

*Exceptional service in the national interest*



# Hyperspectral Confocal Fluorescence Microscopy of *H. Hongdechloris*

Imaging conducted on 10/01/14

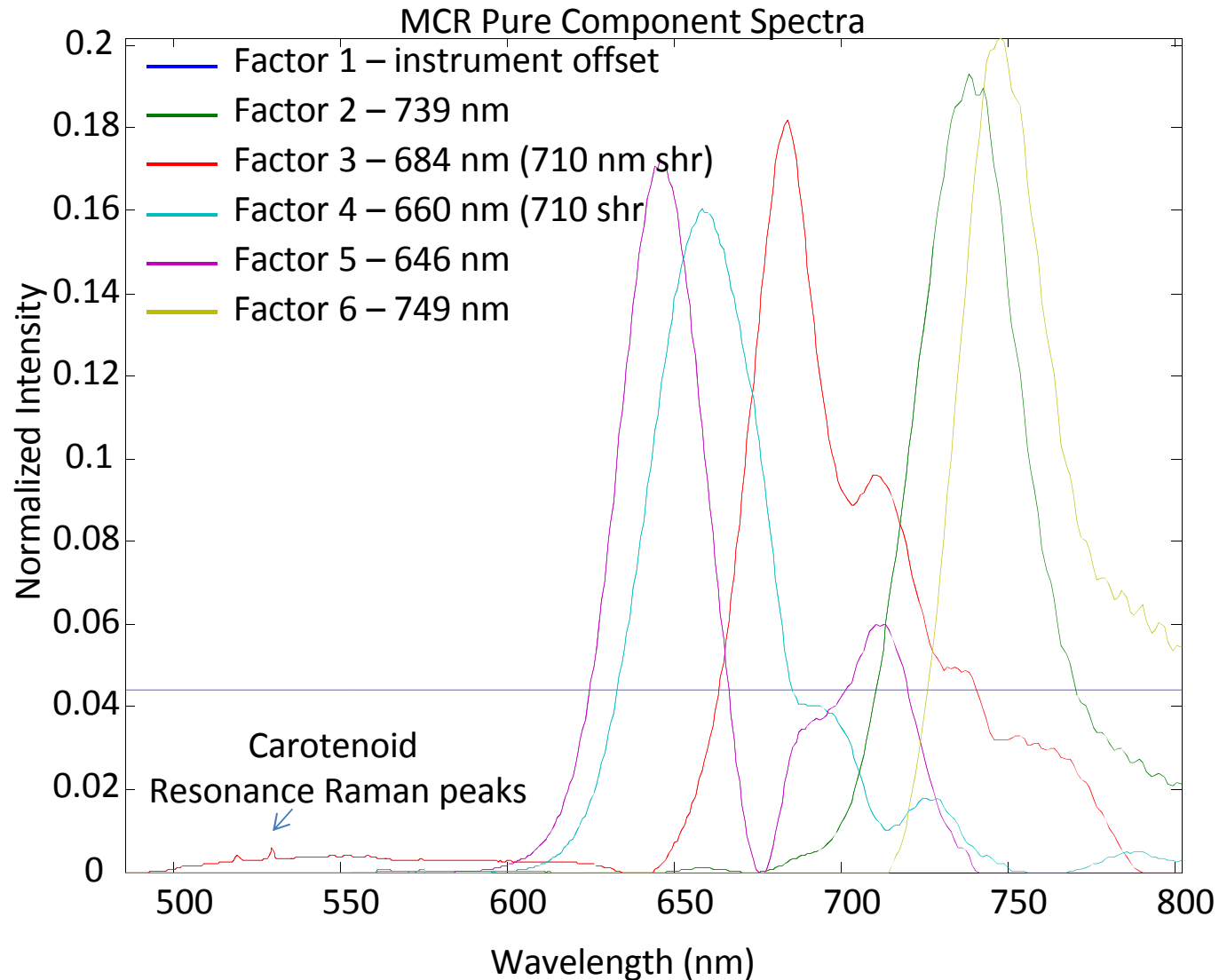


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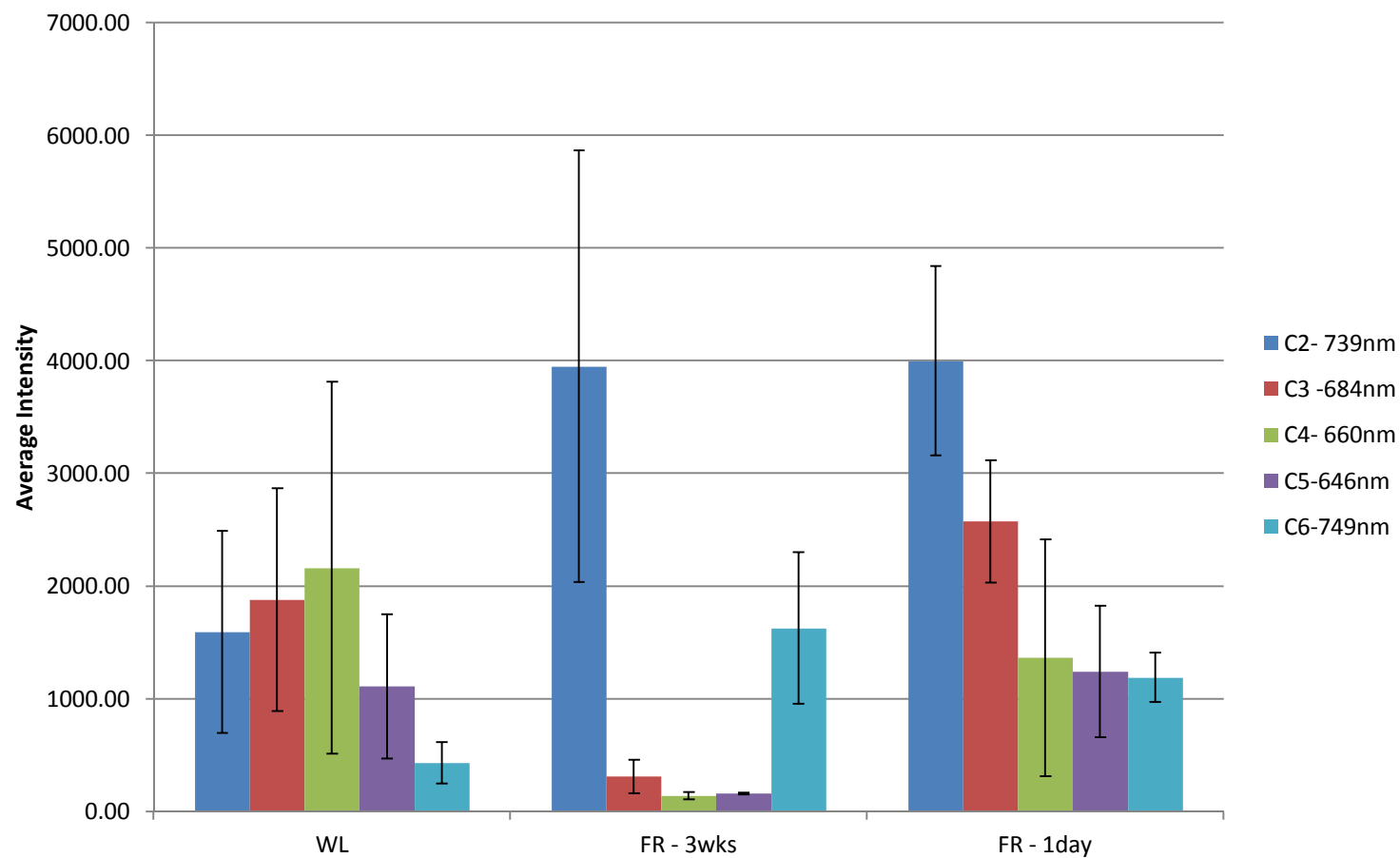
# Imaging/Analysis Details

- Samples arrived on 10-1 and were imaged immediately. Samples were kept in the dark until imaged.
- 488 nm excitation;  $\sim 20 \mu\text{W}$ , 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  $\pm$  the standard deviation of the mean values (may not be the best, but it was quick).

