SAND2012-4847C

Optimizing Algal Cultivation: An Innovative, Multidiscipline, and Multiscale Approach

Jerilyn A. Timlin, Howland D.T. Jones, Thomas A. Reichardt, Scott C. James, Amy J. Powell, Aaron M. Collins, Anne Ruffing, Kylea Parchert, Lindsey Gloe, Brian Dwyer, David T. Hanson

Sandia National Laboratories, Albuquerque, New Mexico
Sandia National Laboratories, Livermore, California
University of New Mexico, Albuquerque, New Mexico

Presented at:
The 2nd International Conference on Algal Biomass, Biofuels and Bioproducts
San Diego, CA
June 10-13, 2012

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

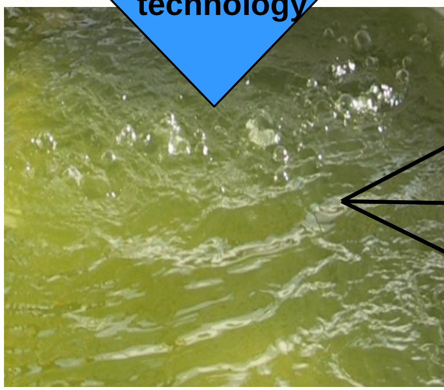
A Challenge of Renewable Fuels

Dynamic response to environment is temporally escalated compared to agricultural crops

Individual Cell Response Dynamics	Population Dynamics
▪ Multiple stressors ▪ Response to single stressor 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

Sandia National Laboratories

Monitoring is a Vital Component of Large-Scale Algal Production



Inexpensive, rapid technology

- **Composition?**
- **Health/growth?**
- **Productivity?**

Decision

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

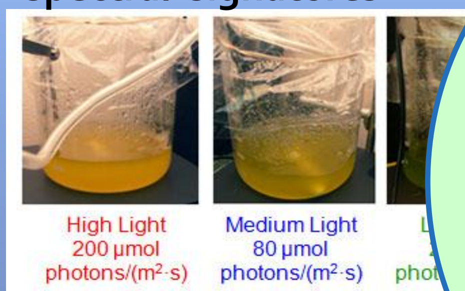
<u>But, major gaps in knowledge of fundamental algal biology limit our ability to "engineer" a solution</u>

Sandia National Laboratories

Innovative, Differentiating Approach

Benchtop Stress Expts

- Basic science on cell level physiological & molecular responses
- Determine cell & culture spectral signatures



Stressors

- CO_2
- Light

Greenhouse Stress Expts

- Validate algal function at meso-scale outside of lab
- Test spectral signatures

Algal Physiology

Molecular Biology

Chemical Imaging / Analysis

Bioanalytical Spectroscopy

Computational Modeling

Remote Sensing

Statistics

eway Verification

- emonstrate validity of approach at raceway scale

Computational Mode

- Translate experimental work into a scalable mathematical model for *in silico* testing of concepts

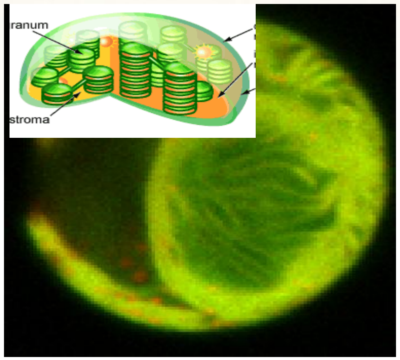
$$\frac{\partial}{\partial t}B(\mathbf{x},t)=\left(P-B_M-P_R-W_S\frac{\partial}{\partial z}\right)B(\mathbf{x},t)$$

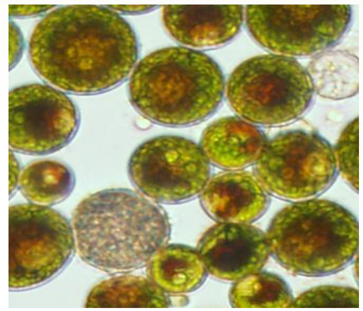
Mechanistic, multi-scale understanding of algal productivity

