Fiji script Cytoplasm/Plasma membrane signal quantification

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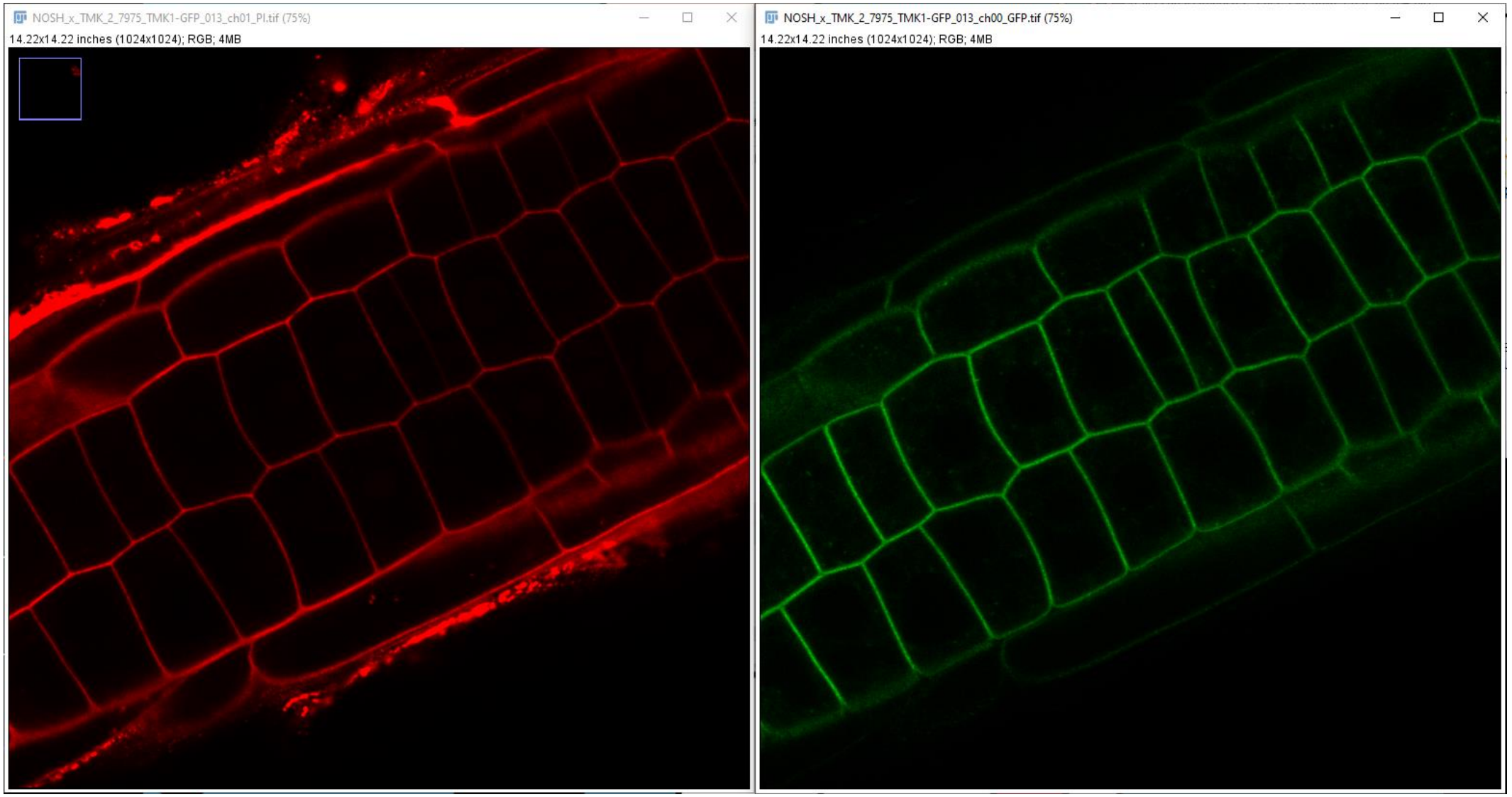
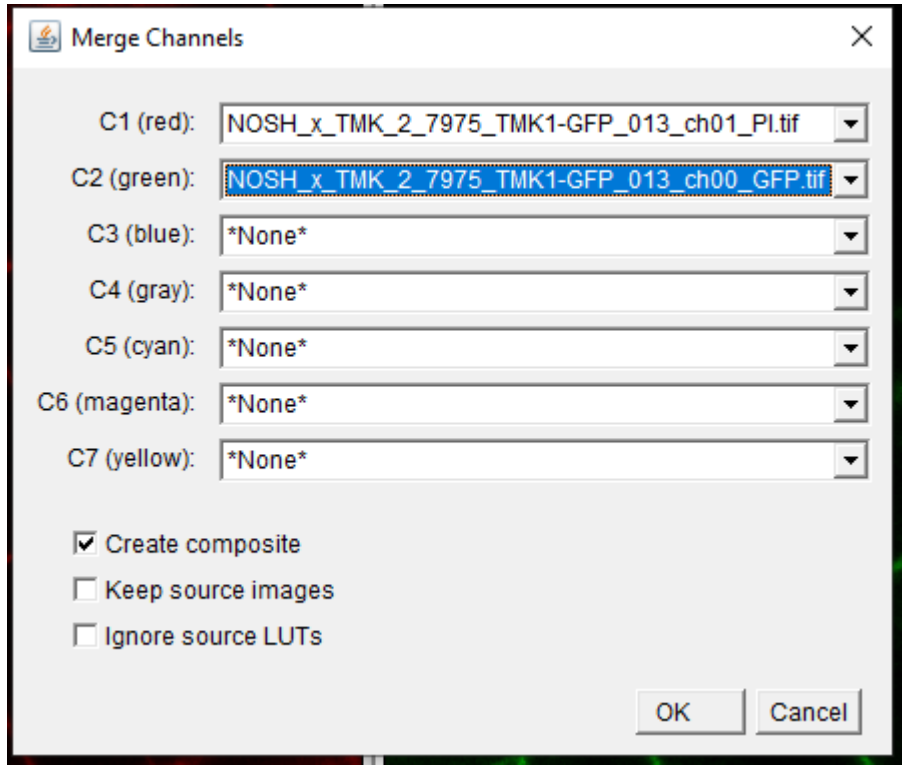
VIB BioImaging Core
Please acknowledge us if you use this script in a publication.

