SAND2012-10356P

Hyperspectral Imaging of Fixed Dendritic Cells in collaboration with Aaron Neumann and Matt Graus

Sample Preparation: 11-06-2012 (Matt/Anita)

Saccharomyces cerevisiae, strain S288C

Fixed dendritic cells, labeled with CMO (Cell mask orange)

Imaging: 11-06-2012 (Jeri)

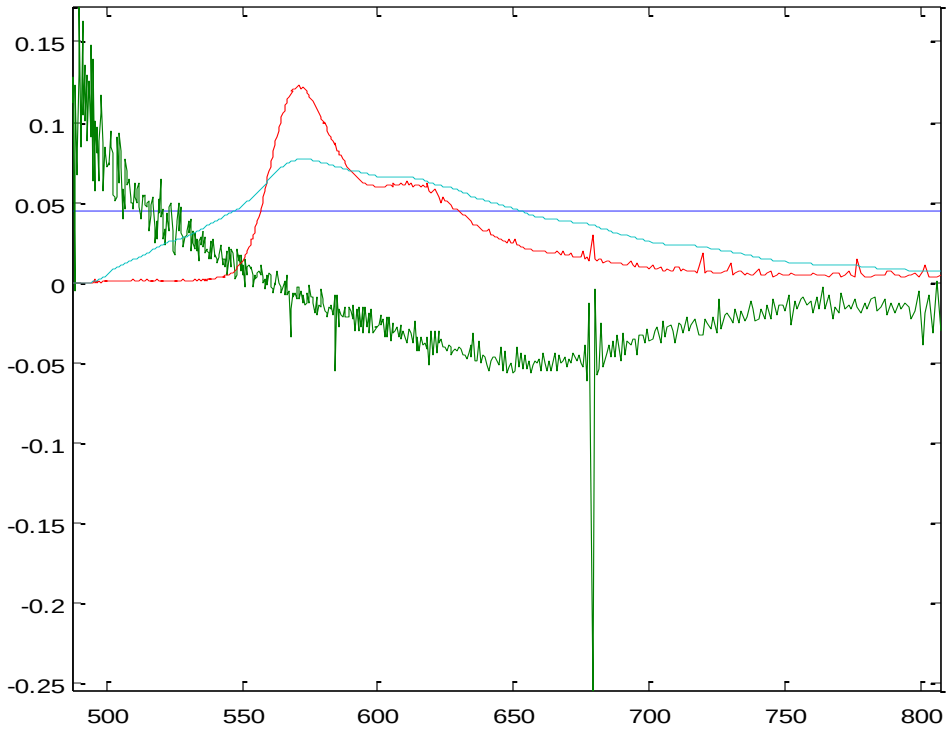
Analysis: 11-11-2012 (Jeri)

Sample prep: either live or fixed fungi or fixed dendritic cells were placed on slide, covered with #1.5 coverslip, and sealed with nail polish.

Acquisition parameters: 488 nm excitation, 60x oil objective, 25 µm x 25 µm field of view, 0.24 msec/pixel, gain = 210, OD=0.

Results for CMO 10000 Dilution

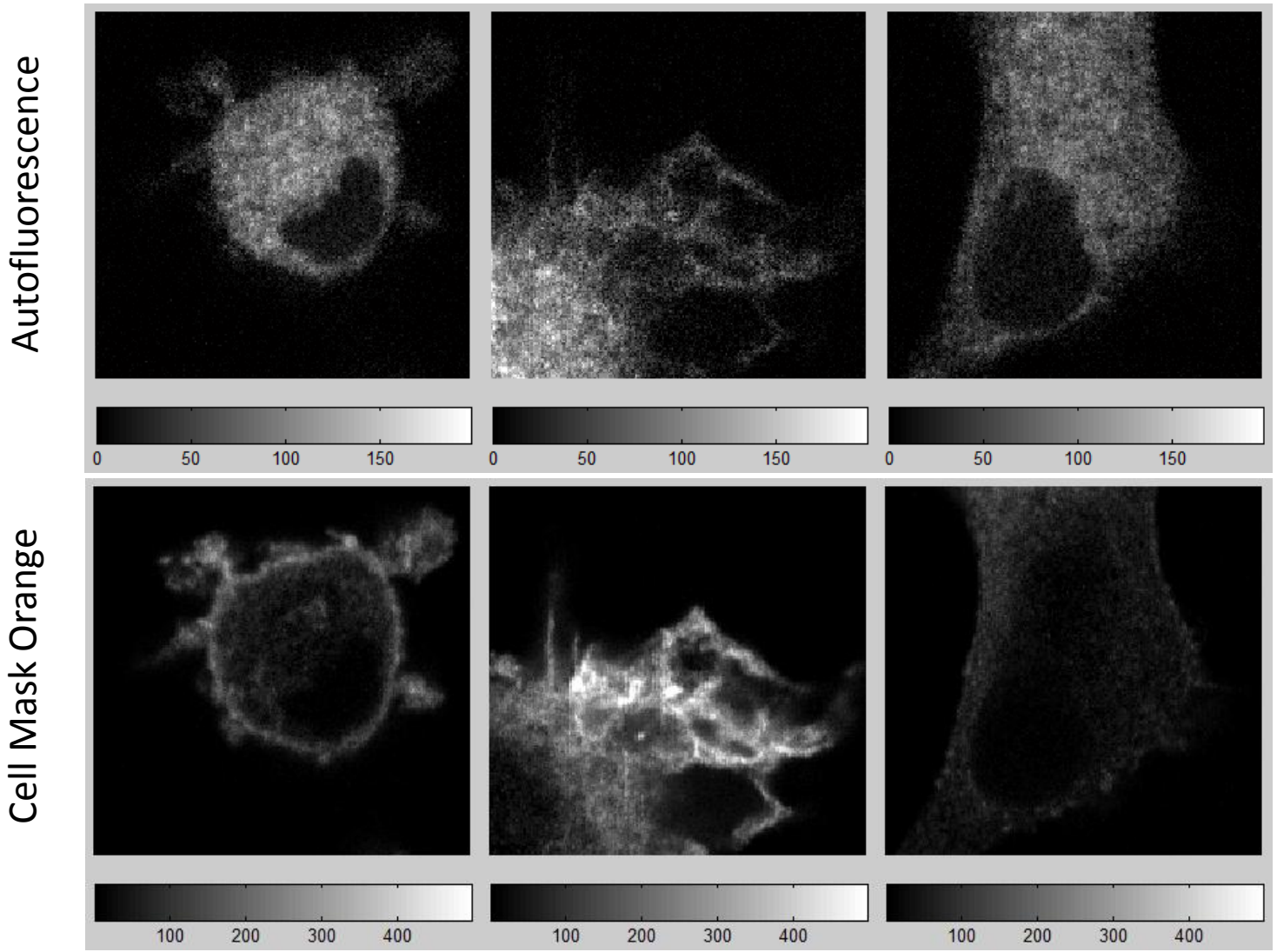
Goal: determine dilution factor necessary for CMO labeling of cell membrane



