

Developing new tools to analyze neuronal morphology, spine and synaptic density

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Abstract

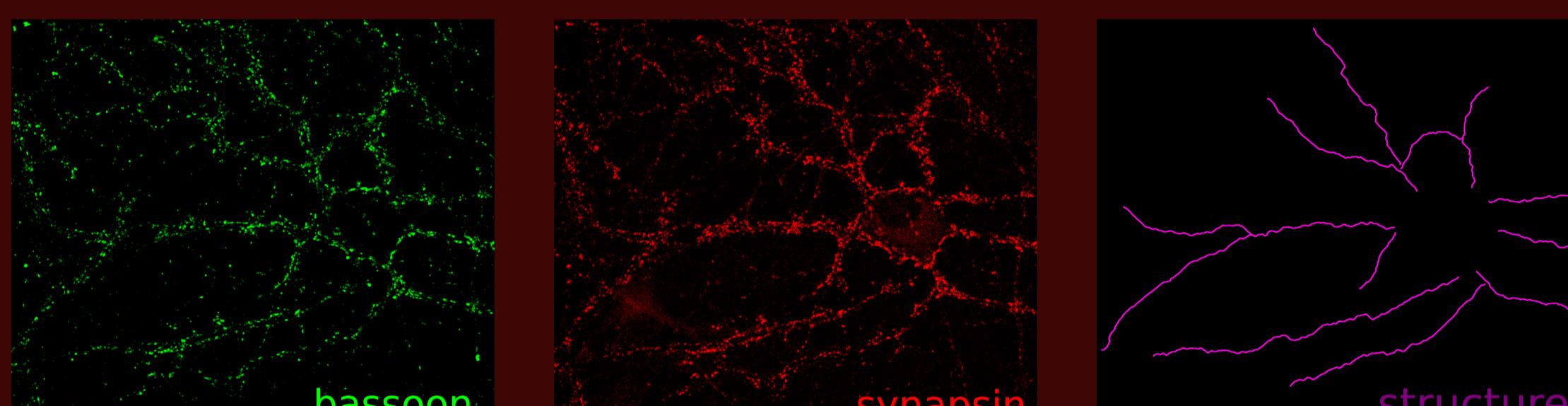
The aim of our work consists in developing software systems which allow a full automation of research, integrating from microscope movement management to final analysis of spine and synaptic density. The underlying algorithms of these programs are based on both homological methods for digital imaging and geometric persistence models.

All our plugins have been implemented in Java for the systems *ImageJ/Fiji*, and can be executed in Windows (XP/Vista/7), Mac OS X and Linux.



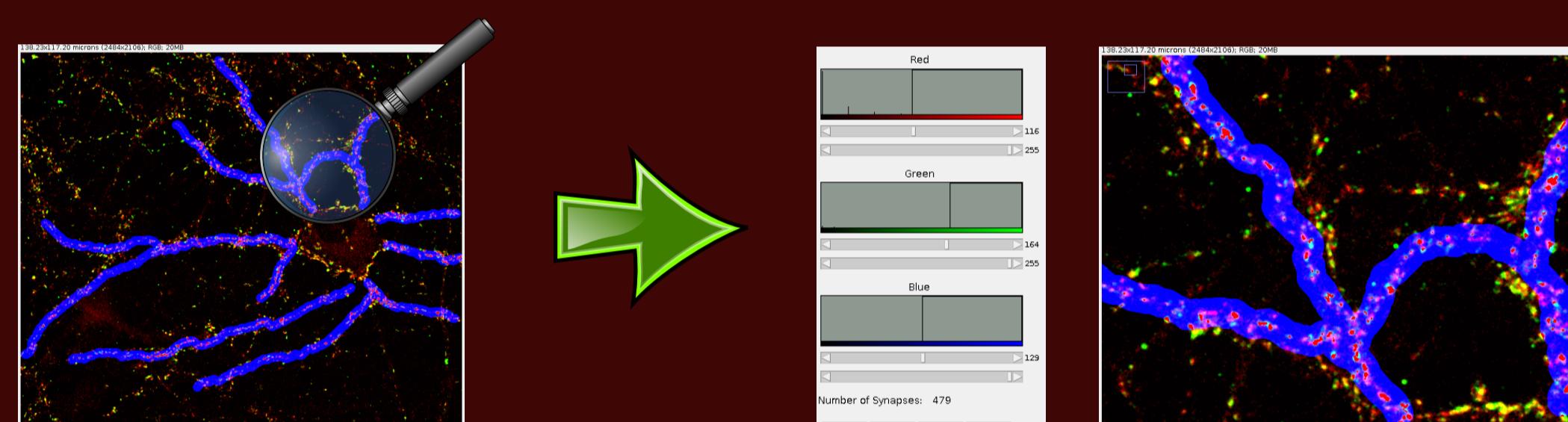
1.- SynapCount

This plugin provides an automatic solution to measure synaptic density. The underlying method of this plugin is able to identify synapses thanks to a triple criterion: two synaptic markers and a neuronal morphology marker.



