MACROglia

MORPHOLOGICAL ANALYSIS OF CELLS

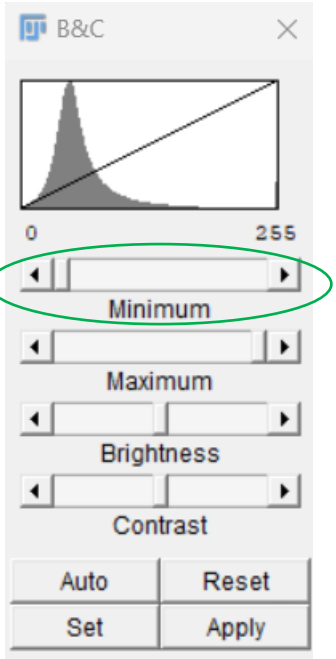
** Previously, install FracLac plugin in ImageJ FIJI **

** The steps done by the macro are in grey*

**This macro works with .JPG and .TIFF images*

MACRO (AUTOMATIC)

1. Open the image in FIJI/ImageJ: **Drag&drop the image** to the tool bar of the program or **File > Open… (Ctrl+O)**
2. Open (drag&drop) the **macro "MACROglia.ijm"** and click on **RUN**
3. Transform the image into 8 bits image: **Image > Type > 8bit**
4. Transform the image into a frequency domain and remove high frequency signals: **Process > FTT > Bandpass Filter > OK**
5. Transform the image into grayscale image: **Image > Lookup Tables > Grays**
6. Adjust the brightness and the contrast: **Image > Adjust > Brightness/Contrast**

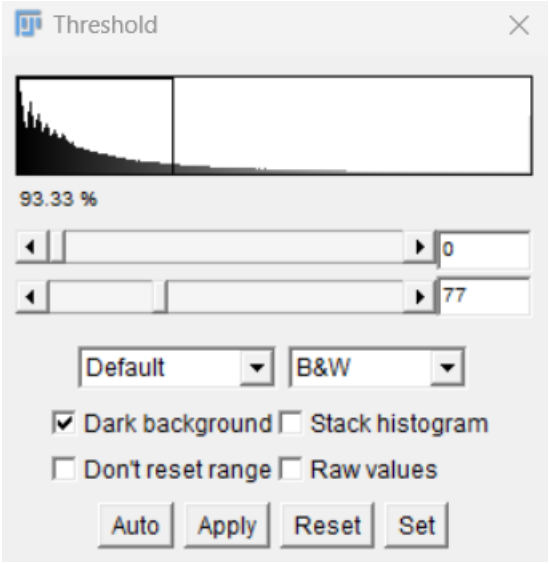


Increase the "Minimum" to have more contrast

> Apply to confirm

MACRO (AUTOMATIC)

7. Once it is adjusted, close "B&C" window and click on **OK** in the window **"Adjust brightness and contrast"**
8. Improve the contrast: **Process > Filters > Unsharp Mask > OK**
9. Remove background noise: **Process > Noise > Despeckle**
10. Duplicate the image and, hereinafter, work with that duplicated image: **Image > Duplicate** or **Ctrl+Mayus+D**
11. Adjust the threshold: **Image > Adjust > Threshold**



DO NOT change these numbers, it's an "automatic section" of threshold settings

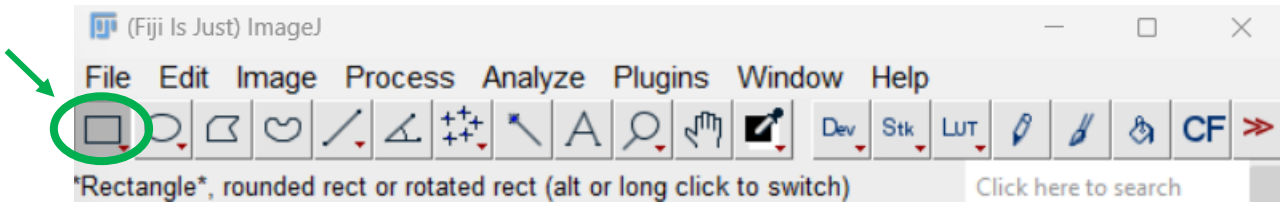
Check that you have set these parameters

