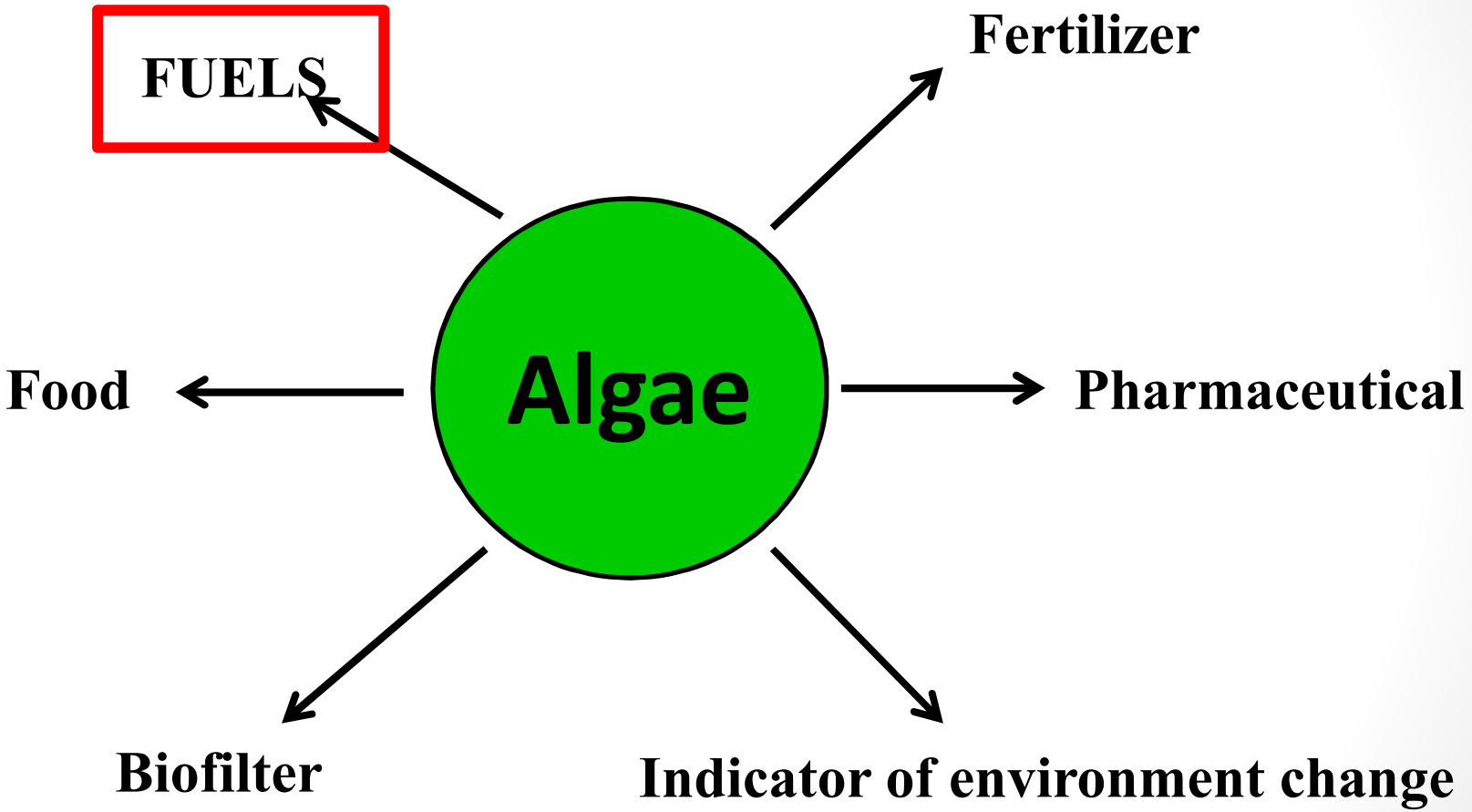
SAND2011-9196C

Does the algal CO_2 concentrating mechanism affect lipid production?

Samuel Lopez-Nieves
University of New Mexico
PSNA 2011
December 15, 2011
Kohala Coast, Hawaii

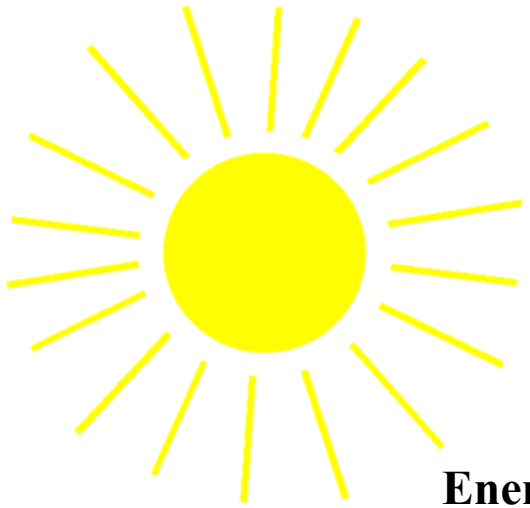
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000