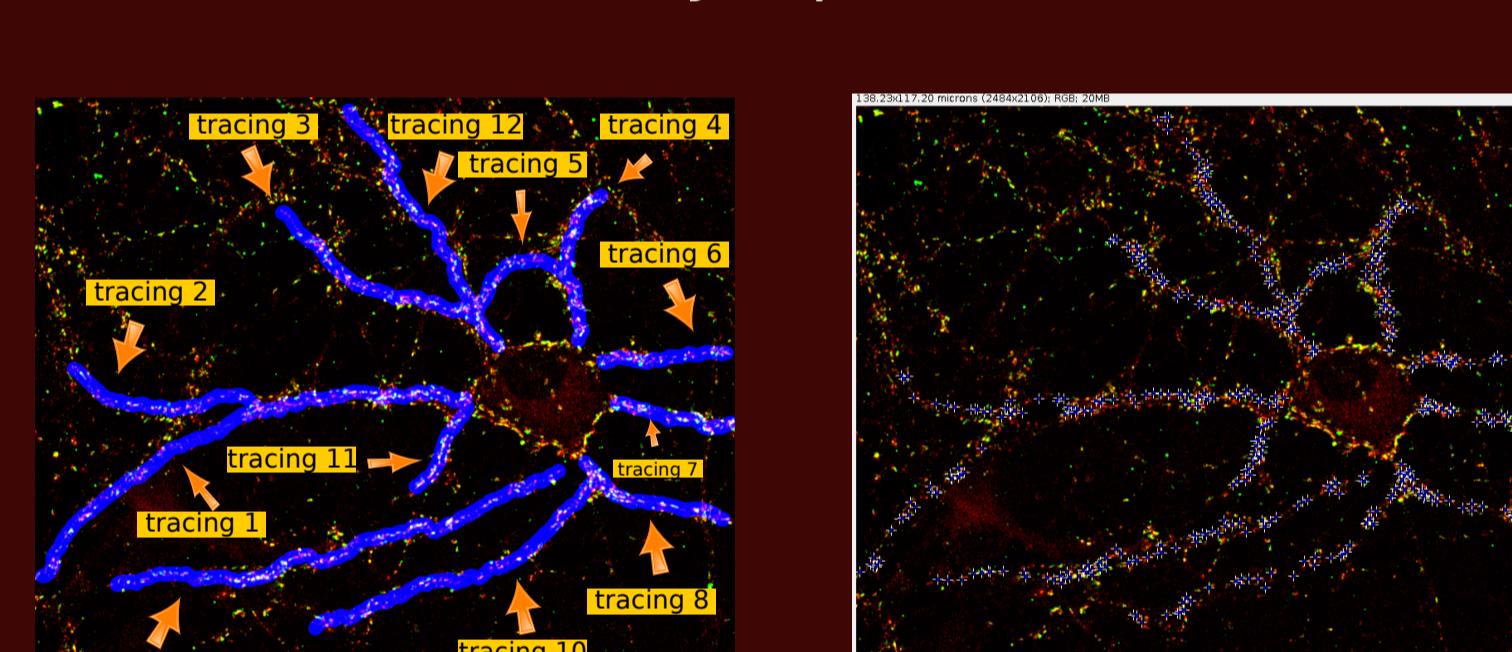
We select the regions where the measurement are going to be performed. In this manner we remove the background. To this aim, we use the NeuronJ plugin.

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



Eventually, *SynapCount* 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	93.6553	71	77.4641	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	49.2688	45	99.4063	116 164
13	Total Neuron	10474.9103	523.7455	479	91.4566	116 164

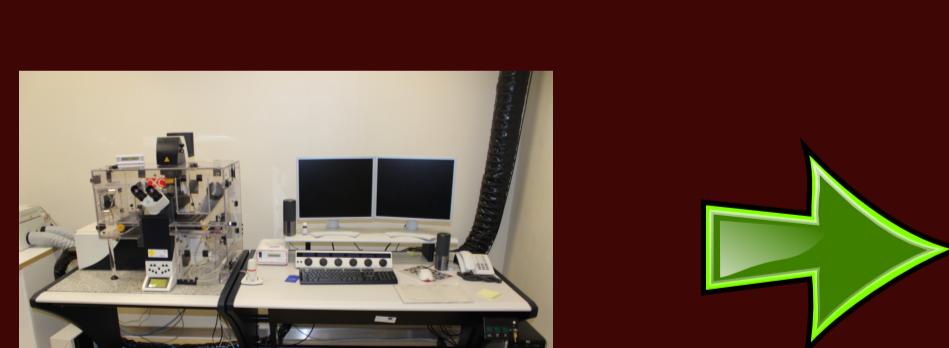


From the threshold data obtained from a individual neuron, the program generates a file with the information which can be applied in batch processing for all images. Notice, that pictures obtained from the same experiment have similar settings. In this way, batch jobs can be carried out inside *SynapCount*.

