THE OHIO STATE UNIVERSITY

Advanced monitoring technologies for cyanobacteria

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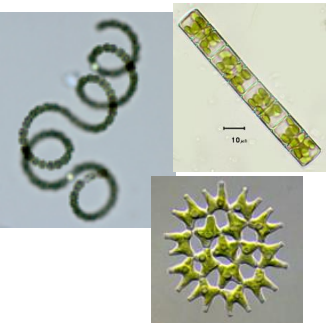
Algae come in all different colors, sizes, and shapes

How do we identify algae and what advances tools are available?

Most algae are not "bad" for lakes

- Algae are tiny plant-like organisms
- 50% of Earth's oxygen produced by algae
  - Every other breath you take, thank algae
- Base of the lake food web
  - "Good" Algae are food for zooplankton
  - Zooplankton are food for small fish

Algae



Zooplankton (small shrimp-like creatures)

Food

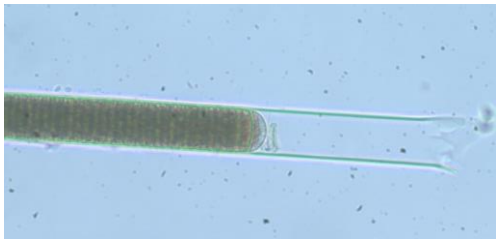
Oxygen

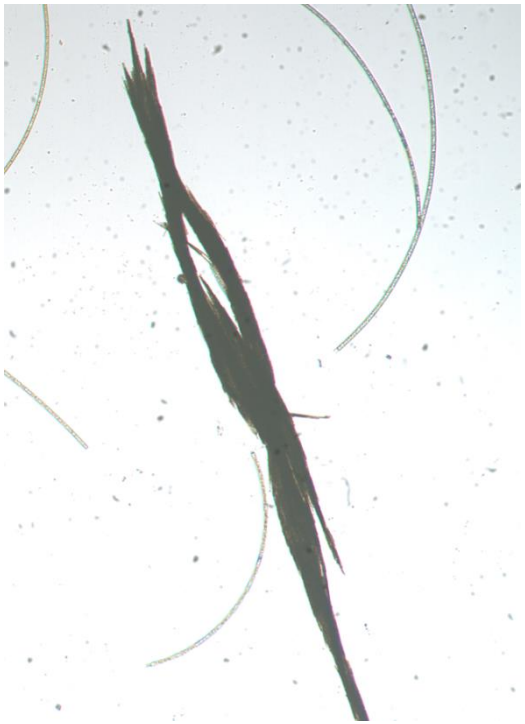
Small fish

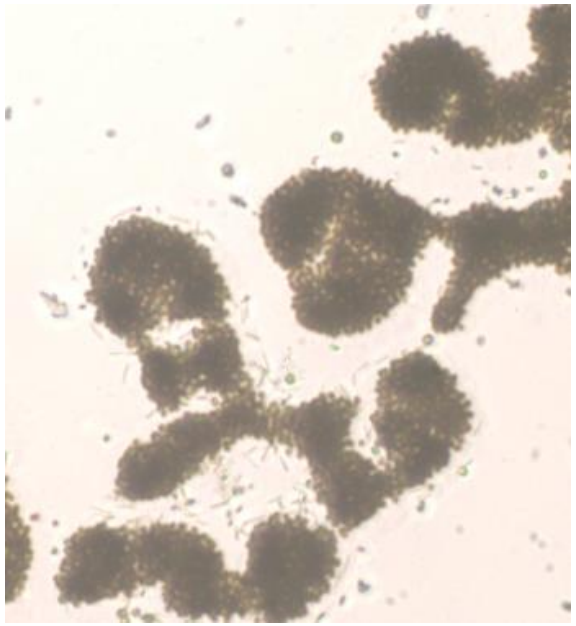
Major algae groups

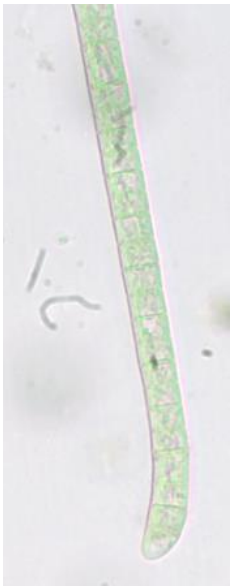
Cyanobacteria

- Blue-green algae, HABs
- Plankton and benthic
- Bloom-forming, some produce toxins





