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Pond-Scale Diagnostics in Algae Production

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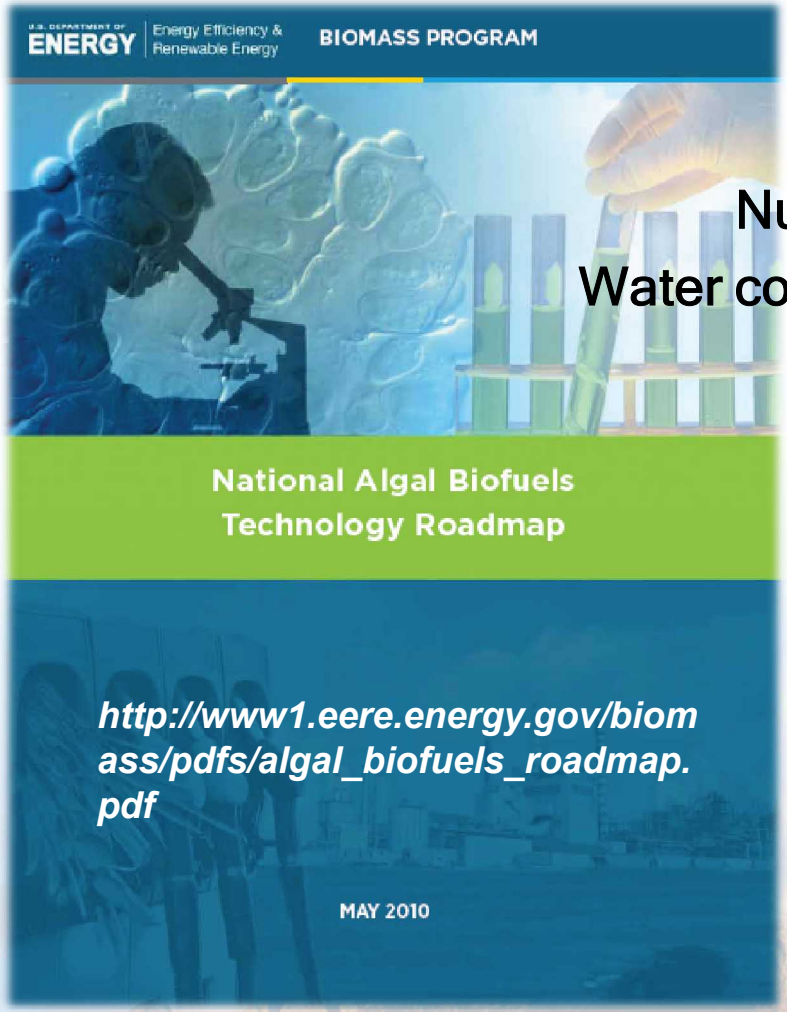
Outline

- **Motivation**
- **Approach**
  - Spectroscopy-based tools
  - Multivariate techniques
- **Results**
  - Reflectivity
  - Fluorescence Lidar
- **Summary & Future Directions**

Challenges in Production, Optimization



Culture sustainability --

System productivity --

Nutrient source scaling and sustainability --

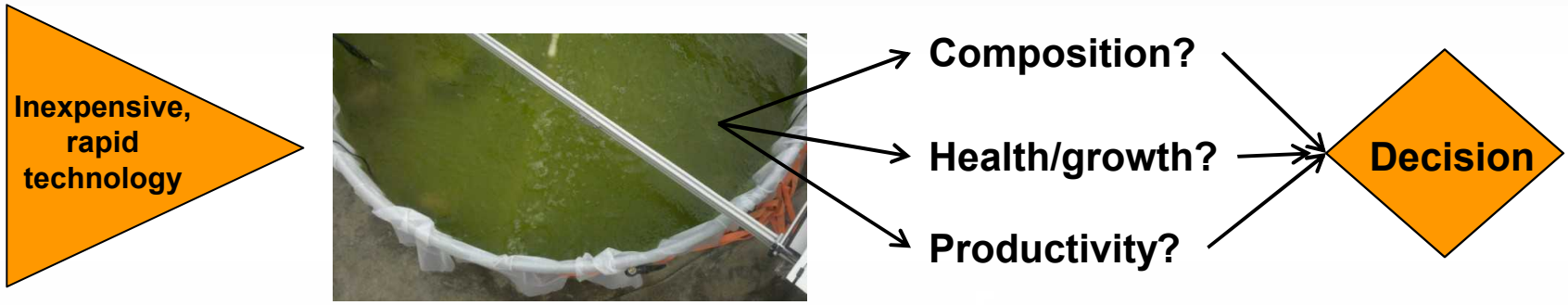
Water conservation, management, and recycling -

A Living System is a Dynamic System

Dynamic response to environment is temporally escalated compared to agricultural crops

Cell Response Dynamics	Population Dynamics
■ Multiple stressors ■ Individual response is still poorly understood, yet multivariate interactions are likely to be key ■ Species diversity: even similar species can possess distinct responses	■ Monocultures are unnatural ■ Difficult to maintain ■ Natural variations with season and stress

Detecting Dynamics



Sensitive, selective, automated methods for early detection of fluctuations in algal communities.

