

Exceptional service in the national interest



Hyperspectral Confocal Fluorescence Microscopy of *H. Hongdecholoris*

Imaging conducted on 10/01/14

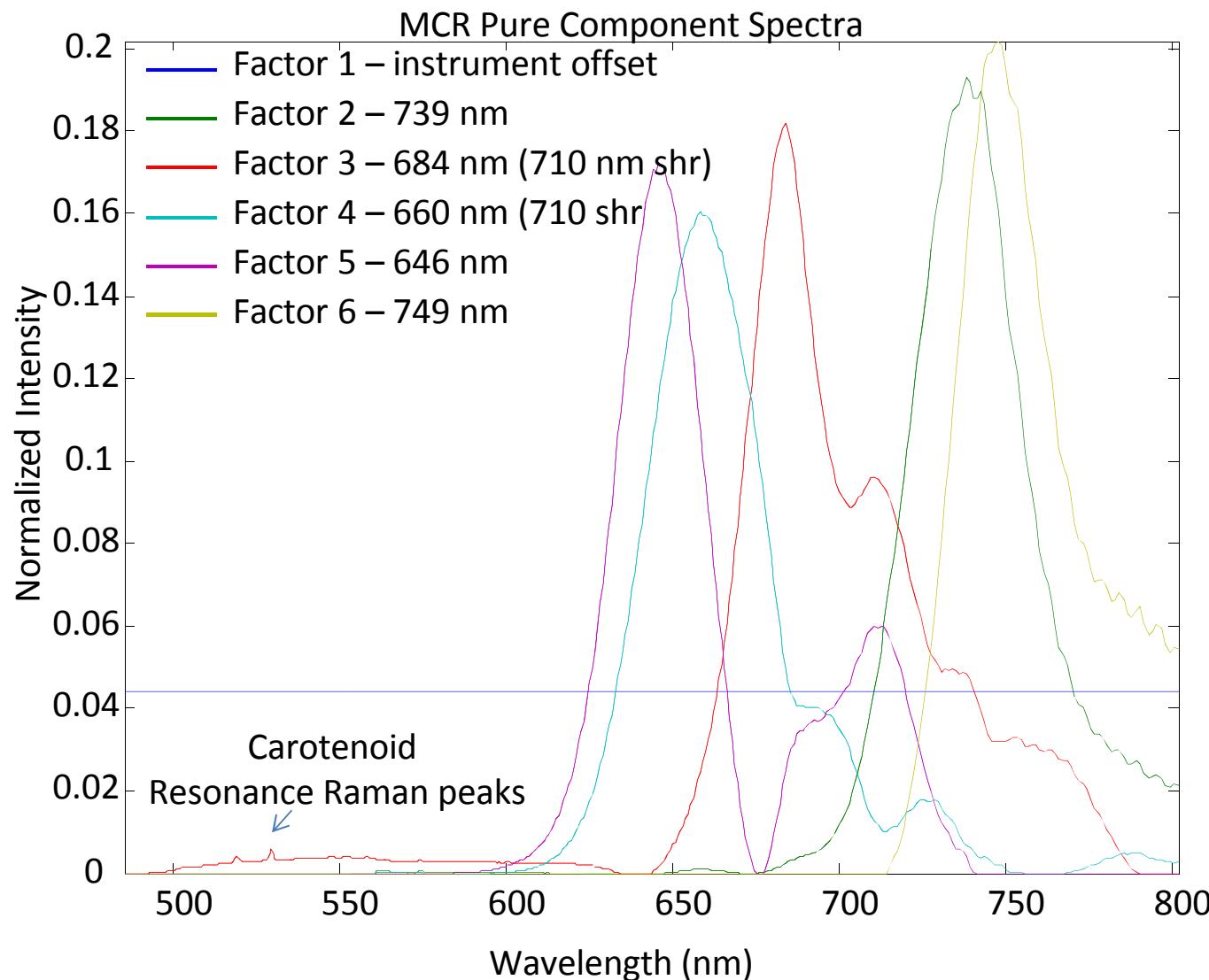


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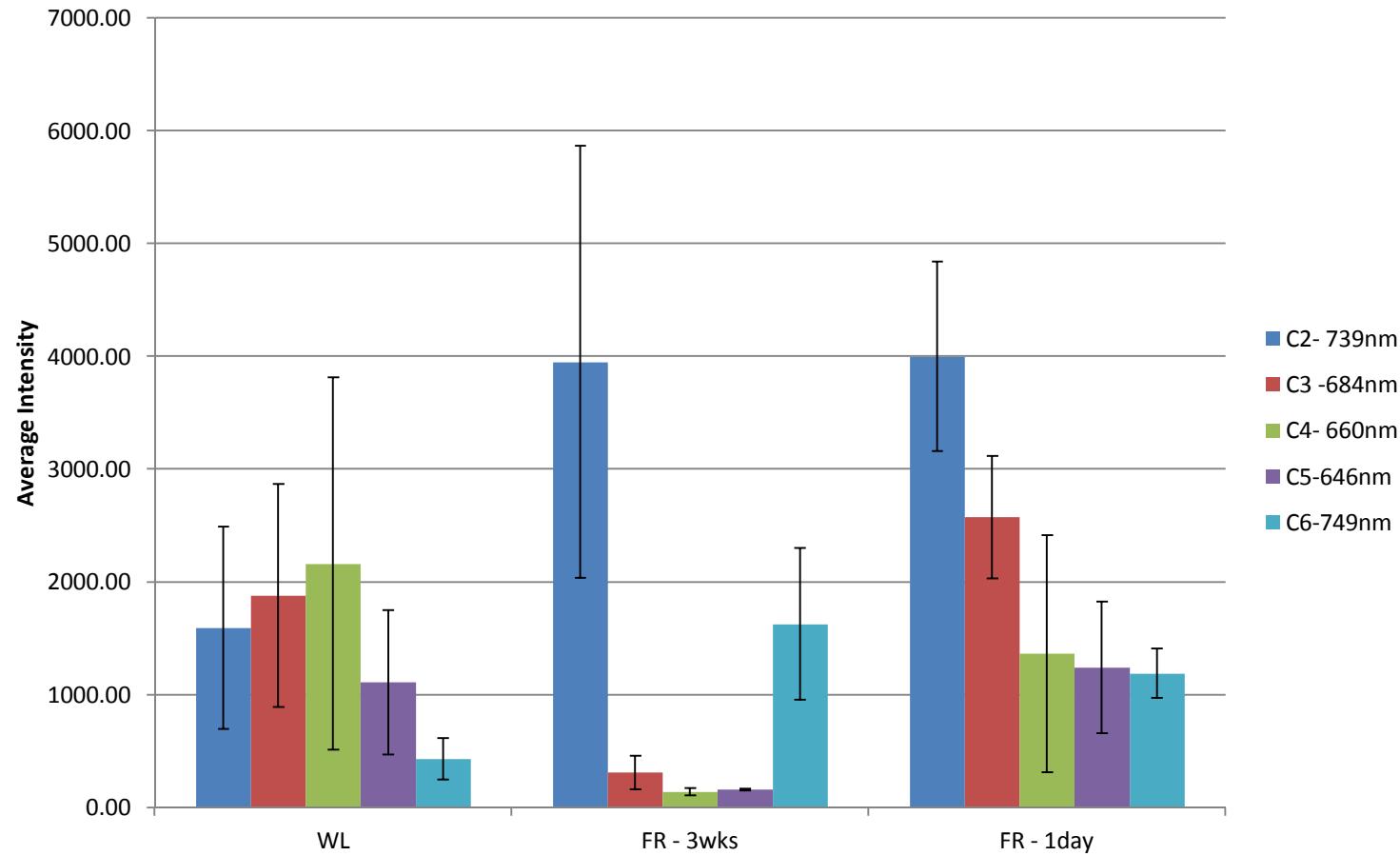
Imaging/Analysis Details

