Saving each Segmented Cell as an Individual Mask

Goal: The goal is to identify and segment cells and then save each cell as an individual mask. We also make a single mask of all cells. These saved masks can be used later within CellProfiler or in other image analysis applications.

Images: Single image with nuclei and cell signal.

Pipeline: This example pipeline shows the identification and segmentation of cells. The individually identified objects are then saved as both individual binary masks and as a single binary mask containing all objects. The workflow is as follows:

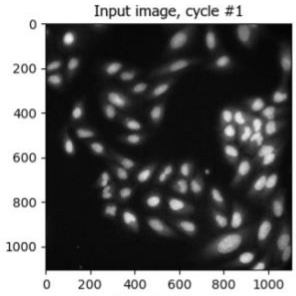
1. Open **CellProfiler**.
2. Click on **Images**. Highlight the image listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the three images in the appropriate CellProfiler window. The original image maintain the folder structure of the original computer used to create the pipeline. If the image is not reloaded from your computer an error will occur.
3. Ignore the **Metadata** step. It is not used in this tutorial and "Extract Metadata" can be set to "no".
4. **NamesAndTypes** module is used to name the single image "DNA".
5. **IdentifyPrimaryObjects** is used to identify and segment Nuclei.
    a. **Typical diameter of objects, in pixel units** was set to 30-80 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.

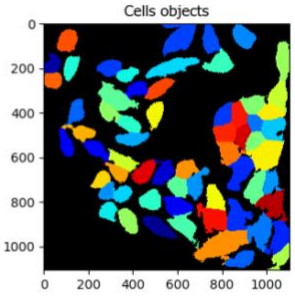
b. **Thresholding strategy** “Adaptive” is used since there are more than two types of intensities in the image; nuclei, some signal in cytoplasmic region, and background.
c. **Thresholding method** “Otsu” is a good general thresholding method to use when the signal to background ratio is high, such as the case with this image.
d. **Two-class or three-class thresholding** The option “Three class” is chosen. In this case the middle ground (cytoplasmic signal) is considered as background since the interest is only Nuclei.
e. **Threshold smoothing scale** was increased slightly to 1.5 to slightly increase smoothing to improve segmentation later.
f. **Threshold correction factor** was also increased slightly to make the thresholding a bit more stringent, reducing slightly the amount of pixels selected.
g. **Lower and upper bounds on threshold** limit were set based on the intensity in the nuclei region. In this case the lower threshold limit was slightly increased to reduce background from being selected.
h. **Size of Adaptive window** is set to 50 which is slightly larger than the smallest expected object (in this case the nuclei) but should be lower the background size.
i. **Method to distinguish clumped objects** was set to intensity because the signal from the center to the edge of each object was much more prominent compare to shape when distinguishing objects.
j. **Method to draw dividing lines between clumped objects** was set to shape since the circular shape of the nuclei, was more prominent than the uneven signal around the nuclei.

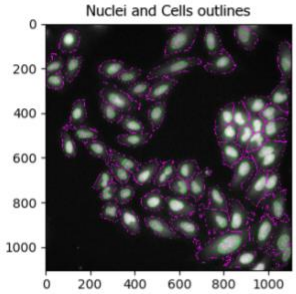
6. **IdentifySecondaryObjects** module was used to identify and segment the Cell using the nuclei as a starting point.
    a. **Thresholding Strategy** “Adaptive” is used since there are more than two types of intensity in the image.
    b. **Ostu** was the most appropriate thresholding method with the good background and foreground signal.

c. **Assign pixels in the middle intensity class to the foreground or the background** was set to foreground this time since we are now interested in the cytoplasmic signal, which is the middle intensity signal.

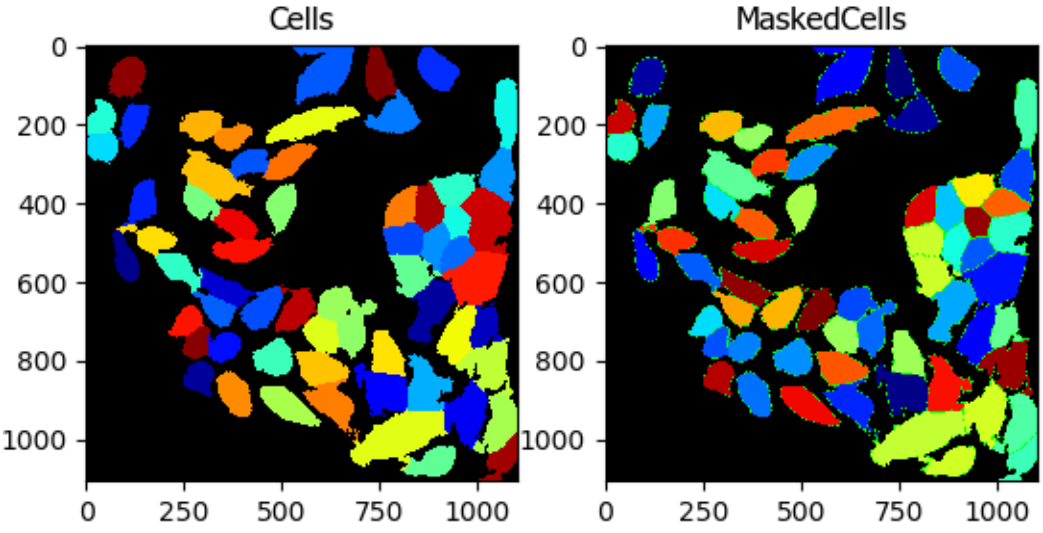
d. All other variables in this method worked well at their default value.







7. **MaskObjects** module is used to create a mask for each segmented Cell. In this case "Select object to be masked" and "Select the masking object" are both set to the identified objects, "Cells". This will create mask objects from the identified cells objects (visually it creates an outline for every cells). These individual masks will be saved in a later step.



8. **MaskImage** module is used to create a mask for all of the segmented Cells. In this case "Select input image" is set to the original "DNA" image and "Select object for mask" is

set to the identified objects, “Cells”. “Invert the mask” is set to yes, so that the object mask will be saved as black. If set to no, the object mask is saved as white. This will create a single mask image consisting of the identified cells objects. This masks of all cell objects will be saved at a later step.

9. **OverlayOutlines** was used to create an image of the identified cell and nuclei objects with cells outlined in grey and nuclei outlined in blue.
10. **SaveCroppedObjects** module is used to save each masked object, created in the “MaskObject” step, individually to your hard drive.
11. **SaveImages** saves the mask created in the “MaskImage” step. A single mask image consisting of all identified objects is saved to your hard drive.
12. **SaveImages** saves the OverlayOutline image to your hard drive.