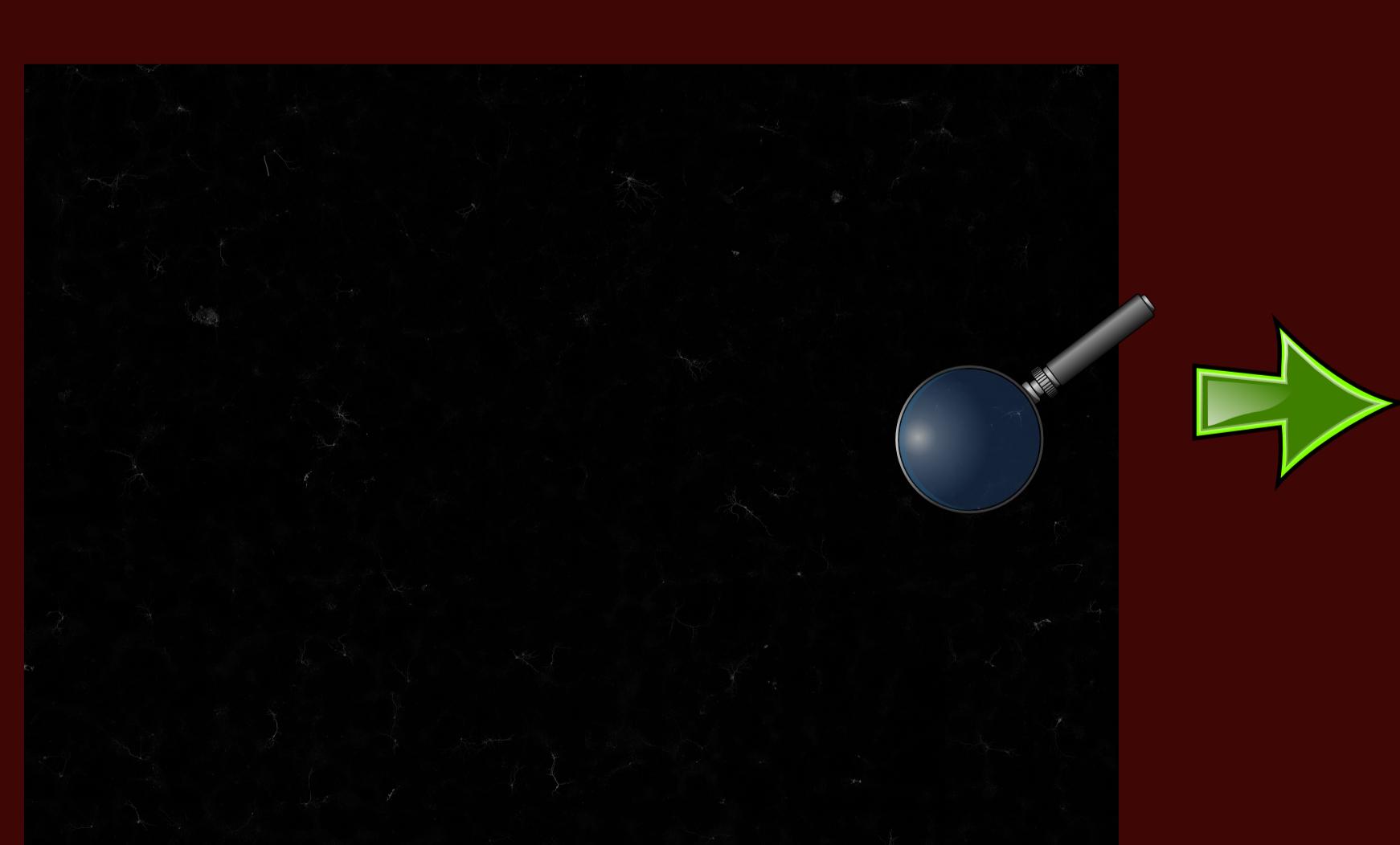
You can get more information and downloading from here:
<http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:synapsescountj:start>