- Samples arrived on 10-1 and were imaged immediately. Samples were kept in the dark until imaged.
- 488 nm excitation; ~20 μ W, 4096 spectra/sec
- All resulting image files were put into a composite data set and pure spectra were identified using multivariate curve resolution that described this composite data set
 - Looked at full spectral region
 - Looked only at carotenoid region
- Let data define the factors isolated, no library spectra were used to seed the analysis
- Note: our imager does use a brief exposure to green light during focusing of sample.
- Order in the image file –
 - Images 1-19: white light
 - Images 20-34: FR 3 weeks
 - Images 35-46: FR 1 day

MCR Isolated Pure Spectra



Relative Intensities of Components



Mean values were determined using a mask (per component) that thresholds the minimum based on full image histogram. Error bars are just $=/-$ the standard deviation of the mean values (may not be the best, but it was quick).

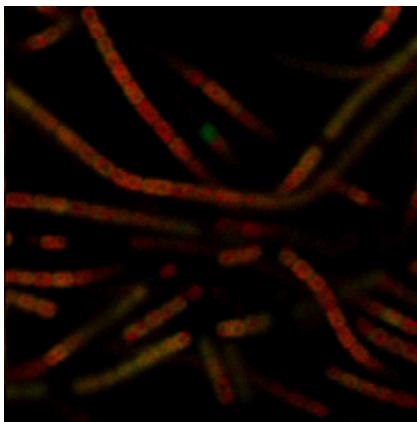
Images Comparing Component 2 & 6

Red – Chl F 739 nm (Comp 2)

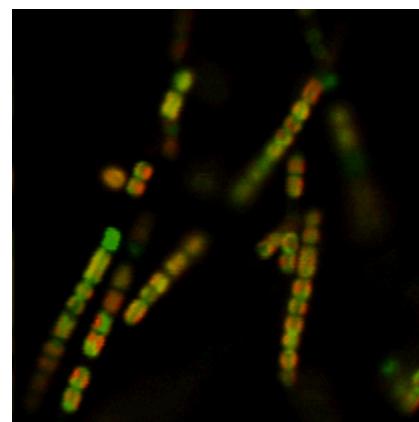
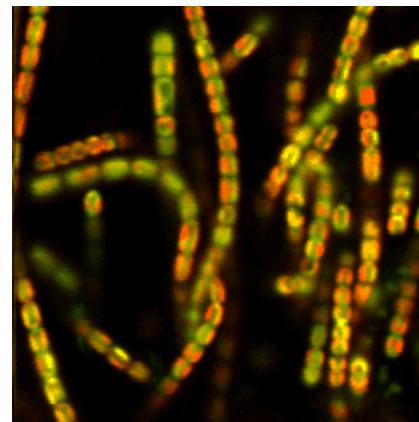
All images are shown on same color scale

Green – Chl F 749 nm (Comp 6)

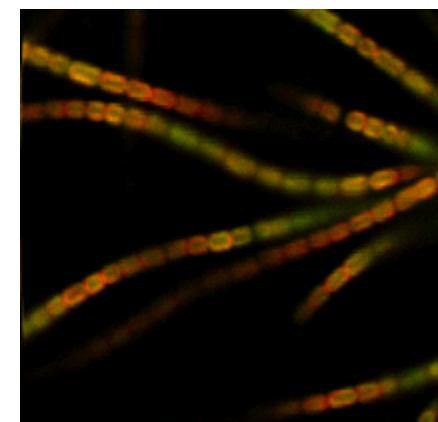
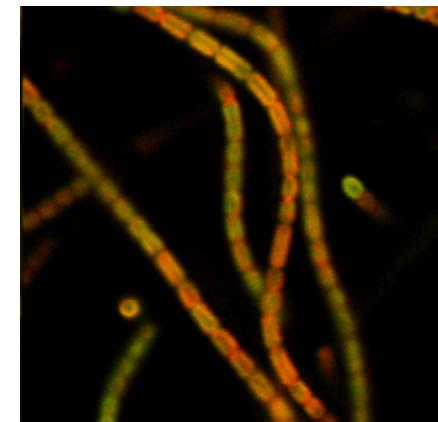
White Light



