Imperial College London

Facility for Imaging by Light Microscopy

FILM

Observing Life As It Happens

Ratiometric imaging with

Volocity Acquisition and FIJI processing

on WF1

Acquiring the images

- acquire the 2-channel image, stack or time series using normal Volocity Acquisition settings

- export the image or time series as ITEM AS OME-TIFF

Analysing the images in FIJI

Opening the files and generating the ratiometric image

- start FIJI

- import OME-TIFF file using the plugin LOCI / BIOFORMATS IMPORTER, using the following options:

  - VIEW STACK WITH HYPERSTACK

  - SPLIT CHANNELS

- to generate a ratiometric image, use the plugin RATIO PLUS:

- use the imported datasets for the two images
- use a multiplication factor that results in a good image brightness (the value of the ratio might be much lower or higher than the actual brightness levels for each pixel)
- to make small variations in the resulting ratio image easier to see, change the display colours using IMAGE / LOOKUP TABLES / SPECTRUM

Quantification

To quantify values in a single image:
- draw a Region Of Interest (ROI) using the FIJI drawing tools
- use ANALYZE / MEASURE; this will open the RESULTS window with the values of the ROI, as defined in ANALYZE / SET MEASUREMENTS
- you can add as many ROIs

To measure values across a whole time series
- start the PLUGIN / TIME SERIES ANALYZER
- this which will open two windows, the ROI MANAGER (also available through ANALYZE / TOOLS) and the TIME SERIES
- create the required ROIs using the FIJI drawing tools and ADD them to the list in the ROI MANAGER
- when finished, use the tool GET AVERAGE in the window TIME SERIES to quantify the intensity of the ROI over time and export it to a table as well as a time plot

Exporting the results
- to export the data, simply use the normal COPY, PASTE and SAVE AS menu items in the respective windows
- to export the plot:
  - click on the TIME TRACE AVERAGE window to make it active
  - copy the content with CTRL-C
  - create a new image using FILE / NEW / INTERNAL CLIPBOARD, save image in GIF format