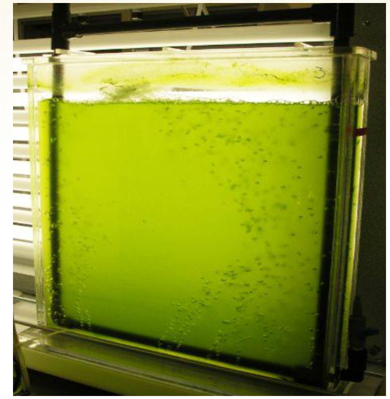
Sandia National Laboratories

Goals Encompass Multiple Scales

Biomarker Discovery & Validation







Subcellular nm → **Single cell µm** → **Ensemble m**

Technology Optimization & Validation

Lab scale 0.01 – 3 gal → **Greenhouse scale 100 – 150 gal** → **Raceways* 1000 – 10,000 gal**

* photo courtesy of collaborators at ASU

Chemical Imaging = Unprecedented View of Cell Processes

Light Microscopy

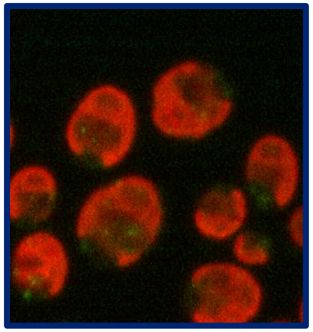
Each pixel in the image is a combination of 3 (RGB) colors (morphology, refractive properties)

Pretty picture →

Fluorescence Microscopy

Each pixel in the image corresponds to integrated bandpass within fluorescence emission (chloroplast, lipid with stain)

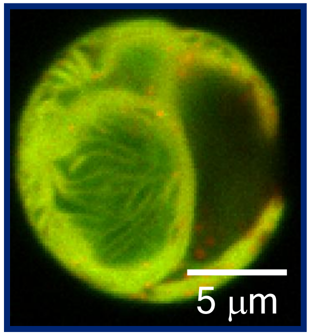
Limited chemical information →

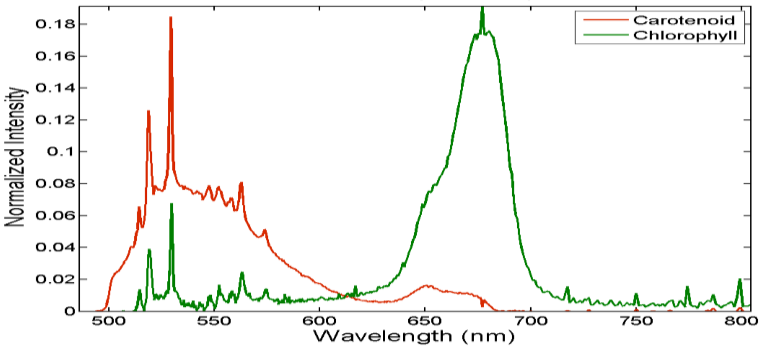


Spectral Imaging

Each pixel in the image is a spectrum relating to chemical and/or molecular structure within

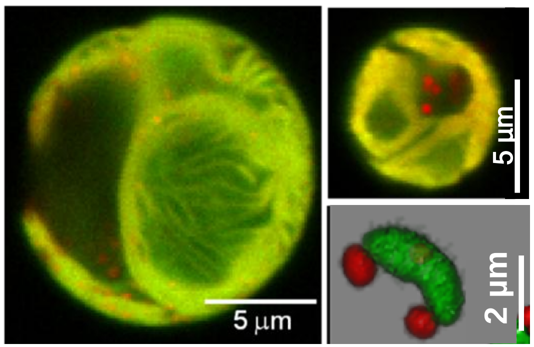
Detailed chemical information →





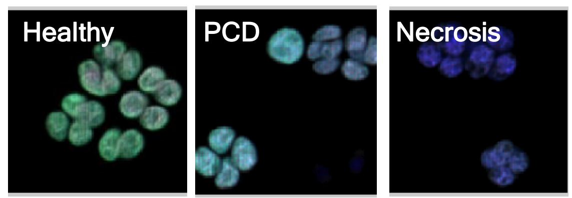
Chemical Imaging in Algal Research

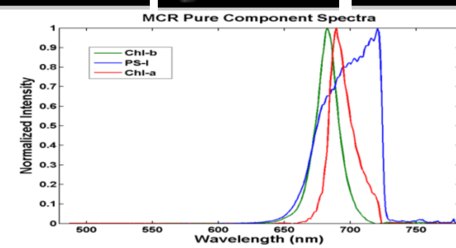
Hyperspectral Confocal Fluorescence Microscopy