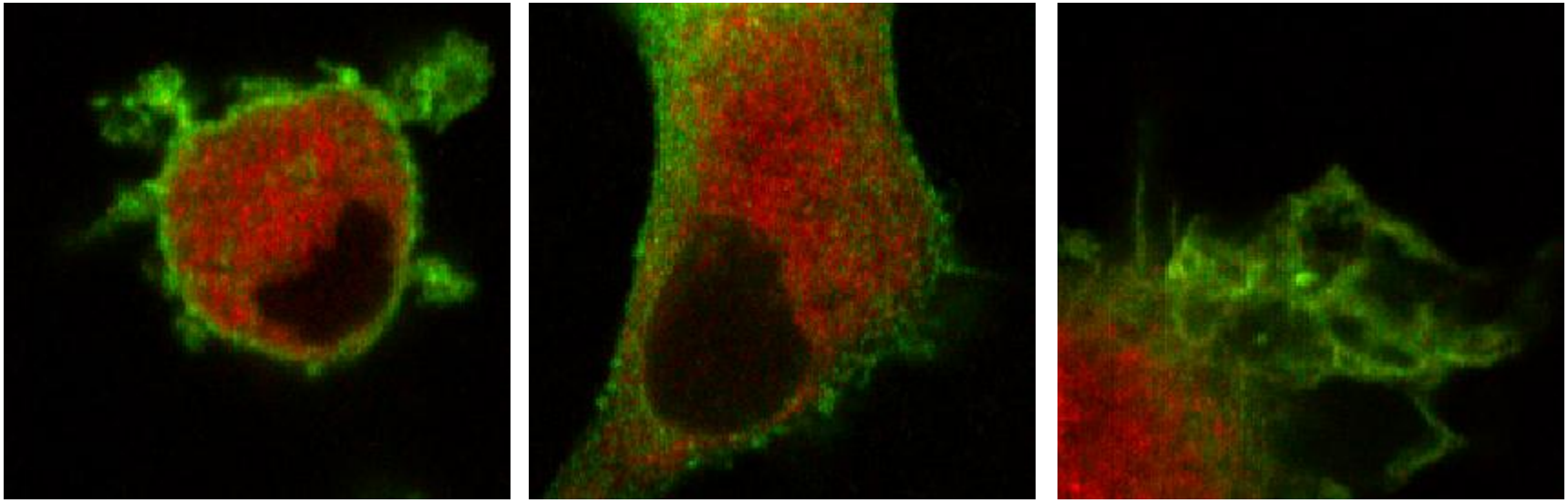
Dilution suggestions:

- 10000-fold dilution works well
- Did not analyze the 1000-fold dilution results, high intensities led to detection non-linearities
- 3D image is not great – photobleaching evident

Results for CMO 10000 Dilution



Results for CMO 10000 Dilution



Hyperspectral Imaging of Fixed Dendritic Cells in collaboration with Aaron Neumann and Matt Graus

Sample Preparation: 11-13-2012 (Matt/Anita)

Saccharomyces cerevisiae, strain S288C

Fixed dendritic cells labeled with one or two of the following: CF488, Cy3.5, or CF532 and/or CMO (Cell mask orange). Unlabeled fixed dendritic cells were also imaged.

Imaging: 11-13-2012 (Aaron Collins)

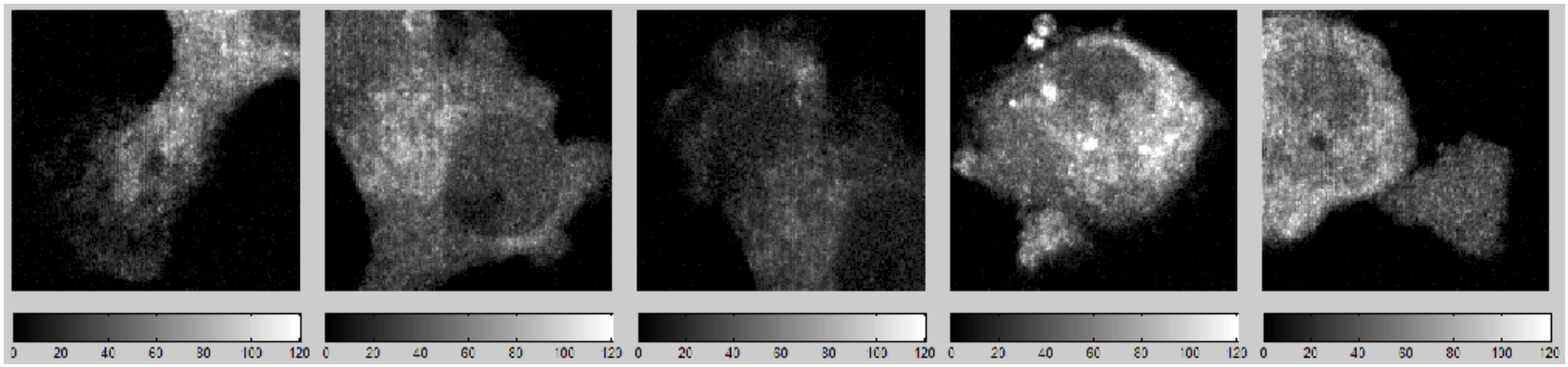
11-17-2012 (Jeri)

Analysis: 11-24-2012 (Jeri)

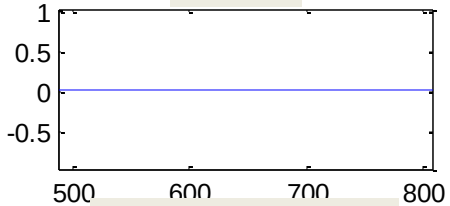
Sample prep: either live or fixed fungi or fixed dendritic cells were placed on slide, covered with #1.0 coverslip, and sealed with nail polish.

Acquisition parameters: 488 nm excitation, 60x oil objective, 25 µm x 25 µm field of view, 0.24 msec/pixel, gain = 210, OD=0.