> Apply to confirm

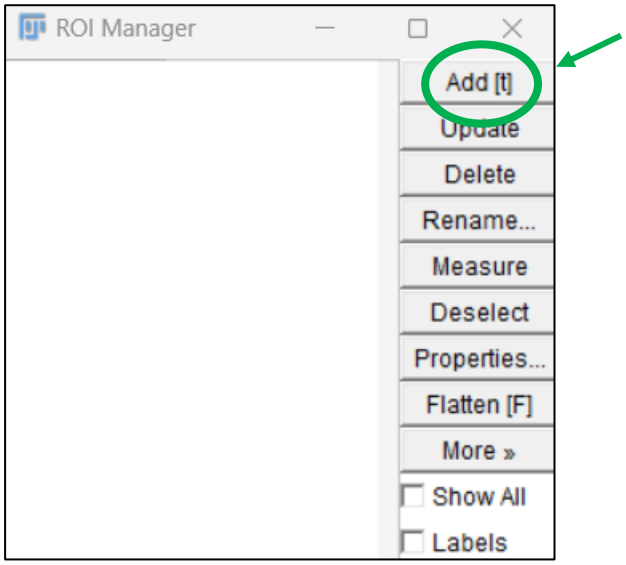
12. Once it is adjusted, close "Threshold" window and click on **OK** in the window **"Adjust threshold"**

MACRO (AUTOMATIC)

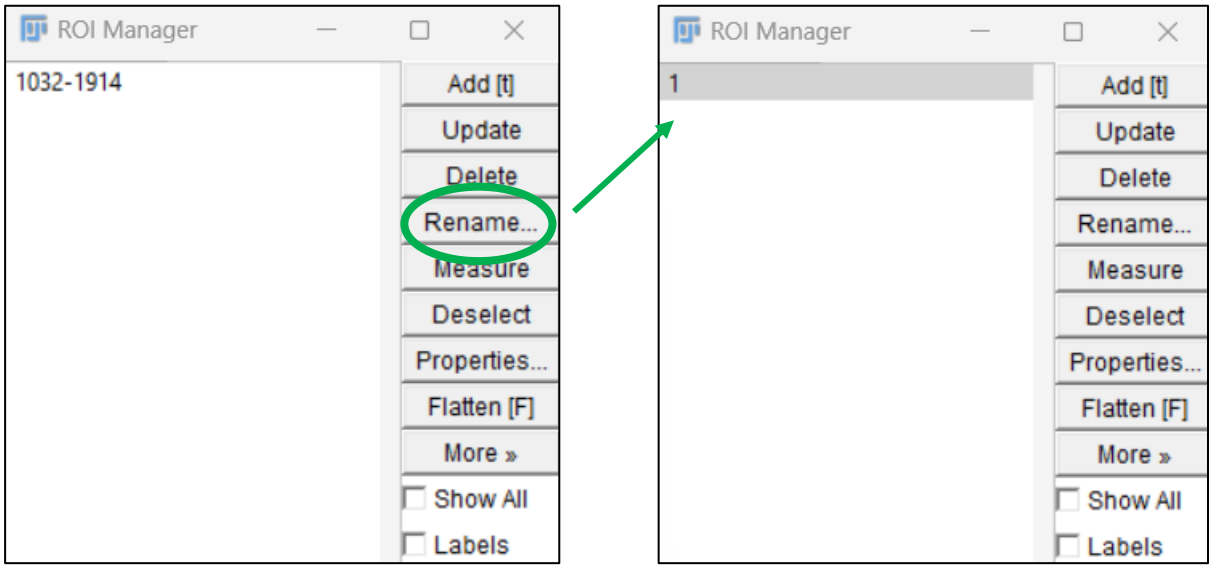
13. Remove the background noise: **Process > Noise > Despeckle**
14. Apply binary image filter (background in black and cells in white) to transform the black pixels adjacent to a white pixel into white too and vice versa, to transform the white pixels adjacent to a black pixel into black too: **Process > Binary > Close**
15. Ascribe the value of neighbouring pixels to those pixels that go through the roof continuously: **Process > Noise > Remove Outliers > OK**
16. Select the cell (microglia, astrocyte, whatever) that is going to be quantified: Tool ***Rectangle***