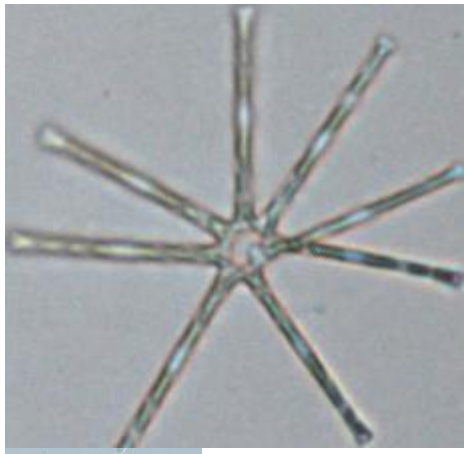


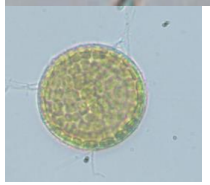


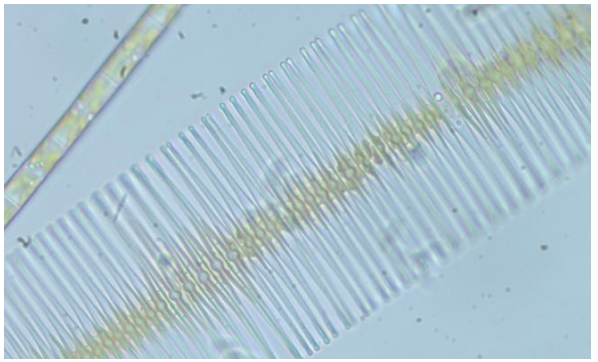
Major algae groups

Diatoms

- Golden color
- Taste & Odor
- Clog filters
- No toxic freshwater diatoms



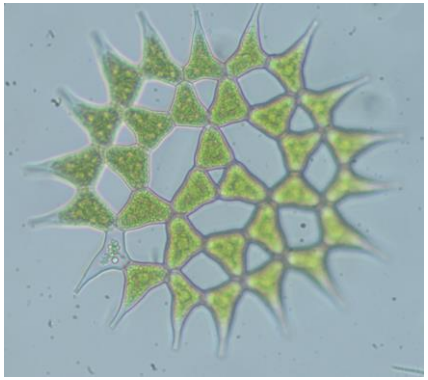


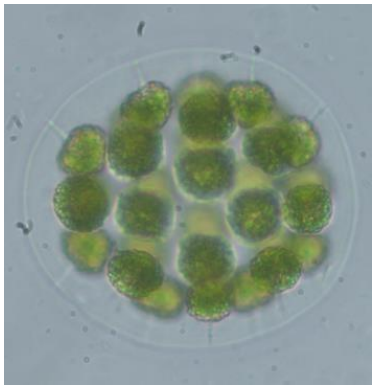


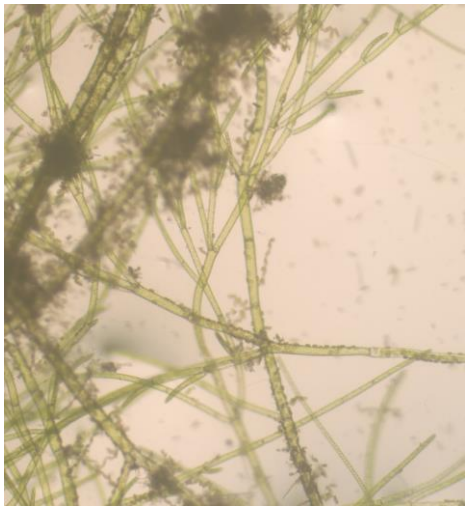
Major algae groups

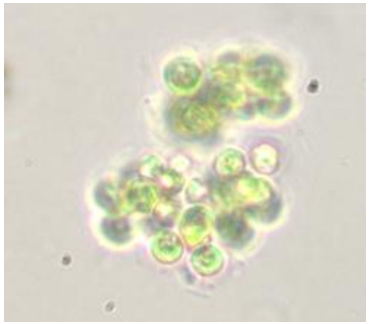
Green algae

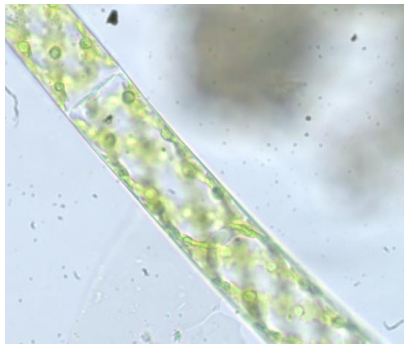
- Very diverse
- Not toxic
- Plankton and benthic

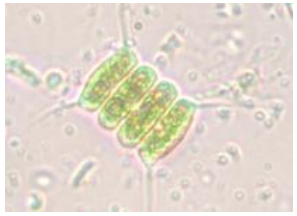


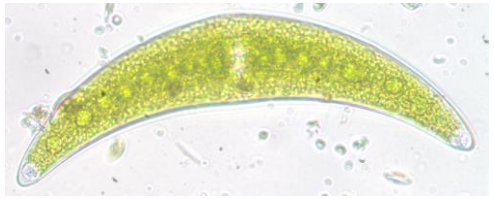








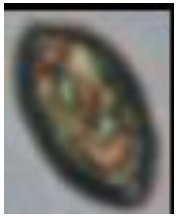


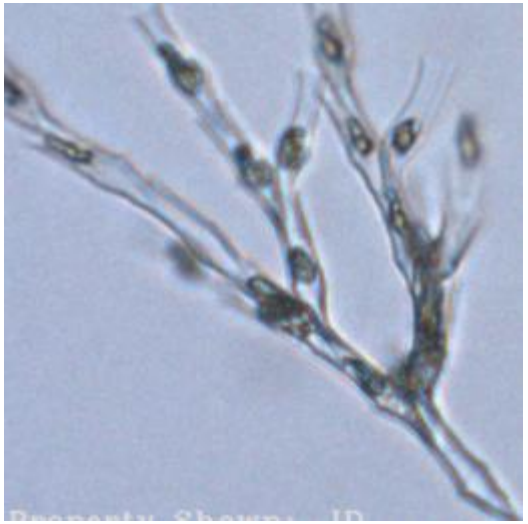


Major algae groups

