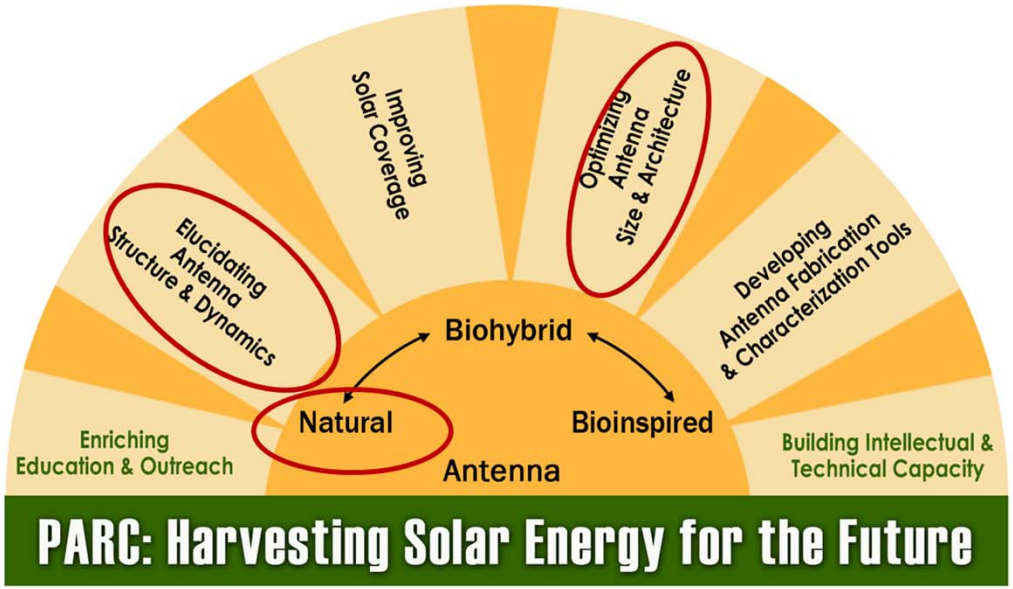
SAND2012-1641C

Large-scale reorganization of thylakoid pigments in *Synechocystis* 6803 phycobilisome mutants

Aaron Collins
Sandia National Laboratories
March 6th 2012



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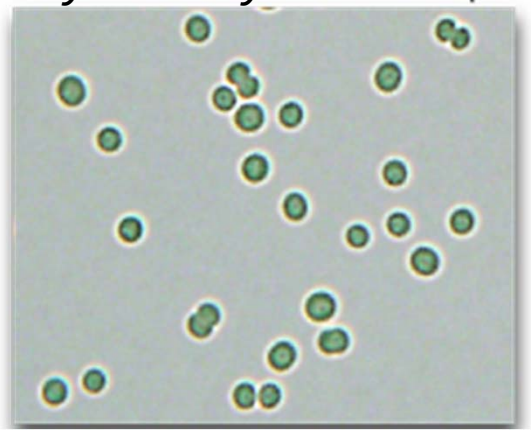
Sandia National Laboratories

Question – Does a diminished light-harvesting antenna change the pigment distribution and organization in thylakoid membranes?

Synechocystis ~1.5-2 µm



Pretty picture

Color (RGB), morphology

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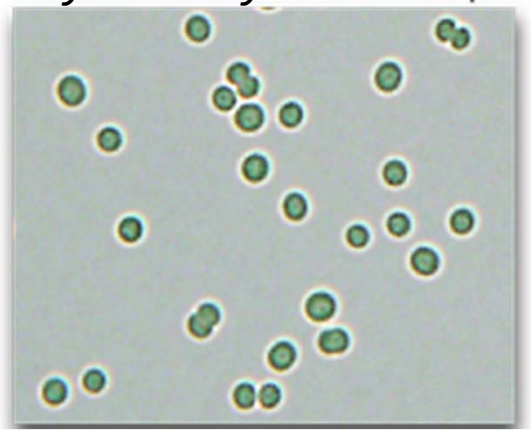

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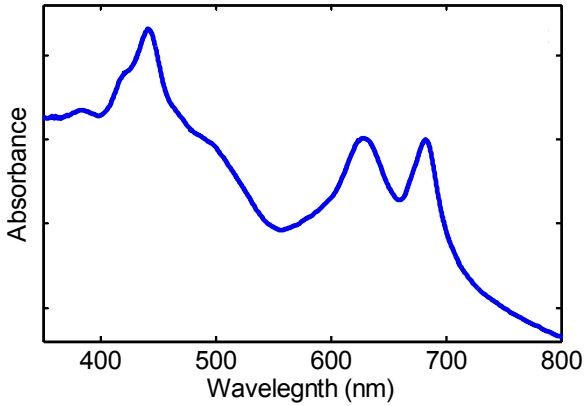

Spectral resolution

Pigment energy, spectral coverage

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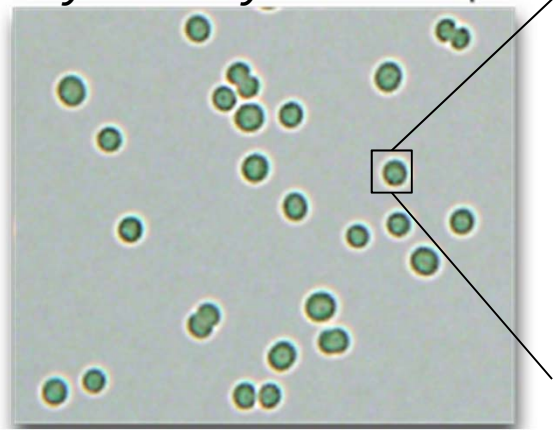
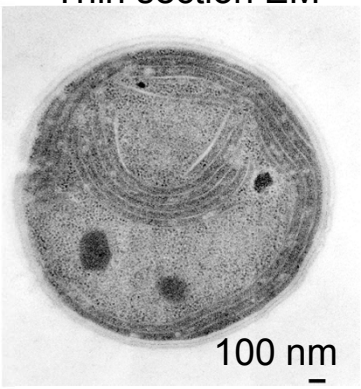


Thin section EM



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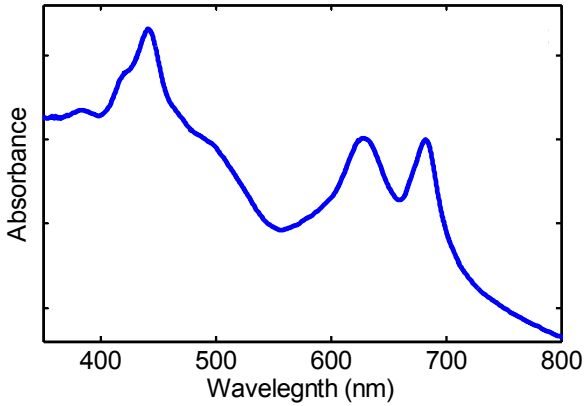
Color (RGB), morphology

Spatial resolution

Structural information



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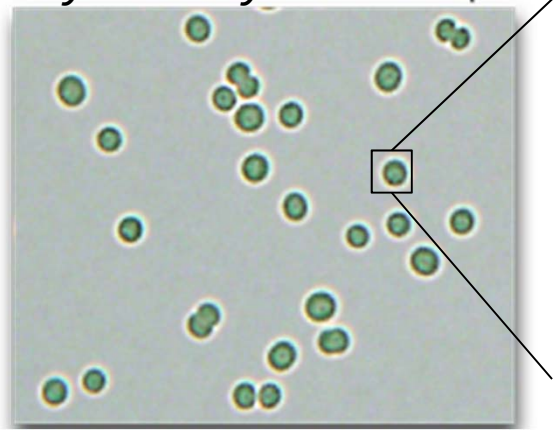

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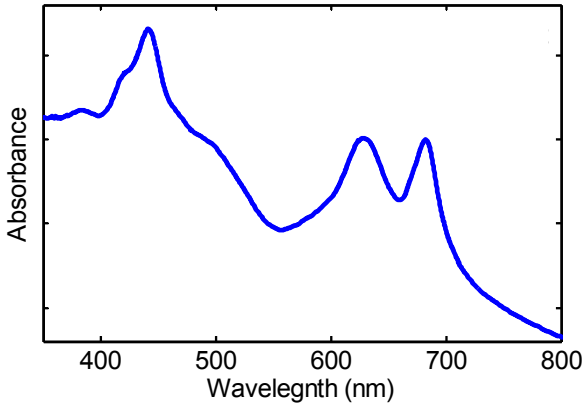