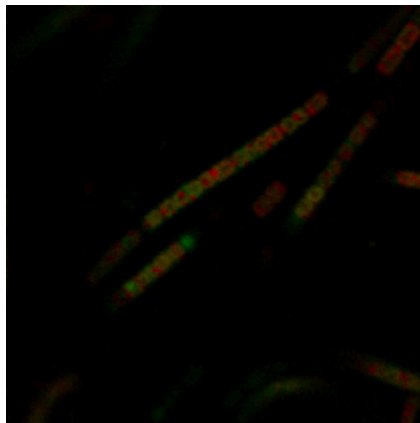
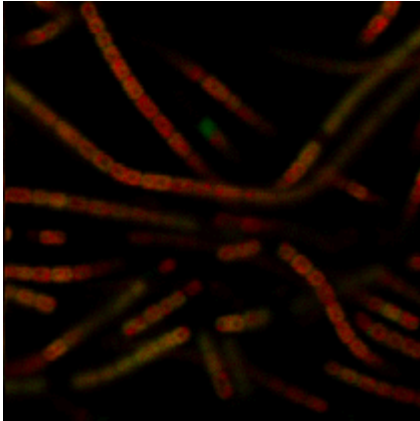
# Images Comparing Component 2 & 6

All images are shown on same color scale

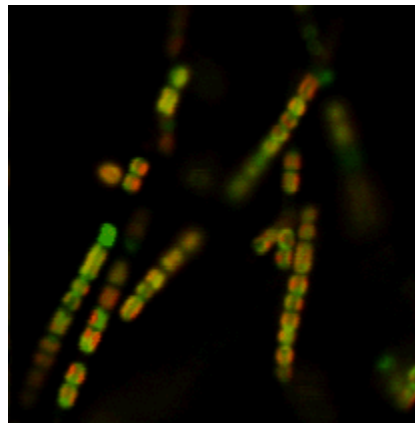
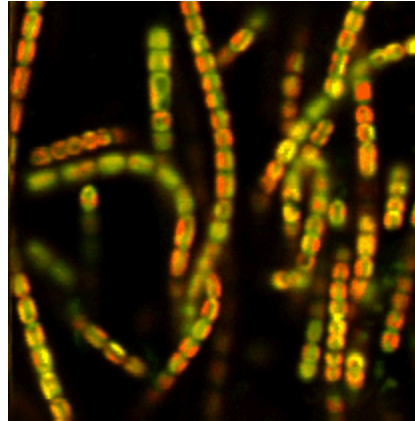
Red – Chl F 739 nm (Comp 2)

Green – Chl F 749 nm (Comp 6)

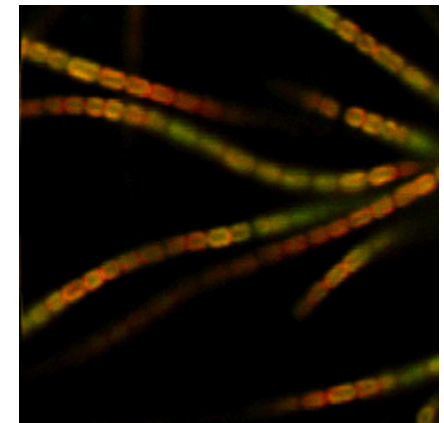
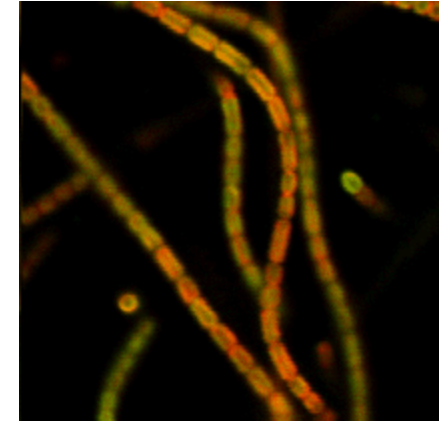
White Light



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.

