

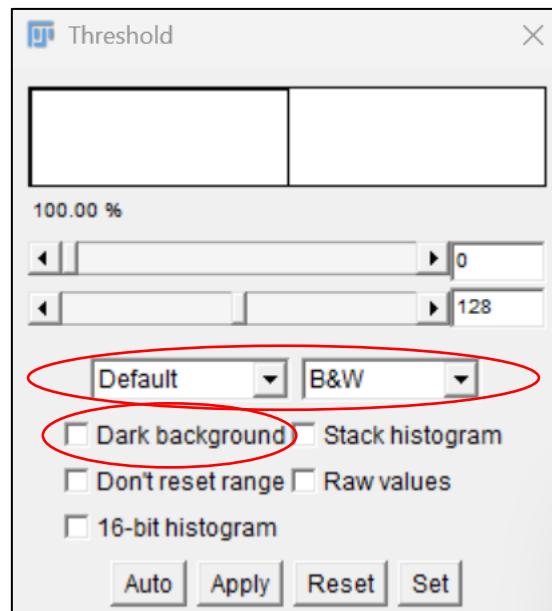
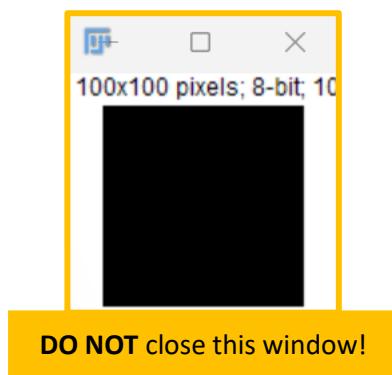


MORPHOLOGICAL ANALYSIS OF CELLS

* Previously, install FracLac plugin in ImageJ *

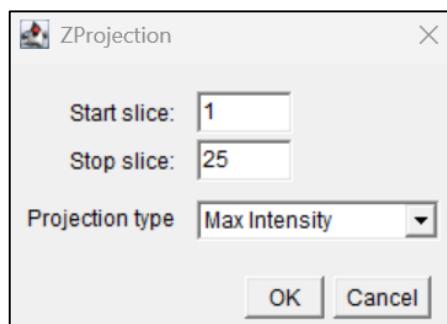
*This macro works with any image format supported by ImageJ or FIJI

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_vXXX_X.ijm”** and click on **RUN**
(If you already created a shortcut, run the desire ‘MACROglia’ version: or)
3. Select the “Default + B&W” mode and untick the “Dark background” box, in case it is ticked. Click on **OK** in the window “**Select ‘B&W’ and untick the ‘Dark Background’ box (if it is ticked)**. Press **OK**”



4. For images containing Z-stacks and/or more than one channel, select the slice(s) that you want to analyze and the preferred projection type

