

Automatic and Near-Real Time Monitoring for Cyanobacteria

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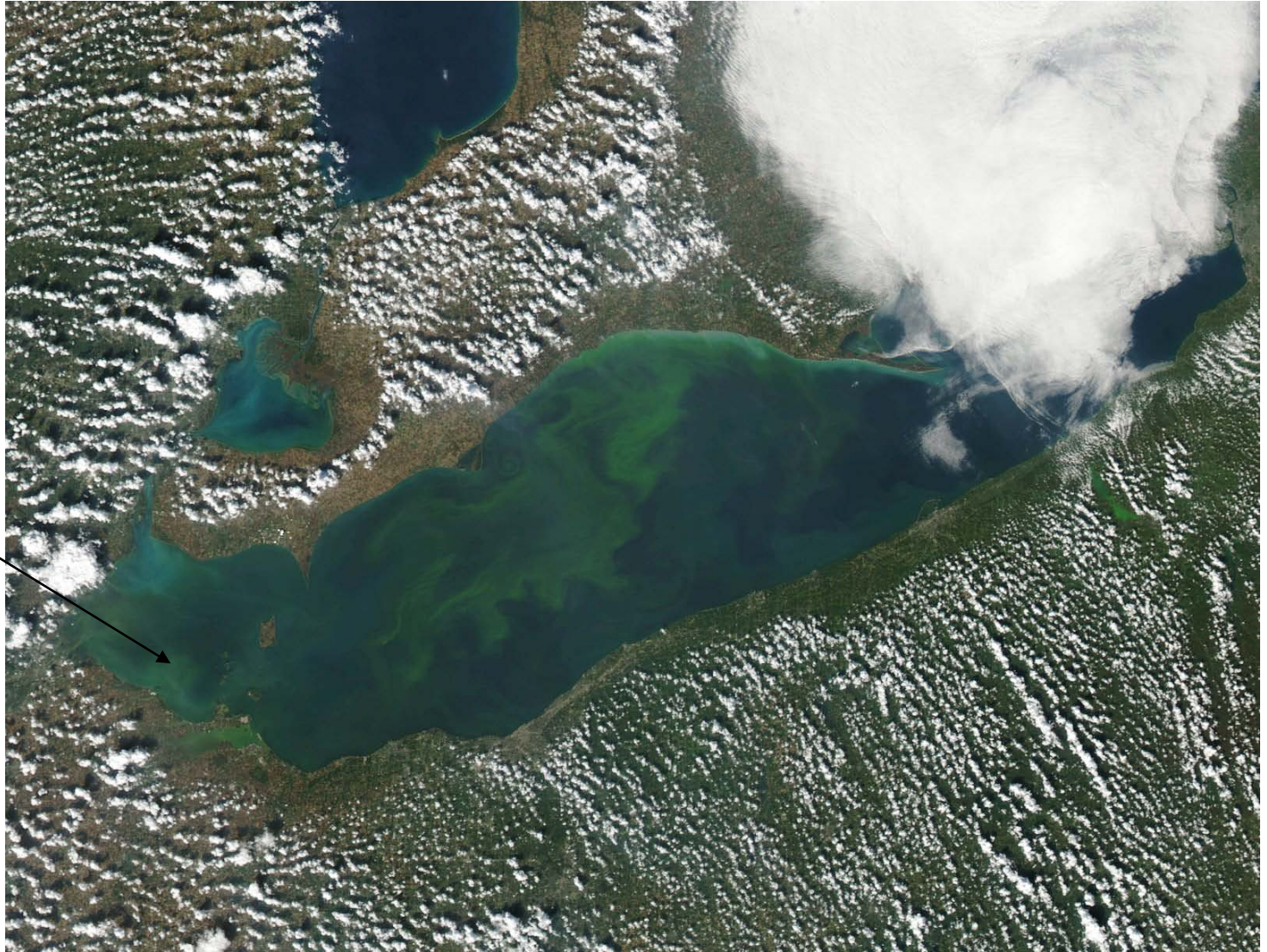
Jeremy Sullivan (PhD)



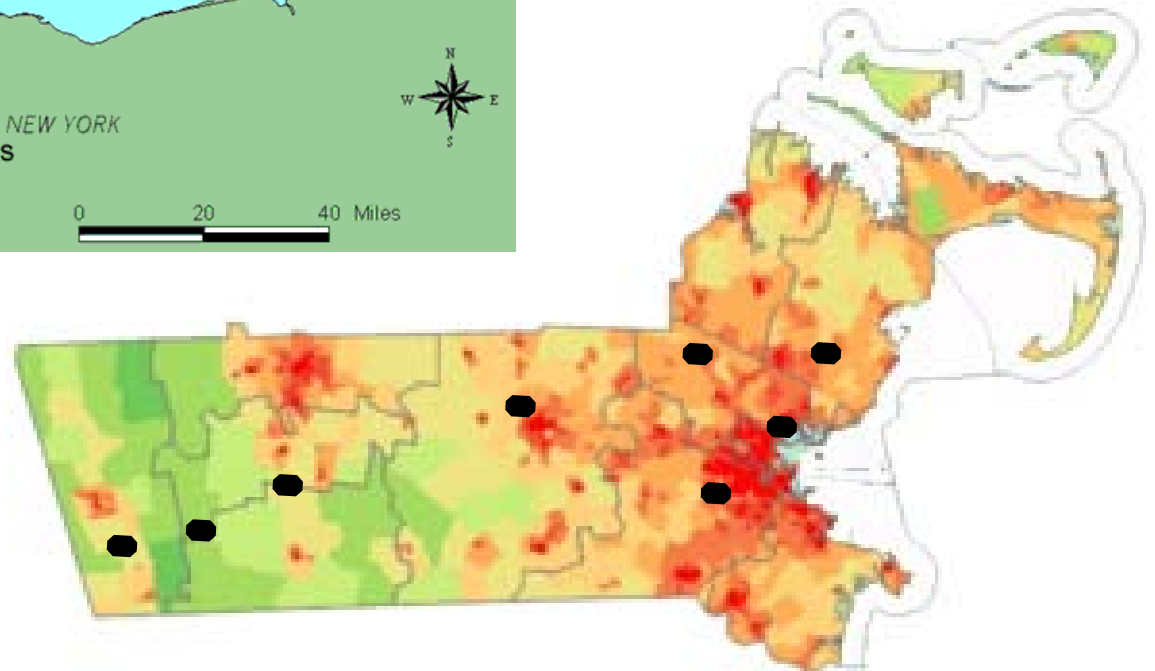
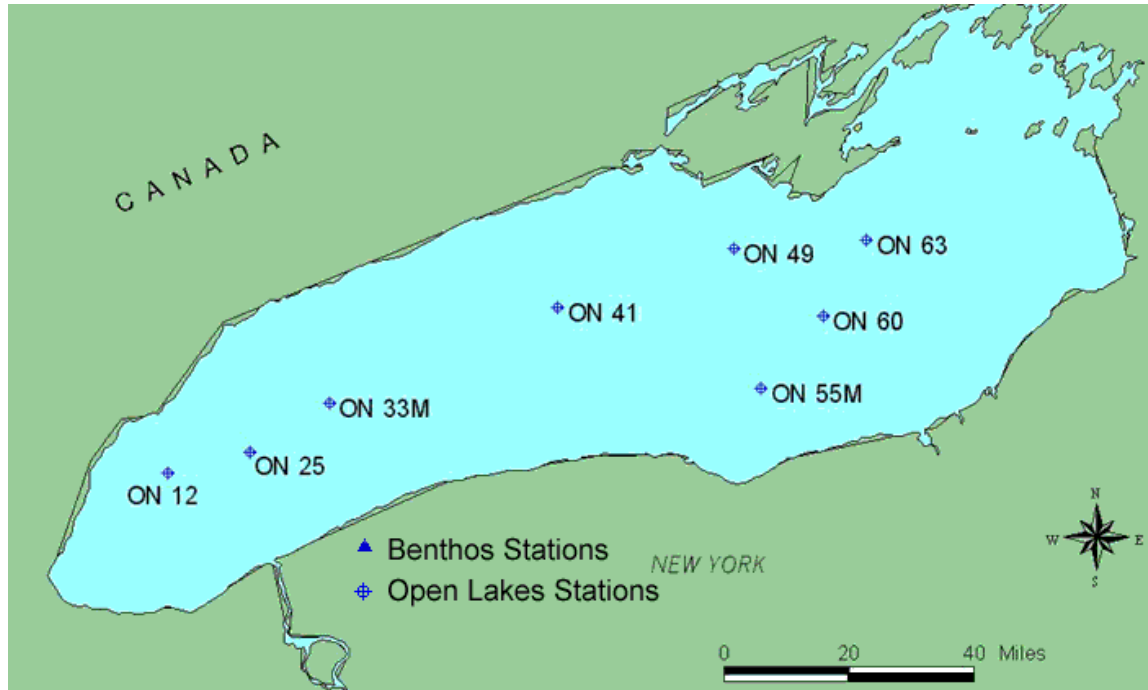
October 7th, 2007



