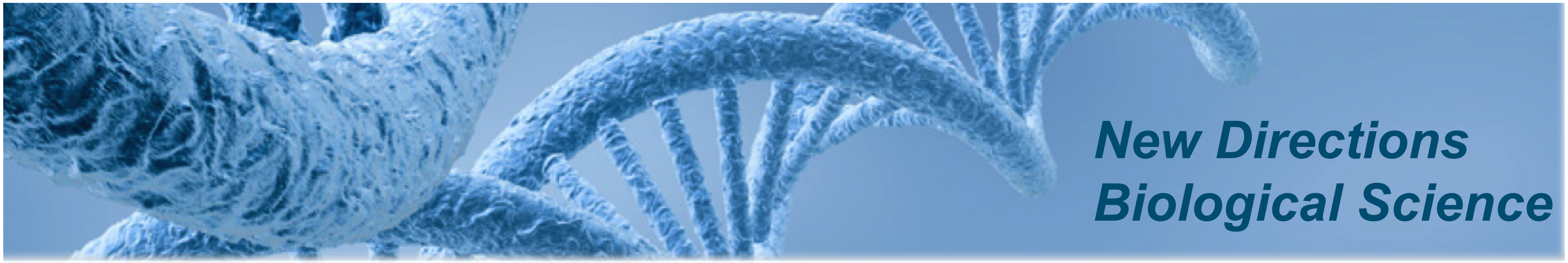

From Benchtop to Raceway: Spectroscopic Signatures of Dynamic Biological Processes in Algal Communities

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Search for New Renewable Energy Sources is On

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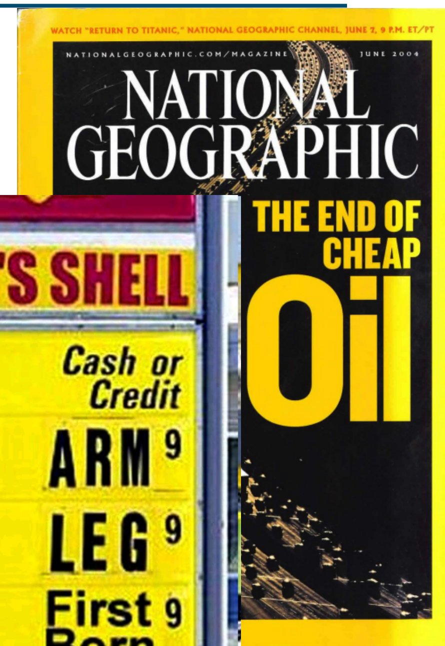
Algal-derived biofuel is a critical piece in the multi-faceted renewable energy puzzle

- 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



How do we make algal biofuels?

Simplified process diagram

Algae biomass growth

Cultivation systems
→ Harvesting / Separation → Dewatering → Drying → Oil extraction/ Conversion

0.1% solids | 1-10% solids | to 100% solids

Open Raceways

Closed Photobioreactors

Problem Definition

"The problem is not making oil from algae, it is making algae with oil, actually it's just making algae … Need to improve current best commercial practice by over a factor of ten" -- John Benneman co-author of "A Look Back at the U.S. DOE's Aquatic Species Program: Biodiesel from Algae"

Problem Definition

Culture sustainability --

System productivity --

Nutrient source scaling and sustainability --

Water conservation, management, and recycling --

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

Technical Approach

Innovative, multidisciplinary, multiscale

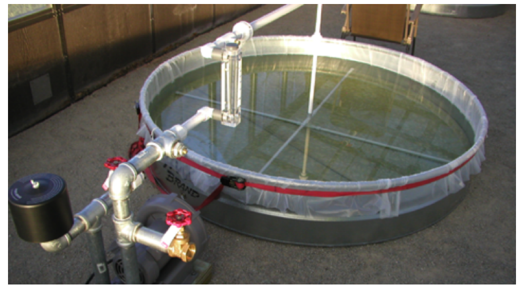
- Goal 1 -- Conduct fundamental research into the effects that dynamic biotic and abiotic stressors have on algal growth and lipid production.
  - *Genomics / Transcriptomics*
  - *Bioanalytical spectroscopy / Chemical imaging*
- Goal 2 -- Discover spectral signatures for algal health at the benchtop and greenhouse scale
  - *Remote sensing, Bioanalytical spectroscopy*
- Goal 3 – Develop computational model for algal growth and productivity at the raceway scale
  - *Computational modeling*