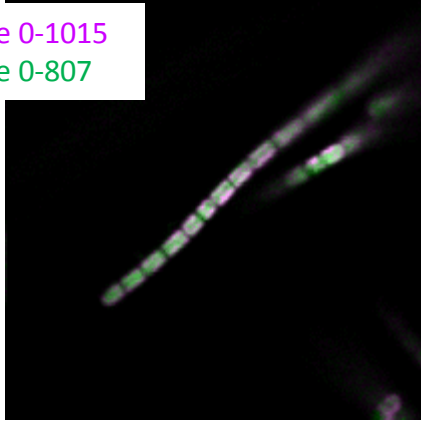
# Images Comparing PBS Components

**Magenta** – PBS 646 nm (Comp 5)    Images are **NOT** shown on same color scale

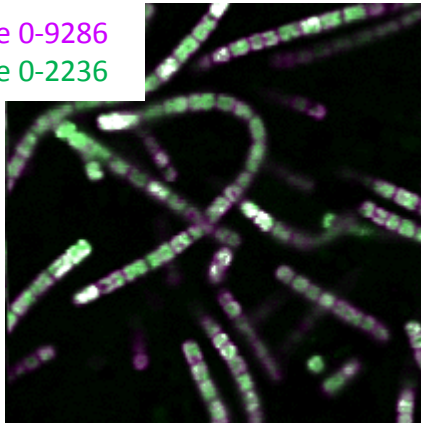
**Green** – PBS 660 nm (Comp 4)

White Light

Scale 0-1015  
Scale 0-807

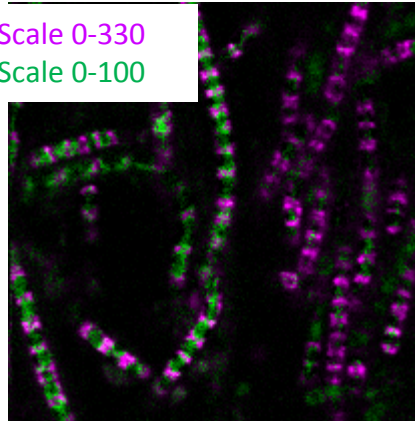


Scale 0-9286  
Scale 0-2236

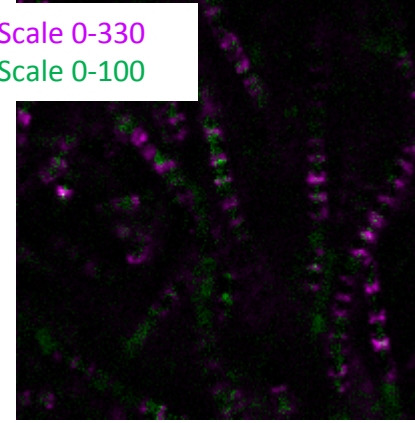


FR (3 weeks)

Scale 0-330  
Scale 0-100

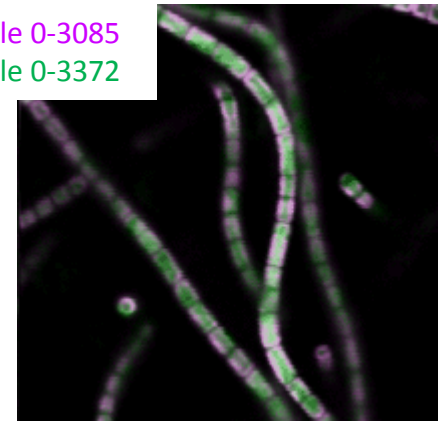


Scale 0-330  
Scale 0-100

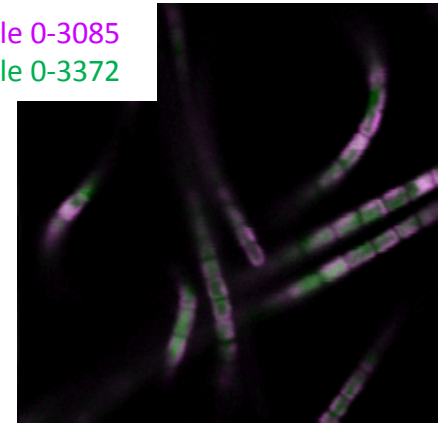


FR (1 day)

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

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

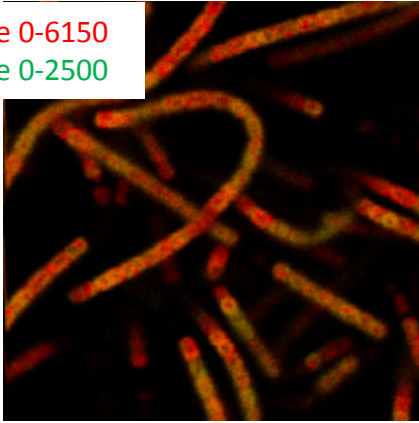
Red – Chl A 684 nm (Comp 3)

Green – Chl F 749 nm (Comp 6)

