SAND2013-5246P

Protoplast Imaging

6-10-2013
Protoplast prep: Maria
Confocal: Aaron
Raman Jeri
Analysis: Jeri/Aaron

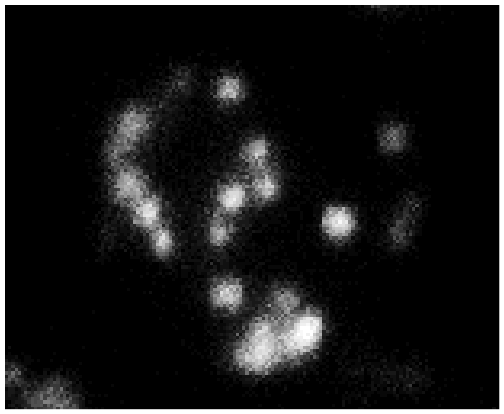
Details

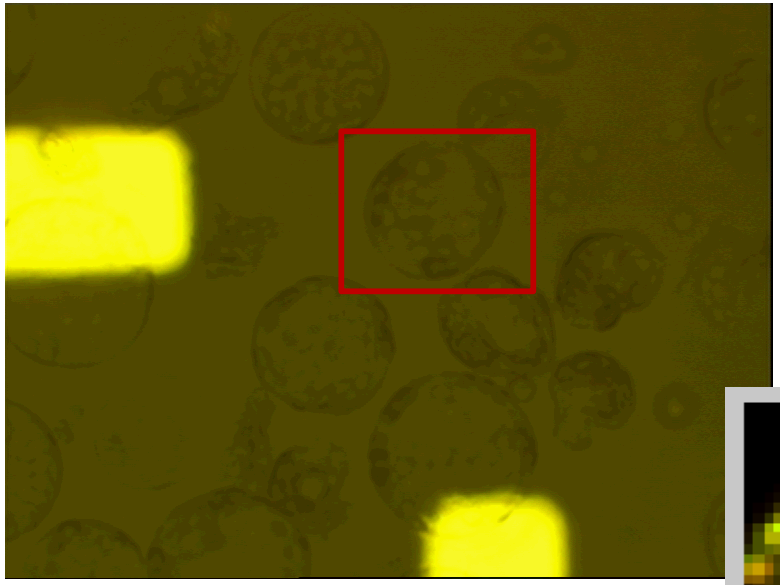
- Samples arrived at 1 pm on 6-10-2013
- Imaging begun immediately , using the Lovens Field Finder slide. Did some Raman, then did confocal on those same cells, then picked a cell using confocal and verified we could image same cell with Raman
- Confocal fluorescence was done with 40x (NA 0.6) objective on Olympus spinning disk microscope
  - Cy5 filter cube (EX=620/60 EM=700/75)
  - 0.1 s integration time, 0.5 um steps in Z to ensure same optical section obtain as in Raman
- Hyperspectral confocal Raman was done with 50x (NA 0.55) objective on Witec Raman microscope
  - 532 nm excitation, 1 mW
  - 25 um FOV images, 0.6 um step size, integration time 0.15 sec

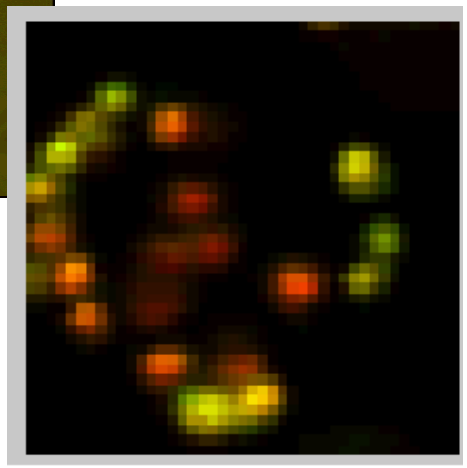
Goals

1. Ensure same cell could be captured by Raman and fluorescence, work out those procedures
2. Determine if we would have sufficient spatial resolution for seeing the spindles under fluorescence
3. Decide on appropriate # of images that could reasonably be acquired in the larger experiment
4. Understand potential heterogeneity in the carotenoid abundances within control protoplasts because this sets our ability to detect changes from the control.

