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Analysis of Live Retina Samples

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Imaging: Mike Sinclair (8-14-14)

Analysis: Jeri Timlin (9-11-14)

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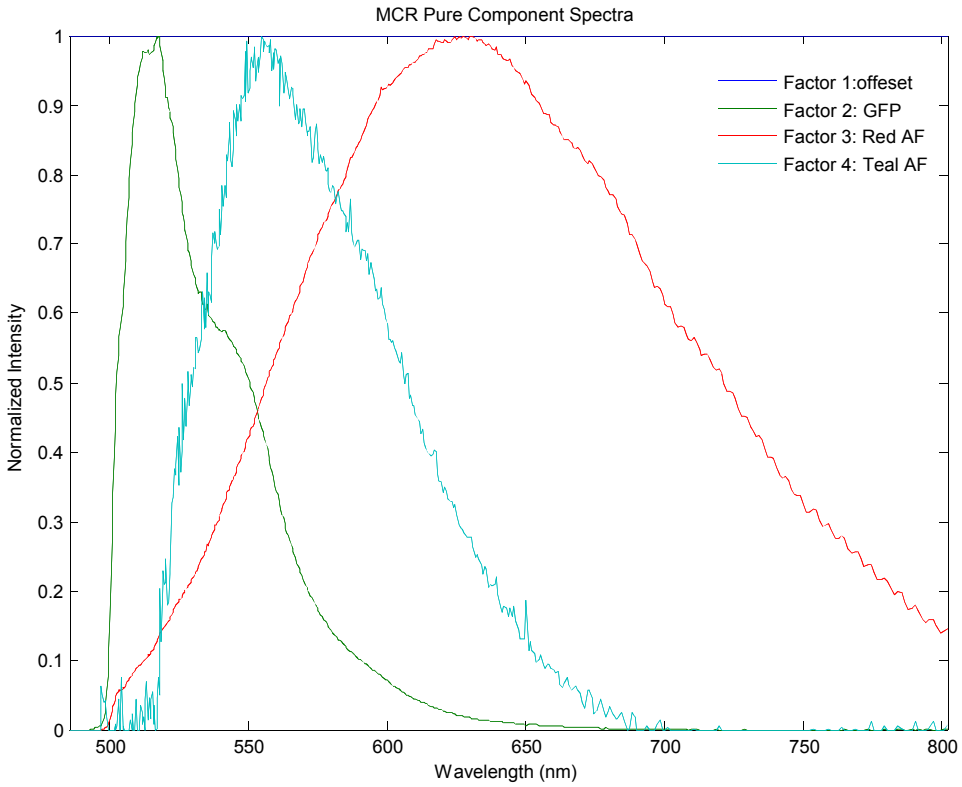
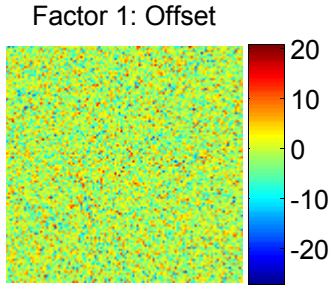
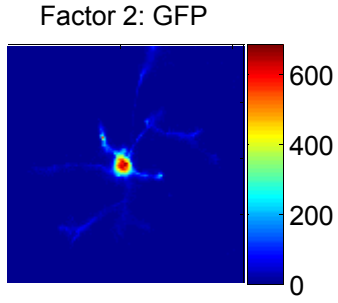
Imaging/Analysis Details

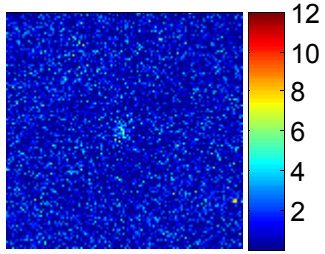
- 488 nm excitation, OD 1 used for GFP expressing samples
- 20x objective, 100 x 100 um field of view
- Spatial resolution: approximately 500nm in X&Y and 1.0 um in Z
- Analysis: MCR analysis (ImageMCR) was run on a composite set of all the images taken (compressed 4x) to identify pure components. Model was clean and easy to obtain. Then CLS was performed on each individual image at full spatial resolution to obtain final concentrations of each component.