3.- Current and Future Work

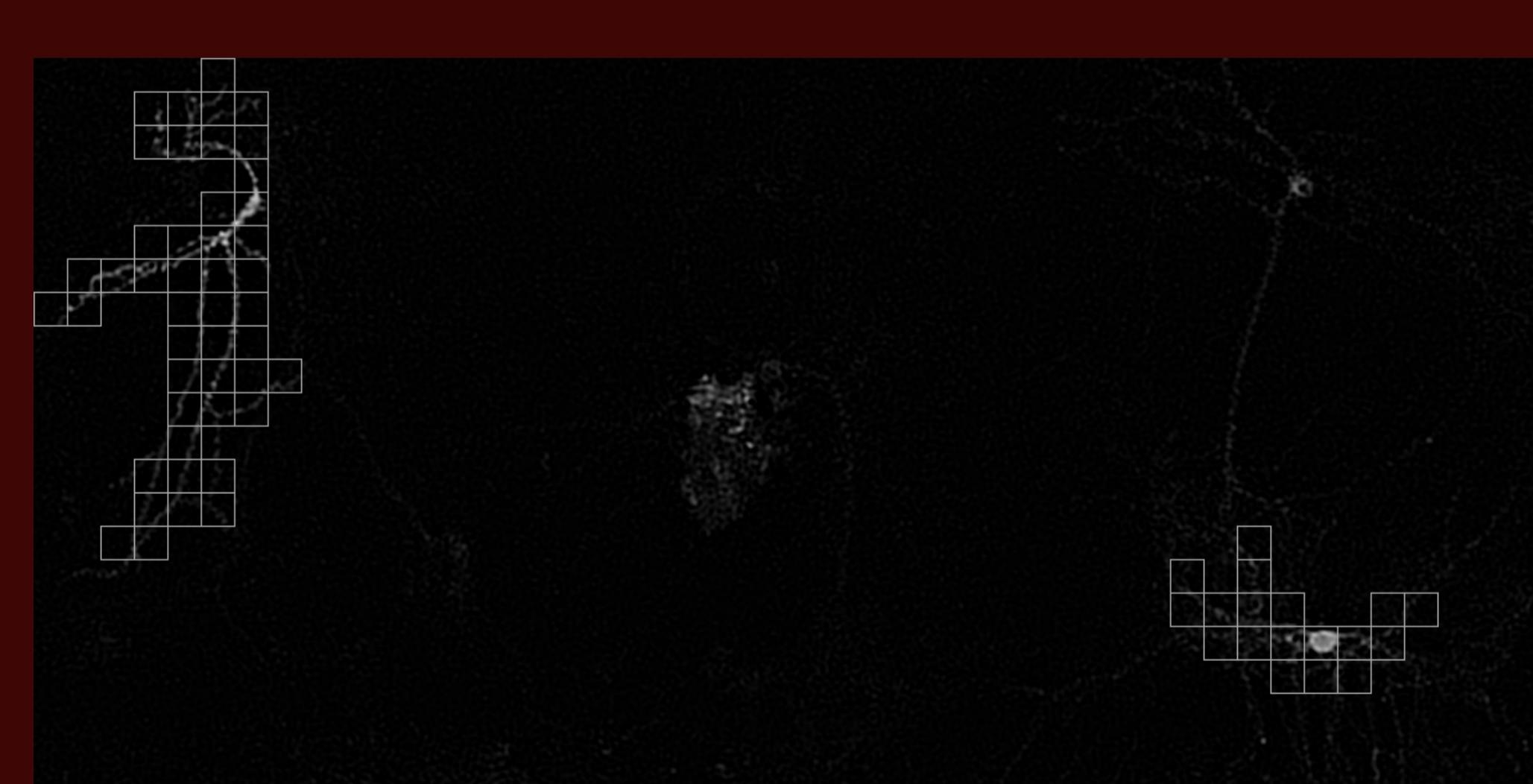
Nowadays, we are developing a new plugin which allows localization of neurons in pictures. The image obtained with the Matrix Confocal Software covers aprox 63620.750mm² of a glass coverslip. In fact, the picture is a mosaic of several images. The final aim of this project is, once neuron is localised, command the microscope movement stage and acquire a second image at higher magnification and resolution. Moreover, we want to develop a new plugin to locate and classify dendritic spines.



Culture plates are obtained with a laser scanning confocal microscope (TCS SP5) using the LAS AF - Matrix Confocal.



Example of a picture mosaic from of a coverslips with neurons transfected with GFP-Actin. This mosaic consist of 12x9 images, each one with a size of 1024x1024px. The images were acquired using 20x objective. The full mosaic measures 9163.013x6943.213μm.



This image is a part of the mosaic showing the result obtained with our plugin. The superimposed squares indicate the area occupied for the neuron. This is achieved using techniques based on tracking algorithms.

4.- Save All plugin

Recently, we have needed a save all functionality when working with *ImageJ/Fiji*. This plugin saves and closes all the open images in the selected folder. If the image is a stack, the plugin separates each slices in a new image, and saves each slice as a new image. "Save_all" can be download from:

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

To obtain this poster:



You can help us to improve our plugins. Please, send comments and questions to: gmata.ext@riojasalud.es