1. Same Cell can be Captured by Fluorescence and Raman

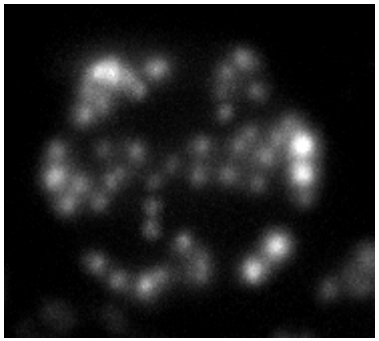


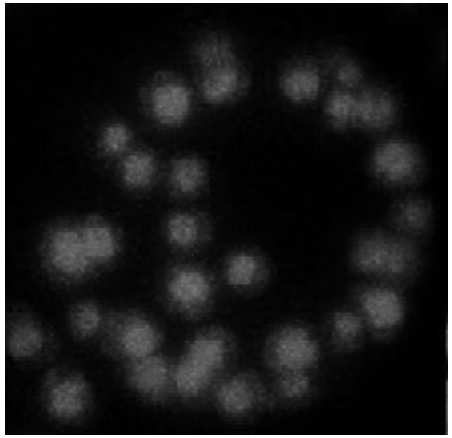




2. Do we have sufficient spatial resolution in fluorescence for seeing the spindles?

- No, likely not as we conducted the experiment with the 40x, NA 0.55. We need a higher NA objective on the fluorescence microscope.
- This is challenging because we don't want to go with an oil objective because of the need to then wipe oil off prior to Raman.
- Found a high NA dry objective (150x NA 0.9) that we could borrow, need to verify it can couple to our microscope (different manufacturer), purchased an adapter, waiting delivery



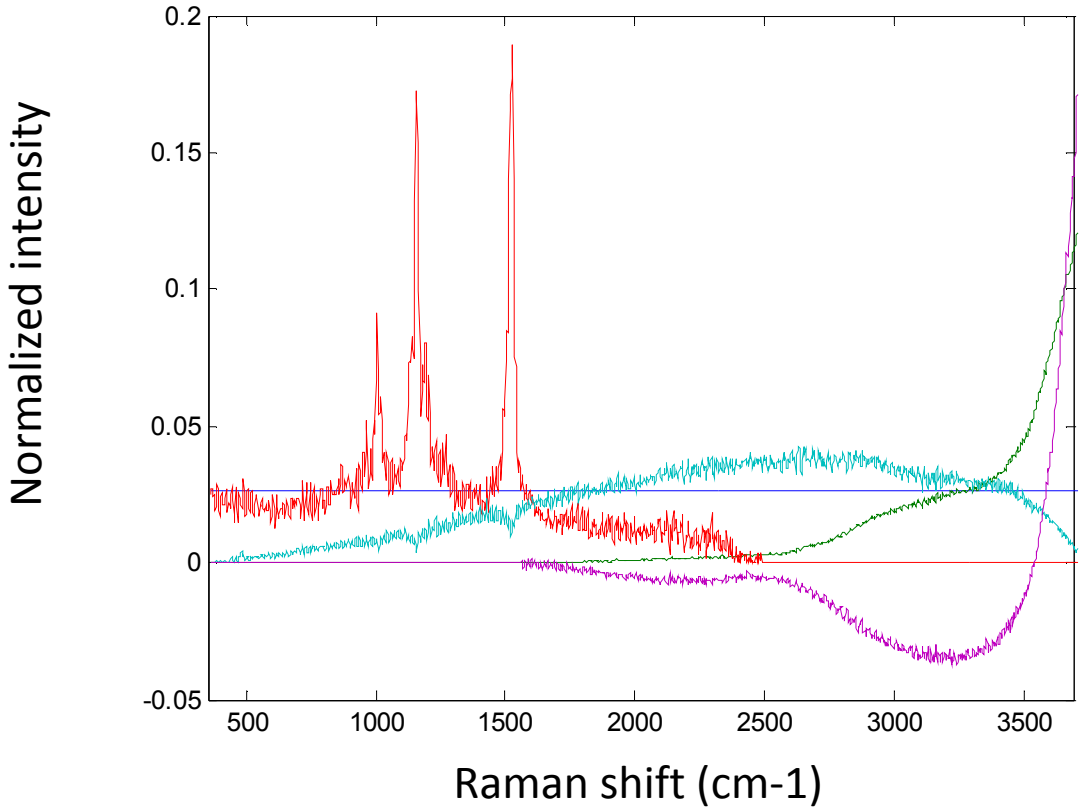


Chl channel from Maria's manuscript, colored gray.