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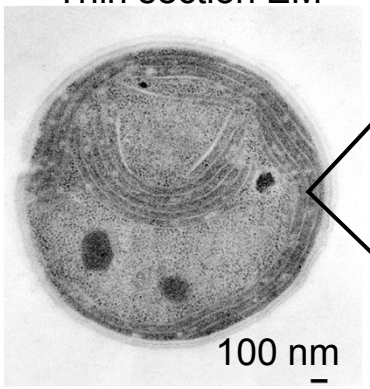

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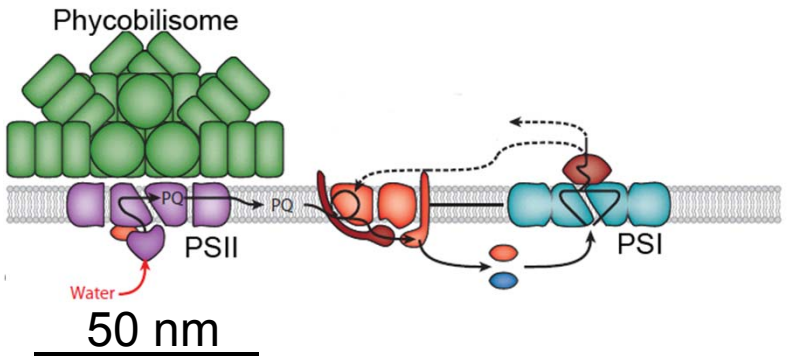

Cartoon model

Inferred organization

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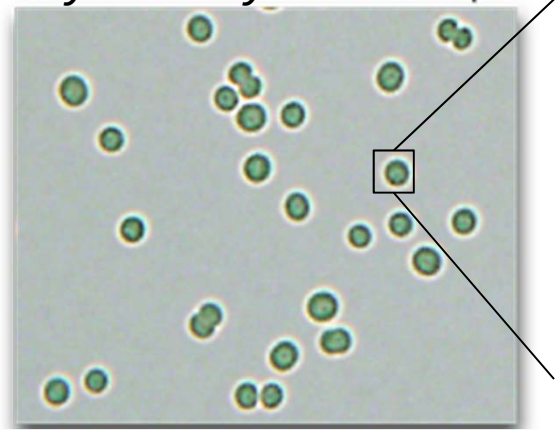

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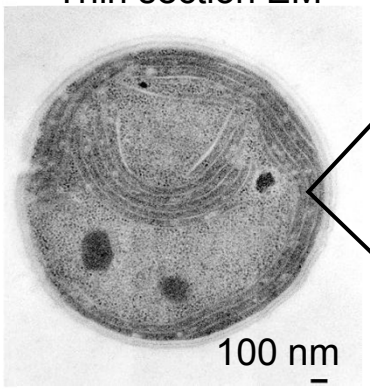

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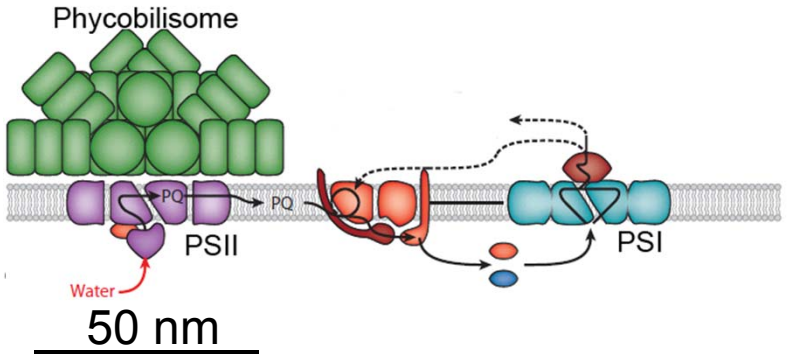

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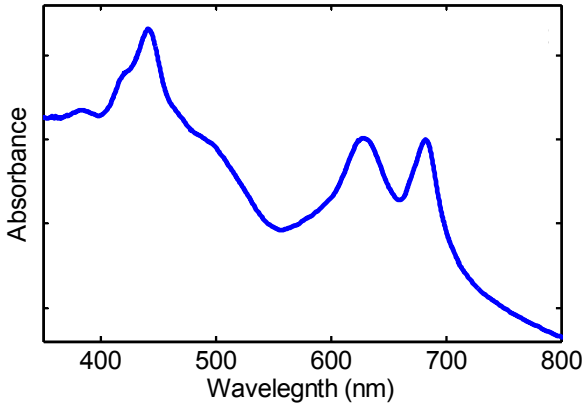
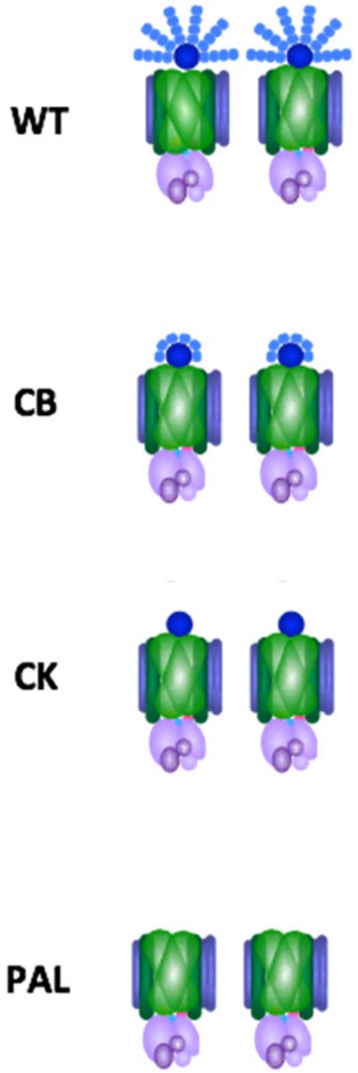


