

# 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)

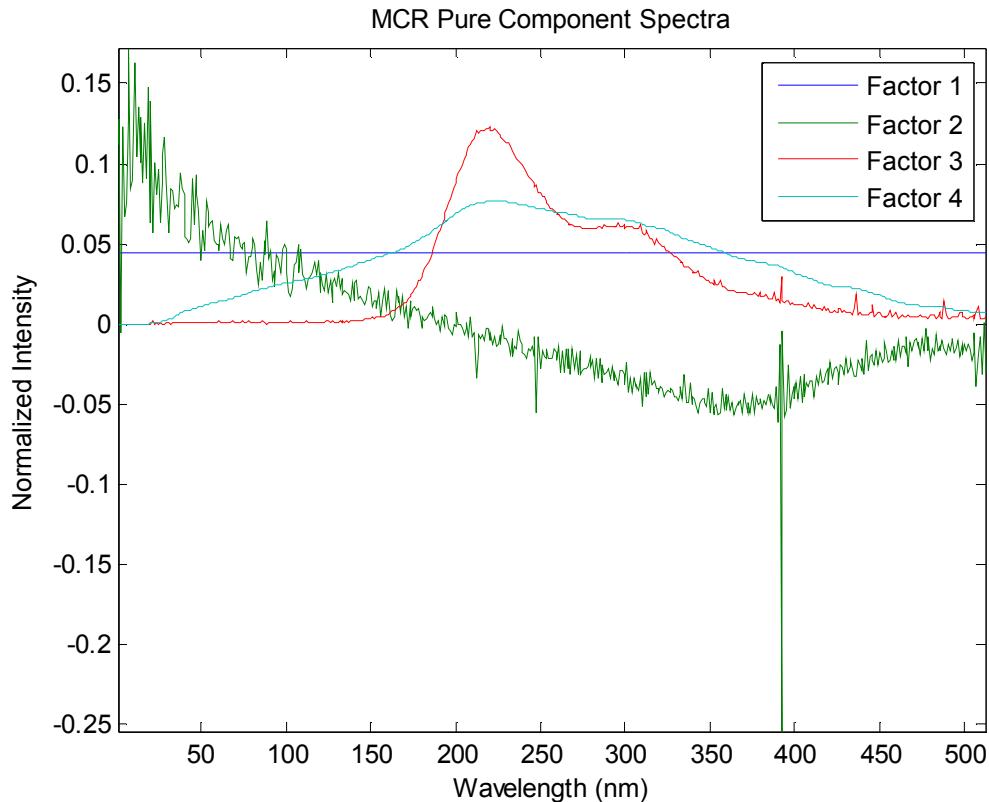
Analysis: 11-11-2012 (Jeri)

*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  $\mu\text{m}$  x 25  $\mu\text{m}$  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

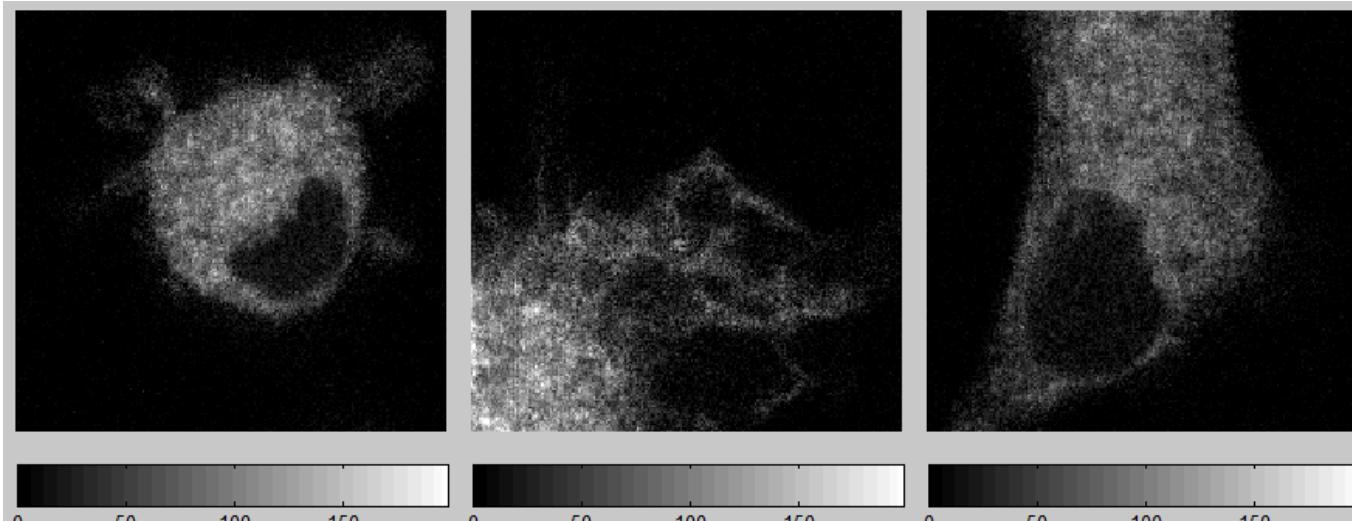


Dilution suggestions:

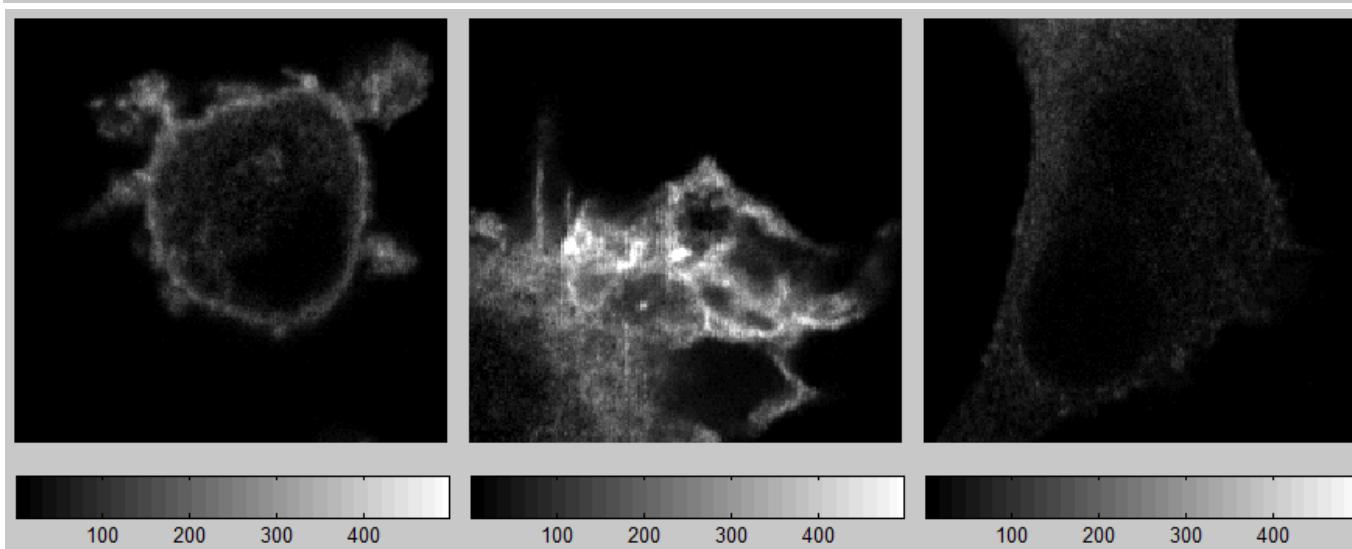
- 10000-fold dilution works well
- Did not analyze the 1000-fold dilution results, high intensities led to detection non-linearities
- 3D image is not great – photobleaching evident

# Results for CMO 10000 Dilution

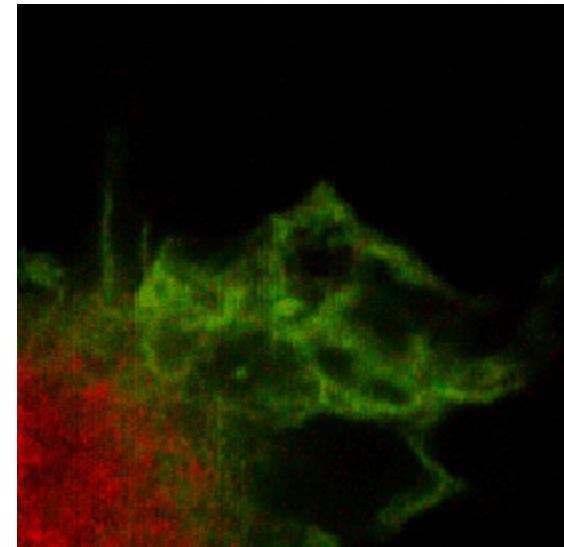
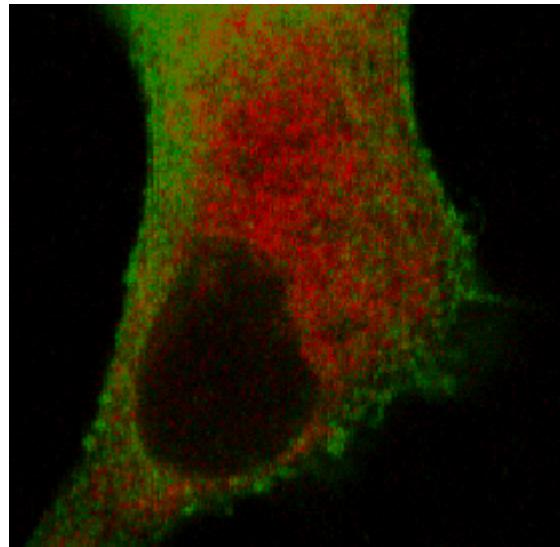
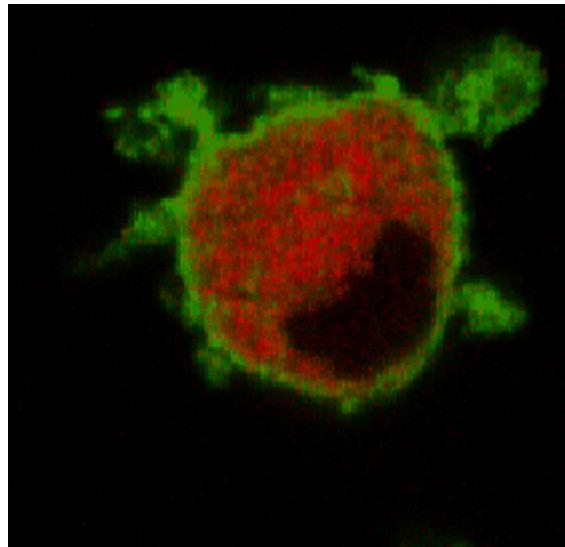
Autofluorescence



Cell Mask Orange



# Results for CMO 10000 Dilution



# Results for CMO 1000 Dilution