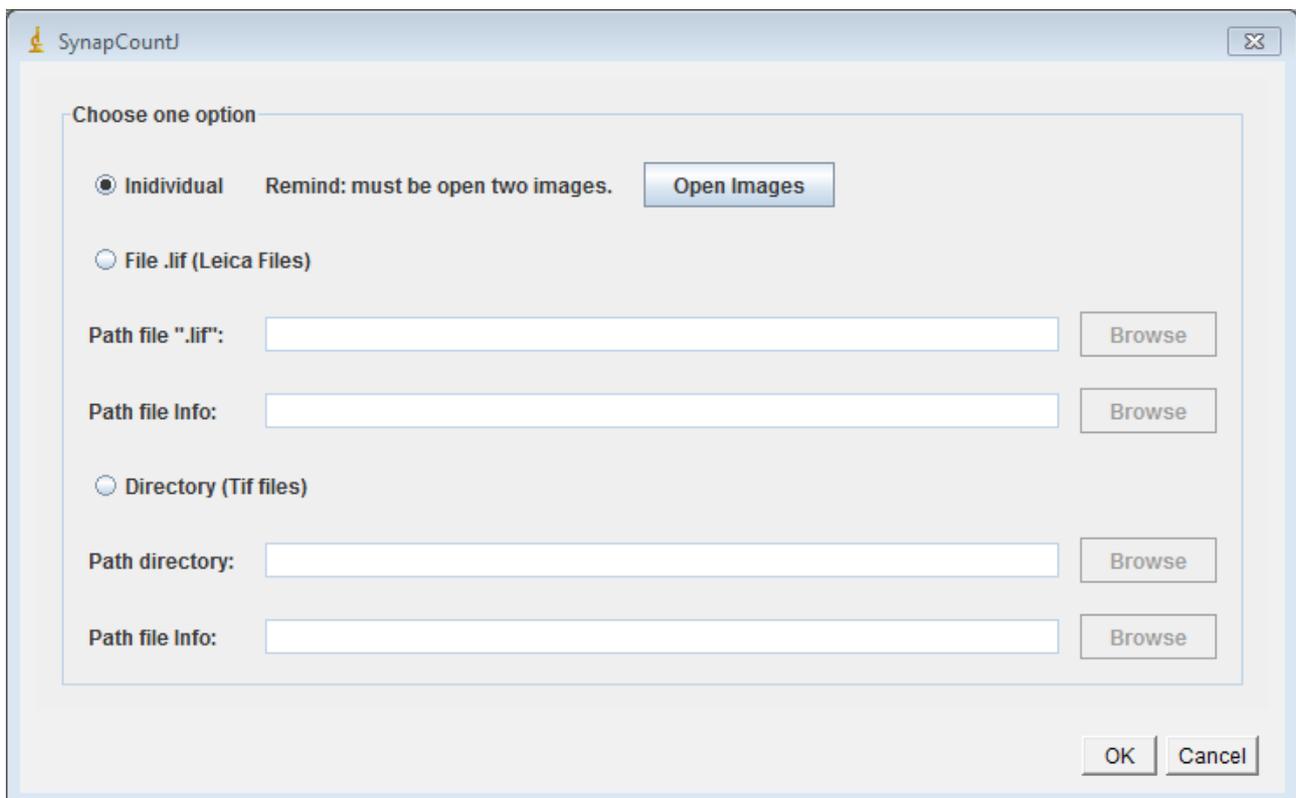
Finally, we close the *NeuronJ* plugin . 



WORKING WITH THE PLUGIN:

Start the plugin *SynapCountJ* (Plugins → *SynapCountJ*).

The following dialog is open:



There are three options:

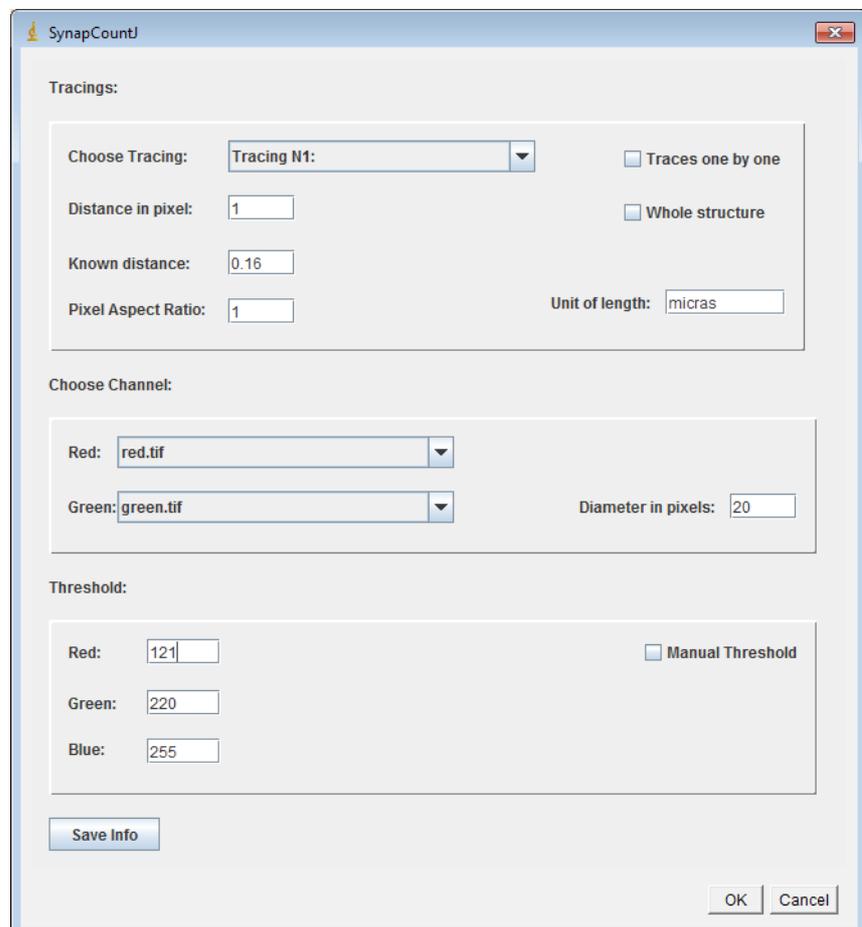
1. **Individual:** Work individually with each pair of photos. This option allows to save the information to work in batch.
2. **File .lif (Leica Files):** Work for batch a set of pairs of images compressed in a file extension '.lif'. It takes the necessary data from a file with '.xml' created before.
3. **Directory (Tif files):** Work for batch a set of pairs of images stored in directories.

Individual analysis

Open the two images with the bassoon and synapsin markers (our examples) . Afterwards introduce the size of images and choose the file which contains the information of the structure of the neuron (it was save before; in our case is call green.txt).



The following dialog is open:

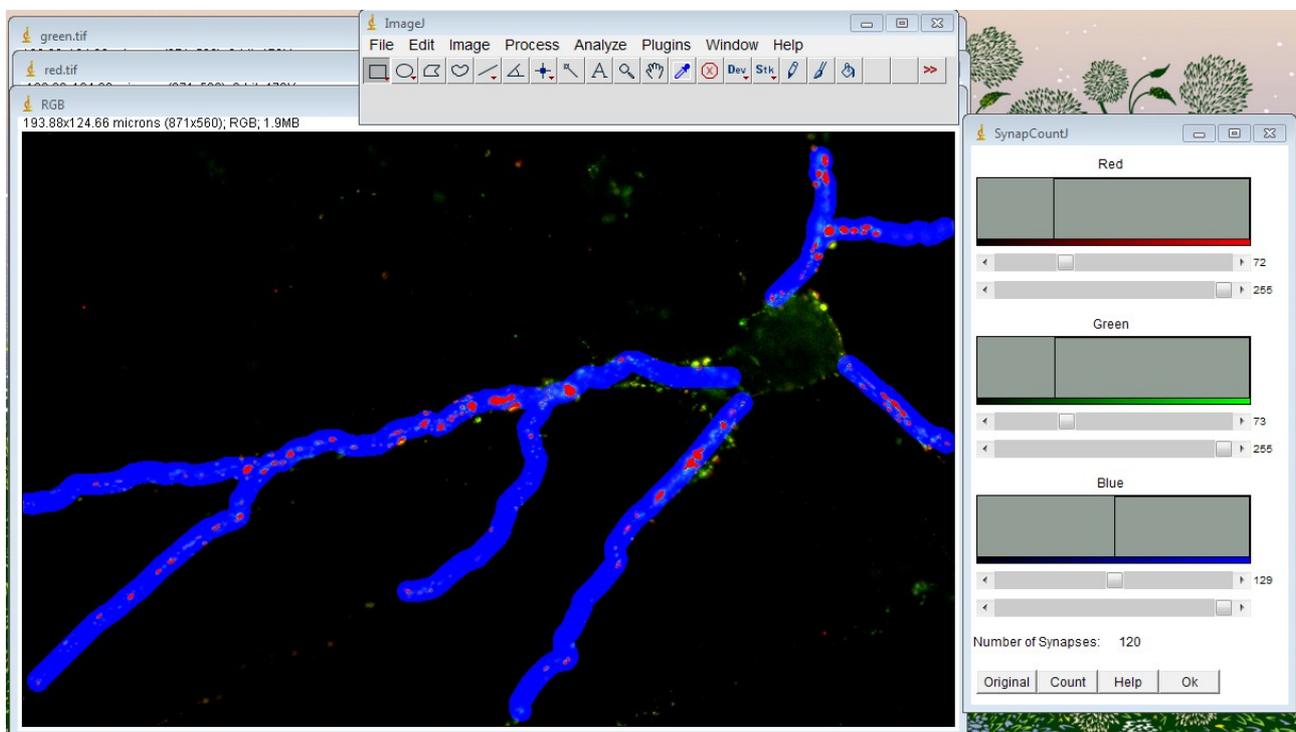


- δ Distance in pixel: which represents the unit in pixels which is going to be taken.
- δ Known distance: shows the measure of the pixel unit taken.
- δ Pixel Aspect Ratio
- δ Unit of length: name of the unit of measure that represents the information before.
- δ Red: select the image for the red channel.
- δ Green: select the image for the green channel.
- δ Diameter in pixels: Set the diameter of the structure of the dendrite. Notice that this value determined the area within the analysis will take place. A value around 20 pixels works fine for us.

φ **Working individually**

- γ To study one dendrite: for doing that, it is necessary to choose one from trace from the (“Choose Tracing combobox”) which depicts all dendrites. Select the option “Manual Threshold”.
- γ To study all dendrites one by one: for doing that, it is necessary to select the option “Traces one by one” and “Manual Threshold”.
- γ To study all the whole neuron: for doing that, it is necessary to select “Whole structure”.

In all this cases, when clicking on the button “OK”, it will show this follow dialog:



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

- ϕ The red channel is for the image with **synapsin** .
- ϕ The green channel is for the image with **bassoon**.
- ϕ The blue channel is for the image with the **structure** of the neuron.

To find the synapses, we should look 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 visual estimation of the synapses.

The buttons which appear in the dialog box, allow you to :

Number of Synapses:

Original Count Help Ok

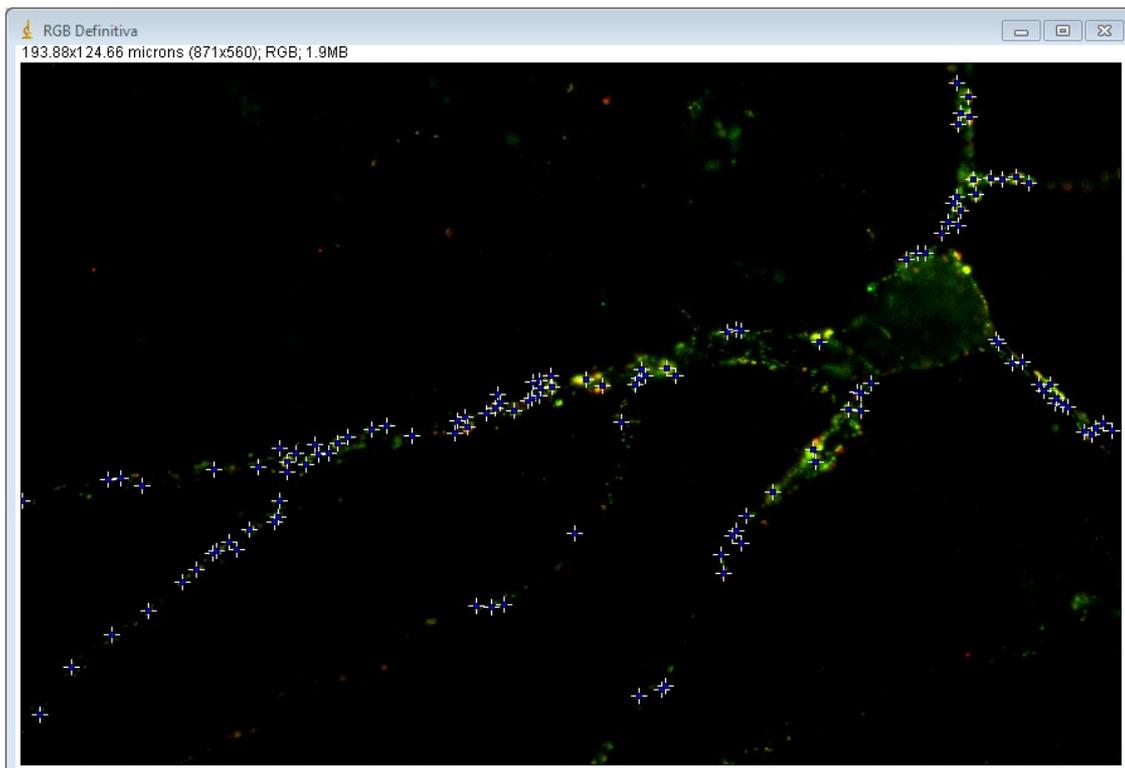
Original: Return to the original image.