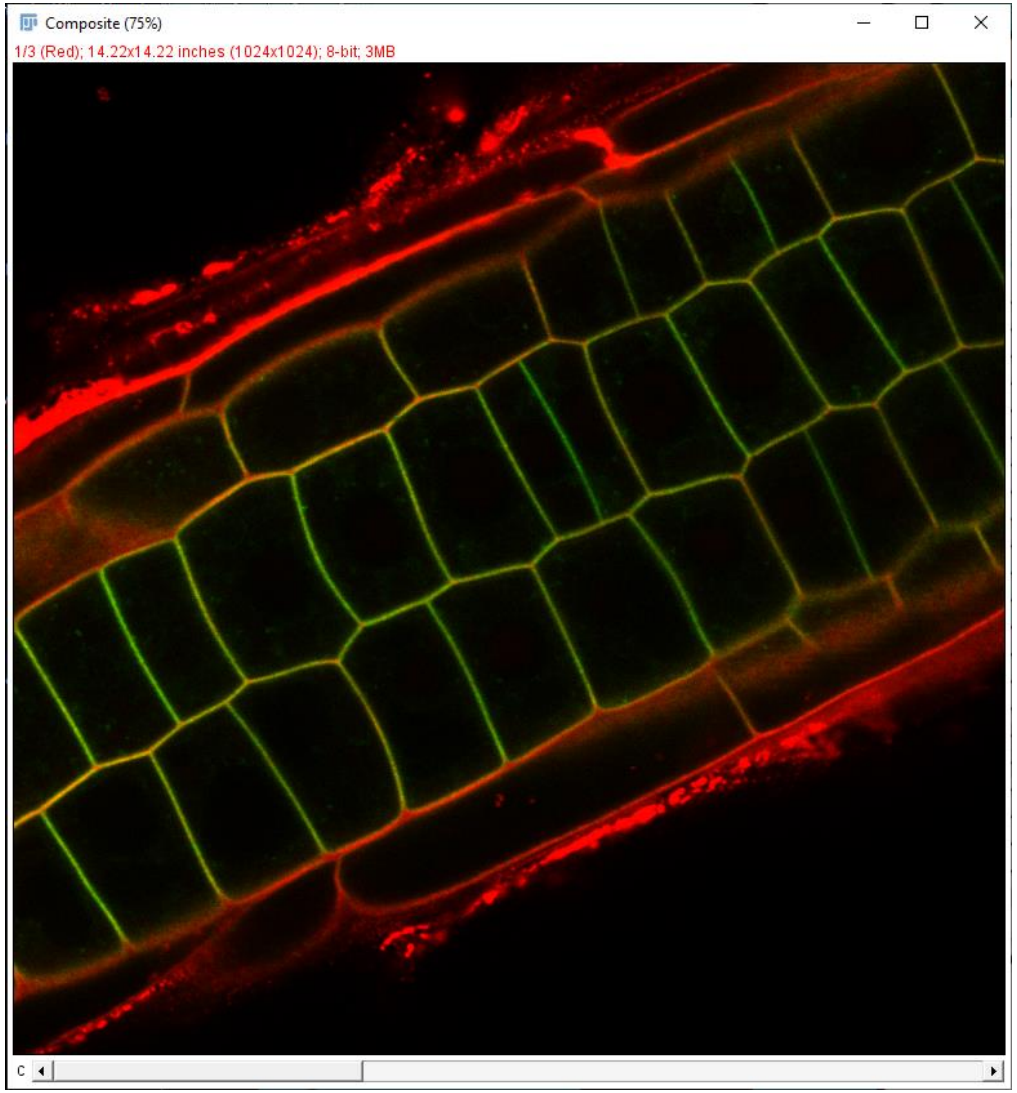
1



Images were acquired using Leica SP8 confocal microscope, objective 40x water, zoom 3x.
Left image is staining with propidium iodide [2µM], Right image is GFP signal.

