LA-UR-15-22320
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Title: Multi-scale Characterization of Improved Algae Strains

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Intended for: Invited seminar at Indiana University at Bloomington

Issued: 2015-04-01

Multi-scale Characterization of Improved Algae Strains

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Bioscience Division

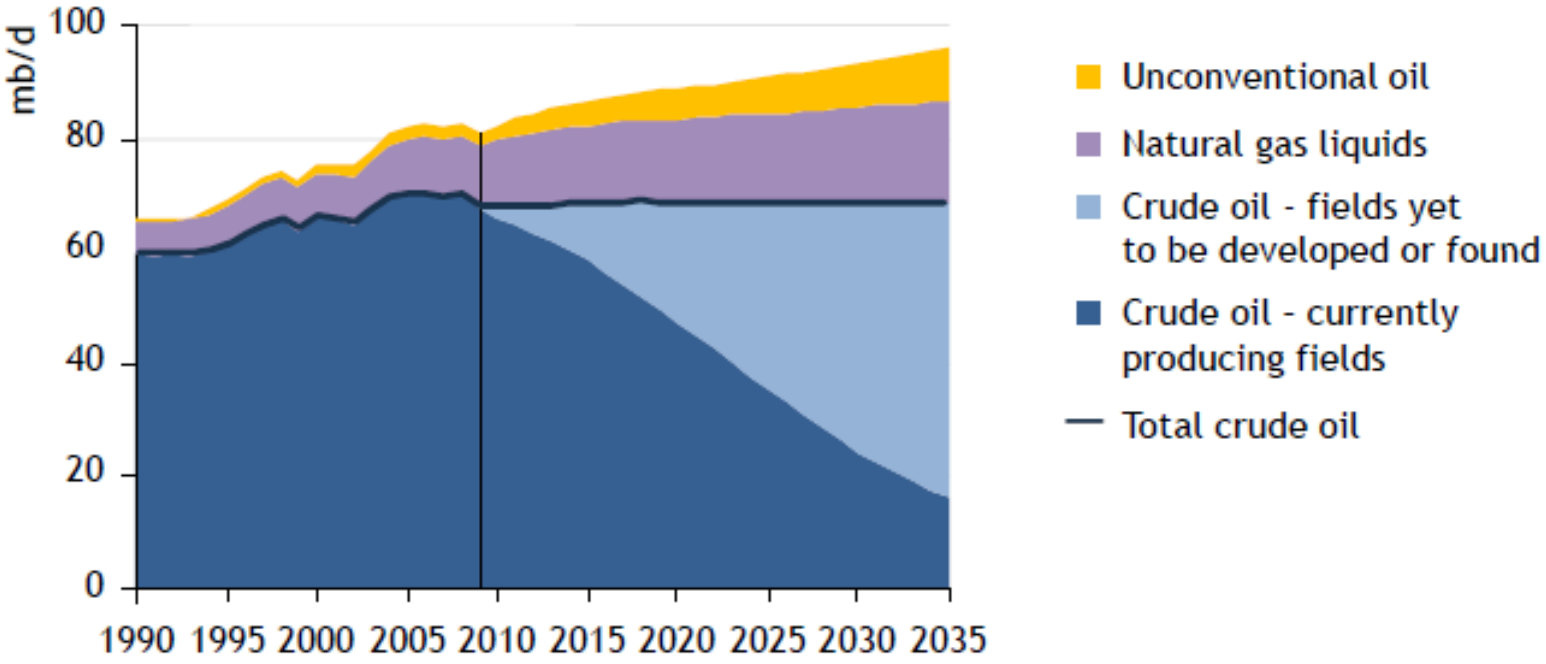
Outline

- Background – Why Biofuels? Why Algae?
- Challenges – Economics, Biology
- Overall Goal & Objectives
- Approach & Results
- Future Work

- Professional Path
- Working at a National Laboratory
- Resources

Why Biofuels?

The 2010 Energy Information Administration Report Indicates Crude Oil Levels Have Peaked



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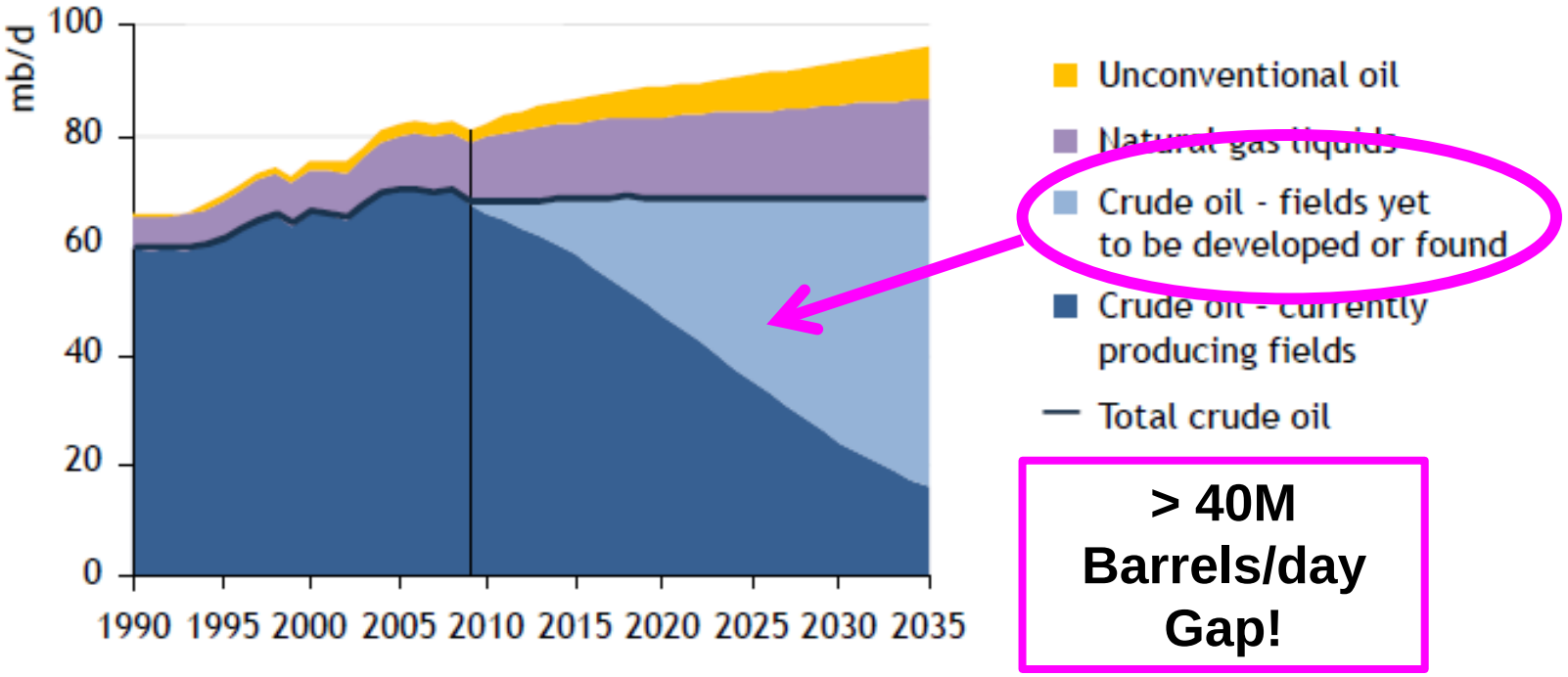


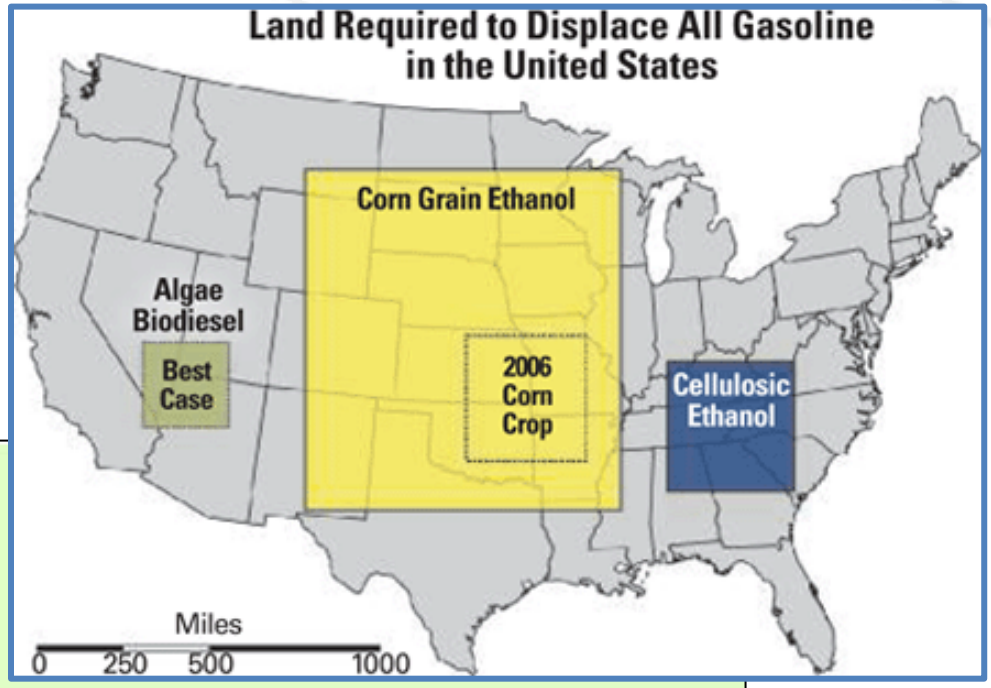
- Petroleum supplies are diminishing, while demand increases (particularly in developing nations)
- Greenhouse gas emissions have provided additional impetus to reduce petroleum use

