Label-free Measurement of Algal TAG Production using Fluorescence Hyperspectral Imaging

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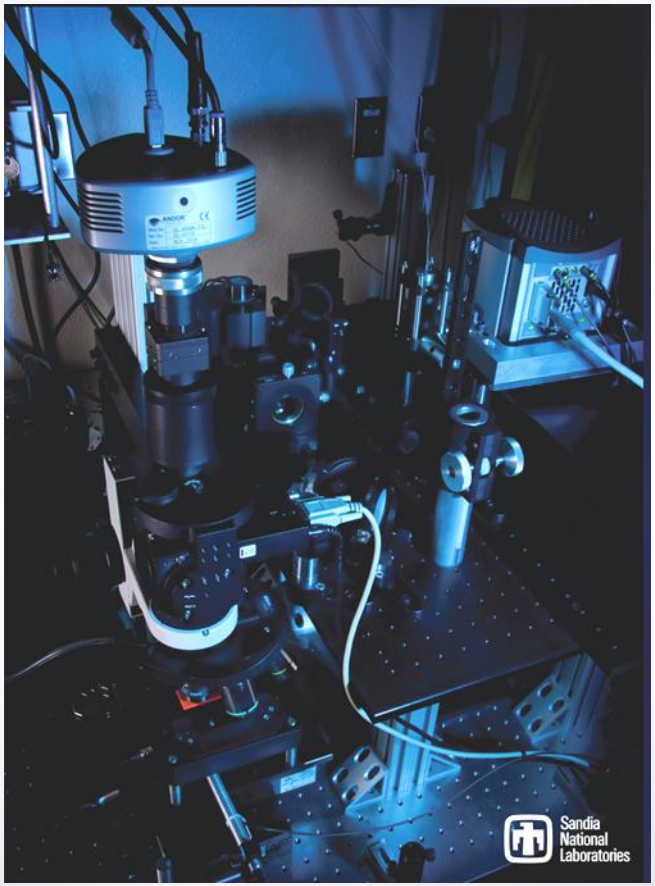
Search for new renewable energy sources

Algal-derived biofuel is a critical piece in the multi-faceted renewable energy puzzle

- 30× more oil than any terrestrial oilseed crop
- Ideal composition for biodiesel
- No competition w/ food crops
- Can be grown in waste water
- Cleaner than petroleum based fuels

Sandia's hyperspectral confocal fluorescence microscope

- Fully confocal design
  - high spatial resolution
  - optical sectioning
- High optical throughput
  - prism spectrometer
  - electron multiplying CCD
- Performance specifications:
  - 488 nm laser excitation
  - 10x, 20x, 60x, 100x objectives
  - Lateral Resolution = 0.25 μm
  - Axial Resolution = 0.60 μm
  - Spectral range 490-800 nm
  - Spectral resolution = 1-3 nm
  - Acquisition rate = 8300 spectra/s