Introduction: Uses of Algae

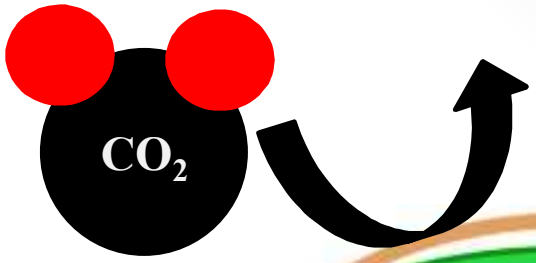


Solar and wind power/ liquid fuels are necessary

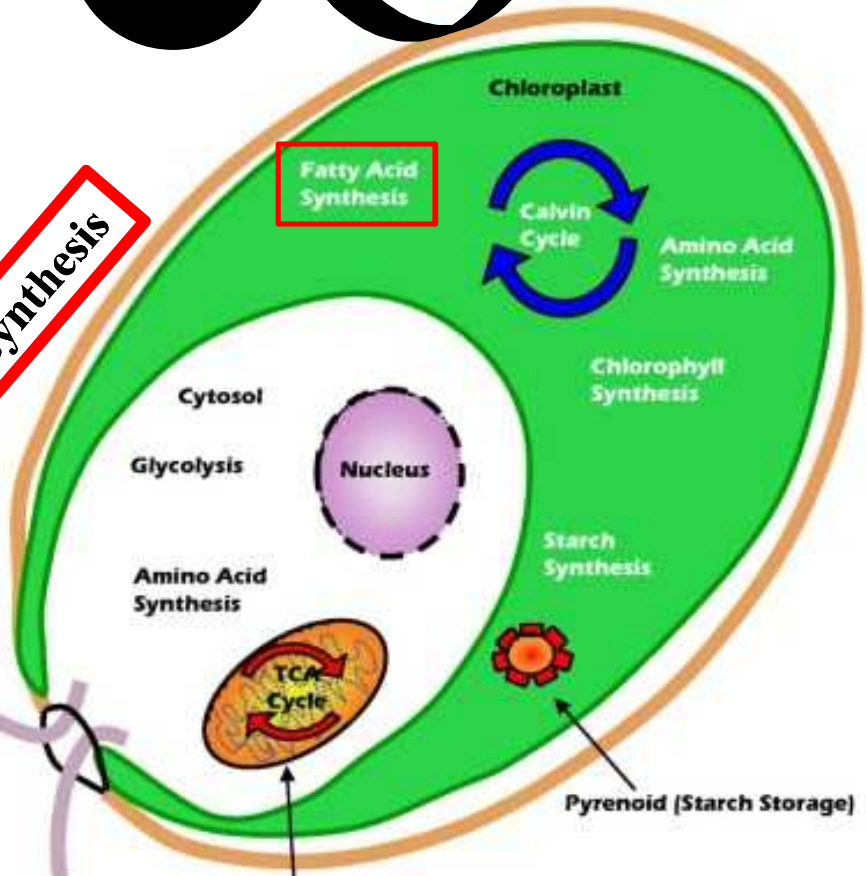
Energy Stored as Lipid in Algae



Energy IN as sunlight



Photosynthesis



Energy Stored as Oils

N.R. Boyle & J.A. Morgan. 2009. BMC System Biology

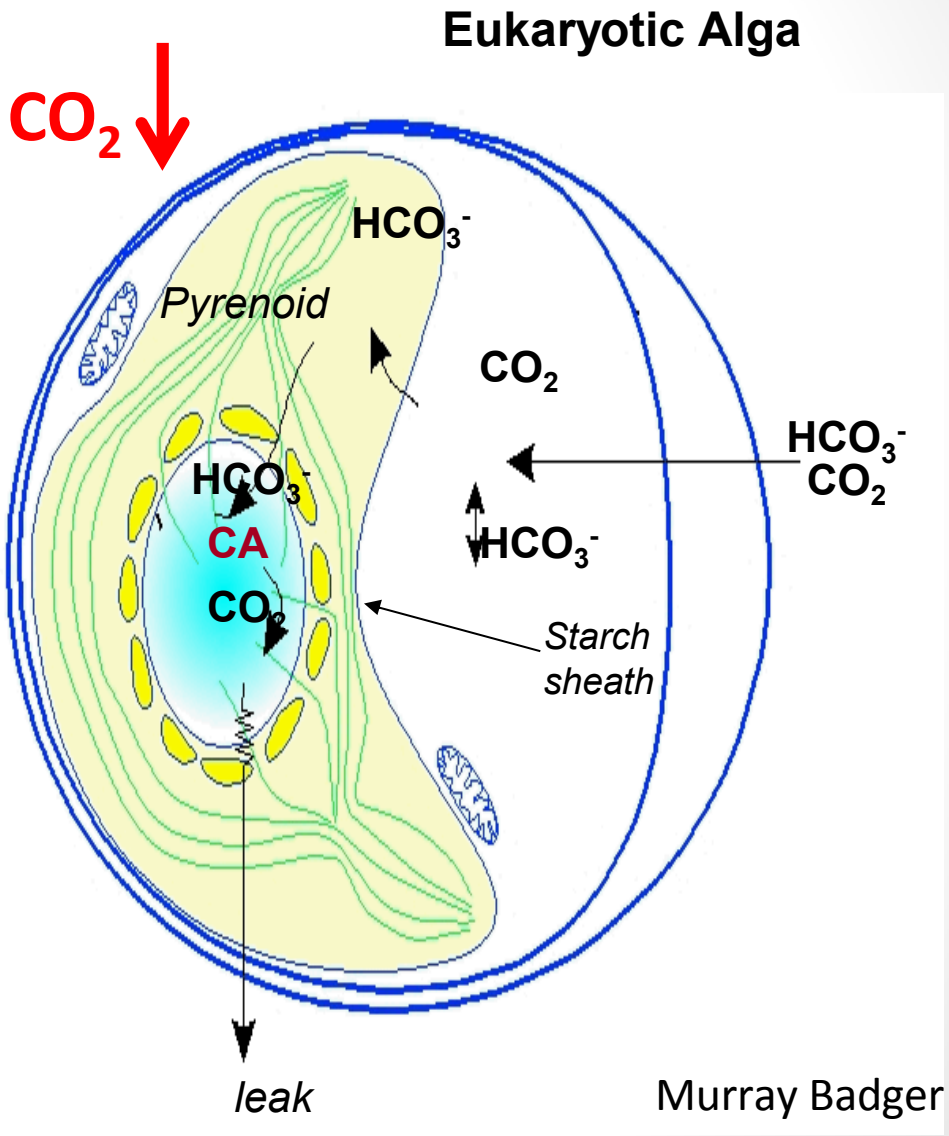
What are the challenges for algal photosynthesis?

- Rubisco has low affinity for CO_2
- O_2 and CO_2 compete for the active site of Rubisco
- Diffusion slow
- pH of the water is important
  - Acidic – CO_2 is higher
  - Alkaline – HCO_3 is higher

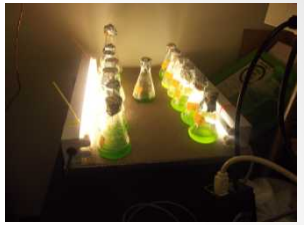
CO_2 Concentrating Mechanism (CCM): Pyrenoids

CO_2 ✖ CCM ← CO_2

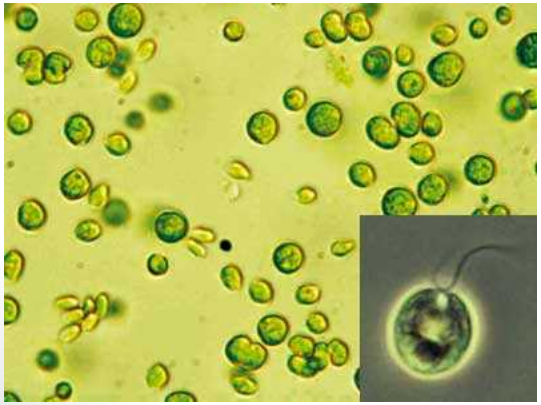
- 4 main components
  - Pump CO_2 and/or bicarbonate into cell
  - Transport CO_2 and/or bicarbonate into pyrenoid
  - Convert bicarbonate to CO_2 for Rubisco in pyrenoid

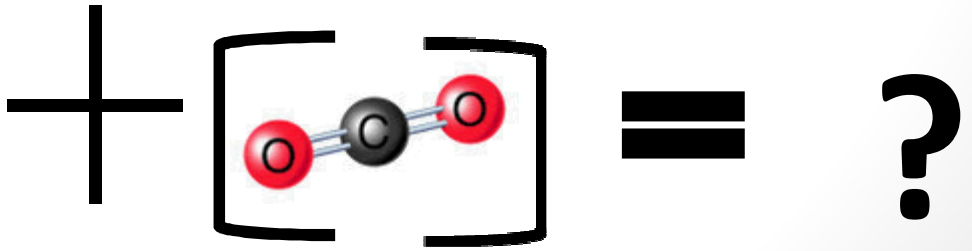


Problem and Purpose

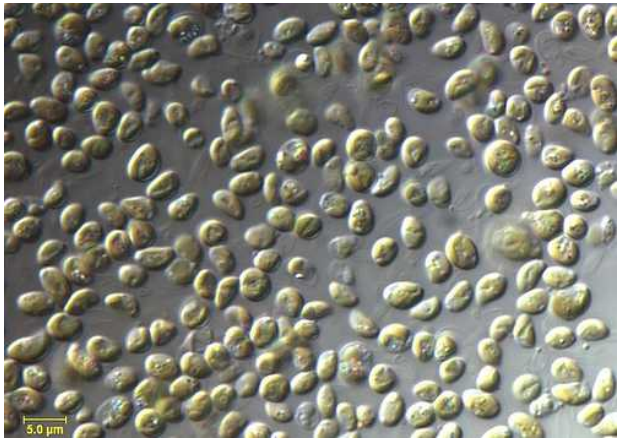


- Problem
  - Does CO_2 concentrate mechanism (CCM) affect the lipid production?
  - Purpose
    - To quantify lipid production and photosynthetic pigments *in N. salina* and *C. reinhardtii*
    - Biofuel lipid production
    - Understand CCM

