



## **Tutorial: BioFlux Montage, Platelet Adhesion**

This tutorial will show how to perform platelet adhesion data analysis in the BioFlux Montage software. The data processing capabilities of Montage can output a number of useful properties relating to platelet adhesion. These include area coverage, fluorescence intensity and other morphological parameters.

In this example, we will use a data set found in the Samples Images directory which is provided with the software. Images can be located in Support Files / Sample Images / "Thrombus – timelapse 2 channels in VW". For this analysis, we will calculate the percent adhesion coverage across a data set which shows platelets adhering. This data was captured using calcein to label the platelets and was imaged using a 10X objective such that two channels could be imaged in the same field of view.

To get started, open the first image in the directory. In this example, each image contains two channels which can be analyzed independently using Regions of Interest. Using the rectangular tool, draw regions which outline the channels of interest. If your particular data set only contains one channel per field of view, Regions of Interest can still be used to exclude background noise, or this step can be omitted altogether if not needed.

From the Taskbar Main Menu, select the Analysis Menu. From the Analysis Menu, select the 'Percent Area' module. For this application, we will process all the images in the directory, although this can also be used to process images one at a time. When asked to threshold the image, we will select yes, and use an interactive threshold to highlight what is to be considered platelet adhesion. Use an inclusive threshold, and adjust the sliders until the adhesion area is properly selected. Click 'Close' to proceed.

When asked to use 'Active ROI' (Region of Interest), select yes. Note that this option will only appear if an ROI has been drawn before starting the module.

When prompted to open a data log, select DDE to feed the results directly into a Microsoft Excel spreadsheet. The data can also be ported into a text file, but using the DDE link directly to Excel will provide the fastest way to analyze the data. The data will now be analyzed and logged directly into an Excel worksheet. The results can easily be plotted into a chart or exported to other database applications.

In addition to percent area, other morphological properties can be analyzed using the Integrated Morphometry Analysis Module.

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