

NucleusJ

**a plugin for ImageJ/Fiji that allows you to count
the number of neurons in an image**

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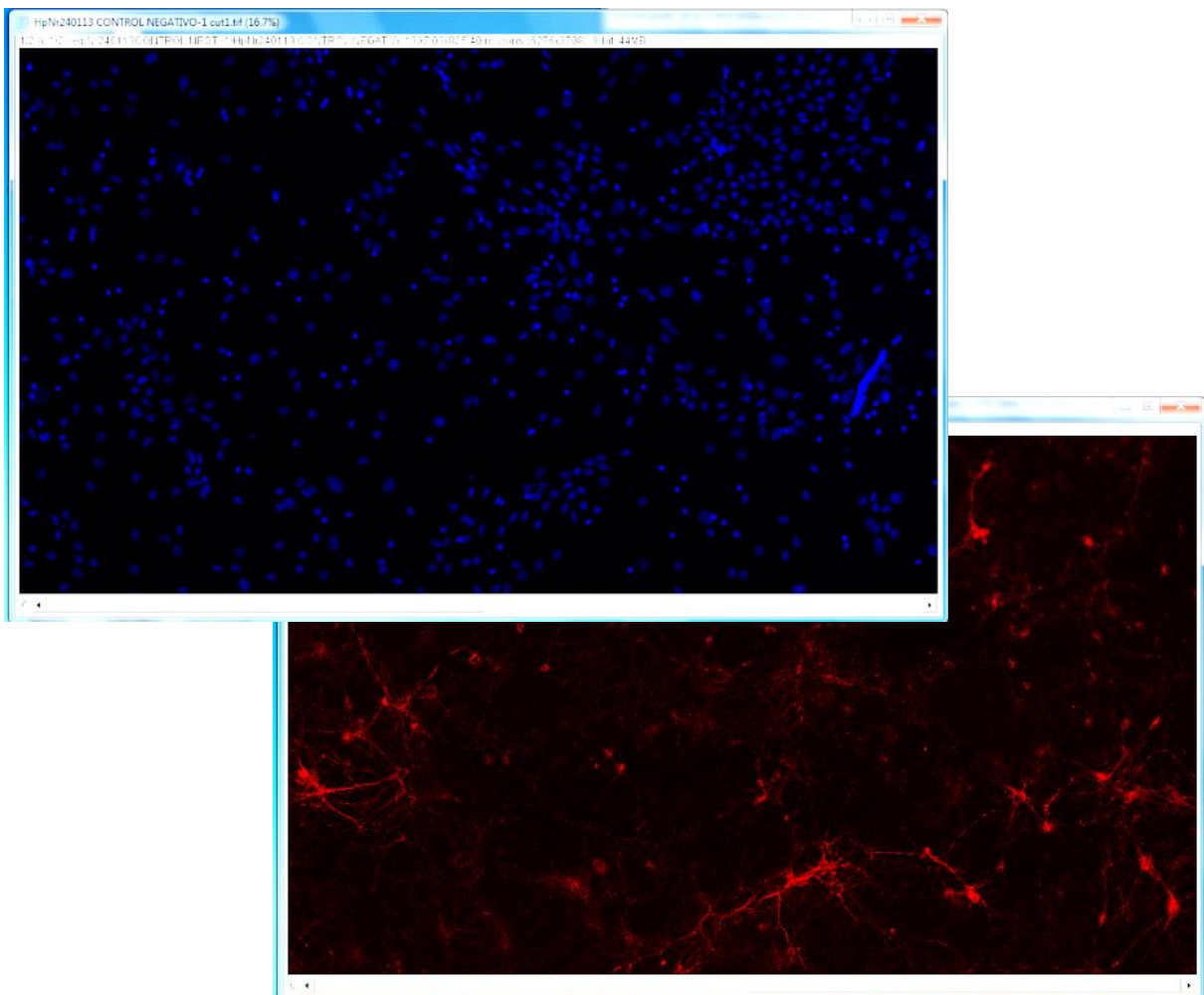
spineup@spineup.es

SpineUp, www.spineup.es

1. Install the plugin “NucleusJ_” by copying it in the folder “plugins” of Fiji.app.

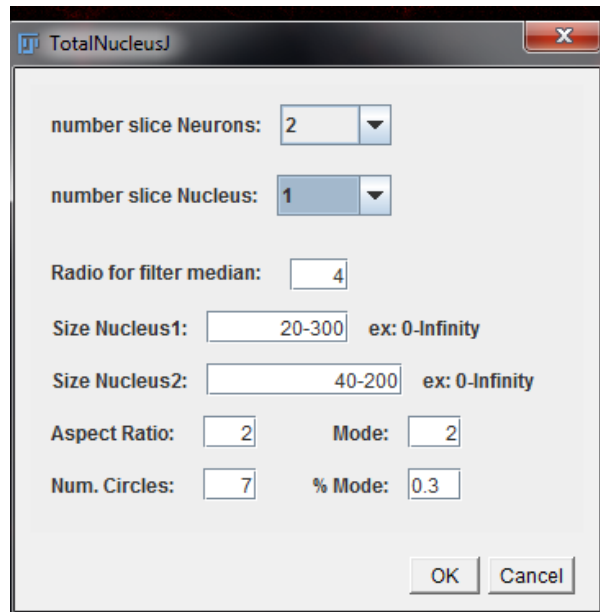
Download NucleusJ_12.rar (<http://spineup.jimdo.com/downloads/>), and unrar. Put NucleusJ_v12.jar into the plugins folder or subfolder. Restart ImageJ (or Fiji) or click “Refresh Menu”, which is at Help Menu. The plugin “NucleusJ_v12” command will be located at Menu/Plugins.

