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Light-Harvesting Pigment Distribution in Algae and Cyanobacteria Determined by Hyperspectral Confocal Fluorescence Microscopy

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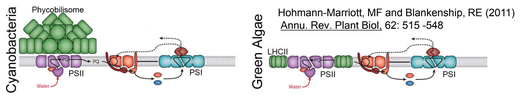
Highlights

- **Hyperspectral confocal Raman and fluorescence microscopy of pigments in living photosynthetic cells**
- **Subcellular localization and quantification of multiple over-lapping pigments**
- **Global distribution and architecture of photosynthetic complexes in native environment**

Introduction

Photosynthetic organisms possess diverse light-harvesting antennas to use various colors and qualities of light. For example, green algae are very efficient at utilizing most of the visible light spectrum with the exception of the so-call "green gap" (500-600 nm). On the other hand, cyanobacteria are often found in nature, living beneath green algae and have evolved unique photosynthetic antennae called phycobilisomes, which absorbs the light that filters through the algal layer above. Understanding the global distribution of natural photosynthetic pigments from various organisms can provide the framework for the next-generation of energy conversion systems.



Experimental Parameters

Fluorescence Microscope

- Custom built
- 488 nm laser excitation
- 2-photon excitation
- 60x Oil objective
- Lateral res. = 250 nm
- Axial res. = ~0.6 µm
- Spectral range 490-800 nm
- Spectral res. = 1-3 nm
- Acquisition rate = 4100 spectra/s
- Sinclair, MB., et al. (2006) Appl. Opt. 45, 3283-3291.

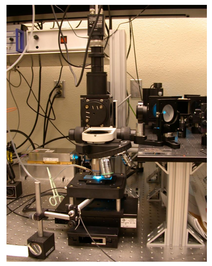
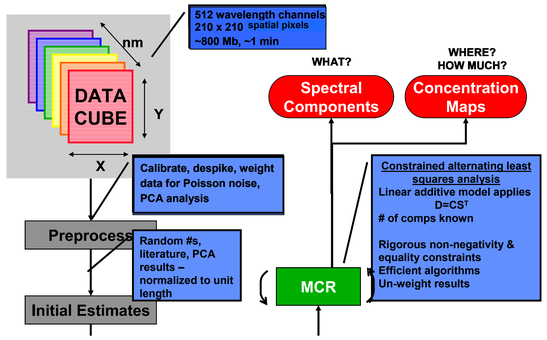


Image Analysis Flow Chart

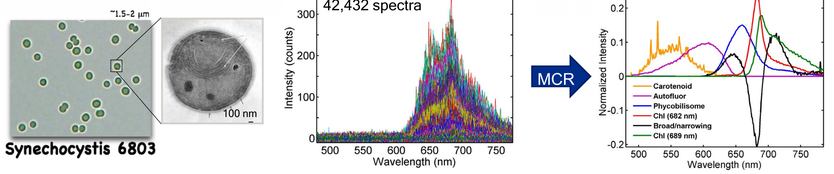
Multivariate Curve Resolution (MCR)

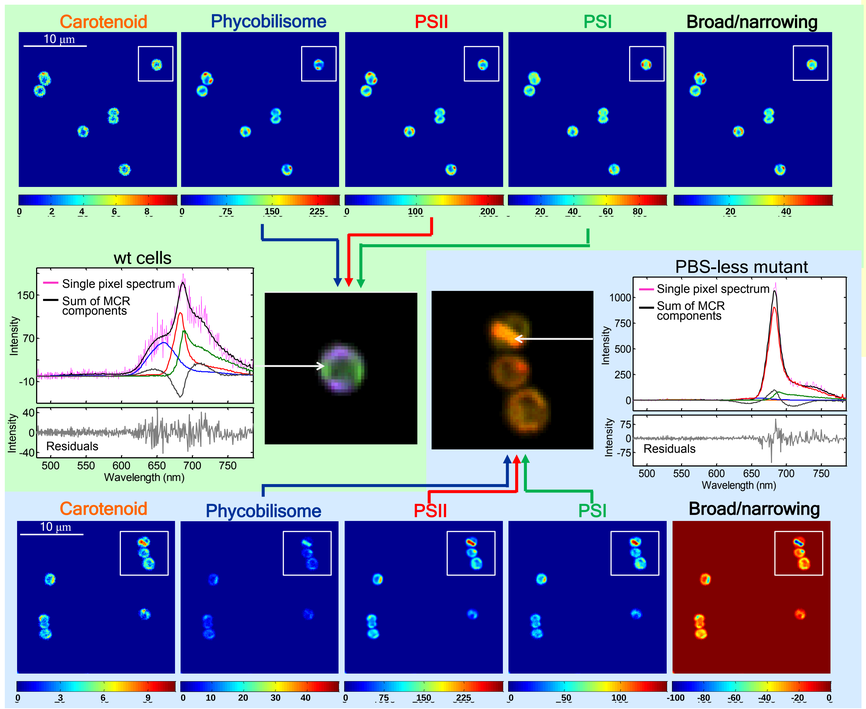
- Discover & quantify all emitting species in a sample simultaneously with no *a priori* knowledge
- Mathematical isolation of pure spectral components, independent concentration maps
- Jones et al., (2008) J Chemom. 22:482-490 and references therein



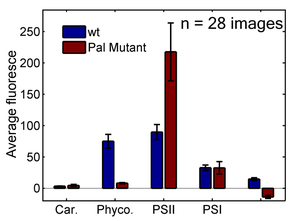
Cyanobacteria

Synechocystis sp. PCC 6803 is a model, genetically amenable cyanobacterium that use a combination of carotenoid, phycobiliprotiens, and chlorophyll to harvest light. To explore the distribution of these pigments *in vivo*, wild-type and a phycobilisome-lacking mutant were compared.





