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SAND2015-5399PE

N Starvation in Synechocystis 6803 Experiment Results

7/22/14, 7/23/14, and 7/24/14

Experiment and initial analysis performed by HAH, imaging & analysis conducted by JAT, single cell analysis completed by JAM & JAT

SAND2015-

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Methods

- Syn6803WT culture was grown in nitrate-containing BG11 media (the normal recipe) for 3 days in an incubator at 30C shaking at 150rpm with between ~30-50uE of constant fluorescent light. After 3 days, two 15ml aliquots of the culture were placed in 50ml centrifuge tubes and spun down at 4500 x g for 10 minutes. The supernatant was discarded, and the pellet was resuspended by vortexing (30s) in 15ml of fresh media (previously warmed to 30C). The control sample was resuspended in BG11+N (normal media formula) and the variable was resuspended in media without nitrate (BG11-N). Centrifugation and resuspension were repeated two additional times to ensure thorough washing of the pellet and that no residual nitrate remained in the variable sample. After the third wash, the pellet was resuspended in 15ml of fresh media, the contents of each tube was transferred to a sterile 500ml baffled flask and 35ml of additional BG11 media (with or without NO_3^-, for control and variable samples, respectively) were added for a total volume of 50ml per flask. 1mL aliquots were taken from each flask to measure absorbance (400-800nm) immediately upon final resuspension. Samples were placed in the incubator (at 30C, 150rpm, ~30-50uE) and absorbance was measured using an Agilent 8453 Spectrophotometer at 0, 8, and 24 hrs . Absorbance was used to calculate chlorophyll (Chl) and phycobilin (PCB) content in each sample according to the following formulae:
  - Chl = 14.96(A678-A730)-0.616(A625-A730)
  - PCB = 0.139(A620-A730)-0.0355(A678-A730)
- After 48h, the N starved culture was split in half, spun down, washed 3 times with BG11+N or BG11-N media using the same protocol as above and transferred to sterile flasks to observed recovery of the N-starved Syn6903WT culture in nitrate-containing BG11 media. One sample remained in BG11-N media as a negative control. Again, absorbance was measured at 0, 8, and 24 hrs to calculate Chl and PCB content of each sample. Results were plotted against time.
- At each time point an aliquot of culture was pulled and 4-7uL were placed on an agar coated slide. A coverslip was applied and the sample was imaged immediately on the HCFM.

Note: We also have a separate set of data with just absorbance measurements (no imaging) where the sampling was done every 4 hrs.

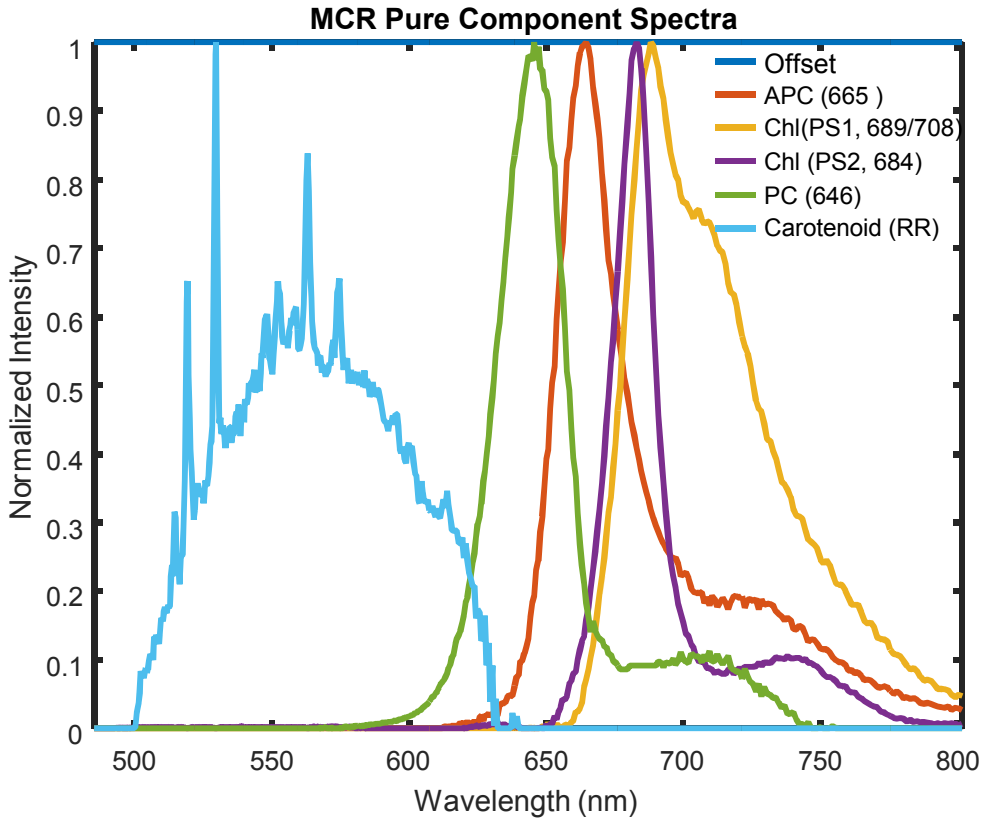
Hyperspectral Confocal Fluorescence Microscopy (HCFM) Parameters