Subcellular localization, quantification of lipid and chlorophyll

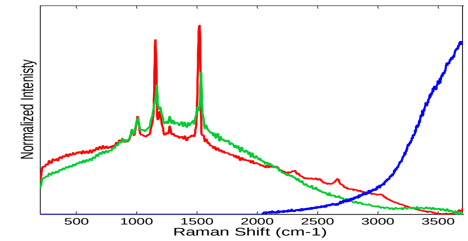
Two-Photon Hyperspectral Fluorescence Microscopy

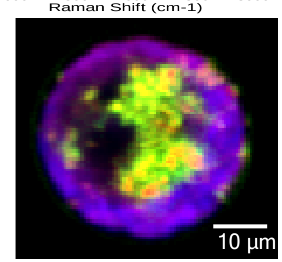




Discrimination between cell death mechanisms at early stage

Hyperspectral Raman Microscopy

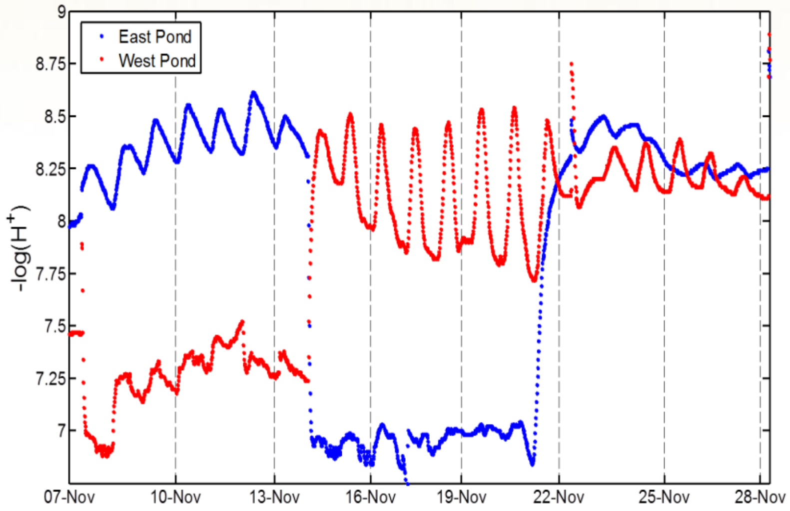


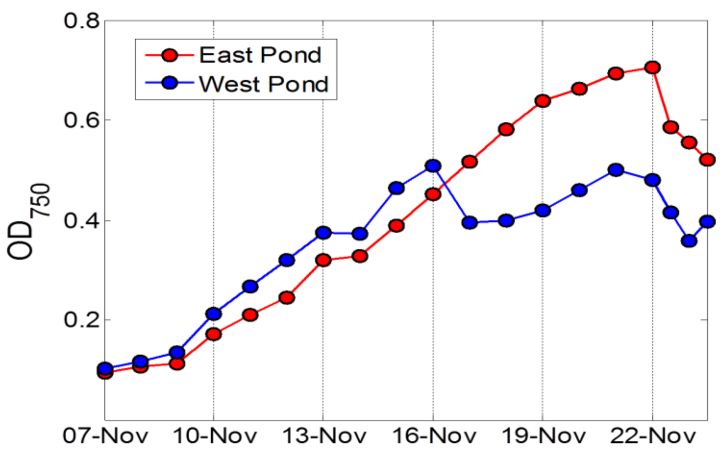


Subcellular localization, discrimination of carotenoid, lipids, and precursors

Sandia National Laboratories

Experimental Design





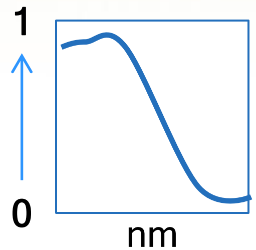
- Variation in CO2 delivery, crash induced at end of experiment
- Automated measurements:
  - pH, salinity, temperature
  - Downwelling irradiance
  - Upwelling radiance
- Daily measurements:
  - pH, absorbance
  - Flow rates
- At key time points:
  - Hyperspectral confocal fluorescence microscopy
  - C:N:P analysis