White Light

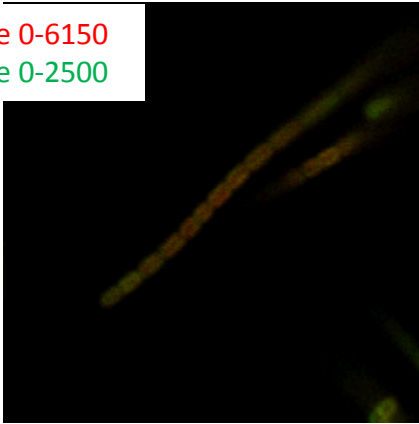
Scale 0-6150

Scale 0-2500



Scale 0-6150

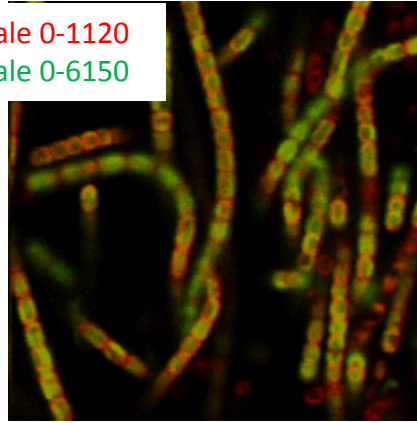
Scale 0-2500



FR (3 weeks)

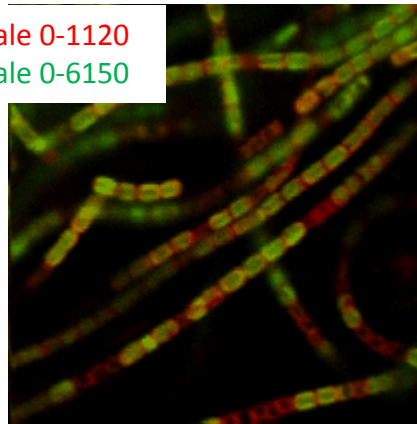
Scale 0-1120

Scale 0-6150



Scale 0-1120

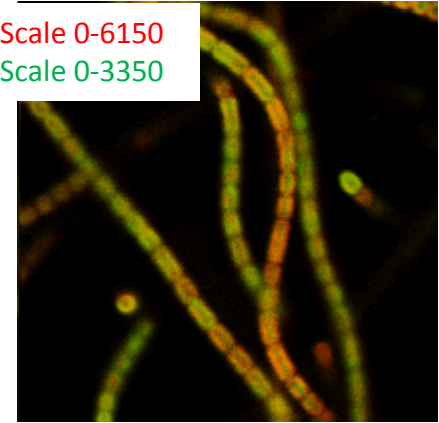
Scale 0-6150



FR (1 day)