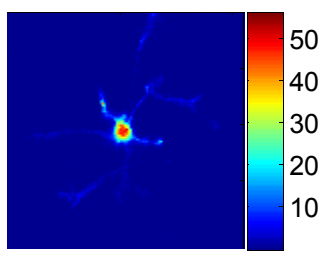
MCR Analysis









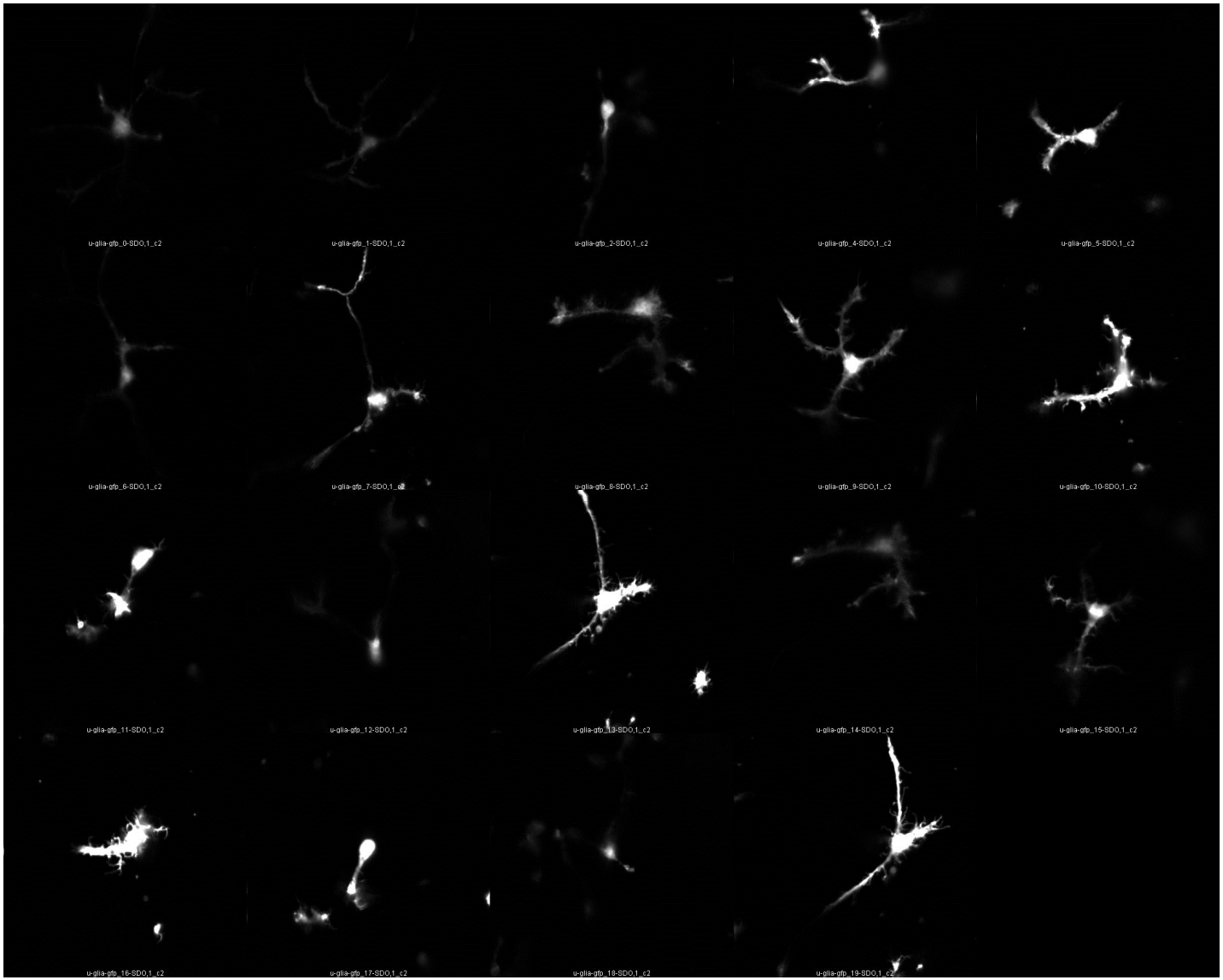


Observation: Factor 3 is the same as the red spots from the previous fixed tissue images.

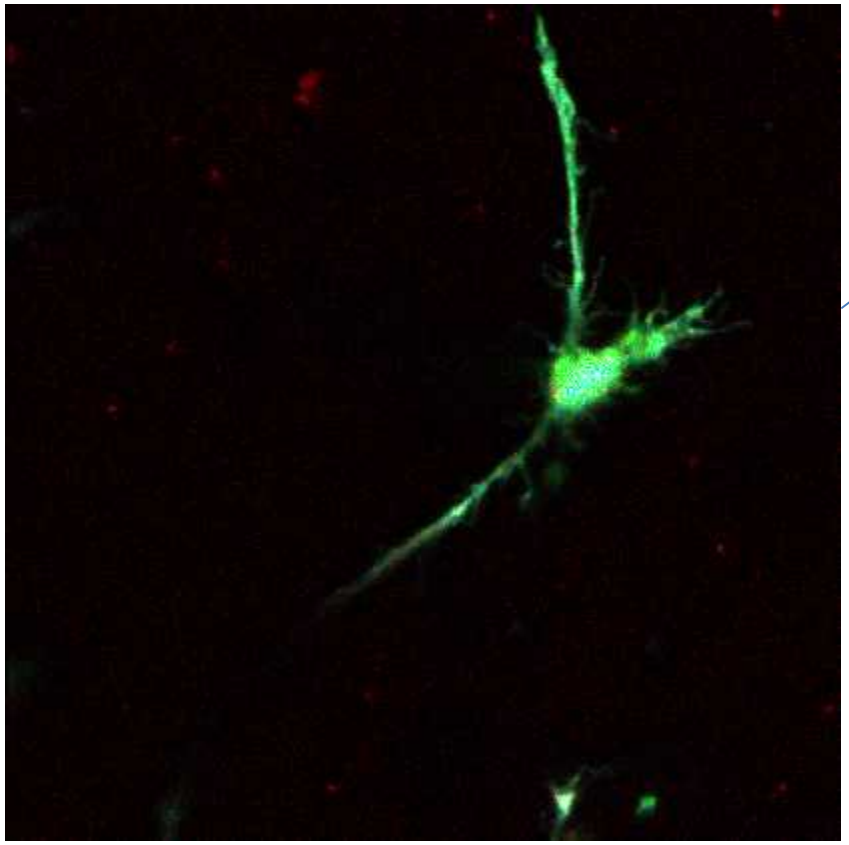
Observation: Factor 4 is a lot noisier than previous data and a bit sharper on the left (blue)edge. It's spatial distribution changes too; its present in the bright GFP regions. This is likely a different component and possibly just a shifted shoulder of the GFP