But, major gaps in knowledge of fundamental algal biology limit our ability to "engineer" a solution

multidisciplinary Our Innovative Approach

- **Determining effects of abiotic & biotic stressors on metabolic networks & pathways (including lipid biogenesis)**
  - *Genomics / Transcriptomics*
  - *Bioanalytical spectroscopy / Chemical Imaging*
- **Monitoring algal culture dynamics at scale**
  - *Remote sensing*
- **Predicting algal productivity & culture behavior at scale**
  - *Computational modeling*

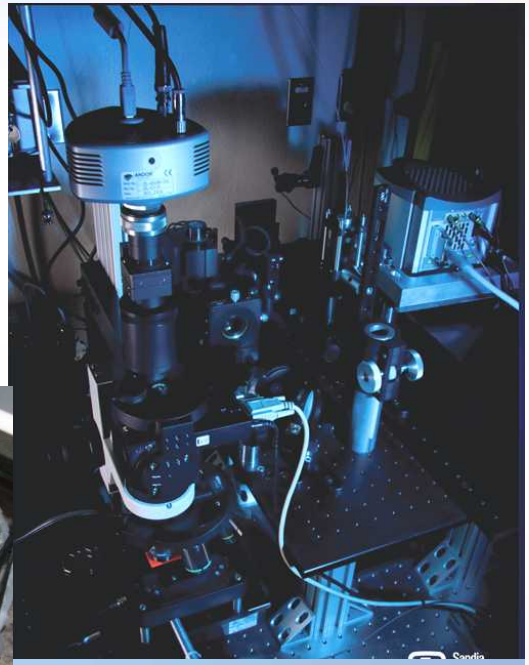
Outcome: Understanding of spatial–temporal variations of biomass growth and lipid production at multiple scales

Why SNL?

- **Strong alignment with DOE and SNL mission in achieving scalable transportation fuels**
- **Niche of algal biology that is poorly understood, particularly relevant for southwest ecosystems**
- **Need multidisciplinary approach; fundamental bioscience R&D w/ “keen eye” on application**
- **Unique combination of expertise unavailable elsewhere**
  - *Spectral signatures for early detection of disease*
  - *Advanced chemical imaging*
  - *Algorithms for robust identification, quantification of minor species in presence of interferrents*

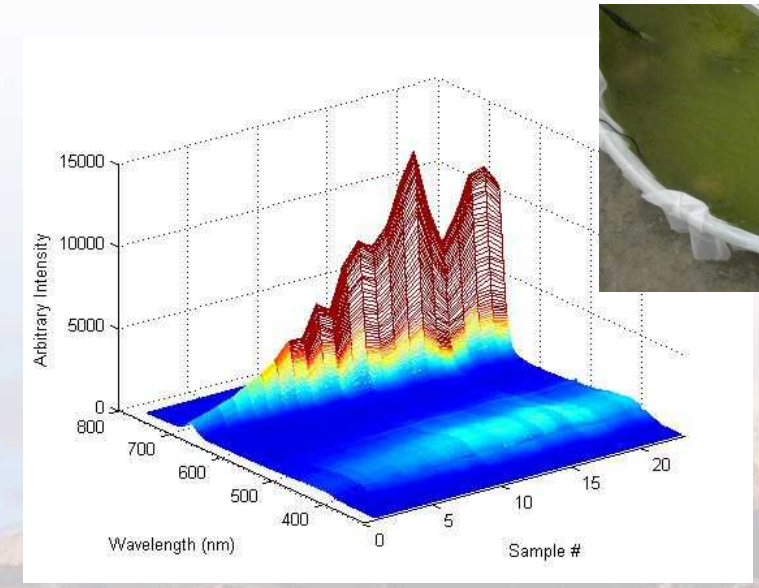
Facilities and Capabilities

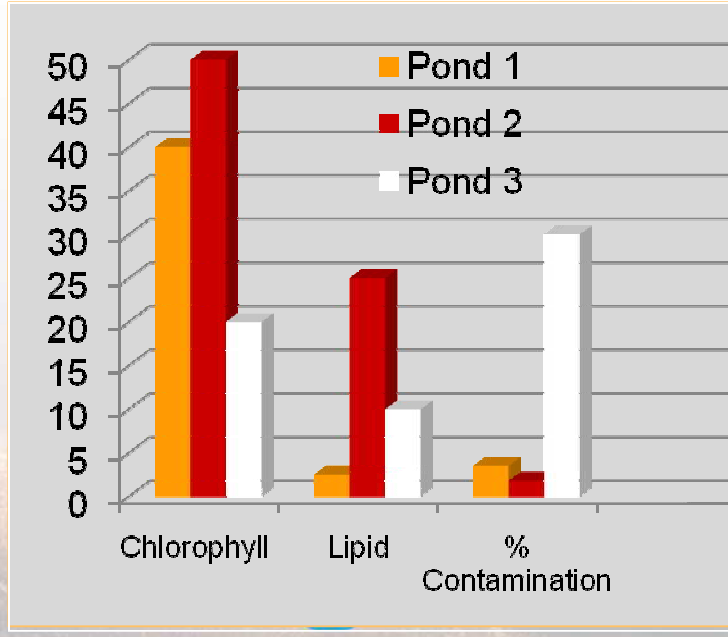
- Multidisciplinary team
- Fully equipped algal biology lab
- Greenhouse
- Advanced spectroscopy and imaging tools
- Extensive expertise in algorithms for spectroscopy



Process Analytical Technology: Revolutionized Pharma and Chemical Industries

- Understand and control the manufacturing process
- On-line or near-line measurements
- Attributes of raw and in-process materials and processed
- Framework for QA/QC
- Tools:
  - Spectroscopy (NIR, reflectance, fluorescence, etc.)
  - Multivariate data acquisition and analysis tools





Not as Simple as it Looks

Variations abound!