17. Save it into the ROIs list in the window “ROI manager”: **Add [t]**

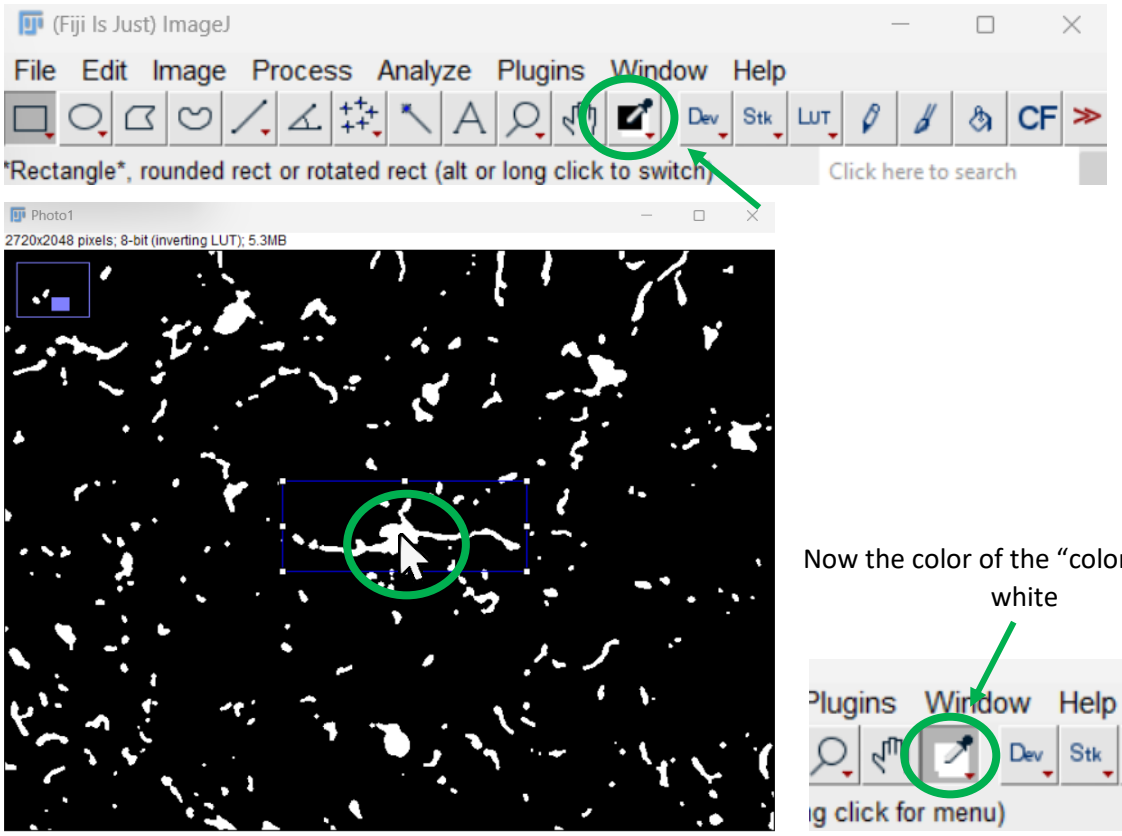


18. In the window “ROI manager”, rename ROI (**Rename…**) and entitled it as “**1”**



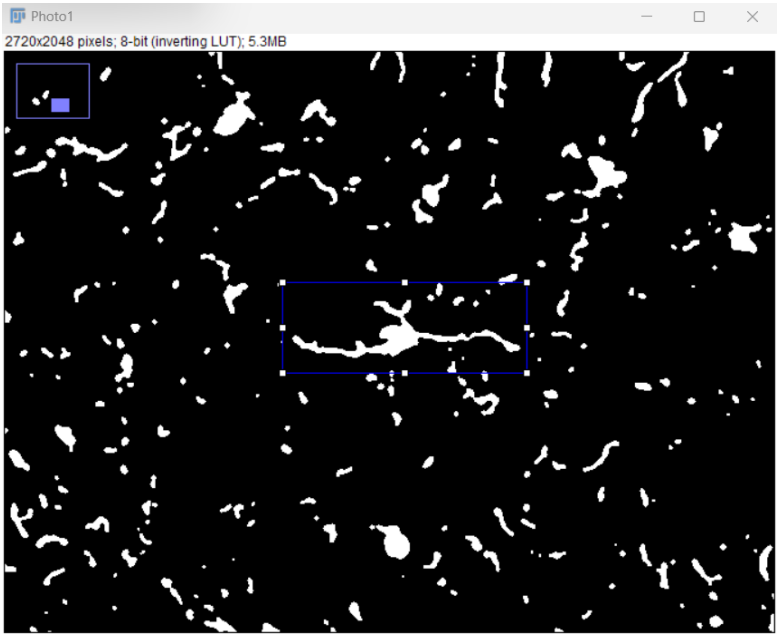
19. Repeat steps from 16 to 18 until you have saved **as many cells as you want to analyse**

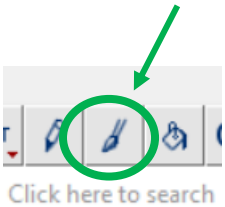
20. Make zoom (tool ***Loop***  , to exit from the loop tool just do double-click over the loop icon) in