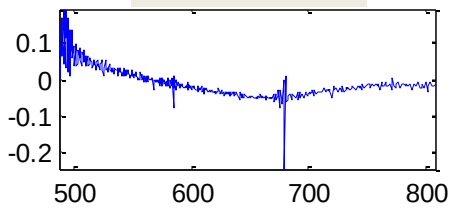
Unlabeled Fixed Dendritic Cells



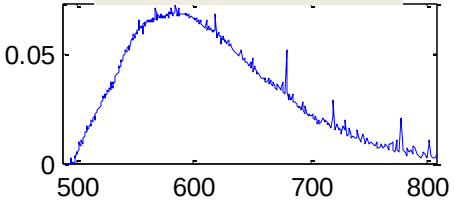
Offset



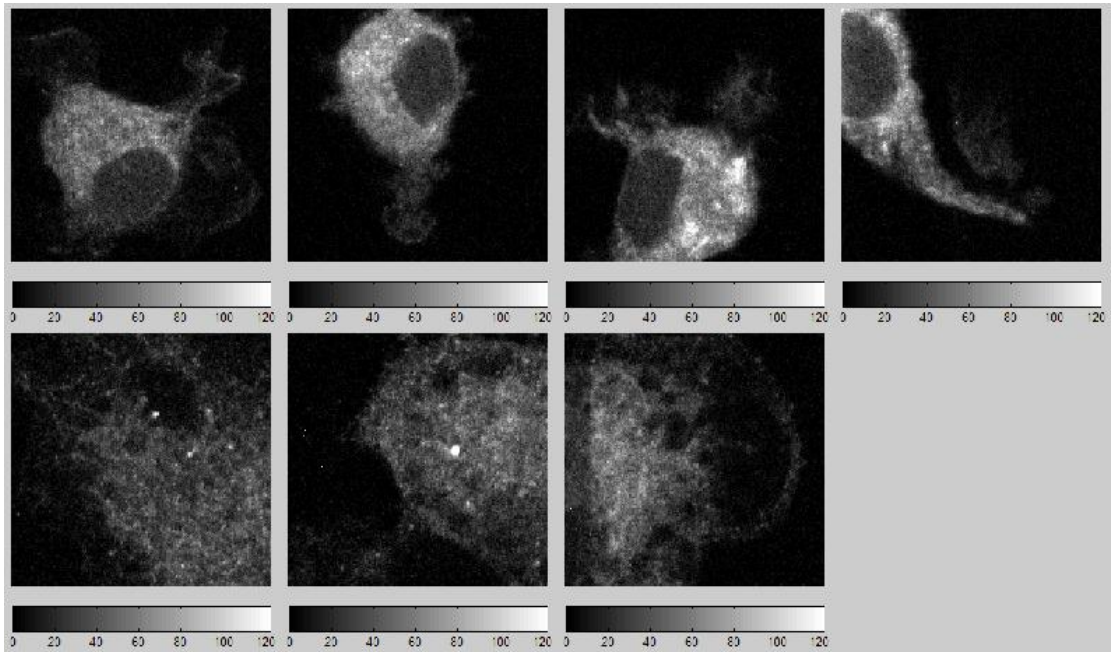
Dark Current



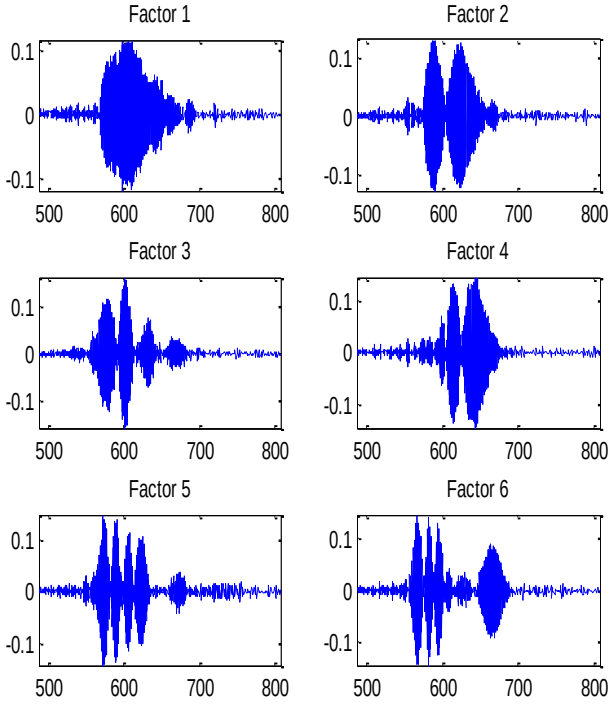
Autofluorescence



Fixed dendritic Cells w/CF532-Dectin1

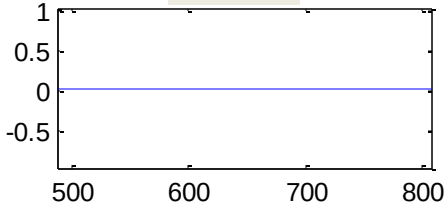


Spectral Residuals

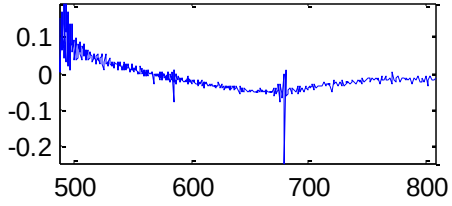


No indication of CF532. Only signal was the cellular AF.