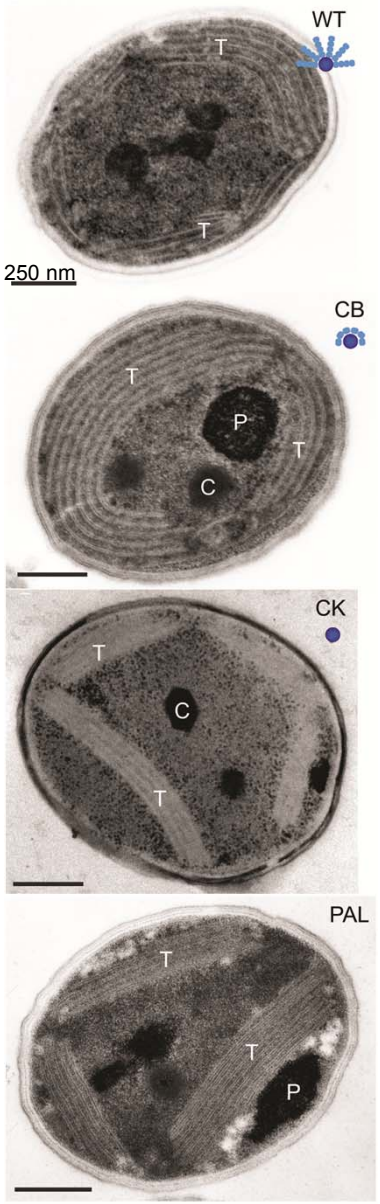
Spectral resolution

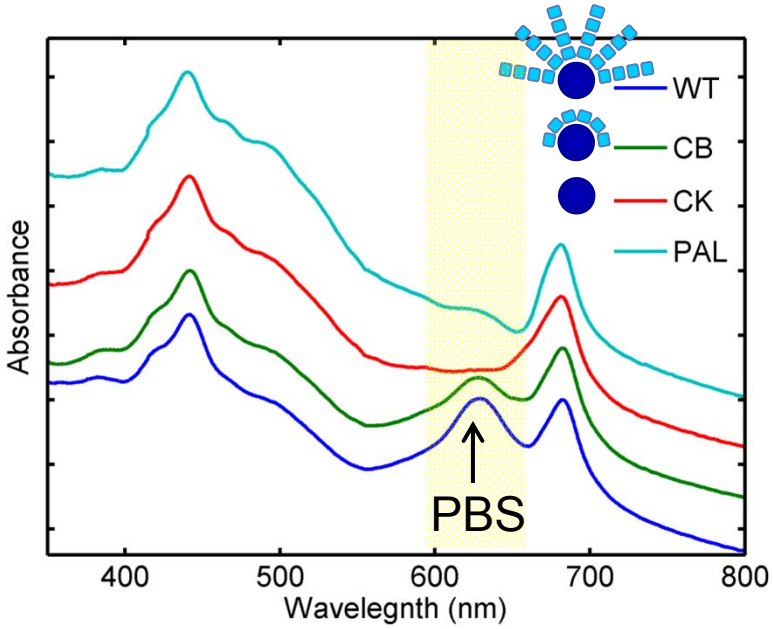
Pigment energy, spectral coverage

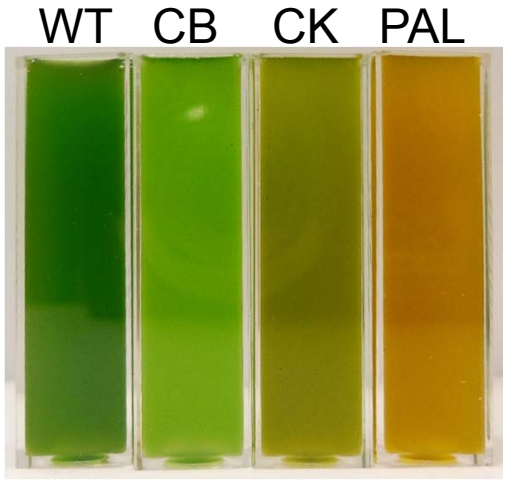
Combine spatial and spectral information at the subcellular location using **hyperspectral confocal fluorescence microscopy (HCFM)**

Synechocystis mutants – Progressive truncation of the phycobilisome antenna

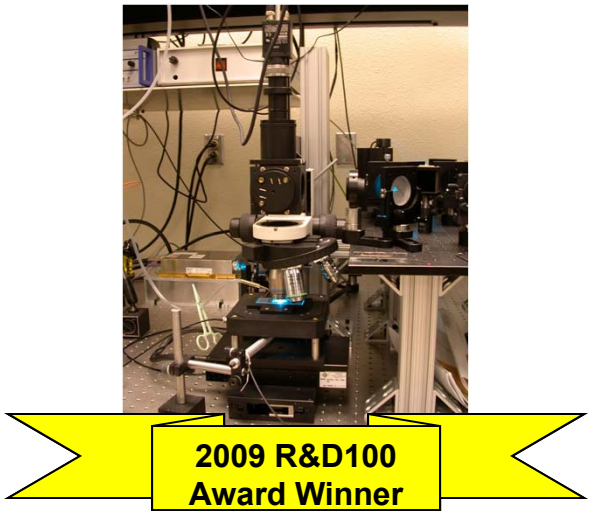








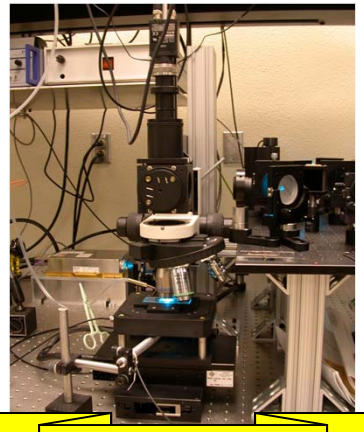
Hyperspectral Confocal Fluorescence Microscopy -



M. B. Sinclair, et. al. Applied Optics, 45, 6283-6291 (2006)

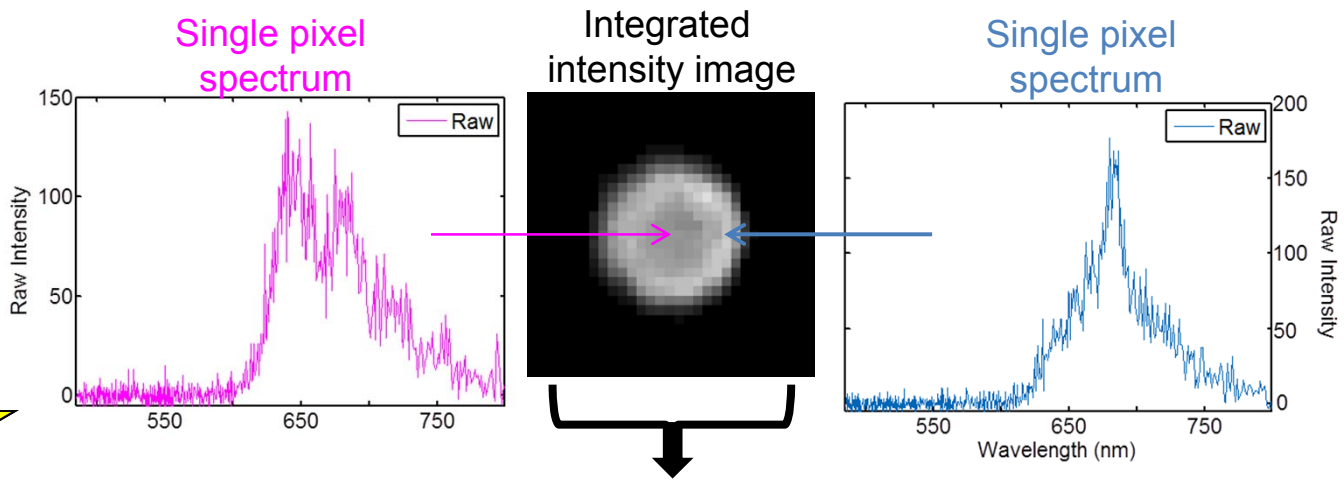
- Custom built
- Lateral res. = 250 nm
- Axial res. = ~0.6 μm
- Spectral range 490-800 nm
- Spectral res. = 1-3 nm
- Acquisition rate = 4100 spectra/s

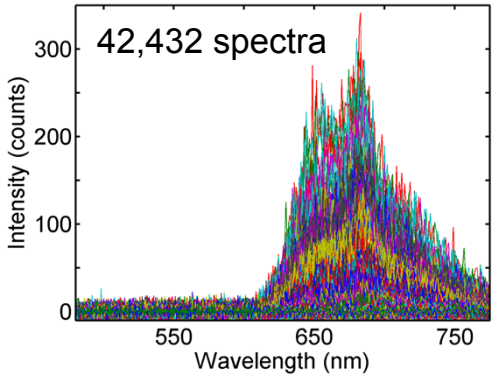
Hyperspectral Confocal Fluorescence Microscopy - Records an entire emission spectrum at each spatial voxel