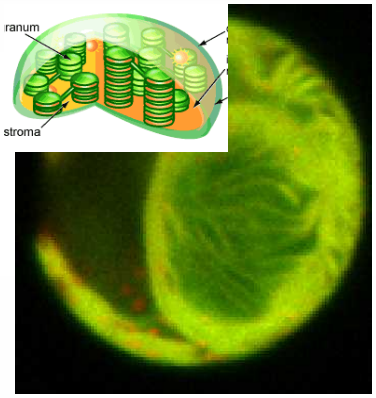
Species specific response
Diurnal and seasonal variation
Temperature, light, salt, nutrients
Predators, pathogens, competitors

Interaction effects are significant too!

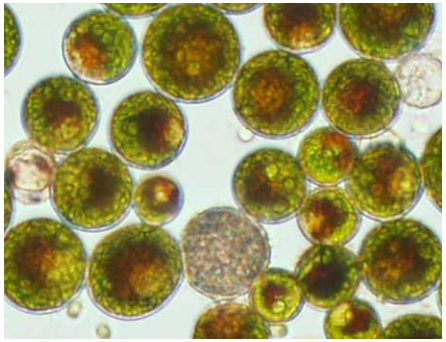
Abiotic & Biotic Effects on Metabolic Networks & Pathways

- **Understanding effect of abiotic stressors on carbon partitioning and lipid production**
  - Temperature
  - Nutrient limitation
  - CO_2 and CO_2 concentrating mechanism
- **Understanding effect of biotic diversity on growth and lipid production**
  - Predators
  - Pathogens

Multiscale – Phenomenon Size



Subcellular
nm



Single cell
µm

Ensemble
m

- ***Biomarker discovery and validation*** at lab, greenhouse, and ensemble scale
- Correlation of spectral signatures to biological variation
- Determine detection limits, robustness with scale-up, feasibility

Multiscale – Culture Size

Lab scale
0.01 – 3 gal

Greenhouse scale
100 – 150 gal

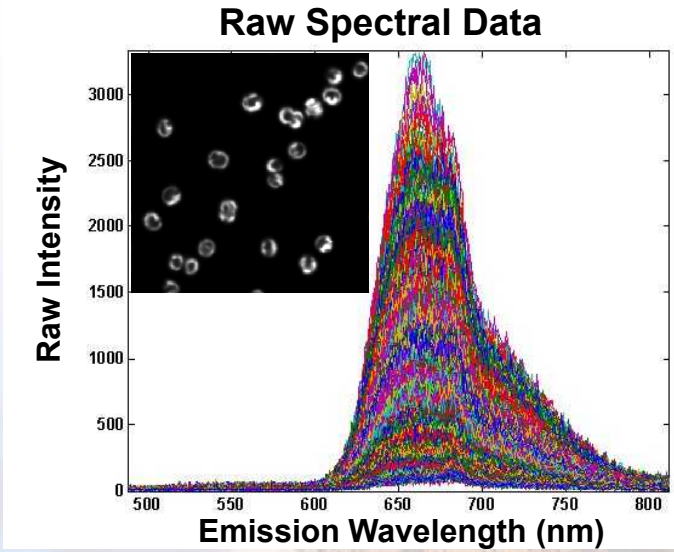
Raceways*
1000 – 10,000 gal

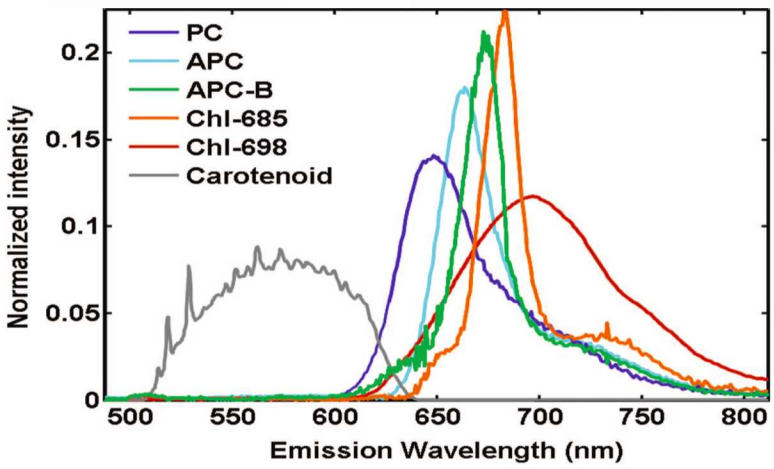
- ***Technology optimization and validation*** at lab, greenhouse, and raceway scale
- Hardware and algorithm/model
- Robust against non-biological variation
- Determine limitations

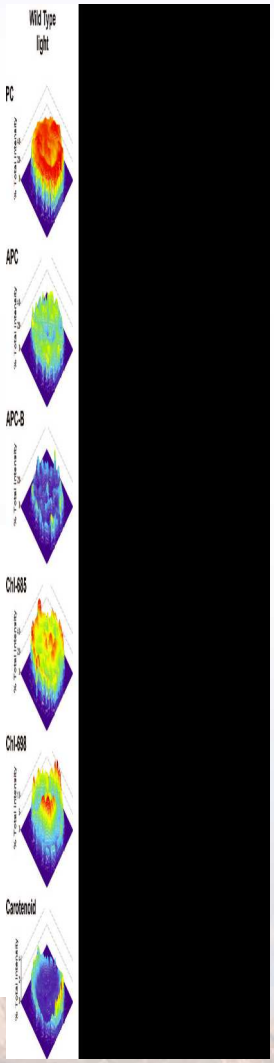
*** photo courtesy of collaborators at ASU**