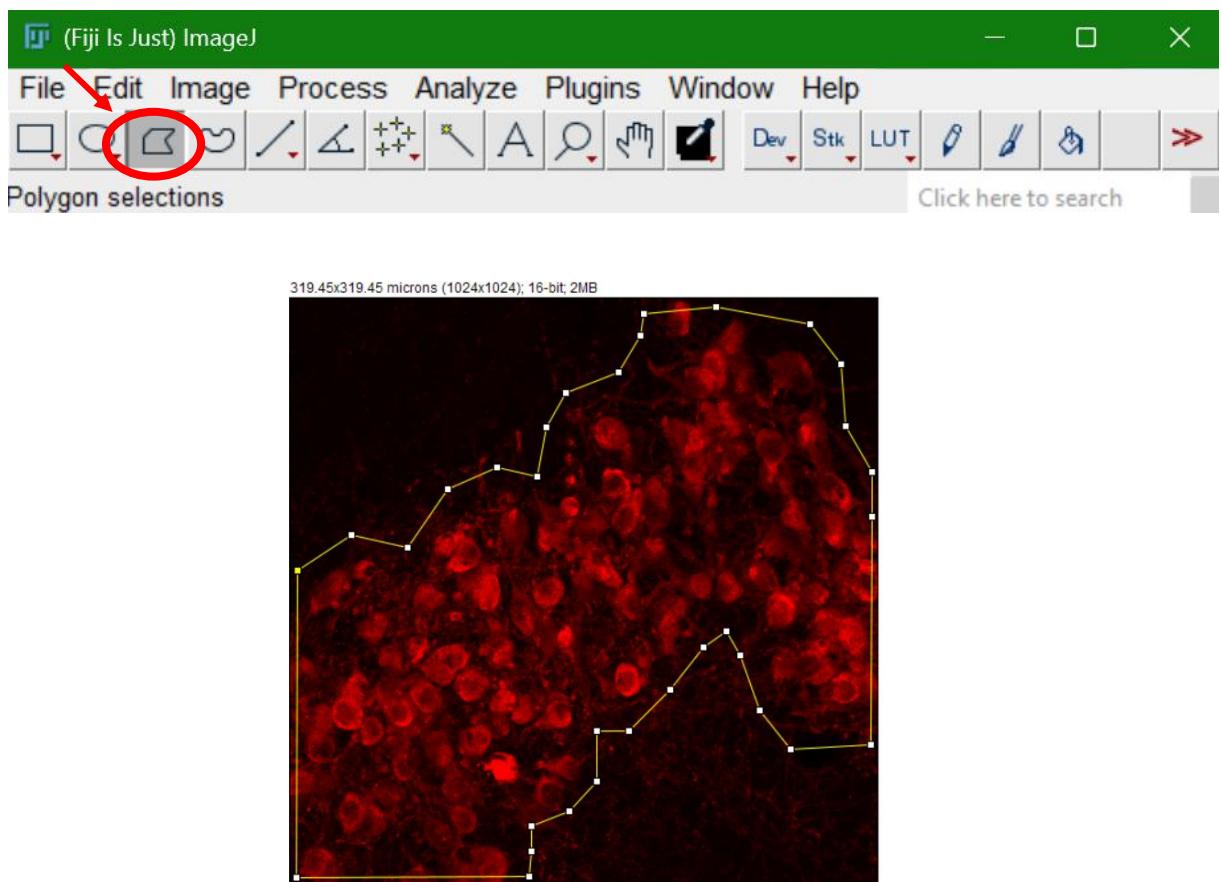
> Press OK to confirm



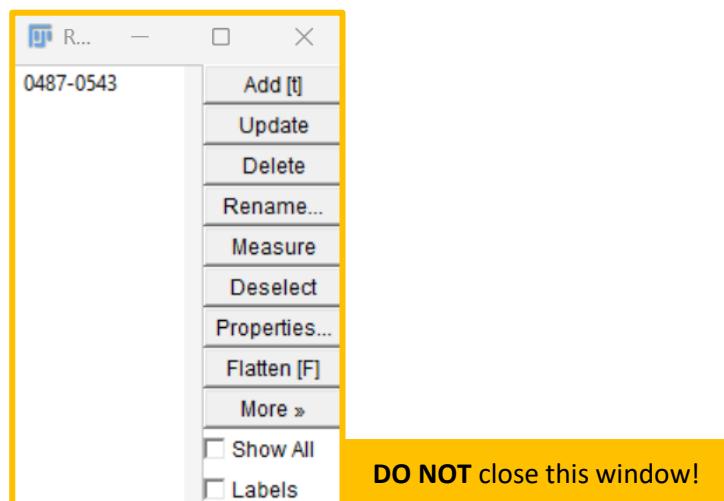
5. In case you want to analyze a specific area or region of interest (ROI) of the image, follow the steps from 6 to 7.

If you do not need to analyze a specific area, select the window that contains the cells that you want to analyze and go to **step 8**

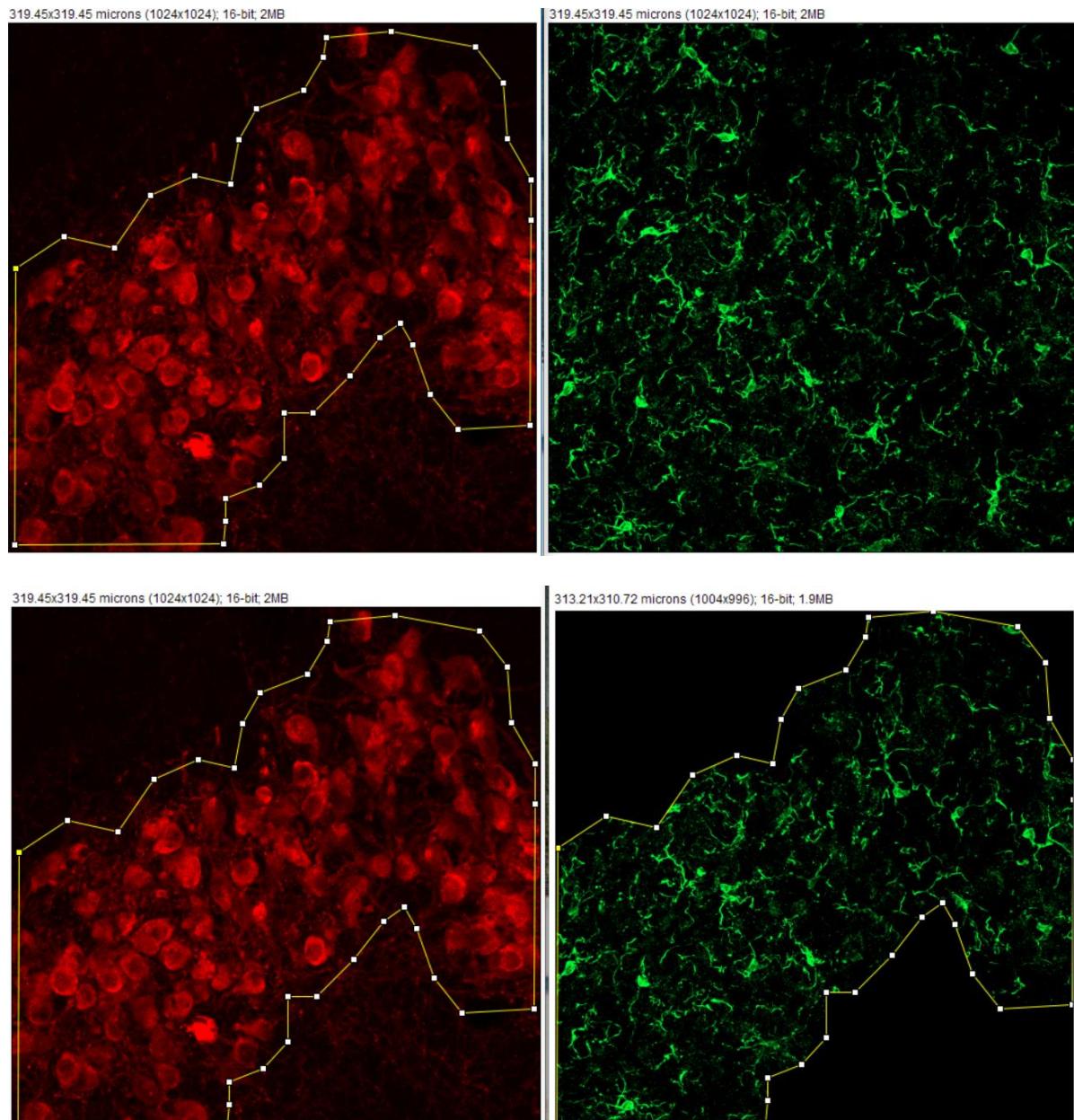
6. To analyze a specific ROI, draw it (use the tool ***Polygon selections***) and click on **OK** in the window “Draw the area that you want to analyze and press OK”