Others

- Euglena
- Dinoflagellates
- Cryptophytes
- Chrysophytes





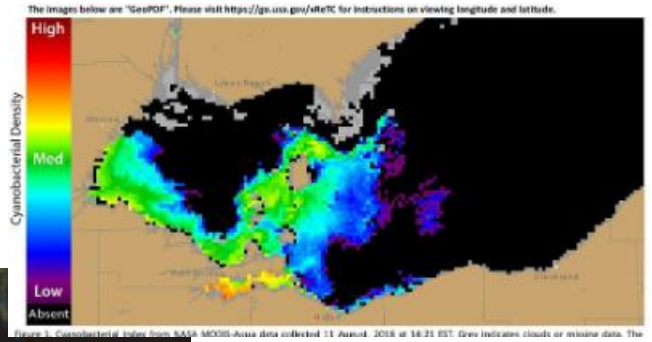
Features used to identifying algae

- Morphology - Microscopy
  - Shape, color
- Pigments present
  - Chlorophylls
  - Carotenoids
  - Phycobiliens
- Genes
  - Taxa level specific
  - Species-specific or division-general
- ‘Behavior’
  - Float vs sink
  - Attached vs phytoplankton

Photos: Ohio Sea Grant

Sample & data collection to identify algae

- Satellites
  - Pigment-based detection
  - Largest spatial scale
- In situ
  - Pigment-based detection
  - Highest frequency
  - Buoys
  - In takes
- Water samples
  - Pigments, genes, microscopy
  - Highest level of detail
  - Grab samples
  - Net samples