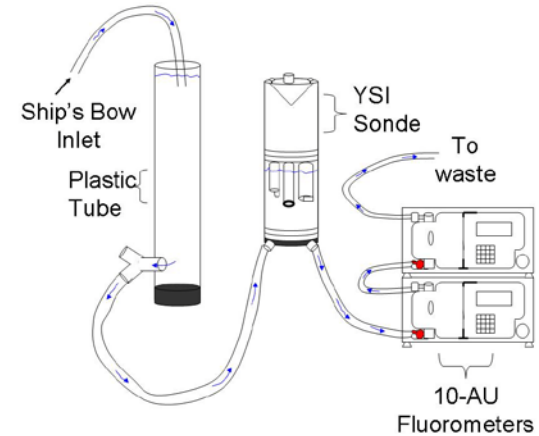
Bloom first detected by our sensor on the monitoring buoy!



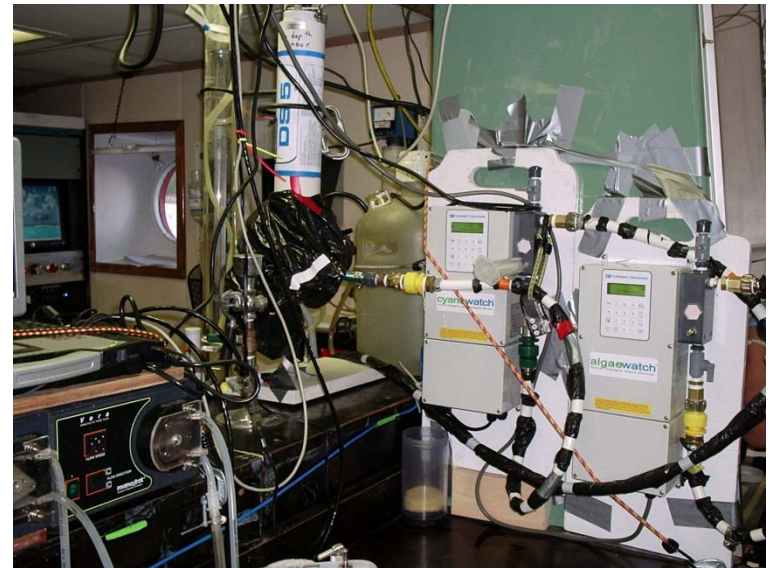
Great Lakes are big!



Ferry boxes offer a solution to this problem:



- Sample continuously while ship is underway:
- 30-60 sec per data point
- 20,000-80,000 points
- 100m resolution



Fluorometer List

Chlorophyll

- TD AlgalWatch
- HydroLab DS5
- YSI 6600 sonde

- BBE FluoroProbe
- Turner Designs 10-AU
- Turner Designs Cyclops

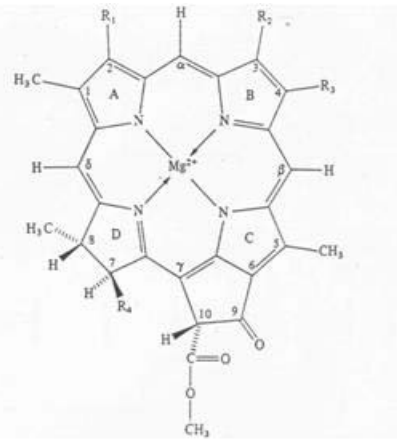
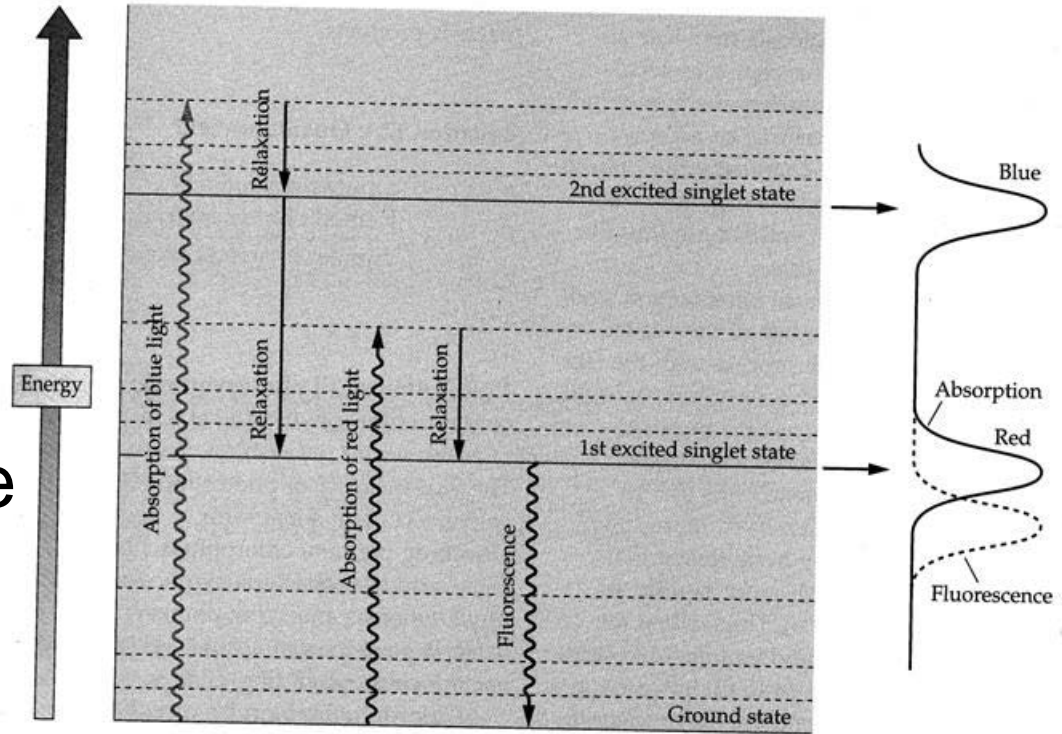
Phycocyanin