2. In one image file there are two pictures, one of the nucleus (usually blue), and another one of the neurons (red or green).



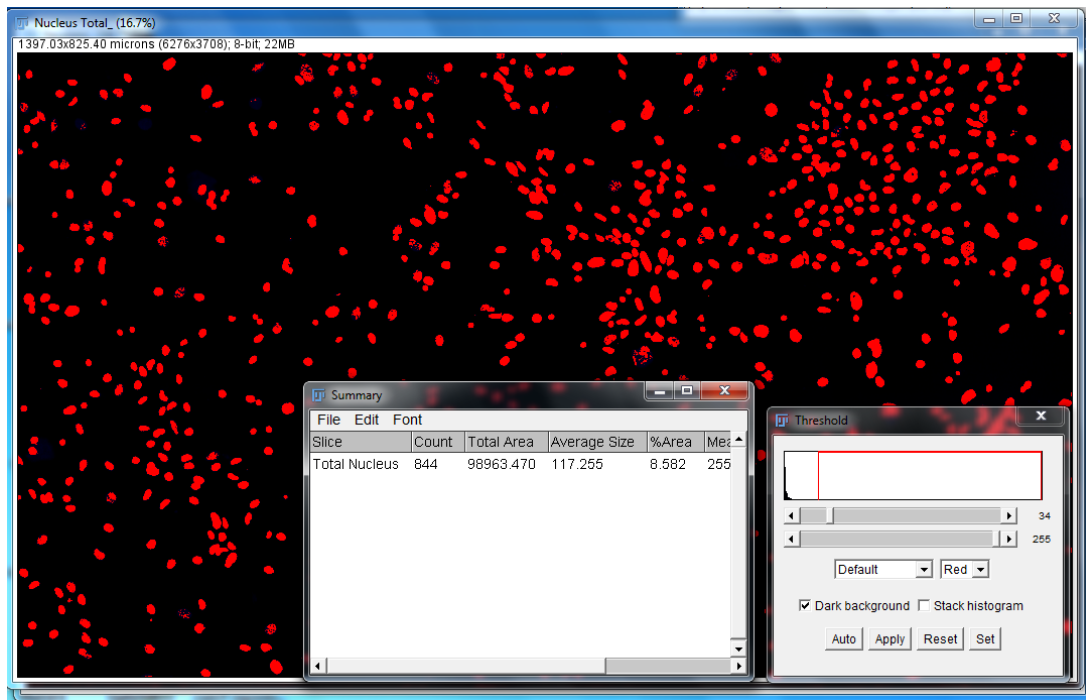
The default values of this plugin are optimized for images like the previous figure, which represents a 12 DIV rat hippocampal culture. Red is MAP2B and blue is nuclear staining with DAPI. The size of the image is about 6mm².

3. Launch the plugin *NucleusJ*. The following dialog is open:



- Adapt the **number slice neurons** and **nucleus** to the good slice picture (1 or 2). It is usually like this, but you have to be careful or the results will be wrong.
- **Radio for filter median**: select the radio for filter median, this filter preserves edges while removing noise. This filter is applied to the image of nucleus.
- **Size Nucleus1**: Set the interval of sizes that are valid for nucleus (of neurons or astrocytes). These values determine the area of nucleus which are going to be studied.
- **Size Nucleus2**: Set the interval of good sizes for nucleus of neurons.
- **Aspect Ratio**: This value determines the maximum relationship between the Major and Minor axis. If the nucleus has an aspect ratio larger than this value, that nucleus is removed.
- **Mode, Number of Circles** and **% of mode** are values to compare with the neuron channel. Usually, in this kind of images, it is almost this way, but you have to be careful or the results will be wrong. The user can change these values.

