Exceptional service in the national interest

SAND2016-0592PE

Hyperspectral Confocal Fluorescence Microscopy of *Chl f Producing Organisms*

Imaging conducted on 11/18-19/15

Timlin & Bryant Labs

SAND2015

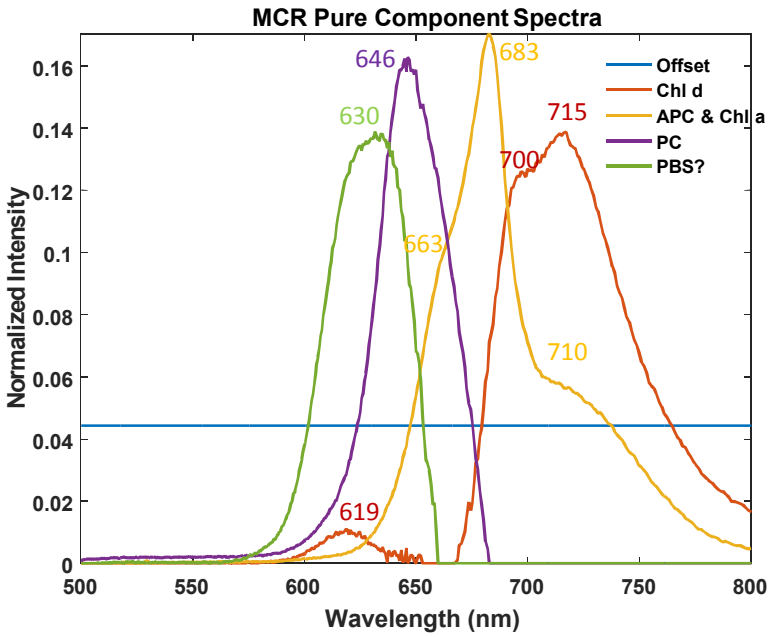
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Experimental Details

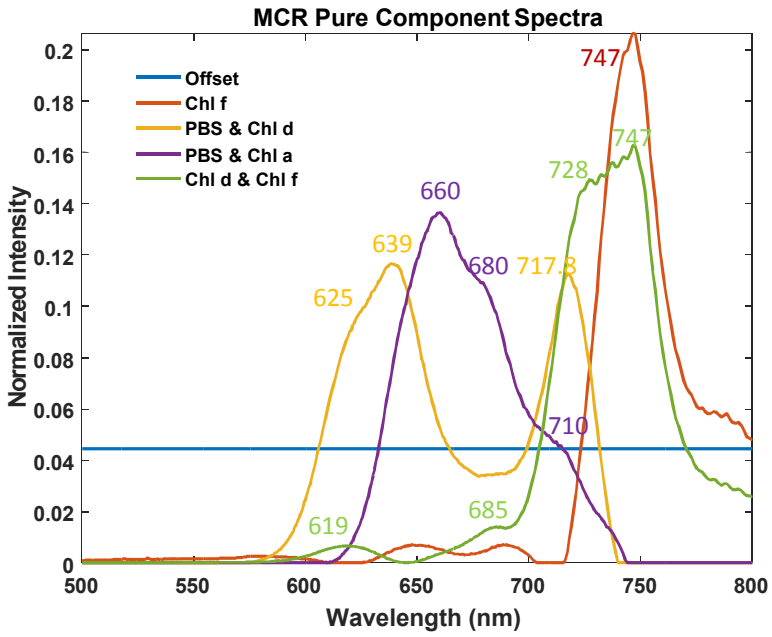
- Samples were received from SNL receiving on 11-18-15 and were placed in an incubator at 30 C & ~50 uE light with shaking. FRL samples were placed on benchtop shaker at rom temperature under about 50 uE light. Note: FRL samples remained wrapped in filters as supplied by Bryant lab throughput the experiment.
- All three FRL samples as well as *Synechococcus* 7335 WL were imaged on 11-18-15
- All six samples were imaged on 11-19-15
- Slides were prepared by the following methods:
  - *Synechococcus:* 1 mL culture was placed in a centrifuge tube and spun down for 1 min at 2500 rpm to concentrate. The majority of the supernatant was removed and the resulting pellet was gently shaken to resuspend in remaining media. 4uL of the concentrated culture was placed on an agar coated slide. The slide was coverslipped and gentle pressure was applied to seat cells to agar. Nail polish was used to seal the coverslip and the sample was allowed to sit for 5 min for cells to settle.
  - *Ficsherella:* A sterile inoculation loop was used to grab some of the biomass and place it on an untreated slide. 4uL of the media was placed on top of the biomass. The slide was coverslipped and gentle pressure was applied. Nail polish was used to seal the coverslip and the sample was allowed to sit for 5 min for cells to settle.
  - *Chlorogloeopsis:* Tube was inverted several times to mix and 1 mL was pipetted out into a centrifuge tube. The sample was allowed to settle for about 10 min. At this point the cells had settled to the bottom. The upper media was pipetted off and the resulting culture inverted again for mixing. 4uL of the concentrated culture was placed on an agar coated slide. The slide was coverslipped and gentle pressure was applied to seat cells to agar. Nail polish was used to seal the coverslip and the sample was allowed to sit for 5 min for cells to settle.
- Images were taken immediately after that, for about 15-30 minutes. FRL samples were kept in the dark during any settling or waiting periods.
- 488 nm excitation, OD setting of 2.0 (laser power < 1 uW/um^2)
- Data was compiled several different ways for analysis.

Ficsherella thermalis 7521

Ficsherella thermalis 7521 WL



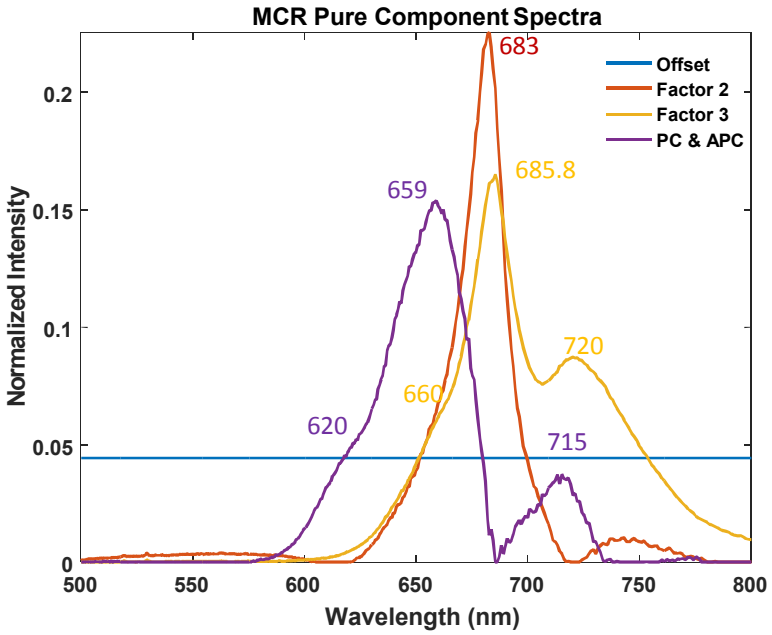
Ficsherella thermalis 7521 FRL



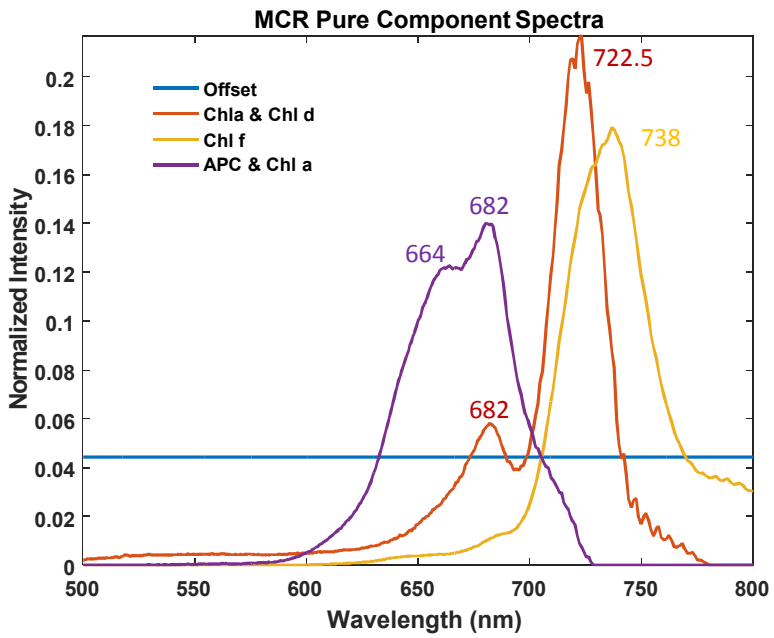
- Significant differences in phycobilin identity and connectivity between FRL and WL growth.
- In FRL conditions there's a component with Chl a, Chl d, and Chl f as well as a separate Chl f component.

