Manual FNMM

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Date: 12-07-2020

Description:

The Fluorescent_Nucleus_Measurement_Macro (FNMM) was designed to estimate a measure of green fluorescent protein (GFP+) cells within a digital fluorescence image using Fiji (ImageJ). This is accomplished by analysing the area of 4',6-diamidino-2-phenylindole(DAPI+) nuclei surrounded by cytoplasmic GFP versus the total area of all DAPI+ nuclei within a given region of interest (ROI).

Requirements:

Fiji (ImageJ)	https://fiji.sc/
FNMM script	https://github.com/J-PTRson/Cell-Image-Analysis

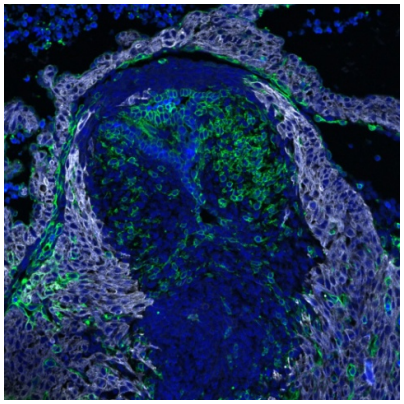
Optional requirements for data visualisation:

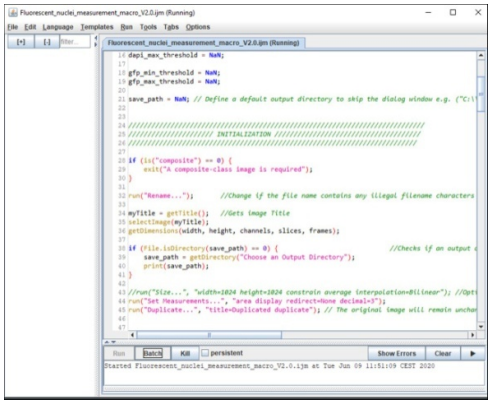
R,	https://www.r-project.org/
Rstudio	https://rstudio.com/
FNMM_analysis_helper.R	https://github.com/J-PTRson/Cell-Image-Analysis

Instructions:

1) ***Load an image***

   Load the example image "Tie2Cre;mTmG_E13.5.tiff" and the FNMM macro into Fiji.
   This is a transversal section of the aortic valve depicting the endothelial derived cell lineage in green (GFP), cardiac tropomyosin in grey, and the nuclear staining is blue (DAPI). For our hypothetical question we would like to estimate an percentage of GFP+ cells relative to all cells within a single leaflet of this image.





2) ***Execute the script***

Upon executing the macro script a dialog window will appear asking whether you would like to rename the file but be mindful of entering invalid filename characters.

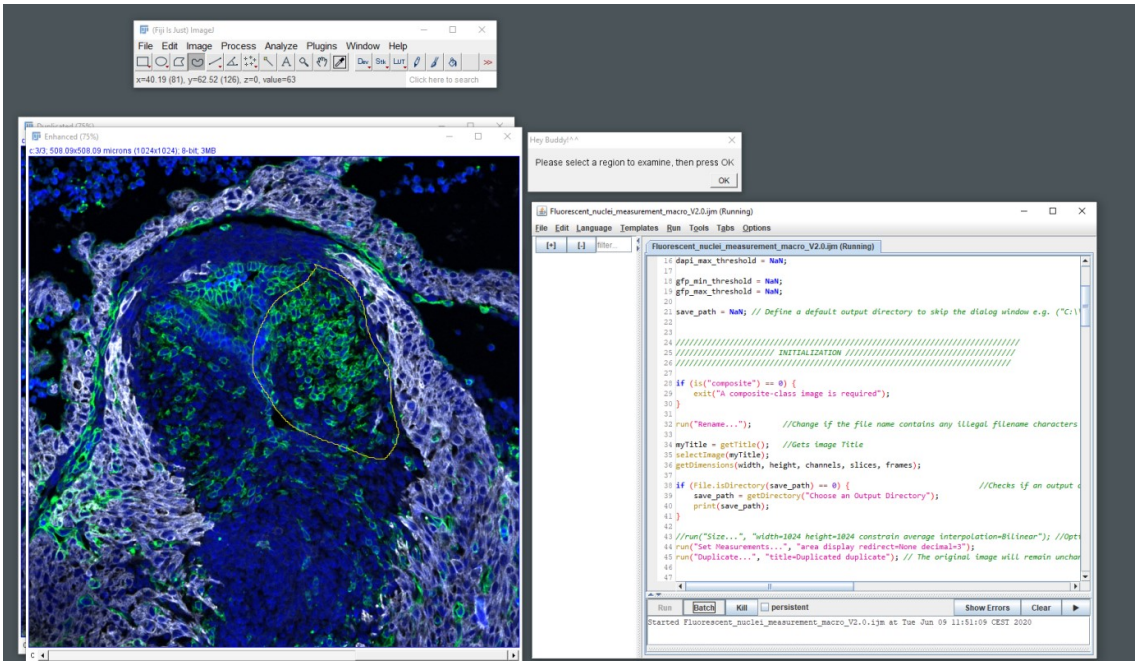
3) ***Do you want to apply linear contrast normalization?***

This is an optional step but it allows imageJ to evaluate the colour saturation within the image and adjust the contrast automatically to a user defined saturation level if desired (0.4% default).