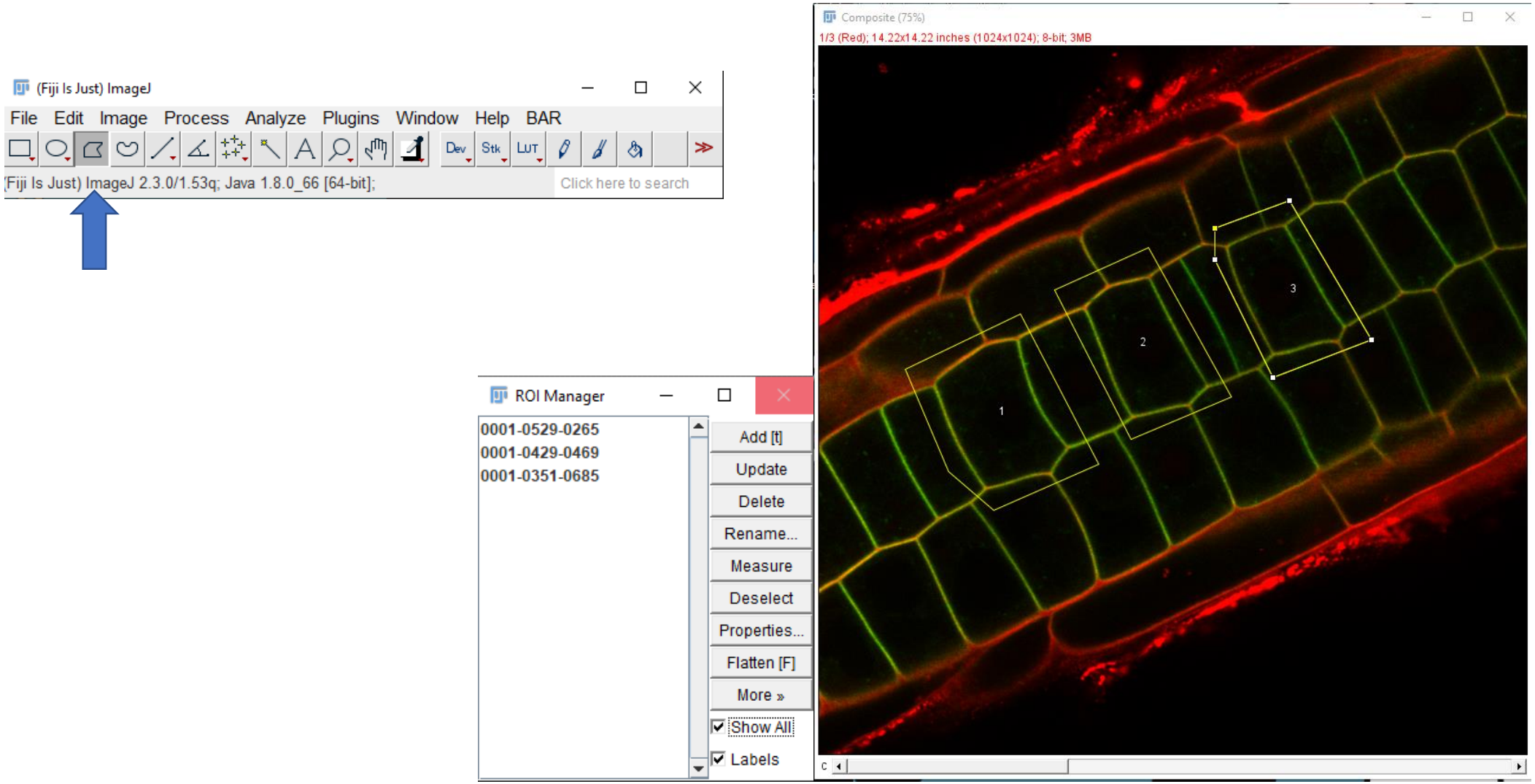
2





Images are open in ImageJ/Fiji and merged together: Fiji/Image/Color/Merge channels…
creating a composite image
Channel 1 is set for the PI staining image and Channel 2 is set for the GFP image.
Channel 1 is used to create a mask for cell outlines and Channel 2 is used for signal quantification

