

Segmenting A Viral Plaque From A Brightfield Image

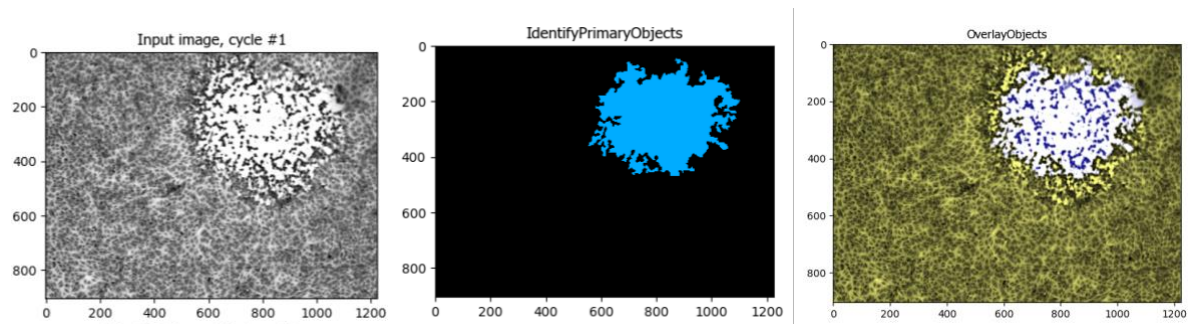
Goal: The goal is to identify & segment a viral plaque from the background of a brightfield image.

Images: Single Differential Interference Contrast (DIC) image.

Pipeline: This example pipeline shows the segmentation of a large region, in this case a viral plaque, from a brightfield image. 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. **IdentifyPrimaryObjects** is used to identify and segment the cells. Since this is a DIC image you could use the “EnhanceOrSuppressFeatures” module to enhance the region from the image based on the structure. In this case region inside the plaque is brighter than the background so this function will not be effective. Instead we adjust the thresholding in the “IdentifyPrimaryObjects” module to achieve the desired results.
 - a. **Typical Diameter of Objects, in pixels** was set to 200-1000. In this example a lower bound of 200 eliminates small objects and prevents the single plaque being divided into multiple objects while the a number above 500 enables detection of the single large object. 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 used in this example, but “Otsu” with “two classes” would also work because the signal to background in this example is quite robust.
 - c. **Threshold correction factor** was lowered to 0.1 so all pixels would be detected. This is because we will control the thresholding manually in the next step by setting the upper and lower threshold boundaries.
 - d. **Lower and upper bounds of threshold** was used to manual set the threshold range. The upper range was set down slightly from the maximum signal to 0.95 while the lower boundary was set to 0.65, the lowest value that enabled detection of the full plaque before too much background was included.
6. **MeasureObjectSizeShape** was used to report the size and shape of the cells.



- 7. **OverlayObjects** was used to overlay the identified objects onto the original image.
- 8. **SaveImages** saves the OverlayObjects image to your hard drive.
- 9. **ExportToSpreadsheet** exports all calculated values for identified objects into a .csv file.

Note: Though this is a DIC image, the “EnhanceSuppressFeatures” module was not used because the region of interest was much too bright to use the enhance feature and the suppress features removes some signal, as shown in the below image panel.