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

Multiscale

Culture Size

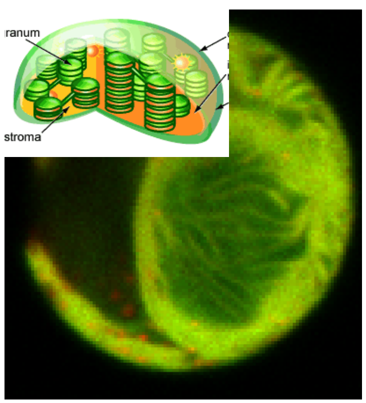
Lab scale
0.01 – 3 gal



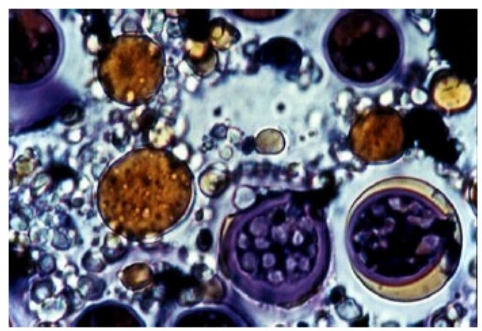
Greenhouse scale
100 – 150 gal

Raceways
1000 – 10,000 gal

Phenomenon Size



Subcellular
nm

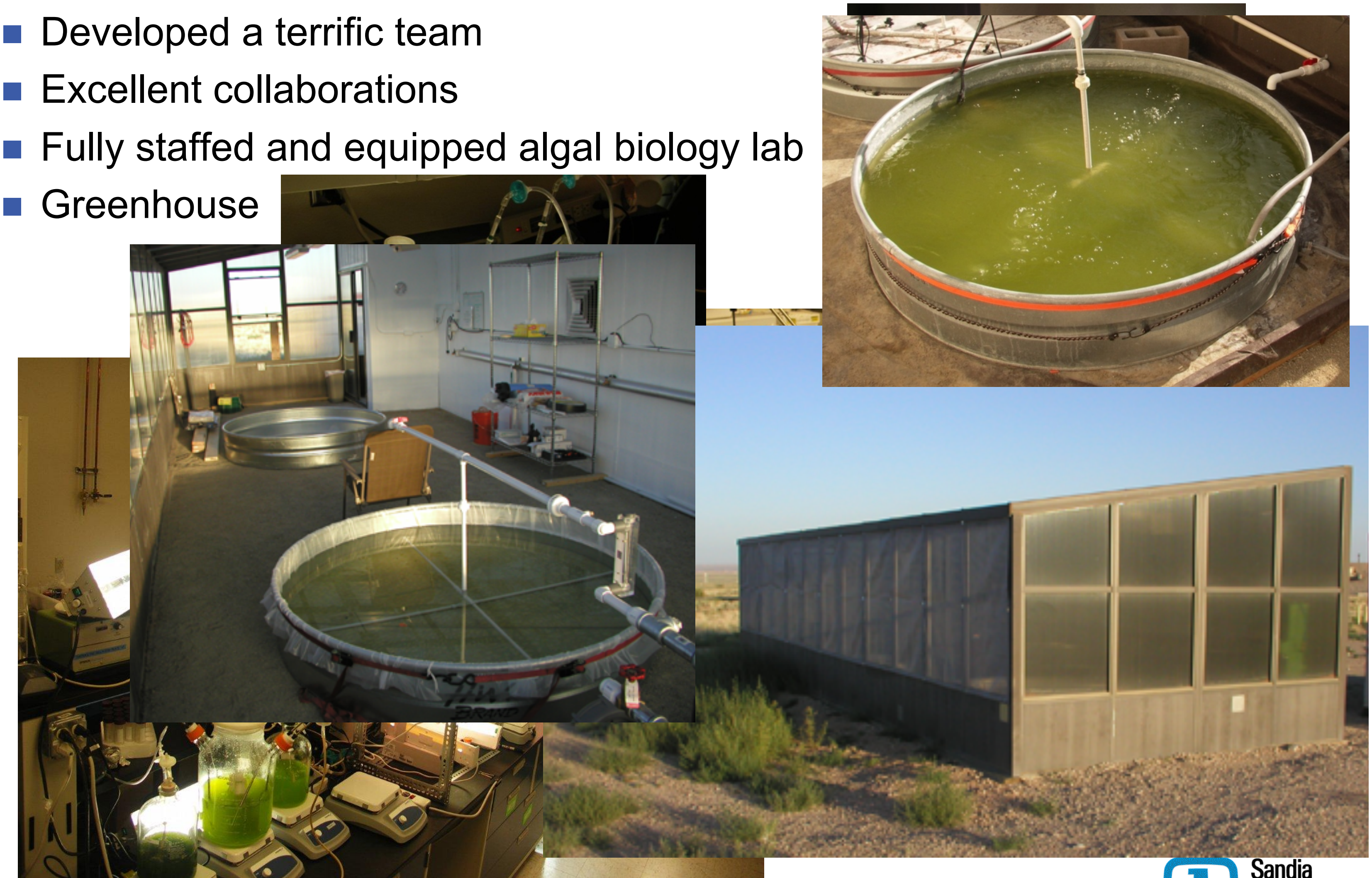


Single cell
µm

Ensemble
m

Overarching Accomplishments

- Developed a terrific team
- Excellent collaborations
- Fully staffed and equipped algal biology lab
- Greenhouse