Chlorogloeopsis sp. PCC 9212

Chlorogloeopsis 9212 WL



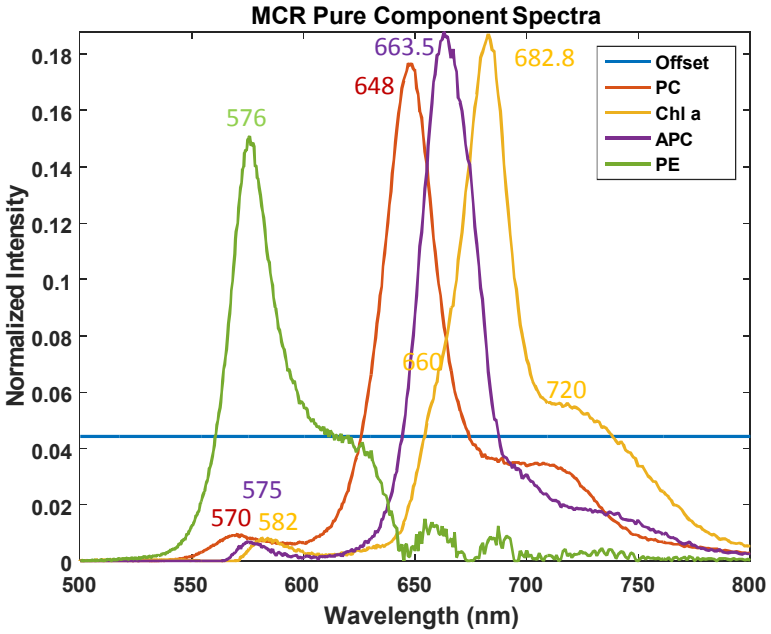
Chlorogloeopsis 9212 FRL



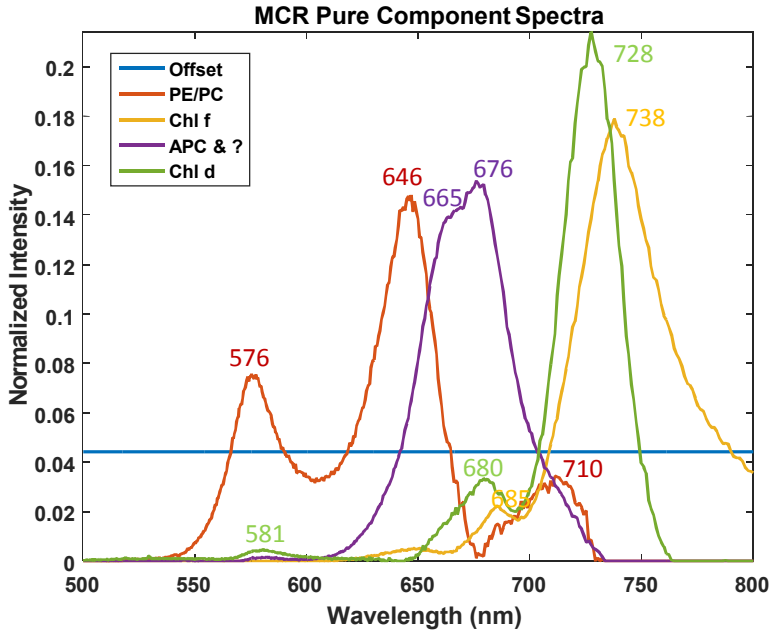
- Very little variation observed between the cells
- Pigment separation is difficult