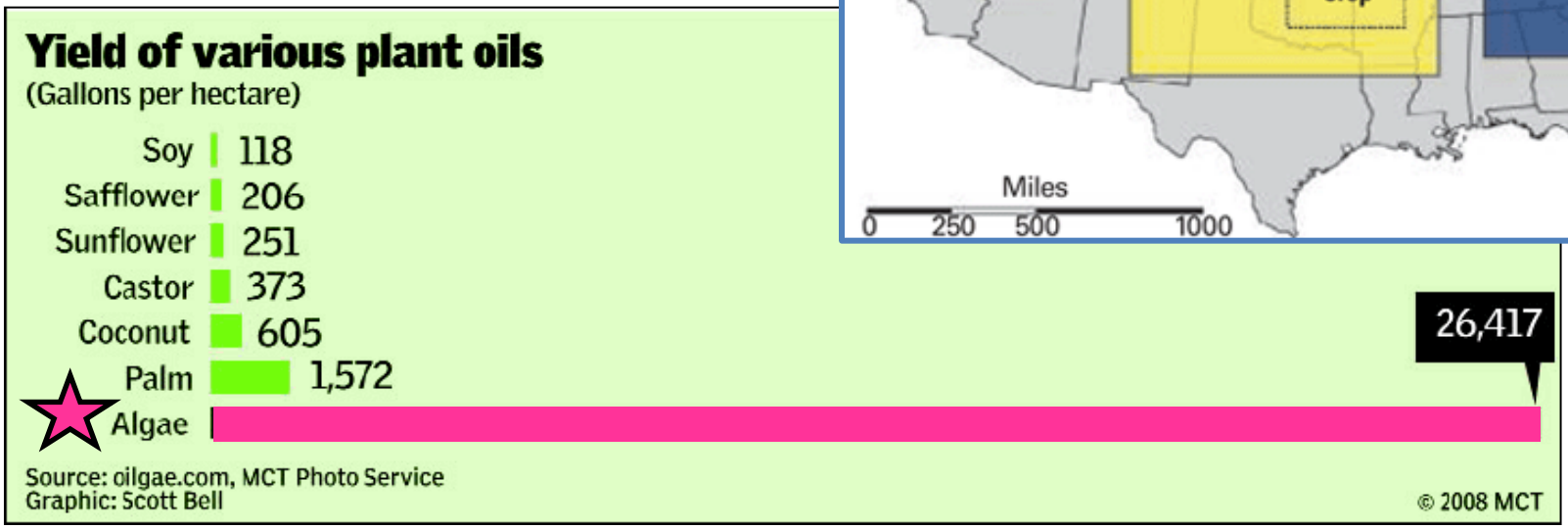
Renewable Energy is Key

But why algae?

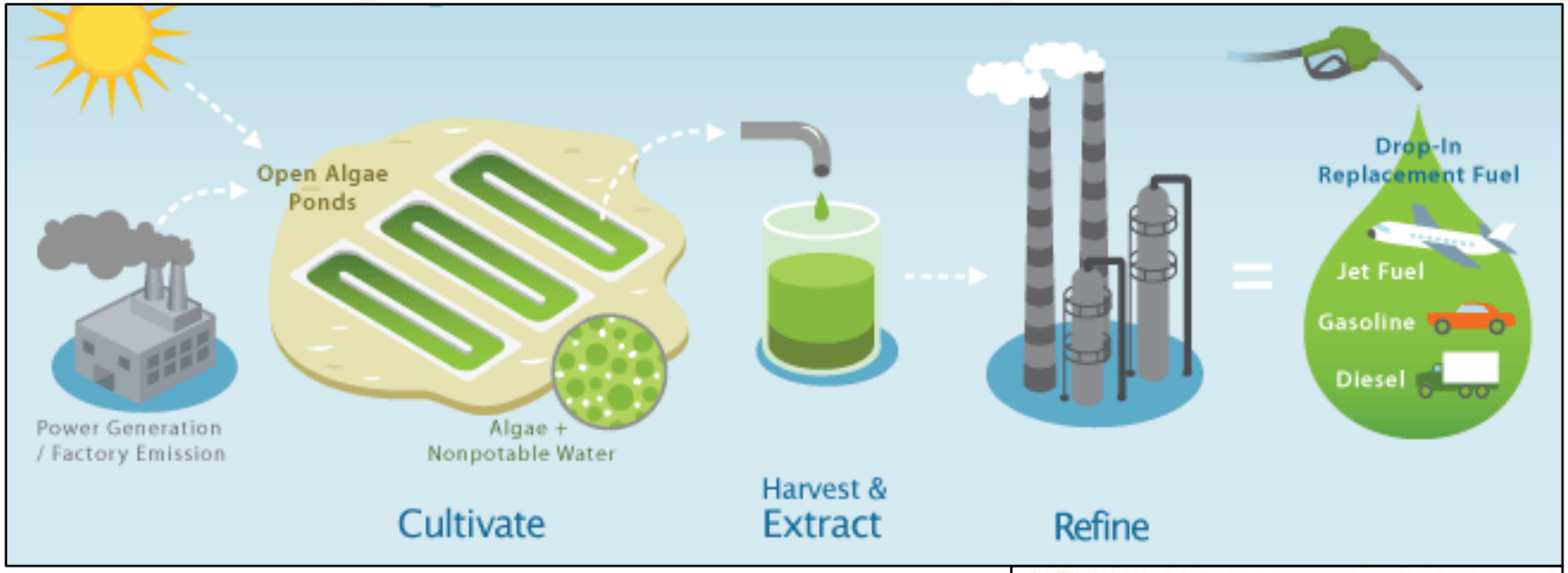
Algae Has Several Advantages Over Other Biofuels

- Can use brackish water and arid land -- Not as competitive with food production as other biofuel feedstocks
- Utilizes existing fuel infrastructure
- High energy density





The Vision for an Algal Biofuels Farm Seems Relatively Simple



Challenges Remain for Creating a Viable Algae Biofuels Industry

1. **Cost**. Although recent economic analyses show that algae biofuels could cost as little as $4/gge, *current costs are >$16/gge*. Additional analyses indicate that the largest drivers of cost are biomass and lipid (oil) accumulation.

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2. **Productivity**. Algae strains do not grow fast enough or accumulate enough lipids to be economically viable. Strains must be improved for productivity.
3. **Consistency**. Strains that have shown improved productivity in the lab environment often perform differently when grown outside.

Project Goal Statement

The overall goal of this project is to develop a streamlined process for improving algae strains and characterizing their performance at multiple scales, from the bench to outdoors.

Project Objectives

1. Generate additional improved algae strains using flow cytometry, adaptive evolution, and transcriptome analyses
2. Establish a pipeline for evaluating improved strains under conditions that directly simulate outdoor climate conditions
3. Transition strains to outdoor ponds for testing

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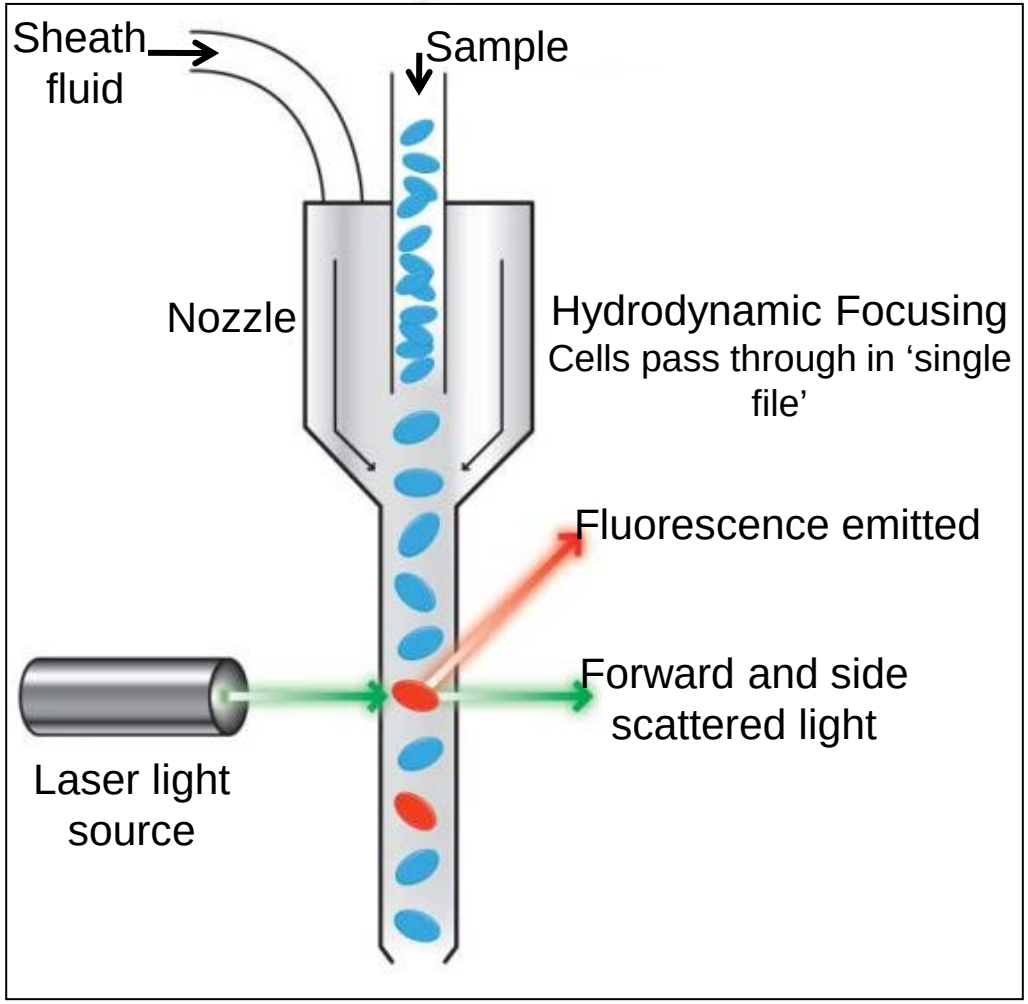
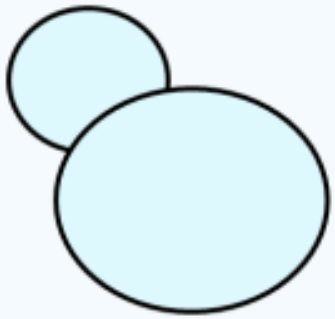
Flow Cytometry is Used to Characterize Individual Cells