7. Select the window that contains the cells that you want to analyze and click on **OK** in the window “Select the window of the channel you want to analyze with MACROglia and press OK”

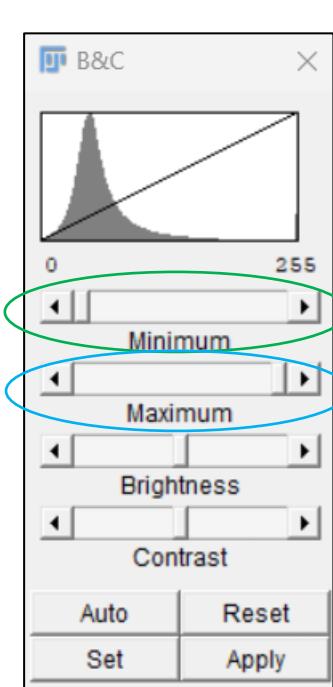


IMPORTANT: may your ROI is determined by a specific staining in a specific channel but the cells that you want to analyze with 'MACROglia' are in a different channel. Do not worry! Draw your ROI and then select the window of the channel containing the targeted cells. Here there is an example:



8. Everything is ready! Click on **OK** in the window “Press OK to start MACROglia”

9. Adjust the brightness and the contrast: **Image > Adjust > Brightness/Contrast**



FOR DAB STAINING

Increase the “Minimum” to have more contrast

*Adjust as needed, up to the edges of the histogram but no further

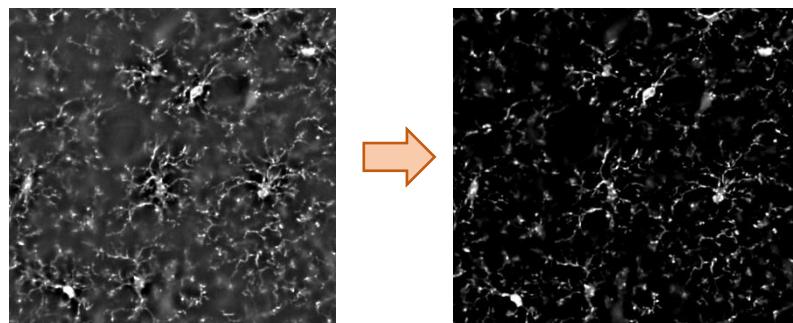
FOR FLUORESCENT STAINING

Decrease the “Maximum” to have more contrast

*Adjust as needed, up to the edges of the histogram but no further

⚠ What is the aim of this step?

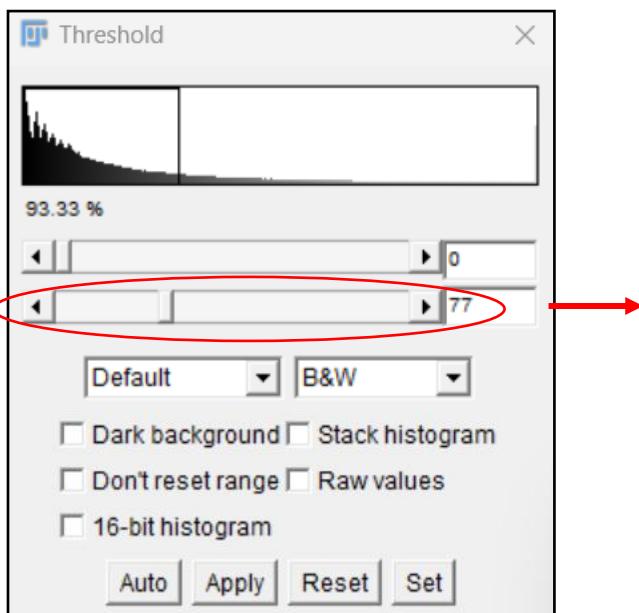
You need to define as good as possible your image



> Press **APPLY to confirm**

10. Once it is adjusted, click on **OK** in the window “**Adjust brightness and contrast (Press **APPLY** and then **OK**)**”

11. Adjust the threshold: **Image > Adjust > Threshold**

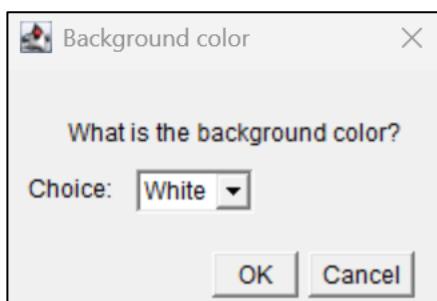


In case you consider that previous steps erased important ramifications, adjust the second slider as needed

