Visualization of Photosystem II Distribution in Encapsulated Chlamydomona reinhardtii

SAND2014-16650PE

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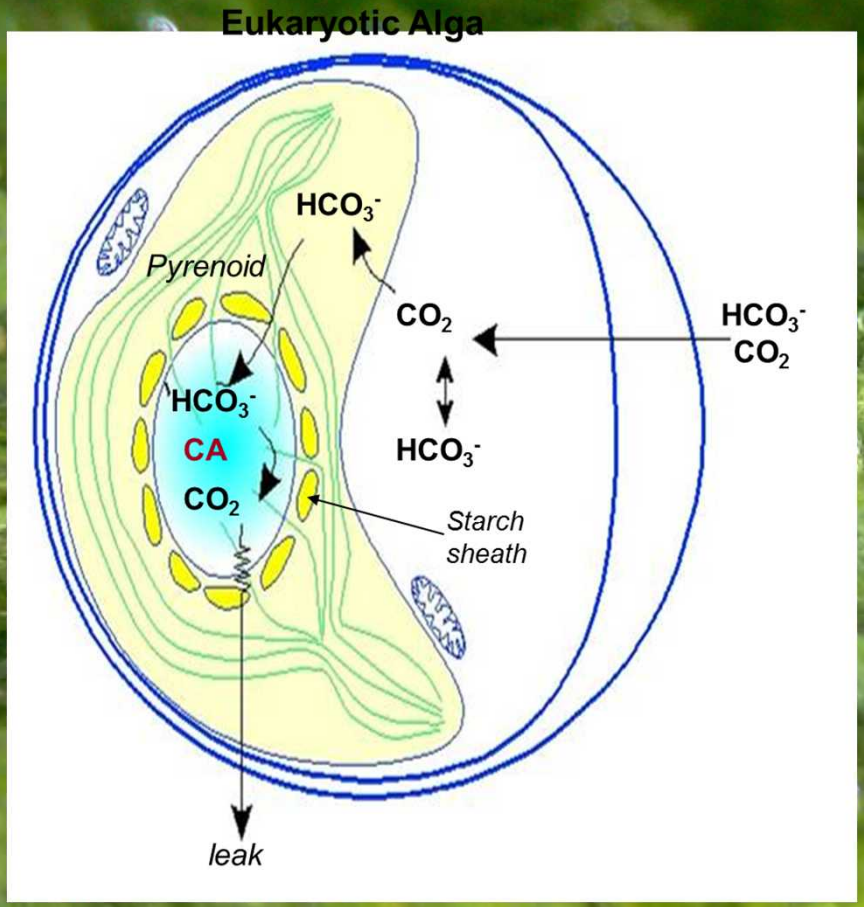
Light: The Drive of Photosystem II

C.hlamydomonas rheinhardtii



Hyperspectral Microscopy

*.hsi files were converted to s3d files using batch script that reads in da
with Lidke code, then calls Creates3d.m. Expansion factor was utilized
improved precision.

*.s3d files were opened individually in ShowMe3D.exe and checked for
integrity/quality.

Sets of *.s3d files that were to be compared were combined into a compos
data set using <Preprocess> function in ImageMCR.

MCR was run on resulting composite files, excluding any individual
traces known to contain saturated pixels (more on this later – Slide 4).