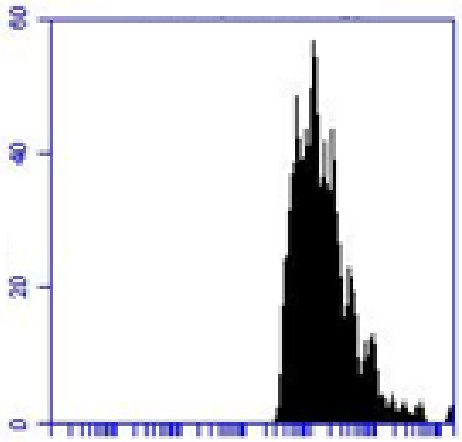
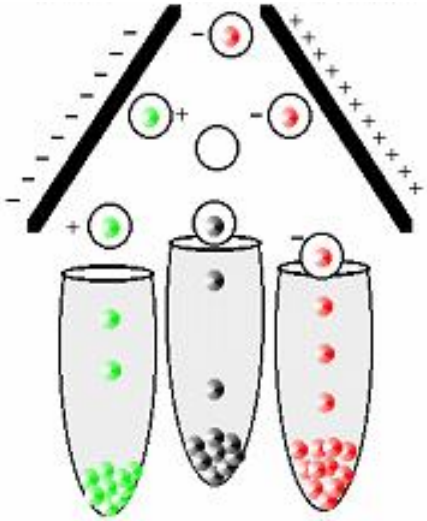


- Cells are focused in a flowing sample stream for individual interrogation by a laser
- Light scatter and fluorescence by the cell is detected & utilized for characterization
- Different populations within heterogeneous samples can be identified and sorted

Algae Cell Properties are Monitored in a Multi-Parameter Flow Cytometry Assay

Forward Light Scatter	Side Light Scatter	Red Fluorescence	Green Fluorescence
Size	Granularity	Chlorophyll	Lipids (when stained)
			

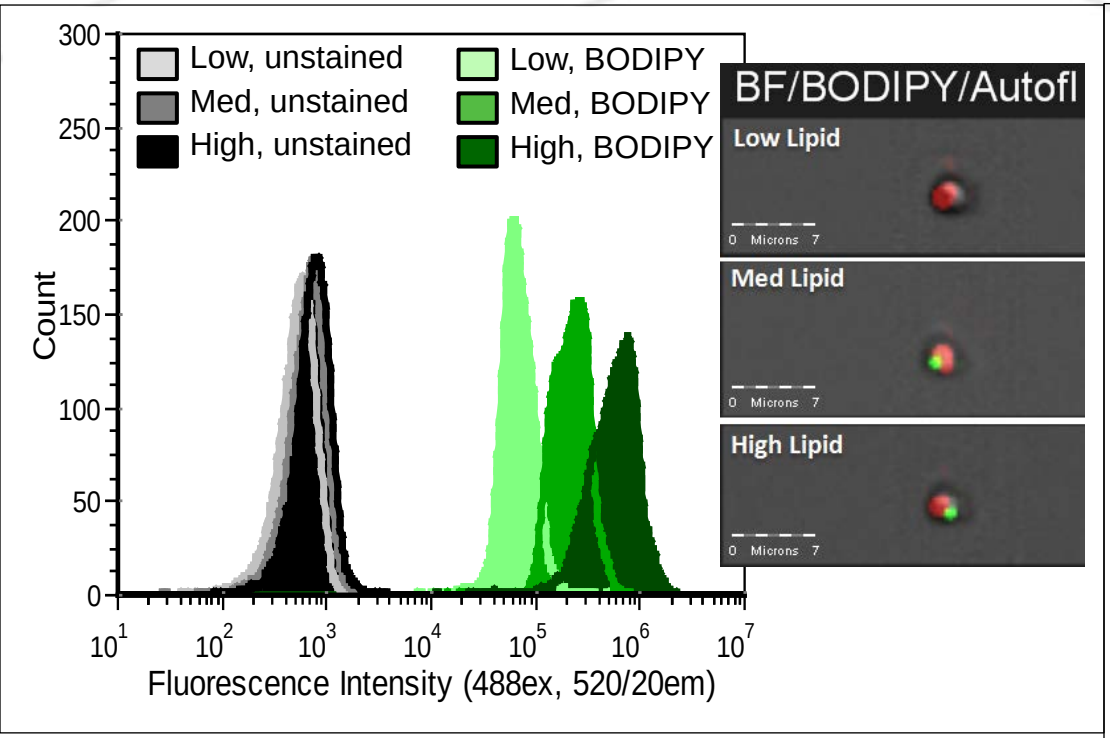
Three Flow Cytometers With Different Specialties Were Used

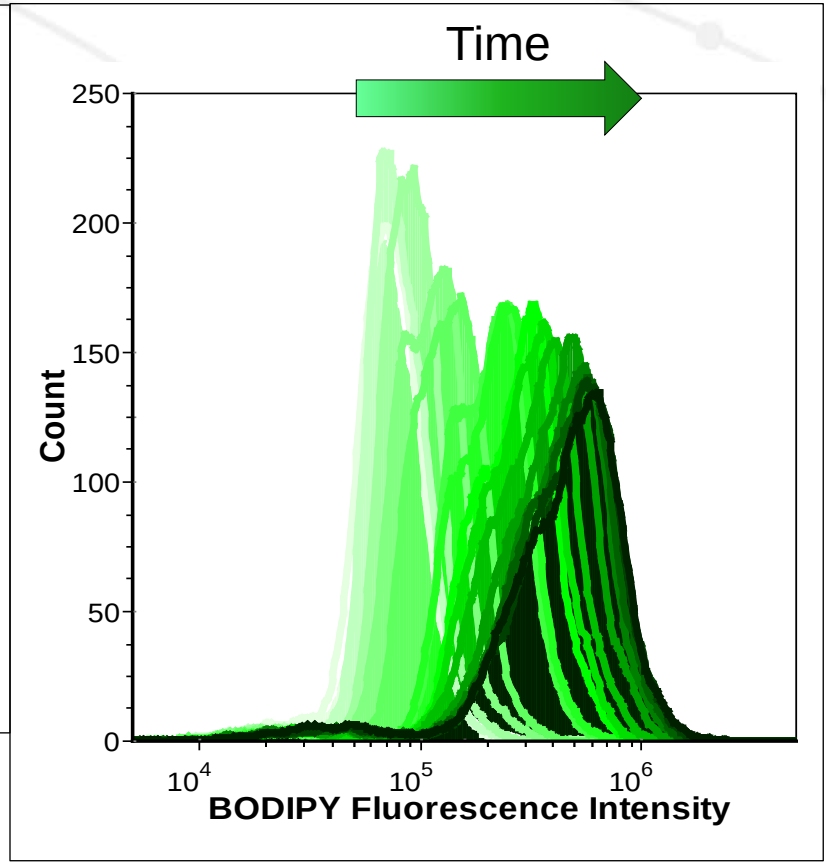
Accuri C6	Amnis ImagestreamX	BD FACSAria
Large dynamic range – can assay samples of v. different fluorescence w/ a single setting	High throughput imaging: Takes images of each cell	Sorting capability
	 X 1000s	