each cell and **redraw the projections of the cell that have been erased** during the image processing by comparing it with the image of the step 7: Select the color with which you want to paint using the tool ***Color picker***



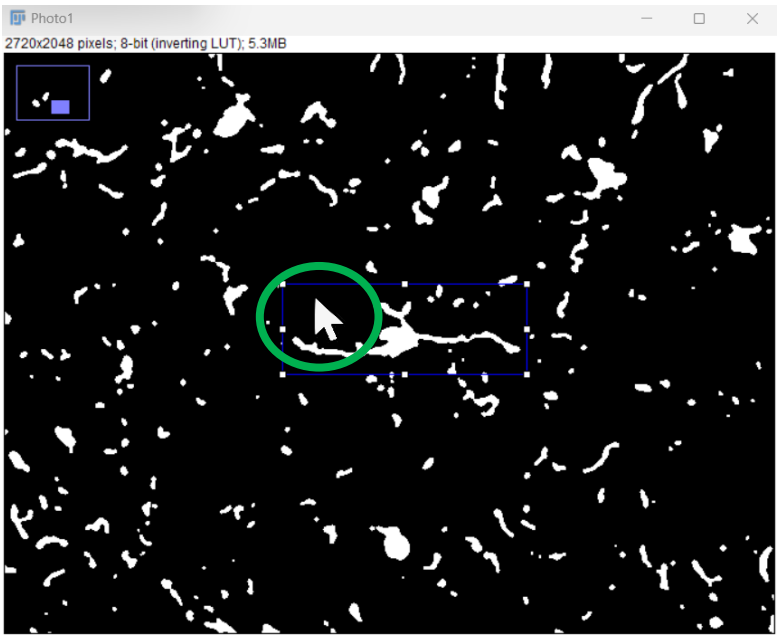
Now the color of the "color picker" is white

> Draw using the tool ***Paintbrush Tool*** (do double-click or press the right click of the mouse to change the width of the paintbrush -**brush width: 5**-)