Photos: Ohio Sea Grant

Tools used to identify algae

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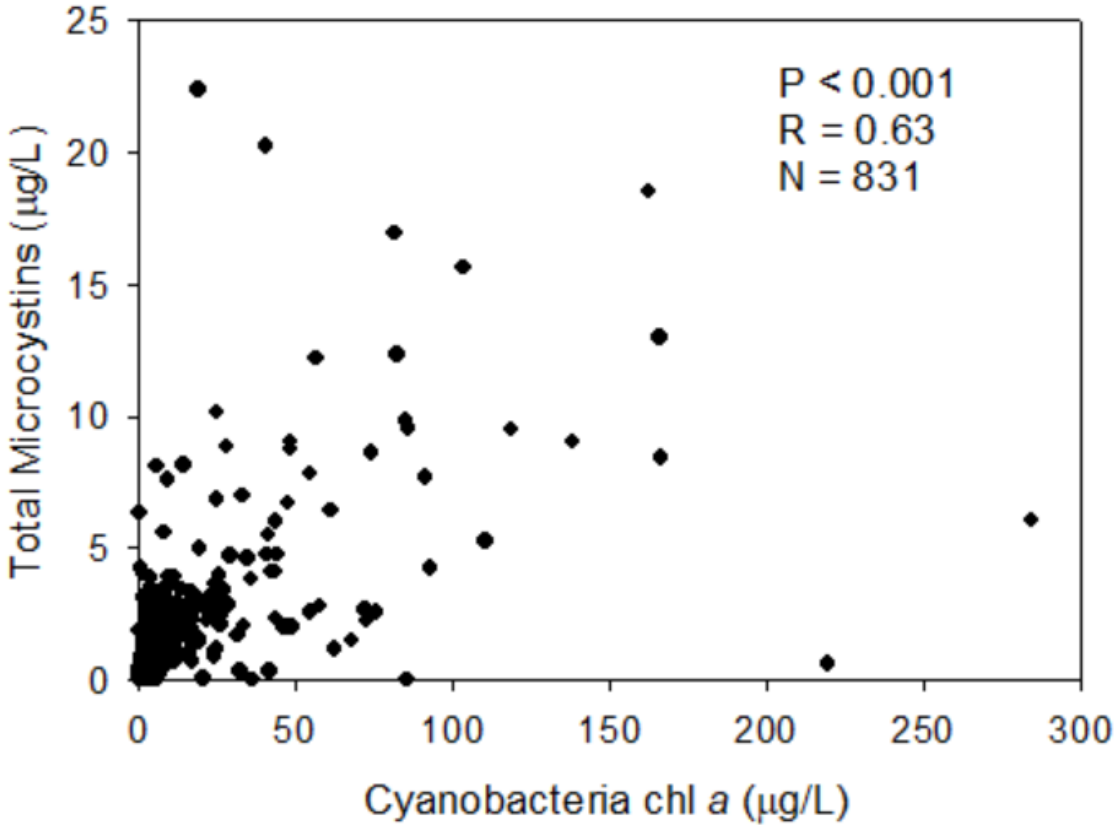
Concerns for cyanotoxins: No correlation between biomass and toxin concentration