- 488 nm laser, OD=1.0 (Power level ~ XX uW)
- 60x oil objective
- 0.24 msec/pixel integration time
- EMCCD, Gain = 95, Detector temp = -60
- Image size 25 x 25 um
- Step size = 0.12 um x and y (240 nm lateral spatial resolution)
- 512 wavelengths collected
- 164 images collected each containing 44100 spectra for a total of 3.5 x 10^9 spectra collected

MCR Analysis

- Hyperspectral images were preprocessed (Jones, et. al 2012)
- Representative images from each time point and sample were combined into one image data set and MCR was executed with non-negativity and partial unimodality constraints on all image pixels above the background. This resulted in a 5 (or 6) component spectral model tat explained >99.4% of the spectral variance. The model was similar to previous imaging of Synechosystis 6803.
- This model was predicted using classical least squares to the entire data set to generate concentration maps indicating the abundance and location of each component.
- The resulting concentration maps were segmented using a modified watershed transformation algorithm to identify individual cells. Automated cell segmentation was verified and edited manually.
- Single cell statistics were calculated for individual cells.

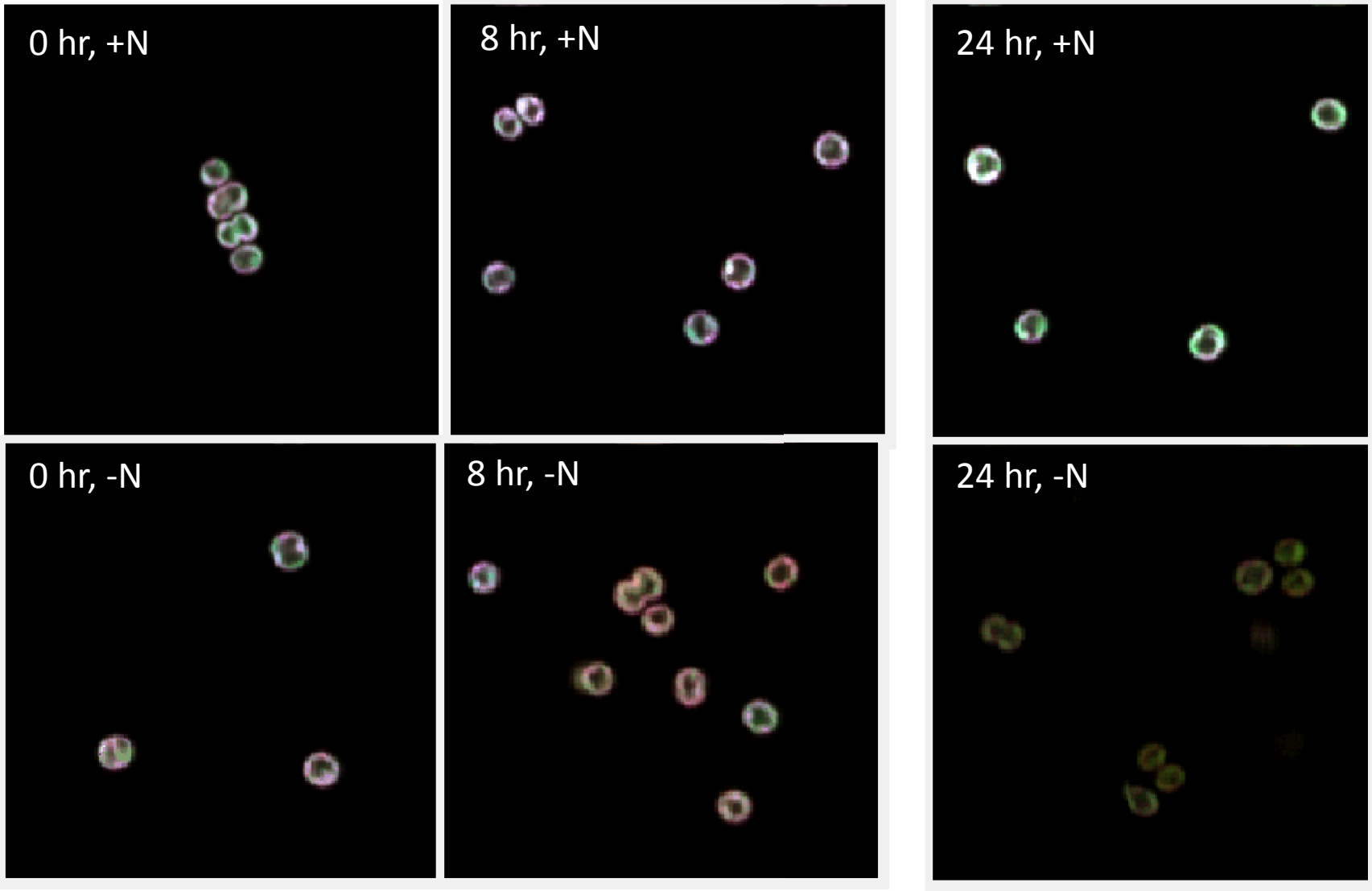
Overall Spectral Model



Note: the intensities in this plot have been normalized and do not represent relative contributions

