SynapCountJ – an *ImageJ* Plugin to Analyze Synaptical Densities in Neurons

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Abstract

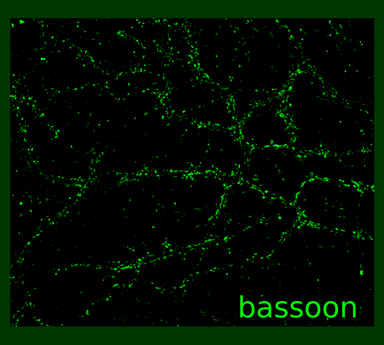
SynapCountJ is a software system which has as goal the identification and quantification of synaptic density from immunofluorescence images. The underlying algorithms of this program are based on homological methods for digital imaging.
This *ImageJ* plugin tries to solve problems such as inaccurate marking, denoise to select the region of interest and unify the criteria when dealing with this kind of images. The final aim of SynapCountJ consists in providing an automatic solution to measure the amount of synapses. This plugin has been implemented in Java and can be executed in *Windows* (*XP/Vista/7*) *Mac OS X* and *Linux*.

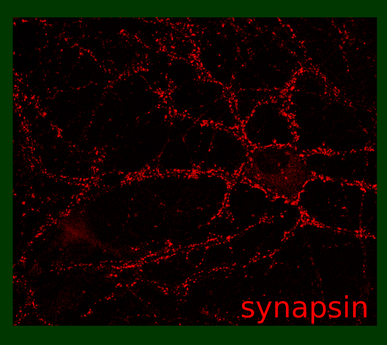
SynapCountJ

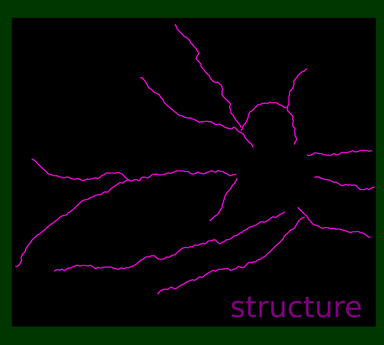
A. Individual treatment of a neuron

We start with two images obtained from a neuron which has been marked with the antibody markers synapsin and basson.

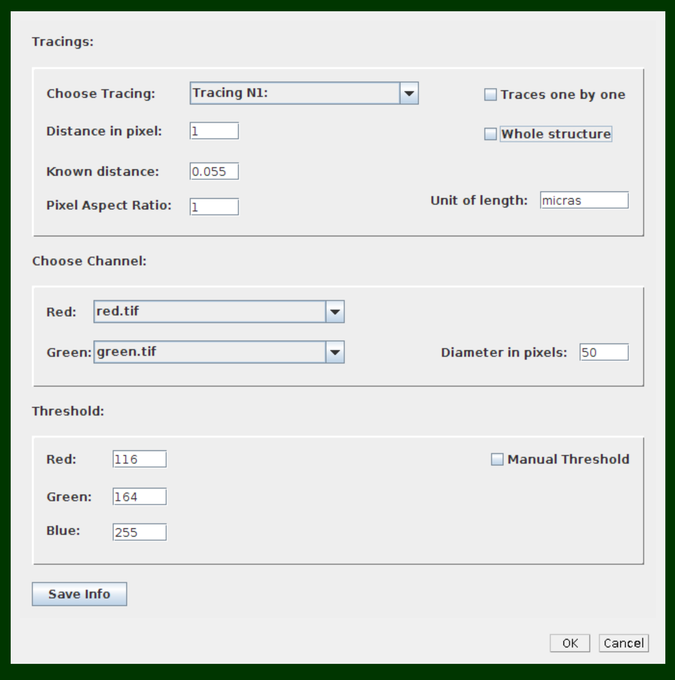
STEP 1:
In this first step the region of interest is specified; namely, we select the regions where the amount of synapsis measurement is going to be performed. In this manner we remove the background. To this aim, we use the *NeuronJ* plugin [1].