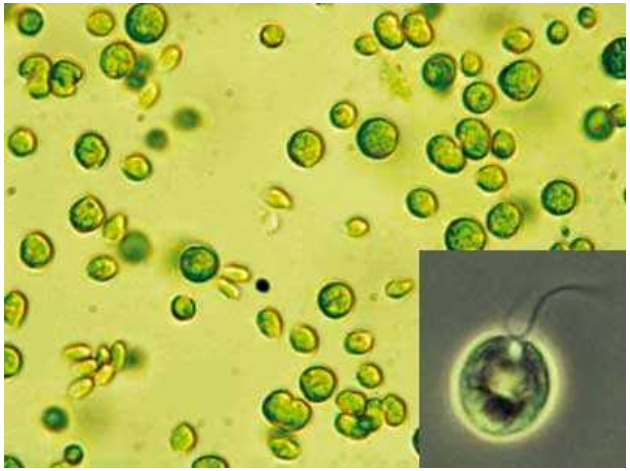




Species of Algae Used for this Experiment



Nannochloropsis salina
(Eustigmatophytes)



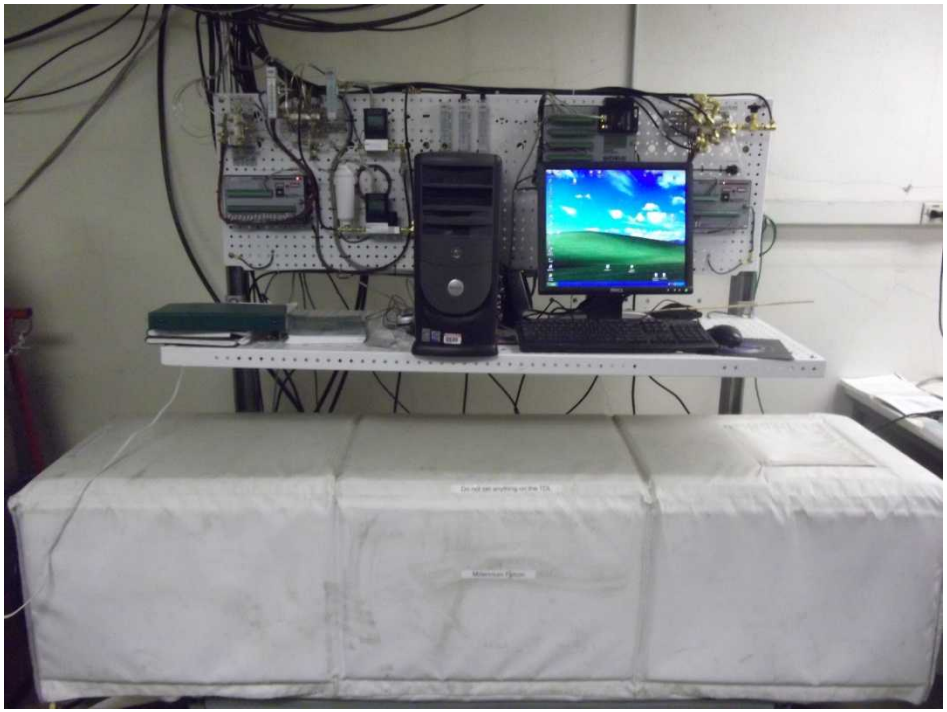
Chlamydomonas reinhardtii
(Chlamydomonadaceae)

Growing Algae: Photobioreactor

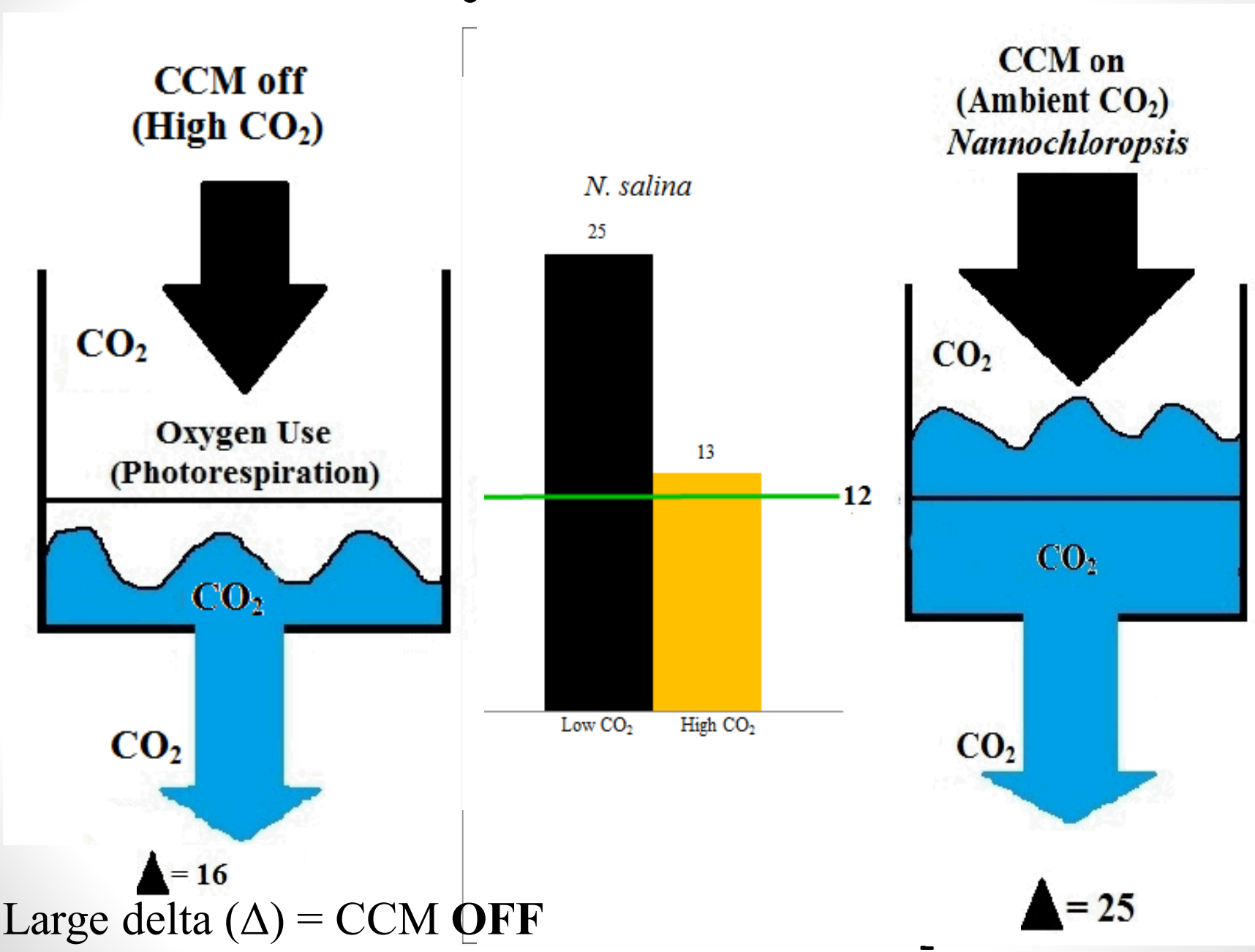
Airlift bioreactors connected to tunable diode laser for $^{13}C\Delta$