> Press **APPLY** to confirm

12. Once it is adjusted, click on **OK** in the window “Adjust threshold (Press **APPLY** and then **OK**)”

13. Indicate the background color of your image and **press OK**.

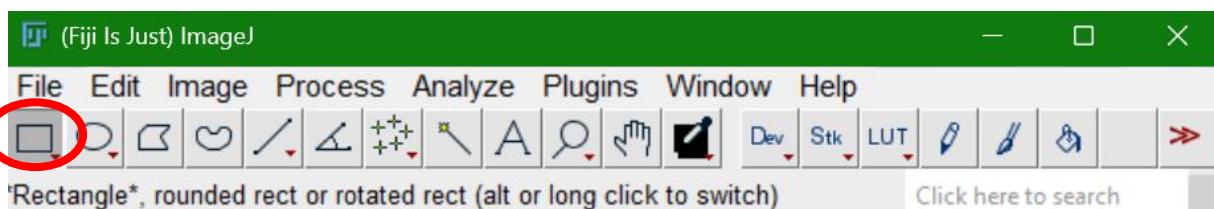


Weird question, right? 🤔

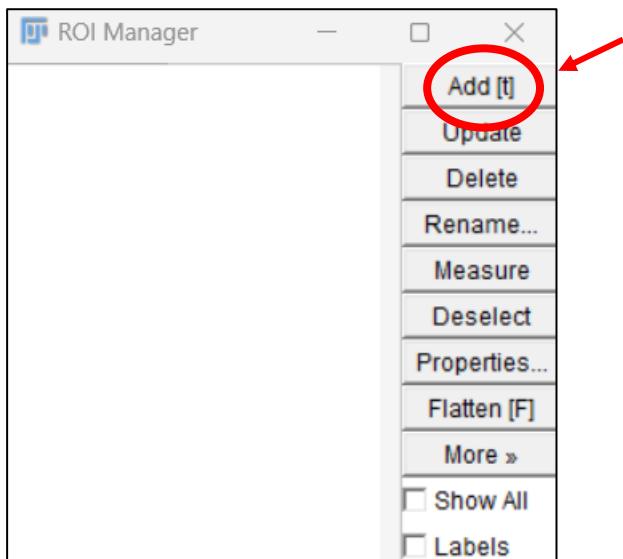
Some images display inverted LUT that will affect the outcome of one of the following automatic steps (specifically the “binary image filter” step)

Thus, this question will avoid any wrong application of this step that could lead into an over-erosion of the image

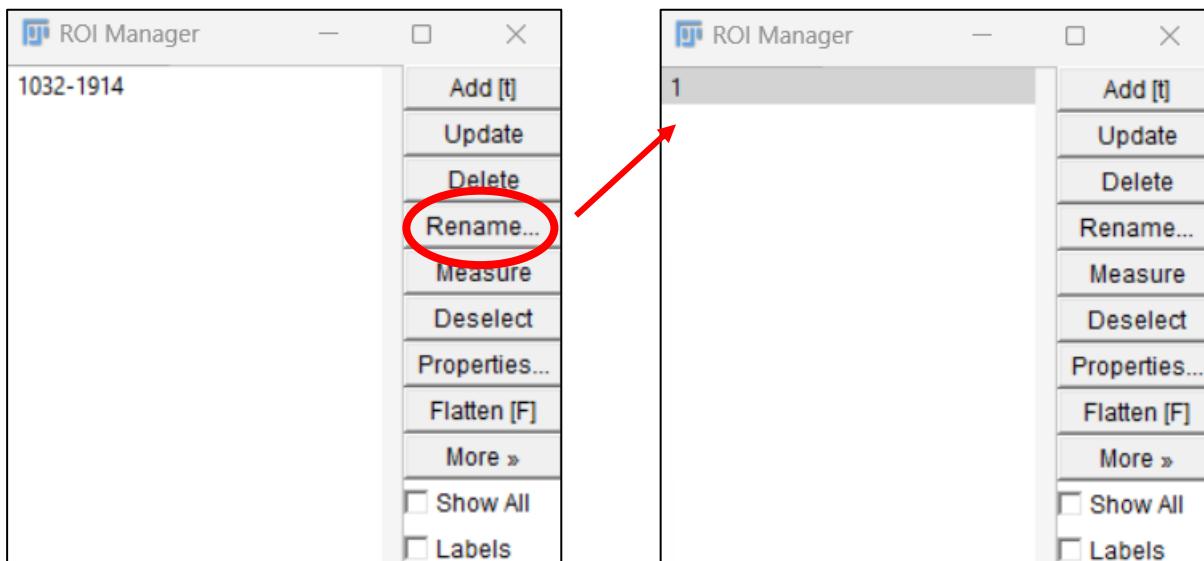
14. **Select the cell (microglia, astrocyte, whatever) that is going to be quantified: Tool *Rectangle***



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



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



17. Repeat steps from **14 to 16** until you have saved **as many cells as you want to analyze**

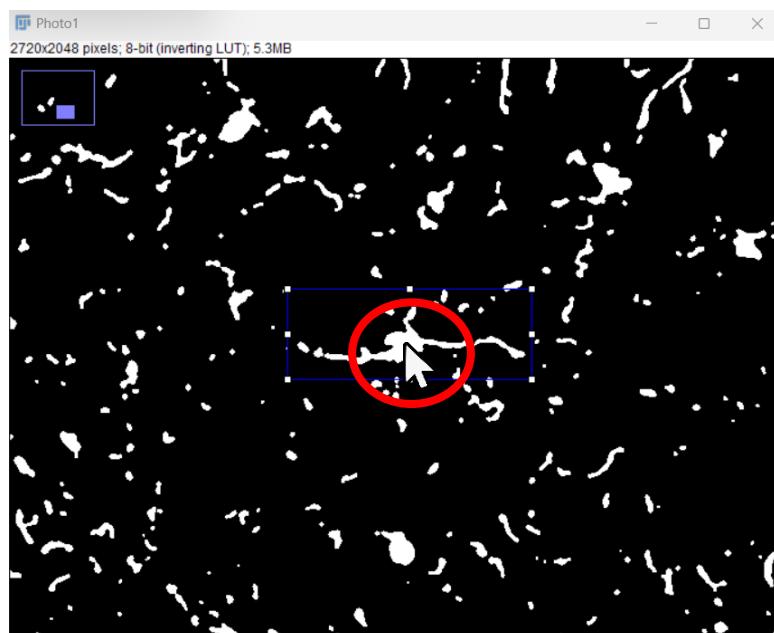
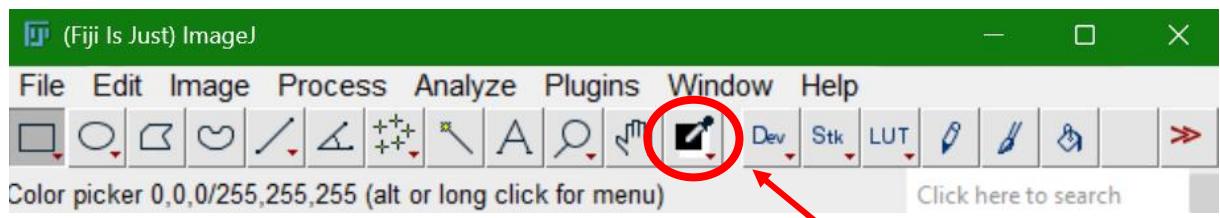