http://www.microscopy.ws/themes/default/img/microscope.jpg

Adapted from http://parts.mit.edu/igem07/index.php/Flow_cytometry

BODIPY Staining of *Picochlorum sp.*

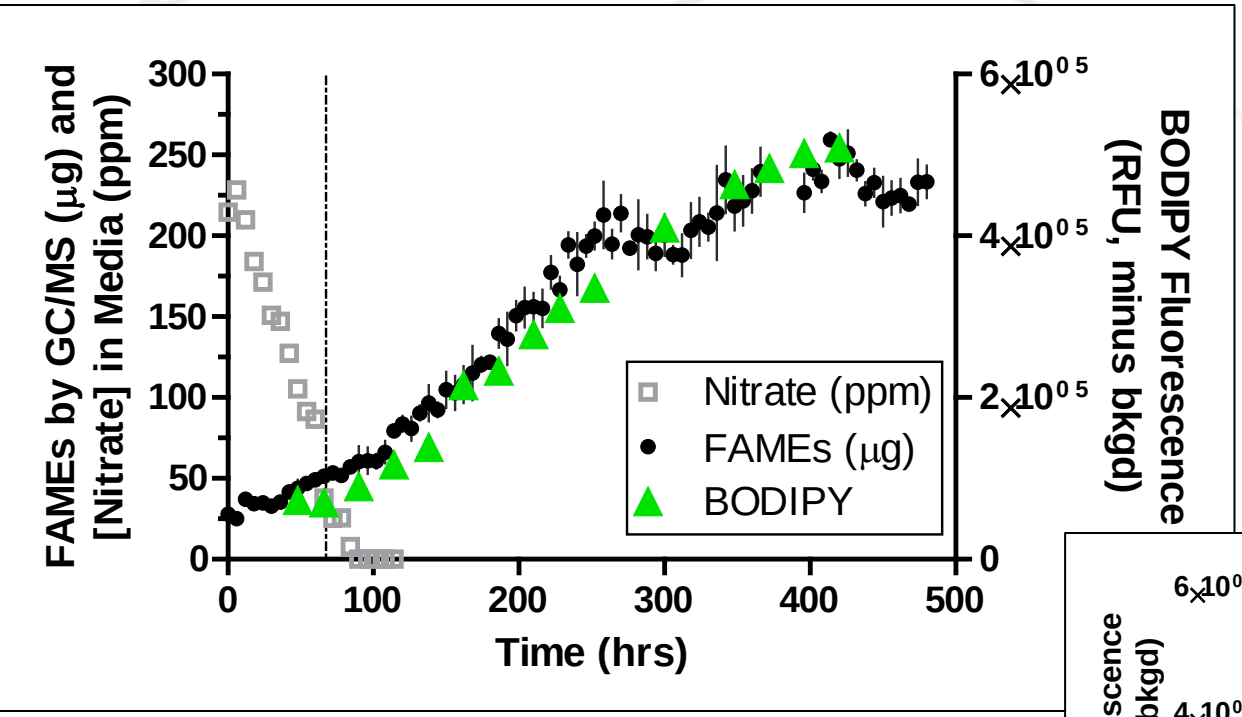


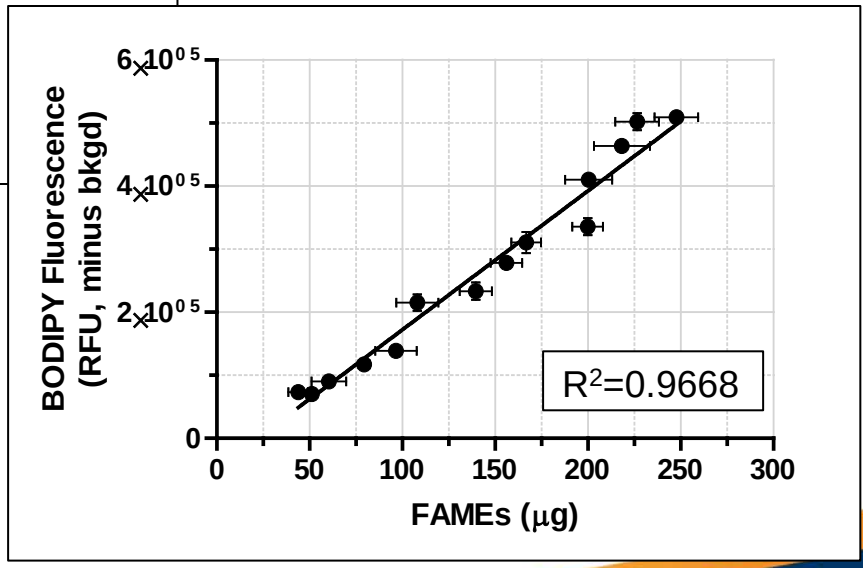


- *Picochlorum sp* is green microalgae that is a strong candidate strain for biofuel production

- BODIPY 505/515 (Invitrogen) is used to vitally stain neutral lipid bodies in microalgae

Fluorescence and Lipid Content Correlate Well

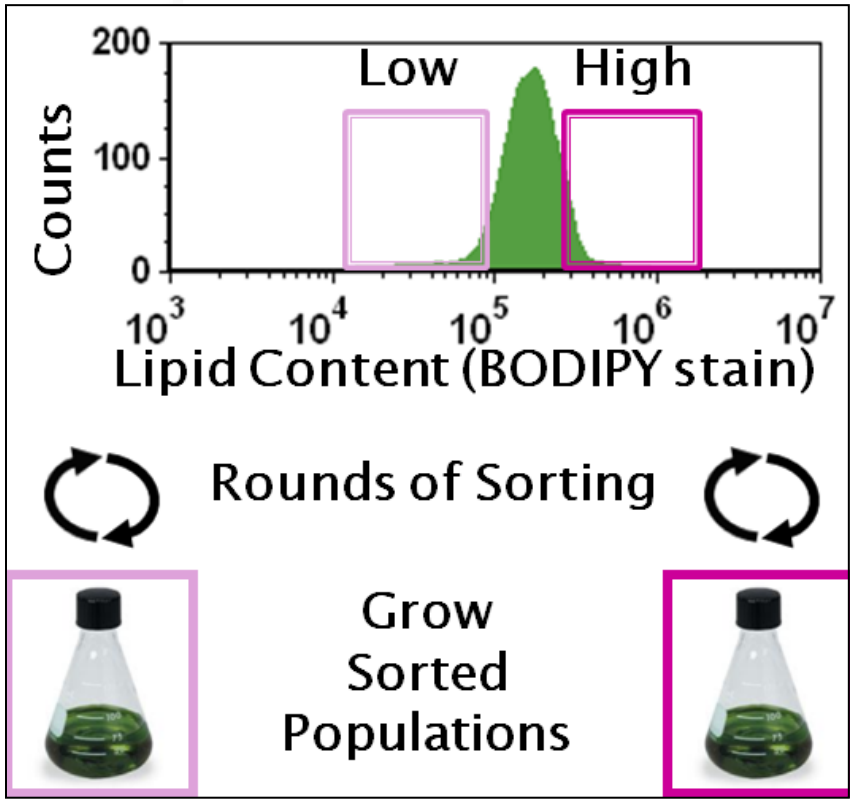


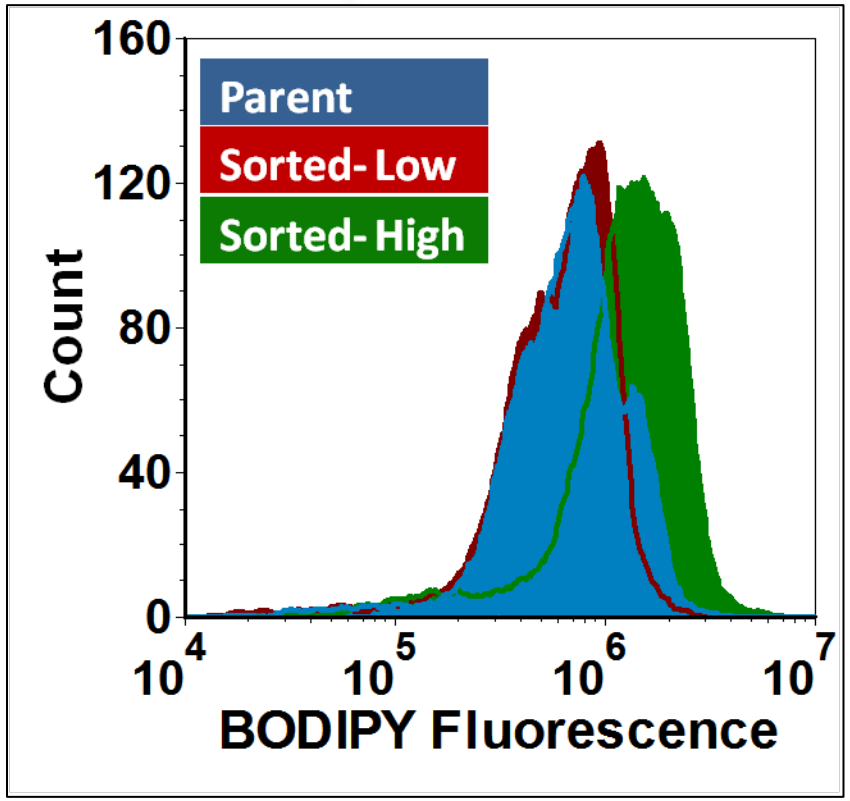


- Lipid accumulation occurs upon nitrogen depletion
- BODIPY fluorescence values correlate well with FAME values by GC/MS