Day period: 14h at 24°C
Night period: 10h at 18°C

Measuring CCM Function and Controlling CO2 with Tunable Diode Laser (TDL)

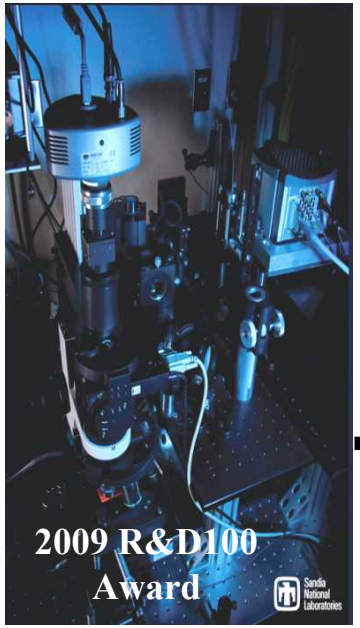


Preliminary Data from the TDL



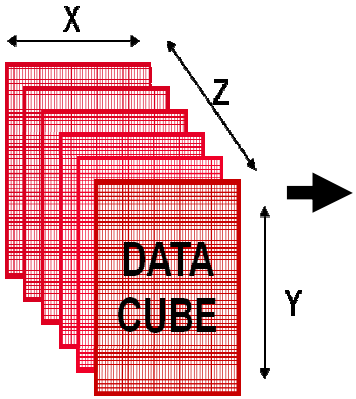
Large delta (Δ) = CCM **OFF**

Image Analysis Software Based on Multivariate Curve Resolution (MCR)

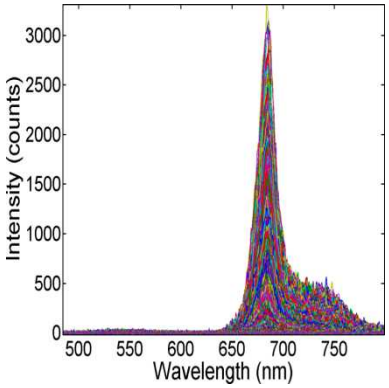


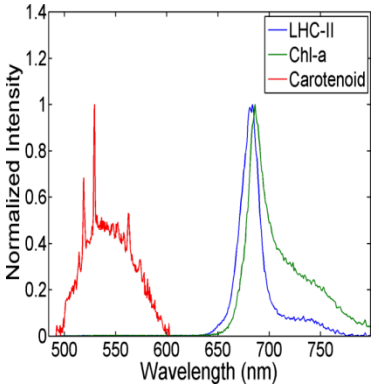
Hyperspectral image data cube

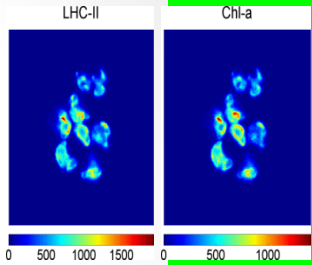
- Three spatial dimensions
- Each pixel or voxel contains 512 wavelengths

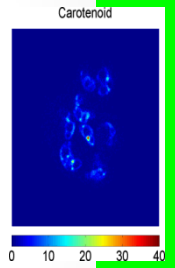


Spectral Data







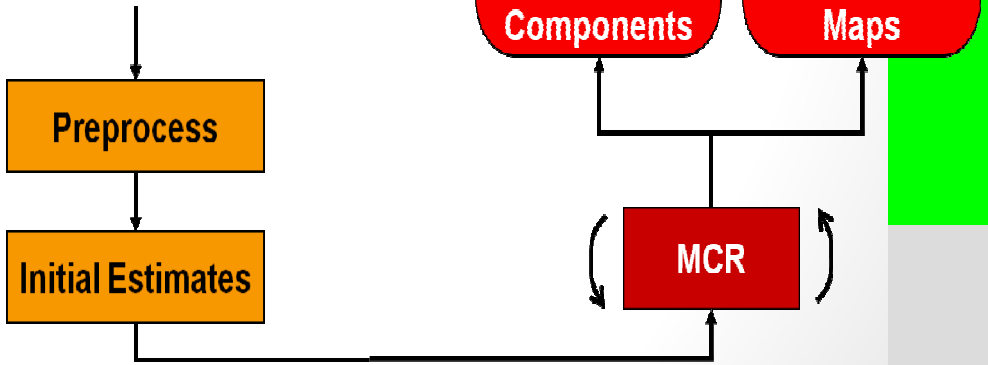


WHAT?

WHERE?
HOW MUCH?

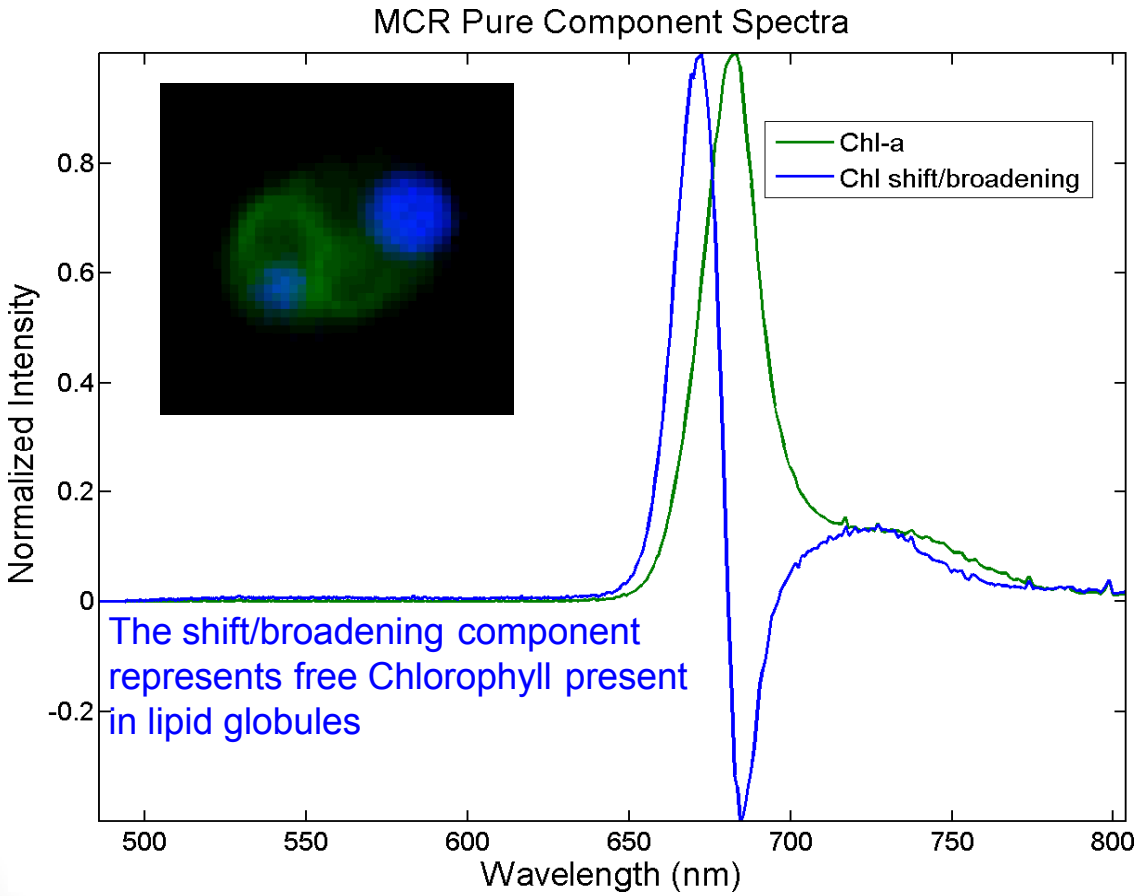
Sandia Designed Hyperspectral Confocal Fluorescence Microscope