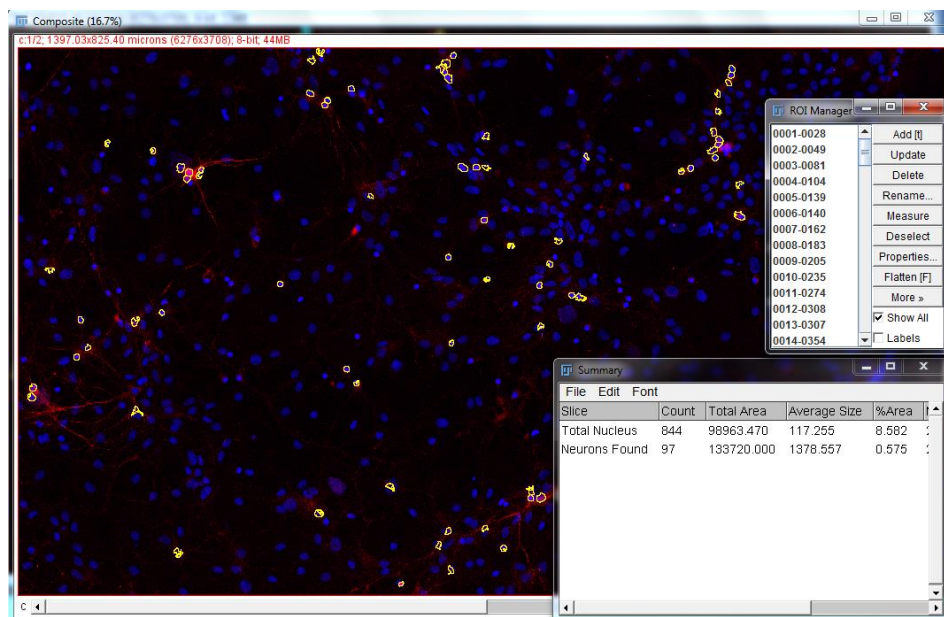
4. In any case, when clicking on the button “OK”, the plugin will show the following dialog and pictures:



- In the summary table, the first value in the ‘Count’ column is a number of total nucleus (of astrocytes and neurons). The second one is a threshold to select a range in which we estimate there are nucleus of neurons. You need to adjust the number of threshold to get red round circles (the best you can).
In this image there are 844 (number of nucleus) and 34 (threshold selected).

- Then click: Apply

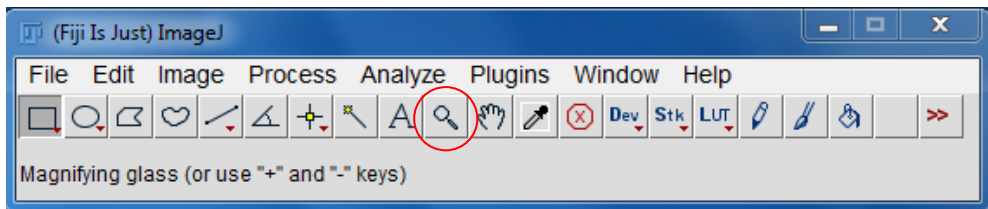
5. Wait for the next result:



In this picture the software surrounds with yellow circles the spots where neurons and nucleus overlap each other.

If the circles are not yellow you can change it in “Properties”, inside the “ROI Manager” window.

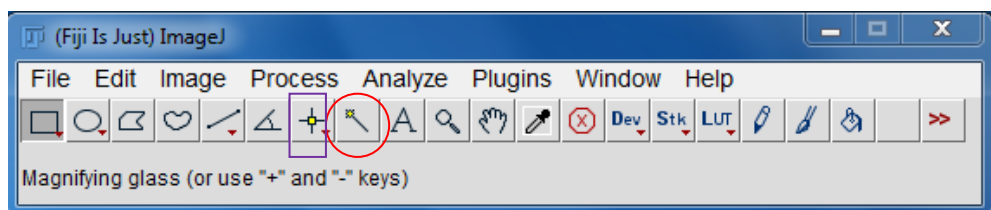
- Then you have to check if the software has worked for the whole neurons and nucleus overlapping. For that use the zoom.



- The plugin allows the user to correct the marked neurons and to add more neurons or nucleus.



- When some circled area is wrong, first click *Labels* on the *ROI manager* window. Then use the tool below to select it, then click delete button to remove it.
- If you want to add another circle, use the cross tool and click on the right spot it will had it in the *ROI manager* window.



In the list on the ROI manager the nucleus added are represented as X-X-X, previous as X-X and the deleted nucleus have been erased.

8. **To study the results**, we write the number of nucleus in an excel sheet of the following form:

1		numero	densidad	%neuro/cls	% neuron/astroci
2	neurons	94	81,51877724	11,14	12,53
3	totals cells	844	731,9345531		
4	astrocitos	750	650,4157759		
5	default threshold	34			
6	you threshold	37			
7	Neurons			PIXELS	MICRONS
8	cells deleted	94	X	6276	1397,03
9	cells added		Y	3708	825,4
10			AREA	23271408	1153108,56
11			0,222598789	AREA mm2	1,15310856
12					

Write the total nucleus and the neurons found number in the excel sheet.

Then finish completing the excel sheet:

- **Neurons** <-> neurons found
- **Total cells** <-> number of total nucleus
- **Default threshold** <-> the first one
- **Manual threshold** <-> the last one
- **Cells deleted** <-> cells manually deleted
- **Cells added** <-> cells manually added
- **Pixels (X,Y)** <-> written on the top of the image
- **Microns (X,Y)** <-> written on the top of the image

The other parameters are calculated automatically.