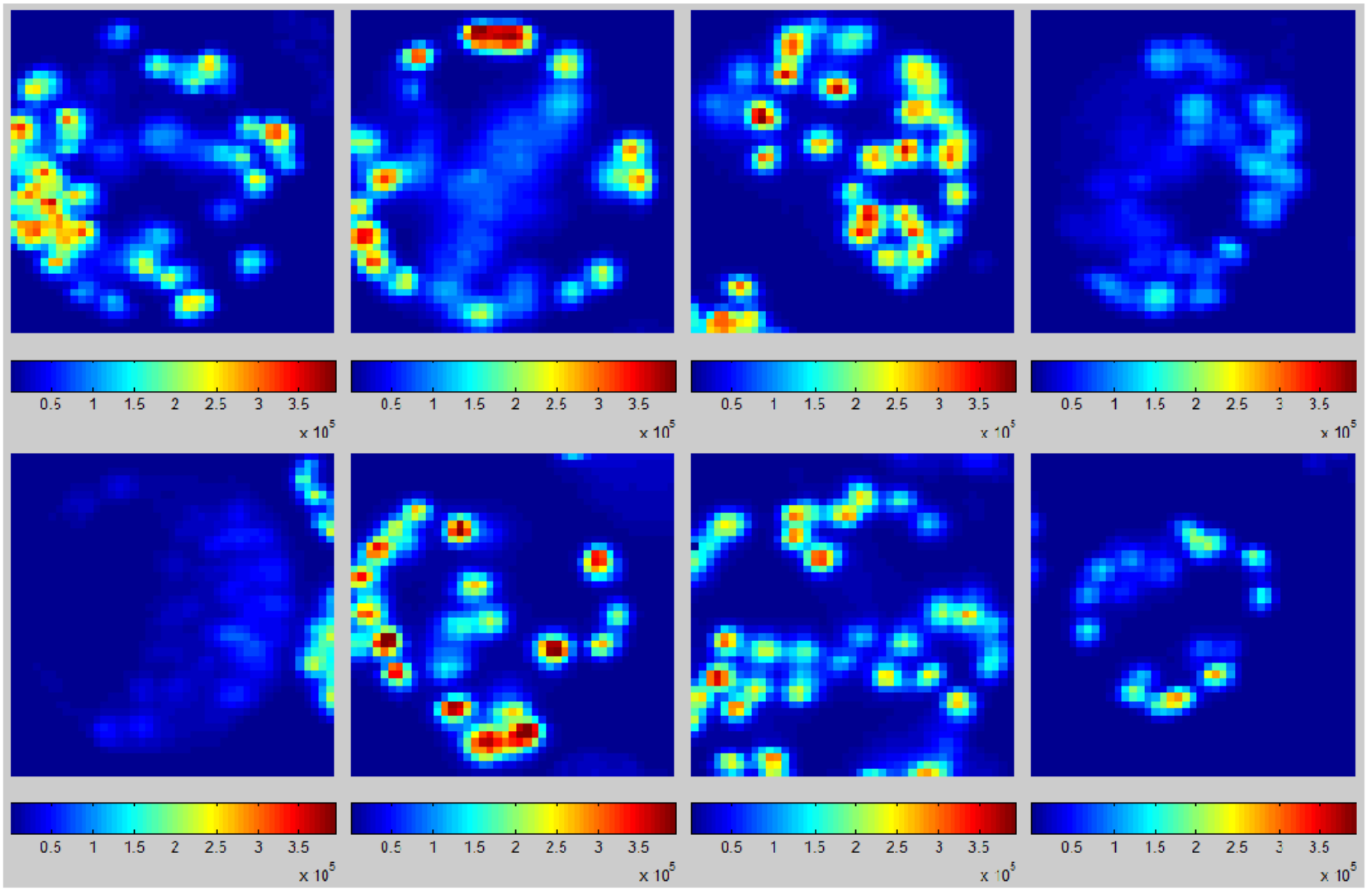
3. How many images can reasonably be acquired in the larger experiment?

- Raman scans themselves are about 5-6 min/image, however it's not trivial to find the cells.
- Given that my estimate is that we can find and scan a specific cell we have picked out form fluorescence at rate of about 1 cell every 12-15 minutes. That's 4 cells and hour.
- Even if we start as soon as receiving the samples 11-1 pm and work into the evening, this is not a high number of cells that can be imaged.
- Is this sufficient?