Inside an Algae Cell w/ Spectral Imaging & Multivariate Curve Resolution

- **Identify and quantify photosynthetic pigments and other fluorophores simultaneously in living cells**
  - Label-free
  - High spatial resolution in 3D (250nm x 250nm x 500nm)
  - High spectral resolution and large spectral range







Vermaas, et. al. PNAS, 2008
Jones et al., J Chemom. 2008 and references therein

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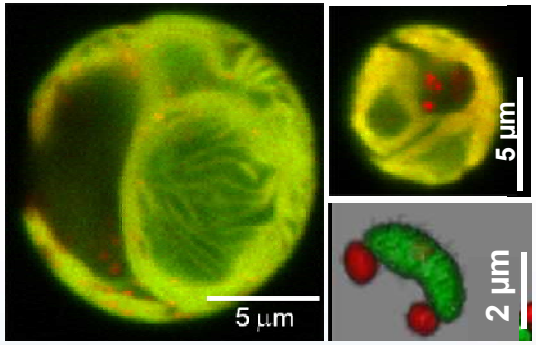
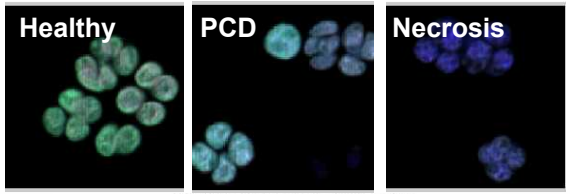
Advanced Imaging = Unprecedented View of Cell Processes

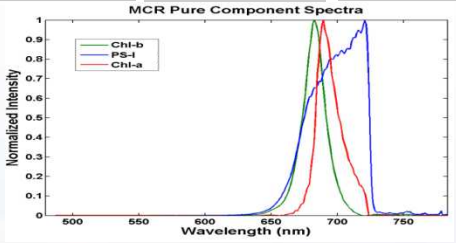
Hyperspectral Confocal Fluorescence Microscopy



Subcellular localization, quantification of lipid and chlorophyll *w/o use of labels*!

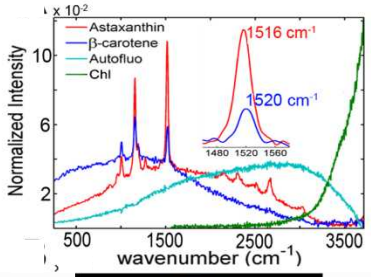
Two-Photon Hyperspectral Fluorescence Microscopy

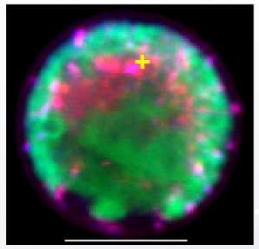




Discrimination between cell death mechanisms *at early stage w/o use of labels*!

Hyperspectral Raman Microscopy





Subcell localization, discrimination of carotenoid, lipids, and precursors *w/o use of labels*!

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Monitoring Algal Culture at Scale

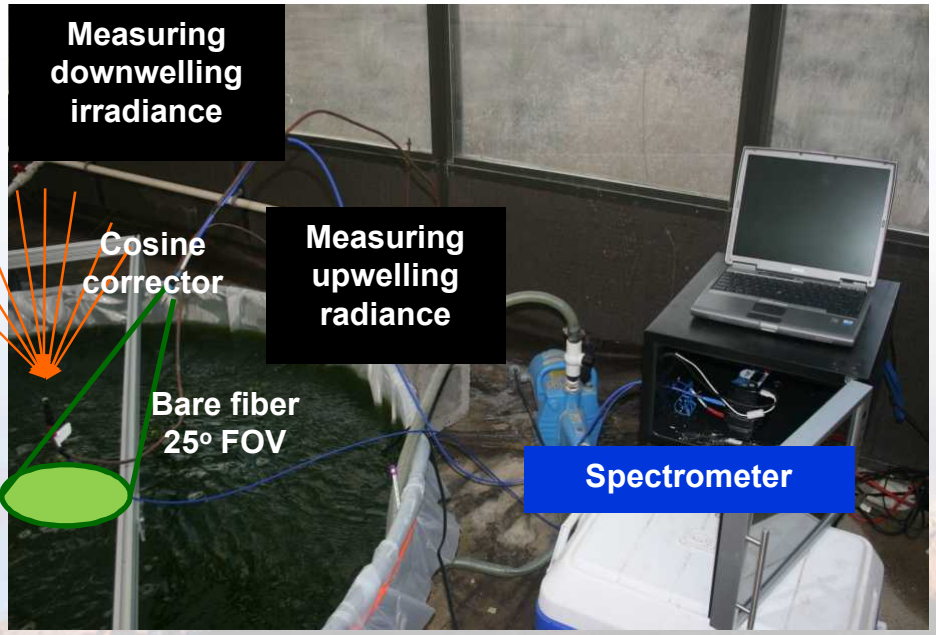
- Reflectivity (passive)
- Lidar (active)

Spectral signatures that are rich in information about algal density, culture health, competitors/predators:
→ Real-time monitoring and quality control