- Lake Erie western basin data 2013-2017



Chaffin unpublished

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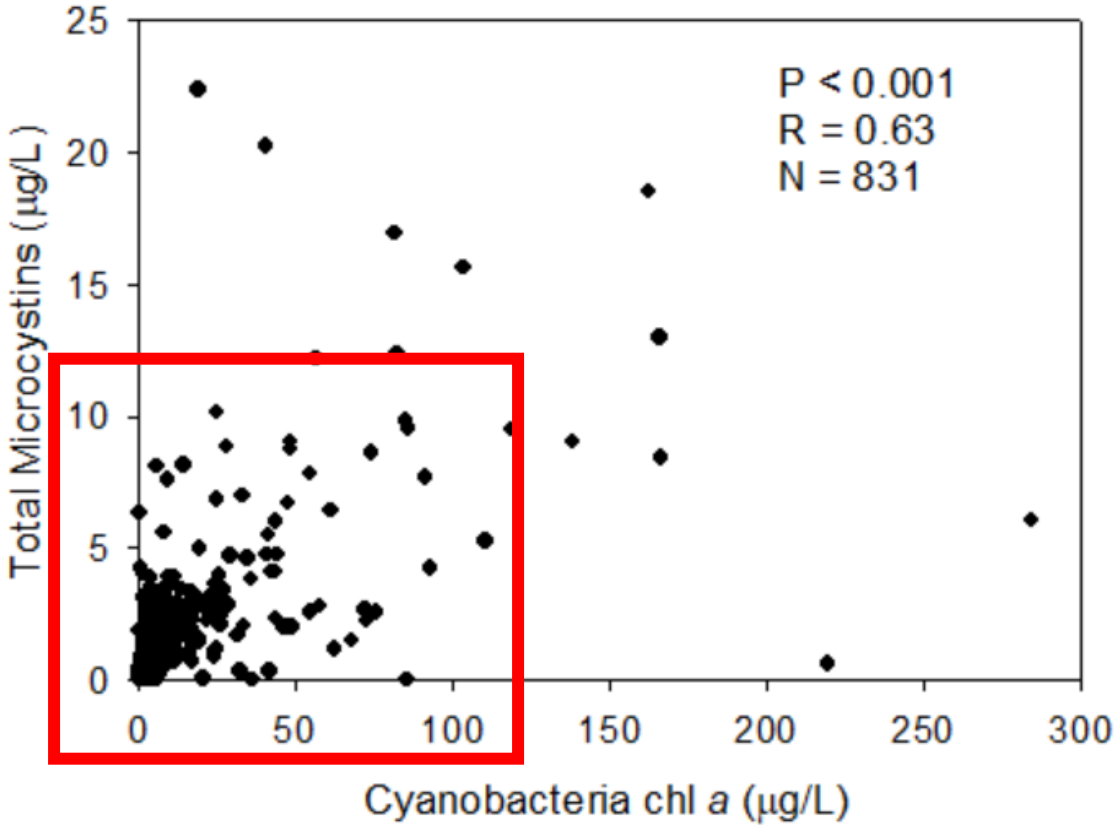

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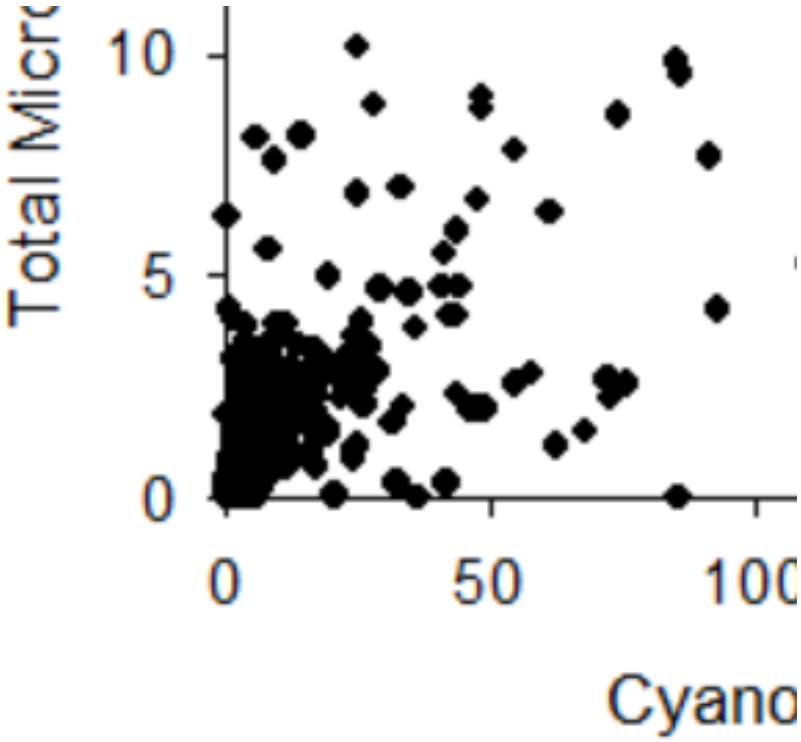