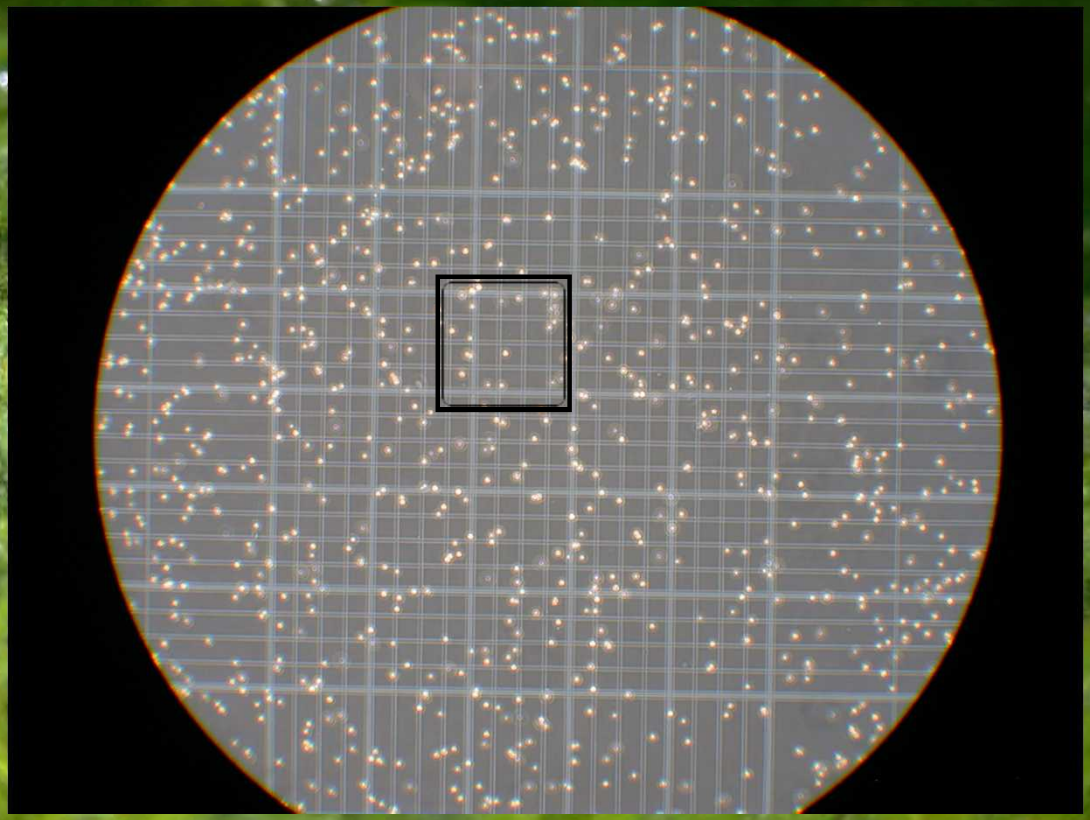
Cell Concentration Hemocytometer

$$C = \Sigma(N/V)$$

0.04 square mm

N is cells in squares

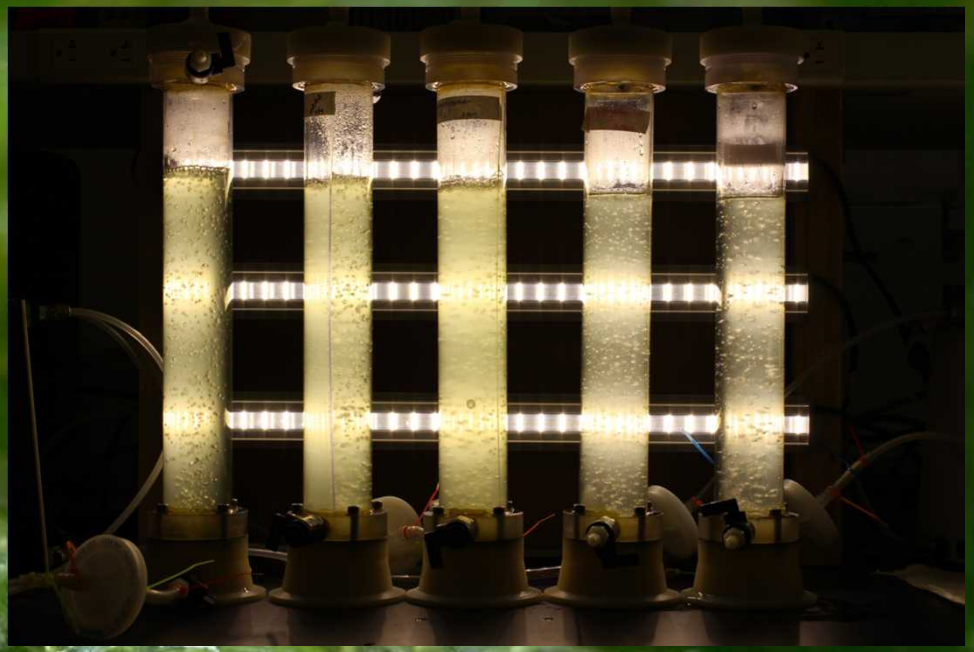
V is volume calculated per # square counted



