Segmentation of Fibroblasts

Goal: The goal is to segment the fibroblasts and compute the correlation between the Myosin and the cells (Actin).

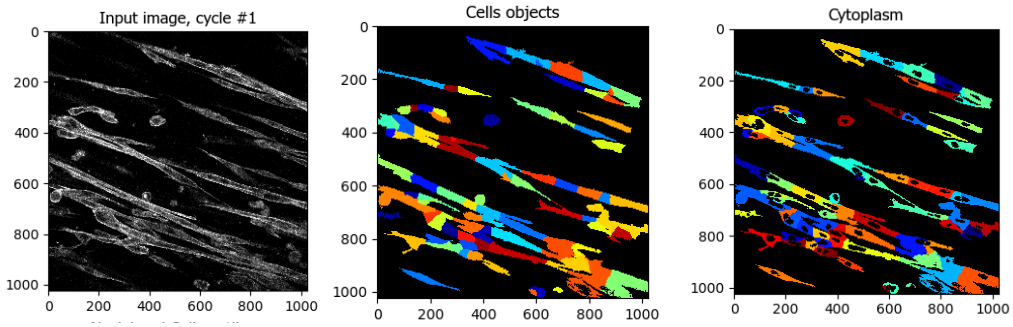
Images: Multichannel image with three channels (DAPI, Actin, Myosin).

Pipeline: This pipeline is used to segment spindle shaped fibroblasts using image thresholding adjustments and smoothing parameters. Nuclei are identified as the primary object. Actin stain and the location of individual nuclei are used to identify and segment the cell body. Intensity based colocalization is then computed between the Actin and Myosin channels using the segmented objects. The workflow is as follows:

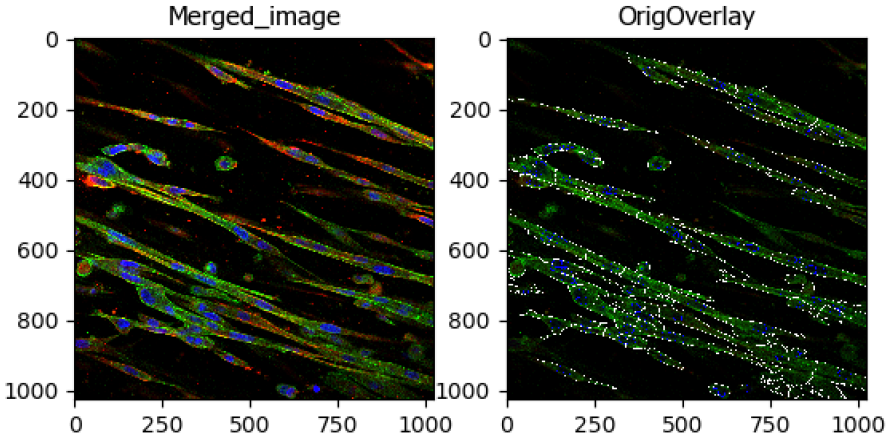
1. Open **CellProfiler**.
2. Click on **Images**. Highlight the three images listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the three images in the appropriate CellProfiler window. The original images maintain the folder structure of the original computer used to create the pipeline. If the images are not reloaded from your computer an error will occur.
3. Ignore the **Metadata** step. It is not used in this tutorial and "Extract Metadata" can be set to "no". The images are identified using the **NamesAndTypes** module. Channel 3 is labelled DNA, Channel 2 Actin, and Channel 1 Myosin.
4. **RescaleIntensity** is used to pre-process the Actin image. This rescales the intensity values within the complete image. This aids in thresholding the pixels of interest based on the intensity values.
5. **IdentifyPrimaryObjects** is used to identify and segment nuclei.
    a. **Typical Diameter of Objects, in pixels** was set to 15-50 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** "Otsu" is a good general thresholding method to use when the signal to background ratio is high, such as the case with this image.

c. **Two classes thresholding** was picked again because of the clear distinction between signal and background, with no middle ground or local background.
d. **Threshold correction factor** was adjusted slightly down to 0.8 to include slightly more pixels when detecting the object.
e. **Intensity** was used to identify and divide objects. In this example, the signal from the center to the edge of each object was much more prominent compared to shape when distinguishing objects.

6. **IndentifySecondaryObjects** module was used to identify and segment the cell using the nuclei objects and the actin channel.
    a. **Minimum cross entropy** was the most appropriate thresholding method based on the intensity distribution within the actin channel, which is much more variable compared to the signal in the nuclei channel.
    b. All other variables in this method worked well at their default value.
7. **IdentifyTertiaryObject** was used to identify the cytoplasm (Cell minus nucleus).



8. **MeasureObjectIntensity** was used to measure the intensity of actin and myosin in the cytoplasm.
9. **MeasureColocalization** calculates the colocalization and correlation of actin and myosin in both the cytoplasm and the entire cell.
10. **CalculateMath** is used to calculate the ratio of the mean intensity between the actin and myosin in the cytoplasmic region.
11. **GrayToColor** was used to assign a color to each image according to its channel and merge them to create a composite image.
12. **OverlayOutlines** was used to create an image of the identified cell and nuclei objects with cells outlined in grey and nuclei outlined in blue.



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