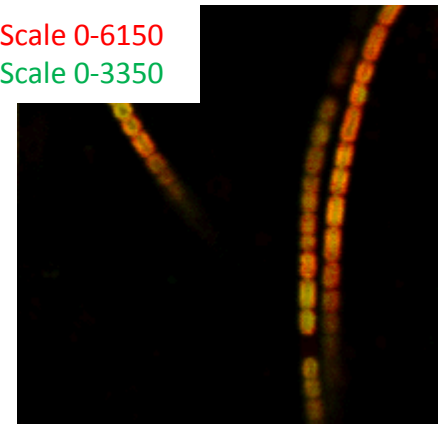
Scale 0-6150

Scale 0-3350



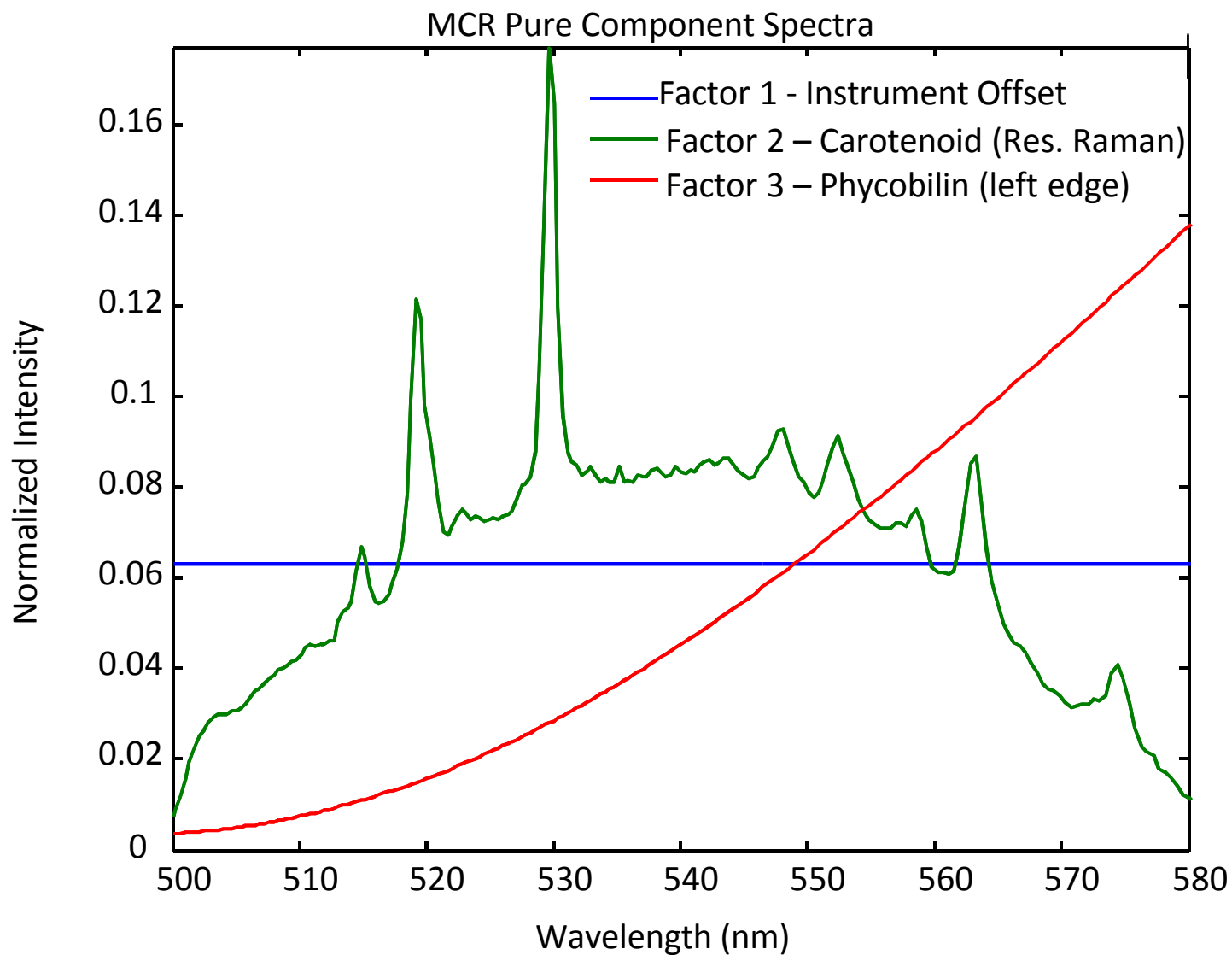
Scale 0-6150

Scale 0-3350



- 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





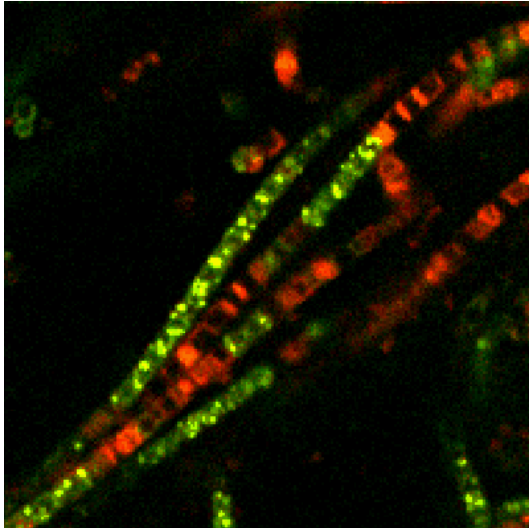
# Carotenoid Distribution

Red – Phycobilisome (left edge)