2009 R&D100 Award Winner

M. B. Sinclair, et. al. Applied Optics, 45, 6283-6291 (2006)





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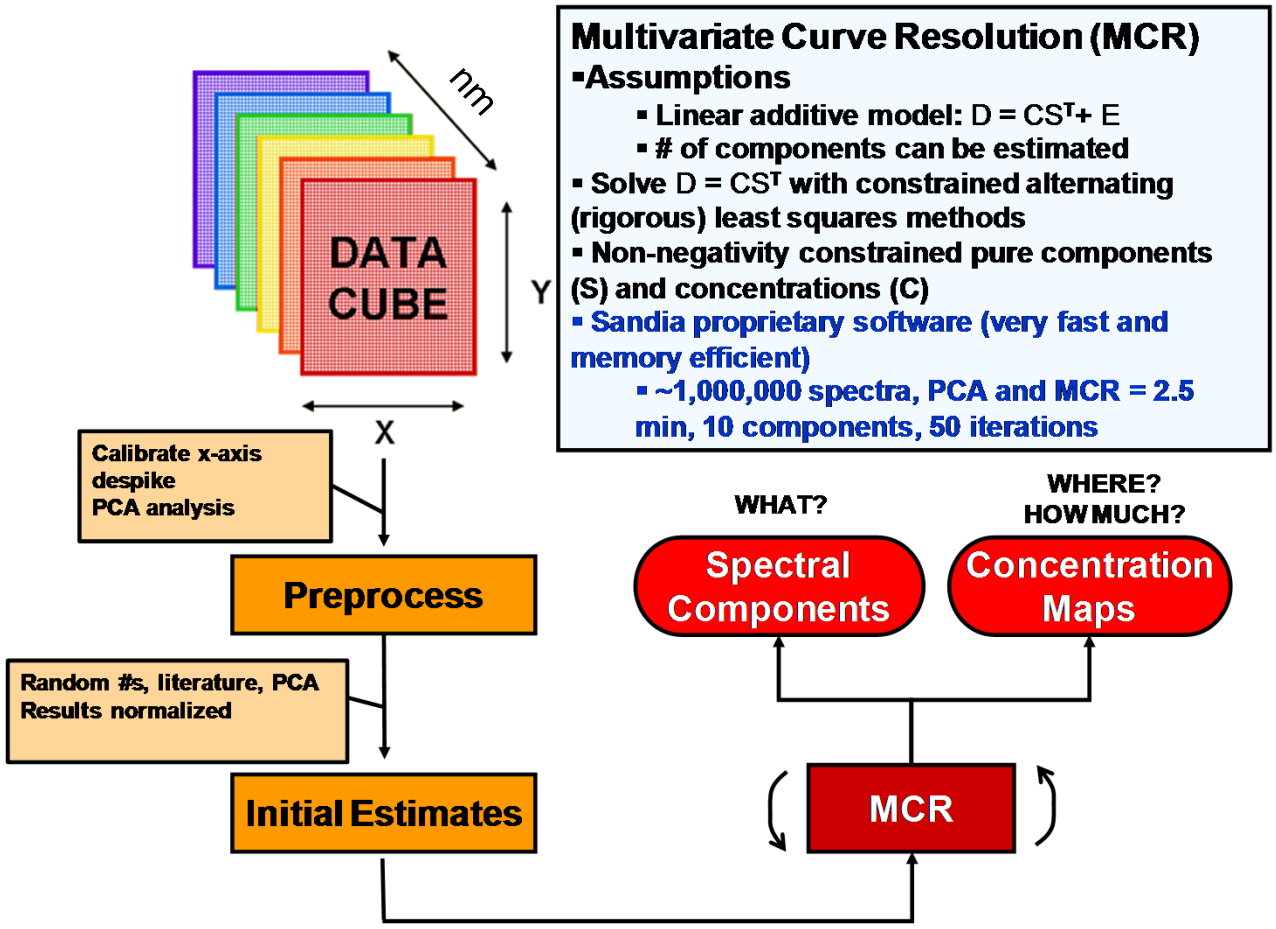
Multiple images combined into a composite image

Need a robust and efficient analysis tool
Multivariate Curve Resolution

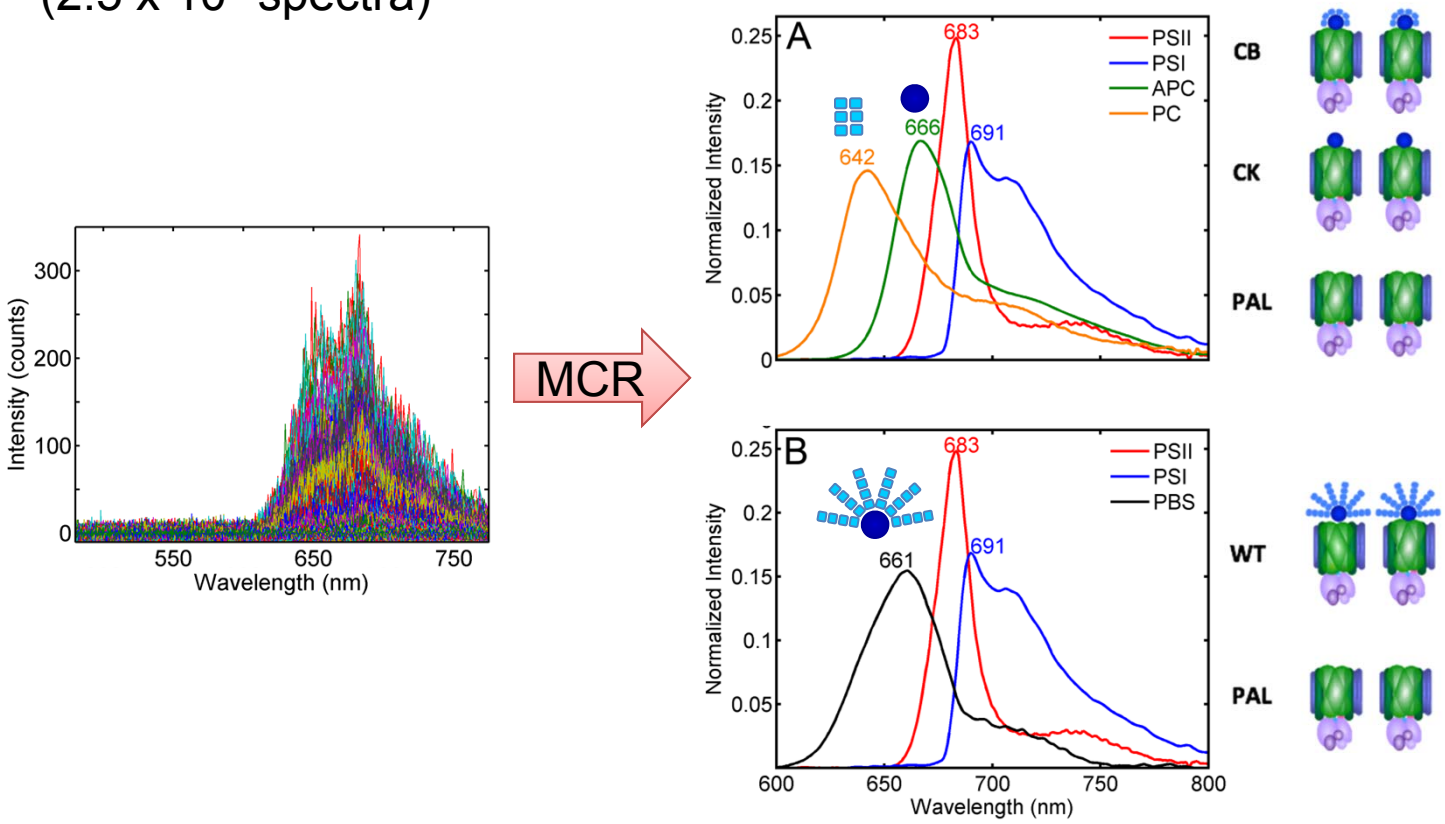
Multivariate Curve Resolution – Mathematical isolation of pure spectral components and generation of independent concentration maps



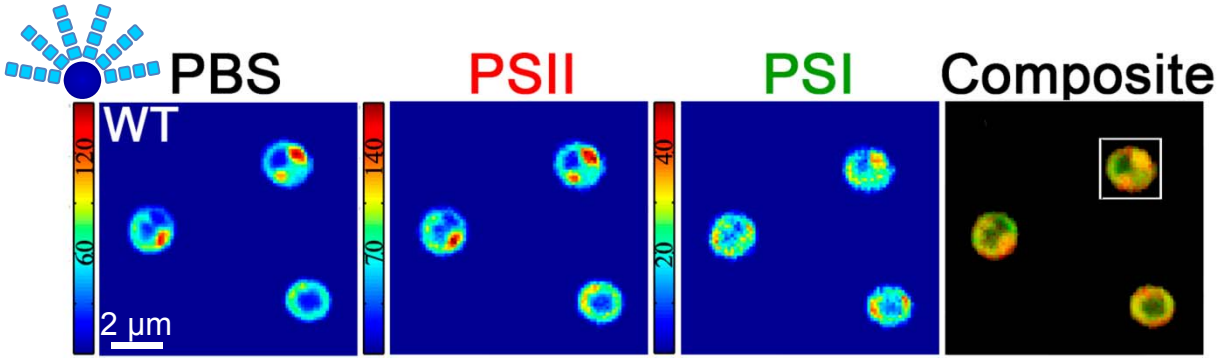
Jones et al., (2008) J Chemom. 22:482-490