Sandia National Laboratories

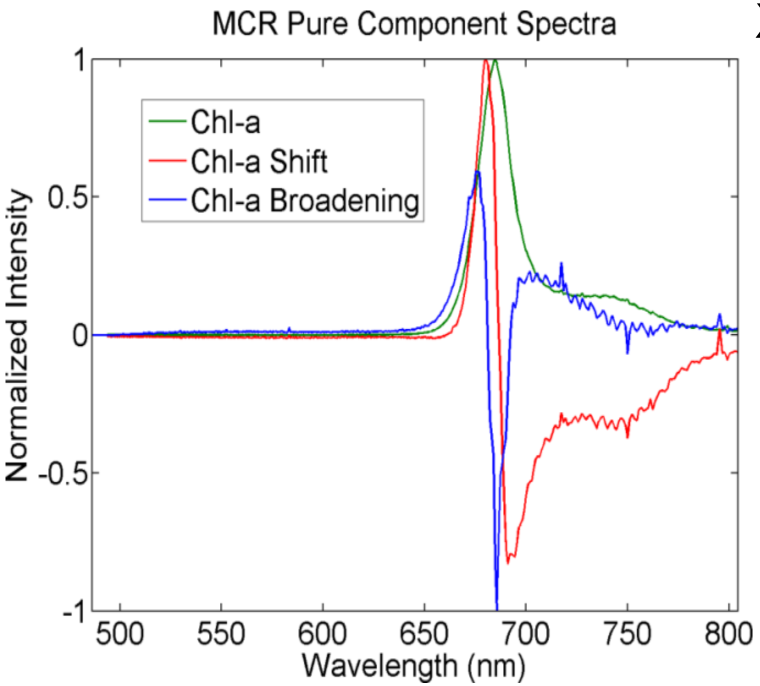
Hyperspectral Imaging Results

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

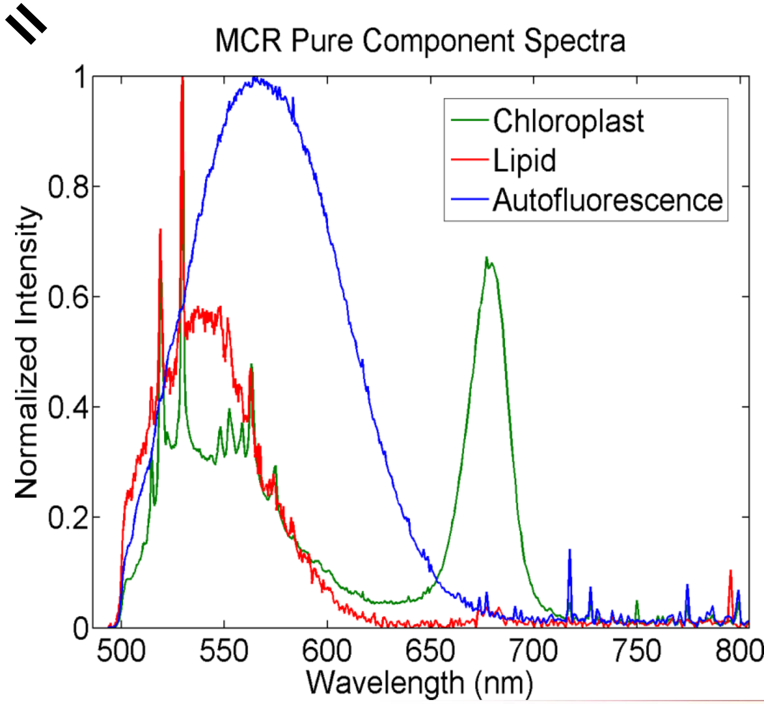
Spectral Emission Filter



X



=

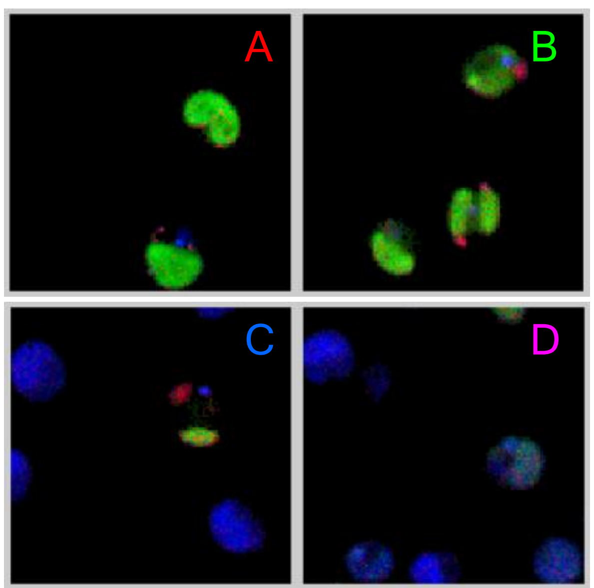


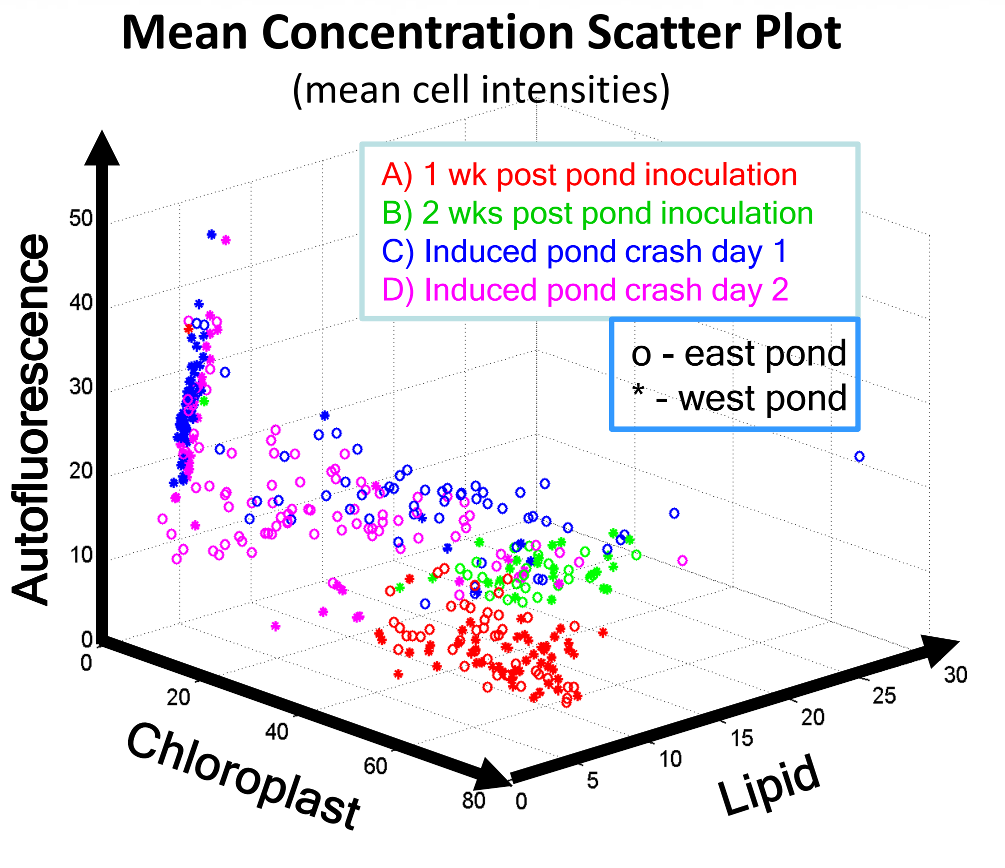
Sandia National Laboratories

Single Cell Analysis

Predictive Capability of Identified Spectral Biomarkers