18. Make zoom (tool *Magnifying glass*) to exit from the magnifying glass tool just do double-click over the magnifying glass icon in each cell and **redraw the projections of the cell that have been erased** during the image processing by comparing it with the image of the step 10: Select the

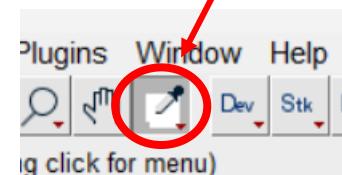


color with which you want to paint using the tool *Color picker*

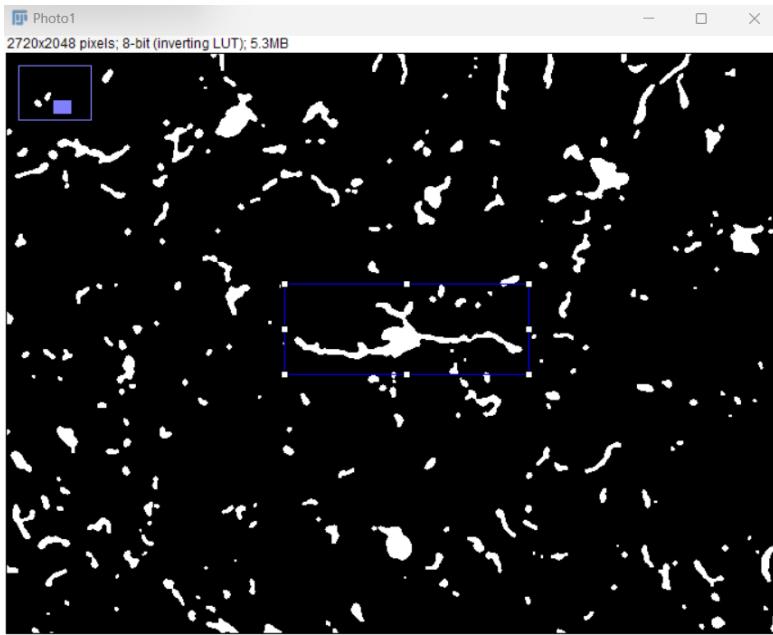
NOTE: The image used in this demonstration is a black background image, the same procedure would be done with a white background image



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-**)

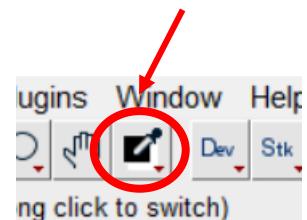


If there are portions of other neighbor cells inside your cell selection rectangle: select the

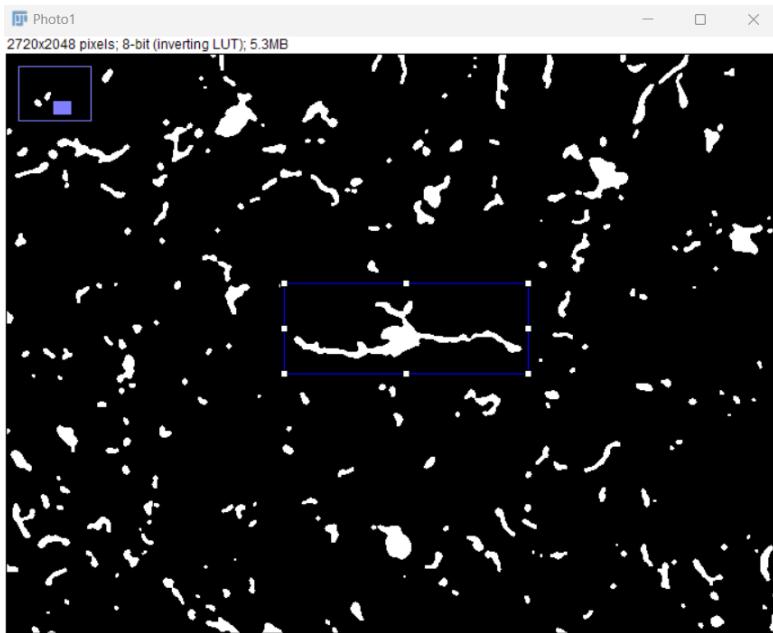
background color with the ***Color picker***



Now the color of the “color picker” is
black



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



19. Once you have it done with all the cells that you want to analyze, click on **OK** in the window “**Select cells and save ROIs**”

**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, in steps from 20 to 26 I will show you the parameters and values that I work with.

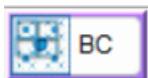
Anyway, I encourage you to read more about this plugin to extract its full potential and adapt it to your cell type/staining.

For more information: <https://imagej.net/ij/plugins/fraclac/FLHelp/Tutorials.htm>

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

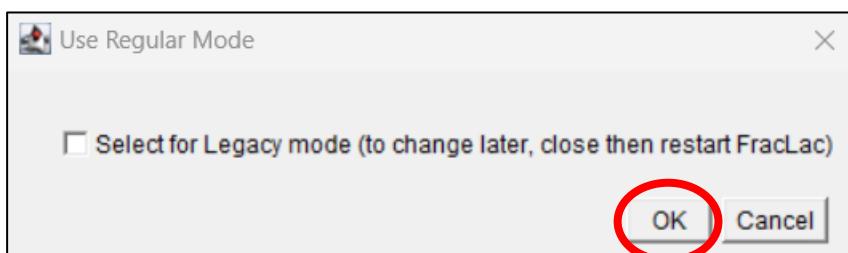
21. Background: *indicate the background color of your image*

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)