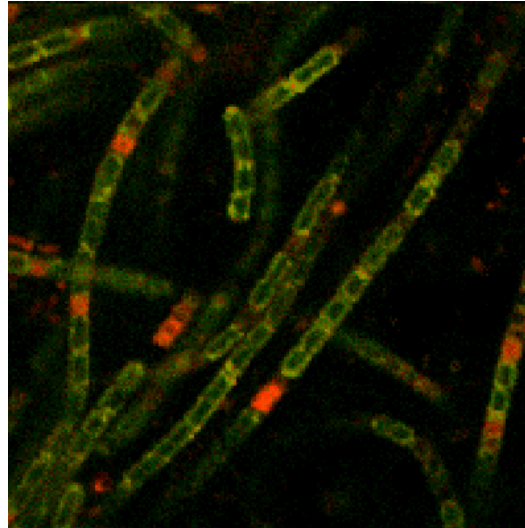
All images are shown on same color scale

Green - Carotenoid – Res. Raman

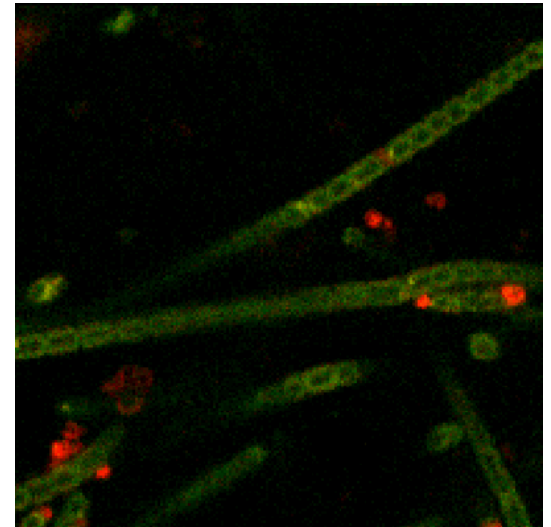
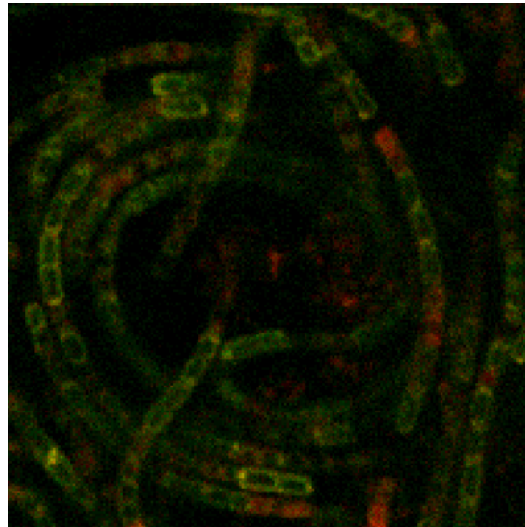
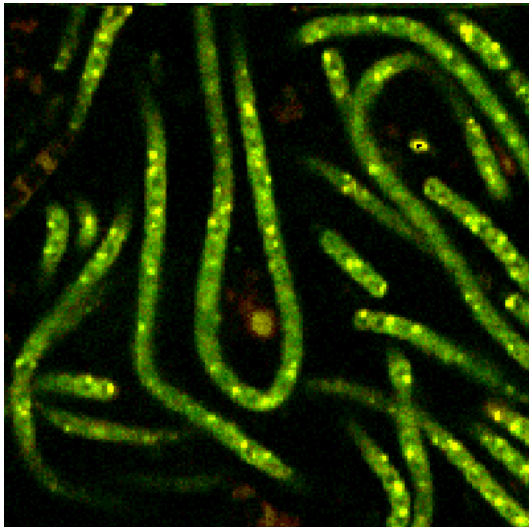
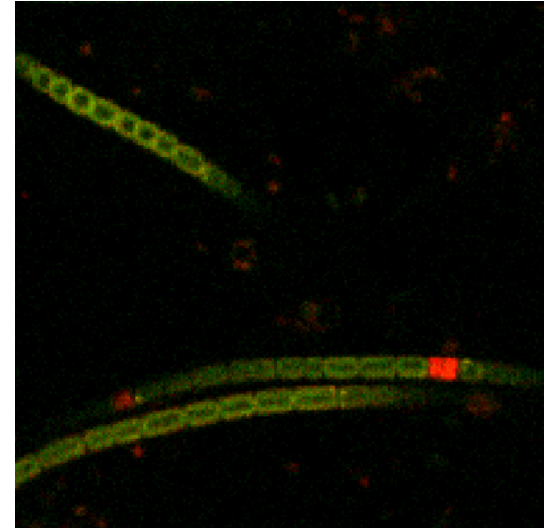
White Light



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.