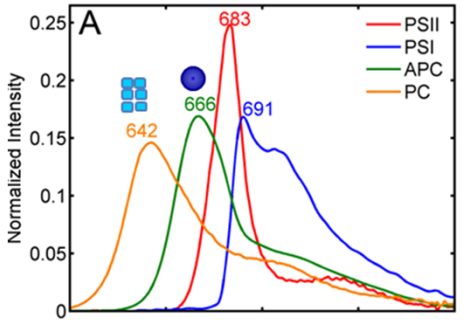
Assignment of spectral signatures

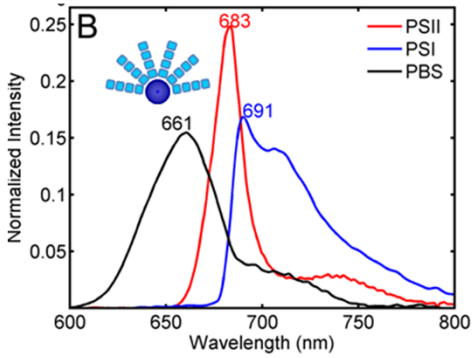
60 images combined
2-3 biological replicates for WT, CB, CK and PAL
303 cells analyzed
(2.5 x 10^6 spectra)



MRC derived concentration maps – Reveals subcellular location and relative quantification of light-harvesting antenna







Collins AM, Liberton M, et. al. (2012). Plant Physiology. DOI:10.1104/pp.111.192849

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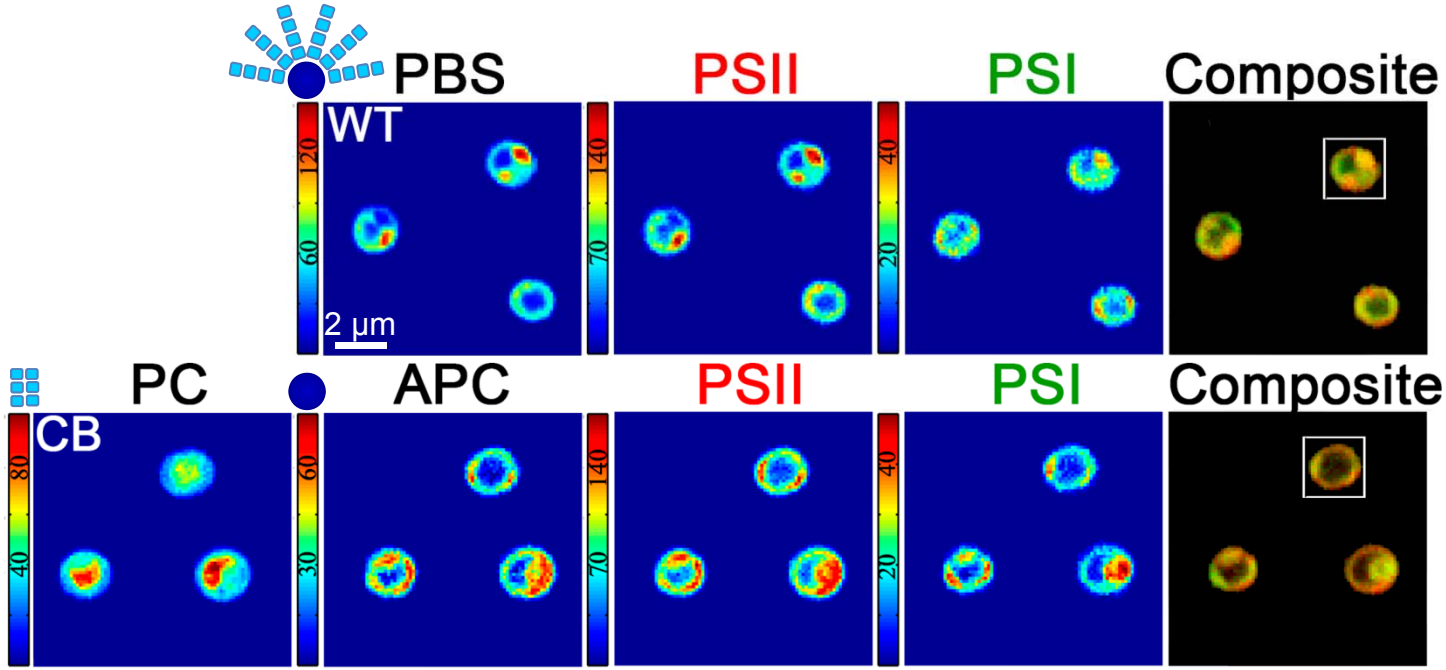

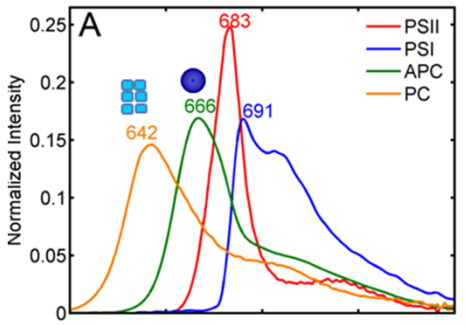

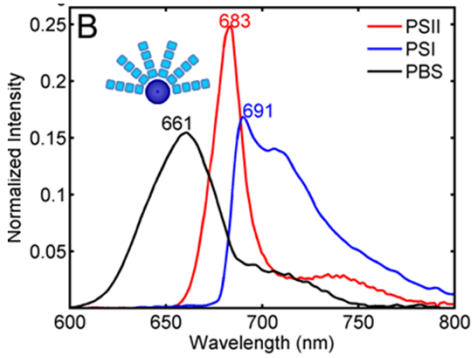

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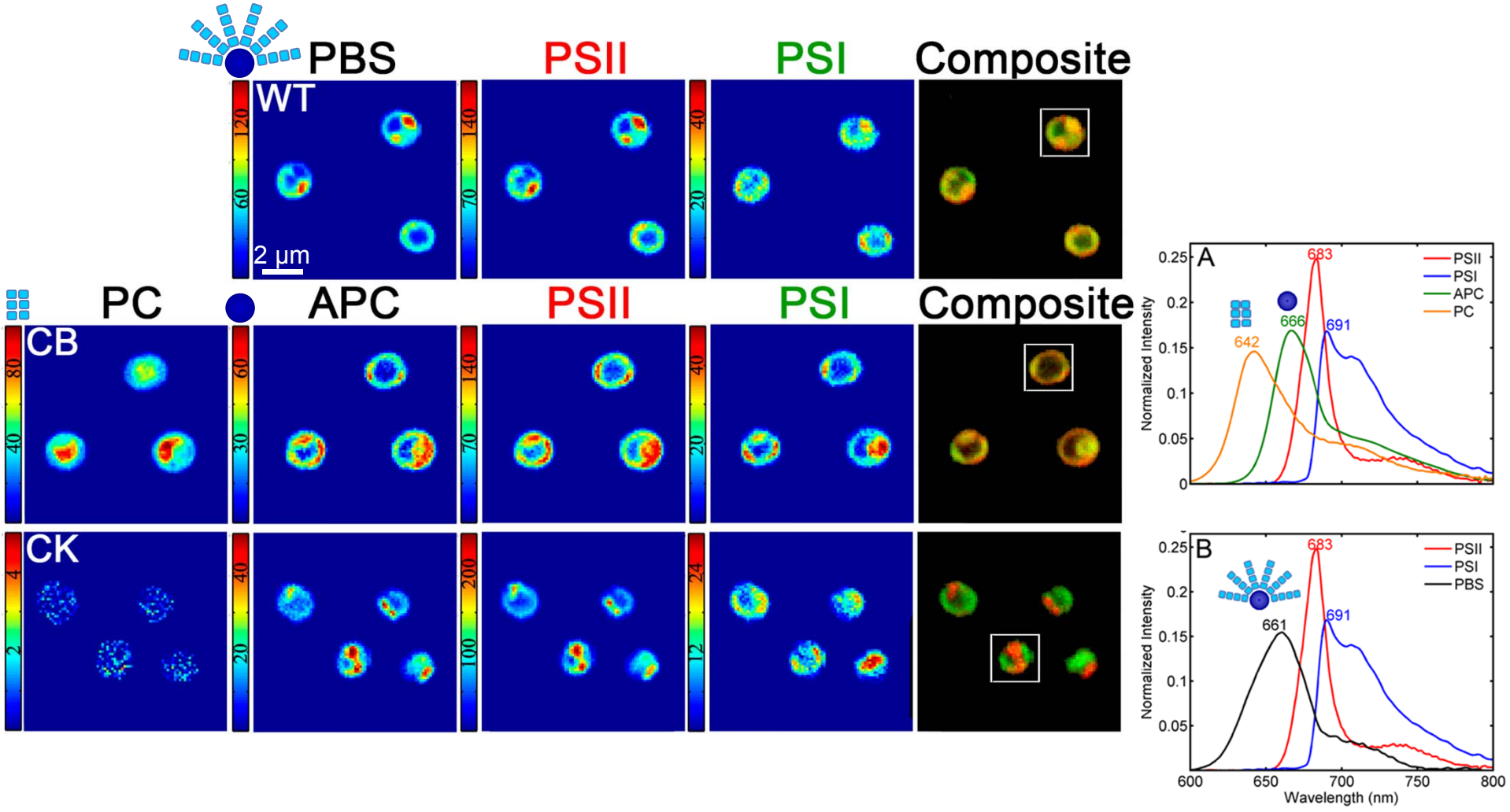