Goal 1: Major Questions

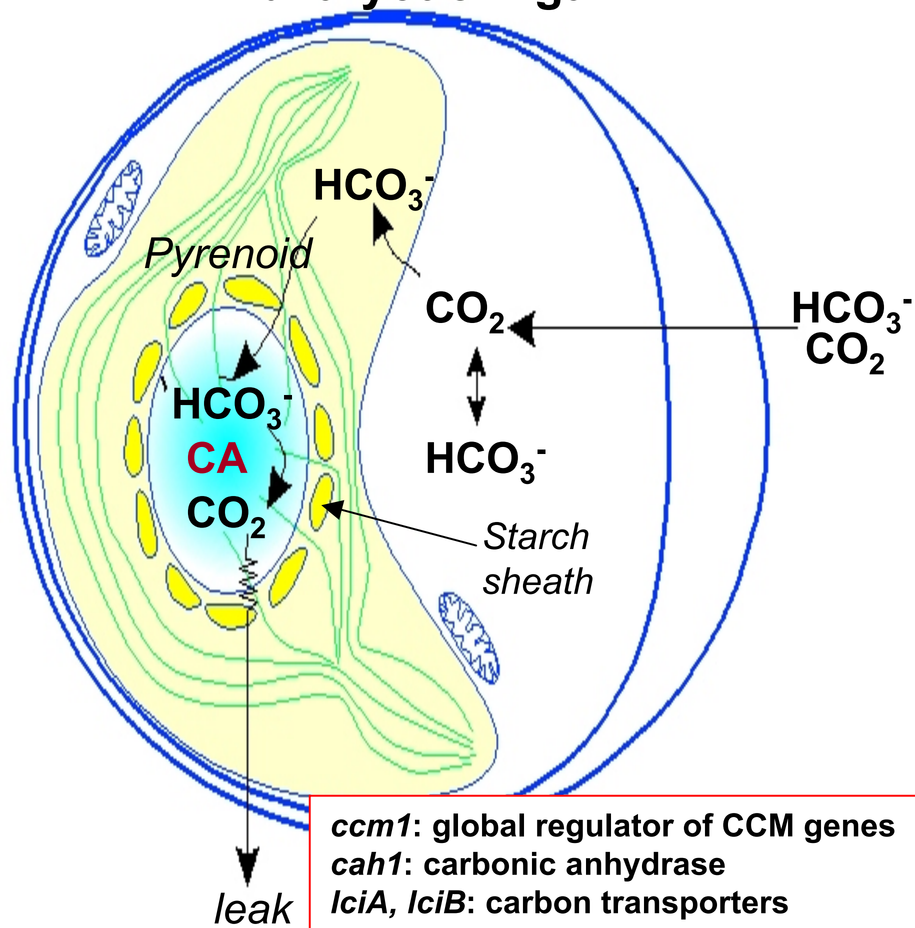
Abiotic & Biotic Effects on Metabolic Pathways

- Understanding effect of *dynamic* abiotic stressors on carbon partitioning and lipid production
    - → Relationship of CCM function to lipid production
    - → Regulation of metabolic networks by CO_2 and oxidative stress
- Understanding effect of biotic diversity *dynamics* on growth and lipid production
    - → Who are the natural pathogens, predators, competitors in arid ecosystem?
    - → What is their potential effects on raceways?

Goal 1 – Abiotic Stressors: Relating [CO_2] to Photosynthesis and Lipid production

- Algae respond dynamically to differing CO_2 concentrations
- Designed, began experiments to determine relationship of CCM to lipid production, spectral composition in 3 diverse species
- Experiments at 0.01, 0.04, and 0.5% CO_2
  1. Rate of photosynthesis
  2. Lipid production
  3. Hyperspectral imaging
  4. Targeted RT-PCR (Chlamy only)

Eukaryotic Alga



ccm1: **global regulator of CCM genes**
cah1: **carbonic anhydrase**
lciA, lciB: **carbon transporters**
sta2, sta3: **pyrenoid biosynthesis**

D Hanson, A Powell, B Ricken, A Ruffing, A Collins

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Goal 1 – Biotic Effects: Understanding Natural Diversity

