FLUXION

Tutorial: BioFlux Montage, Integrated Morphometry Analysis (transmigration example)

This tutorial will show how to use the Integrated Morphometry Analysis module in the BioFlux Montage software. The IMA module is a powerful tool for processing images based on morphological characteristics. An example will be shown here for an image acquired during a transmigration experiment. The concepts shown can be applied to a wide variety of BioFlux data sets. We will demonstrate the processing of a single image, although everything you see her can be fully-automated for larger data sets.

To get started using the IMA, open the IMA module and the image you will be working with.

The first step of any image analysis is to think through what parameters will be analyzed. In this case, we have an image which shows cells both above and below the monolayer, and we know intuitively which is which is which by the way they look. Cells which are phase bright are on top of the monolayer, and cells which are phase dark are beneath the monolayer. For the purpose of this analysis, we'll count both types of cells and characterize their morphological parameters such as area and shape.

We will first run a screen to count the cells on top of the monolayer. We will threshold the image until we have isolated the cells of interest. These settings can be saved and used again if processing large data sets.

Note here that although we've isolated the cells on top, we've also selected some objects which are not cells. We'll now switch over to the IMA module to further refine our selection. We have a wide range of morphological parameters to select from in this module. Here we will set up a filter to screen out objects which are not cells. The parameter called shape factor describes how round an object is. The value of 1 is considered to be a perfect circle, while values less than 1 indicate a trend towards being a more amorphous shape. To activate this filter, we'll select the checkbox and type in our values and our comparison type. When we're finished, we select the Measure button and see the results. This process can be repeated as many times as needed to obtain the desired results. Note that the objects in orange represent the initial threshold, and the green objects represent the ones which will be counted in the data analysis.

Once we're finished with the analysis, we can open a log and save the data in Microsoft Excel or similar databse application. This is done through the Dynamic Data Exchange module. We can also easily export histograms and scatter plots directly into other applications.

Next we'll count the cells which have transmigrated beneath the cell monolayer. We'll threshold the image again, but now select for the dark cells. Notice that we've captured a lot of additional objects which are not necessarily cells, but we can easily deselect these in the IMA. Here we'll set up a screen against Pixel Area, between 1200 and 7000. Like the first analysis, we can record the summary or object data into a database application.

It is possible to further refine your selection using a manual approach. In this case here, we can see that there is a cell which was inadvertently selected as underneath the monolayer, likely a result of a shadow around that cell. We can double click that object and have to removed from the data analysis.

This concludes the IMA tutorial. Remember to plan the analysis out before you start, begin with a good threshold of the image or images, and use the appropriate filters to identify the cells of interest. Please contact a member of Fluxion's technical team if you have any further questions.