Count : show the number of synapses calculated for the selected range of values. The counting will appear at the right of "Number of Synapses" label

Help : Display help about this dialog.

Ok : Does the final quantification, after that the program return the followings results:

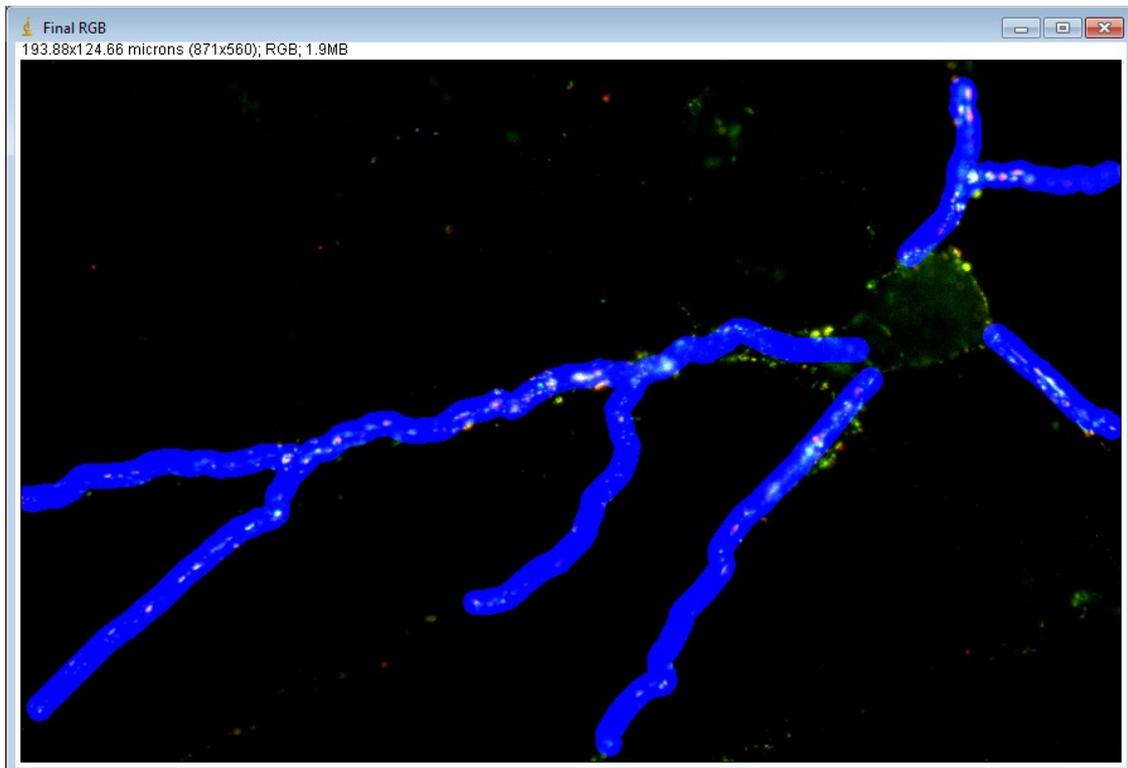
- ϕ The image named "*RGB Definitiva*" shows the synapses which are marked with blue points.



- ϕ the results table shows the number of synapses, dendritic length analyzed and synaptic density by the units of length introduced before.

	Label	Length in pixels	Length in micras	Synapses	Density	Red	Green
1	Tracing N1:	307.8794	6988.8630	20	0.2862	72	73
2	Tracing N2:	725.3956	16466.4790	48	0.2915	72	73
3	Tracing N3:	242.2956	5500.1108	7	0.1273	72	73
4	Tracing N4:	375.0996	8514.7612	17	0.1997	72	73
5	Tracing N5:	193.9476	4402.6116	15	0.3407	72	73
6	Tracing N6:	83.2135	1888.9459	8	0.4235	72	73
7	Tracing N7:	119.1410	2704.5010	16	0.5916	72	73
8	Total Neuron	2046.9724	46466.2726	120	0.2583	72	73

- ϕ In the last image, name "Final RGB", the three channels appears overlapped.