FR (3 weeks)



FR (1 day)



- The 739 nm peak is present in all samples, but to a lower extent in the white light grown. Localization looks similar.
- The 749 nm peak is present in all samples, but it is much lower in the white light grown and isolated to a few cells/spots.

Images Comparing PBS Components

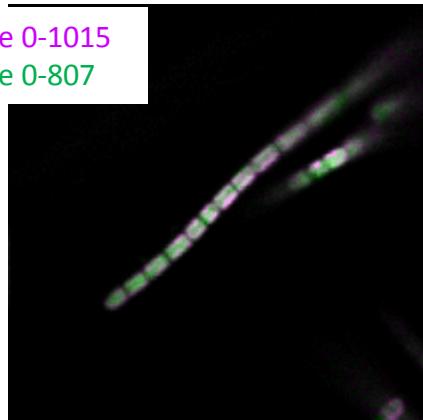
Magenta – PBS 646 nm (Comp 5)

Images are **NOT** shown on same color scale

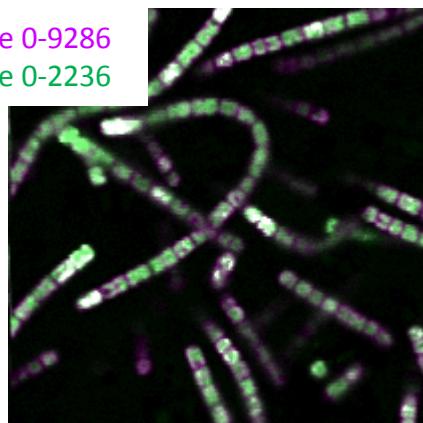
Green – PBS 660 nm (Comp 4)

White Light

Scale 0-1015
Scale 0-807

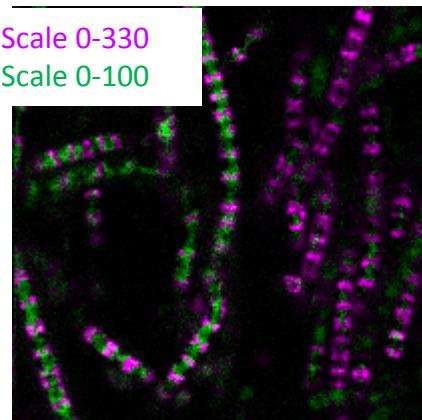


Scale 0-9286
Scale 0-2236

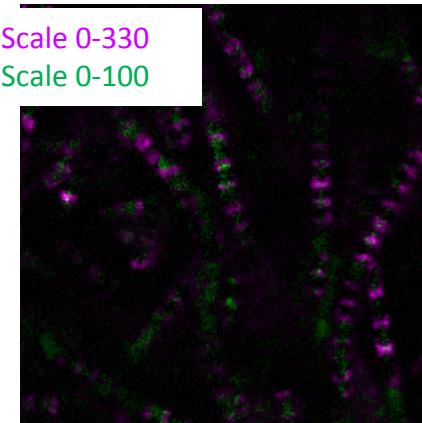


FR (3 weeks)