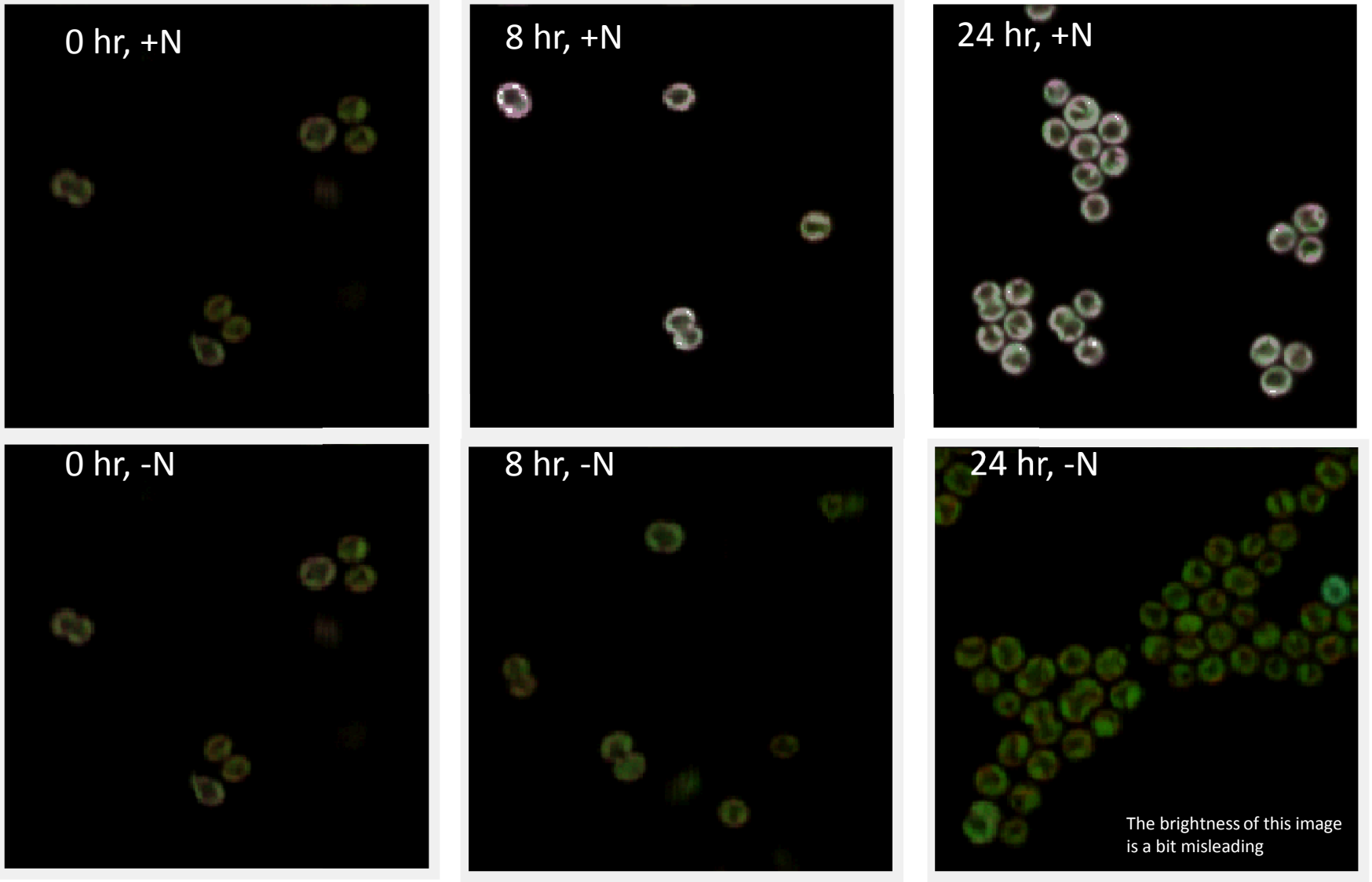
N Starvation Experiment

R=Chl(PS1), G = Chl (PS2), B = PC, APC



N Repletion Experiment

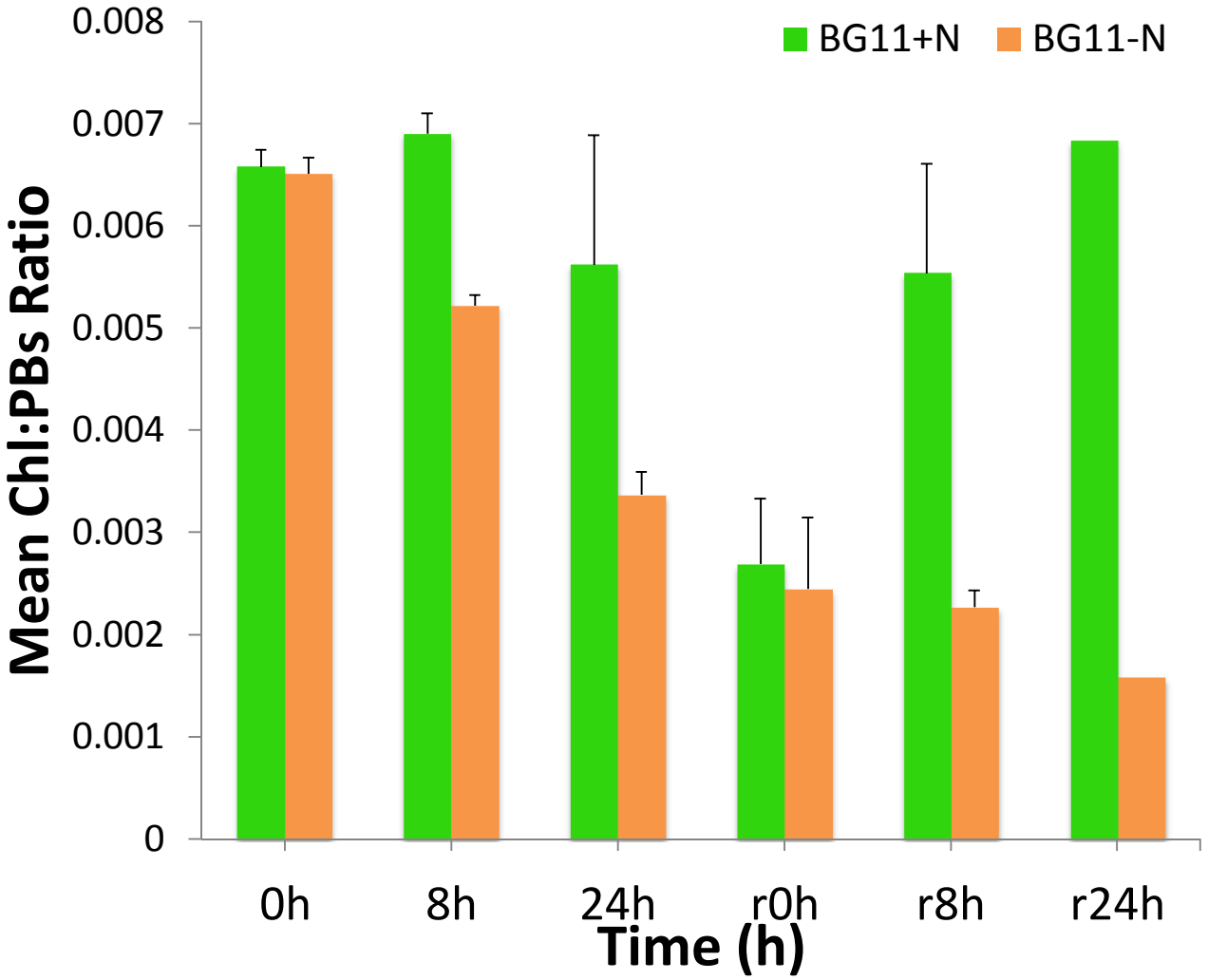
R=Chl(PS1), G = Chl (PS2), B = PC, APC



Single Cell Quantification

Coming soon!

Absorbance Measurements



Error bars are calculated as the standard deviation of three replicate cultures