φ **Working semiautomatically**

- γ To study one dendrite; for doing that, it is necessary to choose one trace (“Choose Tracing drop-down menu”) , and then to introduce manually which are the values of red and green threshold.
- γ To study all dendrites one by one: for doing that, it is necessary to select “Traces one by one” , after that and to introduce which are the values of red and green threshold. This mode works with individual traces, indicating synaptic density from dendrites and whole neuron.

In all cases, after clicking on the button “Ok”, it will show the same images seen in the previous case (individual kind).

φ **Save information**

To further process several images in batches, we can now save the preselected setting such threshold , scale and dendrite diameter . All this information is save in a “.xml” file. The settings can be reuse with pictures from the same experiment. Notice, that antibodies concentrations, exposition time, etc., must be similar in order to use a batch processing .

The screenshot shows the SynapCountJ dialog box with the following settings:

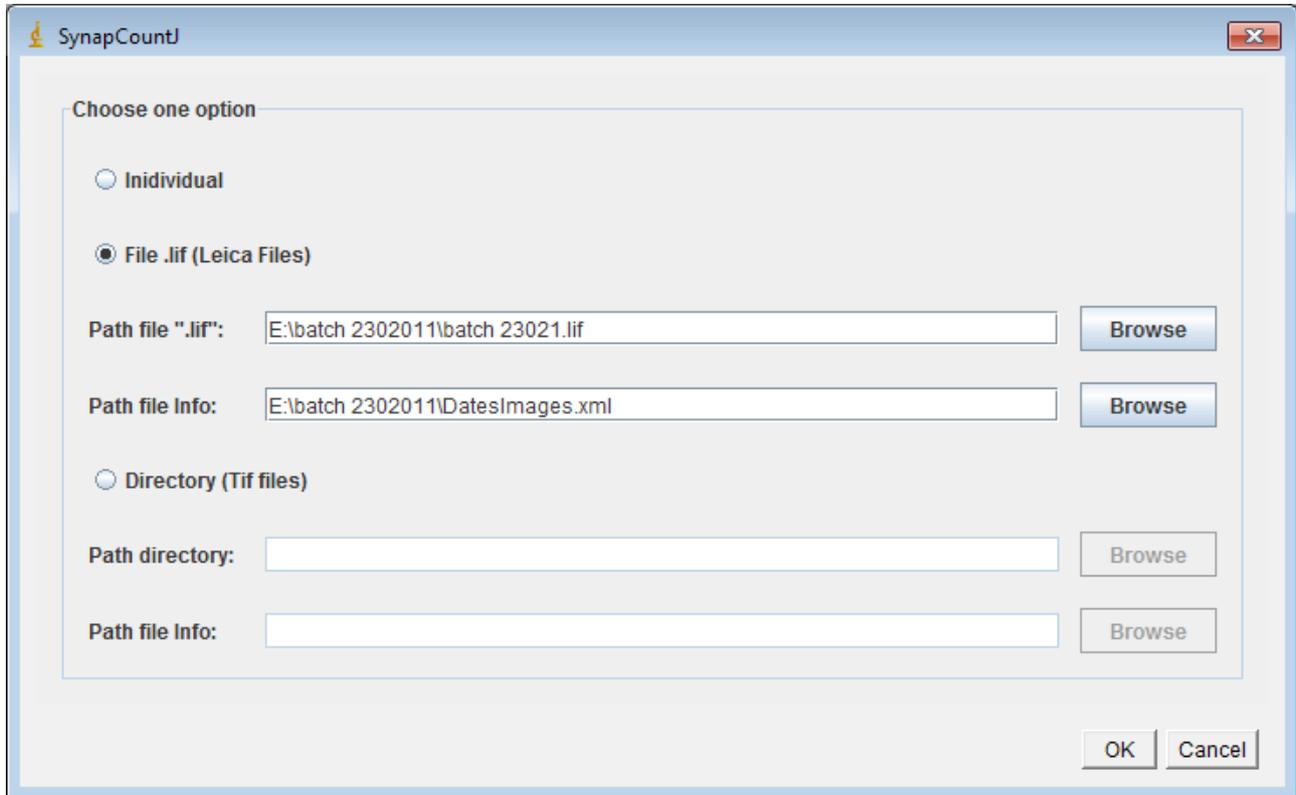
- Tracings:**
 - Choose Tracing: Tracing M1 (dropdown)
 - Distance in pixel: 1 (input)
 - Known distance: 0.16 (input)
 - Pixel Aspect Ratio: 1 (input)
 - Unit of length: micras (input)
 - Traces one by one
 - Whole structure
- Choose Channel:**
 - Red: red.tif (dropdown)
 - Green: green.tif (dropdown)
 - Diameter in pixels: 20 (input)
- Threshold:**
 - Red: 121 (input)
 - Green: 220 (input)
 - Blue: 255 (input)
 - Manual Threshold

Buttons: Save Info, OK, Cancel

Working with files *.lif* (Leica files)

Choose the file ".lif" which stores all images of a batch on "Path file .Lif".