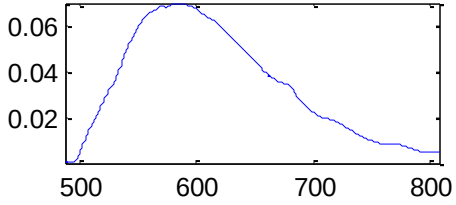
Offset



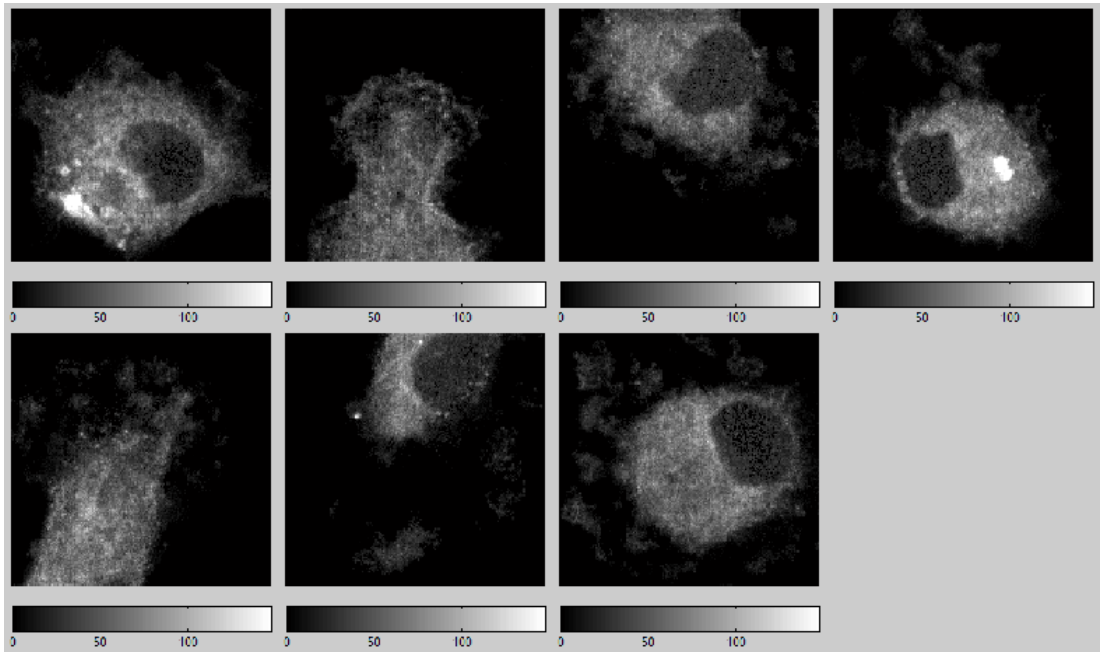
Dark Current

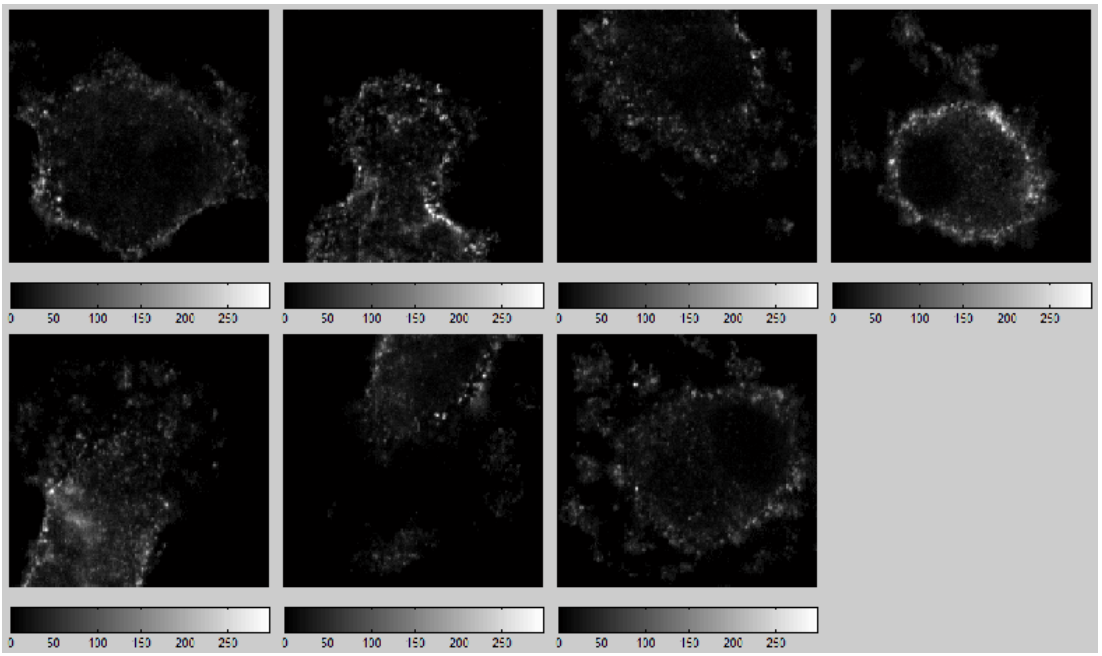


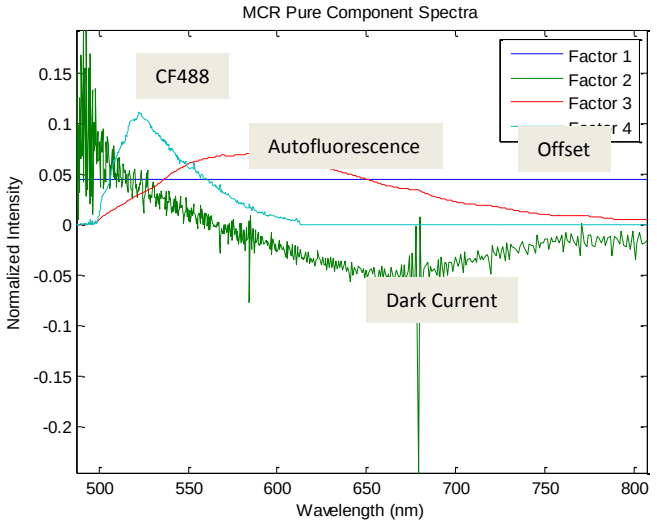
Autofluorescence



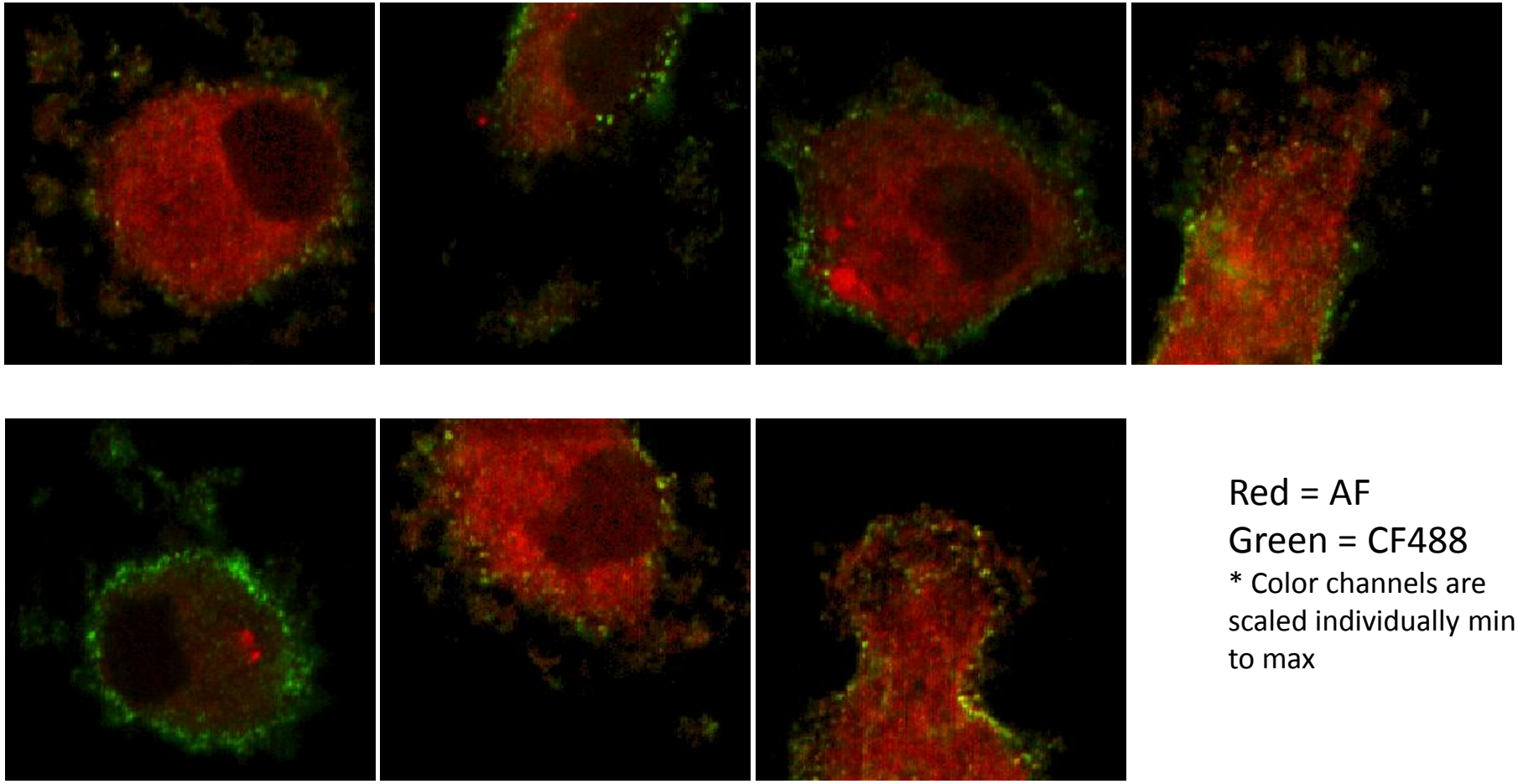
Fixed Dendritic Cells w/CF488-CD206