Synechococcus sp. PCC 7335

Synechococcus 7335 WL

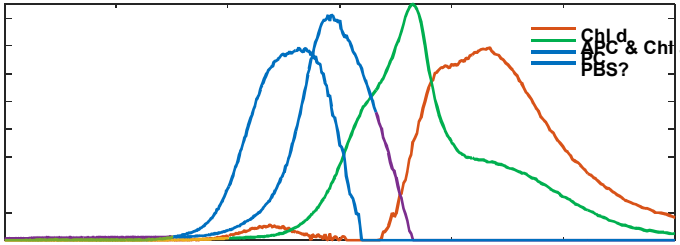


Synechococcus 7335 FRL



- High degree of variation observed between the cells

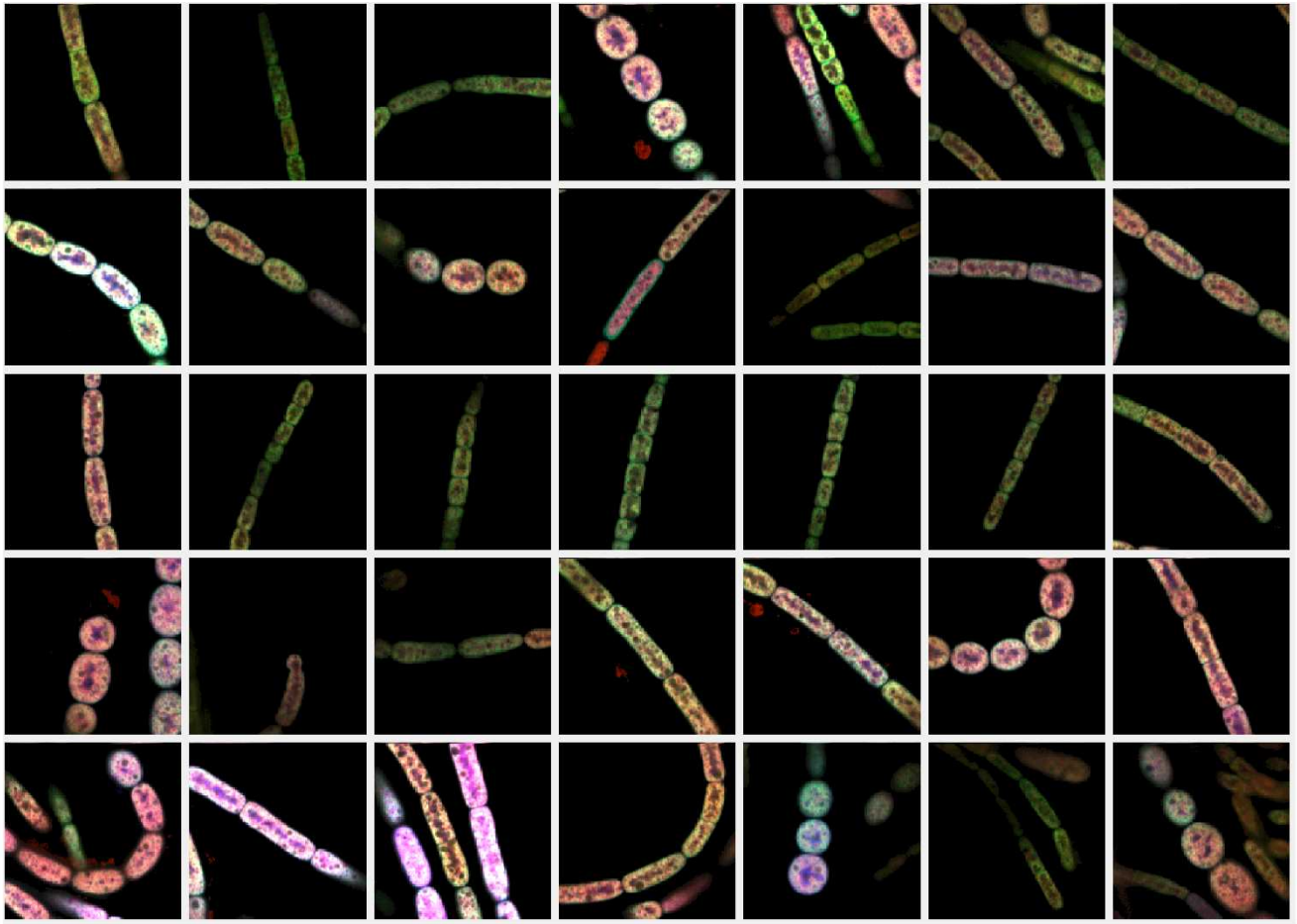
Ficsherella thermalis 7521 WL



Red - Chl d

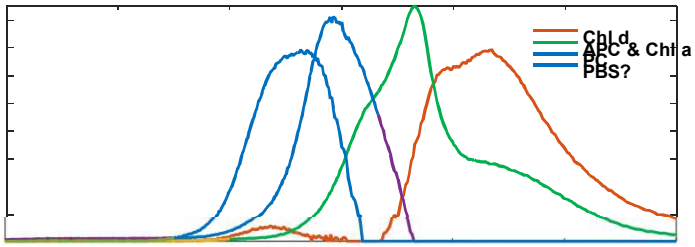
Green - Chl A

Blue – PC (sum of both components)



These images are all plotted on the same colorscale.

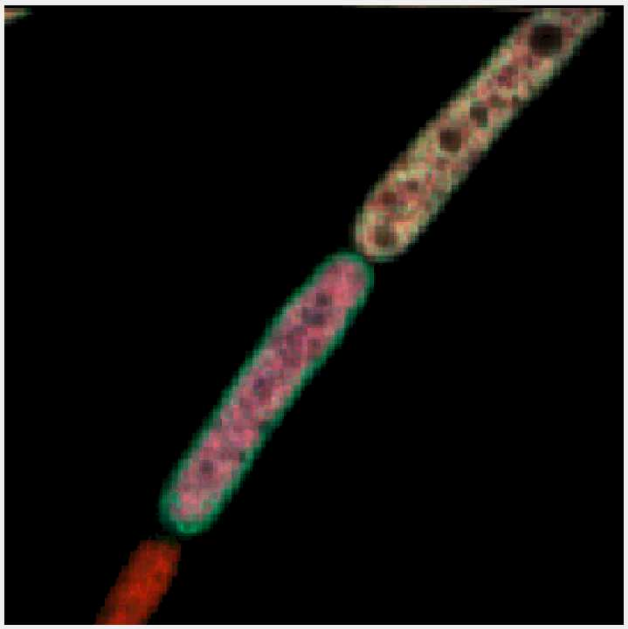
Ficsherella thermalis 7521 WL

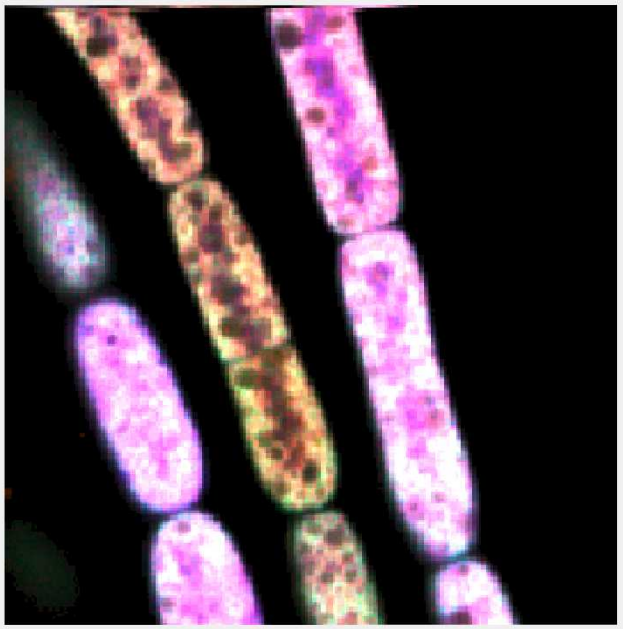


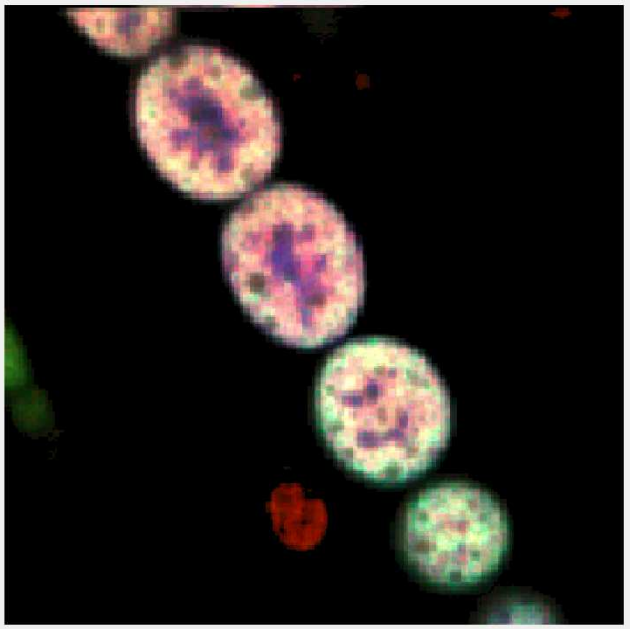
Red - Chl d

Green - Chl A

Blue – PC (sum of both components)

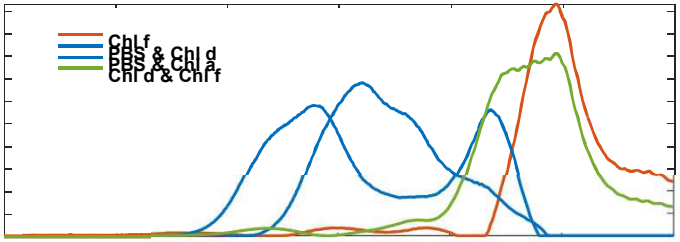






Wide variety of compositions and localizations.