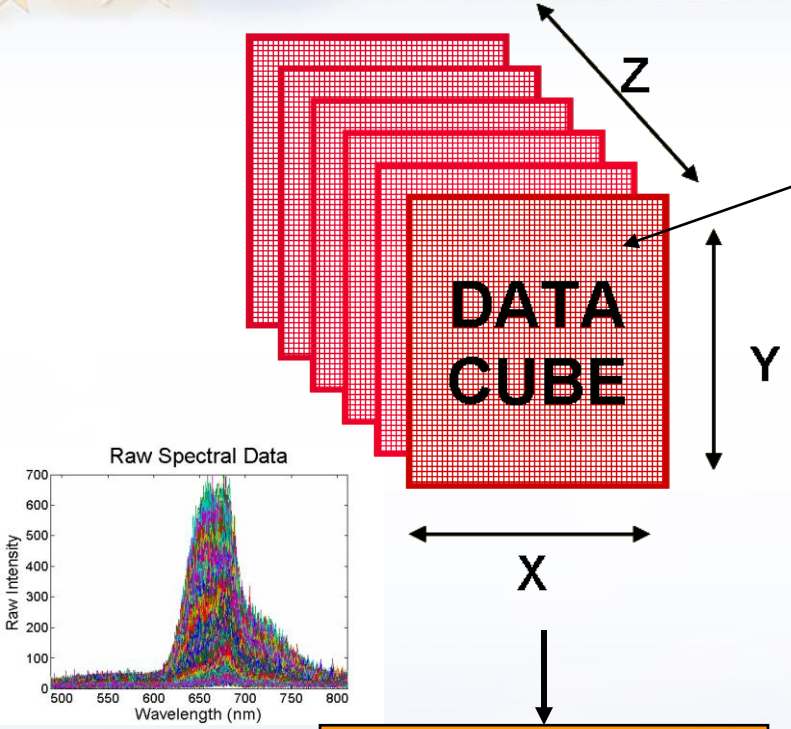


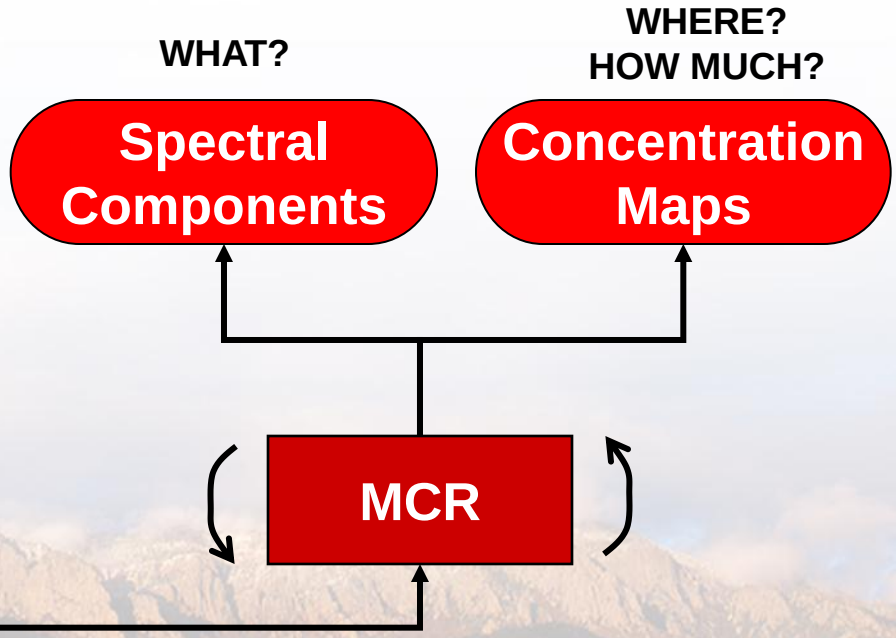
2009 R&D100 Award

M. B. Sinclair, D. M. Haaland, J. A. Timlin, and H. D. T. Jones, "Hyperspectral confocal microscope," Applied Optics, 45, 6283-6291 (2006).

Multivariate Curve Resolution (MCR)

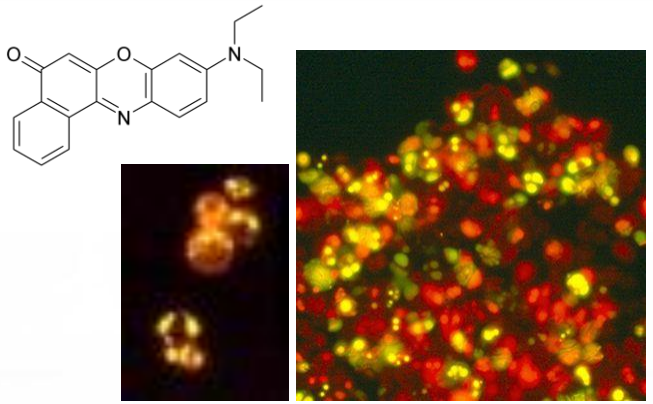
- **Assumptions**
  - **Linear additive model:** $D = CS^T + E$
  - **# of components can be estimated**
- **Solve** $D = CS^T$ **with constrained alternating (rigorous) least squares methods**
- **Non-negativity constrained pure components (S) and concentrations (C)**
- **Sandia proprietary software (very fast and memory efficient)**
  - **~1,000,000 spectra, PCA and MCR < 2.5 min, 10 components, 50 iterations**