- TD CyanoWatch
- HydroLab DS5
- YSI 6600 Sonde

- BBE (Cyano-specific Chl)
- Turner Designs 10-AU
- Turner Designs Cyclops
 - PC, PE, CDOM, CHL

Primer on Chlorophyll fluorescence

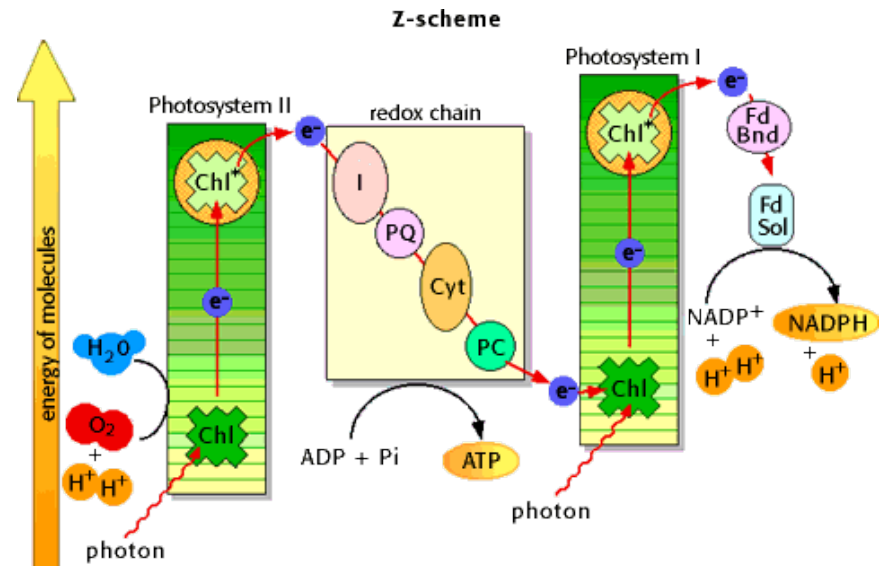
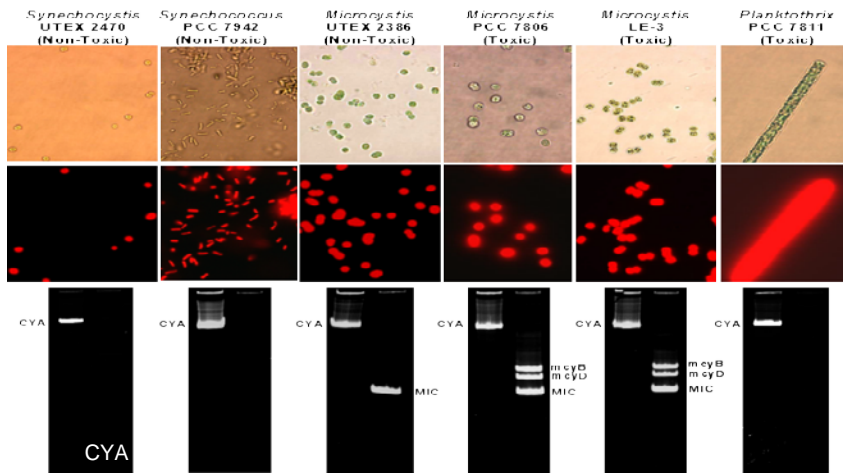
- EPA 445.0
- Extract in acetone
- Excite the chl chromophore.
- Look at the energy given off when the chromophore relaxes.



In vitro (in glass)

In vivo is not *in vitro*!

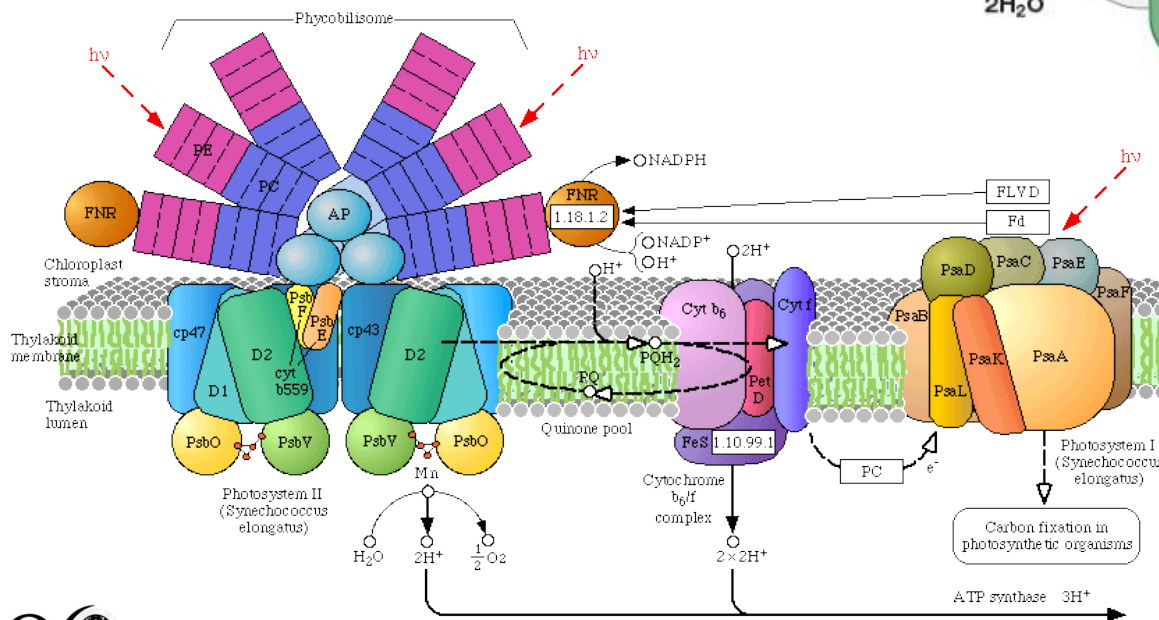
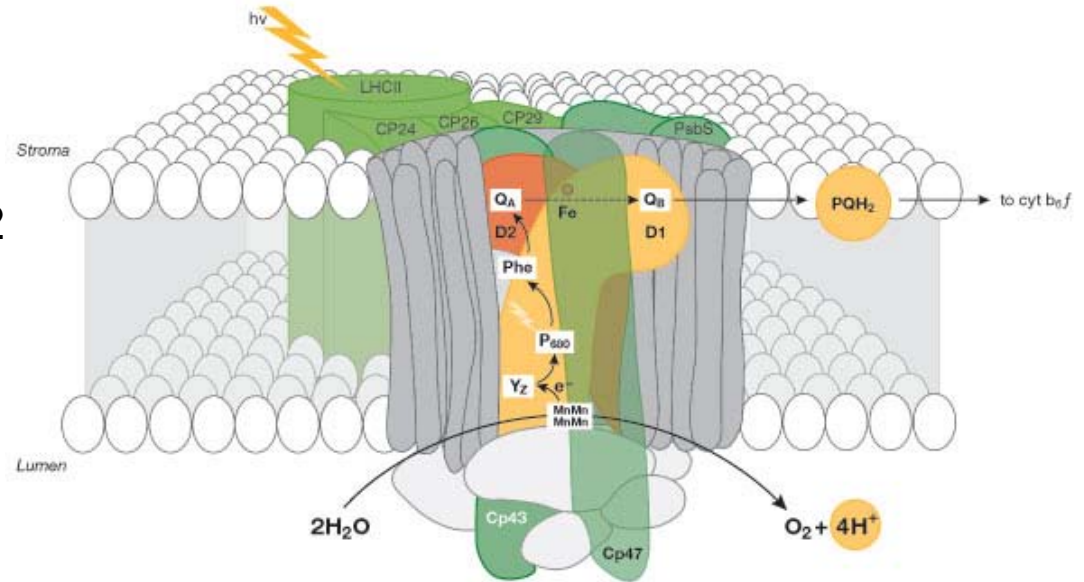
Exciting an intact organism