Chlorophyll assay

Using Centrifuge to separate cells from media then freeze to shock chlorphyll out from cells and vortexing (spinning) methanol to extract chlorophyll from cells. Again later centrifuge down to remove cells body from chlorophyll and methanol. Use methanol as zero, use spectrometer at 750, 665.2, and 650 wavelength

Culturing with Bioreactor

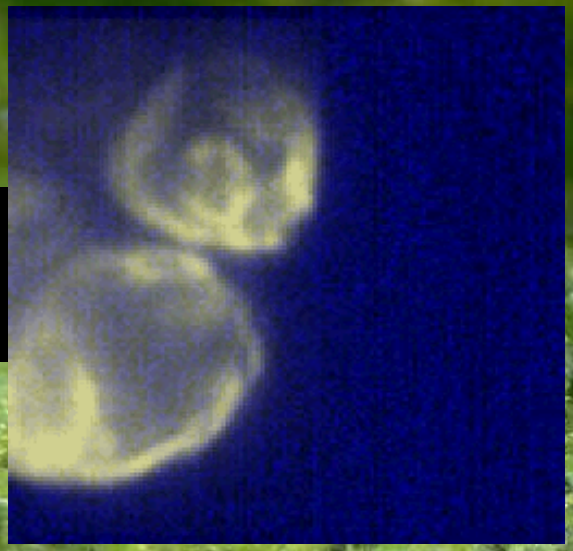


Sample	750	665.2	652
1	-0.1381	0.4889	0.1845
2	-0.1649	0.4489	0.1620
3	0.0311	0.6747	0.3727

0.006708552 mg/mL

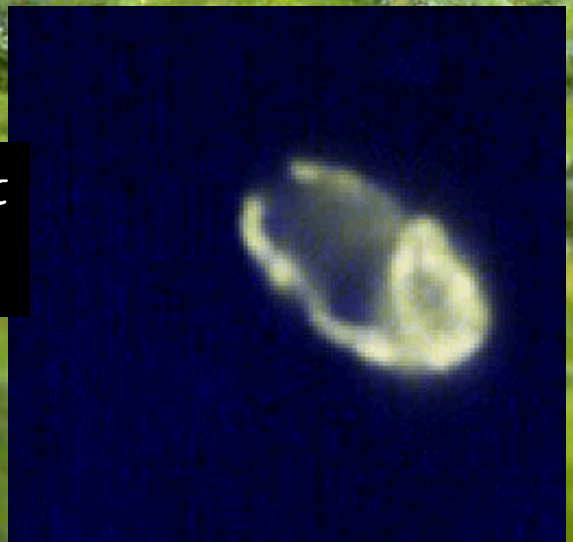
Side	A	B	C	D	E
1	28	30	23	25	36
2	25	28	32	30	22