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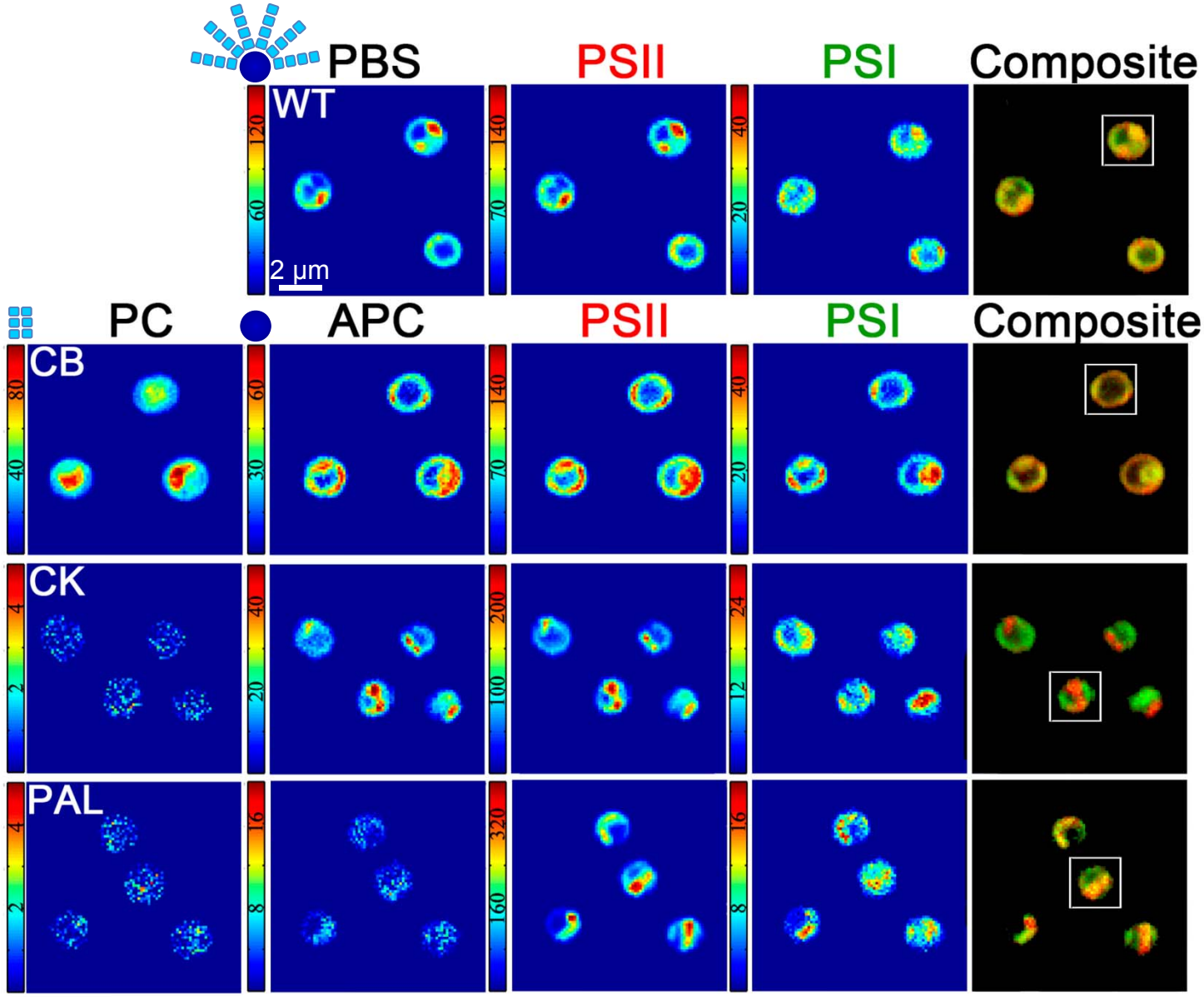

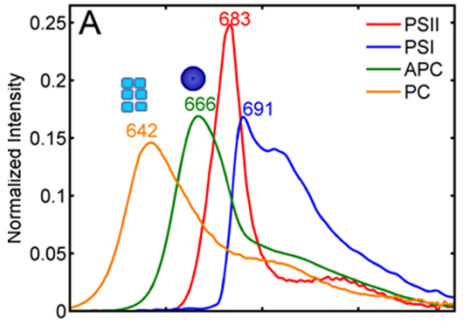

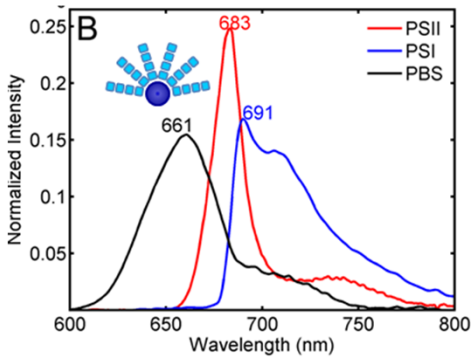

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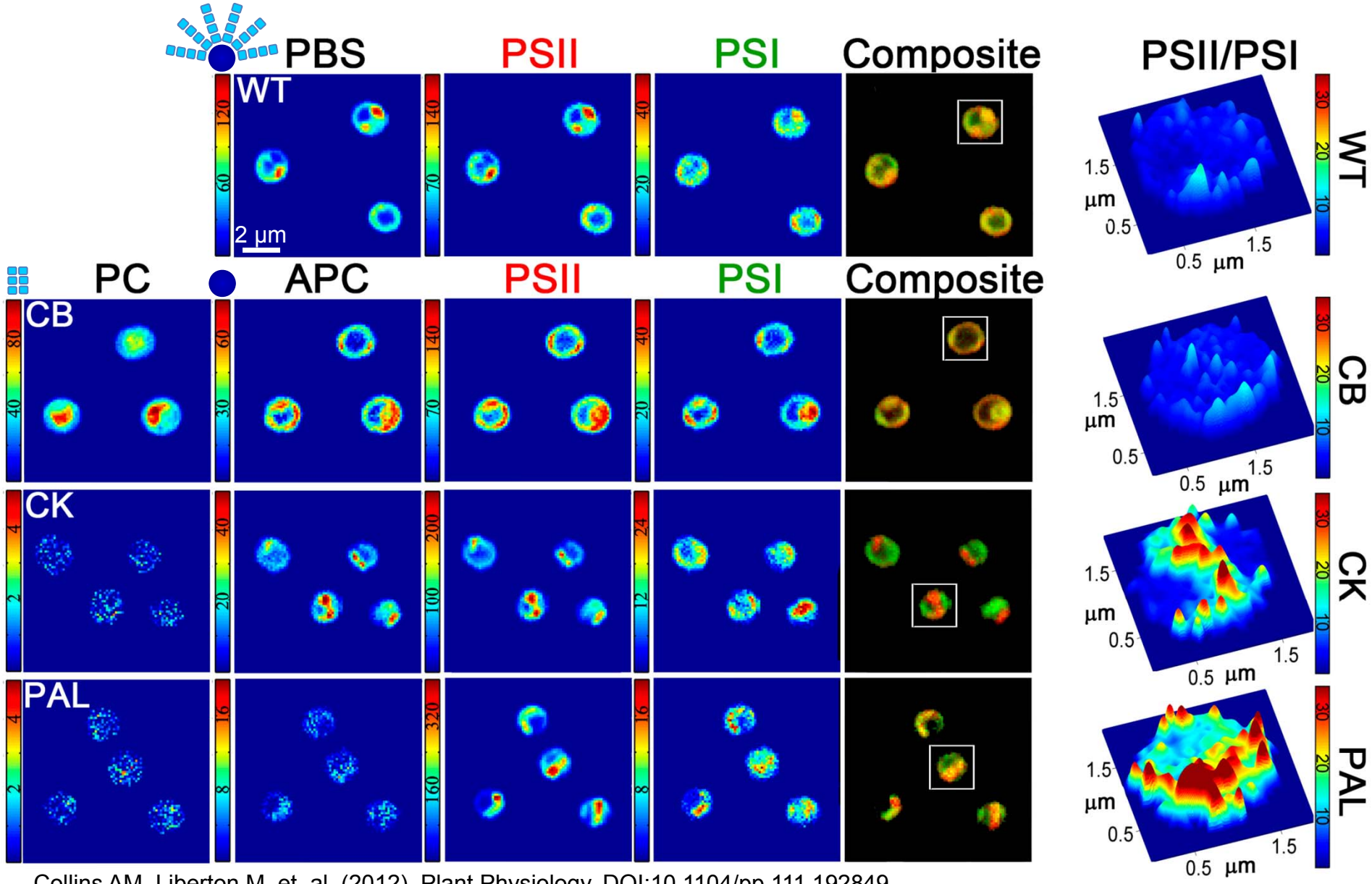
MRC derived concentration maps – PSII/PSI ratio highlights spatial segregation of the two photosystems



