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SAND2014-16302R

Analysis of Hyperspectral Images of Chlamy on Lidke Line Scanner

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Analysis: Jeri

Data taken on 7/9/14, 7/10/14, 7/16/14, 7/17/14

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Analysis Details

- *.hsi files were converted to s3d files using batch script that reads in data with Lidke code, then calls Creates3d.m. Expansion factor was utilized for 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 composite 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).

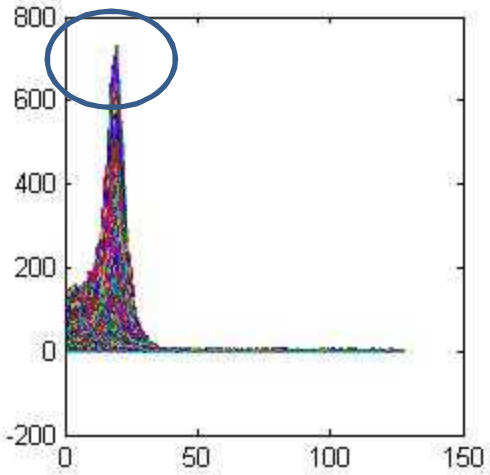
Observation 1

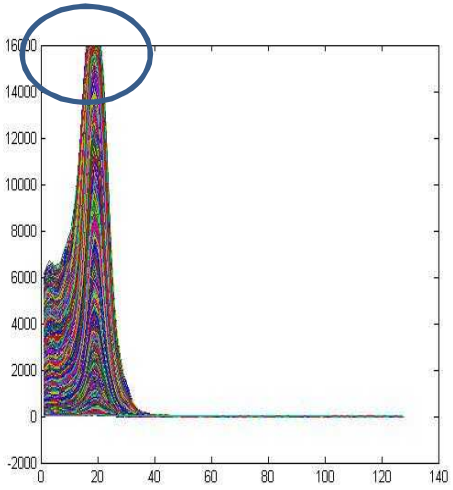
- The baseline does not appear to be a linear offset as we are used to. Instead it seems to slope upward toward the red wavelengths. Note: the data is often quite a bit below zero.
  - This could be adequately accounted for if we had some dark current images of the same size, but these were not present.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

** Future Recommendation: Collect a dark image (no light on the detector at the beginning, middle and end of the each day. Include those in with the data files.

Observation 2

- 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.





** Future Recommendation: Choose a laser power or integration time to ensure no signal is greater than the linear range of detector. In this case I am guessing about 1500 raw cts, but Keith would know better.

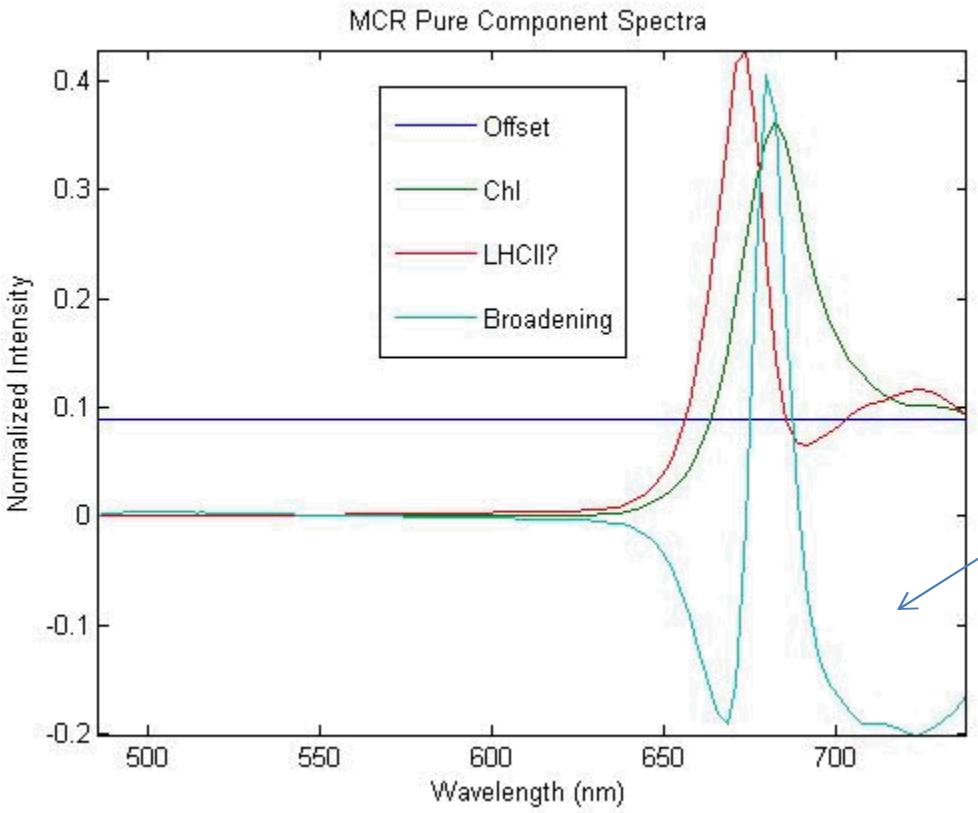
Observation 3

- 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.