Ecapsulation



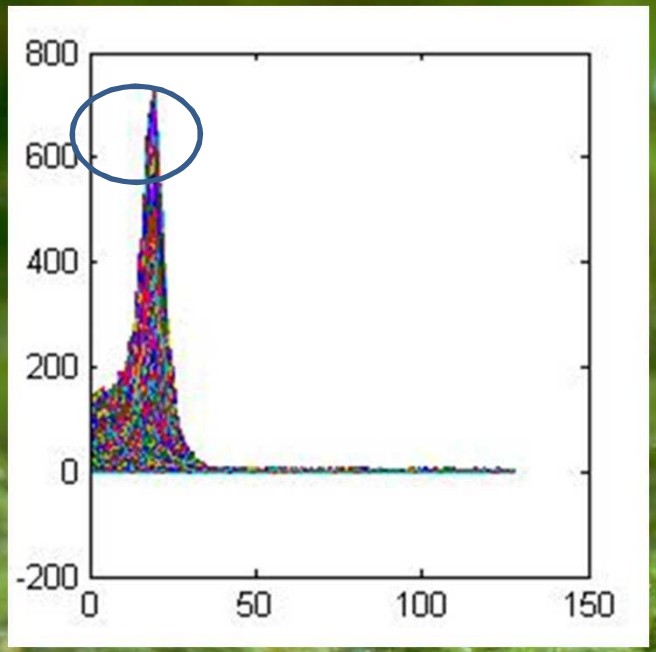
High Light
Thick

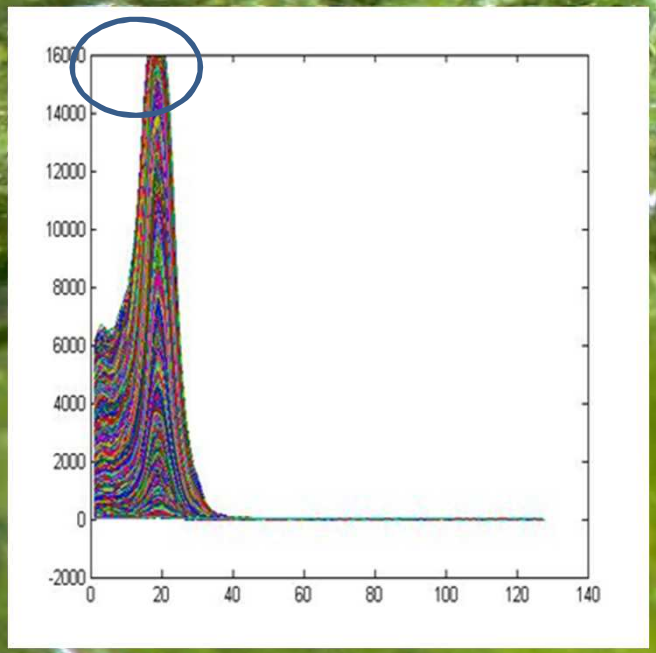
Low Light
Thick



High Light
Thin

Low Light
Thick

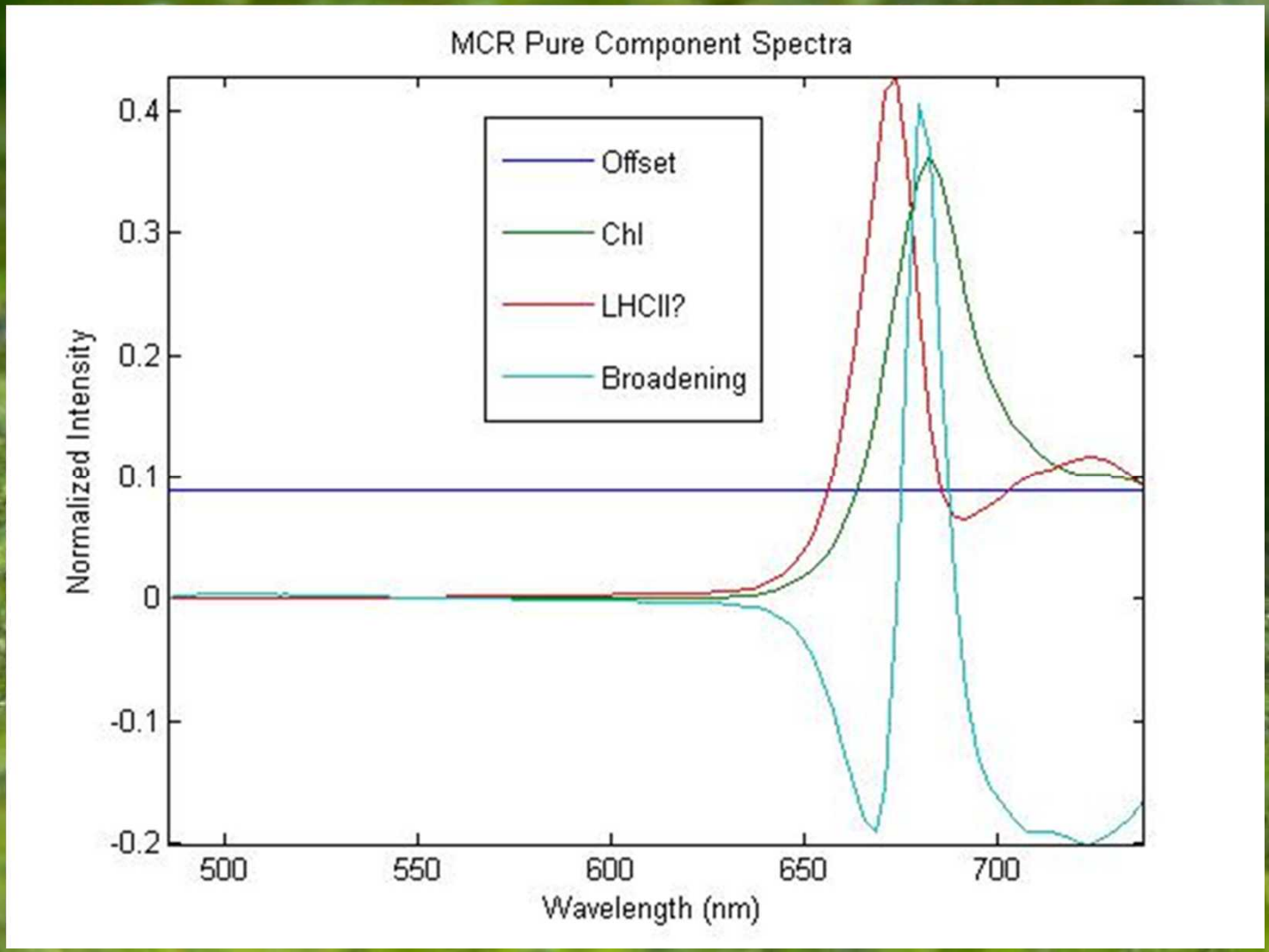


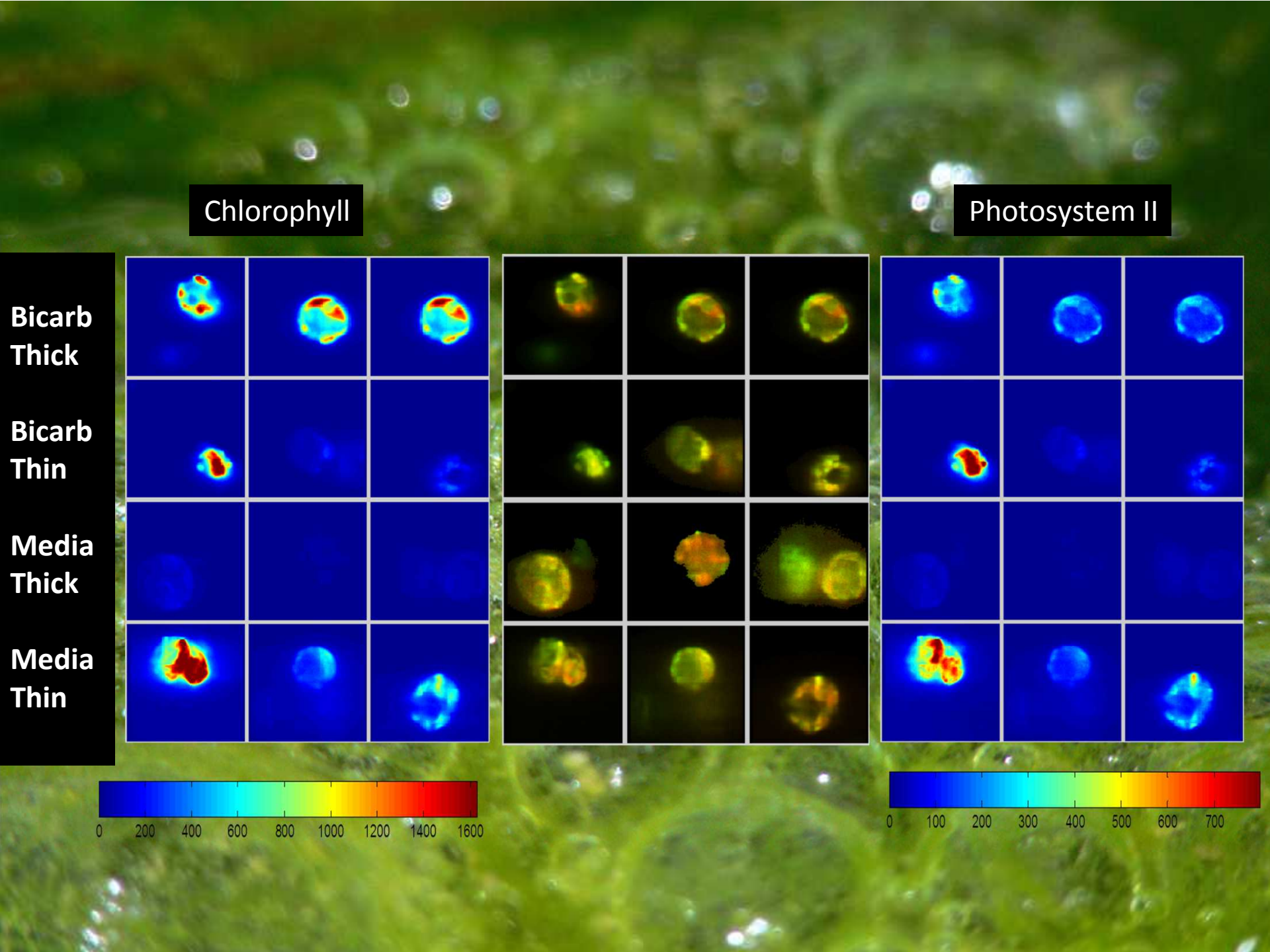


- The baseline does not appear to be a linear offset as we are used to. Instead it seems to slope upward toward the red wavelengths. Note: the data is often quite a bit below zero.
- This could be adequately accounted for if we had some dark current images of the same size, but these were not present.
- Instead a linear offset was modelled and allowed to be negative to improve the results.

- Many of the files exhibited the classic "rolled-over peak) indicative of detector nonlinearity. This was verified by opening raw *.hsi files in hyperview (keith's software).
- This leads to strong broadening effect on data, and in most files is too severe to perform MCR analysis.

- The signal intensity varied greatly from cell to cell even within similar conditions
- Occasionally this was due to the cell being dead
- Sometimes it looked like cell wasn't well focused?
- Was the laser power or integration time adjusted from image to image? If so this is not recommended.


MCR Pure Component Spectra
Offset
Chl
LHCII?
Broadening
Normalized Intensity
0.4
0.3
0.2
0.1
0
-0.1
-0.2
500
550
600
650
700
Wavelength (nm)


Chlorophyll
Photosystem II
Bicarb Thick
Bicarb Thin
Media Thick
Media Thin
0 200 400 600 800 1000 1200 1400 1600
0 100 200 300 400 500 600 700

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QUESTIONS?