Choose the file ".xml" which stores the information about the batch job . Click "Ok".



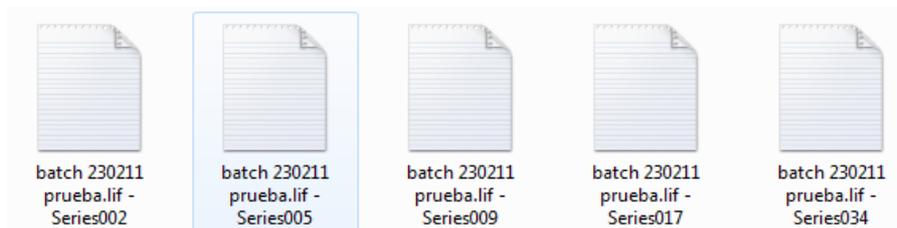
Important note: It is necessary that the structure of each neuron must be previously outlined by NeuronJ. The structural files (".txt") must be inside a folder called "tracings" that needs to be in the same directory where the file extension ".lif" is located. In addition, it is necessary that the names of the files which store the information about structure, are the same than the images obtained from file ".lif".



Example:

If the image generated by Lieca is: batch 230211.lif – Series002.tif

Then, the file which saves its structure has to be named : batch 230211.lif – Series002.txt

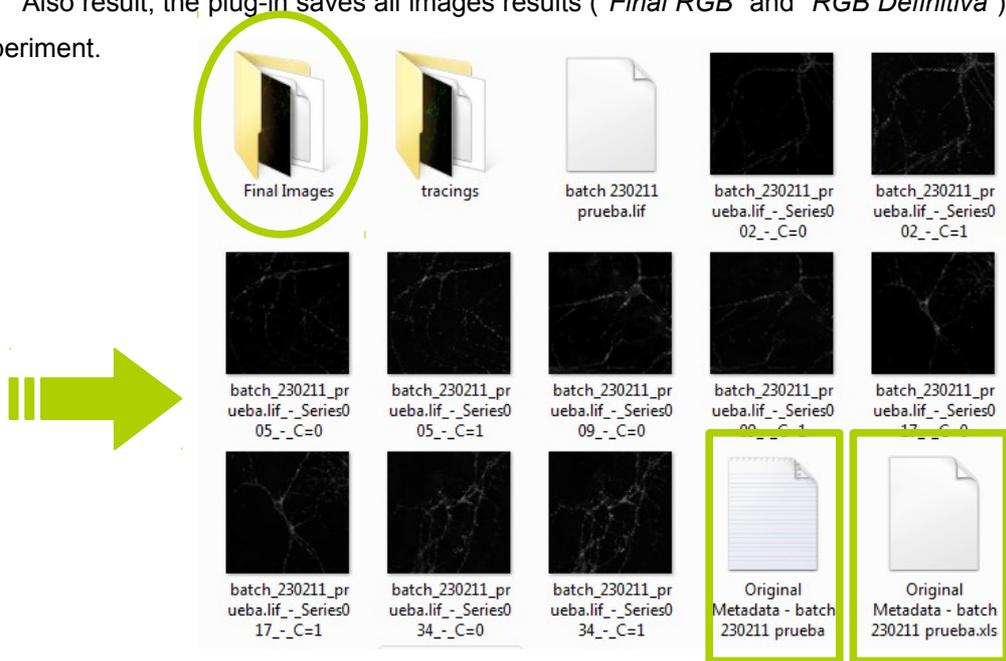


Then press OK and the program will continuous to process all the pictures included in the file.lif. The results table will show the density by traces and whole neurons of all cells included.

