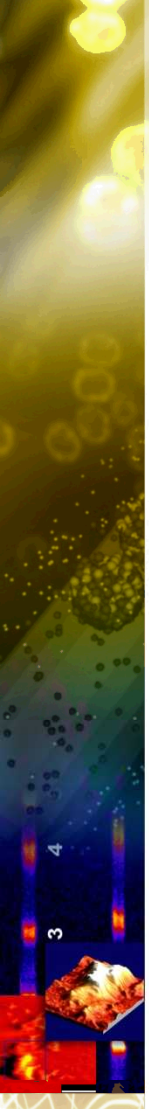
SAND2013-10315P



Hyperspectral Imaging Results
Biophagy Project

PP242 & DMSO treated He-La cells on 11-14-13

Sample prep: Bryan

Imaging: Bryan, Jeri, Stephen

Analysis: Jeri

NMSBA project #: 27202/13.01.081

SANDIA BIOSCIENCE

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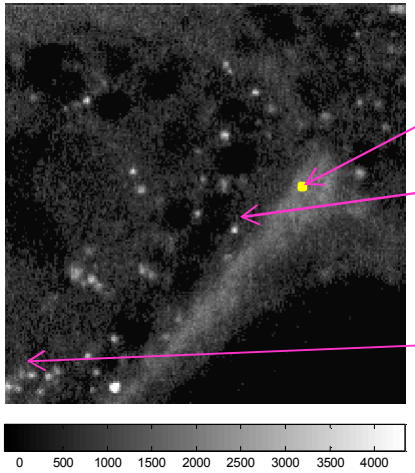
Sandia National Laboratories

Experimental Parameters

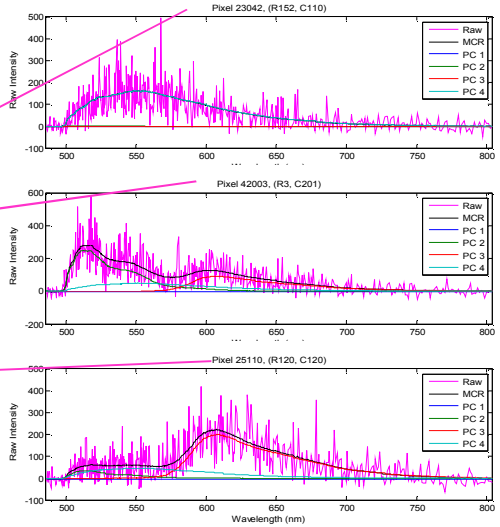
- Samples prepared just as for confocal microscopy
  - DMSO
  - 30 μM PP242
- Using custom hyperspectral microscope. (Sinclair, et. al. Applied Optics, 2006, for details)
  - 488 nm excitation, 60x oil objective (NA=1.4)
  - EMCCD detector (gain =100, temperature =-60)
  - X steps = 0.12 μm, Y steps = 0.12 μm, Z steps = 0.5 μm
  - FOV 25 or 50 μm
  - 0.24 msec exposure
- Used SNL proprietary multivariate curve resolution software for analysis (Jones, et. al. Chemometrics and Intelligent Laboratory Systems, 2013, for details)
- Note: We were trying to image highest expressing cells, but selecting base don brightness is pretty limited on this system right now due to a change in hardware.

Illustration of Multivariate Curve Resolution

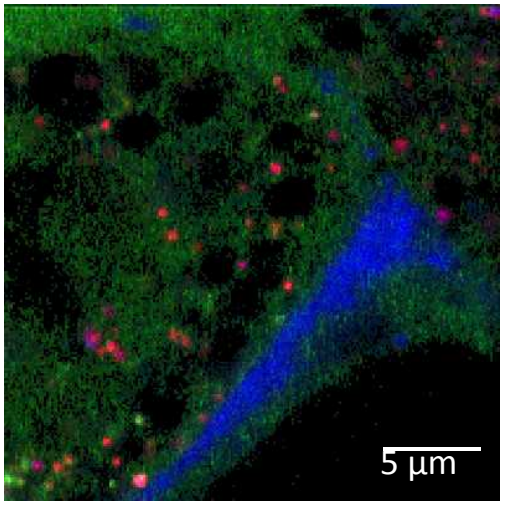
Spectral Image
(integrated over wavelengths for viewing)



