

Quantitative Biochemical Characterization of *Chlamydomonas reinhardtii* Mutants with Altered Antenna Size by Hyperspectral Confocal Fluorescence Microscopy

Aaron M. Collins¹, Sangeeta Negi², Omar F. Garcia¹, Richard T. Sayre² Jerilyn A. Timlin¹

¹Sandia National Laboratories, Albuquerque, NM • ²Donald Danforth Plant Science Center, St. Louis, MO

Highlights

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

Introduction

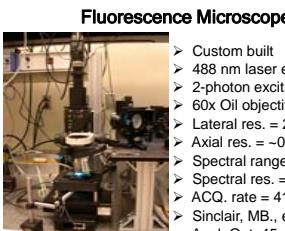
Algae and plants have ability to adapt in different light conditions and have evolved complex and dynamic responses to variable light intensities. Solar energy is balanced by changing the absorption cross-section of the antenna system through state-transitions which involves redistribution of light harvesting complex II between the two photosystems. Under high-light conditions, additional photoprotective mechanisms are employed to avoid photodamage such as non-photochemical quenching of the excited state of chlorophyll as well as regulation of the antenna system at the genetic lever. We present hyperspectral confocal fluorescence microscopy (HCFM) of novel mutants of *Chlamydomonas reinhardtii* with altered antenna size and pigment stoichiometries to probe the global distribution and concentration of their photosynthetic complexes in the membrane. We resolve LHCII, PSII and carotenoid from the analysis of living cells and quantify their relative abundance.

Experimental Parameters

Cell line	Type	Mutation
424 WT	Wall-less	Parent for RNAi lines
118 CR	Wall-less	CAO (Chl α oxygenase) knock down
133 CR	Wall-less	CAO (Chl α oxygenase) knock down
CC2137 WT	Walled	Parent for Chl β -less
Chl β -less	Semi walled	CAO Chl α oxygenase) knockout
CW 15 WT	Wall-less	Parent for PHOT knock out
G5	Wall-less	Photoprotein knockout
UV4	Wall-less	Parent for PHOT knock out
A4	Wall-less	Photoprotein knockout
Chl β -less	Semi walled	CAO Chl α oxygenase) knockout
CC 125	Walled	Parent for FUD mutants
FUD-34*	Walled	psbC knockout
FUD-7*	Walled	psbA knockout

*Grown heterotrophically

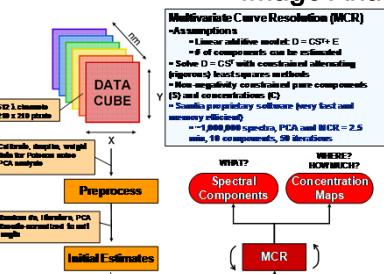
Various cell lines of *C. reinhardtii* were grown on HS media under $100 \mu\text{m m}^{-2}\text{s}^{-1}$ fluorescent light with a 16 hr. photoperiod. Cells were harvested during exponential growth phase, concentrated by gentle centrifugation and loaded onto agar coated slides for imaging.



Fluorescence Microscope

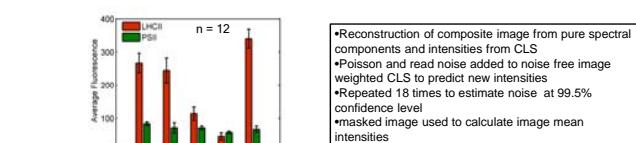
- Custom built
- 488 nm laser excitation
- 2-photon excitation
- 60x Oil objective
- Lateral res. = 250 nm
- Axial res. = ~0.6 μm
- Spectral range 490-800 nm
- Spectral res. = 1-3 nm
- ACQ. rate = 4100 spectra/s
- Sinclair, MB., et al. (2006) Appl. Opt. 45, 3283-3291.