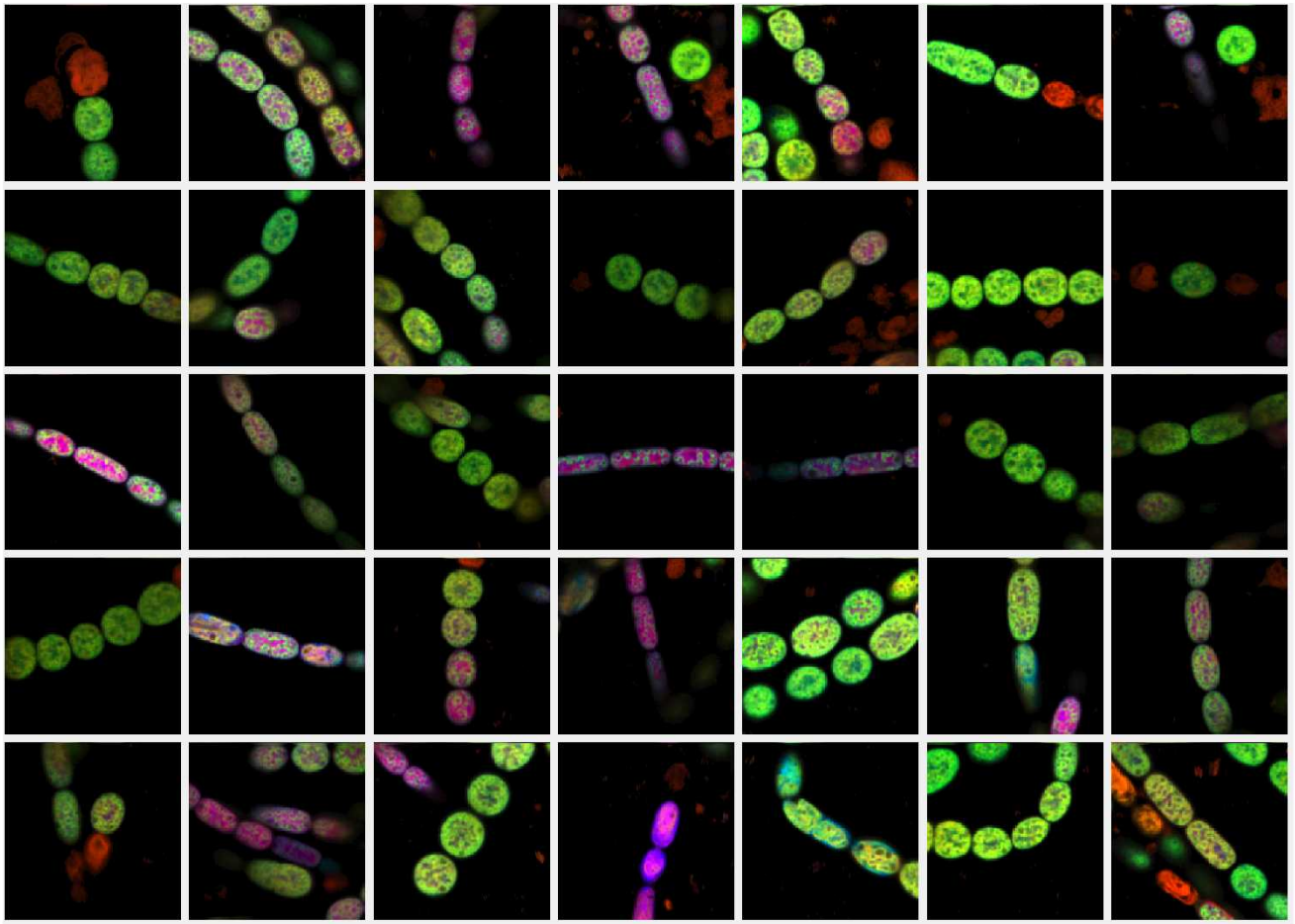
Ficsherella thermalis 7521 FRL



Red - Chl f

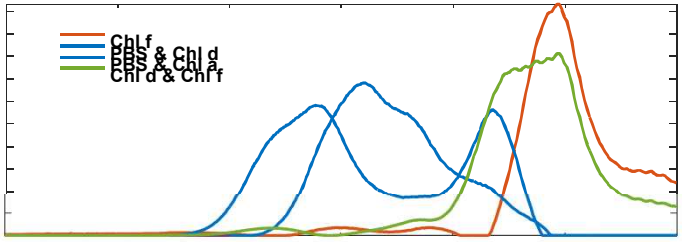
Green - Chl d

Blue – PC (sum of both components)



These images are all plotted on the same colorscale.

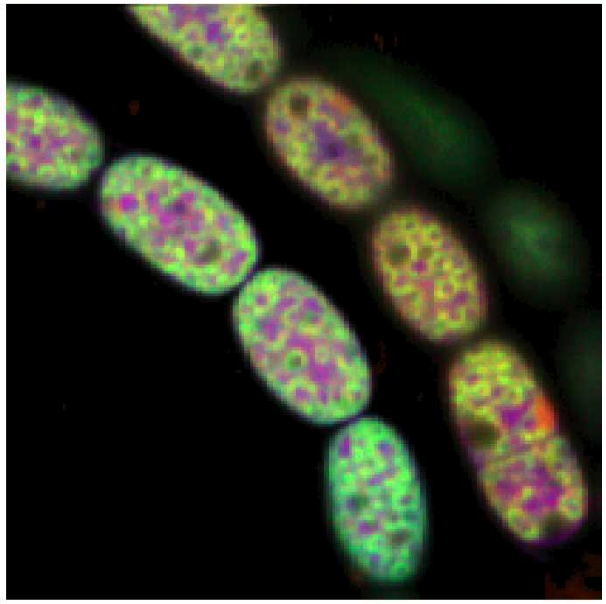
Ficsherella thermalis 7521 FRL

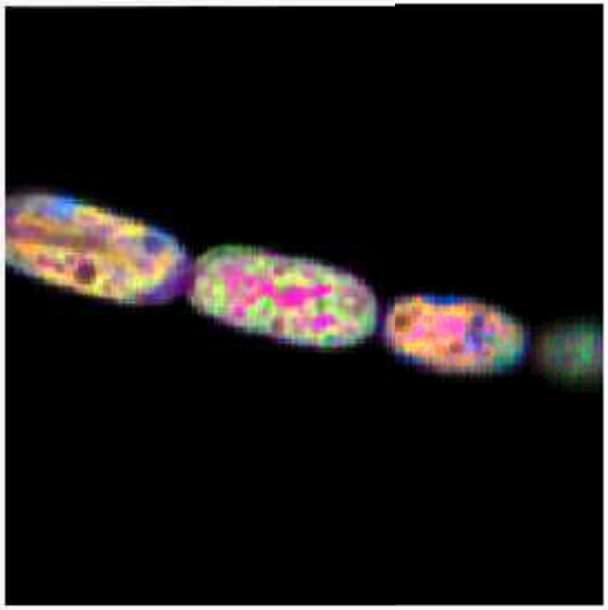


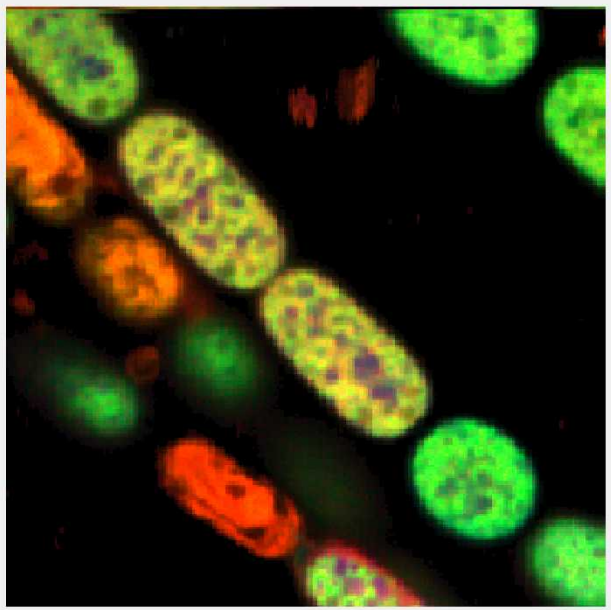
Red - Chl f

Green - Chl d

Blue – PC (sum of both components)