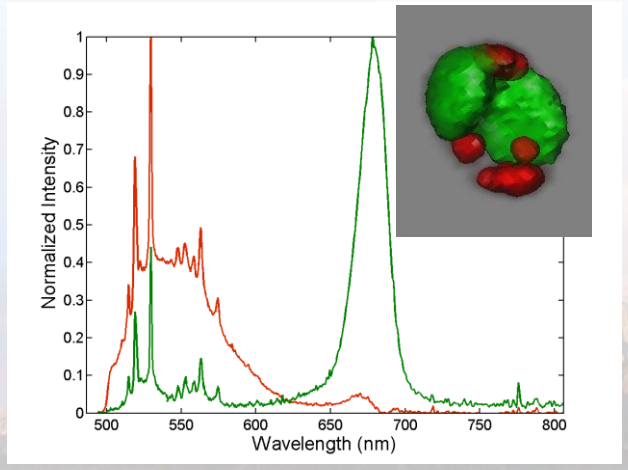




Fluorescence Hyperspectral Imaging of lipid in Algae

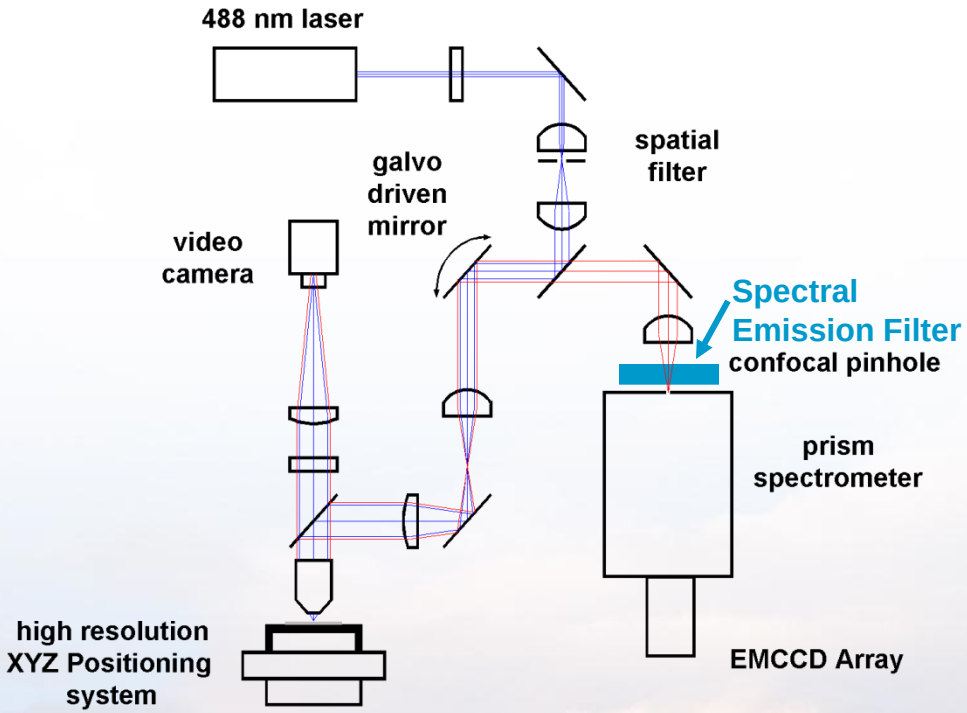
- **Problem**
  - Nile red is the current state of the art for measuring lipid using fluorescence spectroscopy
  - Not ideal for in-situ monitoring of algal cells at the microscopic level
    - Compromises the health of the cell
    - Non-uniform staining
    - Low specficity
  - Filter-based microscopy is limited

