<u>Script Purpose:</u> This script quantifies the neurite density in images taken of dissociated cortical cells.

Inputs:

The inputs of the script are the LVCC images of the cortical neurons taken on the Thunder microscope. The image file names should end with '_im.tif' (but this can be changed in the script). The other input will be the mask images, whose file names should end with '_mask.tif'. The mask images have been modified on ImageJ by using the paintbrush took to colour over the neurites in black, leaving only the nuclei and debris so these can be subtracted from the original image, leaving only the neurites to analyze. These images should all be in one folder which will be input into the script.

This script was designed for Epo-B experiments where 4 images are taken of each well for 4 wells, and each chip is a different condition, so there are 16 images per condition. This can be adjusted.

Outputs:

The outputs of the script will be the neurite densities of each image in each condition, which can be saved as a .csv file to your computer. The script can also plot the neurite densities as boxplots and calculate any significance using an independent t-test between each condition. Plots are displayed in the notebook but can be downloaded to your computer.

How to run the script:

You can hit 'Run all' in the upper left of the screen, or you can run each cell one at a time for troubleshooting. You will have to manually save each csv file and each plot to your computer. Here is an example of the image, mask, masked image and binarized masked image. The outputs should look something like this.