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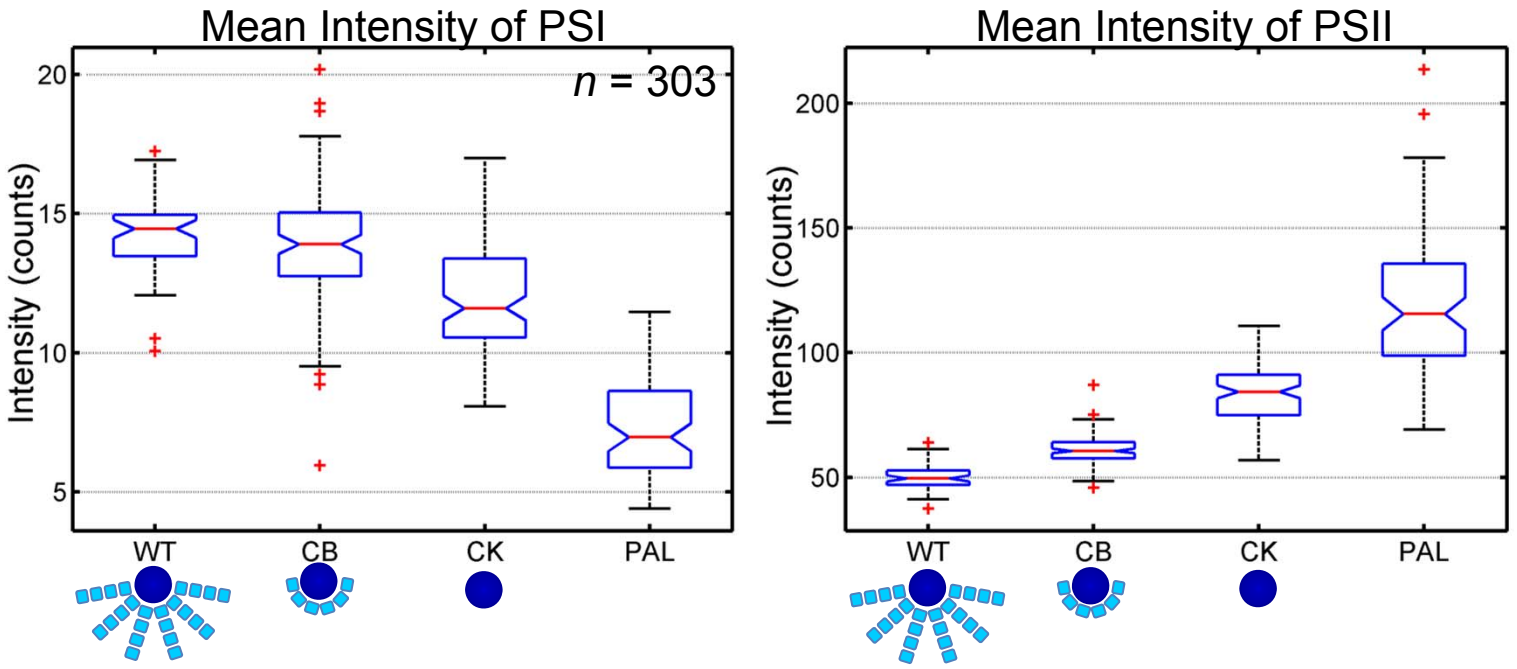
Statistical significant of component intensities

- PSI and PSII intensities were averaged on a per-cell basis
- Nested ANOVA indicated greatest variance is between strains (99% confidence)



As phycobilisome is truncated, PSII ↑ while PSI ↓
PSII and PSI are modulated in response to light sensitization

Conclusions

- Flexibility of *Synechocystis* photosynthetic apparatus to changes in light sensitization
- Shows the importance of phycobilisome antenna to balance excitation delivery
- Unexpected spatial segregation of photosystems
  what is the function of the PSII domains?
- Modulation of photosystems ratio is necessary to ensure linear electron transport – proper electron flux

Where do we go from here?

- Hyperspectral confocal fluorescence microscopy is amenable to a wide variety of systems
  Natural, Bio-hybrid, and Biomimetic
- Allows for elucidation of spatial-temporal-spectral relationships at diffraction limited resolution

Acknowledgements

Sandia National Laboratories

Jerilyn A. Timlin →

Howland D.T. Jones

Omar F. Garcia

Michael B. Sinclair

Michelle Raymer

Washington University in St. Louis

←**Himadri Pakrasi**

Michelle Liberton →

Hyperspectral program development

David M. Haaland

MCR Software & Algorithm Development

Michael R. Keenan

Mark H. Van Benthem

Greg A. Poulter

Funding

Photosynthetic Antenna Research Center PARC

DEPARTMENT OF ENERGY · UNITED STATES OF AMERICA ·

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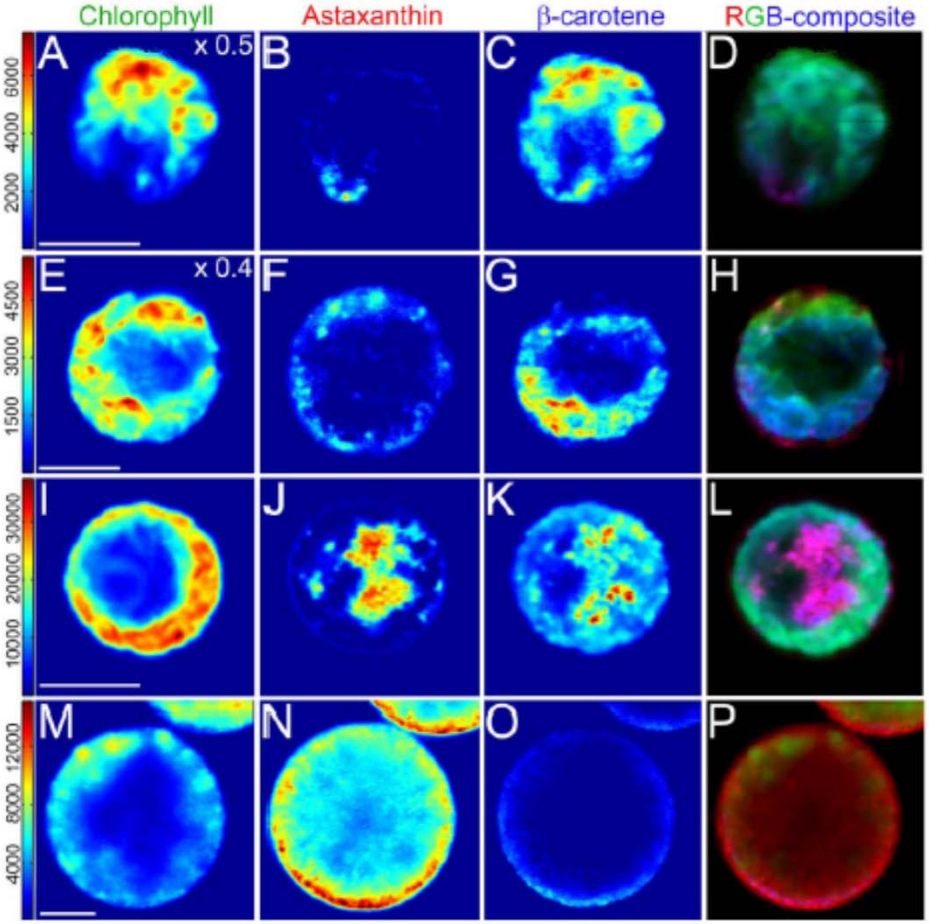
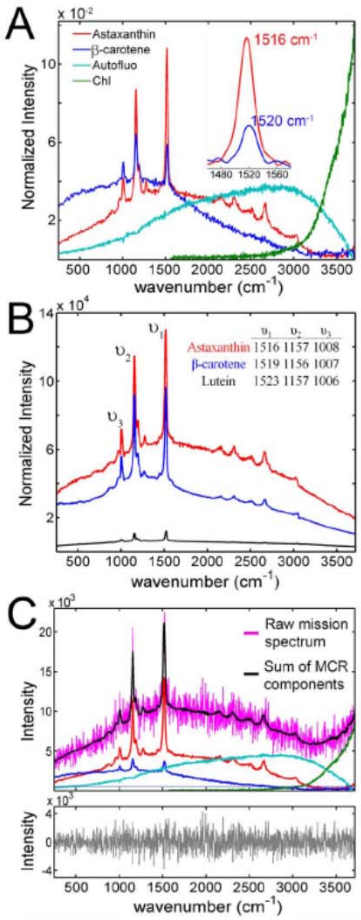
PLoS one