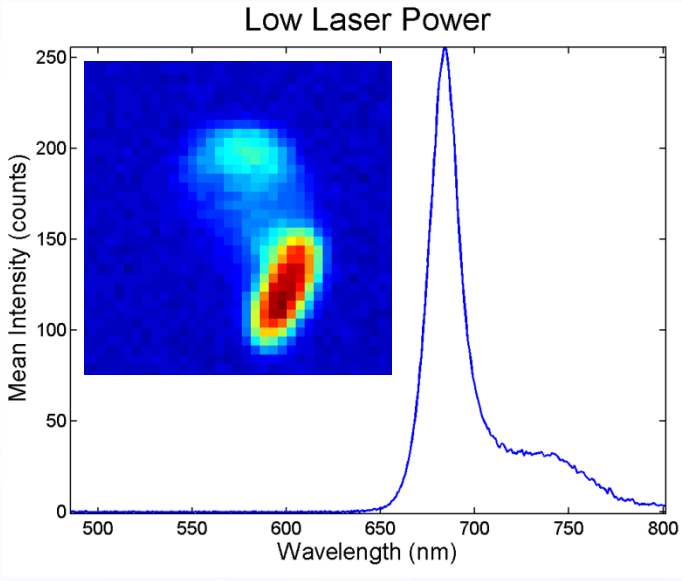


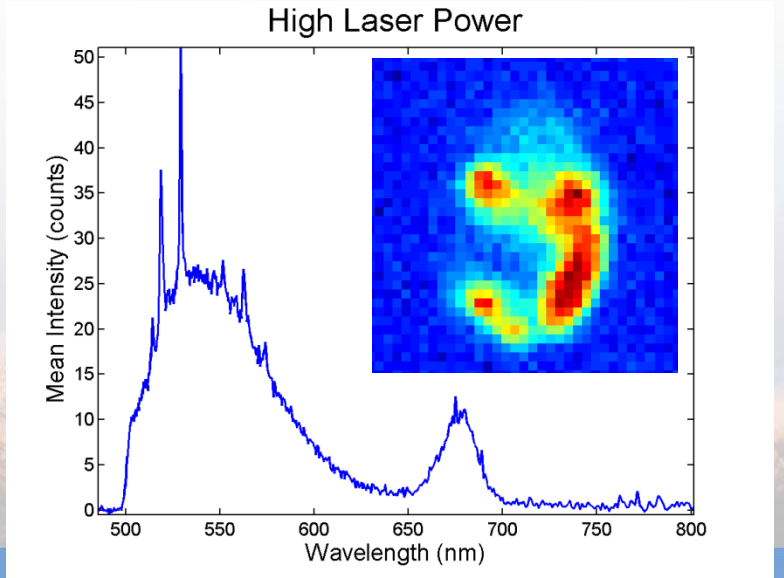


- **Solution**
  - Develop a methodology to use native spectroscopic signatures to monitor lipid production
  - Carotenoid is soluble in lipid and it has a spectroscopic signature
  - Ability to simultaneously monitor photosynthetic pigments