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



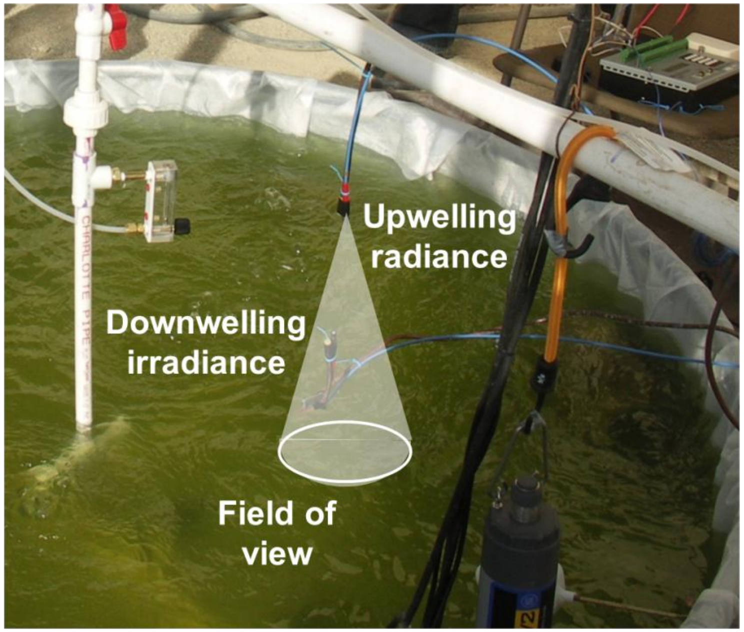


Sandia National Laboratories

Ensemble Measurements Enable Real-time Monitoring

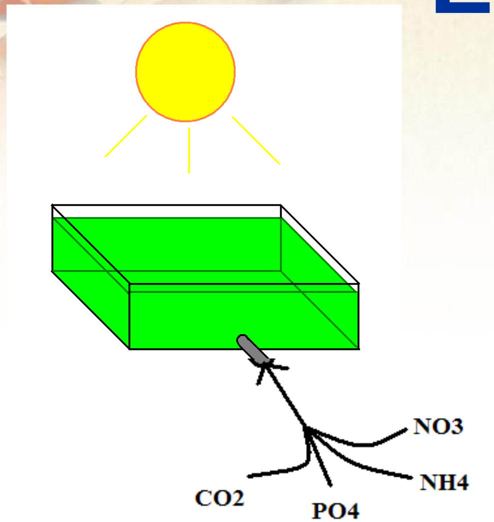
- Additional spectroscopic method: measure bulk optical properties via reflected sunlight
- Recent publication
  - Laboratory-scale
  - Real-time measure of growth rate and Chl content
- Extension to greenhouse-scale
  - Presented later in this session
  - “Remote spectroradiometric monitoring of *N. salina* in a fluidically mixed pond”
- Currently performing outdoor raceway trials

Reichardt, TA, Collins, AM, Garcia, OF, Ruffing, AM, Jones, HDT, Timlin, JA, “Spectroradiometric Monitoring of *N. salina* Growth”, Algal Research, 1(1)*, 22-31, 2012.*