Most energy goes through the electron transport system.
 Some spills out (PS II only!)
 Assume that spill is a constant percentage.

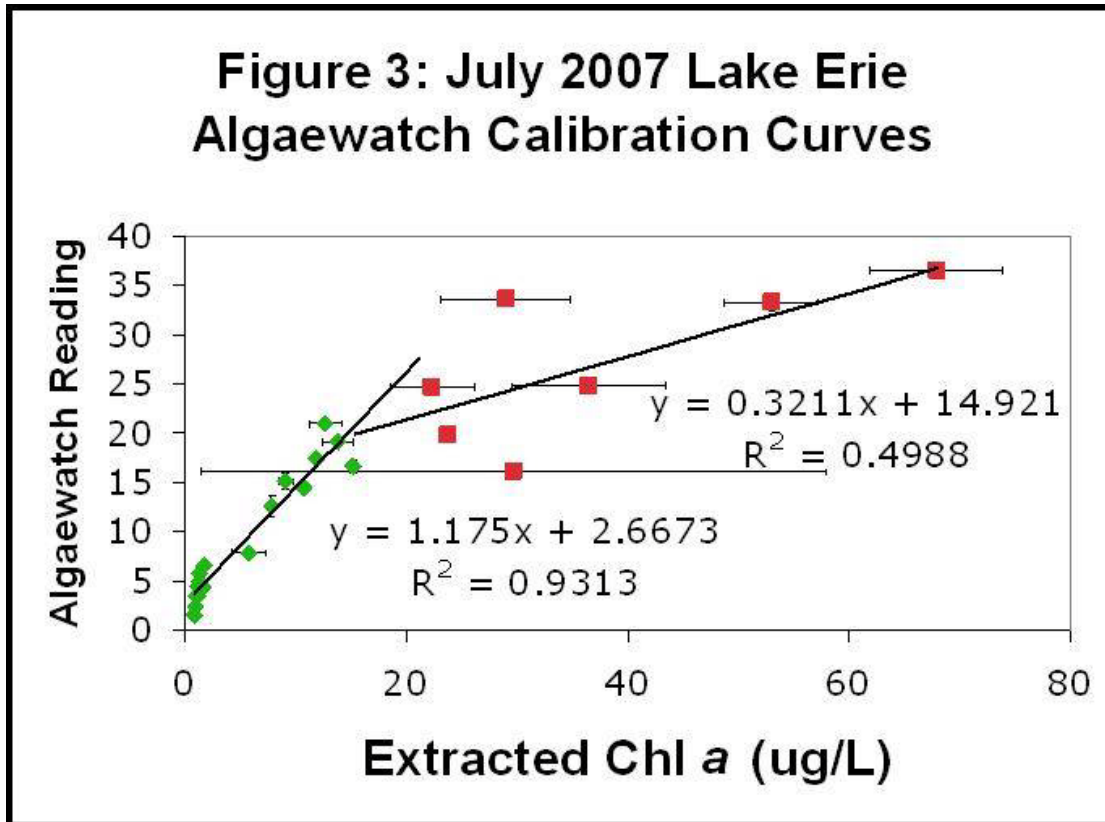
Photosystems aren't equal

Photosystem 2
(higher plant)



Cyanobacteria

Typical Results:

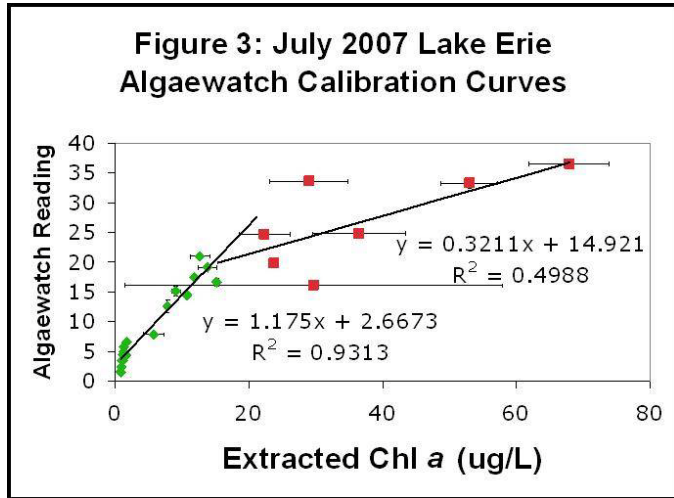


Biphaseic Curve

Eukaryotic Chl

Cyanobacterial Chl

Typical Results:

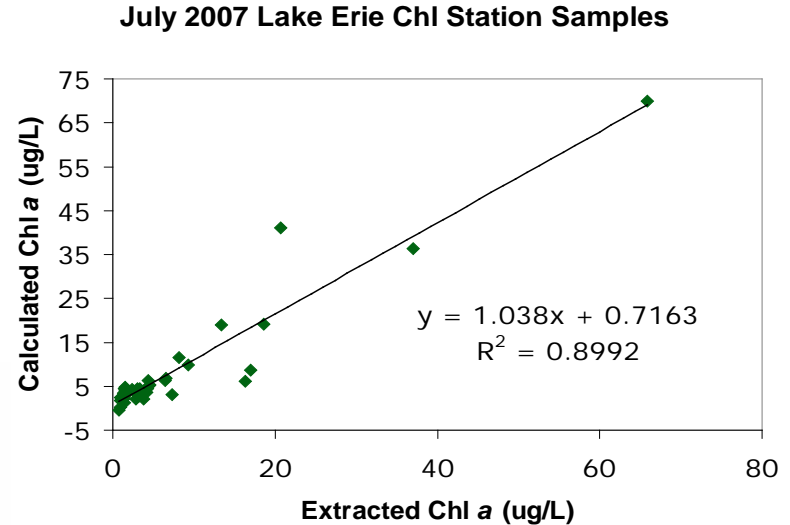


(1) Generate calibration curve



(3) Use that information to generate a chl (or PC) distribution map.

(2) Use the calibration curve used to back calibrate instrument



Start to differentiate between blooms:

Figure 7: Chlorophyll Distribution on Lake Erie, July 2007



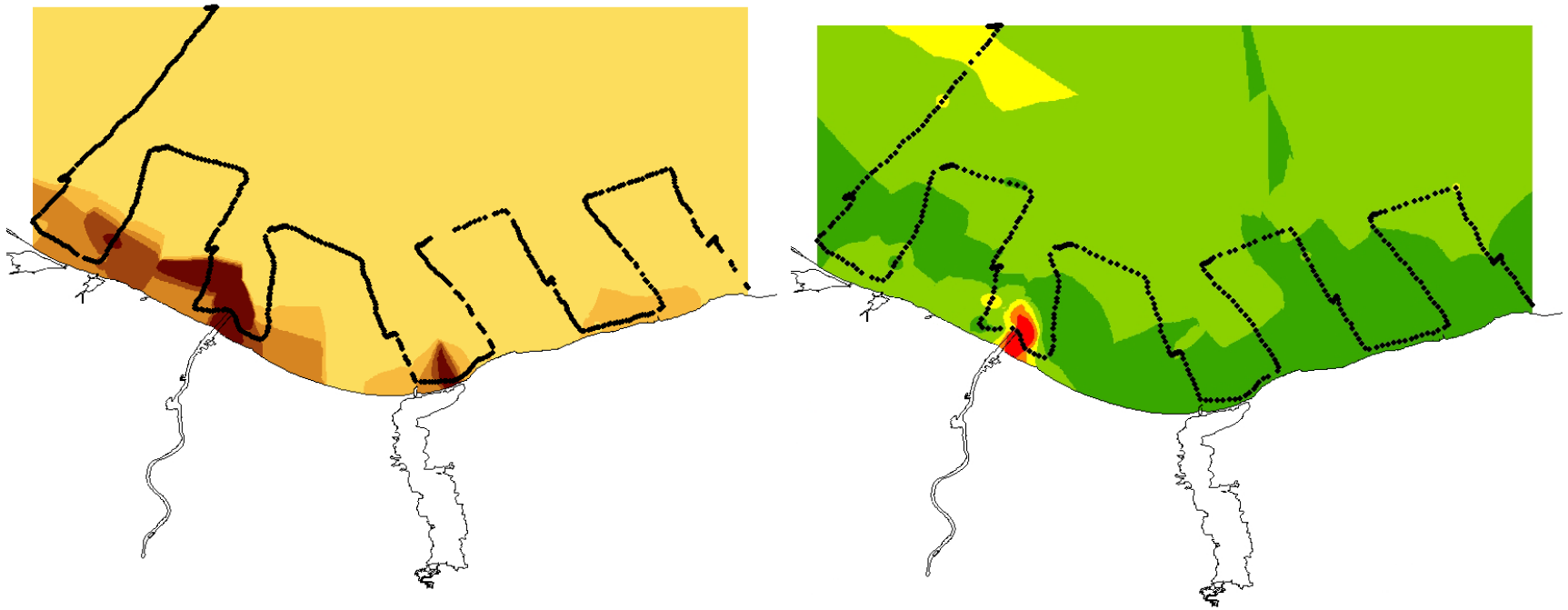
Chlorophyll-a rich blooms occurred in several areas lacking PC; these were likely due to diatoms or green algae

Figure 8: Phycocyanin Distribution on Lake Erie, July 2007



Some high chlorophyll events were associated with cyanobacterial PC

Very high resolution studies Genesee River (Rochester NY)



Specific Conductance

Chlorophyll



Multi-channel sensors provide even more information:

Excite at 5 different Wavelengths

Single emission

- Green Algae
- Dinoflagellates and diatoms
- Blue-green algae
 - Phycocyanin - containing
 - Phycoerythrin - containing
- Others including cryptophytes

Yellow substance correction

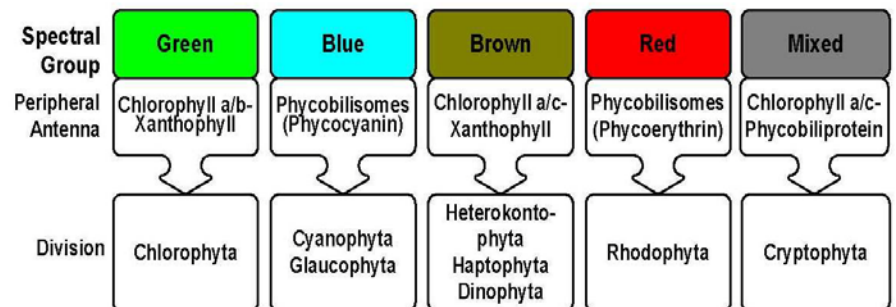
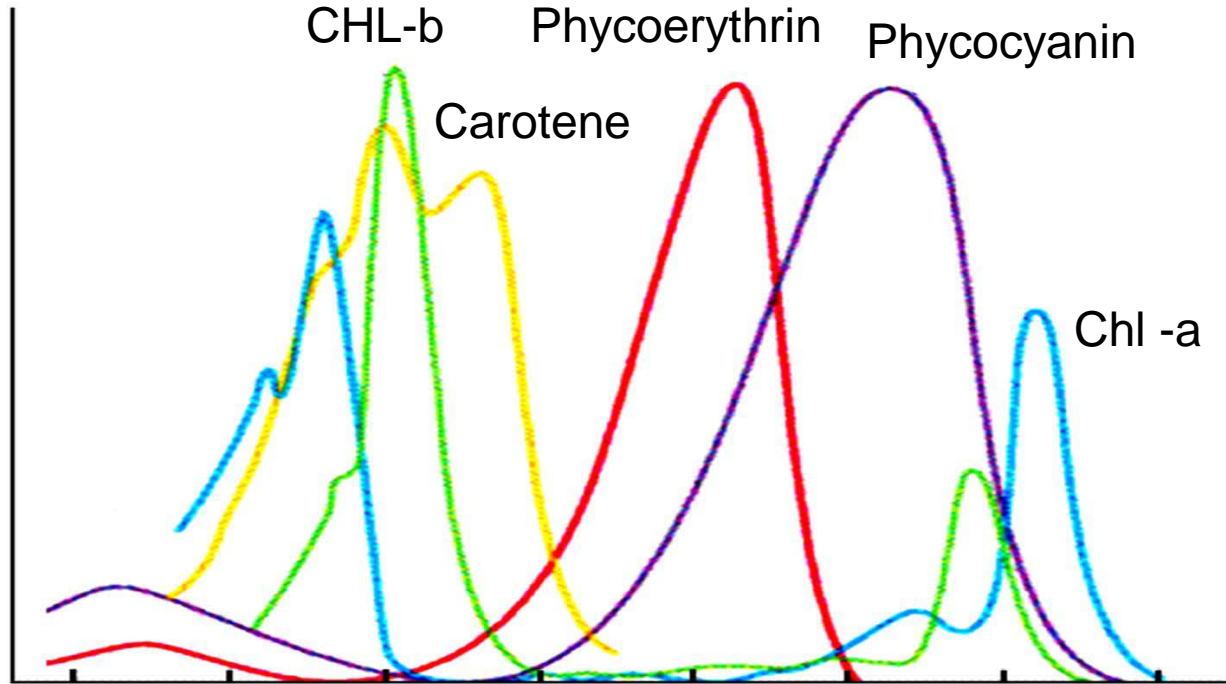