98% of samples were within these ranges

Chaffin unpublished

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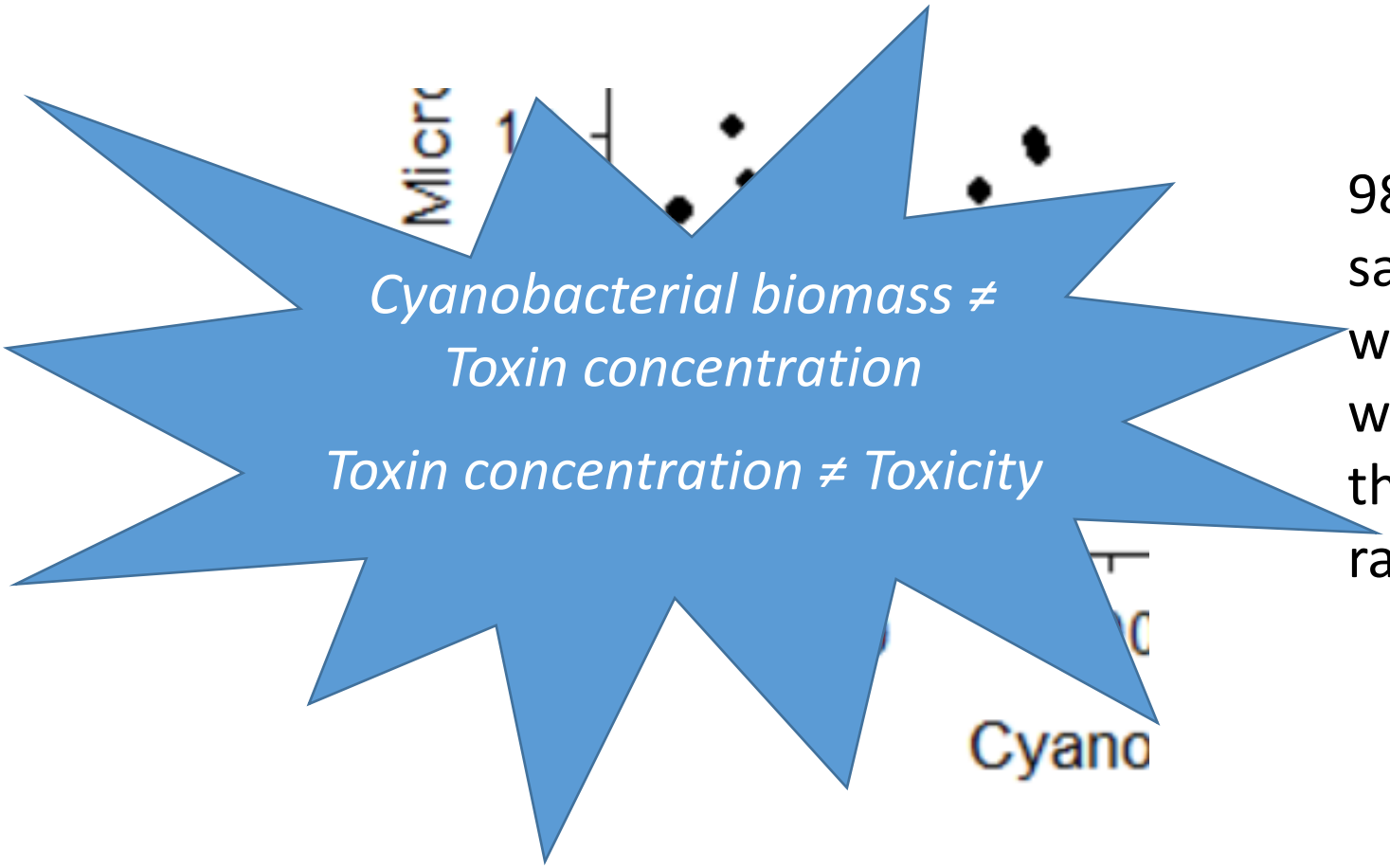

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Chaffin unpublished

Traditional methods can be time consuming

Advanced methods can give real-time data, decrease lab time, and gather more data