Scale 0-330
Scale 0-100

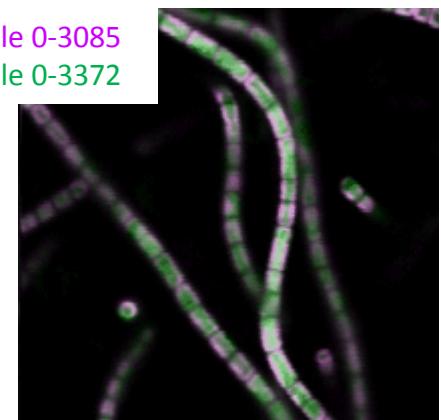


Scale 0-330
Scale 0-100

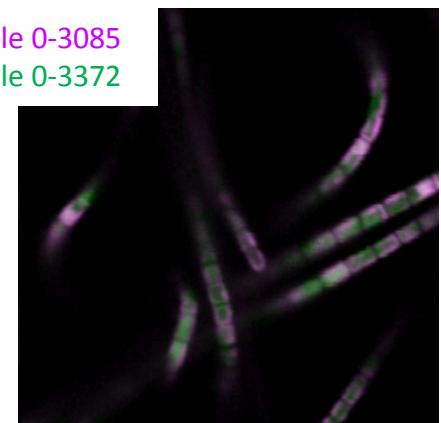


FR (1 day)

Scale 0-3085
Scale 0-3372



Scale 0-3085
Scale 0-3372



- The PBS 660 nm component is pretty much absent in the FR 3 weeks sample, but not in the FR 1 day.
- The PBS 646 nm component is much lower in the FR 3 weeks sample and concentrated to the area between cells. It is localized in that area in white light and FR1 day samples, but not as concentrated.

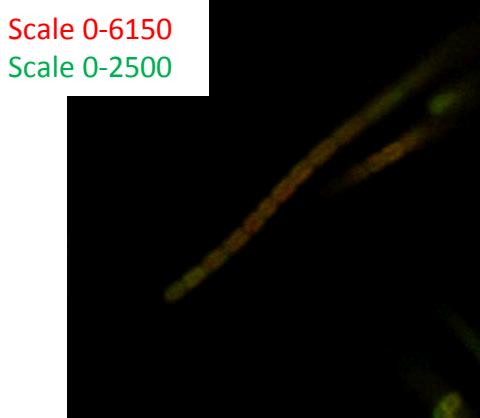
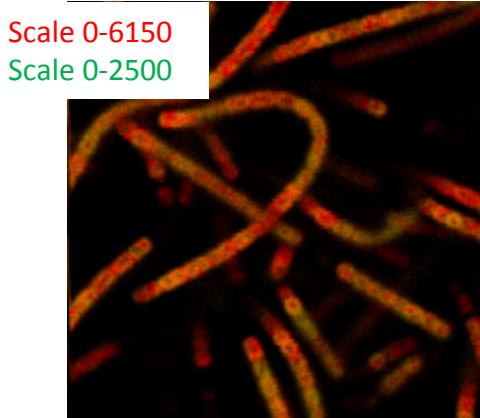
Images Comparing Component 3 & 6

Red – Chl A 684 nm (Comp 3)

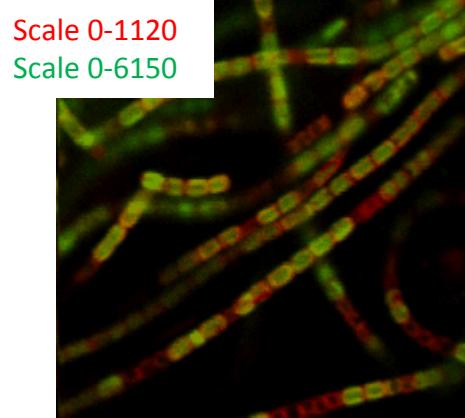
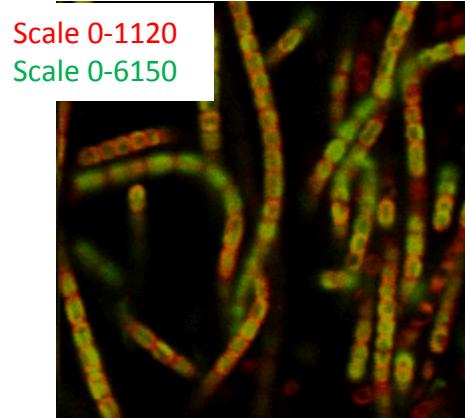
Green – Chl F 749 nm (Comp 6)