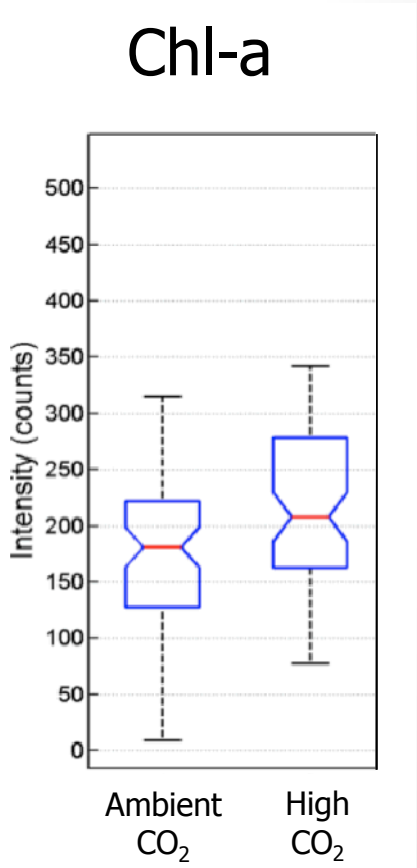
- 488 nm laser excitation
- Spectral range 490-800 nm
- Spectral resolution = 1-3 nm
- Acquisition rate = 8300 spectra/s



Sinclair, et. al. "Hyperspectral confocal microscope", Applied Optics, 45, 6283-6291 (2006).