Fixed Dendritic Cells w/CF488-CD206

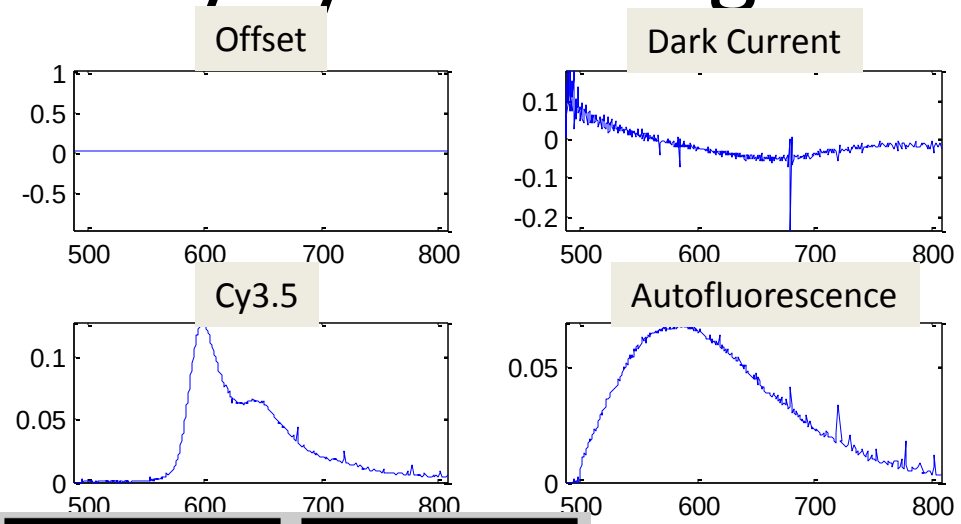


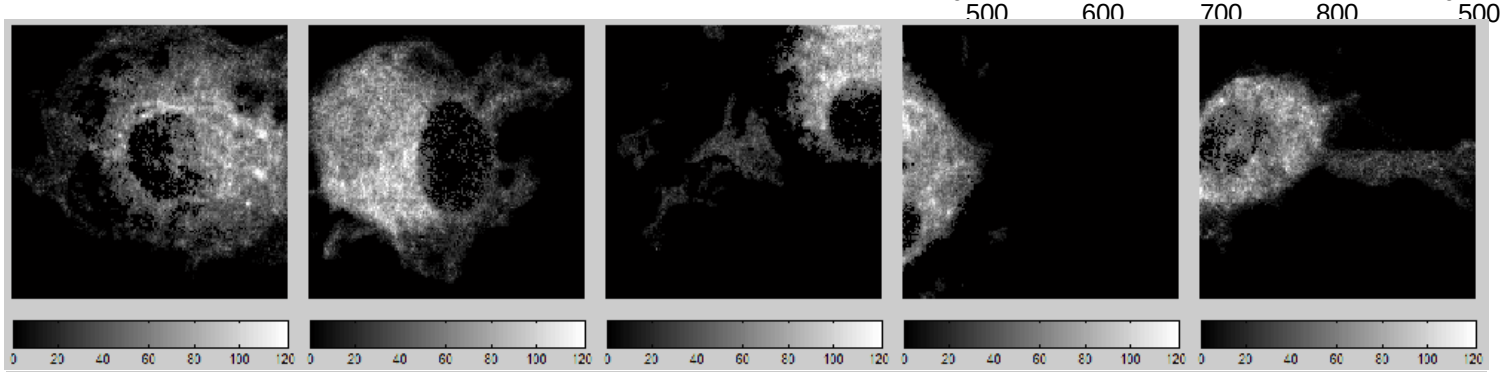
Red = AF

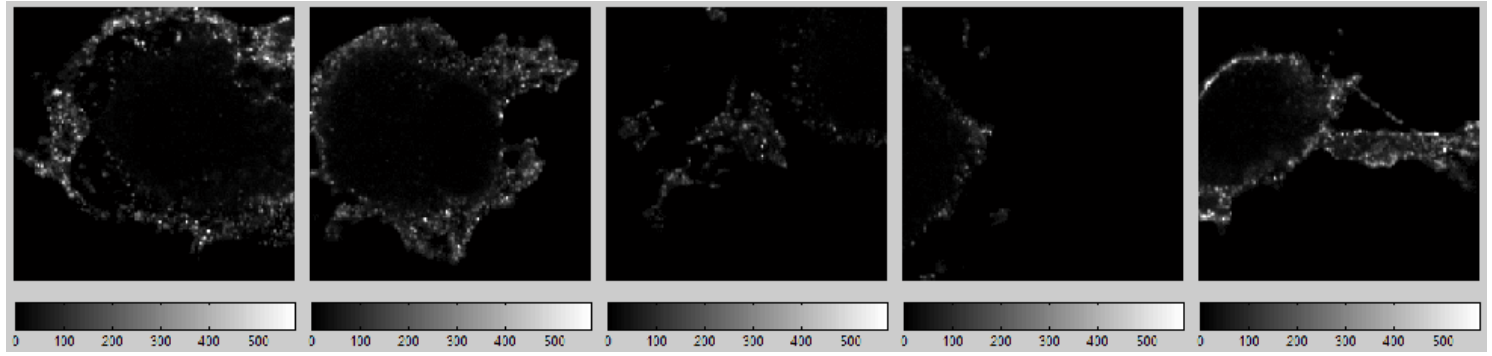
Green = CF488

* Color channels are scaled individually min to max

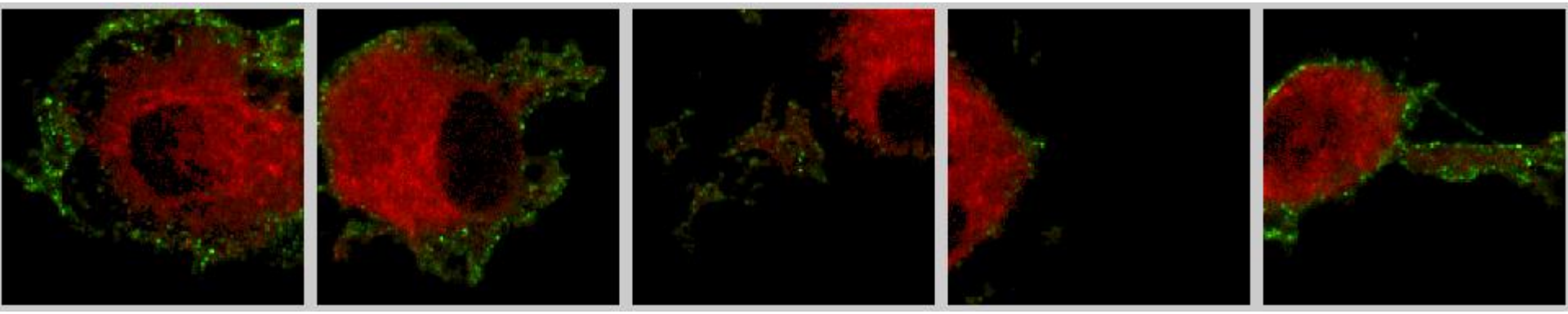
