Protocol for Spheroid Analysis

This protocol allows for quantification of spheroid spread by spread area and shape factor. The important settings used in each module are specified below, and settings not specified were left at their default value.

Basic Object Identification

NamesAndTypes:

- If using .czi images, image type must be set to 'Color image'.

IdentifyPrimaryObjects:

- For spheroid images, the spheroid itself can be identified as a single object. Typically, a size of 500-1500 pixels can be used for spheroid images. The boundary for size is forgiving for spheroid analysis, as the low bound need only be big enough to exclude single cells.
- The thresholding strategy determines the method CellProfiler uses to determine the intensity level to distinguish objects from background. A 'Global' strategy determines a single threshold value across the whole image, whereas the 'Adaptive' strategy determines a threshold at each pixel based on a window of pixels surrounding it. The 'Adaptive' strategy is more robust but more computationally expensive, and is best used for images with inconsistent background intensity, for images with more constant backgrounds 'Global' is as effective and runs more quickly. For most spheroid images, 'Global' is appropriate.
- The threshold correction factor can be adjusted to fine tune the calculated thresholding from above, by multiplying the threshold by the value you set. If a pipeline consistently produces images with identified spheroids that are smaller than can be seen in the original image, a threshold correcting factor less than one can be used. If a pipeline often identifies bright regions of the background as part of the spheroid, a threshold correction factor greater than one can make the threshold more stringent.
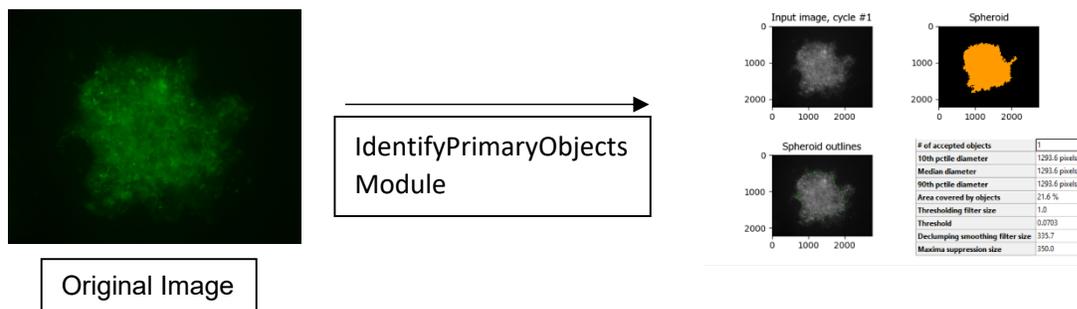
MeasureObjectSizeShape:

- 'Select objects to measure' should be set to the primary object that identifies the spheroid.

ExportToSpreadsheet:

- Export 'SecondaryObjects' -> 'AreaShape'. From here, Area and Perimeter are the only required features, but for different analyses different features could be useful. From the area and perimeter, shape factor can be calculated.