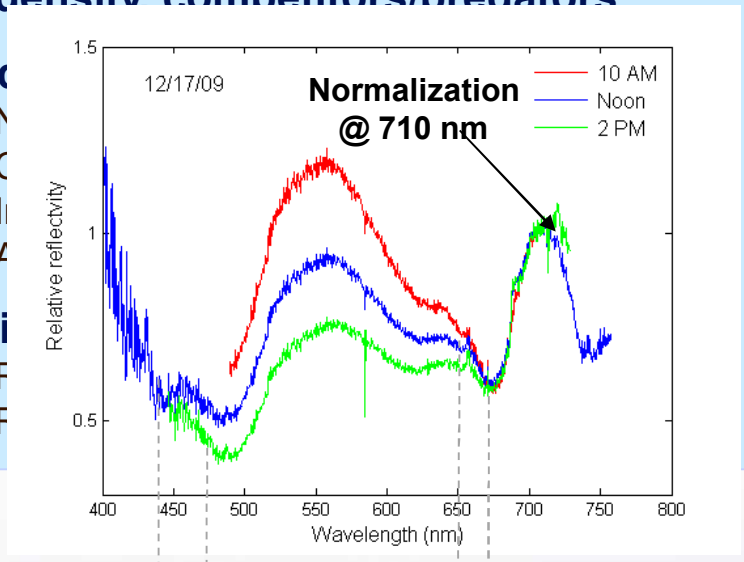
Monitoring Algal Culture at Scale

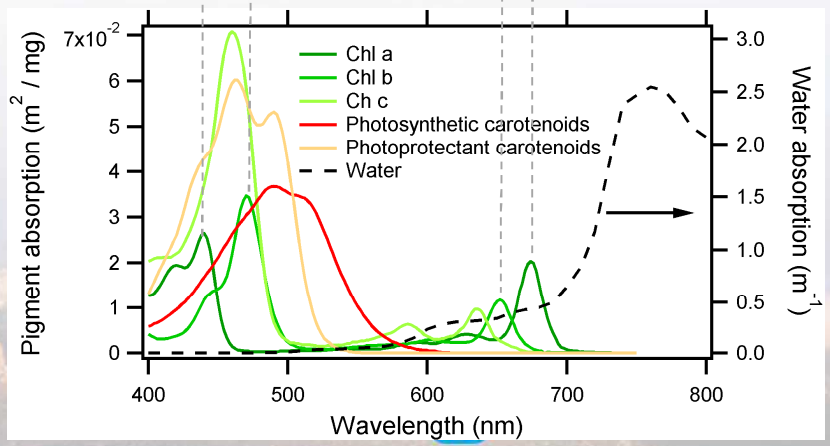
- **Reflectivity (passive)**
- **Lidar (active)**

$$R = \frac{\text{upwelling radiance}}{\text{downwelling irradiance}} \propto \frac{\text{scattering}}{\text{scattering + absorption}}$$



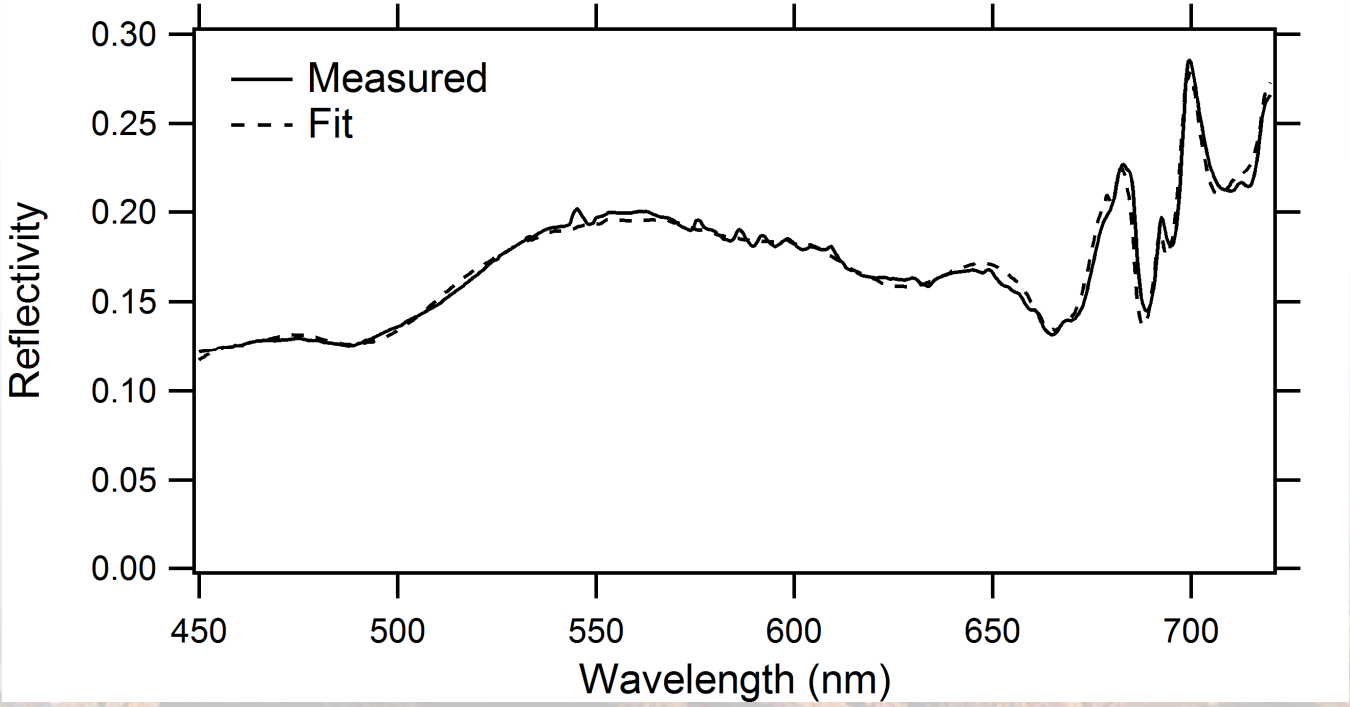
Rapid, wide-area assessment: health, density, competitors/predators