Laser power supplies complementary information

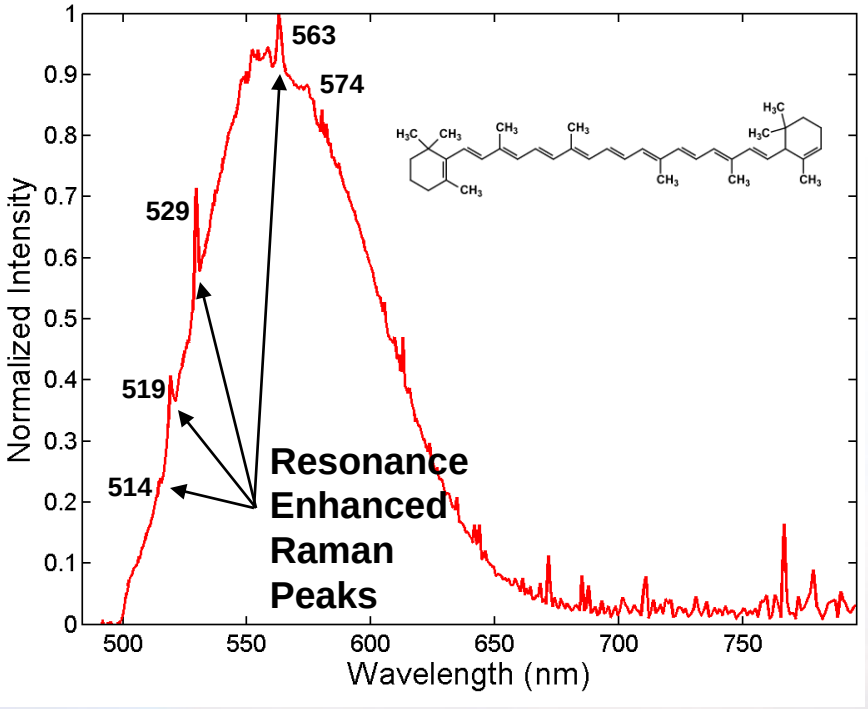




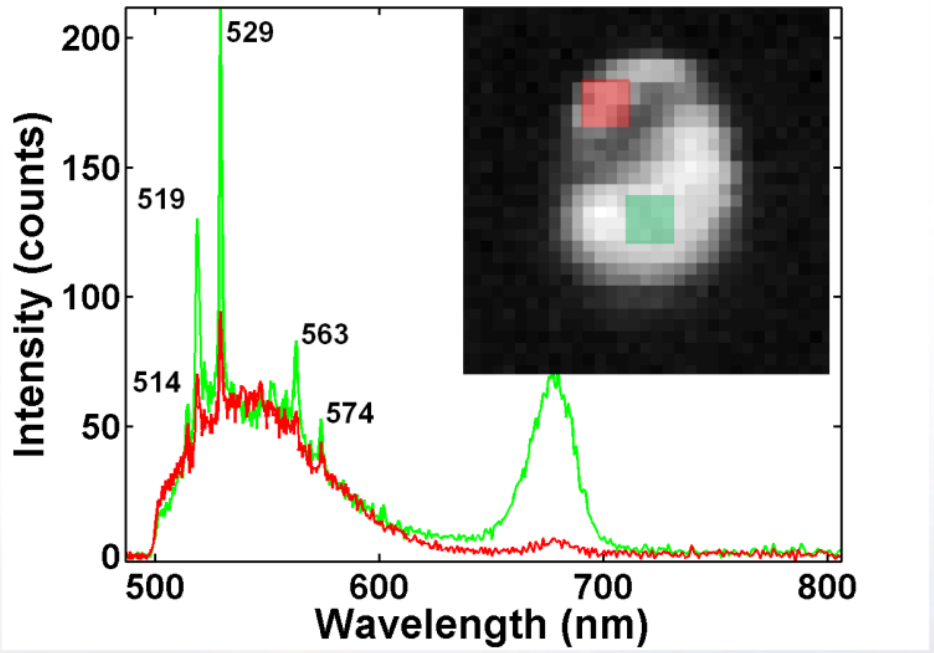


Carotenoid spectral signature

Beta-carotene Pure Spectrum

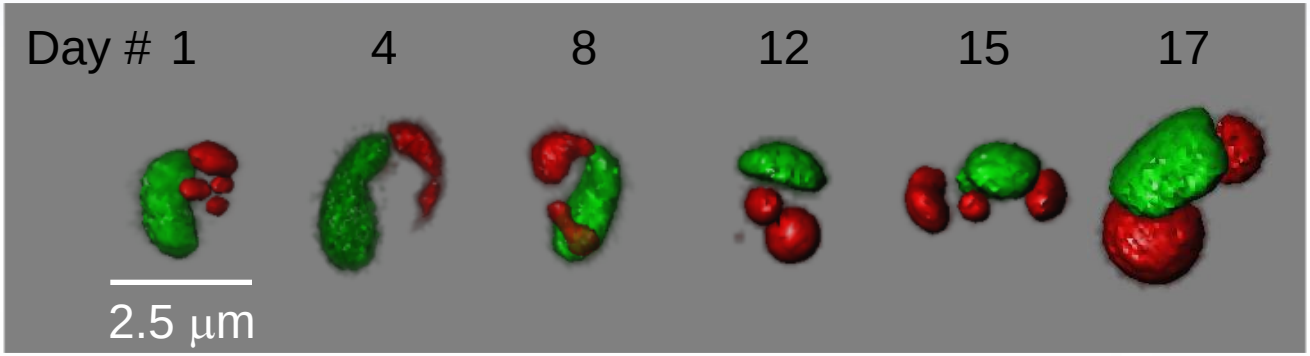


Beta-carotene in Zeiss Immersol 518F
(low fluorescence immersion oil)



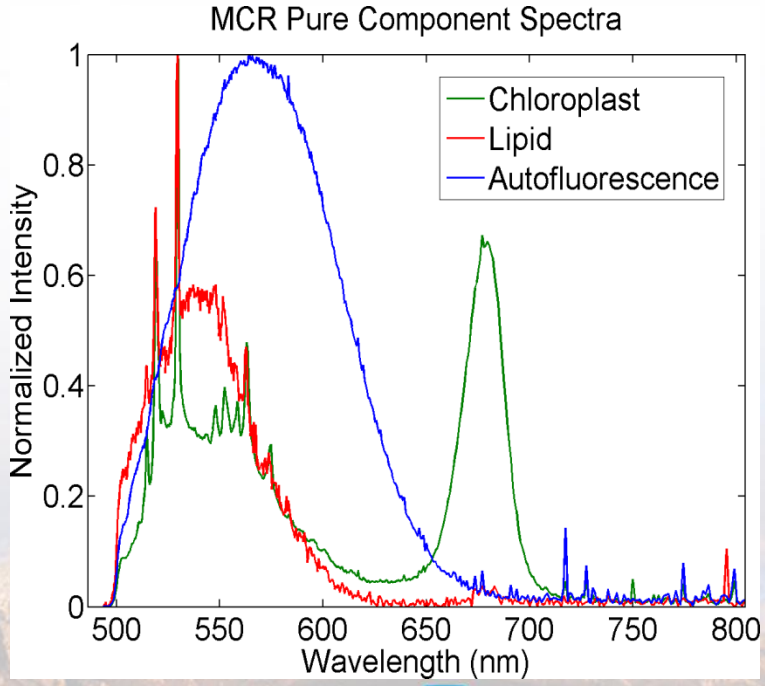
Small changes in the carotenoid signal allows for separation of lipid bodies from the chloroplast