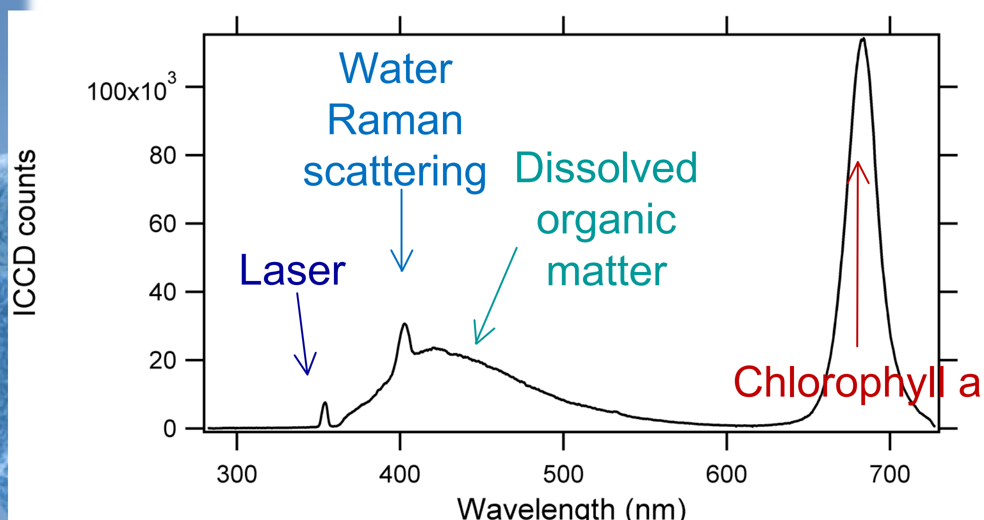
- Leveraging mesocosm experiment to understand dynamic food web in arid ecosystems (June/July 2010)
- Data collected weekly:
  - Temp, DO, turbidity, pH, conductivity, water depth, water column nutrients, soil nutrients
  - Samples for physical counts, Chl *a*, HPLC, isotopes
  - Invertebrate & fish sample (abundance, diversity, isotopes)

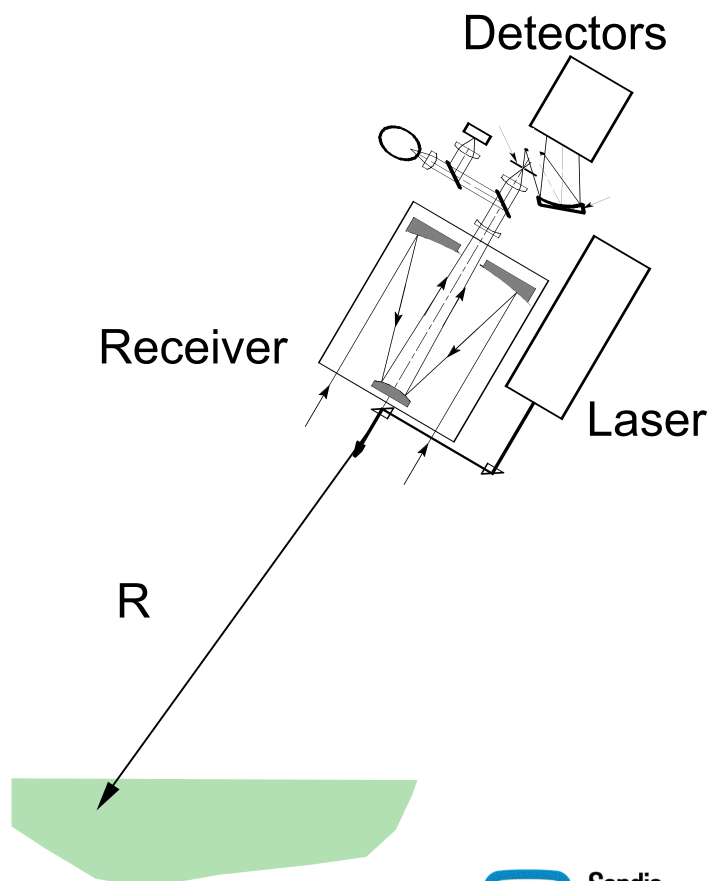
- Pigment extraction, separation w/ reverse-phase HPLC + spectroscopy
- Retention time and abs max identify taxonomic groups via unique, diagnostic pigments
- Hyperspectral fluorescence imaging