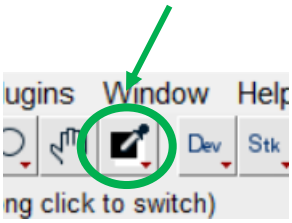




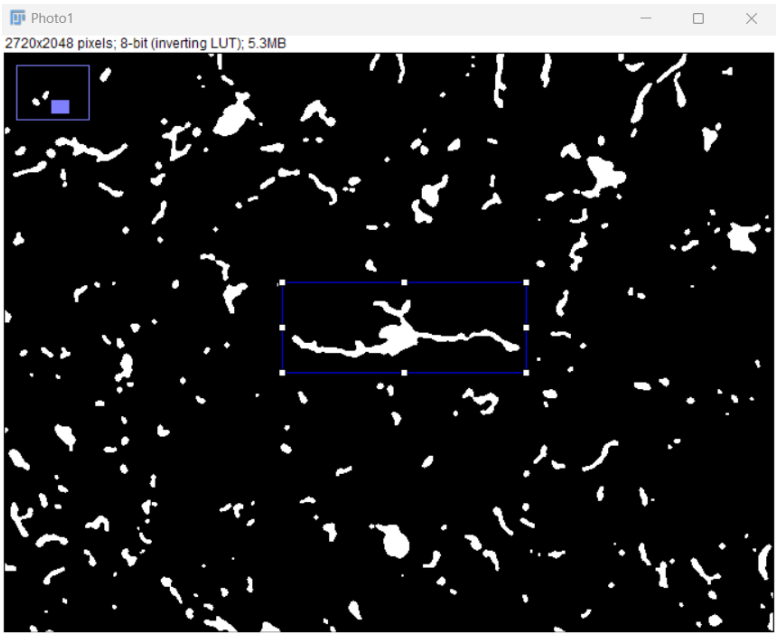
If there are portions of other neighbour cells inside your cell selection rectangle: select the background color with the ***Color picker***

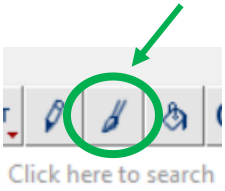


Now the color of the “color picker” is black



> and then delete those pieces with the ***Paintbrush Tool***





MACRO (AUTOMATIC)

21. Once you have it done with all the cells that you want to analyse, click on **OK** in the window **"Select cells and save ROIs"**
22. Remove the filling to keep only the outline of the cell: **Process > Binary > Outline**
23. Analyse the morphology of microglia with the FracLac plugin that you have installed previously: **Plugins > Fractal Analysis > FracLac**

**May you have already used FracLac plugin and you have set your own parameters. In that case, do not read the following steps.*
***If you have never used FracLac plugin**, from steps 24 to 30 I will show you the parameters and values that I work with.*
Anyway, I encourage you to read more about this plugin to extract its full potential and adapt it to your cell type/samples.
For more information: https://imagej.net/ij/plugins/fraclac/FLHelp/Tutorials.htm

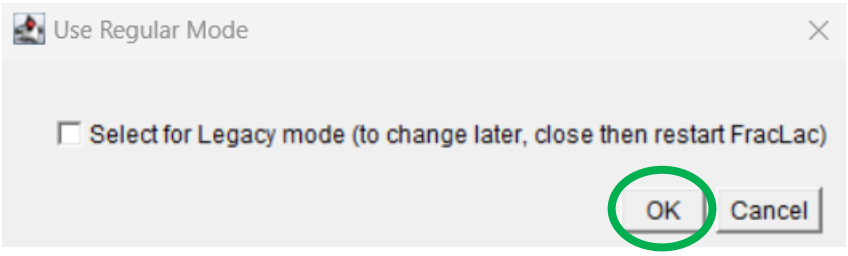
24. In the FracLac plugin  **>** select: **Utilities > Load ROIs as Stack**