Our hyperspectral imaging approach to detect lipid production



14 day study of Nannochloropsis Salina under nitrogen limitation and CO2 stress

- **Understand the lipid production and accumulation at the sub-cellular level by:**
  - Using hyperspectral fluorescence imaging for the *in-situ* monitoring of algal cells
  - Conduct multi-factorial studies varying nutrients
  - Understand spatial/temporal relationship of the lipid and photosynthetic pigments



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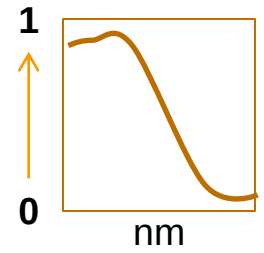
Hyperspectral imaging results

Spatially and Temporally Resolved Biochemical Response of an Organism to Its Environment

Spectral Emission Filter



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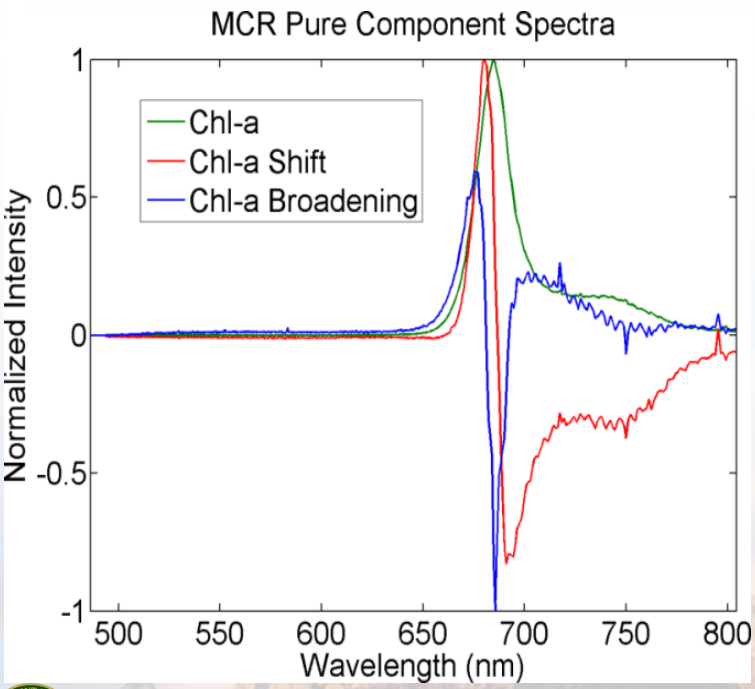