** Future Recommendation: Don't vary power or integration during an experiment. Improvements in focusing were definitely seen as you became more experienced!

Encapsulated Chlamy

(Nadia bicarb experiment)



This 2nd derivative like shape represents broadening and is due to nonlinear effect of bright pixels.

Note: Spectral residuals are still quite structured indicating additional shifts and broadenings that are unaccounted for, but represent only a small portion of the variance with this model, therefore the model is deemed sufficient.

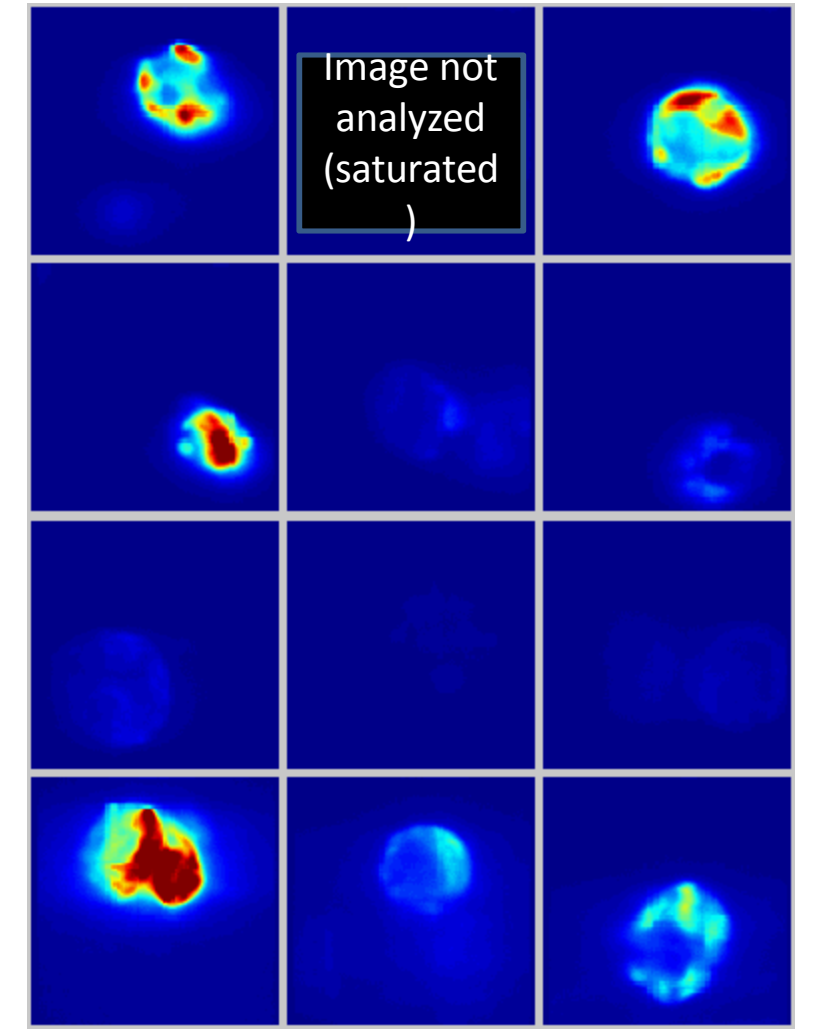
Component 2 (CHL-685)

Nadia – BiCarb Thick

Nadia – BiCarb Thin

Nadia – Media Thick

Nadia – Media Thin



*signal for this row was much weaker than others?