Image Analysis

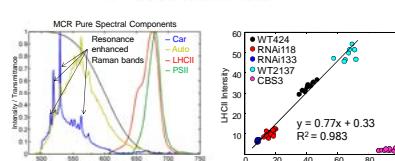


Multivariate Curve Resolution (MCR)

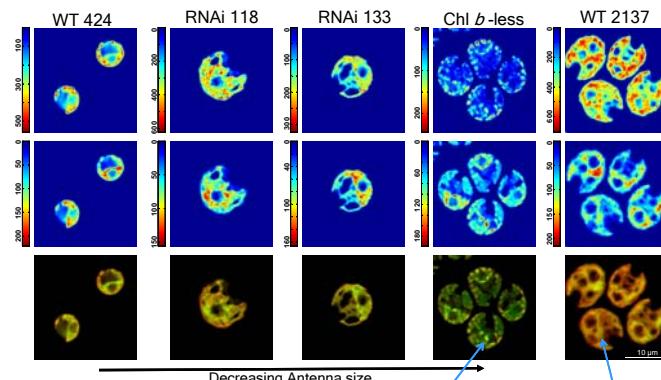
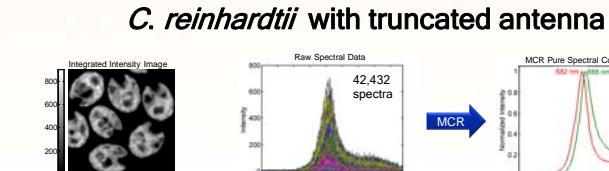
- Discover & quantify all emitting species simultaneously with no *a priori* knowledge
- Mathematical isolation of pure spectral components, independent concentration maps
- Jones et al., (2008) J Chemom. 22:482-490 and references therein



- Reconstruction of composite image from pure spectral components and intensities from CLS
- Poisson and read noise added to noise free image weighted CLS to predict new intensities
- Repeated 18 times to estimate noise at 99.5% confidence level
- masked image used to calculate image mean intensities

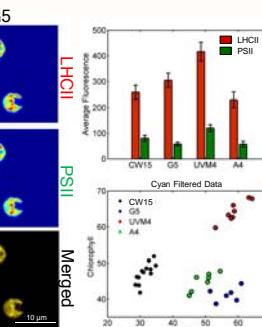


Repeating the above analysis with a filter that down weights the Chl emission allows for information about the carotenoids to become more obvious. A linear relationship is evident between carotenoid and LHCII in the RNAi lines but not for the Chl β -less mutant.



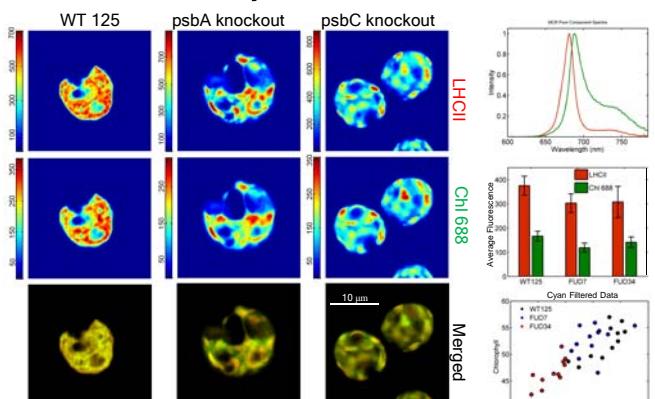
Wild-type cells show typical chloroplast morphology. LHCII and PSII were generally co-located however the chloroplast periphery appeared to be enriched in LHCII and the interconnecting regions had more PSII. The Chl β -less mutant is largely devoid of LHCII and the chloroplast morphology has regions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

C. reinhardtii photoprotein mutants



The G5 photoprotein mutant possesses about half the LHCII and PSII compared to its parent strain (CW15) while the A4 photoprotein mutant has essentially unaltered chlorophyll content. The overall morphology of the chloroplast is comparable across all cell lines.

Photosystem II mutants



psbA and psbC deletion mutants have similar LHCII content compared to their parent strain as well as a MCR component similar to PSII (Chl 688). Despite the similar pigment content, the distribution of these components in the mutant strains are uniquely organized compared to the WT.

Conclusions

- Hyperspectral confocal fluorescence microscopy of pigments in living *Chlamydomonas reinhardtii* cells
- Subcellular localization and quantification of multiple over-lapping components attributed to LHCII and PSII in the chloroplast membrane
- Global distribution and architecture of photosynthetic complexes in native environment for various mutants