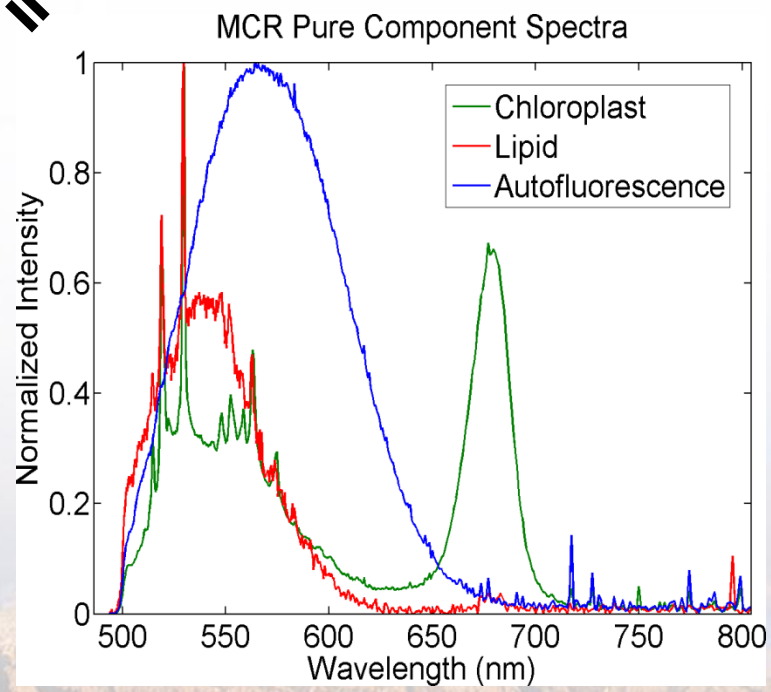

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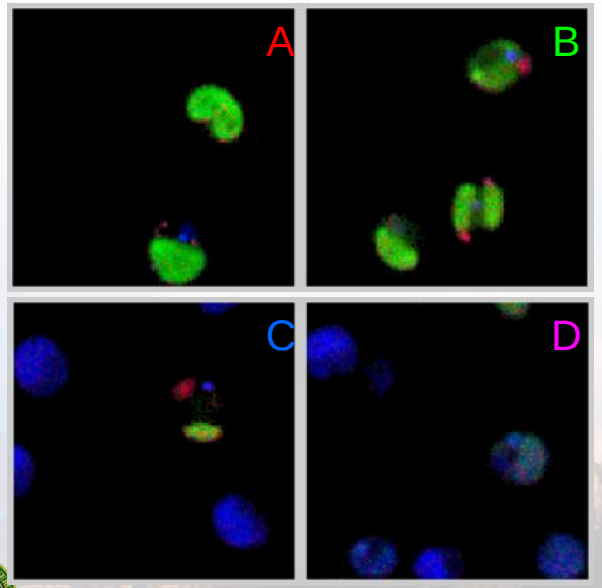
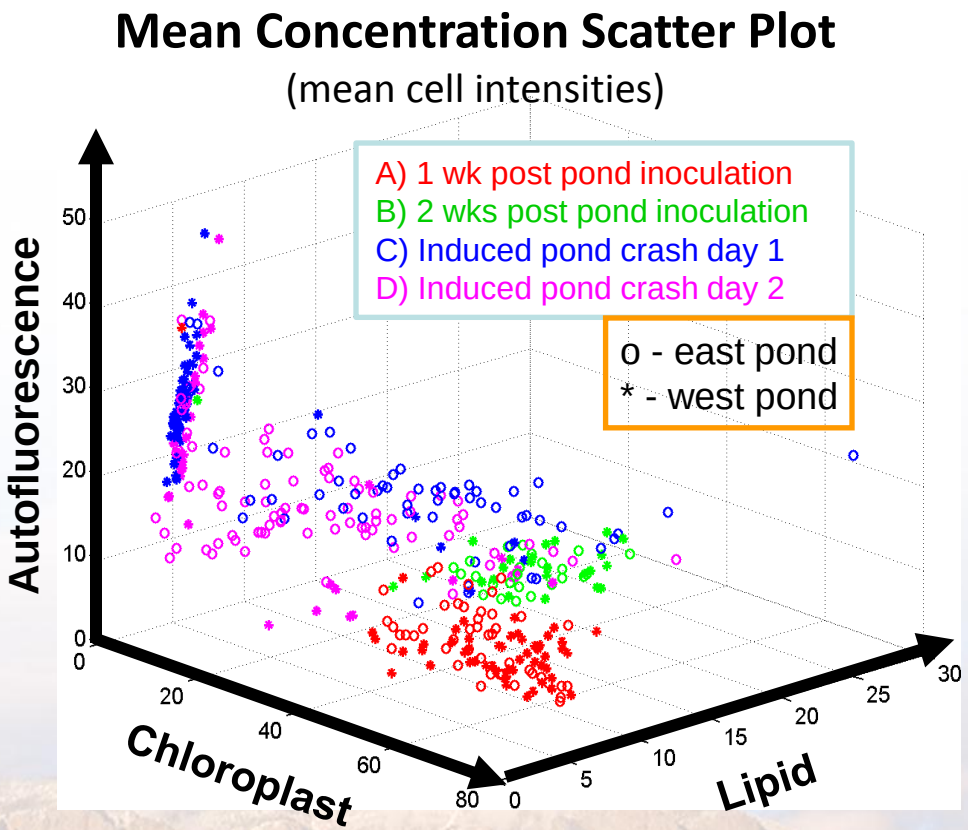
Single cell analysis