Fixed Dendritic Cells w/Cy3.5-DCsign







Fixed Dendritic Cells w/Cy3.5-DCsign

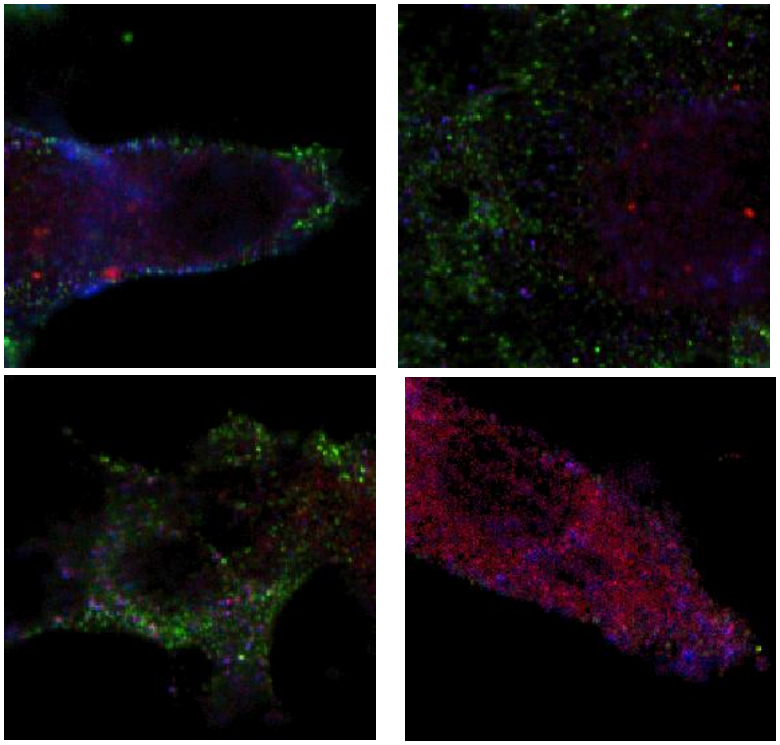


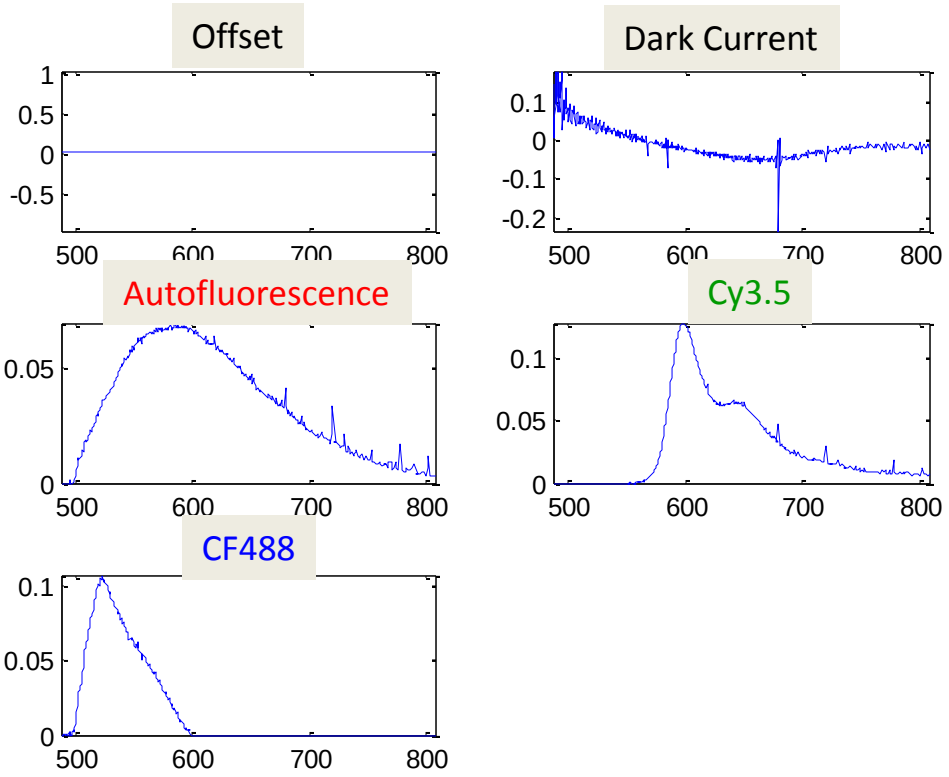
Red = AF

Green = Cy3.5

* Color channels are scaled individually min to max for red and stretched in green due to a few bright pixels.