Fig. 1: Assignment of several algal divisions in spectral groups

BBE FluoroProbe

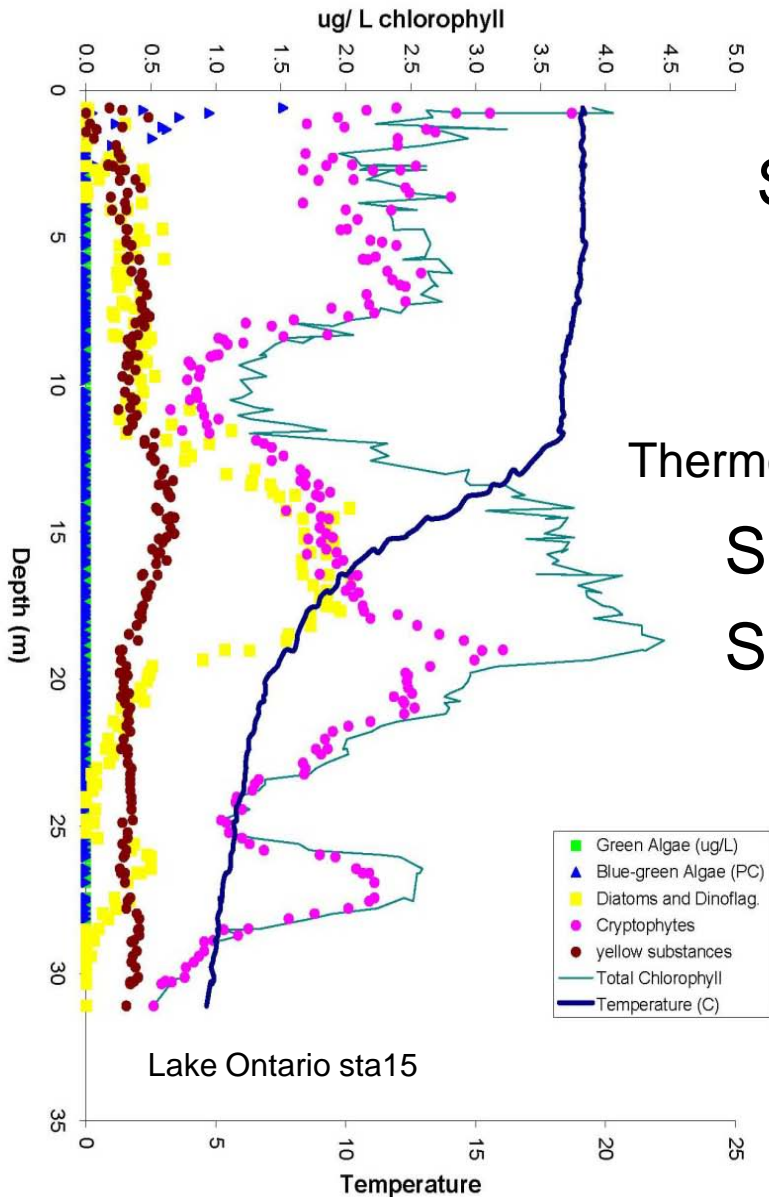
Surface Cyanobacteria bloom

Thermocline

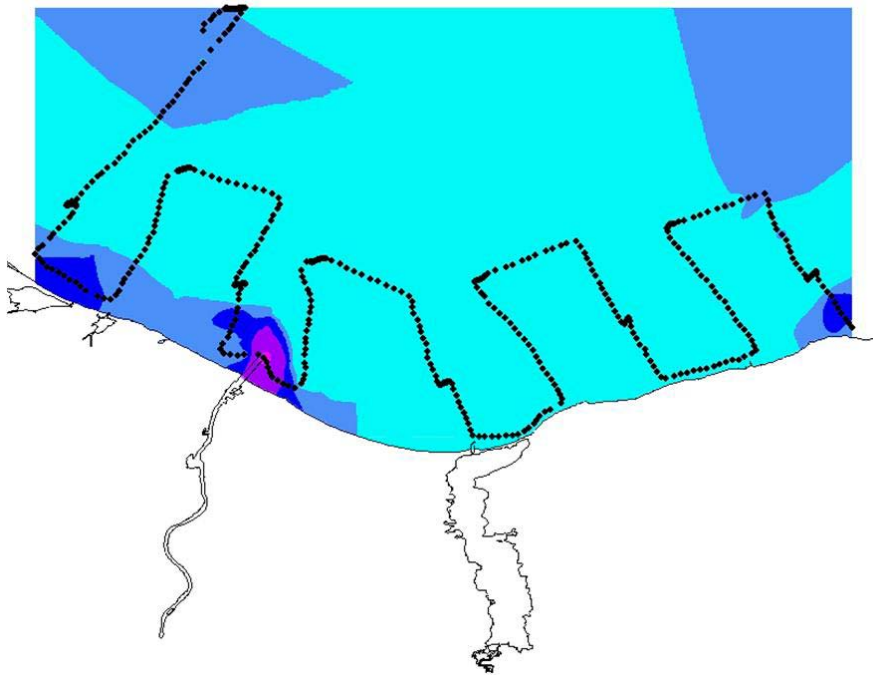
Sub-Surface diatom bloom

Sub-Surface PE bloom

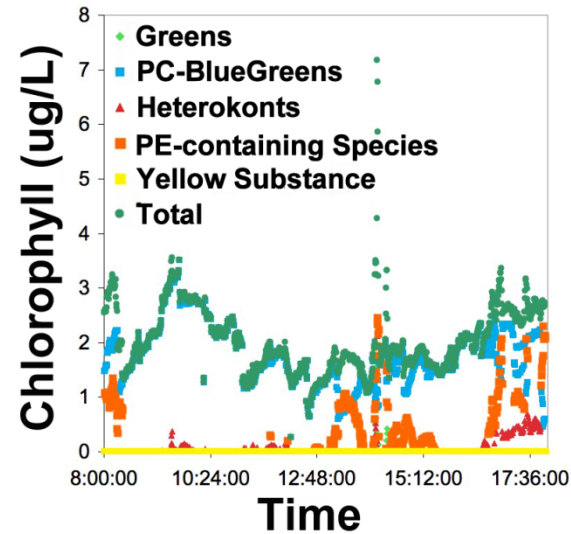
Hypolimnetic Sub-Surface PE-rich bloom



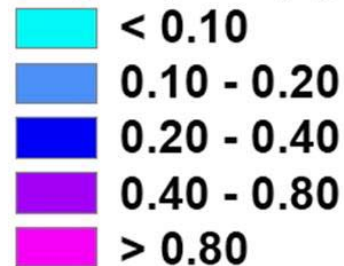