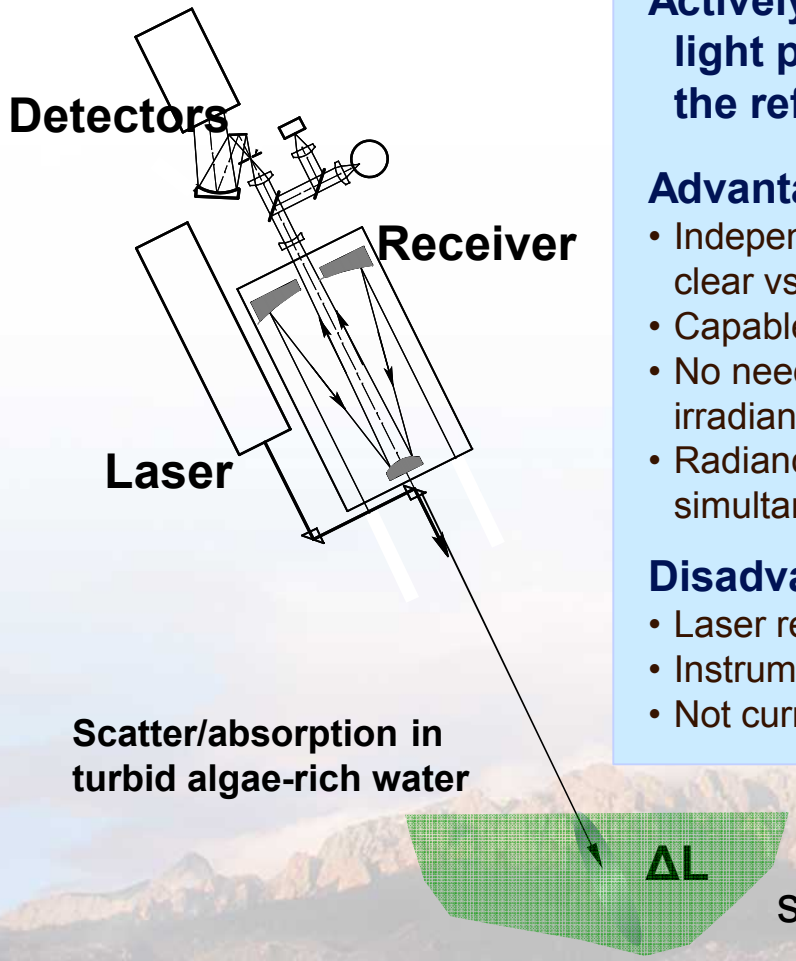
Developed Spectral Reflectivity Model for *N. salina* Growth

- Expanded the equation $R(\lambda) \propto S(\lambda) / [S(\lambda)+A(\lambda)]$
- Includes fluorescence and self-absorption
- Developed at benchtop scale, translation to greenhouse scale in progress



Evaluating Utility of Fluorescence Lidar

- Reflectivity (passive)
- Lidar (active)



Actively probing the water with laser light provides similar information to the reflectivity measurement.

Advantages

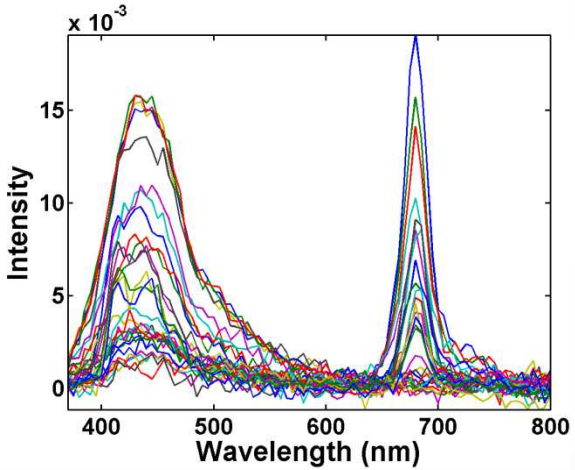
- Independent of weather conditions (i.e., clear vs. cloudy)
- Capable of nighttime operation
- No need to measure/estimate downwelling irradiance for interpretation of results
- Radiance measurement acquired simultaneously