Images are **NOT** shown on same color scale

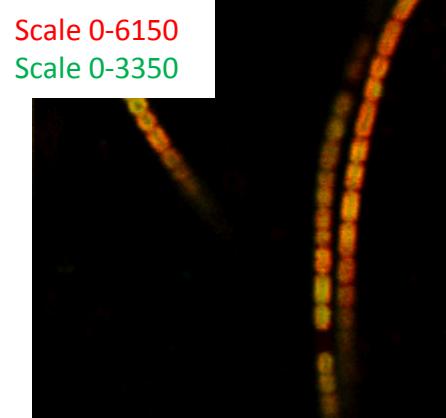
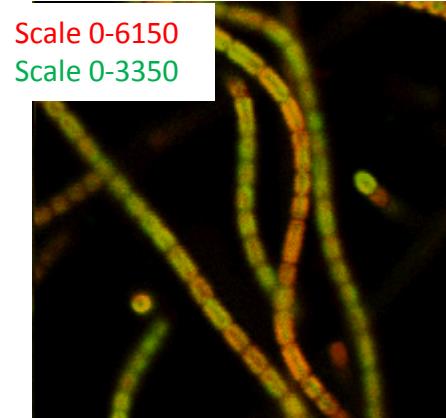
White Light



FR (3 weeks)