Robust MCR algorithms were applied to a composite image that is the sum of spectral images for both wt and mutant cells and pure spectral component model was generated that accounted for **99.94%** of the spectral variance. The pure spectral components were carotenoid, autofluorescence, phycobilisomes (PBS), PSII, PSI, and spectral **broadening /narrowing**. The analysis also generate concentration maps that detail the spatial location and relative abundance of each spectral component. In wt cells, PBS and PSII are co-localized while PSI is often found in regions depleted of PBS and PSII. Carotenoids are located with PSII and PSI but also around the cells periphery. The PBS-less mutant shows dramatically altered pigment distribution. The intensity of PSII was increased >200% and was located in very concentrated regions while PSI remained unchanged.



Acknowledgements

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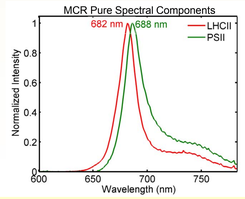
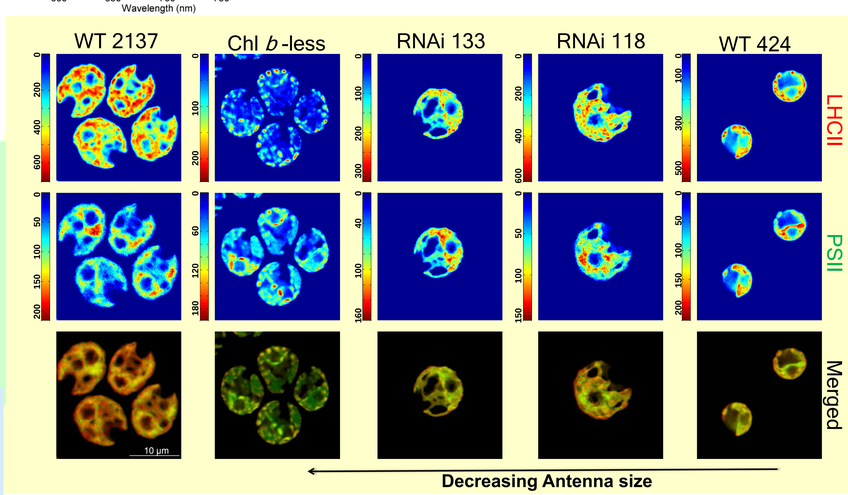
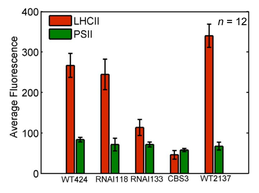
Green Algae

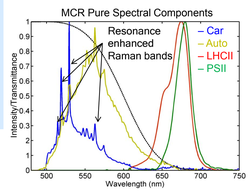


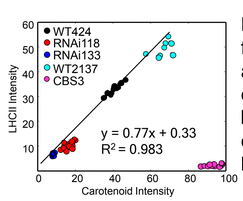
Unicellular green algae lack PBS and harness solar energy using carotenoids, Chl *a*, and Chl *b*. We investigated wt and mutant lines of *Chlamydomonas reinhardtii* with truncated light-harvesting antennas to probe how decreasing Chl content influences global architecture of the chloroplast and pigment distribution. MCR analysis of these cells lines yields two spectral components representing LHCII and PSII.



Wild-type cells show typical chloroplast morphology. LHCII and PSII were generally co-located however the chloroplast periphery appeared to be enriched in LCHII and the interconnecting regions had more PSII. The Chl *b*-less mutant was largely devoid of LHCII and the chloroplast morphology had regions that were punctate. The two intermediate-sized antenna mutants (RNAi188 and RNAi133) had spectral component distributions similar to wt cells however the overall chloroplast morphology was disorganized. In all cells lines, the concentration of PSII is constant.







Repeating the above analysis with a filter that down weights the Chl emission allows for information about the carotenoids to become more obvious. A linear relationship is evident between carotenoid and LHCII in the RNAi lines but not for the Chl *b*-less mutant.

Conclusions

- **Hyperspectral confocal fluorescence microscopy and multivariate analysis demonstrates the ability to distinguish the subcellular localization and identity of multiple, overlapping photosynthetic pigments *in vivo*.**
- **Quantification and tracking of pigment distribution in wild-type and mutant cell lines**
- **Segregation of PSII and PSI in wt cells of cyanobacteria and compositional reorganization of the photosynthetic complexes in thylakoids when phycobilisomes are absent**
- **Amount of PSII is constant in *Chlamydomonas* cells when light-harvesting antenna complexes are diminished or depleted. Amount of carotenoid is linearly related to the amount of LHCII in RNAi mutants.**