Disadvantages

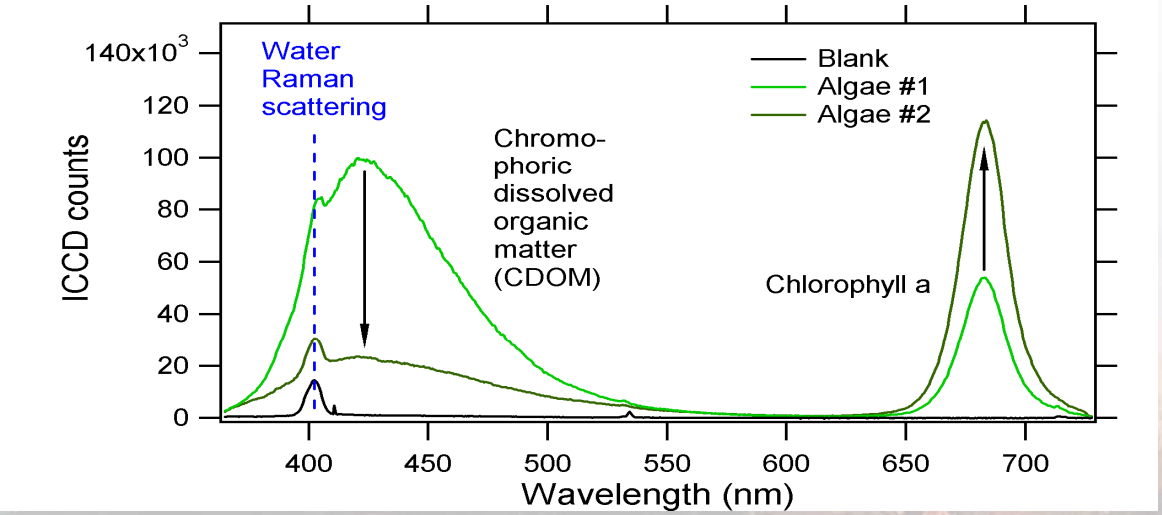
- Laser required
- Instrument not commercially available
- Not currently capable of imaging

$$\text{signal} \propto \frac{\text{concentration} \times \Delta L}{R^2}$$

Identified Spectral Signature for Algal Health @ Two Scales



- **Algal health studies**
  - **Algal Species**
    - **Nannochloropsis**
    - **Chlamydomonas**
    - **Dunaliella**
  - **Nutrient and oxygen starved**
  - **Fluorescence (355 nm excitation)**



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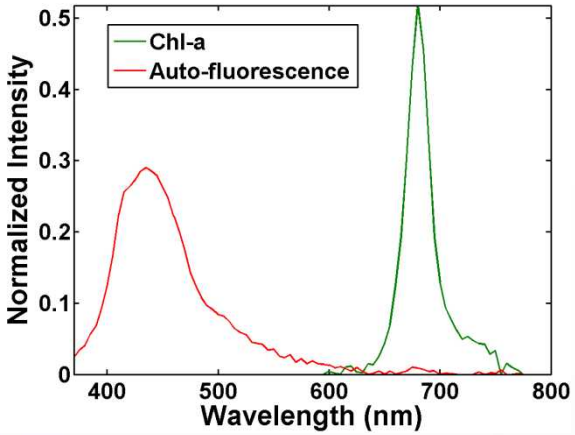
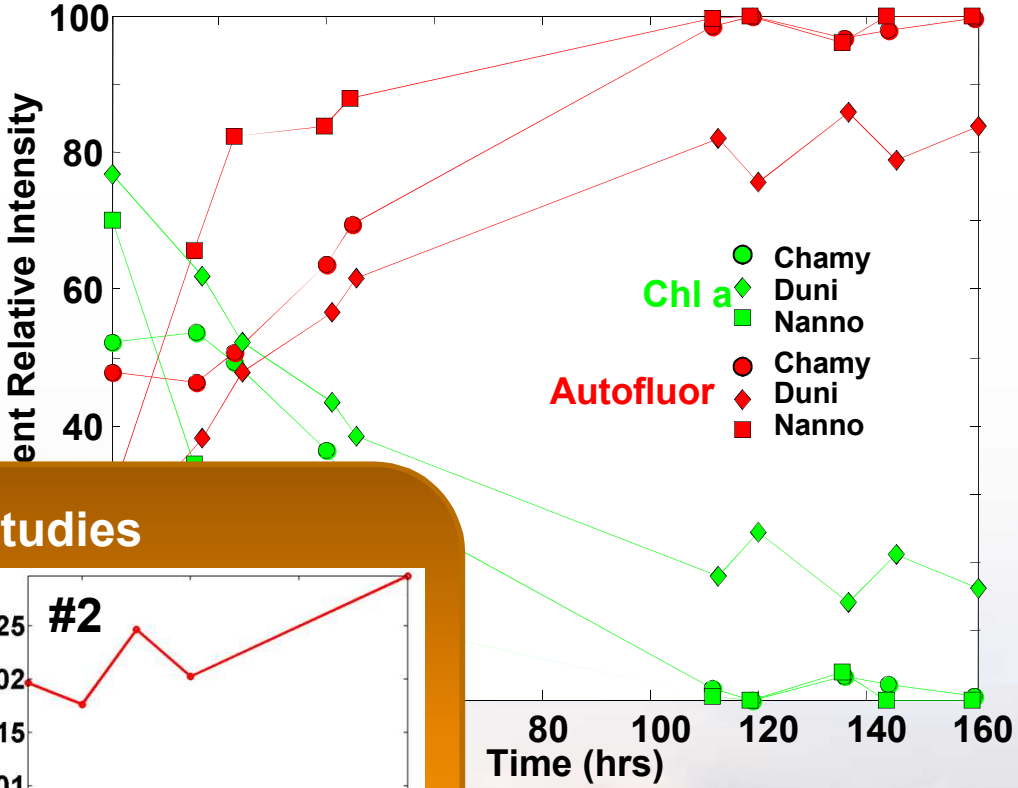
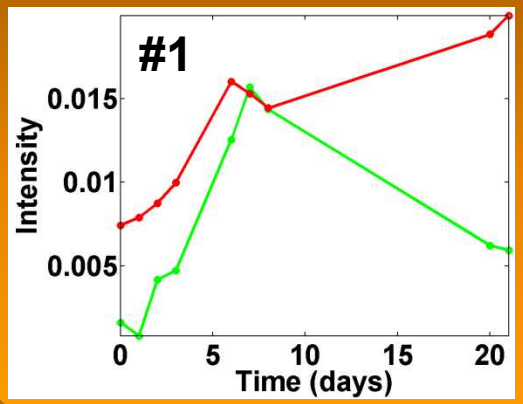
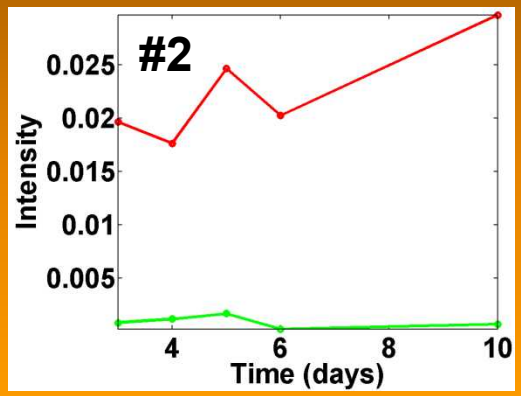