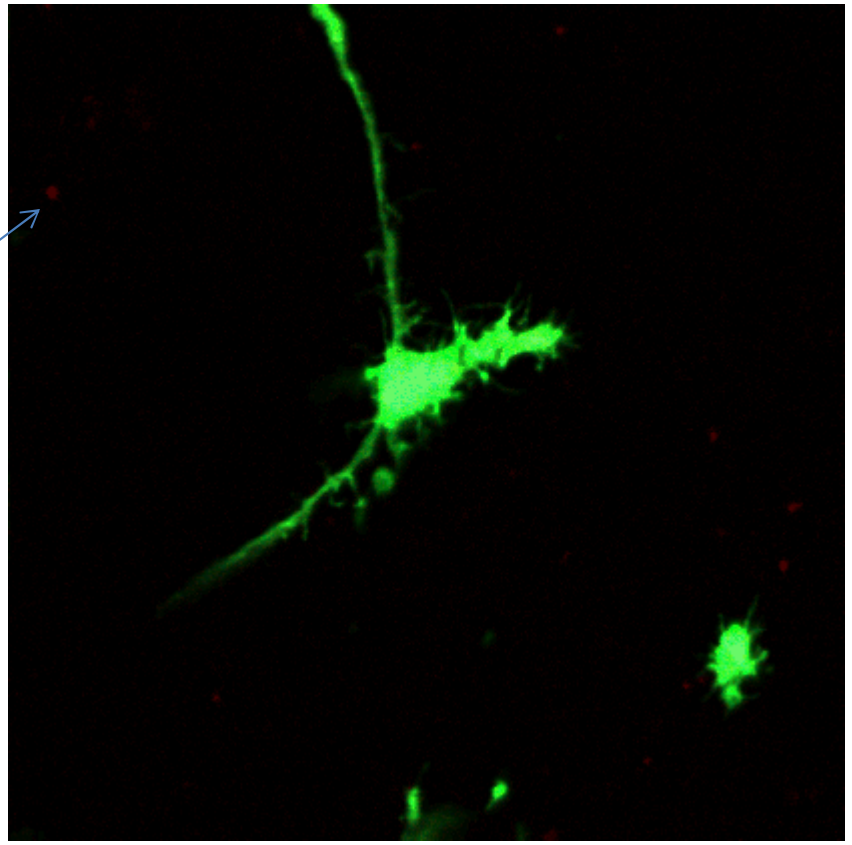
Montage of All images

GFP Component Only



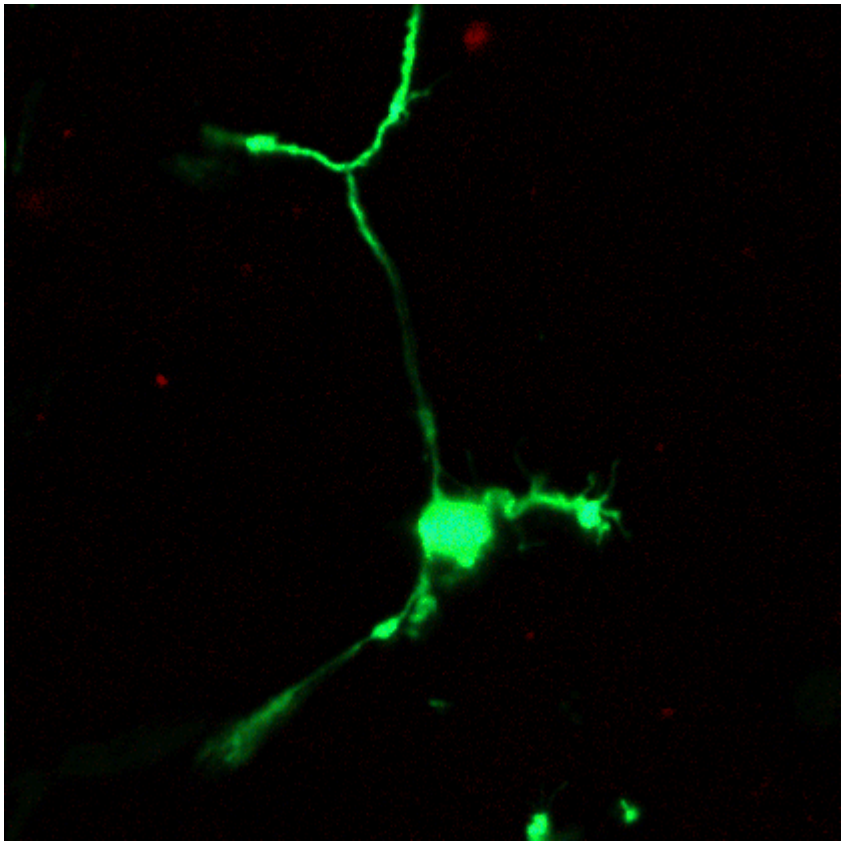
Some Color Overlays

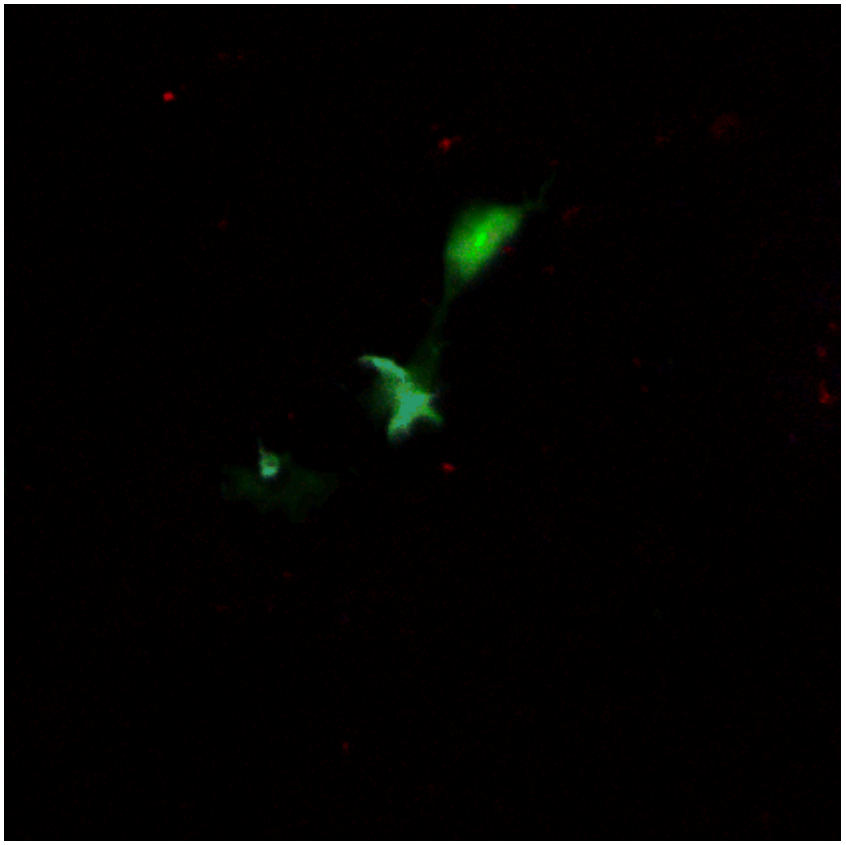




Spots are about 1-2 microns in diameter, therefore most likely not macrophage cells, but they could be an organelle inside the cells.

Some Overlays





Thoughts

- There's no utility in counting the red AF spots because these are control images only, nothing to compare to. However the spots numbers seem consistent with previous images.
- Images seem reasonable and on par with fixed cell images. GFP is a bit brighter in the living cells than in the fixed cells.
- Identity of spot pigment: see literature
  - *Invest. Ophthalmol. Vis. Sci. March 1995 vol. 36 no. 3 718-729.*
    - The authors show lipofuscin which has native pigment features ~620 -650 nm and is known to accumulate in certain parts of brain and opthalmic tissue. Lipofuscin is know to accumulate in dense regions in disease state
  - Biomed Opt Express. 2011 Jun 1;2(6):1494-503. doi: 10.1364/BOE.2.001494. Epub 2011 May 11. "Two-photon excited autofluorescence imaging of freshly isolated frog retinas."
    - The authors here show a nice depth section of the retina and there are spots visibel in two of the layers. They wave their hands around a definitive assignment, but mention Rhodopsin. This is unlikely in our excitation because we are exciting too far in the visible I believe.