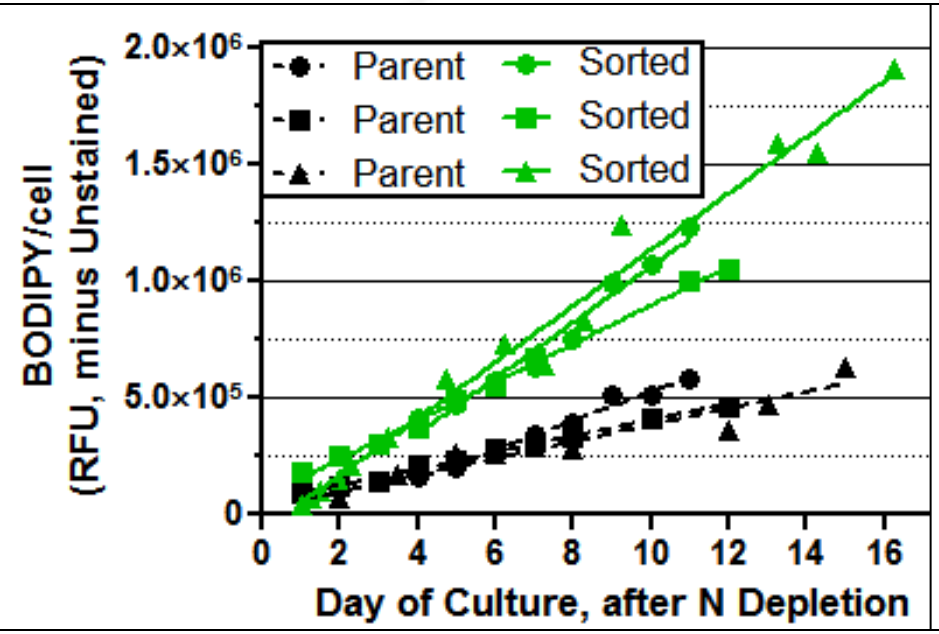
A Subpopulation of *Picochlorum sp.* with Increased Lipid Content Was Isolated Using FACS

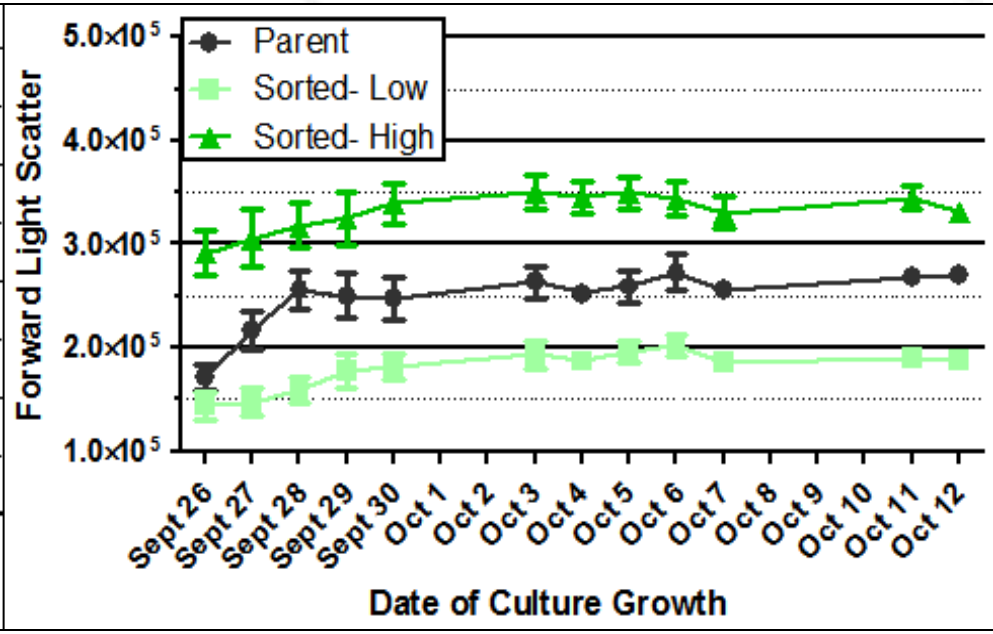
Fluorescence-Activated Cell Sorting





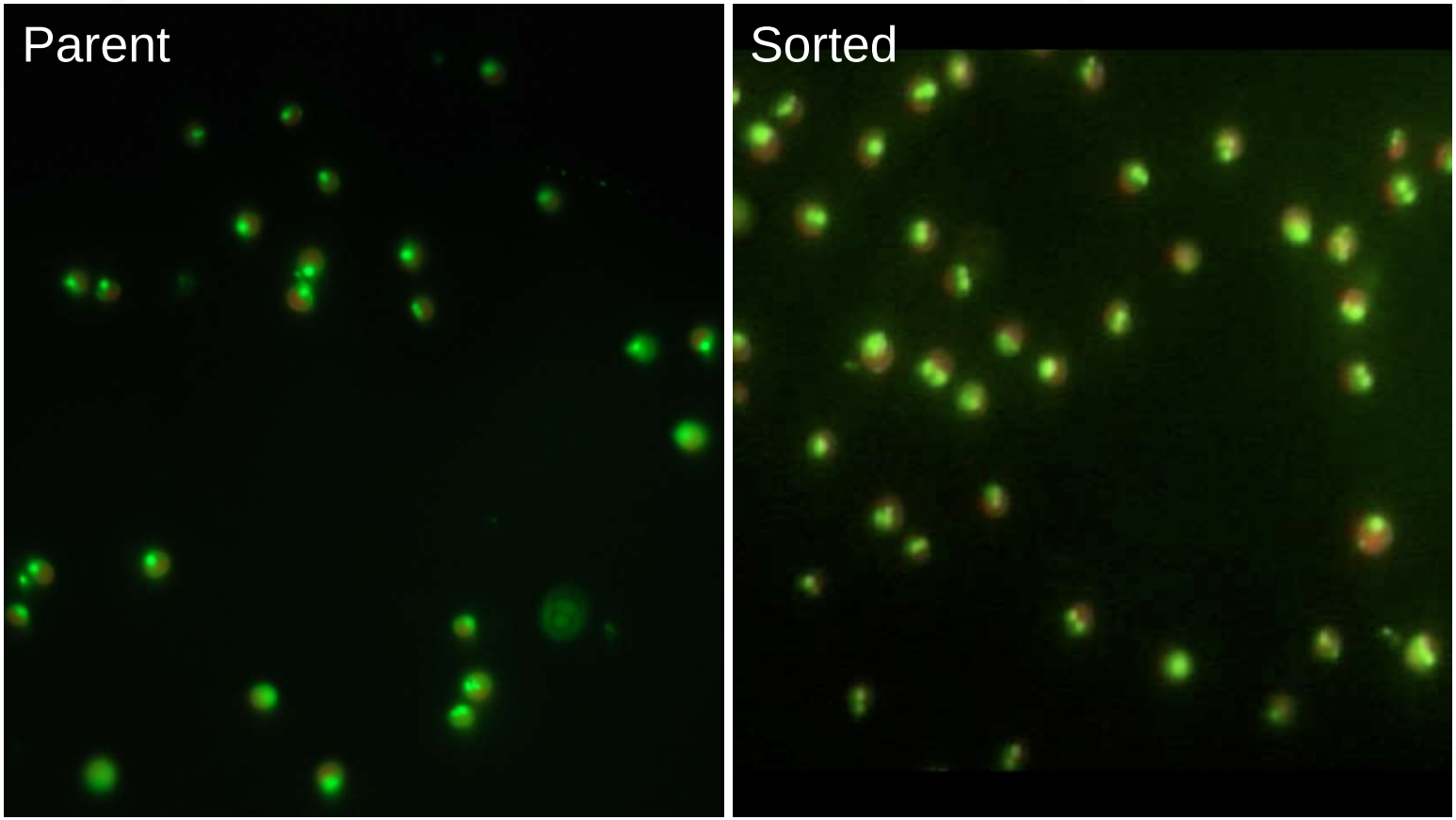
Rate of Lipid Accumulation and Cell Size Are Increased