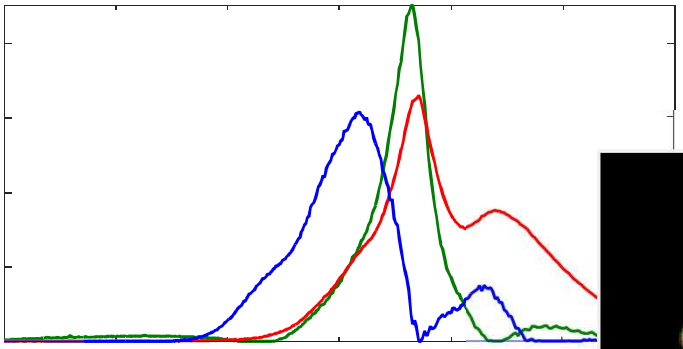


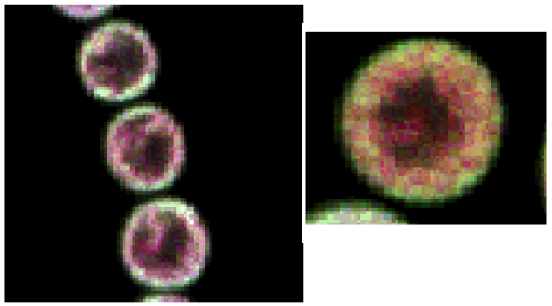


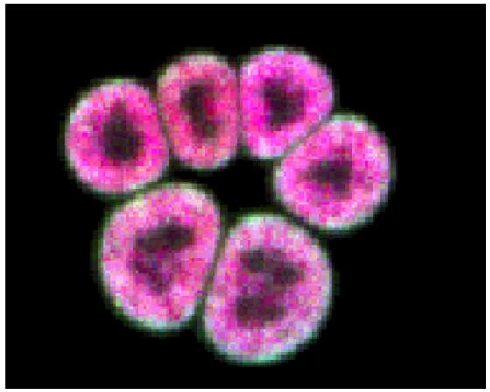


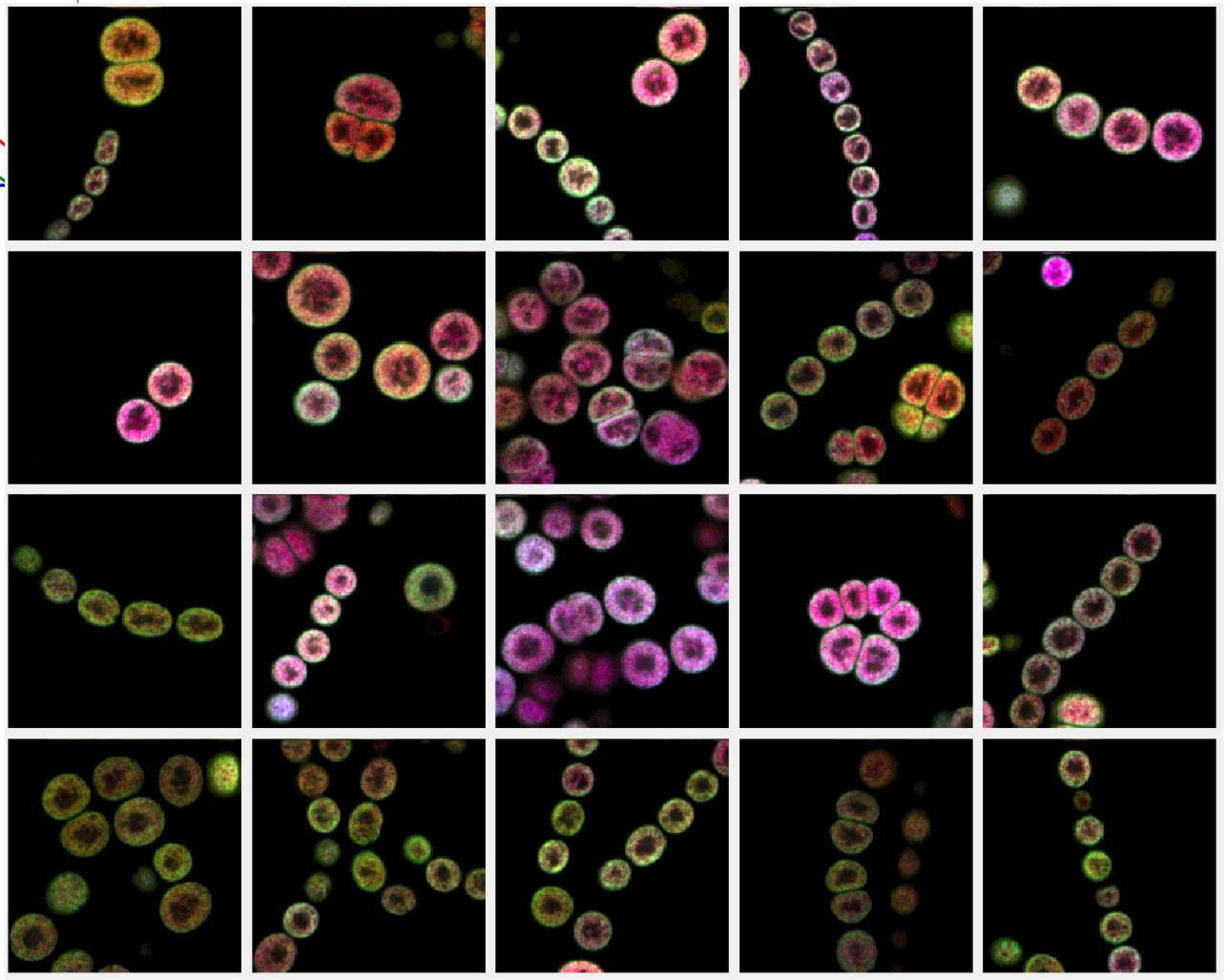
Chlorogloeopsis sp. PCC 9212 WL

Red - Chl red
Green - Chl A
Blue – PC ?