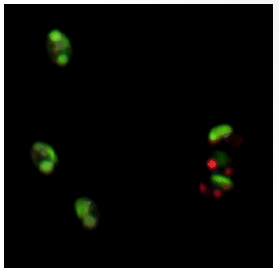
N. salina, No Cyan Filter

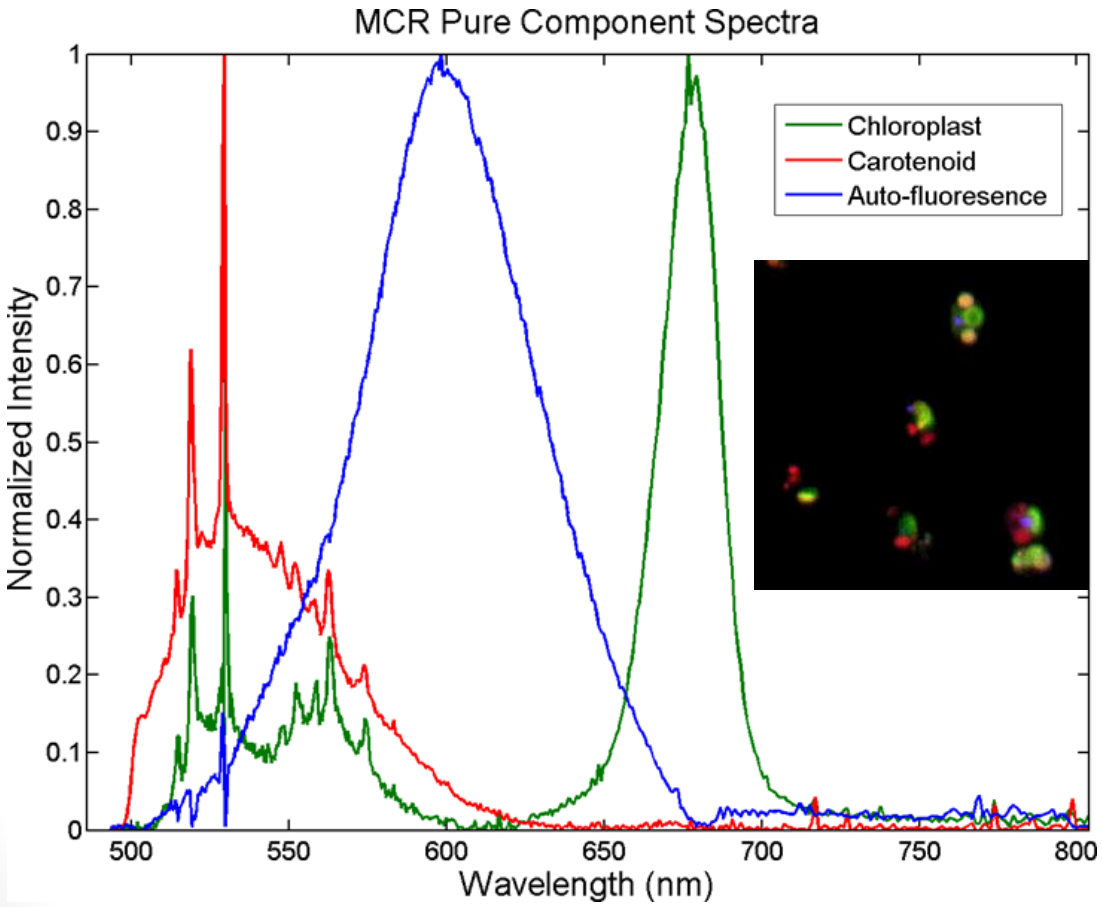


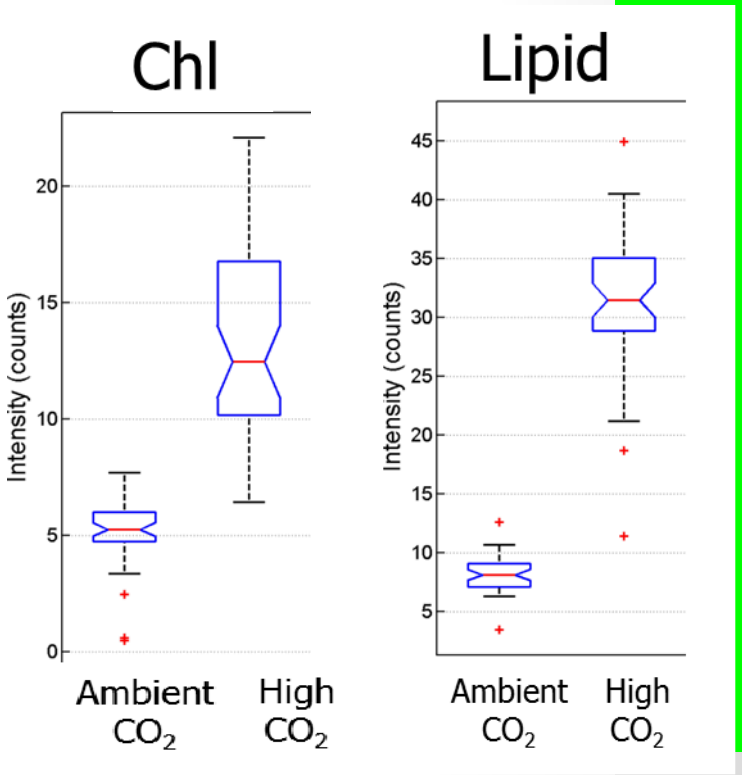


Preliminary Data

N. salina, Cyan Filter



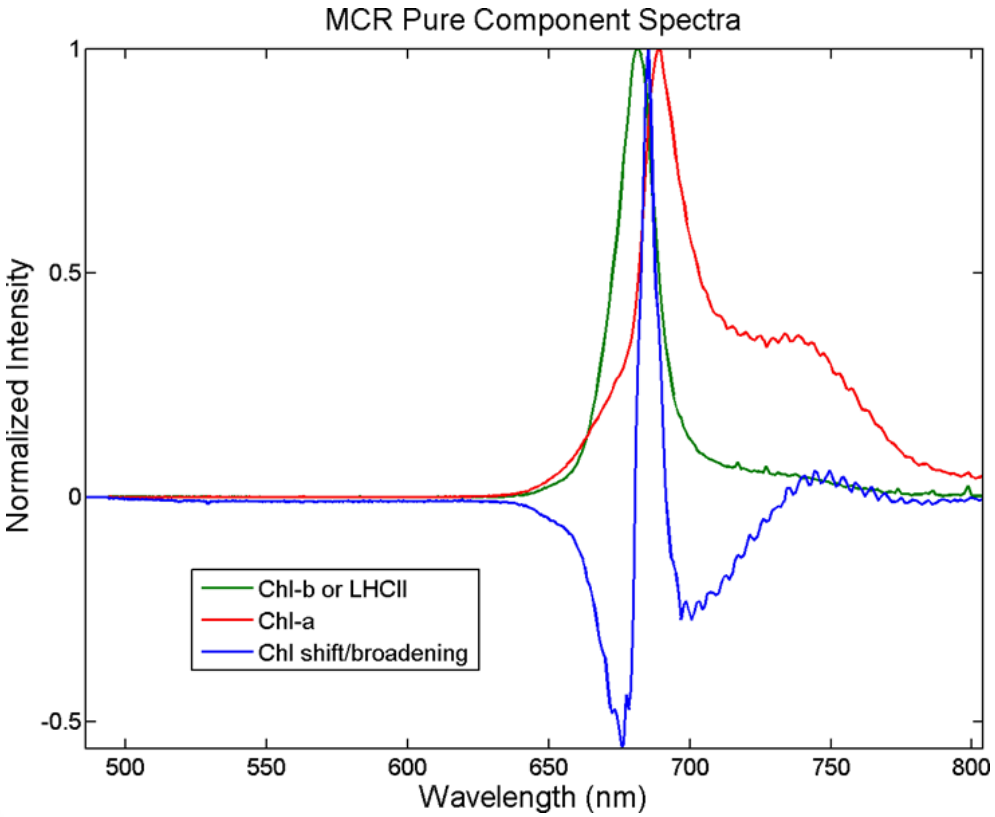


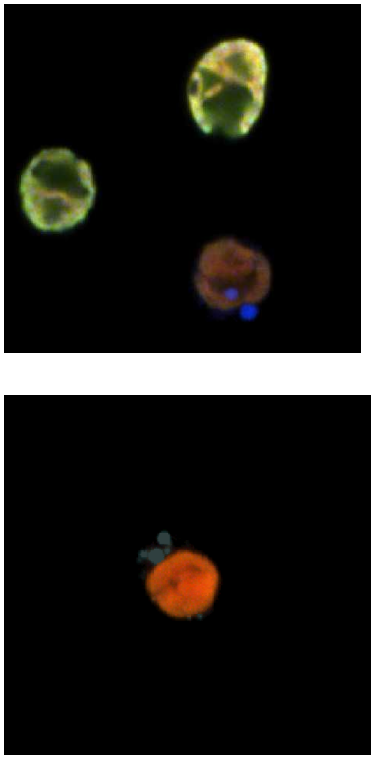


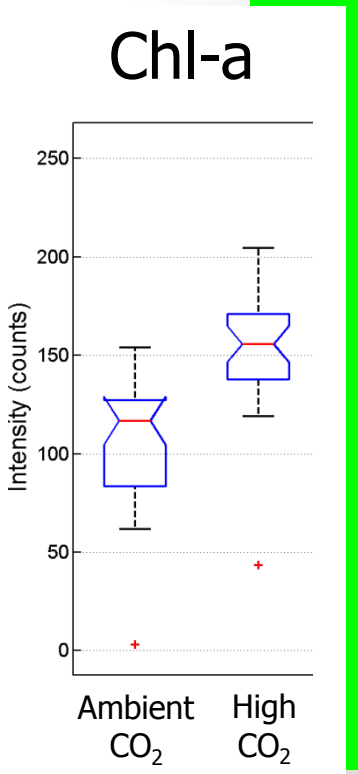
Very unique spectral characteristics of the carotenoid pure components.