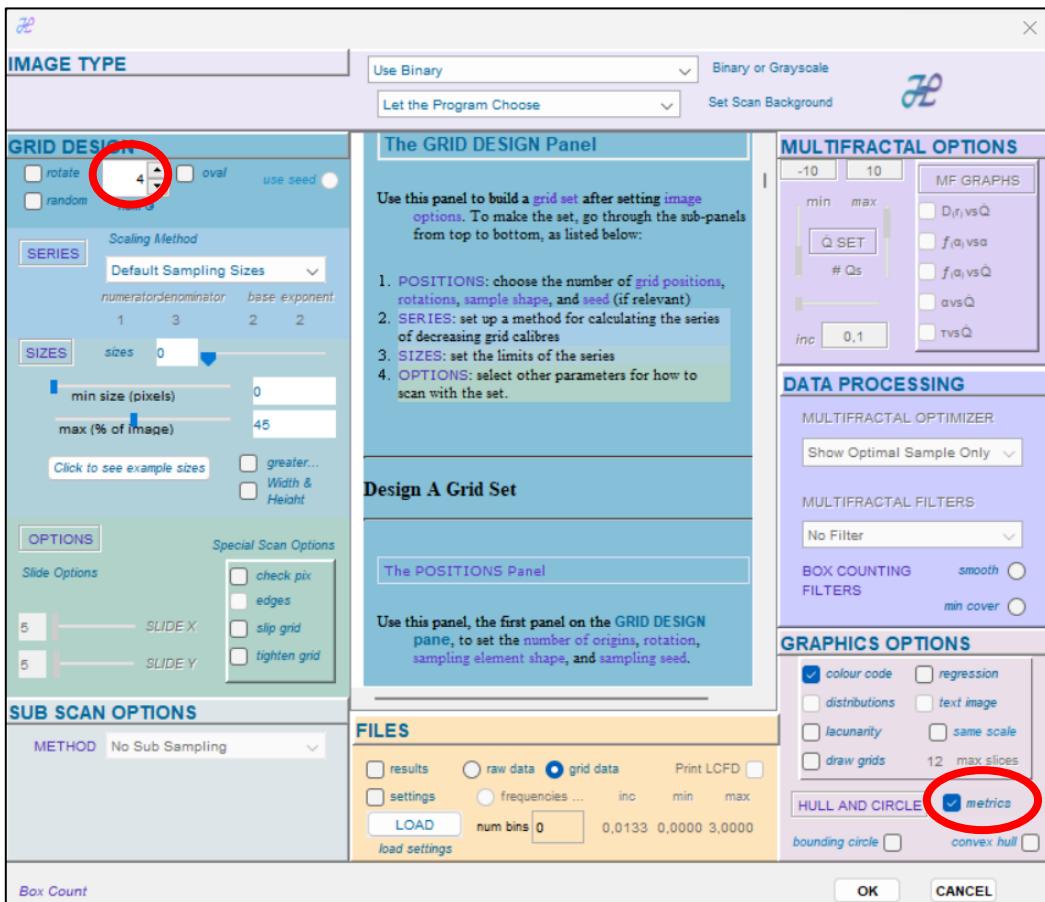
22. In the FracLac plugin, select the box: **BC**

23. **DO NOT** mark the box "**Select for Legacy mode**" and click on **OK**

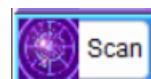


24. Measure the morphology:

- In the tab **GRID DESING** > numG = 4
- In the tab **GRAPHICS OPTIONS** > mark box “Metrics”
- Press **OK**



25. Select the type of fractal dimension to color code: **Db**



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

Then, different emerging windows will appear. For instance, here I show you where to find the fractal, lacunarity, density, area and perimeter parameters (I also indicate the specific location of each value in each window):