S Scholle, T Turner, D Hanson, A Powell, H Jones

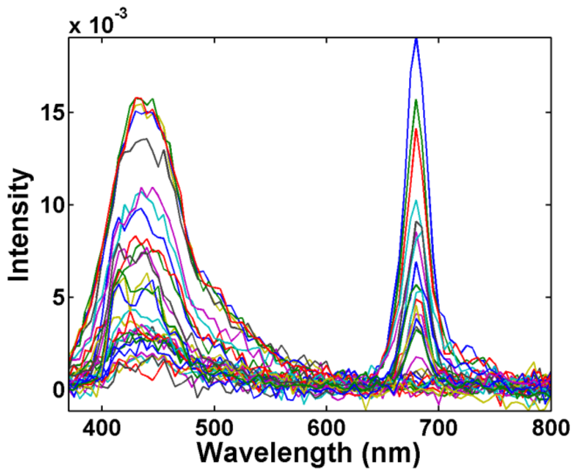
Goal 2: Major Questions

- Can we identify spectral signatures that correlate with algal culture health?
  - Within single cells?
  - At the benchtop level?
  - At standoff distances?

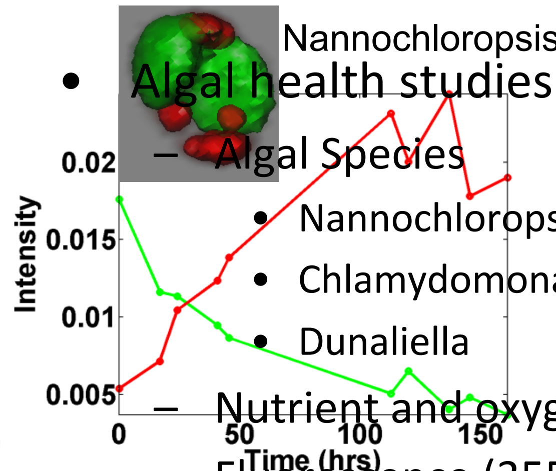


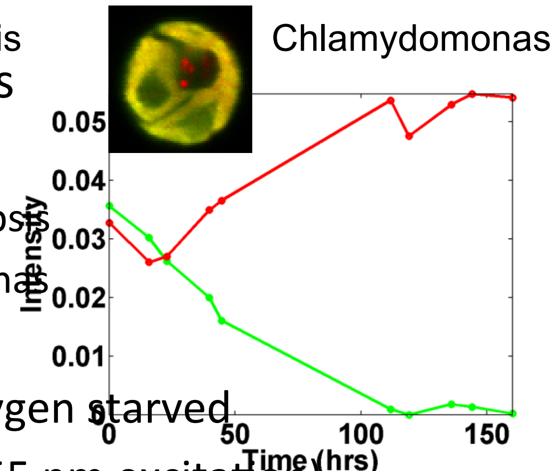


Goal 2 – Discover spectroscopic signatures for algal health and growth

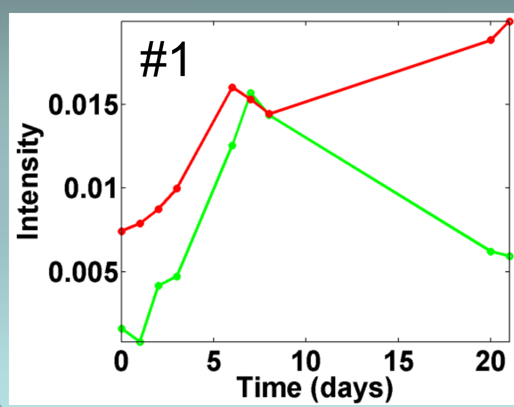


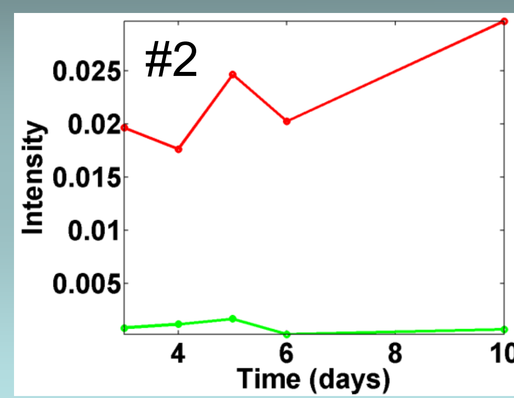
- Algal health studies
  - Algal Species
    - Nannochloropsis
    - Chlamydomonas
    - Dunaliella
  - Nutrient and oxygen starved
  - Fluorescence (355 nm excitation)