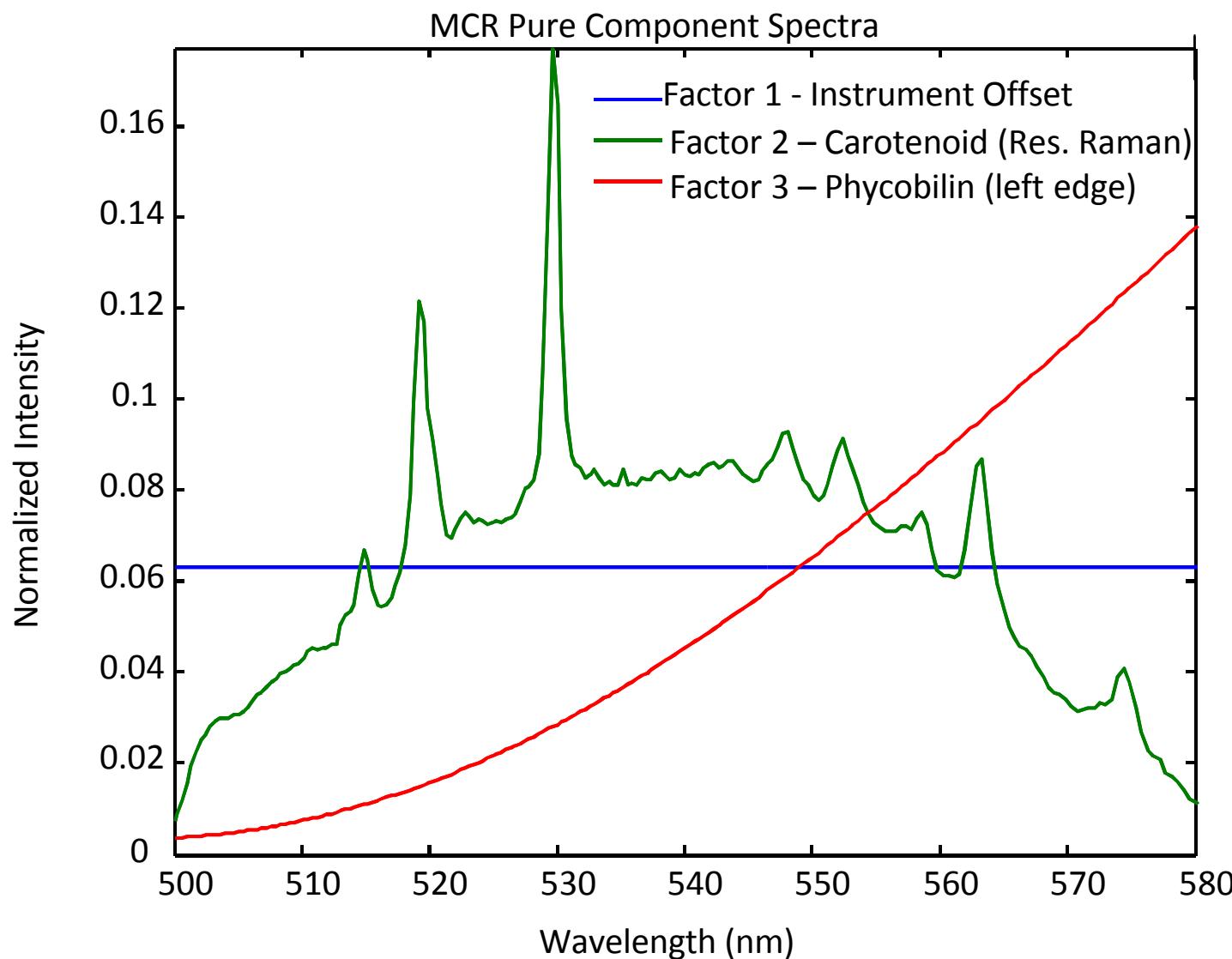


FR (1 day)



- The white light images are pretty variable with the isolated filaments being much weaker in signal.
- Chl A decreases in both FR samples, dramatically in FR 3 weeks sample. Localization appears similar.
- Chl F localization does not mimic Chl A, instead is more in center of cell. Some cells are void of Chl F.

Analysis of Carotenoid Region



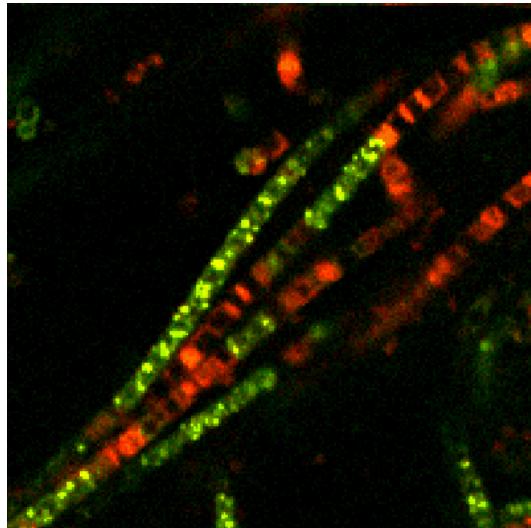
Carotenoid Distribution

Red – Phycobilisome (left edge)

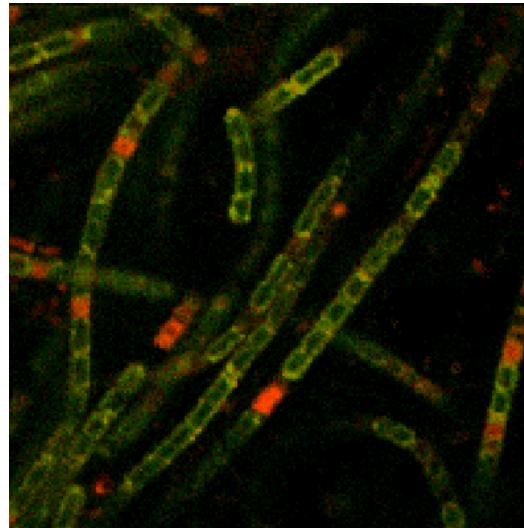
All images are shown on same color scale