a. **Box Count Summary**

- D (fractal)** → position 6
- Lacunarity** → position 87

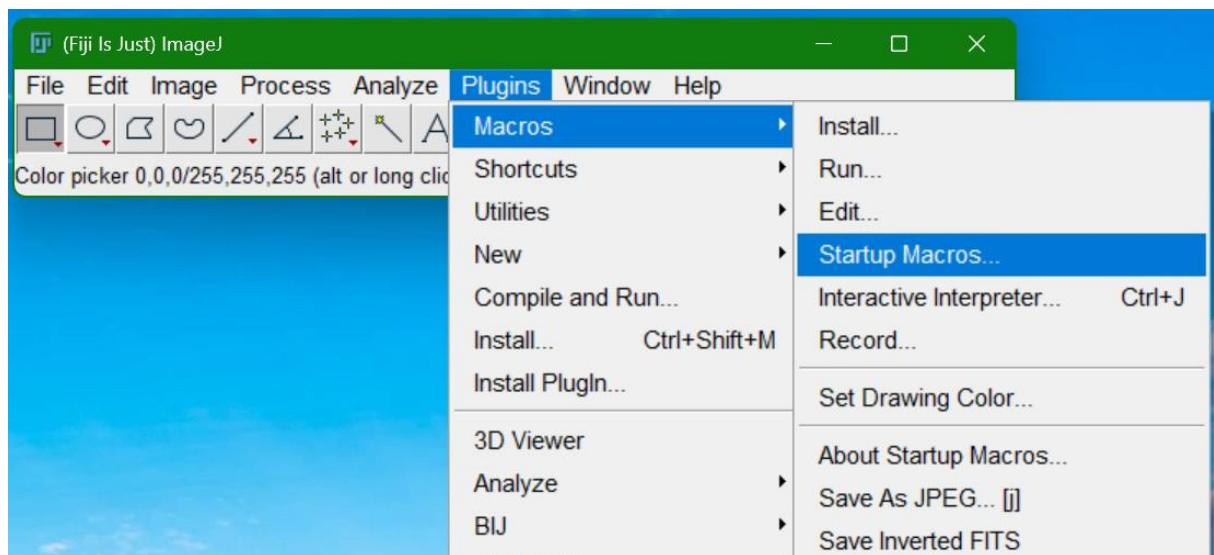
b. **Hull and Circle Results**

- Density**
- Area**
- Perimeter**

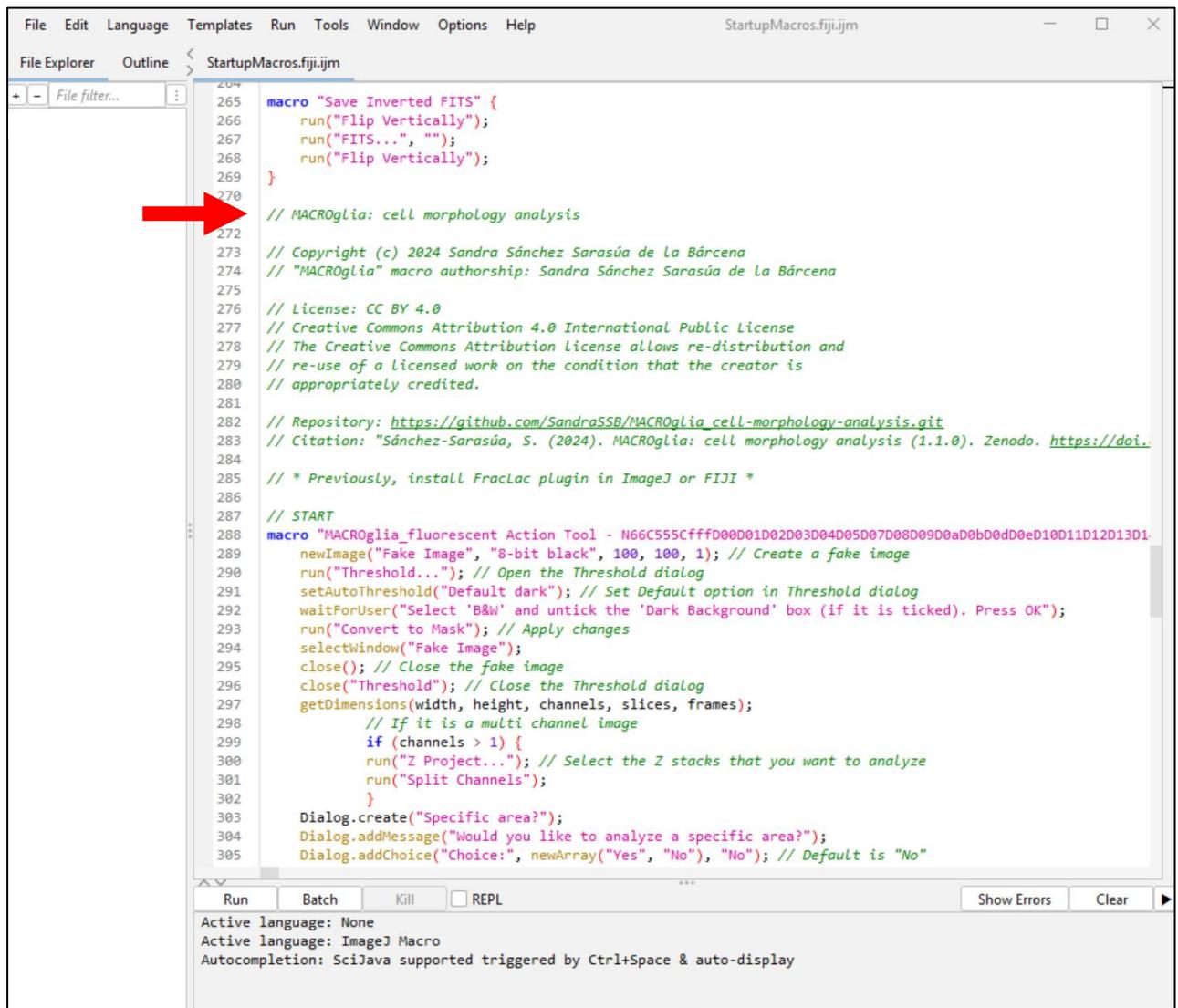


CREATE A SHORTCUT IN THE IMAGEJ TOOLBAR

1. Go to 'Macro' folder and download the .txt file called 'Shortcut_MACROglia_vXXX_X.txt' (please notice that there is a 'Shortcut_MACROglia_v1.2.0_F_DAB.txt' file that contains both versions in case you plan to use 'MACROglia' with the two different stainings)
2. Open ImageJ and go to Plugins > Macros > Startup Macros...



3. Open the .txt file, go to Edit > Select all, copy the text and paste it at the end of the “Startup Macros...” window



```

File Edit Language Templates Run Tools Window Options Help
StartupMacros.fiji.ijm
File Explorer Outline < StartupMacros.fiji.ijm
+ - File filter...
204
205 macro "Save Inverted FITS" {
206   run("Flip Vertically");
207   run("FITS...", "");
208   run("Flip Vertically");
209 }
210
211 // MACROglia: cell morphology analysis
212
213 // Copyright (c) 2024 Sandra Sánchez Sarasúa de la Bárcena
214 // "MACROglia" macro authorship: Sandra Sánchez Sarasúa de la Bárcena
215
216 // License: CC BY 4.0
217 // Creative Commons Attribution 4.0 International Public License
218 // The Creative Commons Attribution license allows re-distribution and
219 // re-use of a licensed work on the condition that the creator is
220 // appropriately credited.
221
222 // Repository: https://github.com/SandraSSB/MACROglia\_cell-morphology-analysis.git
223 // Citation: "Sánchez-Sarasúa, S. (2024). MACROglia: cell morphology analysis (1.1.0). Zenodo. https://doi.org/10.5281/zenodo.1000000" * Previously, install FracLac plugin in ImageJ or FIJI *
224
225 // START
226 macro "MACROglia_fluorescent Action Tool - N66C555CffffD00D01D02D03D04D05D07D08D09D0aD0bD0dD0eD10D11D12D13D1"
227   newImage("Fake Image", "8-bit black", 100, 100, 1); // Create a fake image
228   run("Threshold..."); // Open the Threshold dialog
229   setAutoThreshold("Default dark"); // Set Default option in Threshold dialog
230   waitForUser("Select 'B&W' and untick the 'Dark Background' box (if it is ticked). Press OK");
231   run("Convert to Mask"); // Apply changes
232   selectWindow("Fake Image");
233   close(); // Close the fake image
234   close("Threshold"); // Close the Threshold dialog
235   getDimensions(width, height, channels, slices, frames);
236   // If it is a multi channel image
237   if (channels > 1) {
238     run("Z Project..."); // Select the Z stacks that you want to analyze
239     run("Split Channels");
240   }
241   Dialog.create("Specific area?");
242   Dialog.addMessage("Would you like to analyze a specific area?");
243   Dialog.addChoice("Choice:", newArray("Yes", "No"), "No"); // Default is "No"
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```

Run Batch Kill REPL

Show Errors Clear ►

Active language: None
 Active language: ImageJ Macro
 Autocompletion: SciJava supported triggered by Ctrl+Space & auto-display

4. Go to File > Save