These can be installed in Ferry Box systems also...



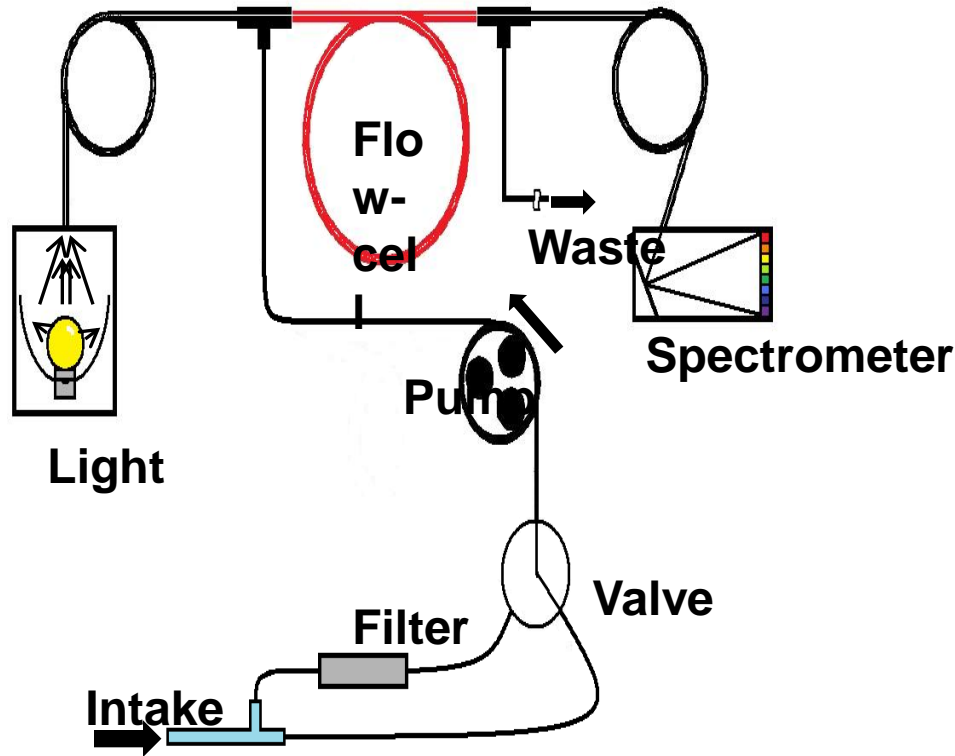
Genesee River
Phycocyanin



Calculated
Extracted
Phycocyanin (ug/L)

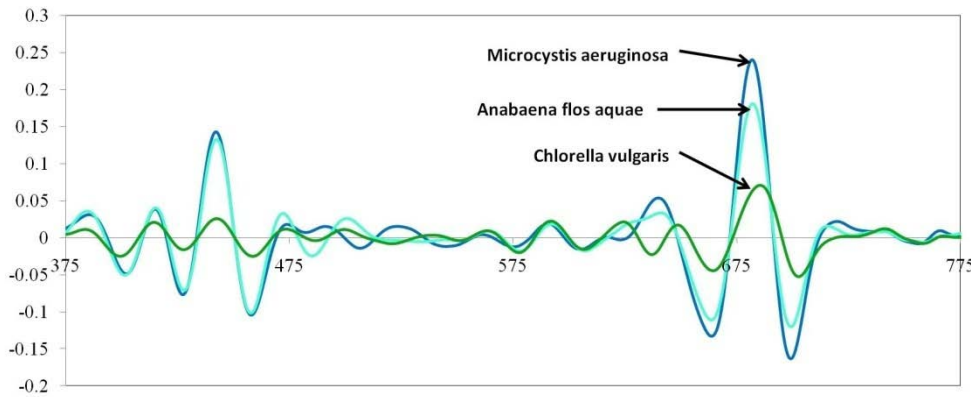


Can we differentiate at the genus level?



- OPC (aka brevebuster)
- Tuned for *Microcystis*

Figure 3: Diagram of the Optical Phytoplankton Discriminator.



