



Tutorial: BioFlux Montage, Multi-Wavelength Cell Scoring

This tutorial will show how to use the Multi-Wavelength Cell Scoring Module in the BioFlux Montage software. This module automatically detects the presence of up to 7 fluorescent stains within each cell. The module identifies a variety of useful measurements including the number and percent of cells scored positive for each marker, as well as individual cell scoring profiles across the set of markers. In this example, we will look at stem cells stained with a nuclear and cytoplasm stain.

To get started, open the image for each wavelength. Each experiment can use multiple fluorophores to stain either the nuclei, cytoplasm, or the entire cell. The first marker should stain and identify all of the nuclei. The second through seventh marker(s) should stain either nuclei, cytoplasm, or both. Calibrate all images according to the objective used during acquisition. This enables the use of real world units such as microns, rather than pixels.

Prior to starting the analysis, it is sometimes helpful to view the images overlaid. To do this, go to the MMStandard Menu/Display/Color Combine and assign each open image to a color channel.

From the Apps menu, open the Multi-Wavelength Cell Scoring Module. Select the number of wavelengths to be analyzed, in this example we will use 2. Assign each open image to one of the tabs, making sure the all nuclei image goes first. Set the parameters for identification of the nuclear stain. The minimum and maximum widths help the software determine what is considered to be a nuclear stain. You can enter these values in an iterative manner to help determine the optimal range, or you can use the Integrated Morphology Analysis module to measure the cells and extract an appropriate range. Here we will use 10um and 30um for min and max. The intensity above background refers to how bright the fluorescence signal is. Most fluorescent stains will be significantly brighter than the background. Here we will use 10 gray levels. Use the Preview button to get an idea of how the segmentation will look prior to running the full analysis.

Repeat the process for the 2nd wavelength, which in this case is a cytoplasm stain. We will use 28um and 65um for min and max. We'll set the threshold to 30 gray levels. For all of the markers which get scored, there is an additional parameter to describe the minimum stained area, which is used to eliminate false positive scoring from smaller dye spots. These settings can be saved at any time for repeating the analysis with additional images from the data set.

Prior to running the analysis, set up a Data Log and Summary Log using the Dynamic Data Exchange (DDE) module. Configure each log from within the Module to determine which data will get recorded.

Once all the settings have been set, click Apply to run the analysis. All of the data will be logged to the previously indicated database application such as Microsoft Excel.

For large data sets, the settings of each analysis can be saved and the entire process can be recorded into a journal for automatic processing.

For additional questions, please contact a member of Fluxion's technical team.