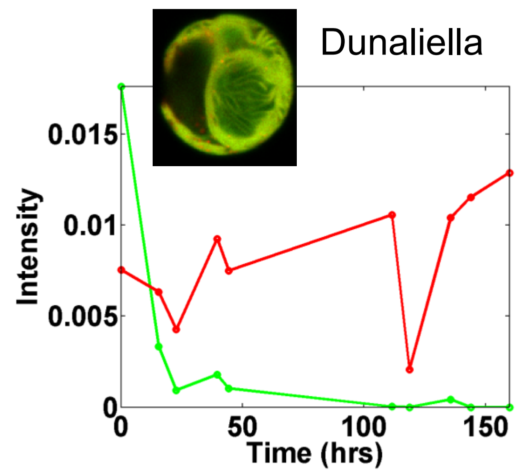




Greenhouse Studies







H Jones, T Reichardt, A Collins, O Garcia, B Ricken, B Dwyer

Goal 3 – Develop, Validate Model of Algal Growth, Productivity at Raceway Scale

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Research Article

Modeling Algae Growth in an Open-Channel Raceway

SCOTT C. JAMES and VARUN BORIAH

ABSTRACT

Cost-effective implementation of microalgae as a solar-to-chemical energy conversion platform requires extensive system optimization; computer modeling can bring this to bear. This work uses modified versions of the U.S. Environmental Protection Agency's (EPA's) Environmental Fluid Dynamics Code (EFDC) in conjunction with the U.S. Army Corp of Engineers' water-quality code (CE-QUAL) to simulate hydrodynamics coupled to growth kinetics of algae (*Phaeodactylum tricornutum*) in open-channel raceways. The model allows the flexibility to manipulate a host of variables associated with raceway-design, algal-growth, water-quality, hydrodynamic, and atmospheric conditions. The model provides realistic re-

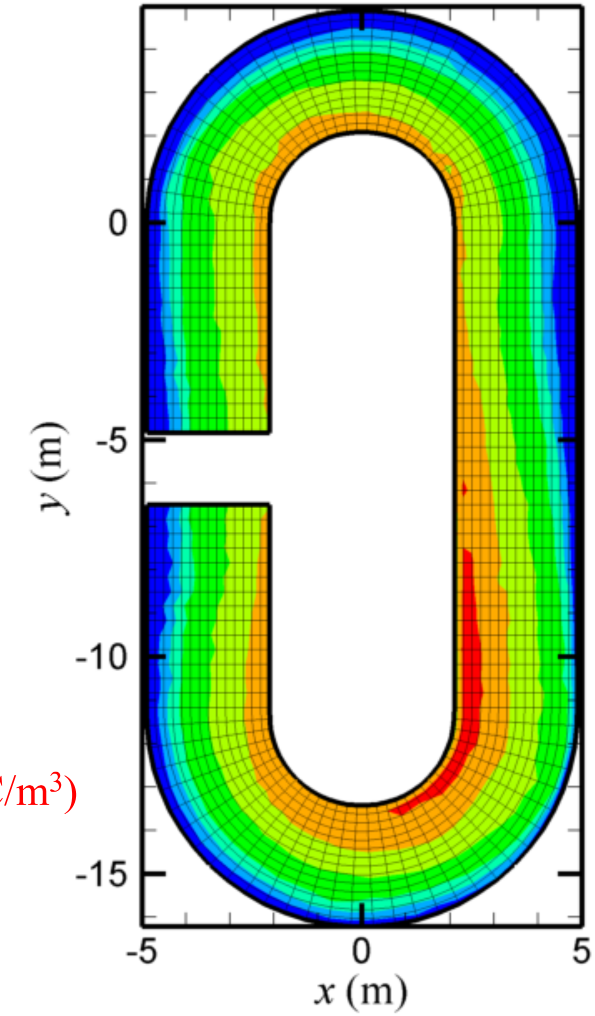
$$\frac{\partial}{\partial t} B(\mathbf{x},t) = \left(P - B_M - P_R\right) B(\mathbf{x},t)$$

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

P is the production rate (1/day)

B_M is the basal metabolism rate (1/day)

P_R is the predation rate (1/day)