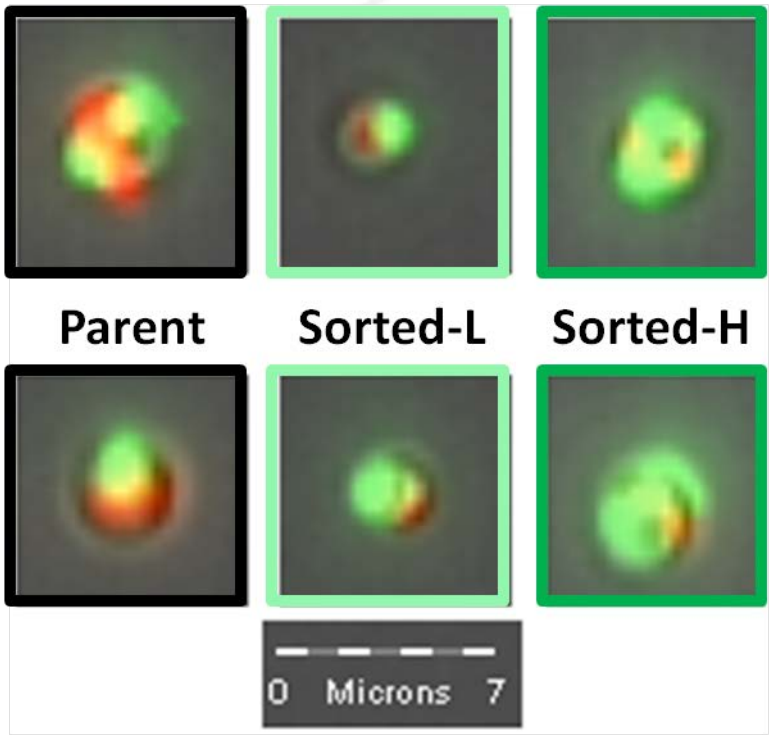


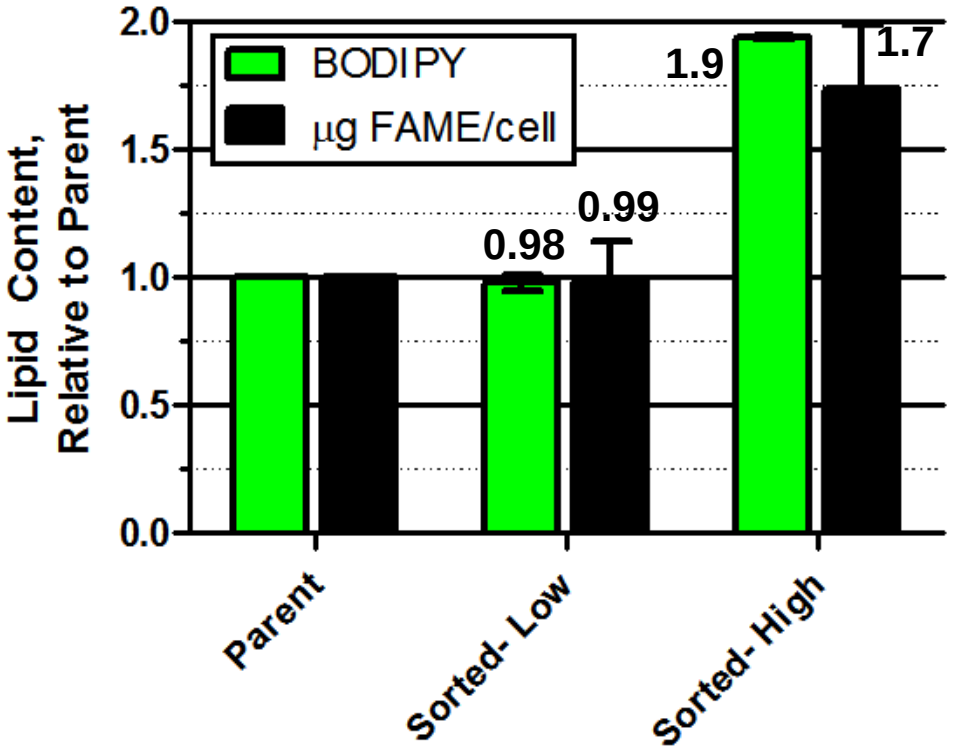
- Rate of lipid accumulation is increased 2.5-fold
- Cell diameter is increased ~20% (~70% increase in cell volume)

Fluorescence Microscopy Shows Larger and A Greater Number of Lipid Bodies Per Cell in the Sorted Population



Results Are Validated by Imaging Flow Cytometry and GC/MS





- Imaging flow cytometry shows larger/brighter lipid bodies in high sorted population, relative to parent
- Increase in lipid levels per cell was validated by GC/MS

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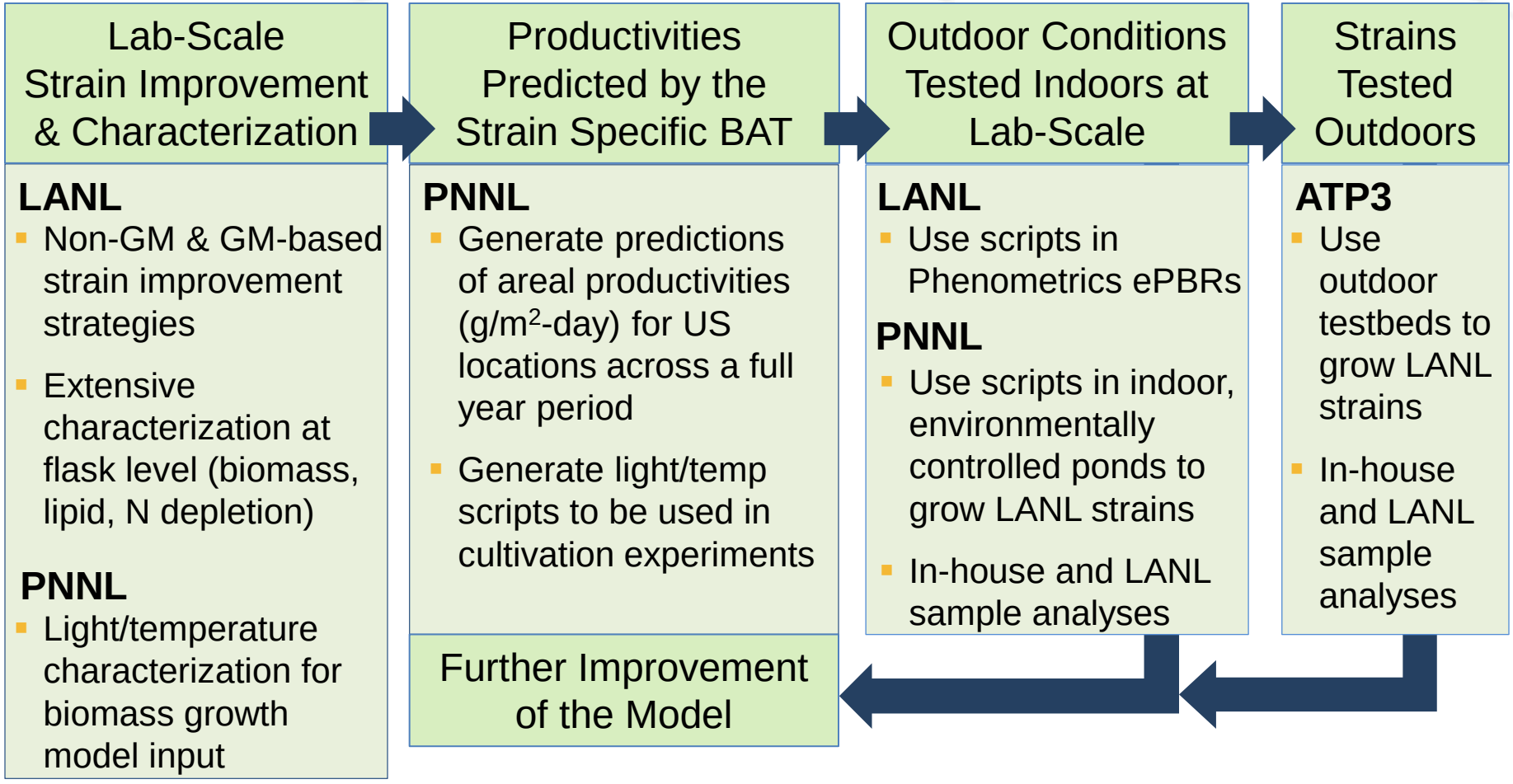
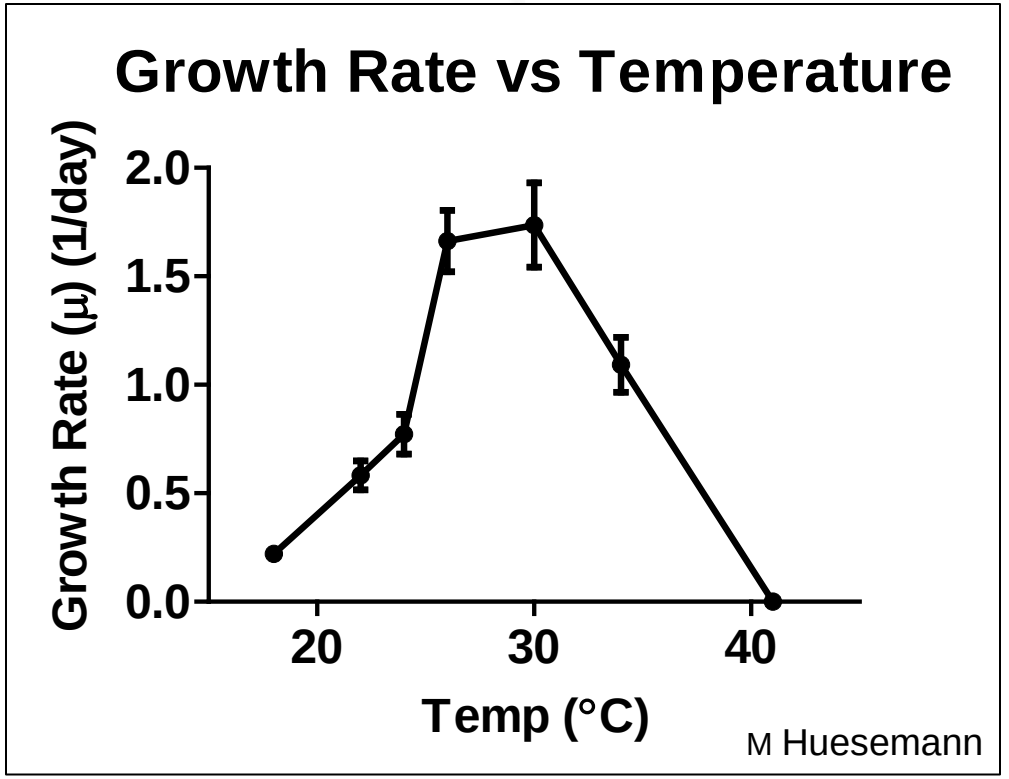
A Strain Performance Pipeline Was Established



