

Data and experimental premise for Preprocessing 1&2

Hypothesis: Vinculin regulates focal adhesion area per cell and promotes cell spreading.

Molecules of interest:

- vinculin is a protein with known force-sensitive roles in cell-extracellular matrix adhesions
- paxillin here is used as a marker of cell-matrix adhesions (focal adhesions)
- F-actin is stained with phalloidin

Cell types:

- WT MEF expressing paxillin-EGFP
- Vinculin knockout MEF expressing paxillin-EGFP

** these cells were a gift from Dr. Brent Hoffman and have been used in Mierke et. al, JBC 2010

Workflow:

1. Image WT and vinc-/- cells with labeled actin and nuclei
 1. Done!
2. Determine the spread area per cell in all conditions
 1. Threshold images of actin channel to make a mask of the cell body (cell body ROI)
 2. Threshold images of nucleus channel to make a mask of nuclei. This is used to count the cells.
3. Determine the focal adhesion size in all conditions
 1. Preprocess pax-EGFP images to reduce noise and subtract cytoplasmic signal
 2. Threshold images of paxillin channel.
4. Compare spread area and focal adhesion size between samples

Data:

Hypothetically, you'd have a folder in your raw data folder full of files like

20170601-vincKO-1.tif
20170601-vincKO-2.tif
20170601-vincKO-3.tif
20170601-vincKO-metadata.json
20170601-WT-1.tif
20170601-WT-2.tif
20170601-WT-3.tif
20170601-WT-4.tif
20170601-WT-metadata.json

...where each file is a confocal fluorescence image of fixed cells in culture. Here, for simplicity, we will only be working with one z-slice. We will want to write a program to analyze all of the data in the same way. Here, we will first tinker with different thresholding and preprocessing algorithms on two sample images to make masks.