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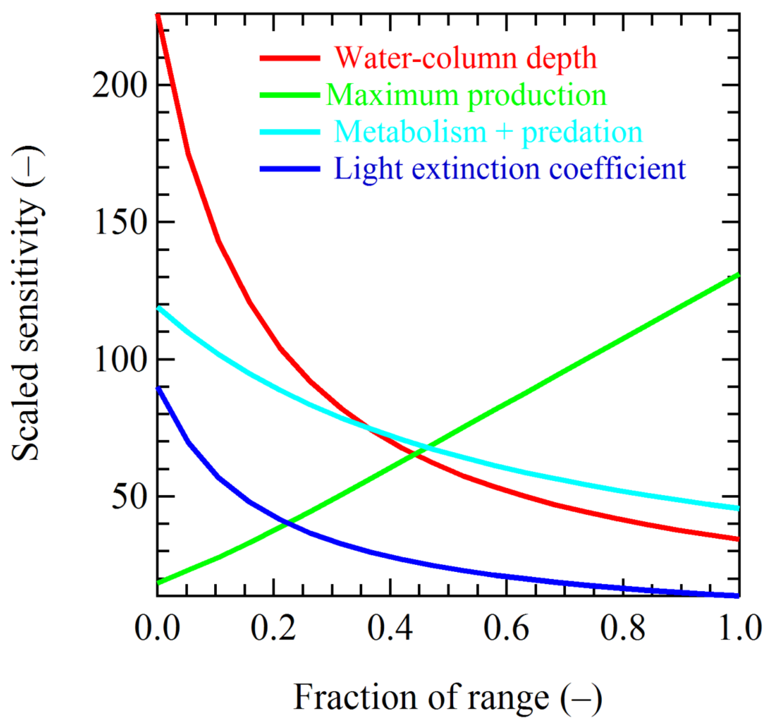
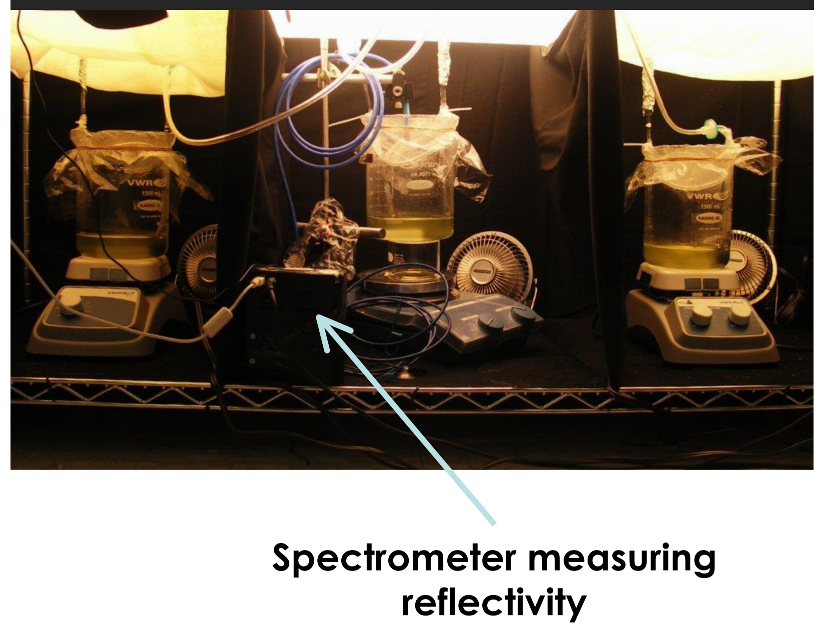
Goal 3 – Develop, Validate Model of Algal Growth, Productivity at Raceway Scale

- Develop species specific constituent relationships for most sensitive parameters



Nannochloropsis growing at 3 light intensities



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Future Outlook

Goal 1: Biological Response

Goal 2: Spectra

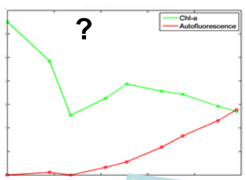
Physical and chemical composition

-Multi-scale analyses
- -rapid non-destructive lab assays
- -continuous and large area in field

-Pigment signals: e.g. chl a

-Novel signals of biological processes
- -lipid production
- -autofluorescence of cell death