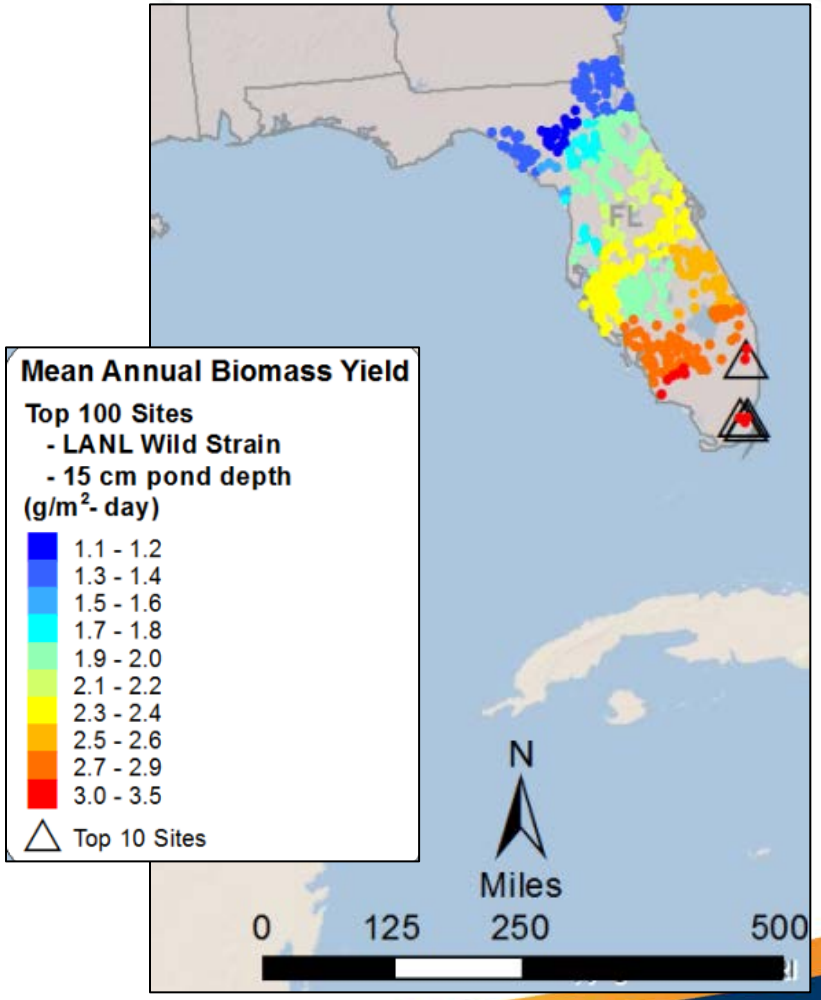
Picochlorum sp. Was Used as an Example Strain

- Extensive lab-scale characterization at LANL
- *Picochlorum* sp. was previously improved in lipid accumulation using FACS
- Additional parameters were measured at Pacific Northwest National Lab for the Strain Specific Biomass Assessment Tool (BAT)



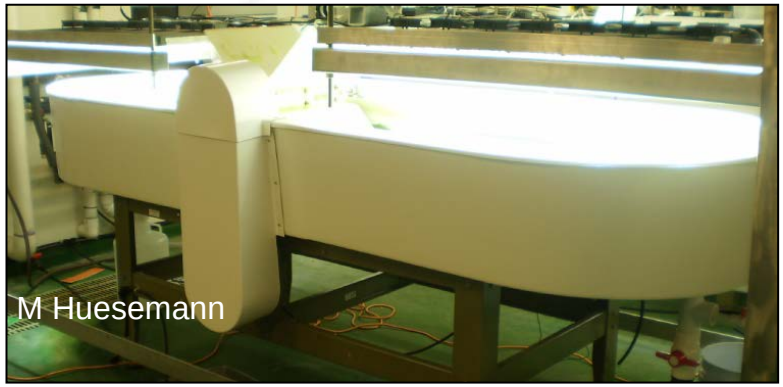
The Biomass Assessment Tool Predicted Biomass Growth for *Picochlorum sp.*

- The BAT predicted areal productivities for 1000s of locations across the US.
- The highest areal productivity for *Picocholorum* was predicted to be in south Florida during the month of May
- A script using 30-yr average light/temp data for this month/location was generated and used for cultivation at PNNL and LANL



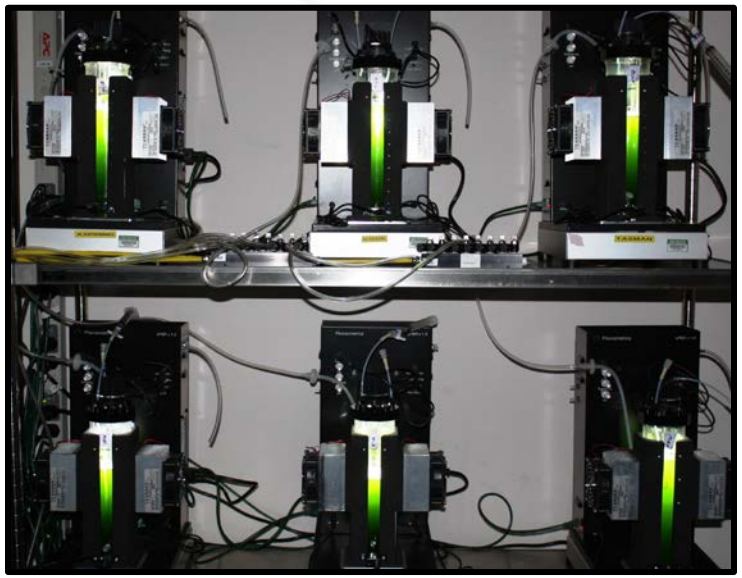
Outdoor Conditions Were Simulated in Two Systems

PNNL Environmentally Regulated Ponds



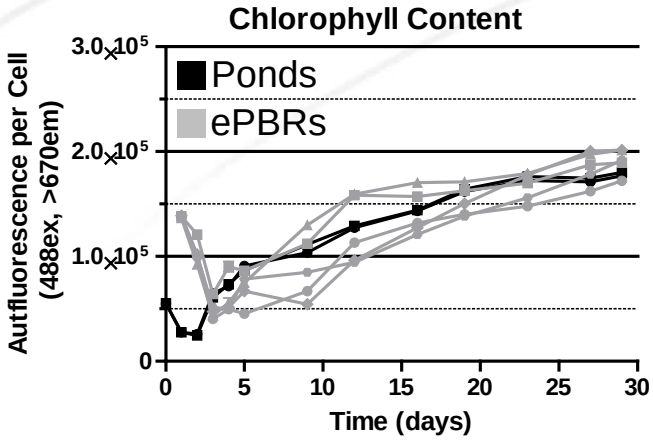
- Light/temperature/pH control
- Full sunlight intensity
- Duplicate experiments, 800L

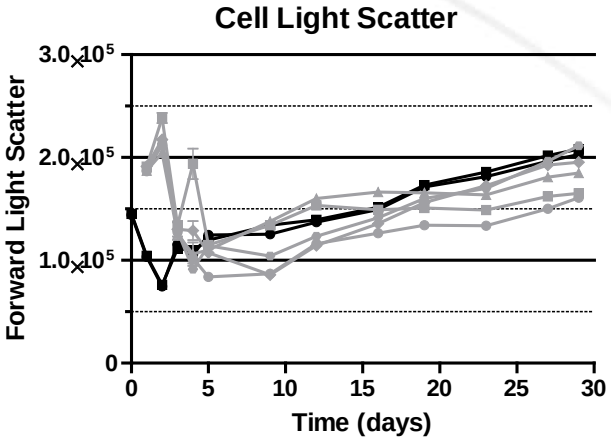
LANL ePBR Matrix Phenometrics Environmental Photobioreactors

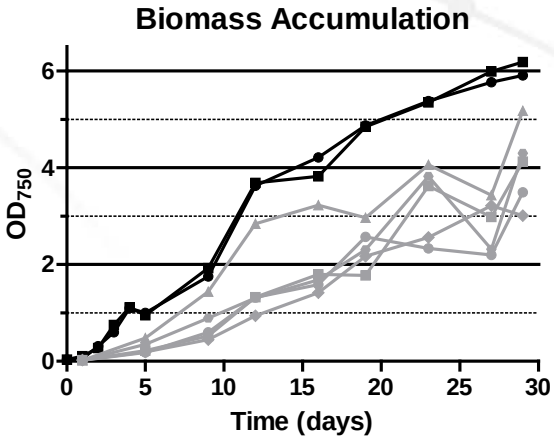


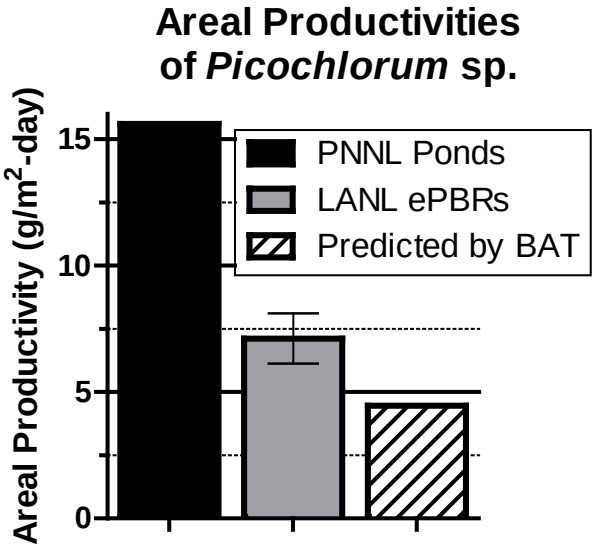
- Light/temperature /pH control
- Full sunlight intensity, light shines from above
- 33 ePBRs (high replicate potential), <0.63L