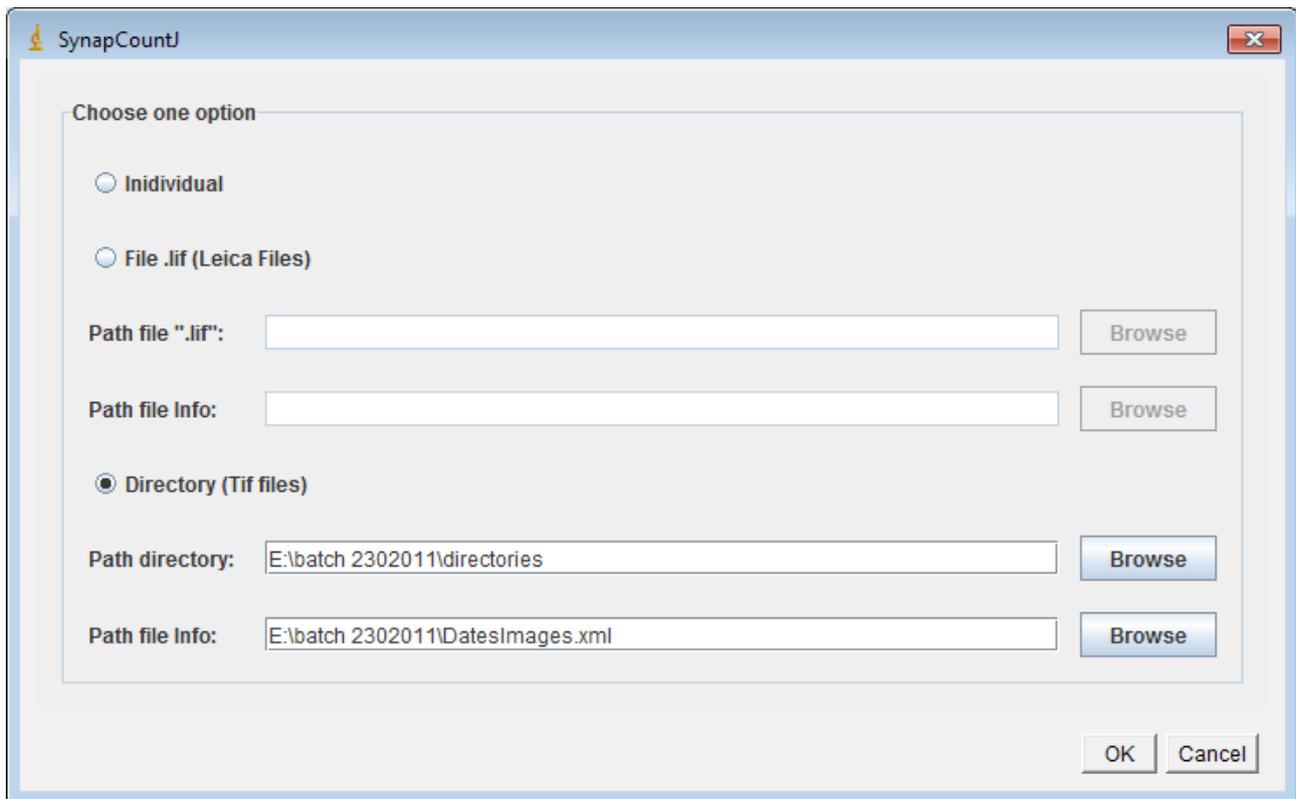
	Label	Length_in_pixels	Length_in_micras	Synapses	Density
1	Total Series002	3911.0846	88781.6202	220	0.2478
2	Tracing N1:	943.5925	21419.5503	76	0.3548
3	Tracing N2:	372.2664	8450.4463	19	0.2248
4	Tracing N3:	109.3606	2482.4856	17	0.6848
5	Tracing N4:	187.1695	4248.7466	29	0.6826
6	Tracing N5:	134.5342	3053.9256	10	0.3274
7	Tracing N6:	1196.8698	27168.9445	50	0.1840
8	Tracing N7:	546.2561	12400.0125	8	0.0645
9	Tracing N8:	301.7864	6850.5508	16	0.2336
10	Tracing N9:	119.2493	2706.9581	2	0.0739
11	Total Series005	4570.3928	103747.9160	251	0.2419
12	Tracing N1:	546.4246	12403.8386	43	0.3467
13	Tracing N2:	1189.6228	27004.4375	62	0.2296
14	Tracing N3:	1046.6659	23759.3167	69	0.2904
15	Tracing N4:	858.8209	19495.2344	45	0.2308
16	Tracing N5:	131.1682	2977.5175	5	0.1679
17	Tracing N6:	797.6904	18107.5713	31	0.1712

Apart from this table, in the same directory where is the file ".lif", the plug-in saves the images contained in the file ".lif". This images are separated by two color channels (red and green). In addition, it stores the dates, about experiment includes in the file ".lif". This dates are saved in ".txt" and ".xls" format.

Also result, the plug-in saves all images results ("*Final RGB*" and "*RGB Definitiva*") for each image from experiment.



Working with directories



SynapCountJ can also work directly with tiff files . To do that you have

γ Select the directory where to find...