Fixed Dendritic Cells w/Cy3.5-DCsign and CF488-CD206





Summary

- The images of labels with CMO were analyzed but not reported here. The concentration of CMO was too high to be optimal so these are not worth detailing.
- There was no indication of CF532 in any of the images.
- The paired image with CF532 were no different the single color so therefore not presented.

Hyperspectral Imaging of Fixed Dendritic Cells in collaboration with Aaron Neumann and Matt Graus

Sample Preparation: 11-27-2012 (Matt/Anita)

Saccharomyces cerevisiae, strain S288C

Fixed dendritic cells labeled with CF488, Cy3.5, and CMO (Cell mask orange). Unlabeled fixed dendritic cells were also imaged.

Imaging: 11-27-2012 (Jeri)

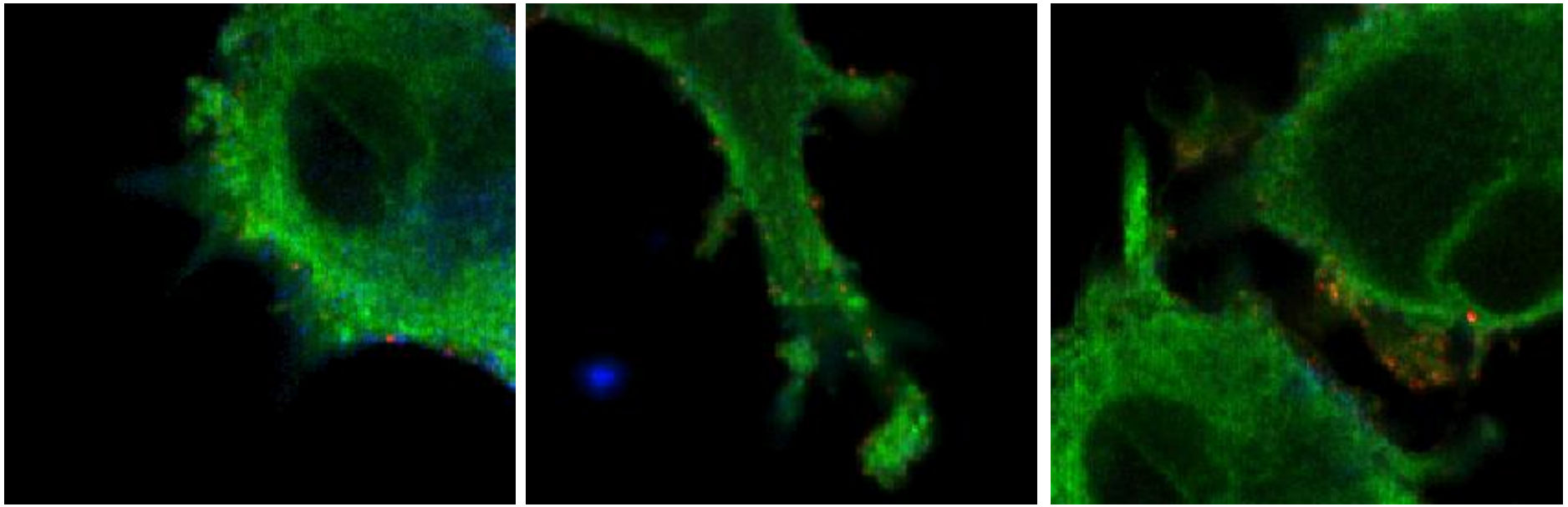
Analysis: 11-29-2012 (Jeri)

Sample prep: either live or fixed fungi or fixed dendritic cells were placed on slide, covered with #1.0 coverslip, and sealed with nail polish.

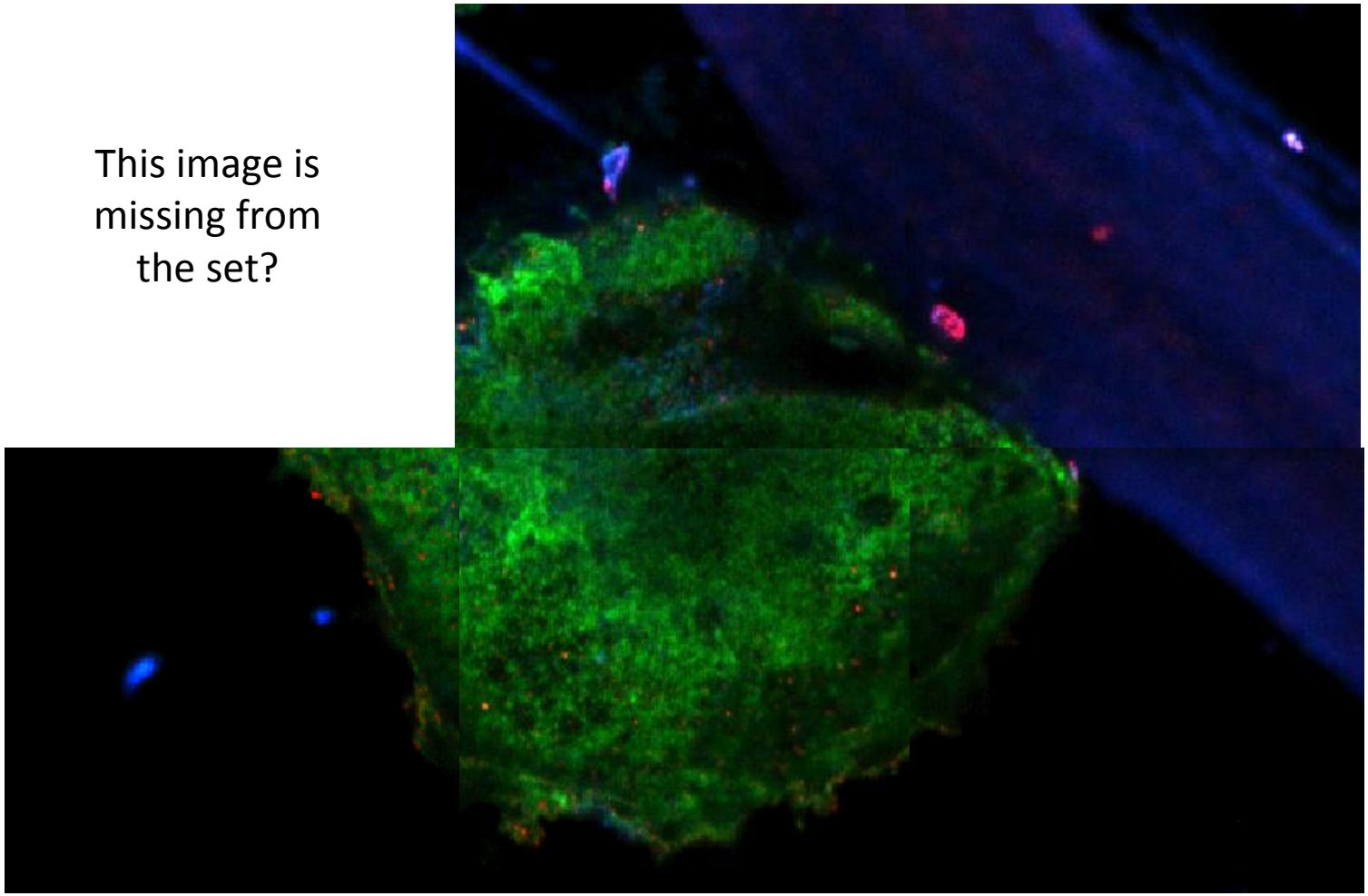
Acquisition parameters: 488 nm excitation, 60x oil objective, 25 µm x 25 µm field of view, 0.24 msec/pixel, gain = 210, OD=0.