Carotenoid Distribution in Living Cells of *Haematococcus pluvialis* (Chlorophyceae)

Aaron M. Collins[1], Howland D. T. Jones[1], Danxiang Han[3], Qiang Hu[3], Thomas E. Beechem[2], Jerilyn A. Timlin[1]*

1 Department of Bioenergy and Defense Technologies, Sandia National Laboratories, Albuquerque, New Mexico, United States of America, **2** Department of Nanomaterials Sciences, Sandia National Laboratories, Albuquerque, New Mexico, United States of America, **3** Laboratory for Algae Research and Biotechnology, Department of Applied Sciences and Mathematics, Arizona State University, Mesa, Arizona, United States of America





In vivo hyperspectral confocal fluorescence imaging to determine pigment localization and distribution in cyanobacterial cells

Wim F. J. Vermaas*†, Jerilyn A. Timlin‡, Howland D. T. Jones‡, Michael B. Sinclair‡, Linda T. Nieman‡§, Sawsan W. Hamad*, David K. Melgaard‡, and David M. Haaland‡

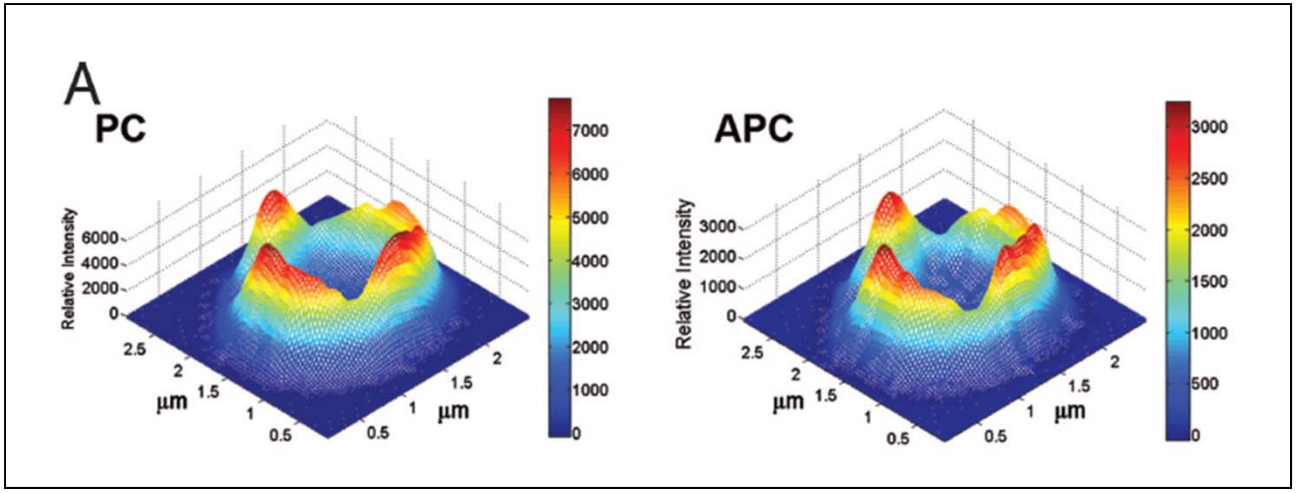
*School of Life Sciences and Center for Bioenergy and Photosynthesis, Arizona State University, Box 874501, Tempe, AZ 85287-4501; and ‡Sandia National Laboratories, MS0895, Albuquerque, NM 87185

Edited by Elisabeth Gantt, University of Maryland, College Park, MD, and approved January 25, 2008 (received for review August 27, 2007)

Hyperspectral confocal fluorescence imaging provides the opportunity to obtain individual fluorescence emission spectra in small (≈0.03-μm^3) volumes. Using multivariate curve resolution, individual fluorescence components can be resolved, and their intensities can be calculated. Here we localize, *in vivo*, photosynthesis-related pigments (chlorophylls, phycobilins, and carotenoids) in wild-type and mutant cells of the cyanobacterium *Synechocystis* sp. PCC 6803. Cells were excited at 488 nm, exciting primarily phycobilins and carotenoids. Fluorescence from phycocyanin, allophycocyanin, allophycocyanin-B/terminal emitter, and chlorophyll *a* was resolved. Moreover, resonance-enhanced Raman signals and very weak fluorescence from carotenoids were observed. Phycobilin

cytoplasmic membrane of cyanobacteria, whereas Chl is not (6, 7). Additional light-harvesting capability, primarily for PS II, is provided by phycobilisomes, which are pigment-binding complexes in the cytoplasm that associate with thylakoids to enable energy transfer to Chl (8, 9). Phycocyanin (PC), allophycocyanin (APC), and allophycocyanin-B (APC-B) are the main phycobilisome pigments in *Synechocystis* sp. PCC 6803 (10).

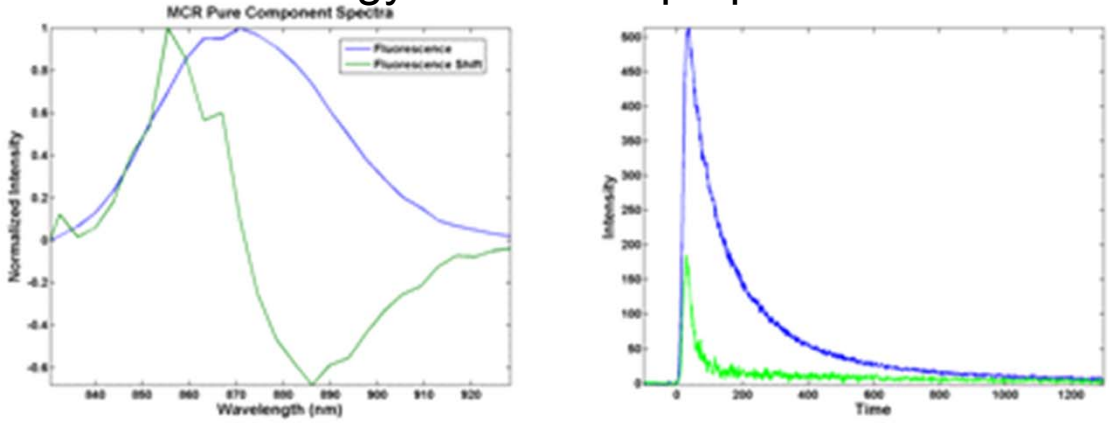
Chl and phycobilisome pigments fluoresce at room temperature with spectral maxima in the 640- to 700-nm range. PC emits fluorescence with an ≈650-nm maximum, APC at 665 nm, and APC-B at 675 nm, and the main emission wavelength of Chl is at 685 nm (11). Phycobilisomes are highly fluorescent in isolated



MCR and Other Modalities

Energy Transfer in purple bacteria

Streak Camera Data



Carotenoid distribution in *H. pluvialis*

Resonance Enhanced Raman