Overview: This macro is useful for finding the fusion index value of an image by subtracting out a mask of the myotubes from the nuclei. This macro was created based on existing procedures used in the lab. This macro was created on Fiji version 1.53c.

Macro output
1. Areas of nuclei out of myotubes 251 μm^2-Infinity
2. Number of nuclei out of myotubes 50-250 μm^2
3. Mode of nuclei out of myotubes 50-250 μm^2
4. One .png file of the final merged image that shows the nuclei that were counted

How to Use:

1. Download the macro called "ViaFuse_F_macOS.ijm" if working with Mac OS or "ViaFuse_F_windows.ijm if working with Windows OS
2. Upload the image in ".Stitch.lsm" format. Do NOT unstack the images. **Note: The macro assumes the following stack order for the following scenarios:**
   a. 4 total slices: myotube image is slice 2 and DAPI image is slice 4
   b. 3 total slices: myotube image is slice 1 and DAPI image is slice 3
      If the order of your images is not like described above or if you have more/less slices, go to the macro and edit lines 13-20 with the correct channel/slice numbers!!!!!! This would be the ONLY place you would need to edit!!
3. Run the macro on the image. (Plugins>Macros>Run>Select Macro)
4. 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"
5. 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
6. The macro will prompt you to type in the multiplication value you wish to use for the myotube, and DAPI images. Type in a number, then click ok when done
7. The remainder of the process will take a few minutes to finish. When the macro is done, the "Calculations Complete!" message will be displayed
8. The .csv file produced will contain the nuclei and their areas sizes 251μm^2-Infinity, followed by the total number of nuclei sizes 50-250 μm^2, followed by the mode area of nuclei sizes 50-250 μm^2
9. Use this .csv file to input numbers into the ViaFuse calculation excel file to determine fusion index