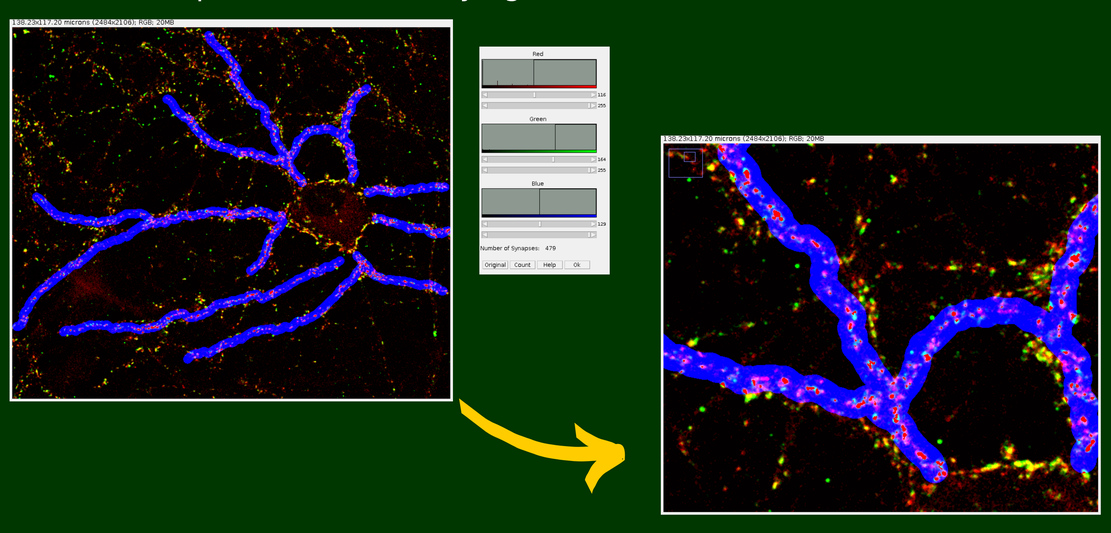




STEP 2:
At this point, the user can decide whether he wants to perform a global analysis of the whole neuron or a local one focusing on each dendrite of the neuron. In both cases, the system requires additional information as the scale and the mean thickness of the region to analyze. This last measure determines the region (blue zone of the image) where the counting process is carried out.

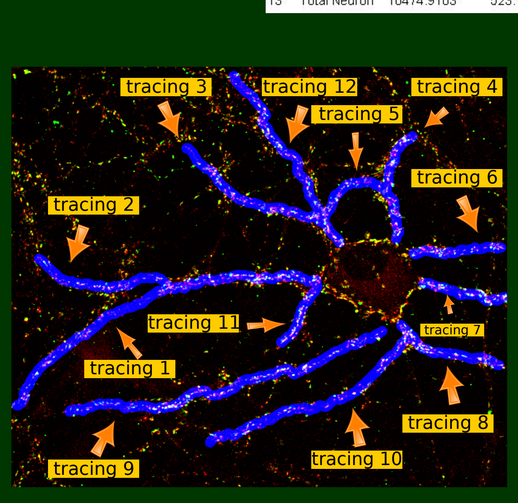


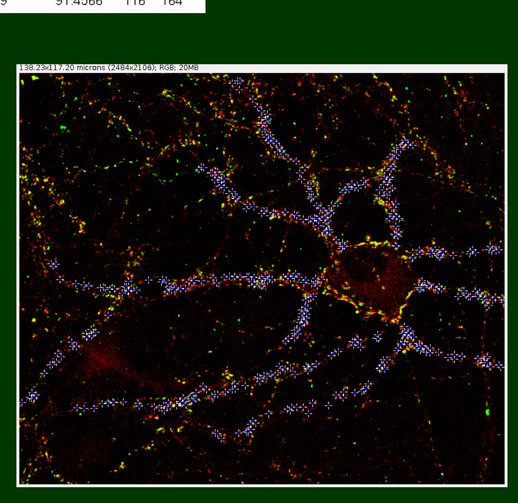
STEP 3:
SynapCountJ overlaps the two original images and the structure (selected region). The plugin identifies the almost white points as candidates to be synapses. The plugin allows one to modify the values of the red and green color in order to modify the detection threshold and obtain a first image where such points are marked (red points in the image) for a further counting. *SynapCountJ* updates automatically the amount of synapsis which has been computed when modifying the threshold.



STEP 4:
Eventually, *SynapCountJ* returns a table with the obtained data and two images showing, respectively, the analyzed region and the marked synapses (blue crosses).

