

Overview: This macro is useful for finding the total nuclei in an image. This macro was created based on existing procedures used in the lab. This macro was created on Fiji version 1.53c.

Note: This macro assumes your units are in μm . If this is not the case, please be sure to update your units accordingly.

Macro output:

1. Areas of nuclei $251 \mu\text{m}^2$ -Infinity
2. Number of nuclei 50 - $250 \mu\text{m}^2$
3. Mode of nuclei 50 - $250 \mu\text{m}^2$
4. The area of the image in mm^2

How to Use:

1. Download the macro called "TotalNuclei.ijm".
2. Upload the image in ".Stitch.lsm" format. Unstack the images. (Image>Stacks>Stack to Images)
3. Close out of all images except for the DAPI image. This is a crucial step in order to ensure that the macro performs the analysis on the correct image.
4. Run the macro on the image. (Plugins>Macros>Run>Select Macro)
5. The macro will prompt you to select the folder where you wish for your results to reside. Once you have selected the desired folder, click "Open".
6. Another dialog box will appear. The first box will have the file path of where your results will be saved based on your previous selection. In the second box, type in the name of the .csv file of your results. Click "OK" when finished.
7. The macro will prompt you to type in the multiplication value you wish to use for the image. Type in a number, then click ok when done.
8. The remainder of the process will take a few minutes to finish. When the macro is done, the "Calculations Complete!" message will be displayed.
9. The .csv file produced will contain the nuclei and their areas sizes $251 \mu\text{m}^2$ -Infinity, followed by the total number of nuclei sizes 50 - $250 \mu\text{m}^2$, followed by the mode area of nuclei sizes 50 - μm^2 , followed by the area of the image in mm^2 .
10. Use this .csv file to input numbers into the ViaFuse calculation excel file to determine total nuclei