25. Background: **White**

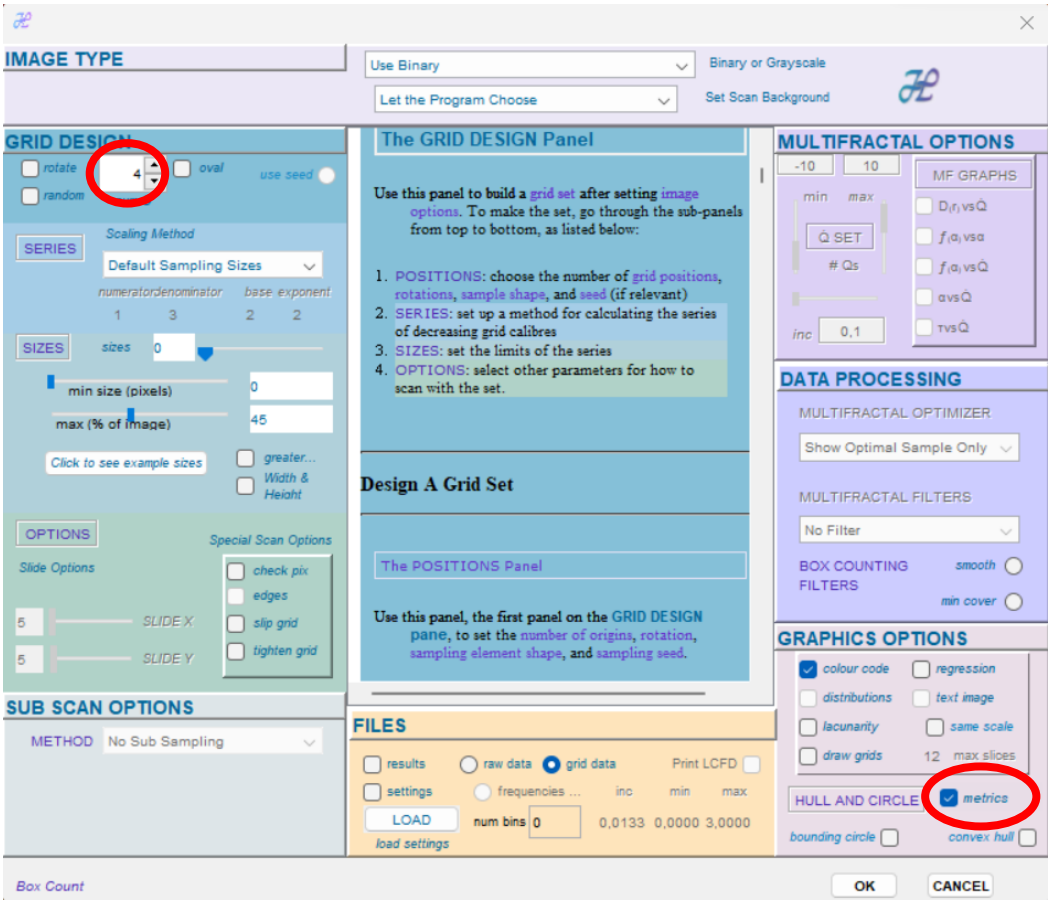
    IMPORTANT: in the emerging window that appears, check that each ROI is only the outline of the choosed cell (e.g. the draw of the cell “1” correspond to the cell that you have entitled as “1” in the window “ROI manager” - step 18)

26. In the FracLac plugin, select the box: **BC** 

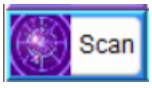
27. **DO NOT** mark the box **“Select for Legacy mode”** and click on **OK**



28. Measure the morphology:
    a. In the tab **GRID DESING > numG = 4**
    b. In the tab **GRAPHICS OPTIONS > mark box "Metrics"**
    c. Press **OK**



29. Select the type of fractal dimension to colour code: **Db**

30. Measure the outline of the cells: **FracLac > SCAN** 

    Then, different emerging windows will appear but I use to focus on the ones that are named here and the values that are in these specific positions in each window:

    a. **Box Count Summary**
        i. **D (fractal)** → position 6
        ii. **Lacunarity** → position 87
    b. **Hull and Circle Results**
        i. **Density**
        ii. **Area**
        iii. **Perimeter**