- Clear segregation between healthy and unhealthy
- Health monitored through autofluorescence
- Increased lipid production evident
- Differences between ponds

Predictive Capability of Identified Spectral Biomarkers





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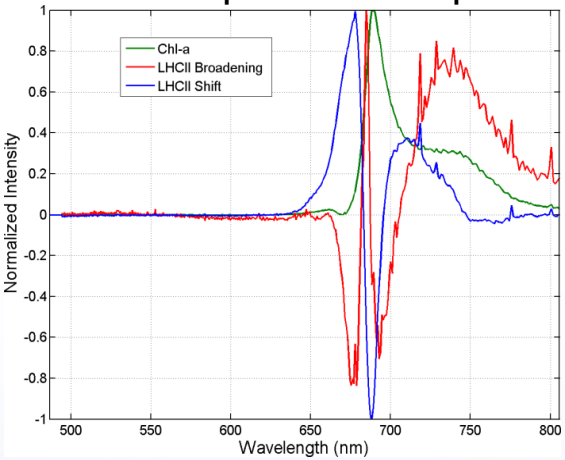
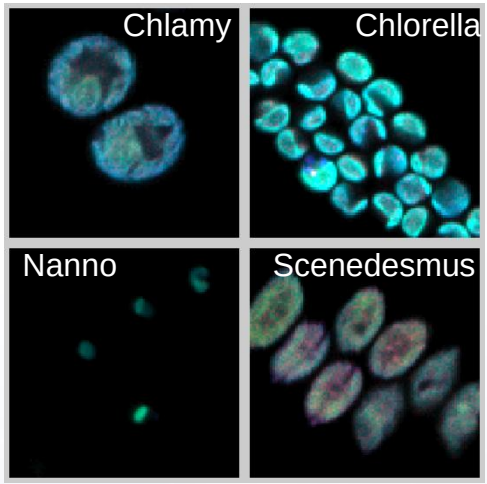
Classification of algal species using only chlorophyll features

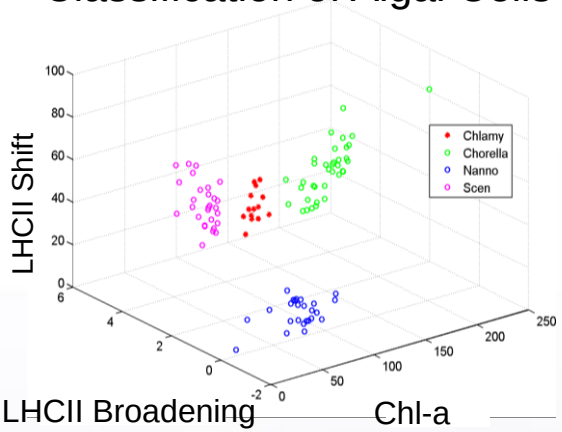
MCR Pure Spectral Components



RGB Images Colored Same as Spectral



Classification of Algal Cells



- **Hyperspectral imaging allows you to detect small subtle shape changes in the chlorophyll signatures.**
  - 100% correct classification based on these signatures
- **Investigate whether these chlorophyll signatures change as a result of mixed cultures over time. Can we use these signatures to examine compatibility between algal cultures?**
- **Investigating the possibility of quantifying species by using these types of signatures**
  - Using data from standard fluorometers or spectrophotometers

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Conclusions

- **Hyperspectral imaging coupled with MCR has been an excellent tool for understanding algal biomass problems**
  - Can provide spectral, spatial and temporal information about the algal biomass systems being investigated
  - Cellular and subcellular hyperspectral imaging can provide insights into the spectral detection signatures and possible spectral interferences
    - Important for the development of spectroscopic algal monitoring equipment
- **Hyperspectral imaging coupled with MCR is an important tool for understanding and discovering unknown biological systems.**
  - CLS can be used when the spectral signatures are well understood

Acknowledgments

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  - *"From Benchtop to Raceway: Spectroscopic Signatures of Dynamic Biological Processes in Algal Communities"*
  - *"Utilizing Biocomplexity to Propagate Stable Algal Blooms in Open System – Keeping the Bloom Going"*
  - *"From algae to oilgae: In-situ studies of the factors controlling growth, oil production, and oil excretion in microalgae."*
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