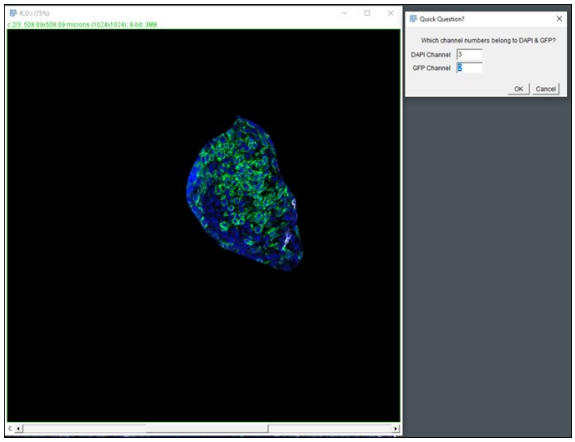
4) **Select a region of interest**

Select a ROI to analyze using your mouse.

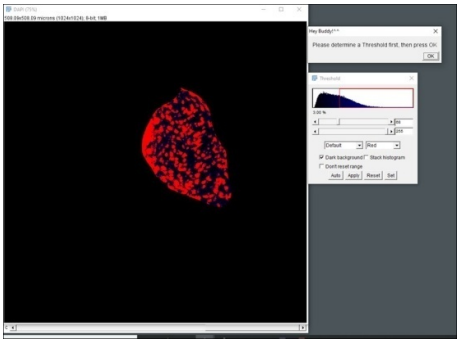
In this example we are interested to measure the relative amounts of GFP+ cells in the left coronary cusp (indicated in yellow).

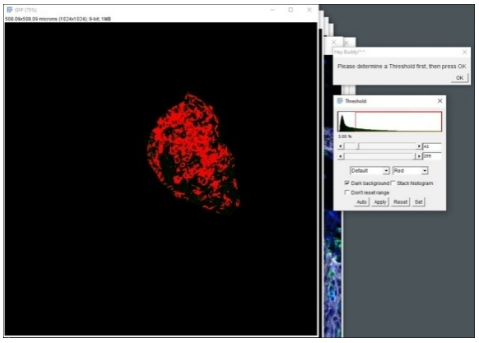


5) ***Attribute the channel number corresponding to each colour***.
In our example we are examining green cells (cytoplasmic GFP)with a blue nuclei (DAPI). Side note: Our composite image is composed out of multiple layers of 8-bit scalar images ranging from pixel intensity values of 0 to 255. The colour green is a visual representation due to the chosen look-up-table (LUT). If we were interested in analyzing the cells in our "grey" channel (e.g cytoplasmic-tropomyosin) we could define that particular colour channel instead of GFP.

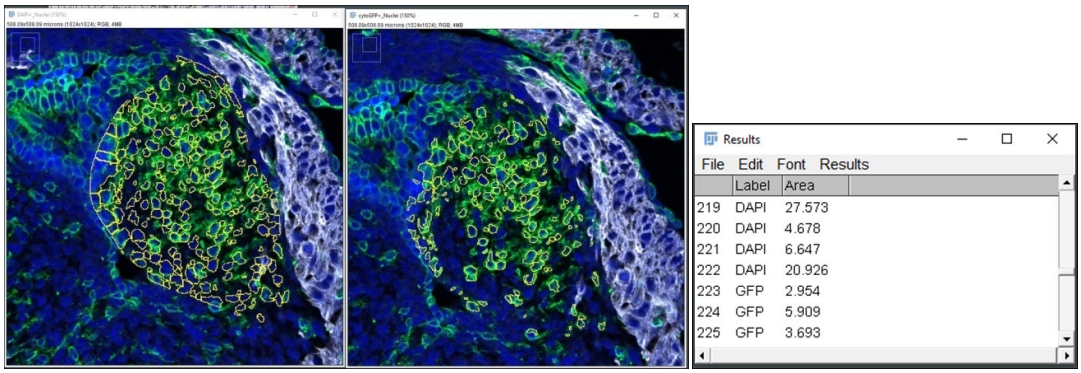


6) **Define the colour thresholds (x2)**
Define the threshold values for the DAPI and GFP+ channels. These values define the limits of pixel intensity values used for analysis. When performing image analysis of multiple images (or multiple ROIs in a single image) it is good practice to keep these values static for each channel throughout your analysis.

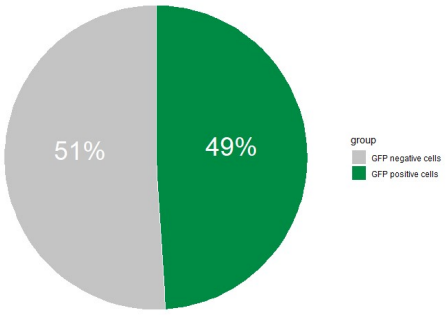




7) **Examine the results of your analysis.**
In our example the measurement generated two groups, DAPI and GFP. In both cases measurements were derived from the nuclear staining. In the case of GFP, the nuclear area was measured cells which were positive for cytoplasmic GFP. In the case of DAPI, the nuclear area was measured for all cells within the ROI.



8) **(Optional)Data visualization.**
To quickly examine the cellular distribution within the leaflet, execute the FNMM_analysis_helper.R script in RStudio (select the .csv result file) to analyze the obtained output file.



Afterword:
Although the FNMM macro is able to output absolute cell numbers as a result, I for one prefer the use of surface area measurements (or nuclear volume). This is because absolute cell number quantification relies on certain (often discipline specific) assumptions regarding cell morphology. Nevertheless, this macro can be relatively easily adapted to your custom needs.

When performing multiple analysis or batch processes it is advised to personalize the "configuration" section of the macro code in order to skip the presented dialog windows.