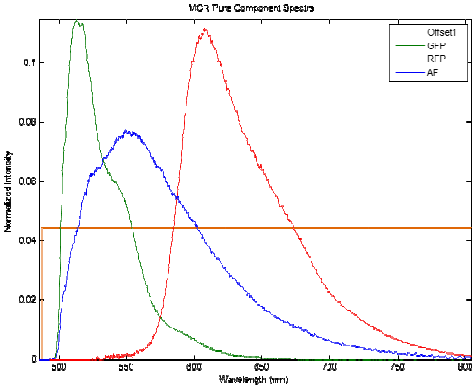
Examples of MCR Model Fits



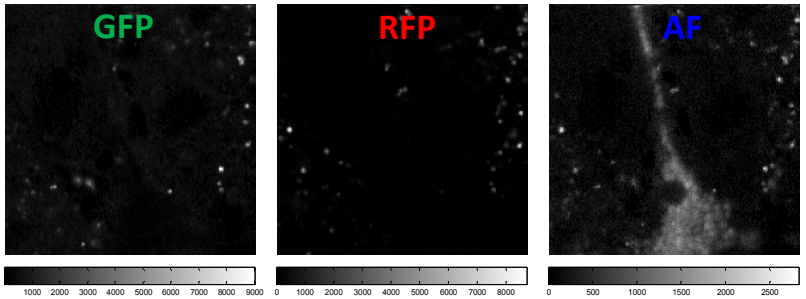
RGB Overlay



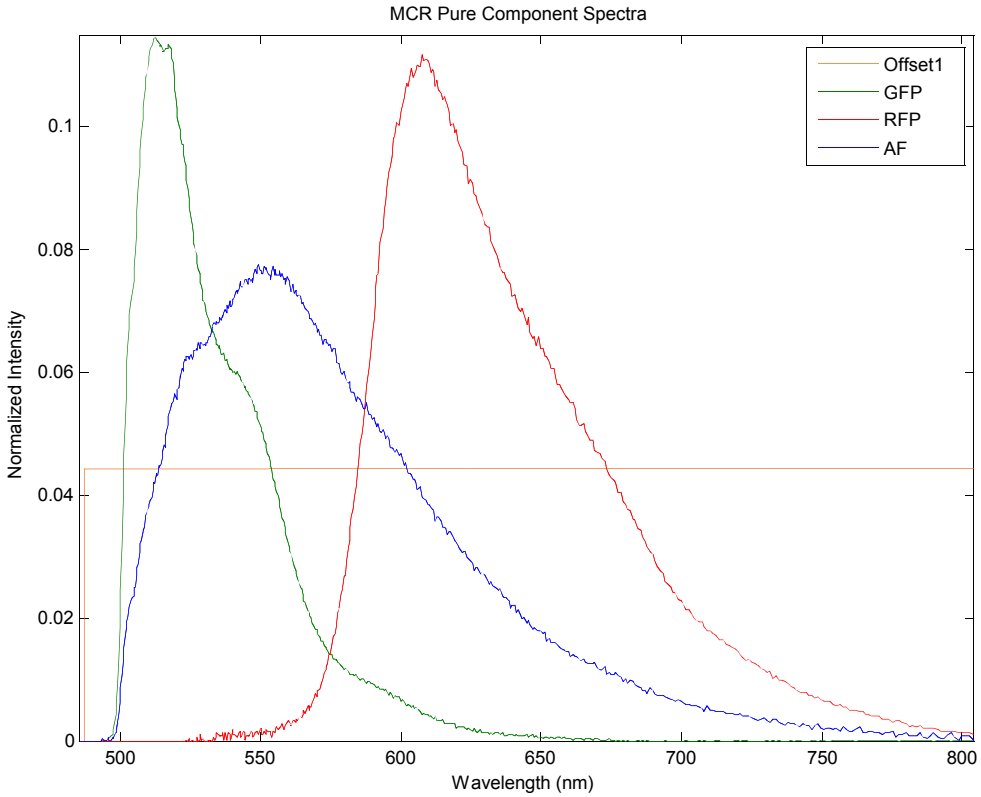
MCR Spectral Model



MCR Generated Independent Abundances



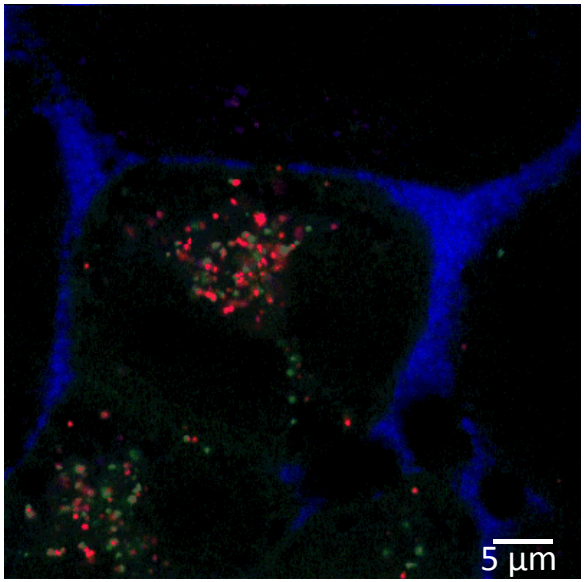
Component Spectra

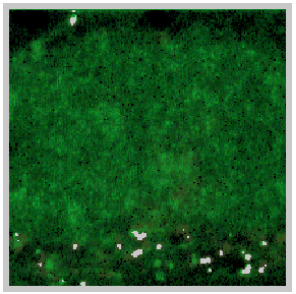


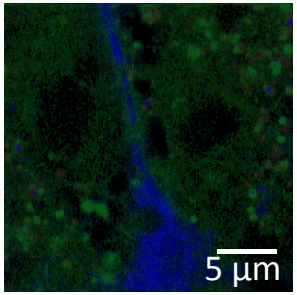
* Slightly incomplete separation of GFP and autofluorescence as evidenced by shoulder on AF peak around 525 nm corresponding to GFP. This is due to the fact that GFP is expressed at low levels throughput the sample. This could be resolved by imaging the parent cell line w/o the FPs to get a pure AF spectrum.

Representative Results

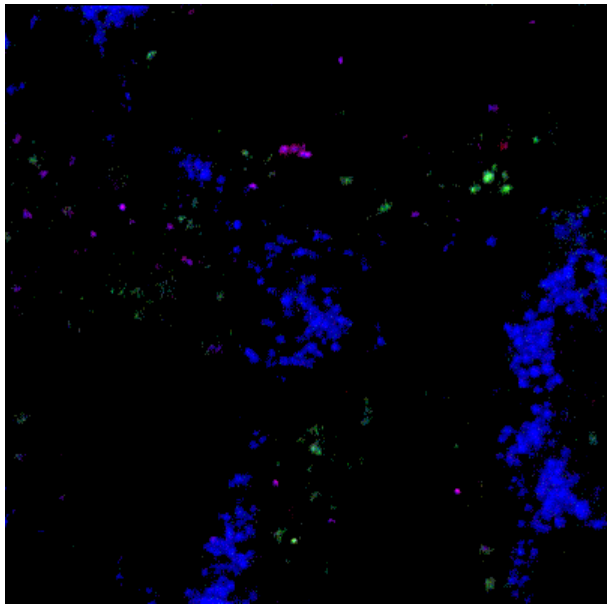
DMSO

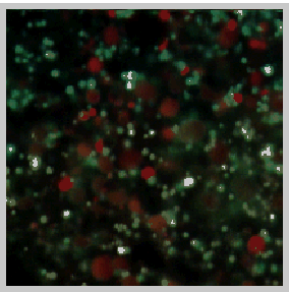






30 uM PP242





** RGB color intensities are scaled independently for improving viewing contrast. Colors are according to spectral plot on previous page. AF= blue, GFP = green, RFP = red.

Conclusions/Summary Thoughts

- Fluorescent protein expression levels are sufficient for hyperspectral imaging
- Autofluorescence can be an issue in this cell system. It probably affects the green and red channels about equally in filter base measurements (evidenced by similar integrated areas under the spectral emission)
  - Predominantly in cell junction areas (extracellular matrix?)
  - Some overlap with late endosomes
- If a third dye were to be used the spectral region of 540 -590 is most promising
- Photobleaching is evident in optical sections