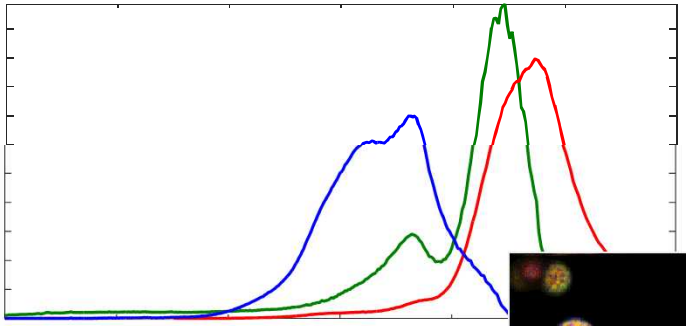




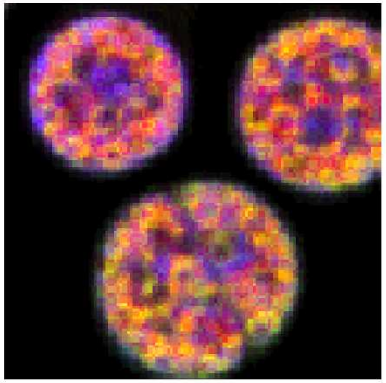


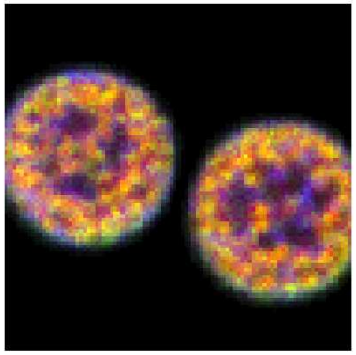


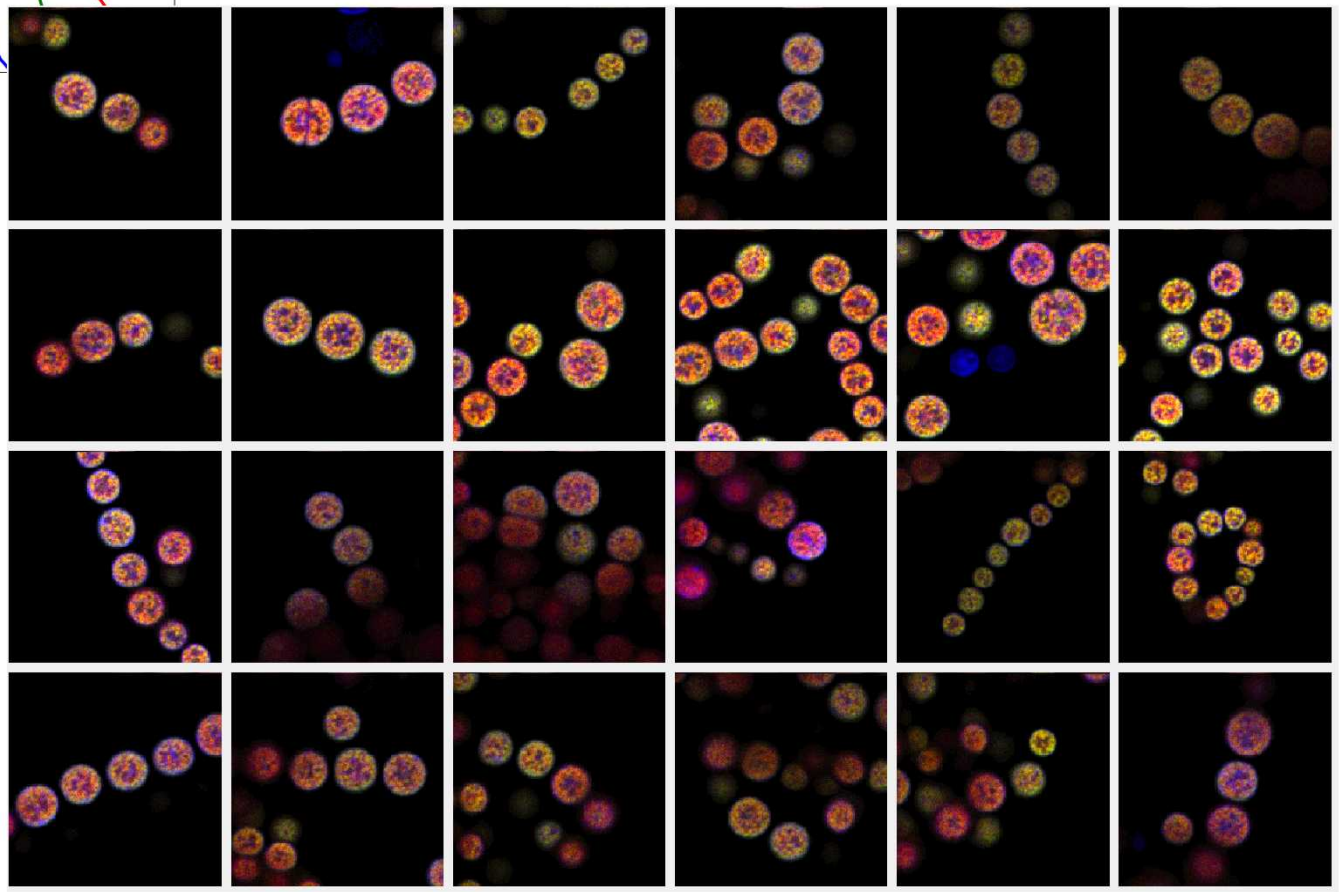
Chlorogloeopsis sp. PCC 9212 FRL



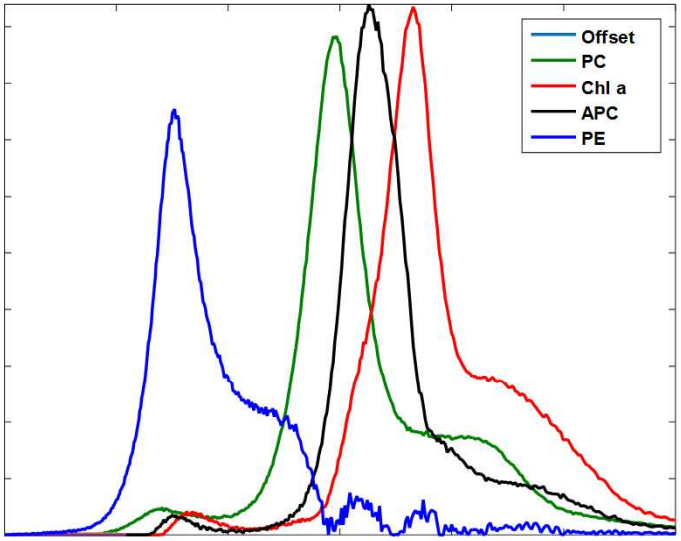
Red - Chl red
Green - Chl A
Blue – PC ?