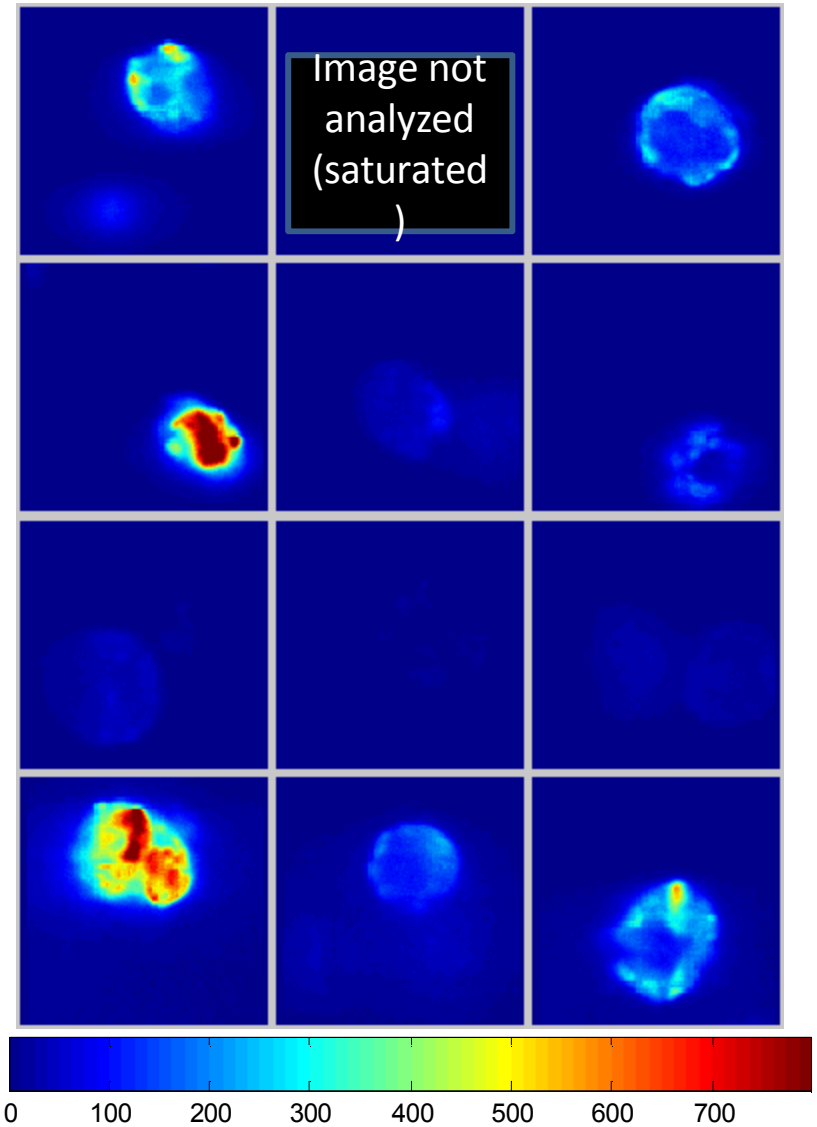
Component 3 (LHCII?)

Nadia – BiCarb Thick

Nadia – BiCarb Thin

Nadia – Media Thick

Nadia – Media Thin



*signal for this row was much weaker than others?

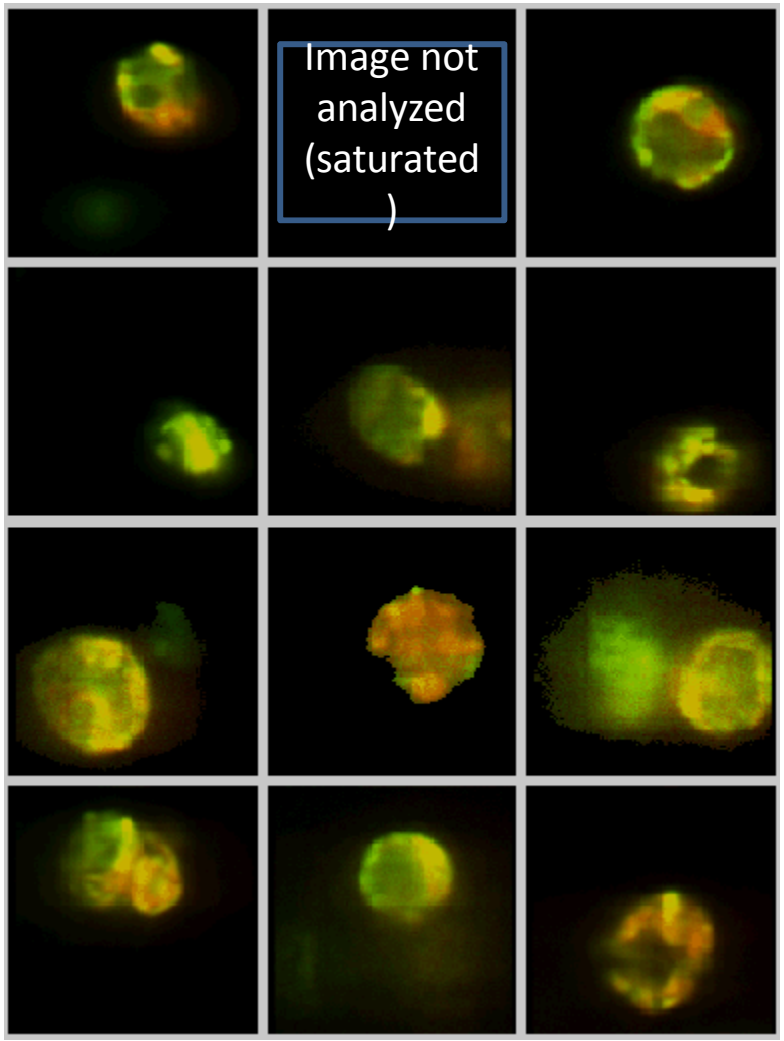
Encapsulated Chlamy

Nadia – BiCarb Thick

Nadia – BiCarb Thin

Nadia – Media Thick

Nadia – Media Thin



*signal for this row was much weaker than others?

Color scales are adjusted independently for each image.

Summary Encapsulated Chlamy

(Nadia bicarb experiment)

- Not enough consistent data to determine if there are any trends.
  - There's some segmentation of the red and green signal, however it doesn't appear to trend with condition.
  - There could be an overall change in intensity, but it is not certain whether this is due to condition or other factors like focusing.

** Future Recommendation: Continue collecting data. We typically like 30-50 cells at each condition for robust statistics.