```

File Edit Language Templates Run Tools Window Options Help
Macros.fiji.ijm
New Ctrl+N
Open... Ctrl+O
Open Recent ...
Save Ctrl+S
Save As... Ctrl+Shift+S
Export as JAR...
Export as JAR (With Source)...
Lock (Make Read Only)
Revert...
Show in System Explorer
Close Ctrl+W
macro "Save Inverted FITS" {
    run("Flip Vertically");
    run("FITS...", "");
    run("Flip Vertically");
}

// MACROglia: cell morphology analysis
// Copyright (c) 2024 Sandra Sánchez Sarasúa de La Bárcena
// "MACROglia" macro authorship: Sandra Sánchez Sarasúa de La Bárcena

// License: CC BY 4.0
// Creative Commons Attribution 4.0 International Public License
// The Creative Commons Attribution license allows re-distribution and
// re-use of a licensed work on the condition that the creator is
// appropriately credited.

// Repository: https://github.com/SandraSSB/MACROglia\_cell-morphology-analysis.git
// Citation: "Sánchez-Sarasúa, S. (2024). MACROglia: cell morphology analysis (1.1.0). Zenodo. https://doi.org/10.5281/zenodo.5555555"
```

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

```

// START
macro "MACROglia_fluorescent Action Tool - N66C555CfffD00D01D02D03D04D05D07D08D09D0aD0bD0dD0eD10D11D12D13D1"
newImage("Fake Image", "8-bit black", 100, 100, 1); // Create a fake image
run("Threshold..."); // Open the Threshold dialog
setAutoThreshold("Default dark"); // Set Default option in Threshold dialog
waitForUser("Select 'B&W' and untick the 'Dark Background' box (if it is ticked). Press OK");
run("Convert to Mask"); // Apply changes
selectWindow("Fake Image");
close(); // Close the fake image
close("Threshold"); // Close the Threshold dialog
getDimensions(width, height, channels, slices, frames);
// If it is a multi channel image
if (channels > 1) {
    run("Z Project..."); // Select the Z stacks that you want to analyze
    run("Split Channels");
}
Dialog.create("Specific area?");
Dialog.addMessage("Would you like to analyze a specific area?");
Dialog.addChoice("Choice:", newArray("Yes", "No", "No")); // Default is "No"

```

Run Batch Kill REPL Show Errors Clear

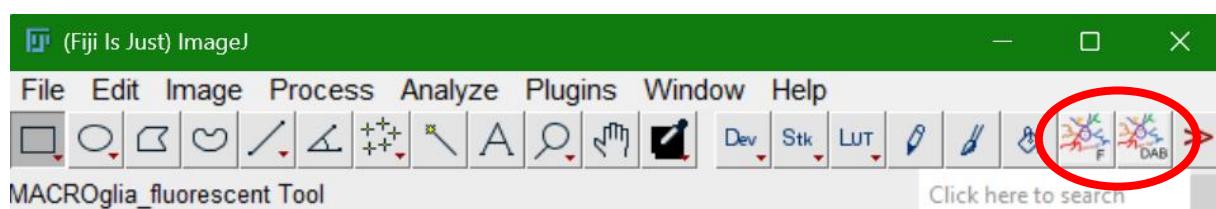
Active language: None
Active language: ImageJ Macro
Autocompletion: SciJava supported triggered by Ctrl+Space & auto-display

5. DONE Every time you need to use the macro you can find it in the ImageJ toolbar!

NOTE: each version of 'MACROglia' has its own toolbar icon:

 **DAB** for “Bright field - DAB staining” version

 **F** for “Fluorescent staining” version



'MACROglia' macro authorship: Sandra Sánchez Sarasúa de la Bárcena

FracLac plugin authorship: Audrey Karperien

'MACROglia' is an extended and semi-automatic version of the protocol described in: "Young K, Morrison H. Quantifying Microglia Morphology from Photomicrographs of Immunohistochemistry Prepared Tissue Using ImageJ. J Vis Exp. 2018 Jun 5;(136):57648. doi: [10.3791/57648](https://doi.org/10.3791/57648)"

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 <https://www.researchgate.net/profile/Sandra-Sanchez-Sarasua>

 https://github.com/SandraSSB/MACROglia_cell-morphology-analysis

 <https://zenodo.org/SandraSSB>

Reference

When using '*MACROglia*', please cite:

Sánchez-Sarasúa, S. (2025). MACROglia: cell morphology analysis (1.2.0). Zenodo. <https://doi.org/10.5281/zenodo.14922374>

To cite '*FracLac*' plugin use:

Karperien, A., FracLac for ImageJ. [Introduction.htm](#). 1999-2013"