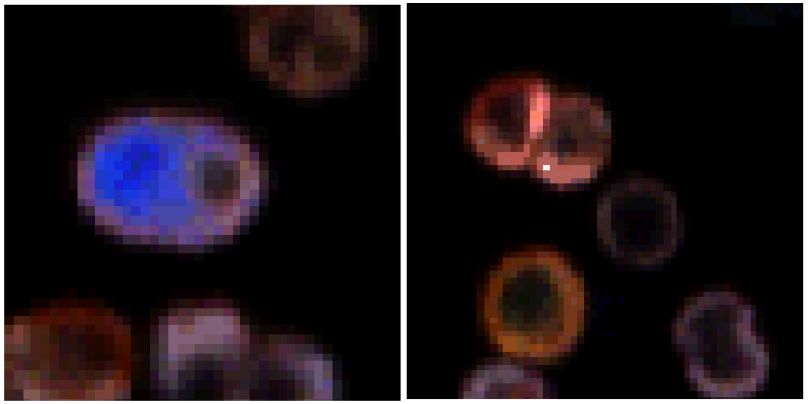


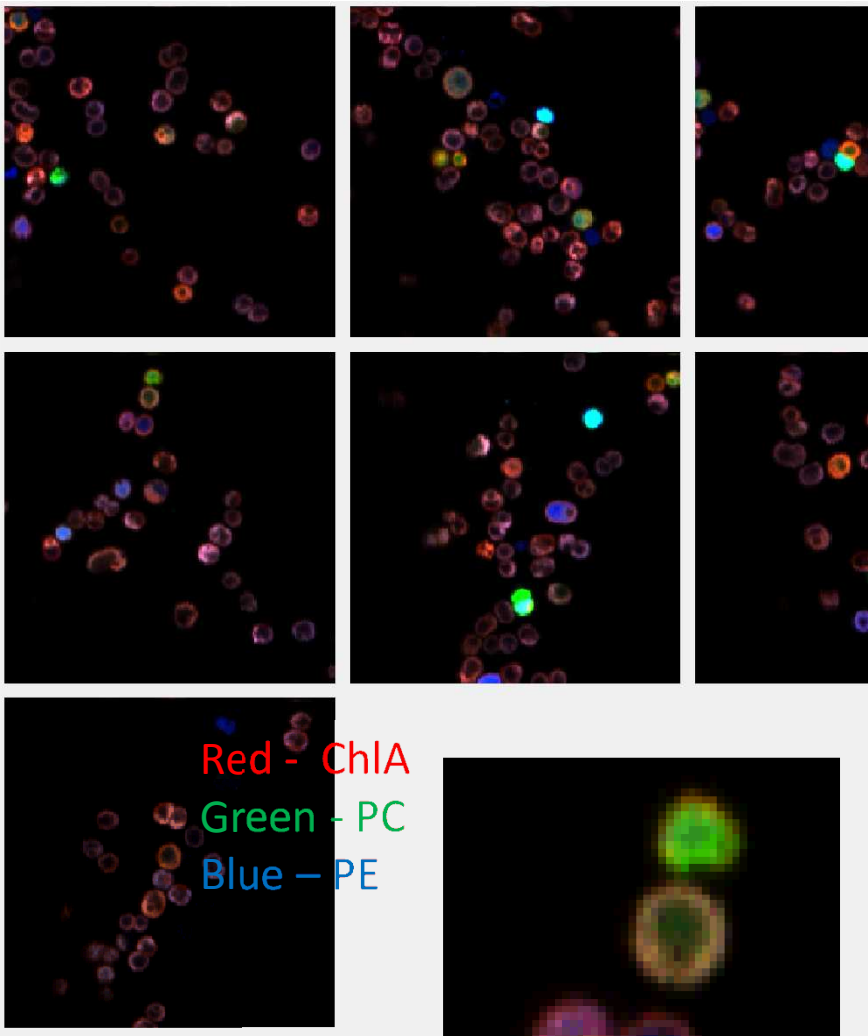


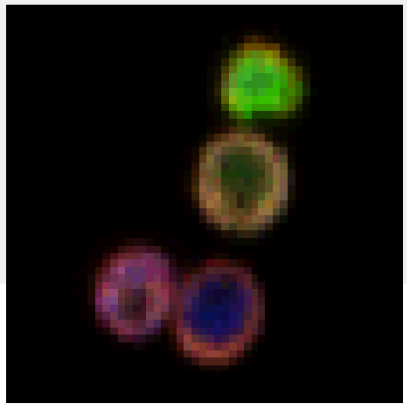


Synechococcus sp. PCC 7335 WL

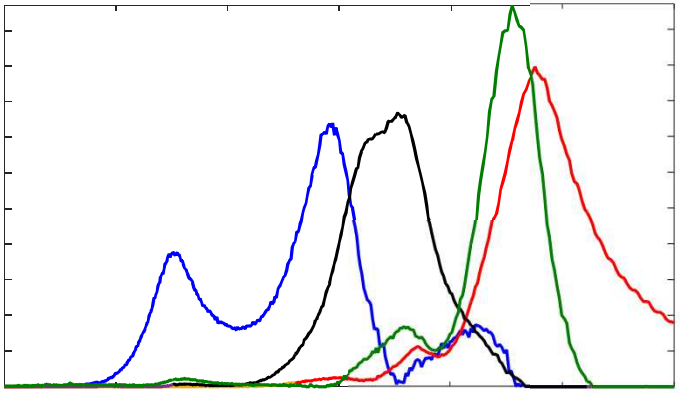


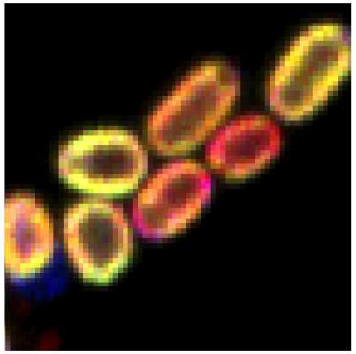


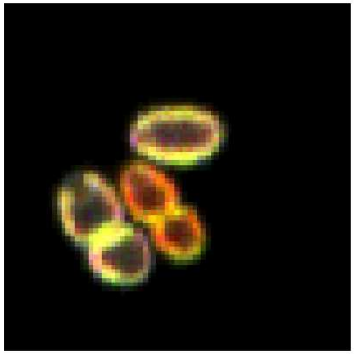


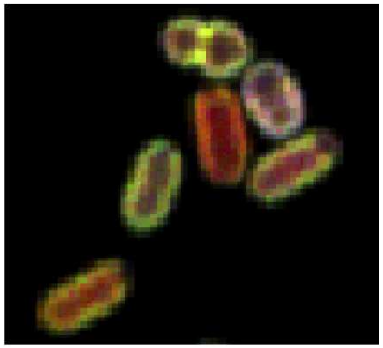


Synechococcus sp. PCC 7335 FRL

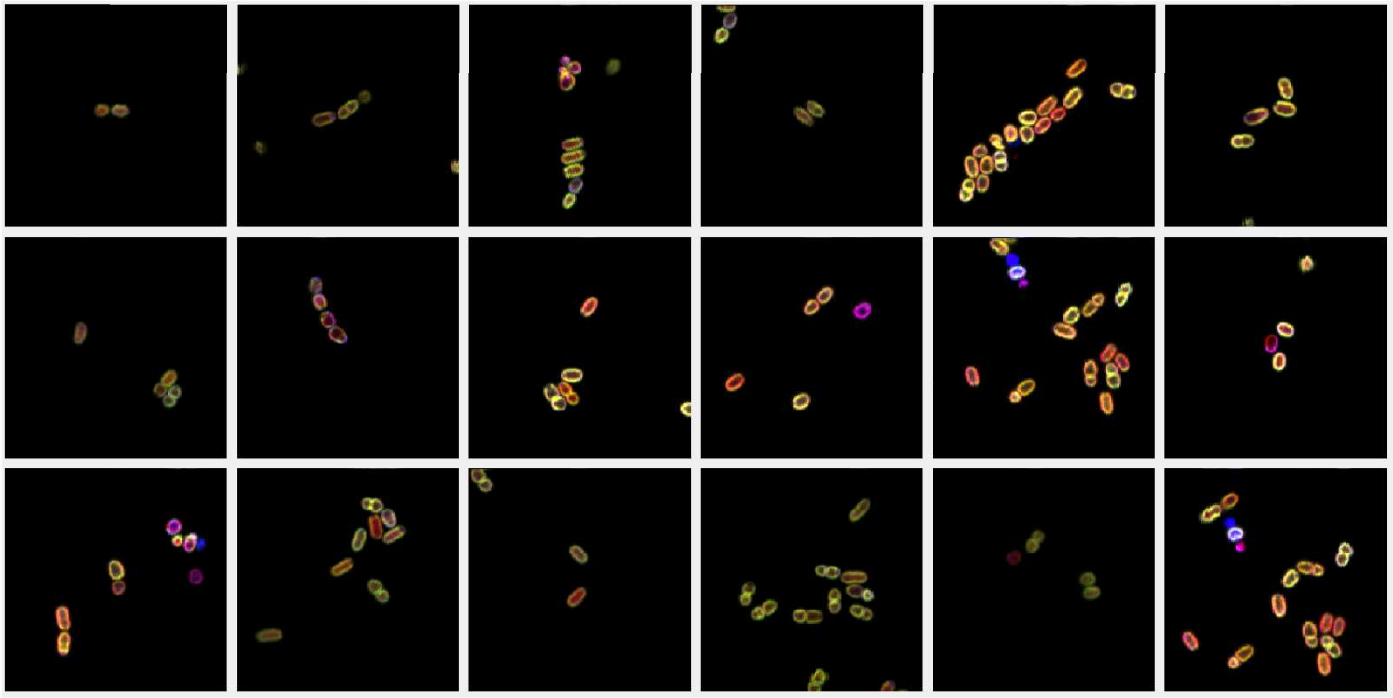