Cultures from Both Systems Were Analyzed





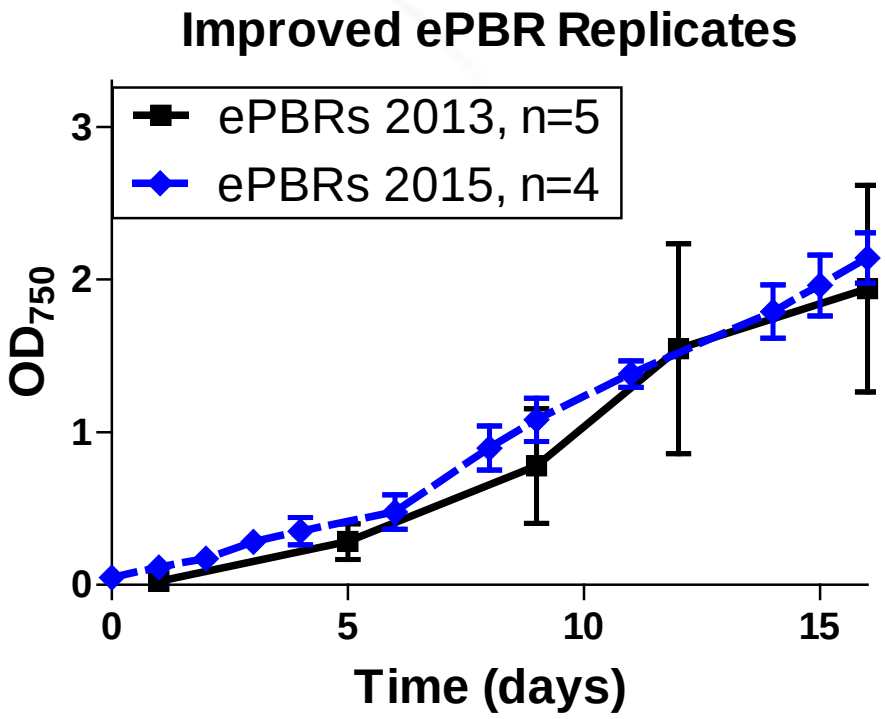




- Single cell characteristics were similar between ponds and ePBRs, but overall growth was quite different
- ePBRs showed just ~50% of the areal productivity of the ponds
- The BAT underpredicted both Ponds and ePBRs: *Picochlorum* grows at 15.6 g/m^2-day, much better than predicted and as productive as the best NAABB strain

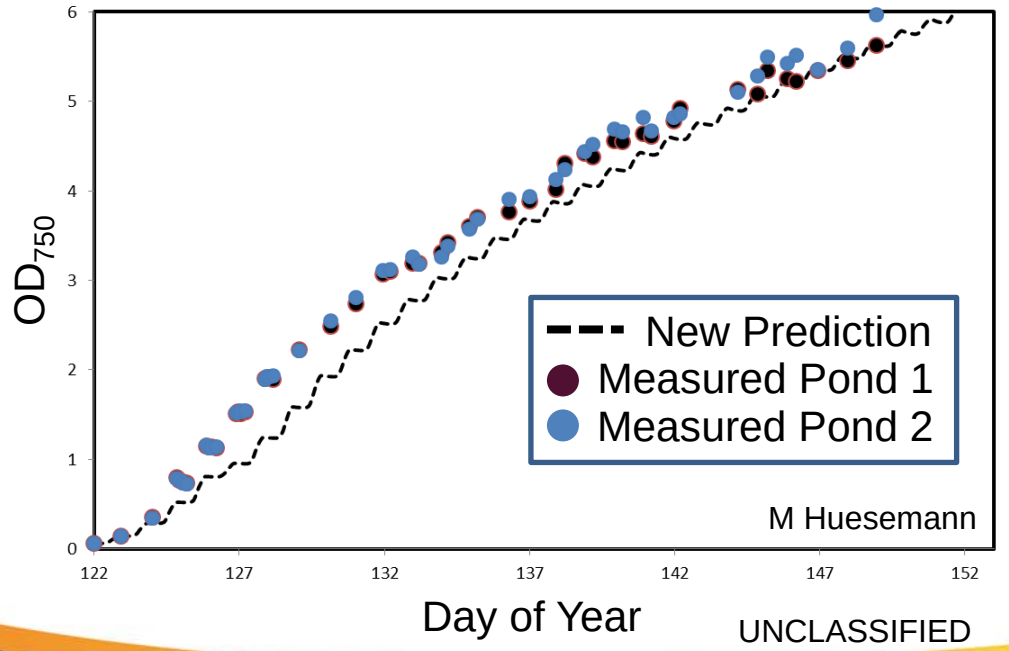
ePBR Modifications Tightened Error Bars

- **ePBR hardware and script modifications, round 1**
  - CO_2 delivery
  - Gas diffusion improved
  - pH control
  - Rapid yet sterile sampling
  - Replenishment of evaporative loss
- **ePBR hardware and script modifications, round 2**
  - Flowmeters to improve consistency of CO_2 delivery
  - 2 gas diffuser stones tested
  - Finer pH control
  - New vortex stir bars



Improvement of the Biomass Growth Model

- Previous strains showed good agreement between the PNNL ponds, the BAT prediction, and directly measured outdoor data
- What is different about *Picochlorum*? → Increased light scattering of the culture
- This parameter was measured for *Picochlorum* and added to the model, leading to an improved biomass growth prediction



This result demonstrates the value of adding more strains to the biomass assessment tool, which was initially developed based on a "generic" algae.

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Parent and Sorted *Picochlorum* Are Being Sent to ATP3 This Week

- ATP3 is the Algae Testbed Public-Private Partnership, a testbed facility funded by DOE
- Our main goal currently is open ponds similar to those used for the PNNL indoor ponds (800-1000L)

J McGowen

Results

1. We have generated 4 improved strains to date, 2 freshwater and 2 marine. We are exploring additional strategies for strain improvement, in conjunction with cell sorting.
2. We established a pipeline for evaluating strain performance, using a predictive tool and light/temperature conditions that simulate outdoor conditions. We aim to use this to screen sets of strains for best outdoor performance.
3. We will be starting our actual outdoor experiments this spring.

Relevance

- We are generating actual areal productivity values at lab scale (this is not typical at lab scale but an important DOE metric).
- Improvements in the model should permit better predictions and testing of outdoor conditions, inside.
- We can:
  - test a desired season/month of interest at any time
  - simulate locations where testbeds are not available
  - simulate testbed environmental conditions to downselect strains
  - improve strains under more outdoor-relevant conditions (high light, sinusoidal light, varying temperature, etc.)
- This process provides a pathway for transitioning or downselecting strains in a fashion that can be fully coordinated with their move to relevant outdoor testbed facilities or industrial partners.

Future Work

- Continue strain improvement work
  - Submit a strain with improved environmental robustness to the pipeline
  - Continue with the GM efforts based on the transcriptomic data
- Execute plan to move strains outside this spring
  - Complete data analysis and work with PNNL for model improvement as needed

Team/Acknowledgments

- Scott Twary
- Claire Sanders
- Amanda Barry
- Hiro Teshima
- Robin Yoshida
- Shawn Starkenburg

- Babs Marrone

- DOE-EERE Bioenergy Technologies Annual Operating Plan 9.1.2.5

My Professional Path – Undergrad & Grad Work

- Allegheny College, Meadville, PA
- Small liberal arts school
- Biochemistry major, research focused on RNA thermodynamic stability

- Univ. of Colorado at Boulder
- Chemistry & Biochemistry Dept
- Aimed to elucidate the mechanism by which aa-tRNAs, with different amino acids and RNA sequences, perform similarly during protein synthesis

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My Professional Path – Postdoctoral Work

- University of Utah
- Dept. of Biochemistry
- Aimed to explore the mechanism by which one protein complex recognizes a diverse pool of miRNA substrates

- Postdoctoral work #1 – Characterization of putative helicases for the development of a handheld pathogen detection device
- Postdoctoral work #2 – Established flow cytometry assays for the characterization and improvement of algae

Los Alamos National Laboratory

- >10,000 employees
- 36 square miles of DOE-owned property
- >2000 individual facilities
- City of Los Alamos is adjacent to Lab Site
- Located 35 miles NW of Santa Fe

- Employment opportunities include summer student positions, post-baccalaureate positions, postdoctoral positions, and occasionally graduate student positions (in collaboration with local universities)
- http://lanl.gov/careers/career-options/index.php

The New Mexico Consortium

- Non-profit organization formed to advance scientific research and education in New Mexico
- Includes partnerships with local universities, industry, and LANL
- Pursues joint initiatives with LANL in Advanced Computing, Plant Biology, Biomedical Engineering and Modeling and Analysis
- Houses a handful of LANL employees, from Bioscience, Theoretical, and Environmental/Earth Sciences Divisions
- Offers jobs independent of LANL, www.newmexicoconsortium.org

Advantages of Working at LANL

- Science is application driven, specifically towards national security problems
- Teams are often highly interdisciplinary – common to work regularly with chemists, engineers, theoreticians
- Close ties/interactions with funding agencies in D.C., continual consideration of what is happening at the federal level
- No teaching requirement but opportunities exist to be a mentor (usually to undergraduates and postdocs)
- Opportunities to work on multiple projects at once
- World-class instrumentation (we have 8 commercial flow cytometers as well as custom-built cytometers)
- Excellent salaries

Challenges Experienced at LANL

- Urgency to solve problems rapidly, strongly tied to Congress legislation
- Highly interdisciplinary teams may mean less interaction with people with your expertise
- High cost of business, with the same pressure to acquire funding as exists in academics
- Typically working on multiple projects at once
- Staff tend to follow their technology/method, rather than their application, leading to movement across fields
- Nice instruments are sometimes housed in old buildings.

Los Alamos, NM

- Small town in beautiful mountain setting
- Year-round outdoor recreation
- 300+ sunshine days a year
- Friendly community
- Good schools

Thanks!!