	Label	Length in pixels	Length in micras	Synapses	Density	Red	Green
1	Tracing N1:	1833.1058	91.6553	71	77.4642	116	164
2	Tracing N2:	867.7840	43.3892	35	80.6652	116	164
3	Tracing N3:	983.5322	49.1766	53	107.7748	116	164
4	Tracing N4:	599.8320	29.9916	41	136.7049	116	164
5	Tracing N5:	437.7388	21.8869	25	114.2234	116	164
6	Tracing N6:	468.8438	23.4422	26	110.9111	116	164
7	Tracing N7:	447.6296	22.3815	31	138.5074	116	164
8	Tracing N8:	574.3691	28.7185	38	132.3191	116	164
9	Tracing N9:	1776.2572	88.8129	69	77.6915	116	164
10	Tracing N10:	1224.7374	61.2369	45	73.4851	116	164
11	Tracing N11:	355.7054	17.7853	26	146.1884	116	164
12	Tracing N12:	905.3750	45.2688	45	99.4063	116	164
13	Total Neuron	10474.9103	523.7455	479	91.4566	116	164





B. Batch Processing

From the threshold data obtained from the individual treatment of a neuron, the program generates a file with some information which can be applied in batch processing of images. Notice, that pictures obtain from the same experimetn have a similar settings.

SynapCountJ is able to read '.tif' files organized in folders or directly from a '.lif' file (the kind of files produced by Leica confocal microscopes). In order to work with '.lif' files it is necessary the *Bio-Formats* plugin [2].

	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

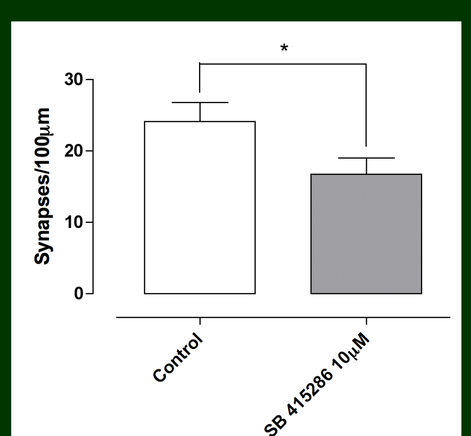
As a final result, a table with the information related to each one of the neurons (both from the global neuron and from each dendrite) is obtained.

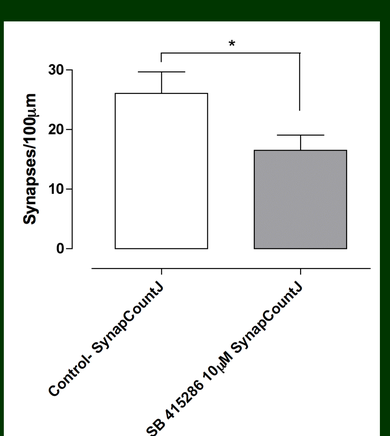
Experimental Results

A comparative study has been performed in order to evaluate the results which have been obtained with *SynapCountJ*.

In concrete, we have studied the effects of two chemical inhibitors of *GSK3 (SB415286)* on cultured rat hippocampal neurons (12 days in vitro). 13 individual images have been analyzed.
In the following graphics we can observe that using a manual method to identify and count synsapses, we obtain a mean of 24.12 synapses in the control cultures and 16.74 in the cultures which have been treated. The results obtained with the plugin are similar, there is a mean of 26.03 synapses in control cultures and 16.50 in the ones which have been treated.

Notwithstanding the differences in the counting, in both procedures we obtain almost the same inhibition percentage, a 36% manually and 36,6% automatically. This shows the suitability of *SynapCountJ* to count synapses, meaning a considerably reduction of the time employed in the manual process.





Conclusions and Further Work

SynapCountJ allows one to automate the task of counting synapses from immunofluorescence images obtained from cultures. The plugin has been tested not only with neurons in development but also with the neuromuscular union of *Drosophila*, therefore, this plugin can be applied to the study of images which contain two synaptic markers and a determined structure.
The next step in our work consists in improving the usability of the plugin and the inclusion of a post-processing tool to manually edit the obtained results.
Our final aim is the achievement of a complete automation of the method, thereby it is necessary the automatic detection of neuron morphology. At this point, topological information will play a key role since they will be used to reduce the amount of information to deal just with the relevant one. Moreover, we want to extrapolate this method to locate and classify in vivo dendritic spines. The plugin is free and can be downloaded from:

http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:synapsescountj:start

Suggestion can be sent to: gmata.ext@riojasalud.es

References

1. *NeuronJ*: http://www.imagescience.org/meijering/software/neuronj/
2. *Bio-formats*: http://www.loci.wisc.edu/software/bio-formats