Segmentation of Cells in Tissue With a High Background

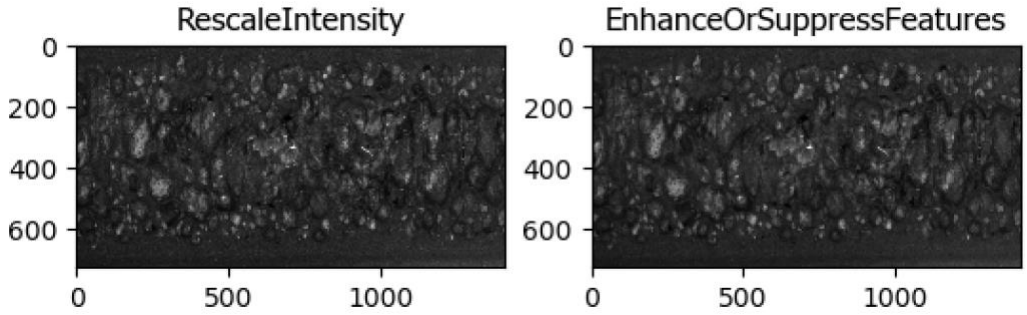
Goal: The goal is to segment cells from a tissue image with high background.

Images: A single tissue image.

Pipeline: This example pipeline shows the segmentation of cells in a tissue image with high background. CellProfiler's **EnhanceOrSuppressFeatures** is used to suppress the high background before segmentation as a part of a pre-processing step. The workflow is as follows:

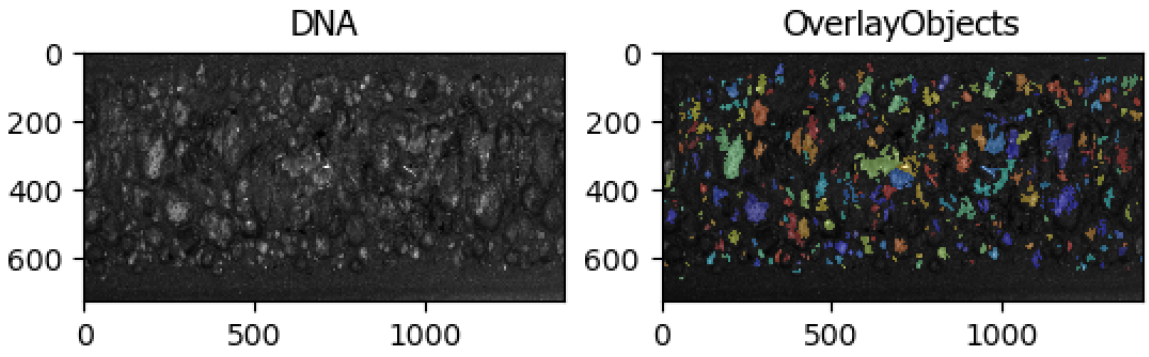
1. Open **CellProfiler**.
2. Click on **Images**. Highlight the image listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the image in the appropriate CellProfiler window. The original image maintains the folder structure of the original computer used to create the pipeline. If the image is not reloaded from your computer an error will occur.
3. **Metadata** information is not used in this example.
4. **NamesAndTypes** is used to name the single Grayscale image used in this example as DNA
5. **RescaleIntensity** is not always necessary but was used in this case to pre-process the DNA image. This rescales the intensity values, increasing contrast, and aids in thresholding the pixels of interest. In cases where signal to background is large the RescaleIntensity step can be skipped.
6. **EnhanceOrSuppressFeatures** is used on the rescaled intensity image to suppress the background features. A **Feature size** of 4 was determined to give the best results for this

image. Some trial and error is necessary to determine the best value for a specific image.



7. **IdentifyPrimaryObjects** is used to identify and segment the cells.
    a. **Typical Diameter of Objects, in pixels** was set to 10-200 since the cell sizes are in a large range. 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 background and the nuclei is not large, and the intensity distribution within the nuclear channel is variable.
    c. **Automatically calculate size of smoothing filter for declumping** was turned off and set to 15 since there are many objects that are clumped and need to be separated. The value is set higher than normal because the size of the objects to be separated are on the larger size.
    d. **Automatically calculate minimum allowed distance between local maxima** was turned off and set to 30 because of the large range of object sizes present. The size was set based on the smallest sized objects to be separated. A rough estimate can be calculated using "Measure length" from "Tools" menu.
    e. **Intensity** is used to identify and divide objects. In this example, object size is variable, making intensity a better option to use to distinguish objects.
8. **MeasureObjectSizeShape** was used to report the size and shape of the cells.

9. **OverlayObjects** was used to overlay the identified objects onto the original image.
10. **SaveImages** saves the OverlayObjects image to your hard drive.



11. **ExportToSpreadsheet** exports all calculated values for identified objects into a .csv file.