Sandia National Laboratories

Experimental Data Refines Predictive CFD Model



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

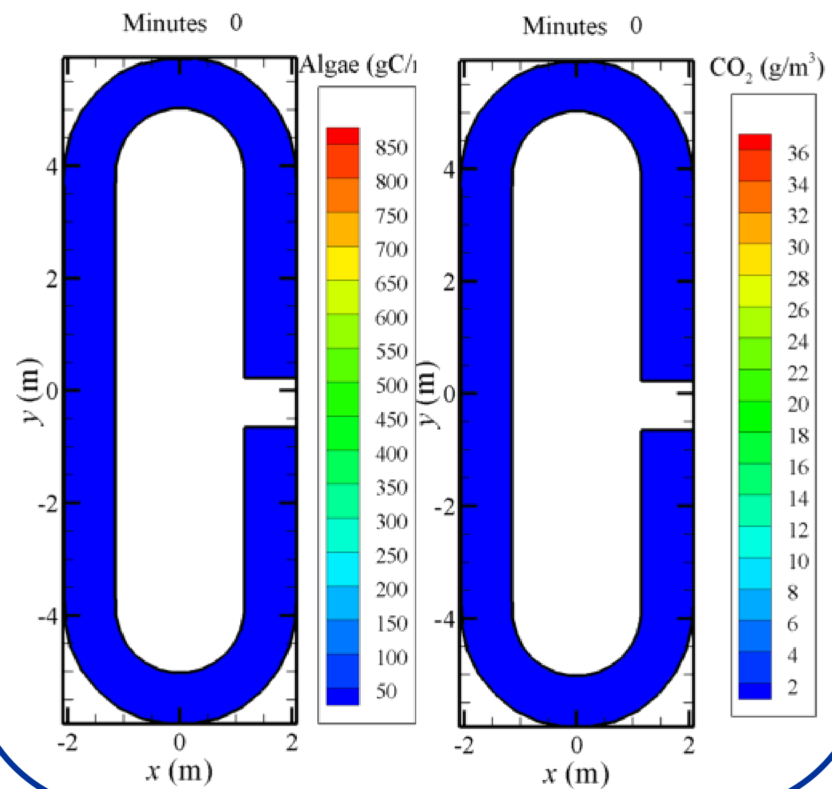
$B(\mathbf{x},t)$: spatio-temporal algal biomass (gC/m^3)

P: production rate (1/day)

B_M: basal metabolism rate (1/day)

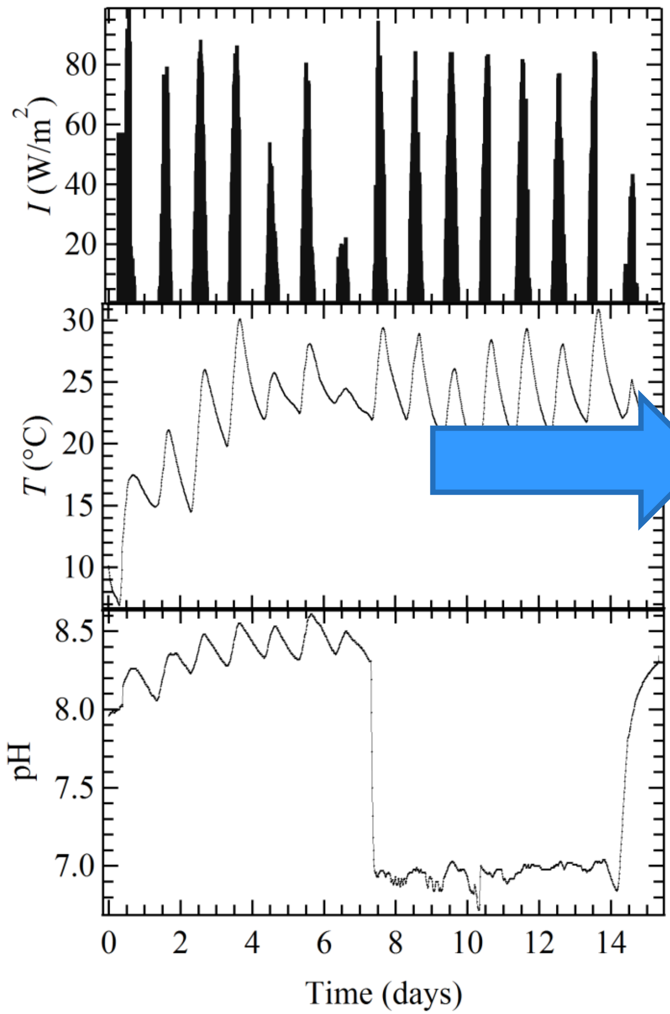
P_R: predation rate (1/day)