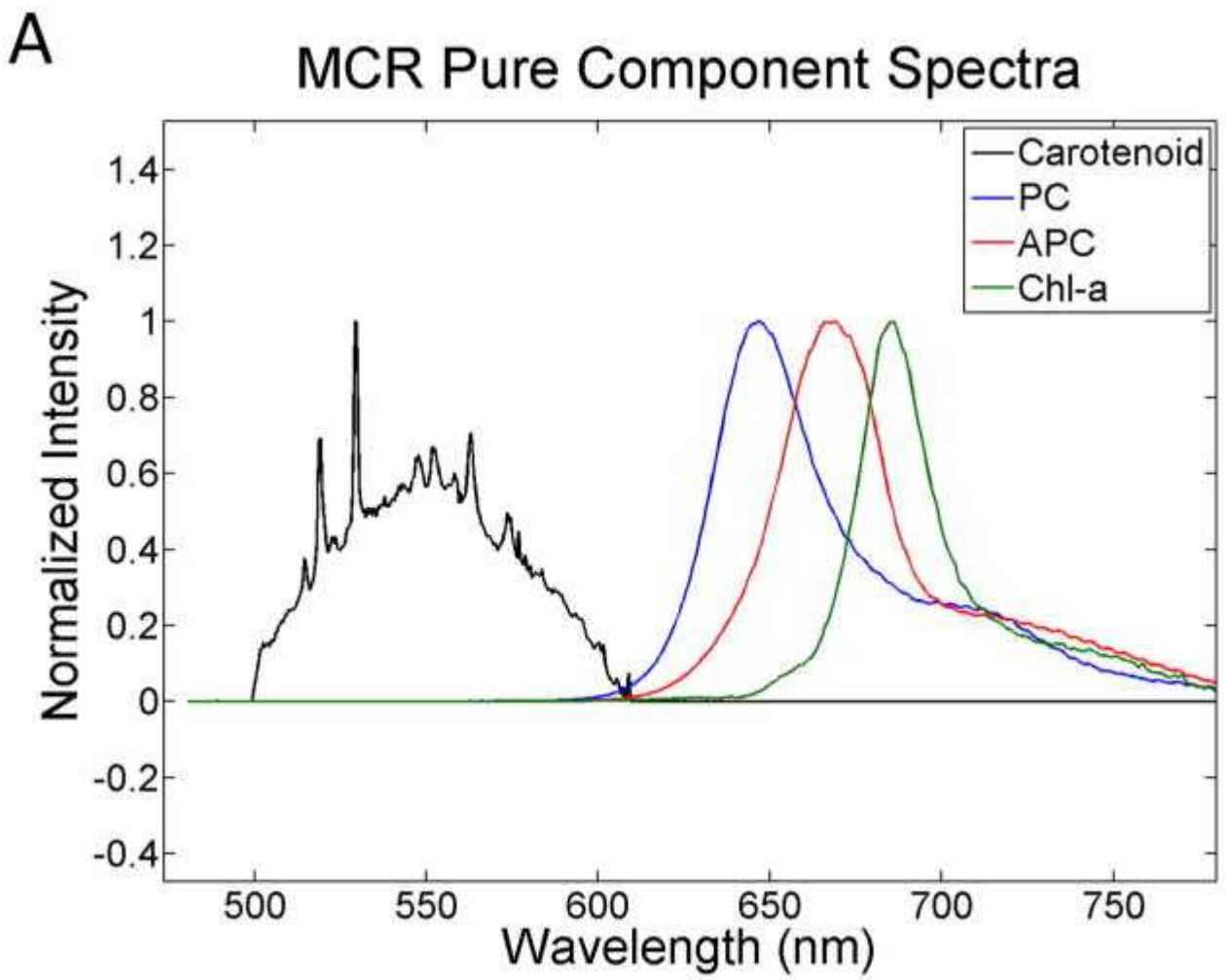


Red - ChlF
Green – Chl D
Blue – PE/PC



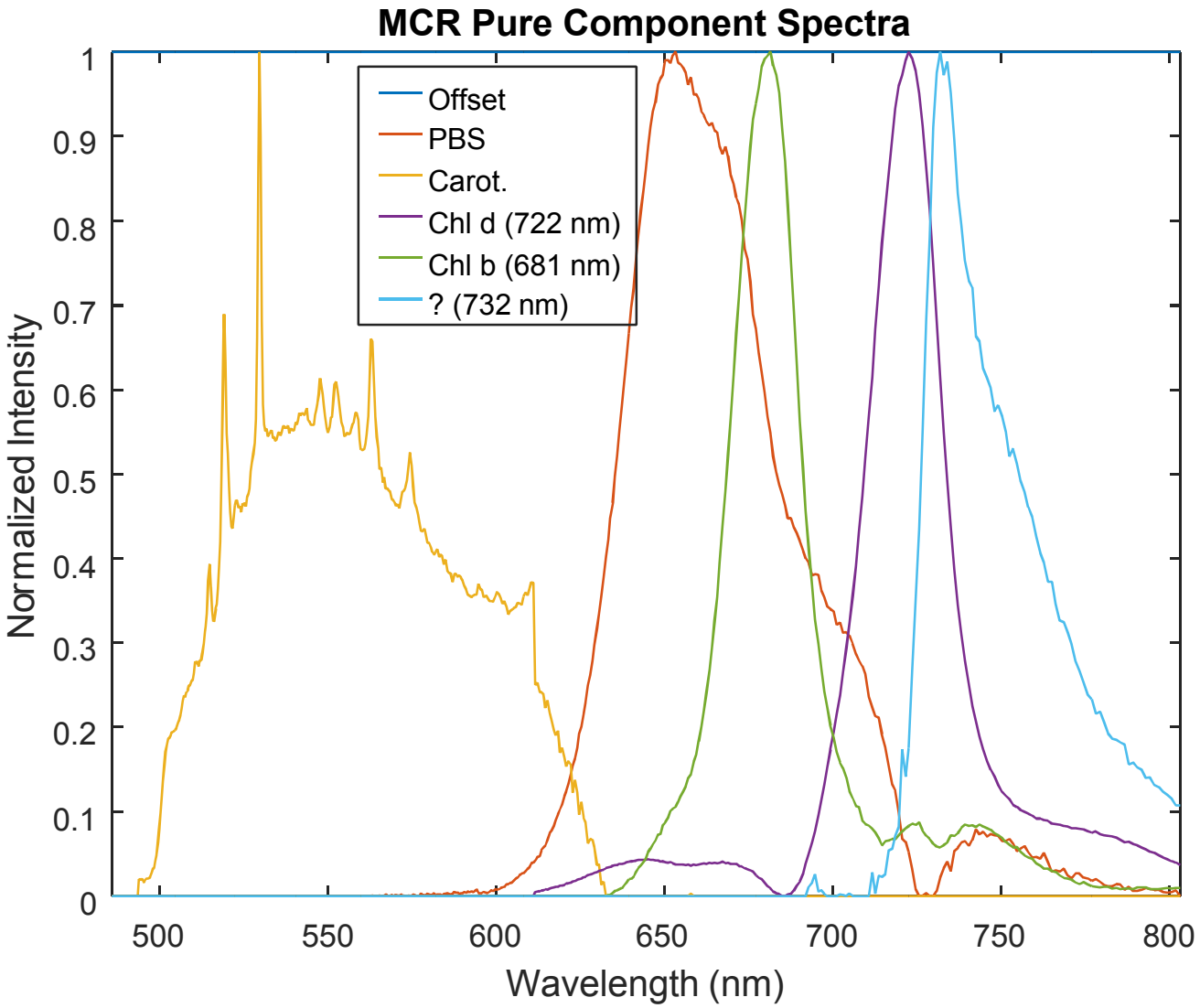
PREVIOUS RESULTS FOR COMPARISON

Synechococcus 7942

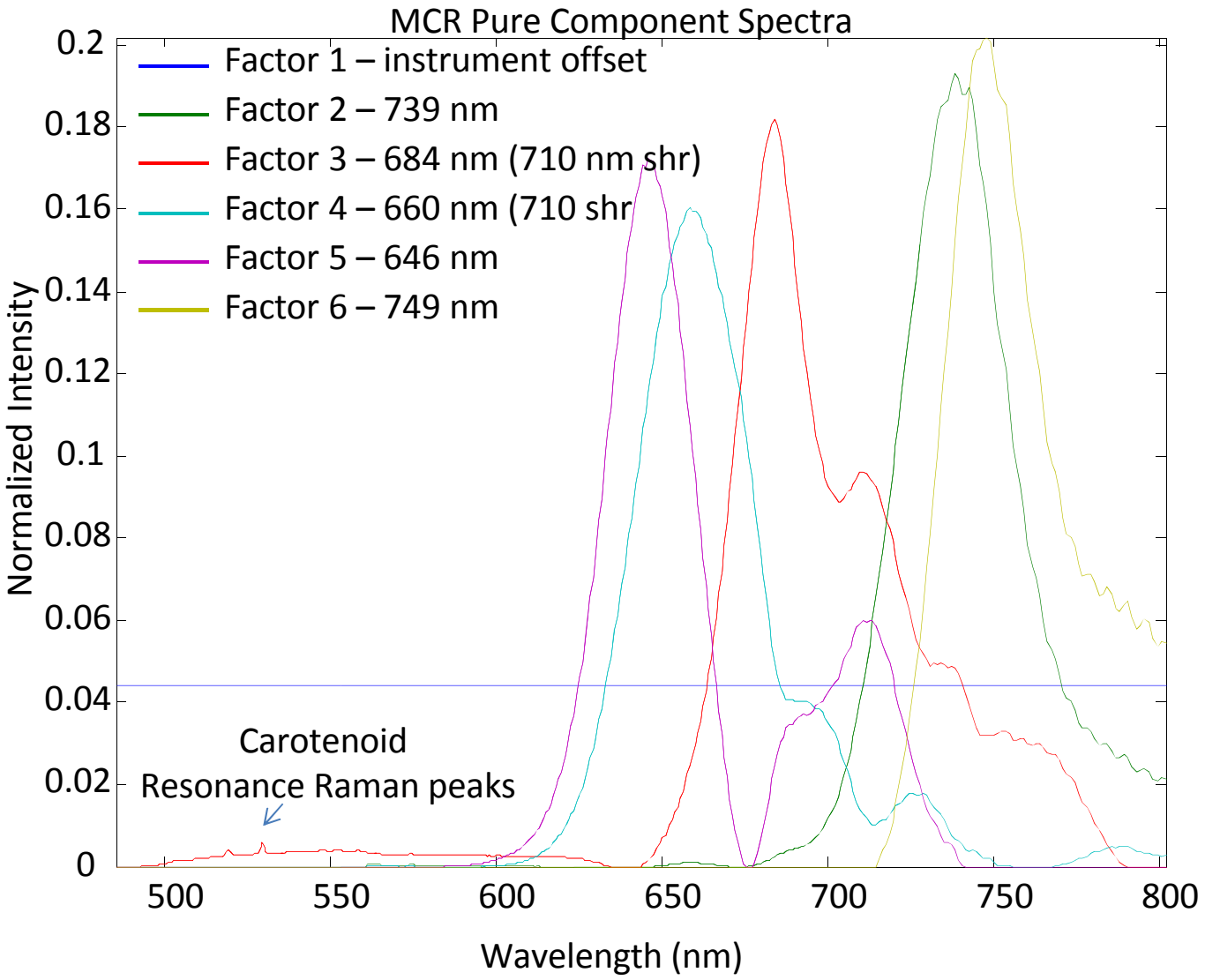


Ruffing and Jones, Biotechnol Bioeng. 2012 Sep;109(9):2190-9. doi: 10.1002/bit.24509. E

Acaryochloris marina & R61



H. hongdechloris



Synechococcus 7335

(WL & FRL combined model, Blankenship Lab)