Segmentation of Microglia

Goal: The goal is to segment the complete Microglia with dendrites.

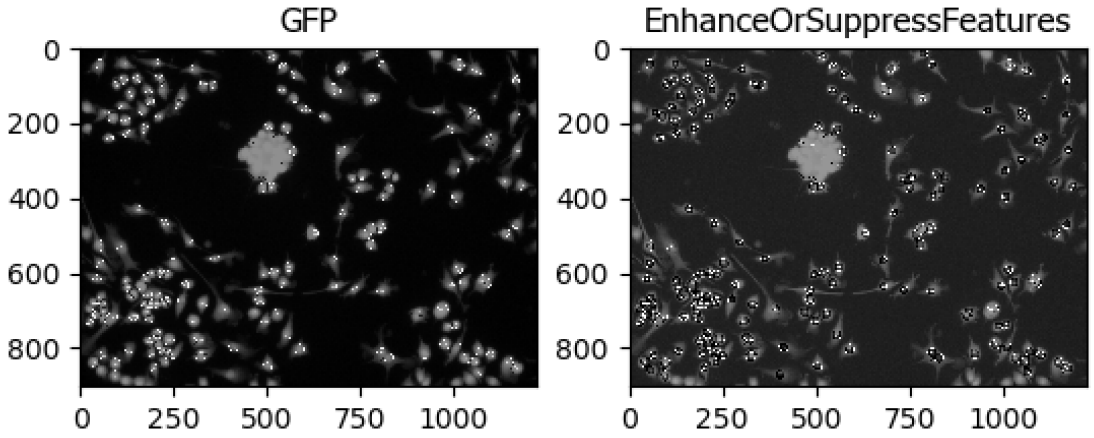
Images: Multichannel images with two channels (DAPI and GFP).

Pipeline: This example pipeline shows the segmentation of Microglia with dendrites, including enhancing the dendritic features in the image. The images for this example have been previously processed. Both contain combined DAPI and GFP channels as well as object outlines. Even using pre-processed images we can demonstrate how the Enhance/Suppress Feature of CellProfiler can improve the identification of weakly fluorescing objects such as dendrites. The workflow is as follows:

1. Open **CellProfiler**.
2. Click on **Images**. Highlight the two images listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the two images in the appropriate CellProfiler window. The original images maintain the folder structure of the original computer used to create the pipeline. If the images are not reloaded from your computer an error will occur.
3. The **Metadata** has an expression designed to extract various information, but only file pattern matching is used in this pipeline in the subsequent step.
4. **NamesAndTypes** is used to tell CellProfiler which images are DAPI or GFP.
5. **IdentifyPrimaryObjects** is used to identify and segment nuclei using the image identified as DAPI.
    a. **Typical Diameter of Objects, in pixels** was set to 10-50 which is the average range for nuclei in this experiment. Tightening this range will result in fewer nuclei identified, while expanding the range will include more objects. To get an idea of object size, go to the “Images” module, right click on an image and select “Show Selected Image”. With the new image window selected, select “Tools”, “Measure length” from the toolbar pull down menu. Drag your mouse over an object and view it’s size in the lower right hand of the image window.

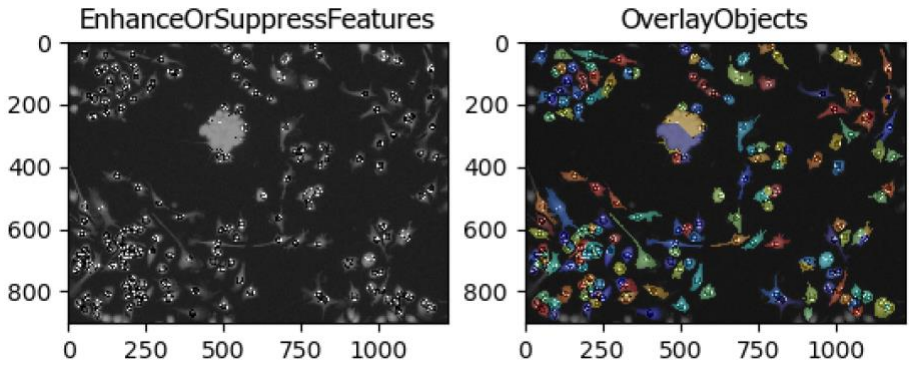
b. **Thresholding method** "Minimum cross entropy" was the most appropriate thresholding method to use since the signal difference between the nuclei and cell is not large, and the intensity distribution within the nuclear channel is variable.
c. **Threshold smoothing scale** was adjusted down to 0.5 to reduce the smoothing of edges and hole filling. This increases segmentation, which worked better for this image.
d. **Threshold correction factor** was adjusted up to 1.6 to reduce the number of pixels detected, separating the nuclear signal from the cell signal and resulting in the ideal representation of segmented nuclei for this image.
e. **Shape** was used to identify and divide objects. In this example, the signal is variable making shape a better options to use to distinguish objects.

6. **EnhanceOrSuprressFeatures** is used on the image identified as GFP to enhance the dendritic features using the "Neurites" feature type.



7. **IndentifySecondaryObjects** module was used on the "enhanced" GFP image to identify and segment the Microglia cells using the nuclei objects.
    a. **Robust Background** was the most appropriate thresholding method because the intensity distribution in the GFP channel is variable, but significantly higher than the background.
    b. # of deviations was increased to 4 to reduce the number of pixels detected.
    c. Thresholding smoothing scale was increased to 1.3 to increase edge smoothing and hole filling, resulting in an ideal representation of segmented Microglia cells.

8. **MeasureObjectIntensity** was used to measure the intensity of objects computed from the GFP channel
9. **MeasureObjectSizeShape** was used to measure the size and shape of the nuclei and Microglia cells.
10. **OverlayOutlines** was used to create an image of the objects overlaid onto the GFP channel image.



11. **SaveImages** saves the OverlayOutline image to your hard drive.
12. **ExportToSpreadsheet** exports all calculated values for each object as separate .csv files.