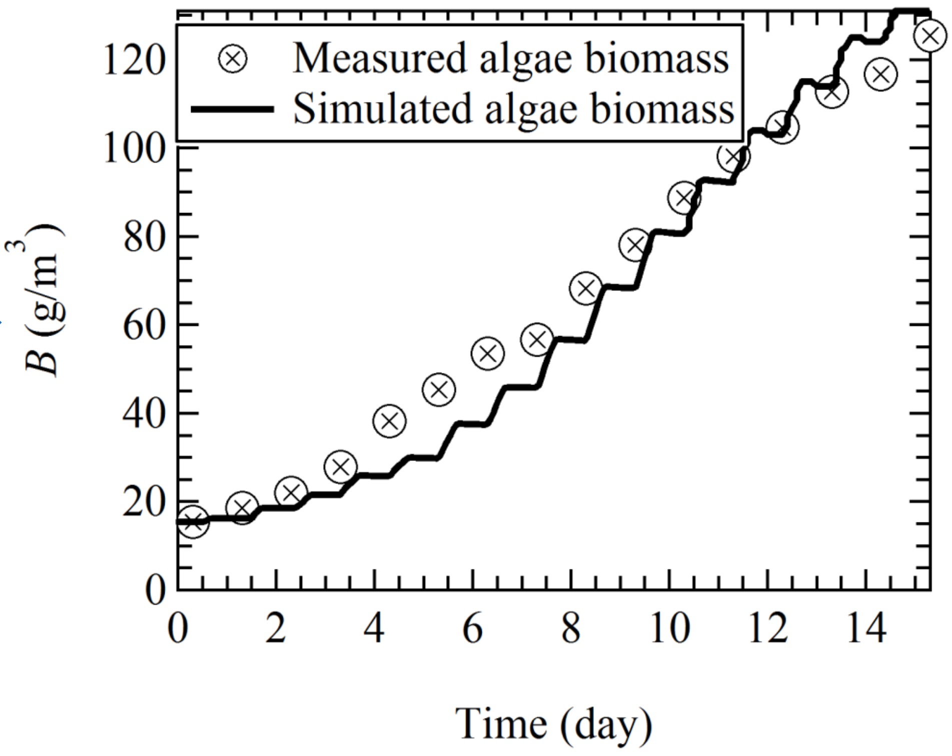
Dynamic Simulations



James, SC, Boriah, V, “Modeling algae growth in an open-channel raceway,” *J Comp Bio*, 17 (4), 1-11, 2010

Experimental Data Refines Predictive CFD Model





- Recent efforts focus on incorporating pH effects into the CFD model
- Work presented by Scott James on Monday

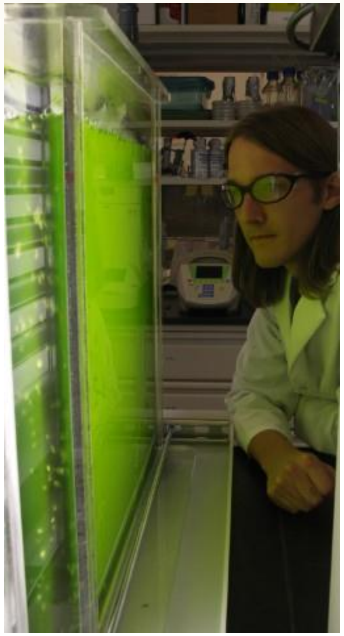
Conclusions

- Novel, integrated approach addresses scale-up challenges
  - Multi-scale experiments inform model, develop optical signatures grounded in understanding of algal response
  - CFD model can reduce experimental burden
- Real-time monitoring of algal culture health at benchtop and greenhouse scales
  - Fluorescence and reflectivity
  - Detection based-on optical/spectral properties of photosynthetic pigments and algae
- Early detection of change in health, culture composition is feasible w/ low-cost optical methods
  - Specificity yet to be determined
- Future outlook - Raceway scale demonstration

Acknowledgements

- Aaron Collins
- Brian Dwyer
- Omar Garcia
- Lindsey Gloe
- Scott James
- Vijay Janardhanam
- Howland Jones
- Todd Lane
- Kylea Parchert
- Amy Powell
- Tom Reichardt
- Anne Ruffing
- Christine Trahan

- Hanson Lab, UNM Biology
- Hu Lab, ASU's Laboratory for Algae Research & Biotechnology



This work was supported by the Laboratory Directed Research and Development program at Sandia National Laboratories.

LDRD

THE UNIVERSITY of NEW MEXICO

ASU ARIZONA STATE UNIVERSITY

Sandia National Laboratories