Preliminary Data

C. reinhardtii, No Cyan Filter

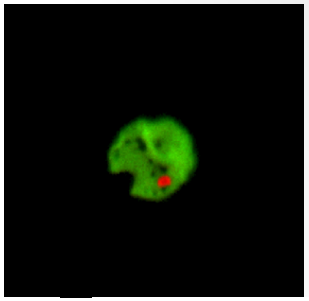


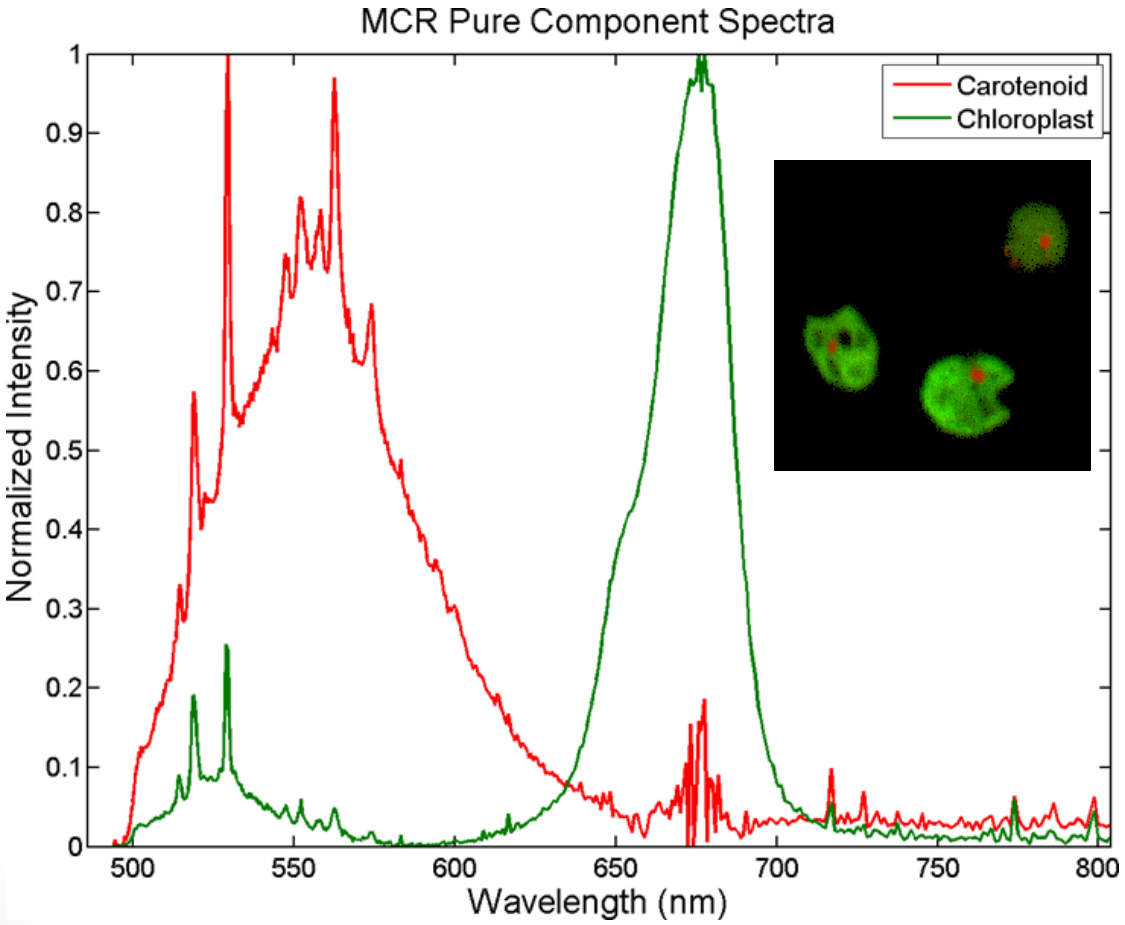


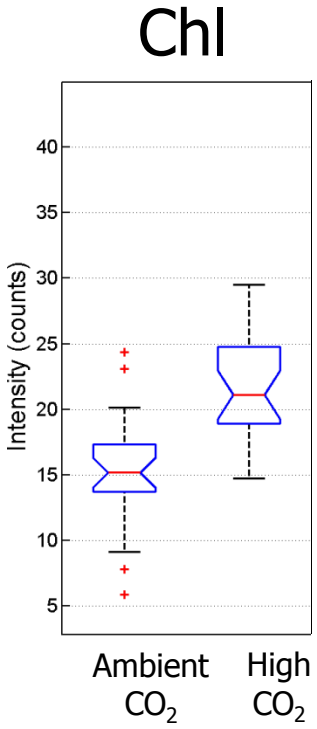


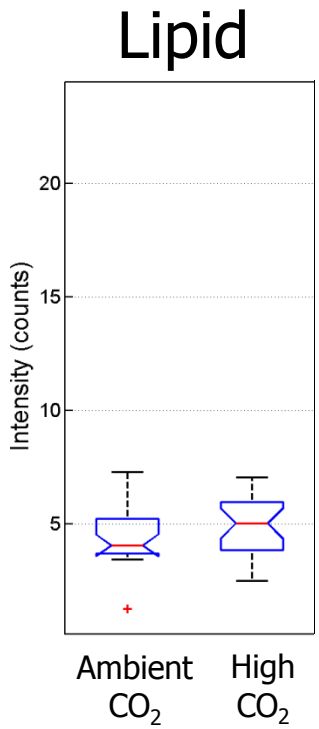
Preliminary Data

C. reinhardtii, Cyan Filter









Preliminary Data

Summary of the preliminary analyses

- CCM function
  - Suppressed in *C. reinhardtii* at high CO_2
  - Probably suppressed in *N. salina* at high CO_2, though the physiology is unusual
- Hyperspectral analyses
  - Chlorophyll content increased in both species after exposure to high CO_2
  - Lipid content increase in *N. salina* but not *C. reinhardtii* after exposure to high CO_2
- The relationship between CCM function and lipid production appears to be species specific.

Acknowledgement

- Dr. Hanson and his lab
- Sandia National Laboratories
  - Howland D. T. Jones
  - Dr. Aaron Collins
  - Omar Garcia
  - Dr. Jerilyn Timlin
- PREP Program/NIH
- Dr. Cripps and Antonio Banuelos