Green - Carotenoid – Res. Raman

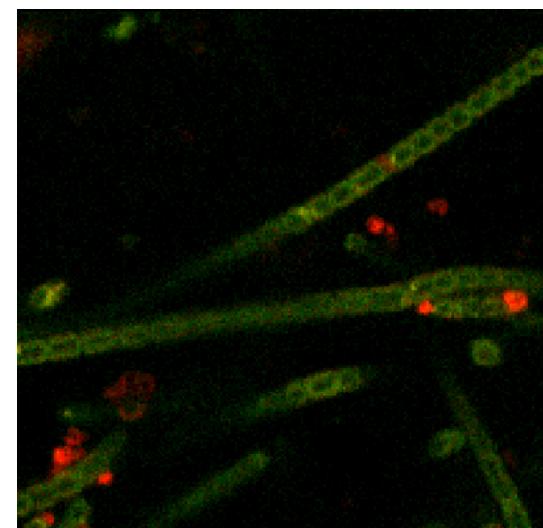
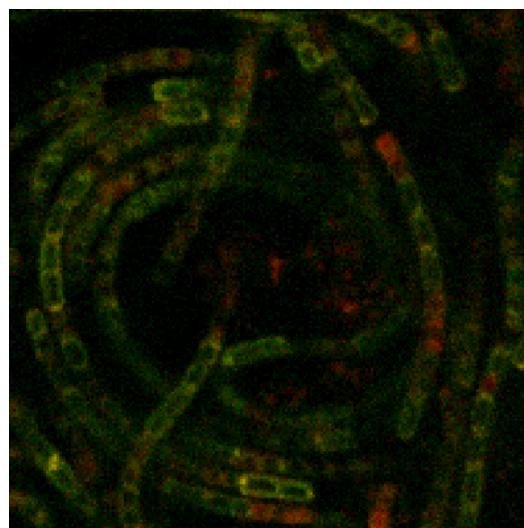
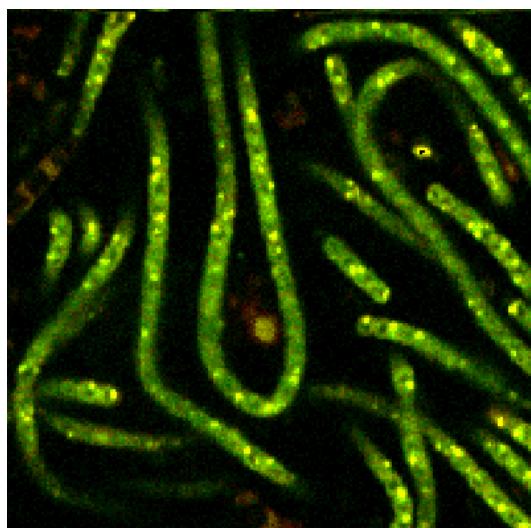
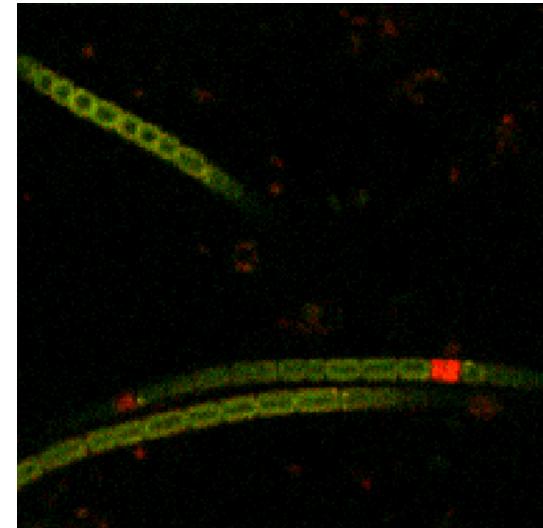
White Light



FR (3 weeks)



FR (1 day)



Random Observation

- Cells that were more isolated (sticking out from edge of microbial mat) seemed to have a lower overall intensity of every pigment.