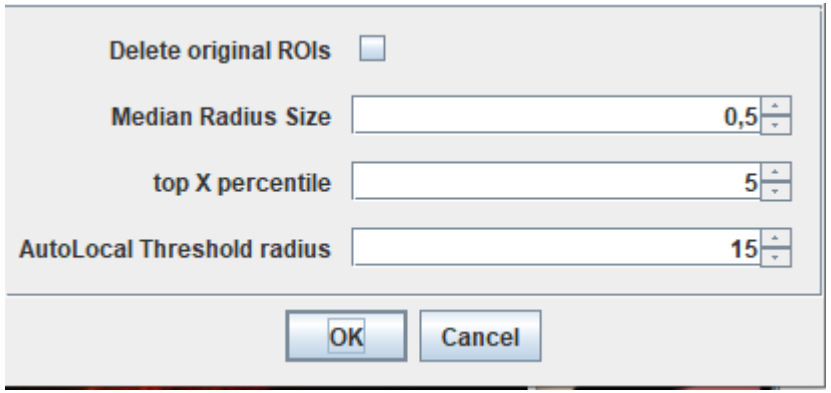
3

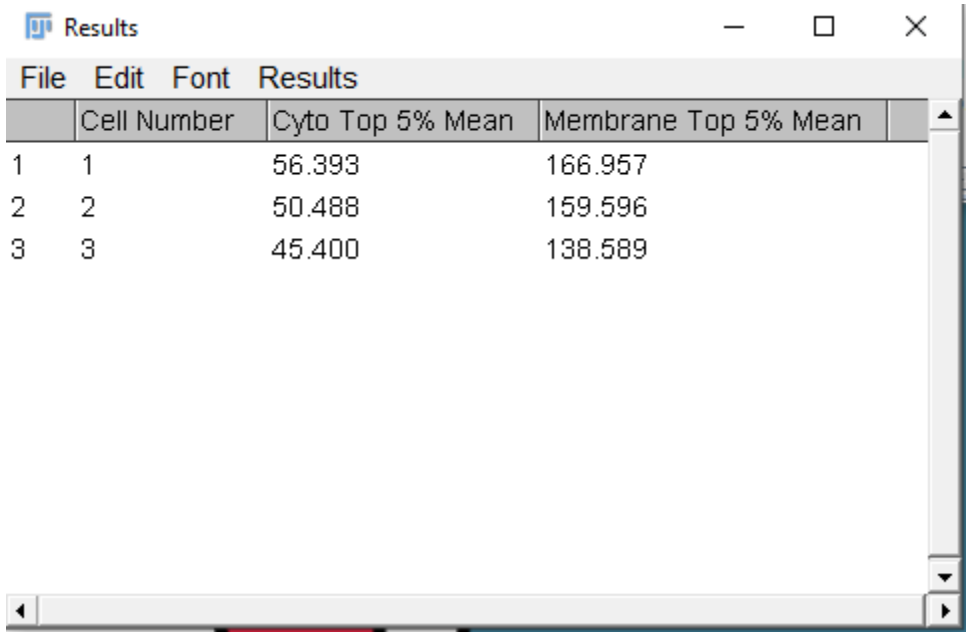


Marking of the region of interest (ROI) is performed by Polygon selection tool and saved in the ROI Manager (the script is automatically detecting the cell outline in the saved ROI).

Once the preferred ROIs are saved in ROI Manager, you can Run the script.