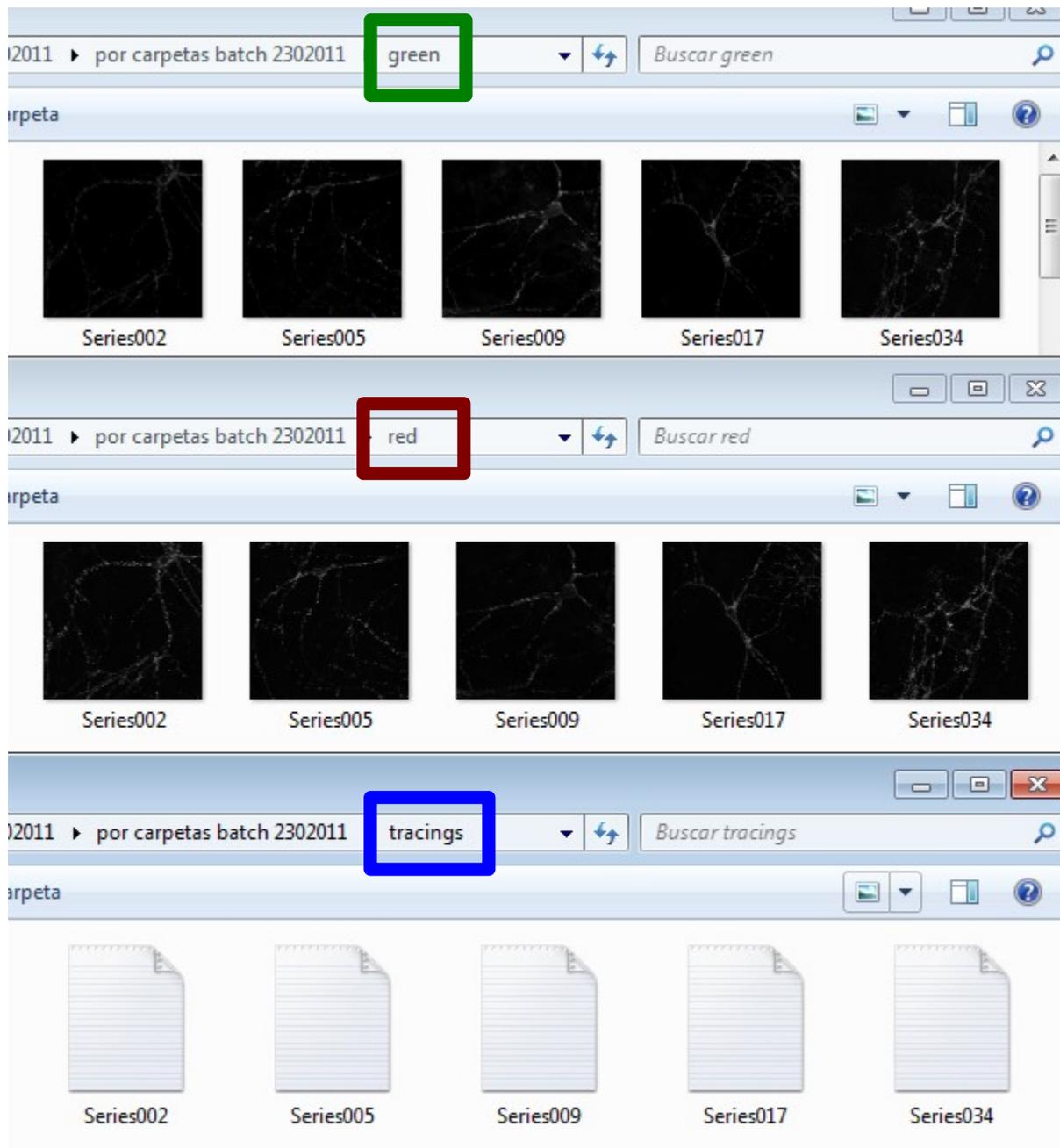
... a folder named “red” which store the images of red channel with your favorite antibody

... a folder named “green” which store the images of green channel with your second favorite antibody.

... a folder named “tracings” which store the structure of each neuron from batch.



Important Note: all files from the same neuron must have the same name. For instance in our experiment the files are call *series00X* , the one in the folder green contains the bassoon immunostainnig, the one in the folder red the synapsin staining and the one saved on tracing folder the structure generated by *NeuronJ*.



Important Note: all files which are of a same neuron, are named in the same way.

"That's all folks".

This is a plugin in working process, I would really appreciate your comments to improve it. Please write me to gadea.mata@gmail.com or gmata.ext@riojasalud.es

In the near future we like to develop an automatic structure recognition plug-in and a dendritic spine recognition algorithm. Stay tuned.