National Nuclear Security Administration

THE UNIVERSITY of NEW MEXICO

U.S. DEPARTMENT OF ENERGY

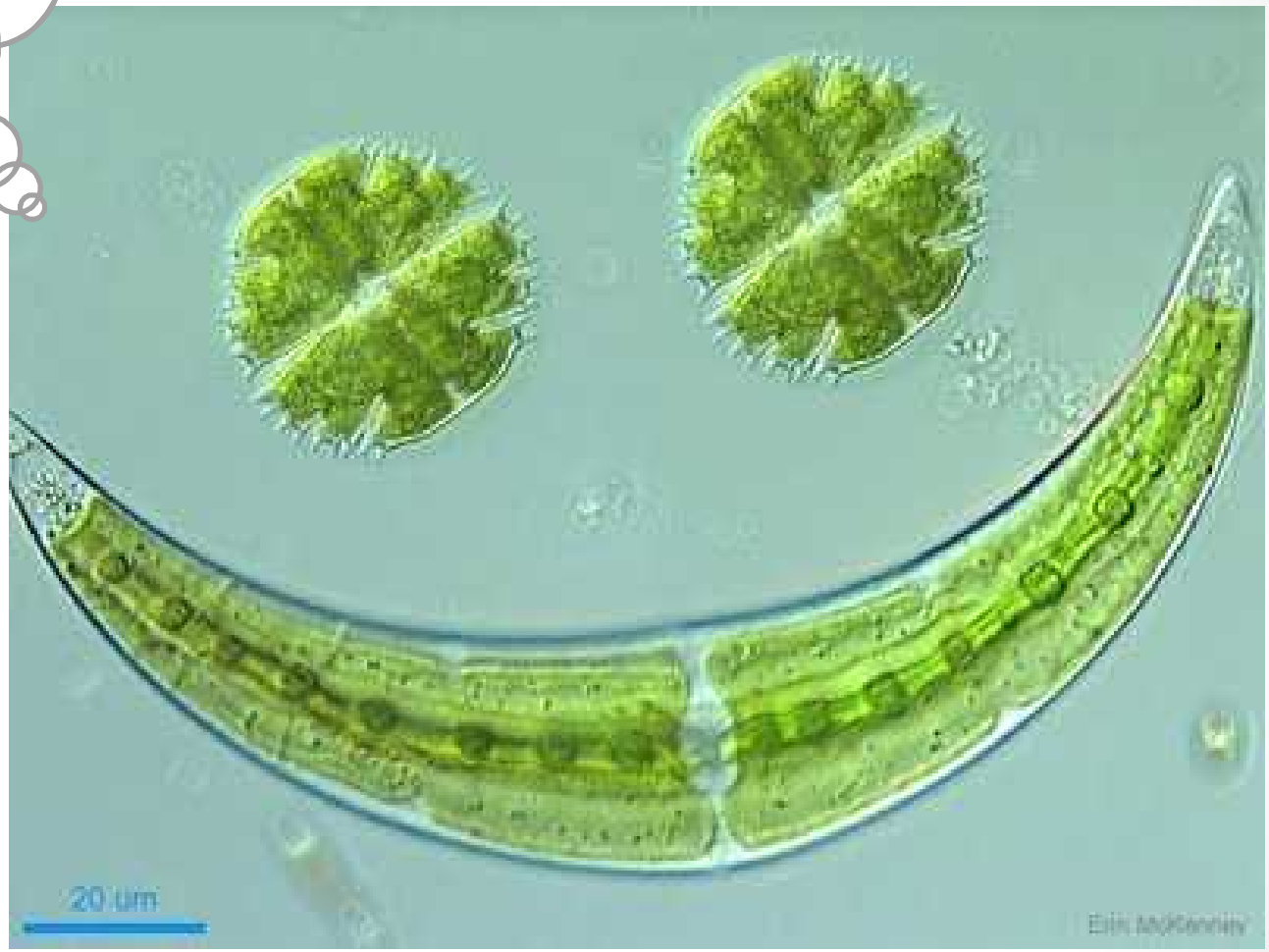
Sandia National Laboratories

NATIONAL INSTITUTES OF HEALTH

PREP @ UNM

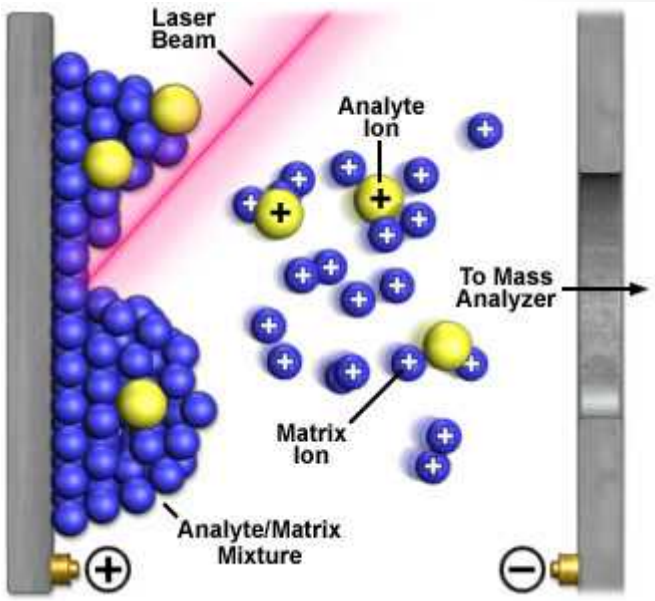
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000

Questions ?

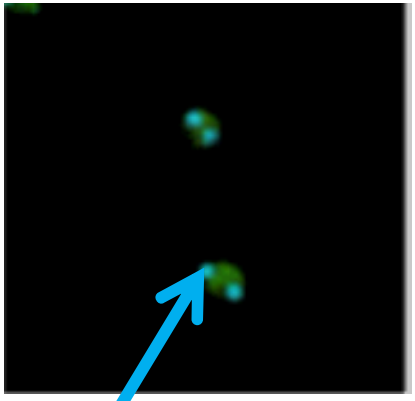


Future Directions

- Quantification and Identification of lipid with the Matrix-assisted laser desorption/ionization (MALDI)-TOF
- Confirm imaging of the microscope with the results of the MALDI
- More analysis from Sandia Laboratories at different stress condition (High CO_2 then Low CO_2)



How are we measuring lipid content?



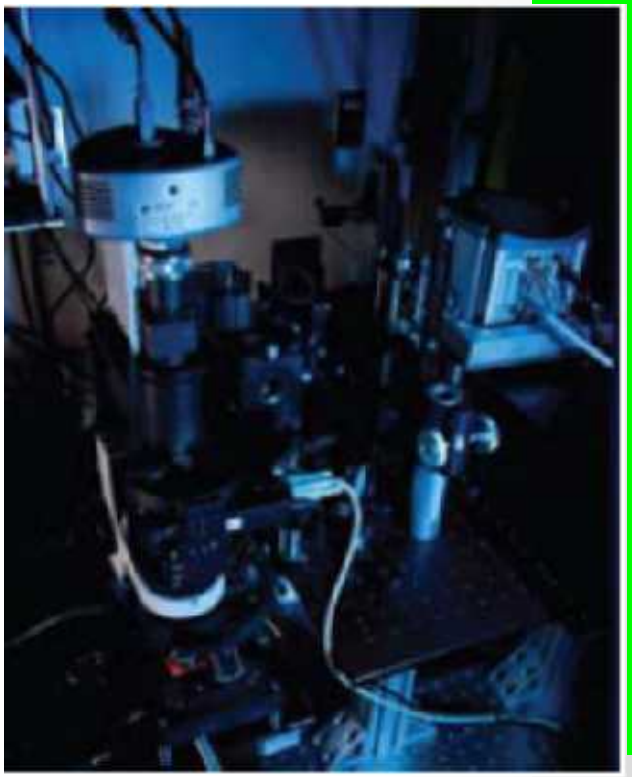
Ratio between Carotenoids

Chloroplast

Hyperspectral Imaging

A new way to measure lipid content: Hyperspectral Imaging

- Is a microscope
  - Excite cells with 488 nm laser
  - Obtain fluorescence emission spectra for each pixel
    - From 400-800nm
  - With and without a cyan filter
    - Chlorophyll A auto fluoresces, so a cyan filter is used to reduce the chlorophyll signal

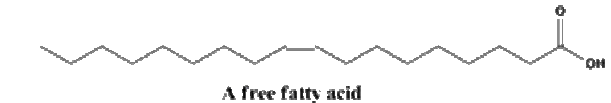


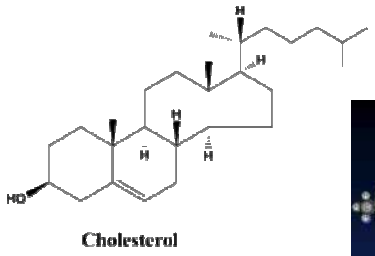
Hyperspectral Imaging

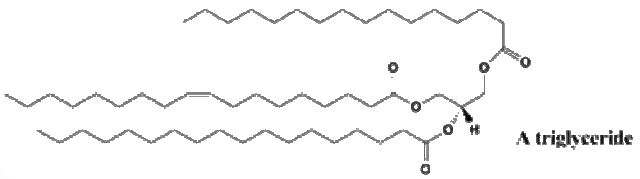
- Approach:
  - Capture a fluorescence spectra for every pixel
  - Chlorophyll a auto fluoresces, so a cyan filter is used to reduce the chlorophyll signal
    - Increases ability to see carotenoids
    - All oil droplets contain some carotenoids due to the shared biosynthetic pathway
  - Multivariate curve resolution (MCR)
    - Compares spectra between adjacent pixels
    - Separates how the number an amount of compounds fluorescing in each pixel
    - Area of fluorescing compounds in cell cross section correlates with the amount in the cell

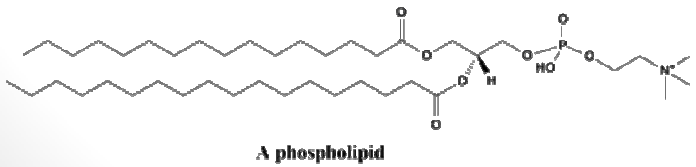
What is oil/lipid and biofuel?

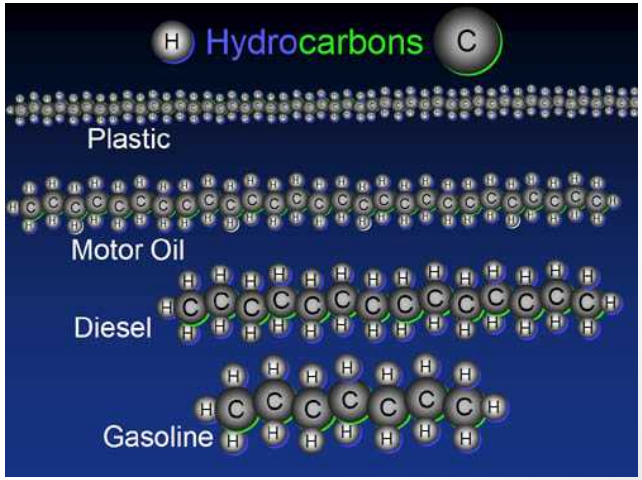
- Long chain of C-H, hydrophobic and soluble in organic solvents
- Biofuel – fuel derived from biomass (biological material)
  - Biodiesel –oil based in diesel from animals/plants and contain long chain of alkyl (methyl, propyl, ethyl) esters.











Growing Algae:
Absorbance, Chlorophyll Assay, pH and Cell Count