Fixed Dendritic Cells w/Cy3.5-Dcsign, CF488-CD206, and CMO (1:100000)

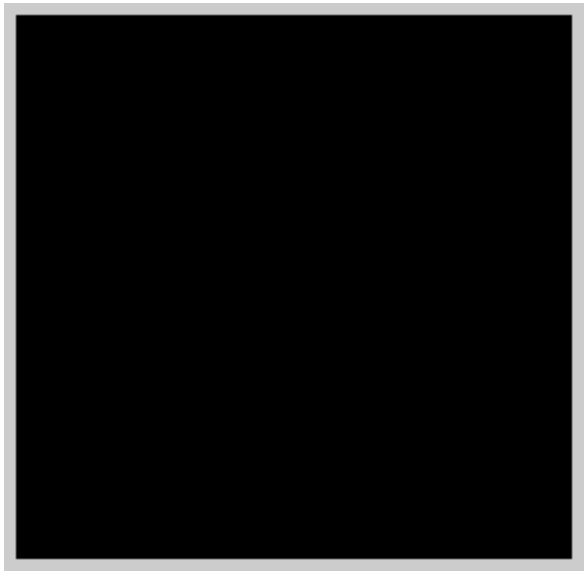
Red – Cy3.5-DCsign
Green – CMO
Blue – CF488-CD206

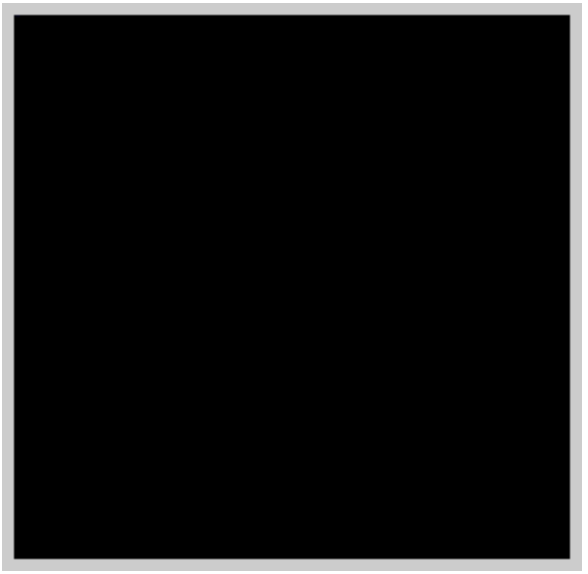


This image is missing from the set?



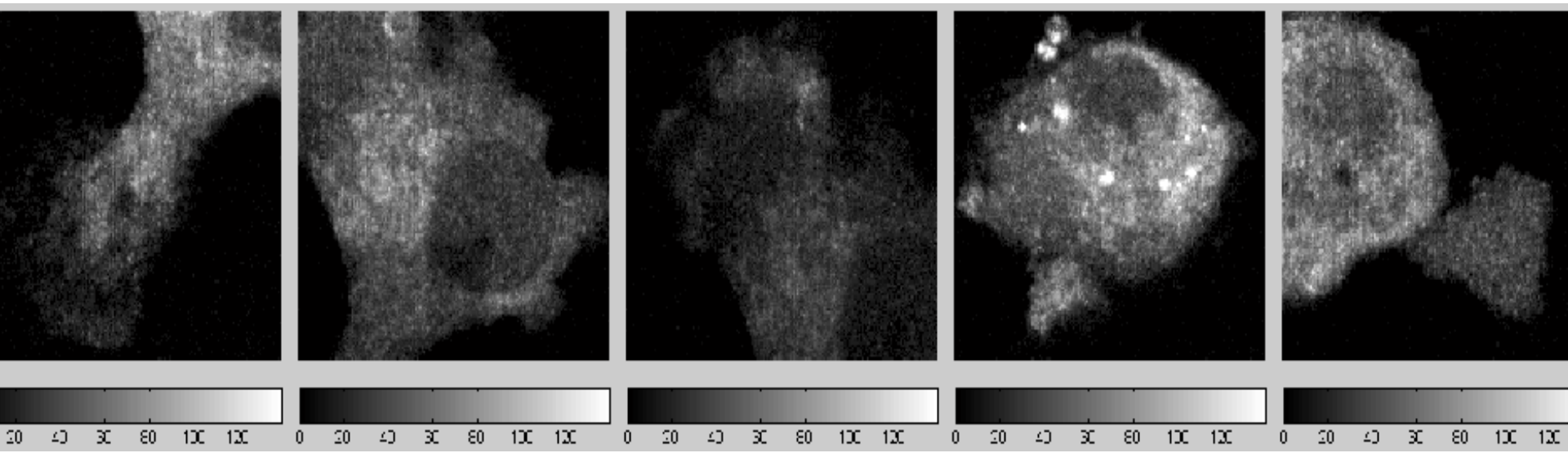
Attempts at Sections





These were fairly crude sections, done at 1 um Zstep. I was looking to see if there was a great deal of photobleaching and I do not think so.

Control Fixed Dendritic Cells (No label)



AF is consistent with previous experiment. Some cells exhibit bright spots of AF with a slightly red-shifted spectrum, however in the presence of labels this will be a wash.