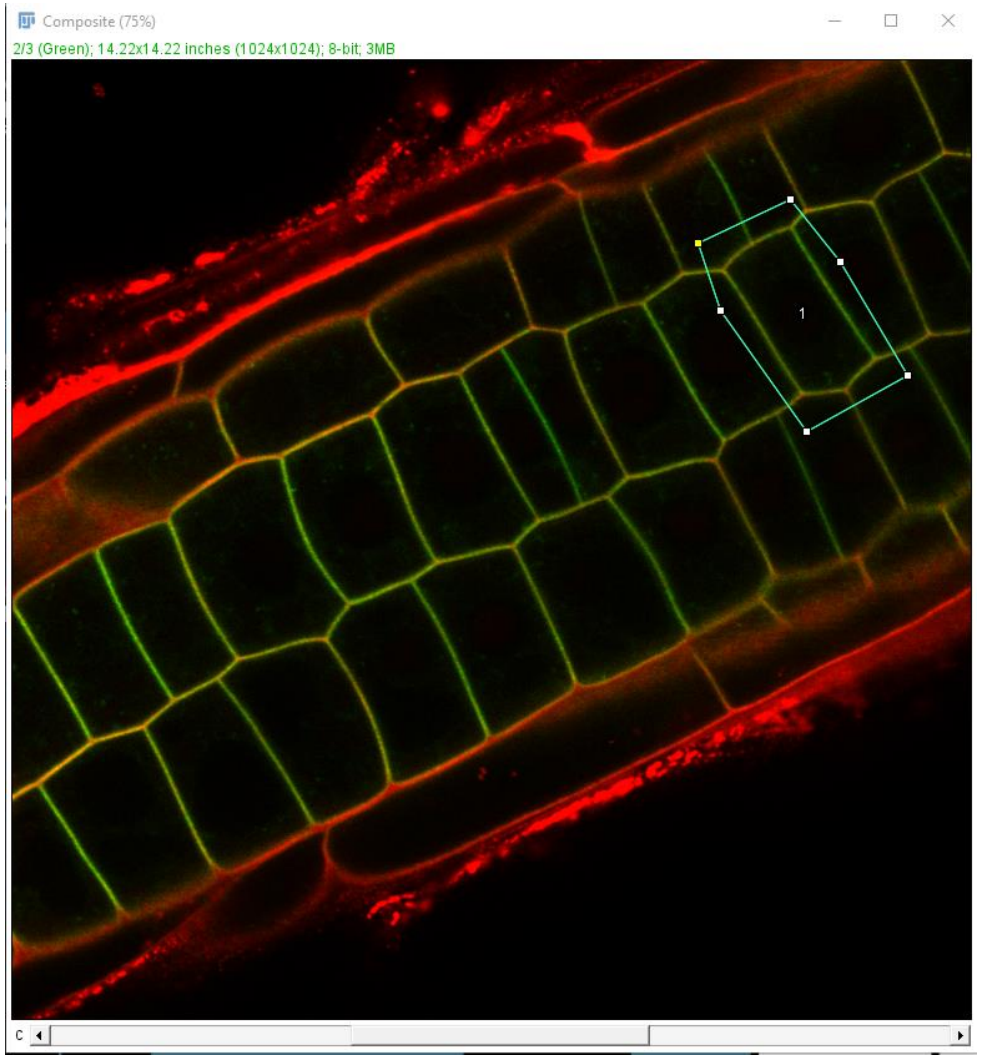
4

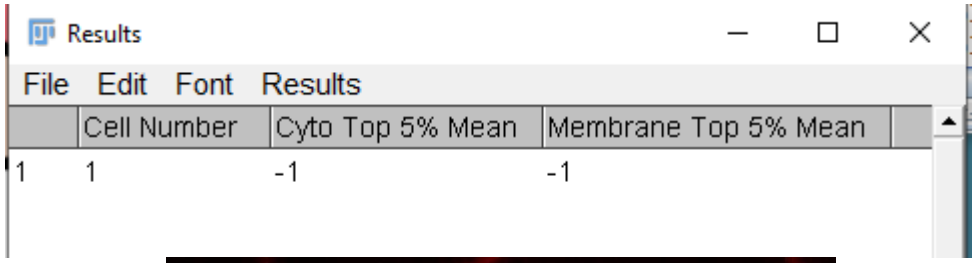


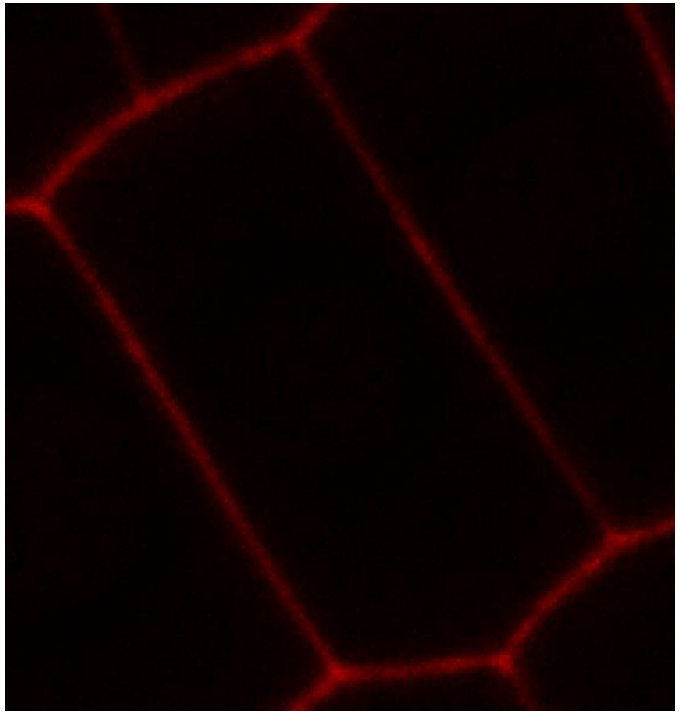


Pop-up window will appear, in which you can change parameters of the analysis.
By pressing OK, the script will run and calculate the signal intensity of the Channel 2 based on the mask from Channel 1. The results table will appear.

5







If analysis of the selected ROI will come back as -1 / -1. This is an error message, caused by inability of the script to create a mask from Channel 1. This can be caused by low quality of Ch1 image, signal not bright enough or gaps in the PM staining creating a discontinued outline). This cell thus cannot be quantified and should be omitted from the analysis.