Greenhouse Studies





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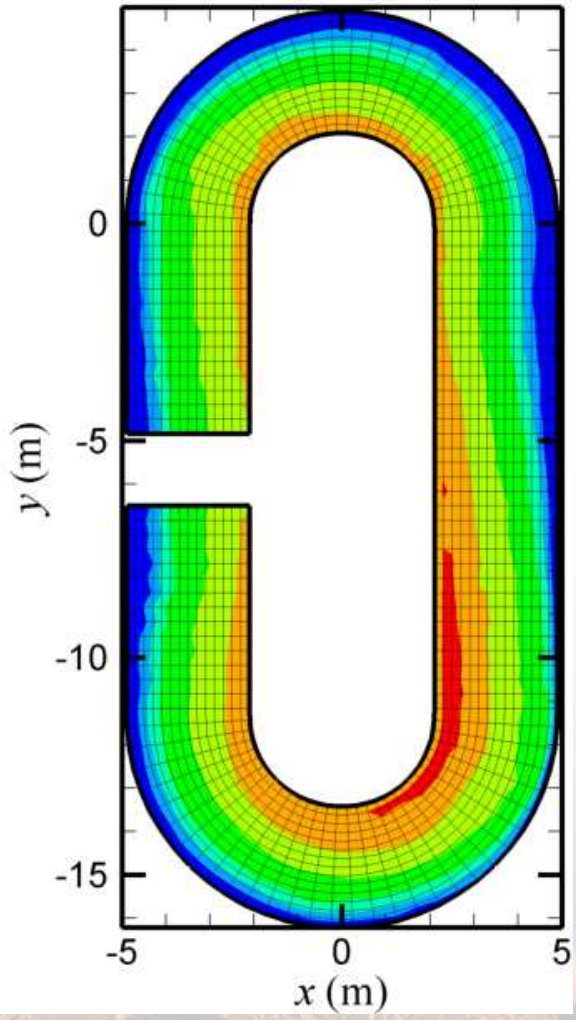
Predicting Algal Productivity

Computational Fluid Dynamics Model

- **Modified EPA and US Army Corp of Engineering Codes**
- **The Environmental Fluid Dynamics Code (EFDC) solves 3D Navier-Stokes equations of open channel flow to model speed, temperature, and nutrient gradients.**
- **CE-QUAL couples nutrient kinetics and 22 independent variables (N, P, Si, O_2…) to model growth rates.**

$$\frac{\partial}{\partial t}B(\mathbf{x},t)=\left(P-B_M-P_R-W_S\frac{\partial}{\partial z}\right)B(\mathbf{x},t)$$

B(x,*t*) is the spatio-temporal algal biomass (gm Carbon/m^3)
P is the production rate (1/day)
BM is the basal metabolism rate (1/day)
PR is the predation rate (1/day)
WS is the settling velocity (m/day)



James, S. C. and V. S. Boriah, Modeling algae growth in an open-channel raceway, Journal of Computational Biology, **17**(*4*), 2010.

Robust validation of CFD Model

- Conducted parameter sensitivity study
- Input species specific experimental data
- Effects of pH

Long Term Outlook:

- Spectral measurements and computational fluid dynamic model can work together in a final process analytical system to make real-time, accurate predictions

Summary

- **Innovative, multidisciplinary, multiscale approach only possible at a national lab**
- **Produce critical knowledge of algal culture response to abiotic and biotic environment dynamics**
- **Provide tools for monitoring algal culture health and productivity at multiple scales**
  - Spectroscopy and chemometrics are key
  - Inexpensive, real-time monitoring is possible
- **Predictive model for algal growth and lipid production in open raceway ponds**

Next Steps: **Expand our spectral and computational models to include additional effects due to species specific response and variable interactions**

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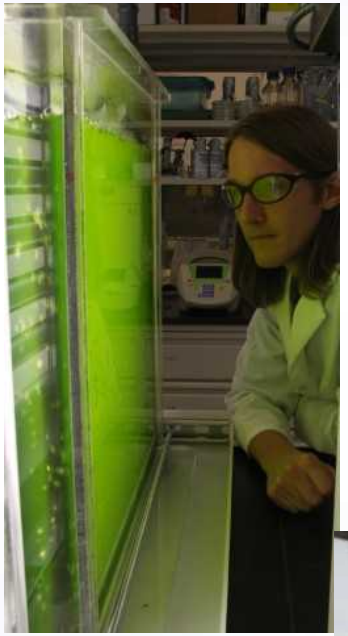

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