- Model validation at multiple scales



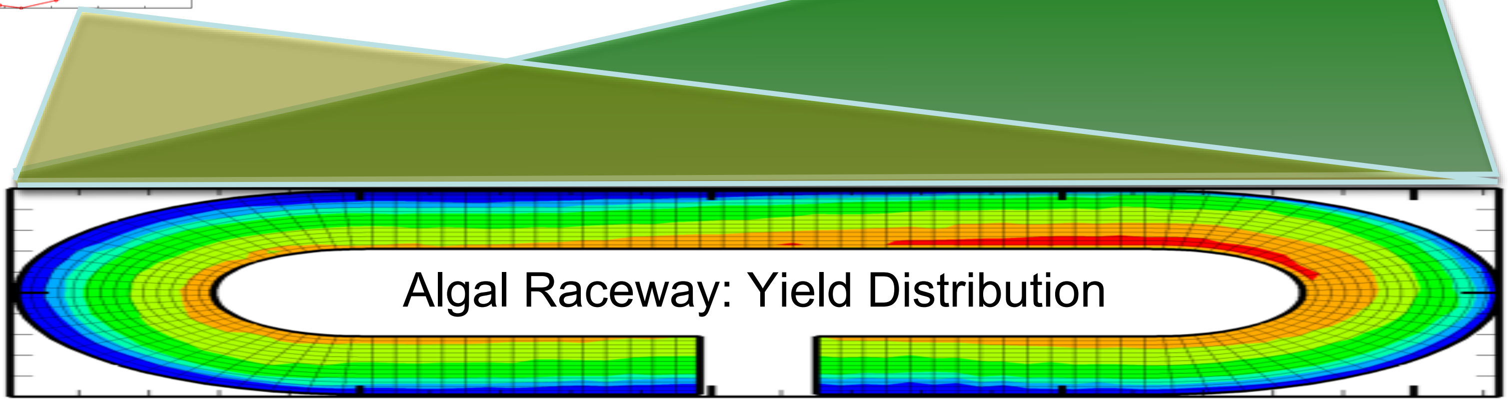
Physiological & molecular information

- Growth rate and lipid content
- Efficiency of light and CO_2 capture
- Gene expression
  - broad assessment of metabolic perturbation
  - may correlate to spectral shifts
  - expected to cause physical and chemical changes

Goal 3: Model

Predict productivity

- Spatially estimate biological function over time
- Distribution of physical resources: e.g. light and CO_2
- Locations for monitoring



Why Us? Why Now?

- Niche of algal biology that is largely unexplored, yet critical
  - *Particularly relevant for arid southwest ecosystems*

- Need multidisciplinary approach; fundamental bioscience R&D w/ “keen eye” on application

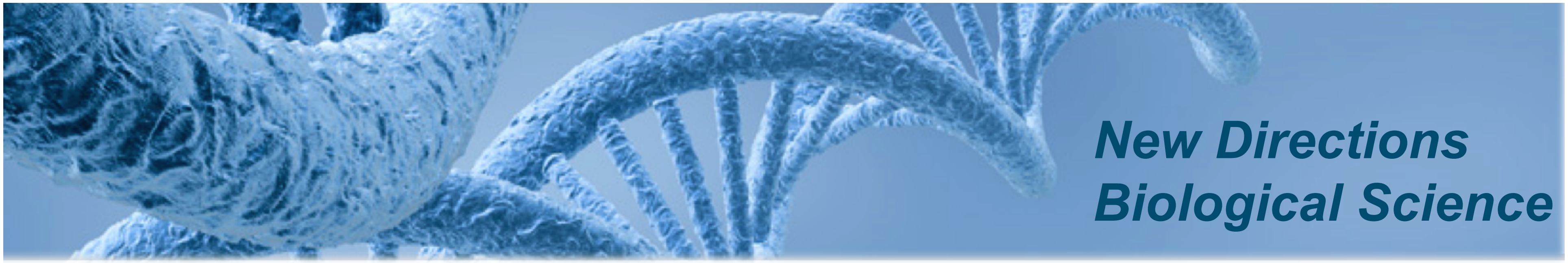
- Unique combination of expertise unavailable elsewhere
  - *Genomics, metagenomics*
  - *Algal growth, biochemistry, and physiology*
  - *Spectral signatures for early detection of disease*
  - *Algorithms for robust identification, quantification of minor species in presence of interferrents.*
  - *Standoff detection of biological signatures*
  - *Computational fluid dynamics of algal raceways*

Directly Aligned with DOE Mission, Bioscience Strategic Thrusts

- Fundamental Bioscience R&D
- Address challenges in achieving scalable transportation fuels, specifically algal cultivation at scale
- Leverages strengths in
  - chemical imaging, multivariate analysis
  - remote sensing
  - systems biology
- Success strategically positions SNL to be competitive in external funded opportunities

Impact & Outcomes

- New knowledge of algal biology/physiology as it relates to cultivation at scale and lipid production.
- Increase our visibility in the fields of Algal Biofuels & Bioscience
  - Peer-reviewed publications
    - *James, SC and V Boriah, Modeling algae growth in an open-channel raceway, J. Comp Bio,* ***17****(4), 2010.*
  - Presentations at regional, national, and international conferences on algal biology, photosynthesis, and more generally, biofuels and bioenergy
    - *4 presentations (2 invited), 2 posters*
- Positions SNL to be competitive in external proposals to DOE
  - Integrated biorefineries
    - *2 submitted, 1 successful – James, Jones, Timlin "Sapphire IBR"*
  - Sustainable Algal Biology Center
    - *Funded – $6M*
  - EERE/OBP lab call
    - *2 submitted, 1 successful – Lane, Timlin, Wu "Pond Crash Forensics"*



Questions?

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