Generate a similarity index based on 4th derivative spectrum

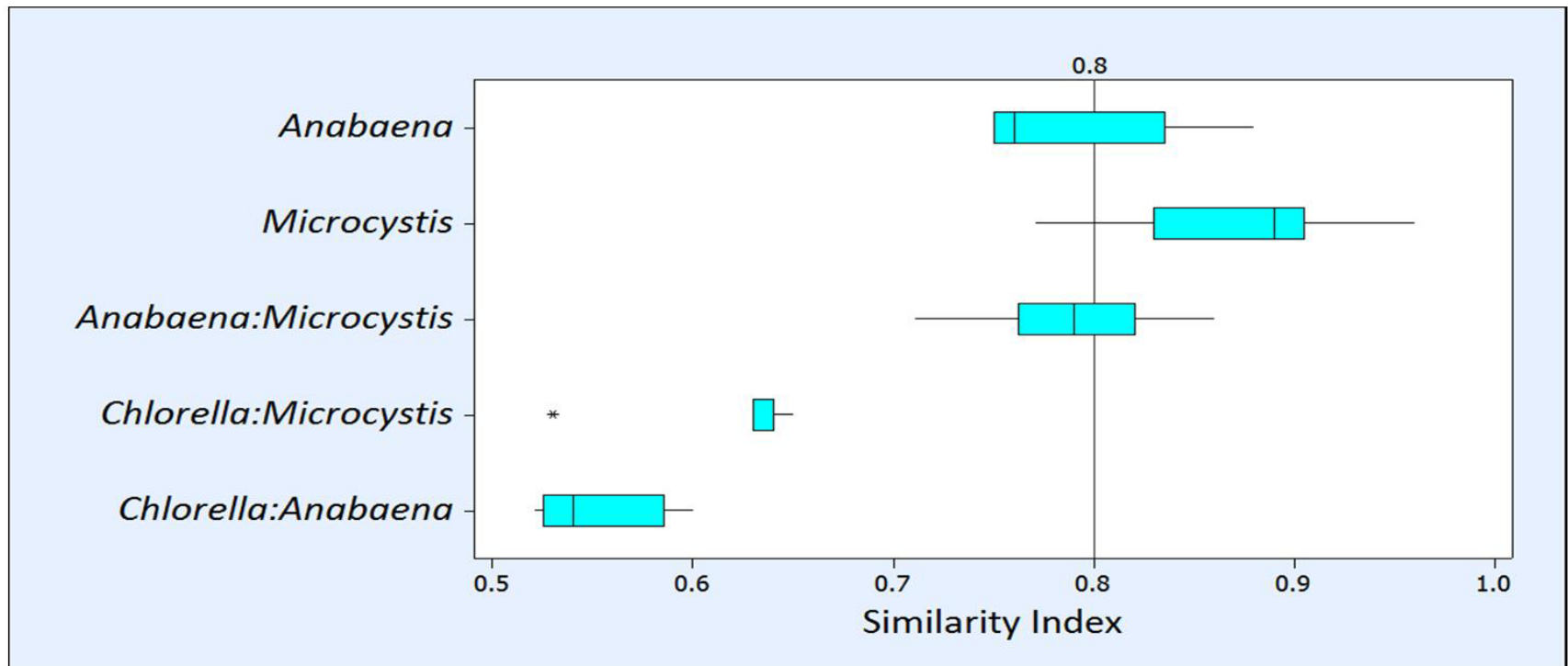
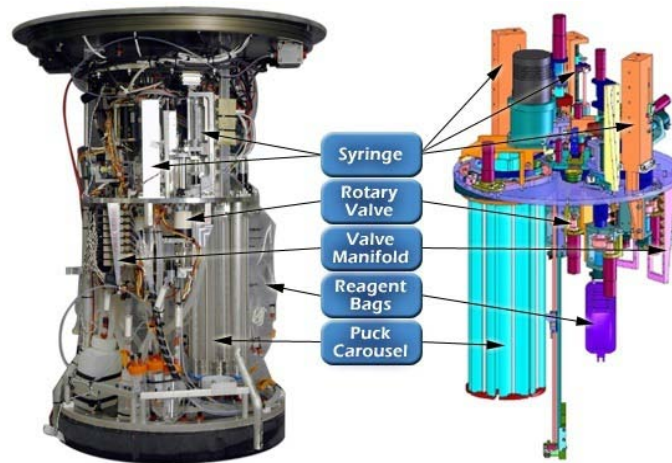


Figure 6: Similarity index results for pairwise comparisons of four *Ananabaena* strains, seven *Microcystis* strains, and *Chlorella vulgaris*.

Next generation of sensors needs to be specific for the toxins themselves....



MBARI Environmental Sample Processor (ESP)

Robotic Multiprobe:

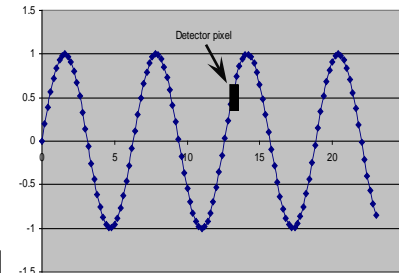
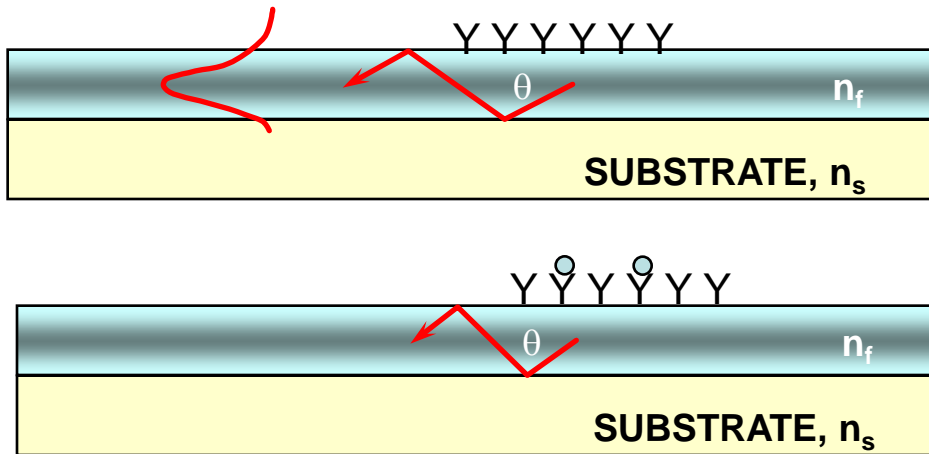
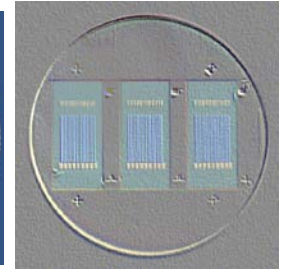
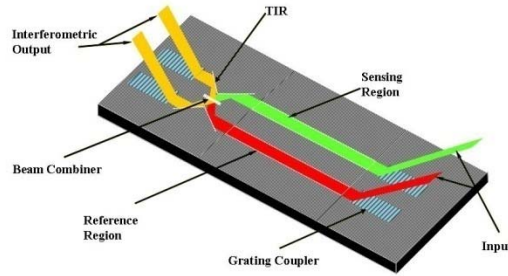
- Species by 16S RNA
- Toxin by ELISA

Suitable for open ocean deployment



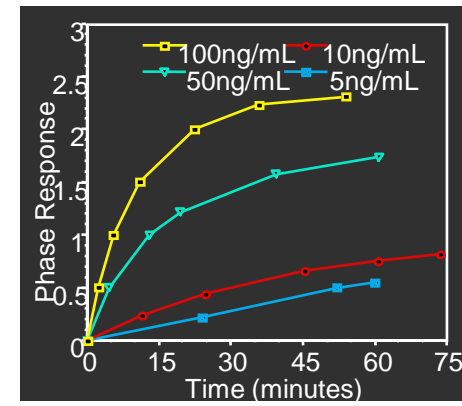
Sensor Functional Description

- Coupled lightwave propagates inside waveguide by total internal reflection



Fringe Pattern

But we have a long long way to go!



Summary

- QUALITATIVE autonomous detection of chlorophyll on buoys and boats is a here.
- QUANTITATIVE detection requires knowledge of the phytoplankton population.
- New techniques allow for detection at the family (FluoroProbe) and Genus (brevebuster)
- Careful of your biochemistry!