Spectral Model

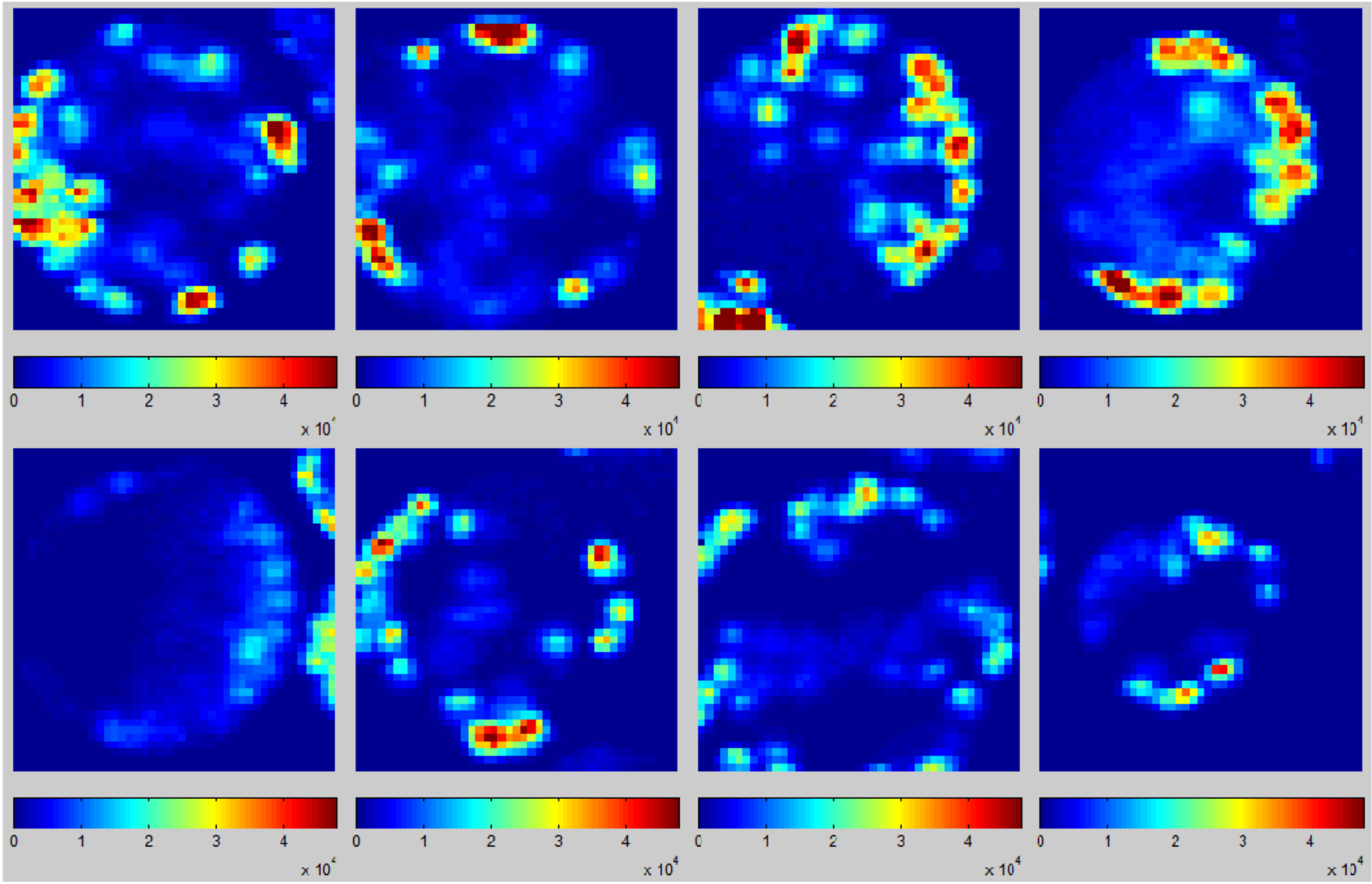


Chlorophyll Localization



Images are 25 um x 25 um

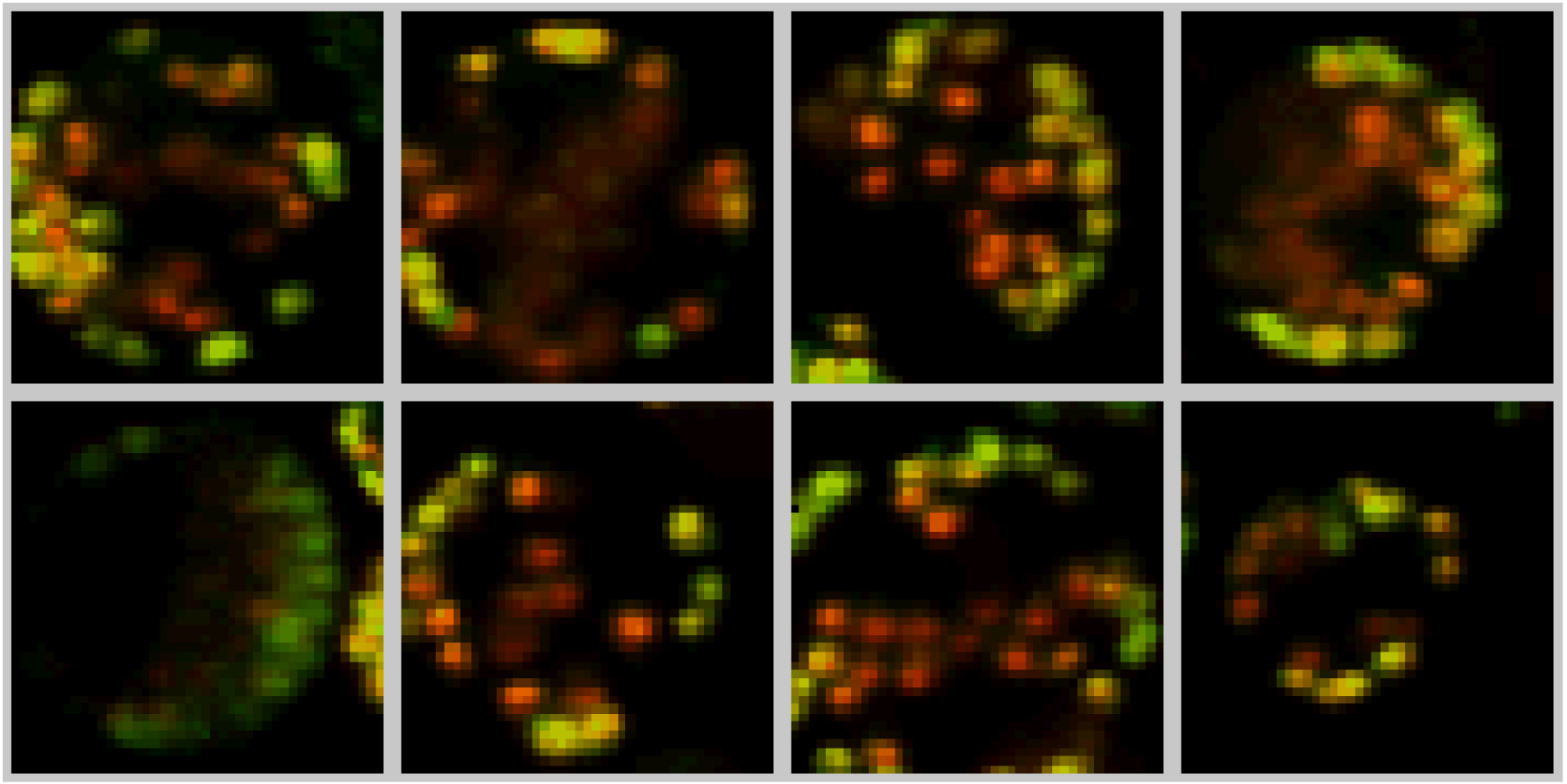
Carotenoid Localization



Images are 25 um x 25 um

Color Overlays

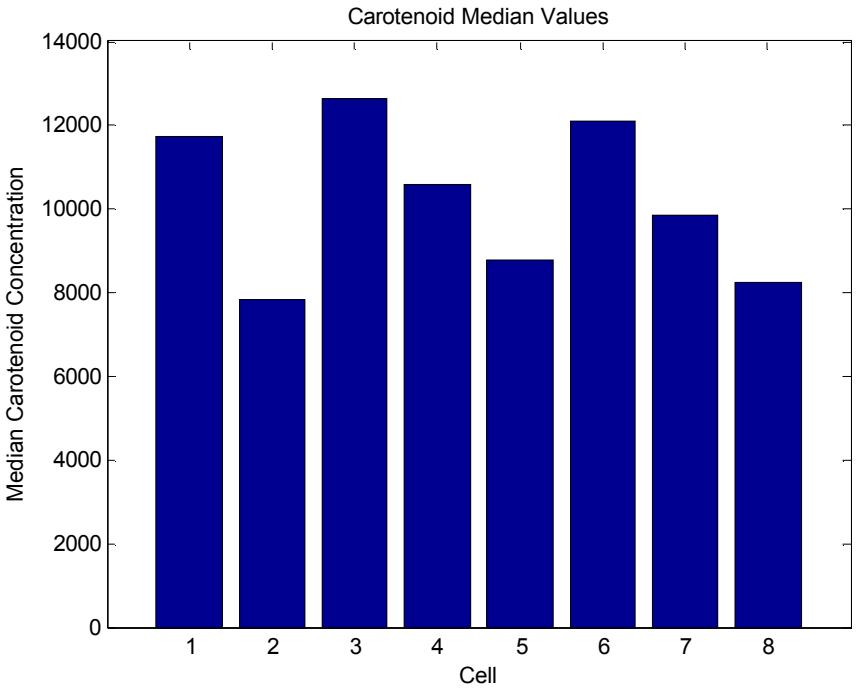
Reveal within Cell Heterogeneity

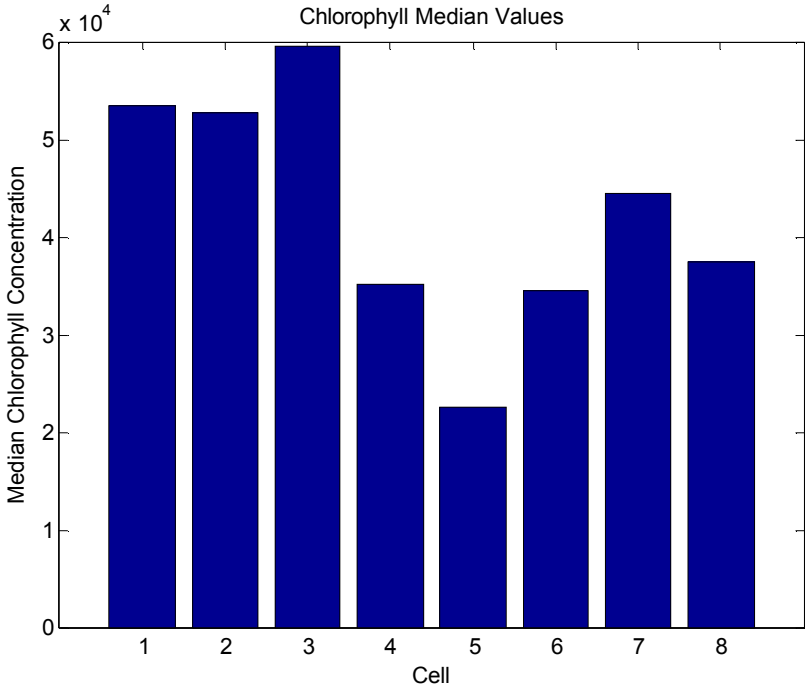


Green = Carotenoid and Red = Chlorophyll

Each color channel is scaled independently (min -> max)
Images are 25 um x 25 um

Single Cell Heterogeneity





Some Statistics

Cell	2	3	4	5	6	7	8	9		Mean	std
Chl_mean	78159.8317	70990.72	88589.82	44433.46	34790.22	79645.63	69385.65	53534.15		64941.19	18770.53
Chl_med	53347.0915	52719.41	59561.73	35162.33	22505.79	34471.3	44545.61	37496.33		42476.2	12308.45
Chl_std	73097.1168	69651.98	83345.79	32316.72	35599.48	90696.01	68249.47	50432.42			
Car_mean	15292.0713	12708.27	17136.91	15435.06	10597.5	15364.4	11659.95	11585.08		13722.41	2371.479
Car_med	11728.0801	7829.361	12641.75	10598.27	8795.966	12097.95	9854.757	8229.493		10221.95	1837.992
Car_std	10443.4743	11049.24	11740.35	10929.92	5554.595	9790.746	6039.776	7323.04			

** These rows represent the within cell standard deviation. Expect this to be high because even with a tight mask there are pixels that represent the entire diffraction limited resolution.

These are the cell-to-cell numbers and represent the level of variance within the control population.

Heterogeneity

- Significant cell-to-cell heterogeneity was observed
- This sets the baseline for what we can determine as statistically significant.
- Is this in the ball park of the expectations?
- Any idea why the within-cell heterogeneity in carotenoid abundance? (Edges greener than centers)
  - We are checking into whether this is an optical artifact from increased chromatic aberration when imaging deep from the designed focal plane of that objective.