Ohio Sea Grant

CDC.gov

Advanced technologies not discussed today

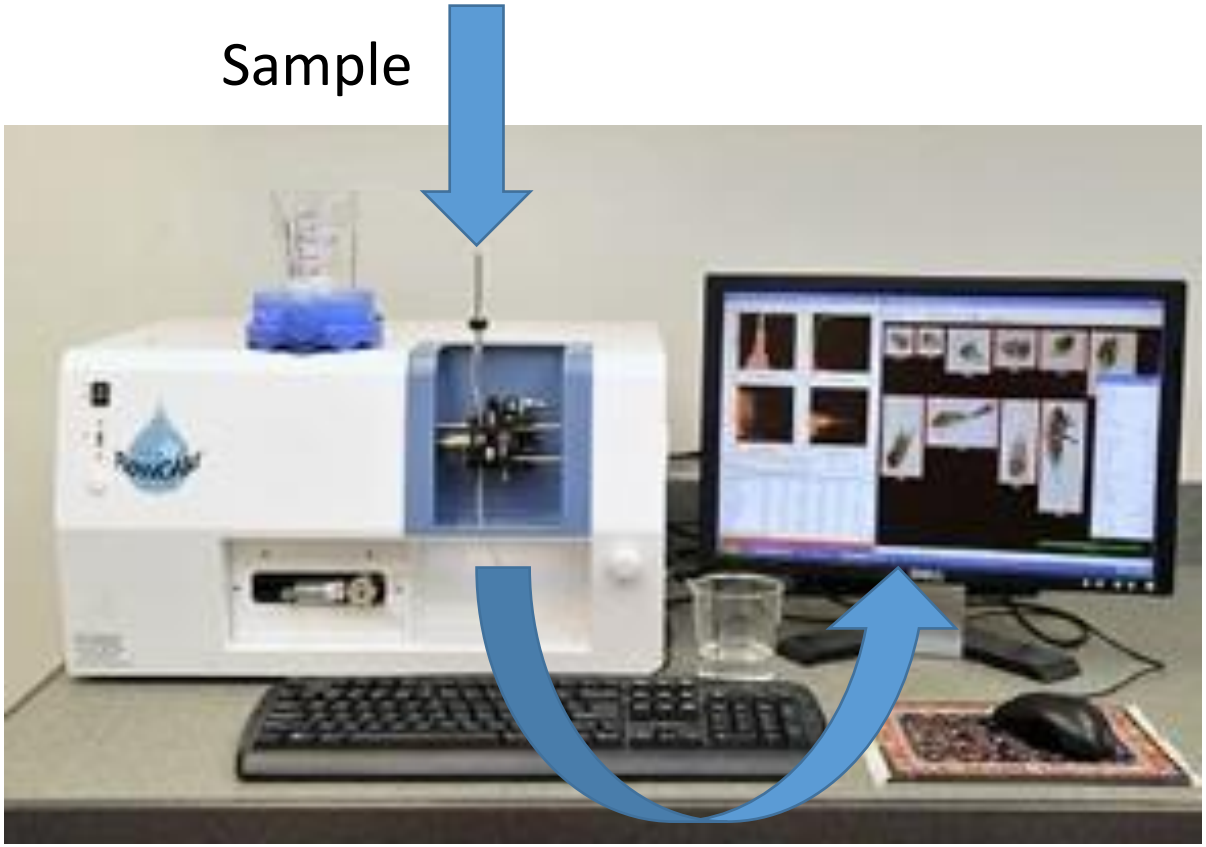
- Remote Sensing
  - Satellites. NOAA HAB Bulletins. Lake Erie, Large inland lakes
  - Drones, small aircrafts
- ELISA – Used for microcystins, required by Ohio EPA for PWS
  - Abraxis does have an automated system for ELISA
- qPCR – Used to detect cyanobacteria and cyanotoxin genes
  - Required by Ohio EPA for PWS
  - Indicate the ***potential*** for toxin production ***in the water column***
  - Benthic cyanobacteria can produce toxins that are released to the water column.

Flow Cam - Fluid Imaging Technologies

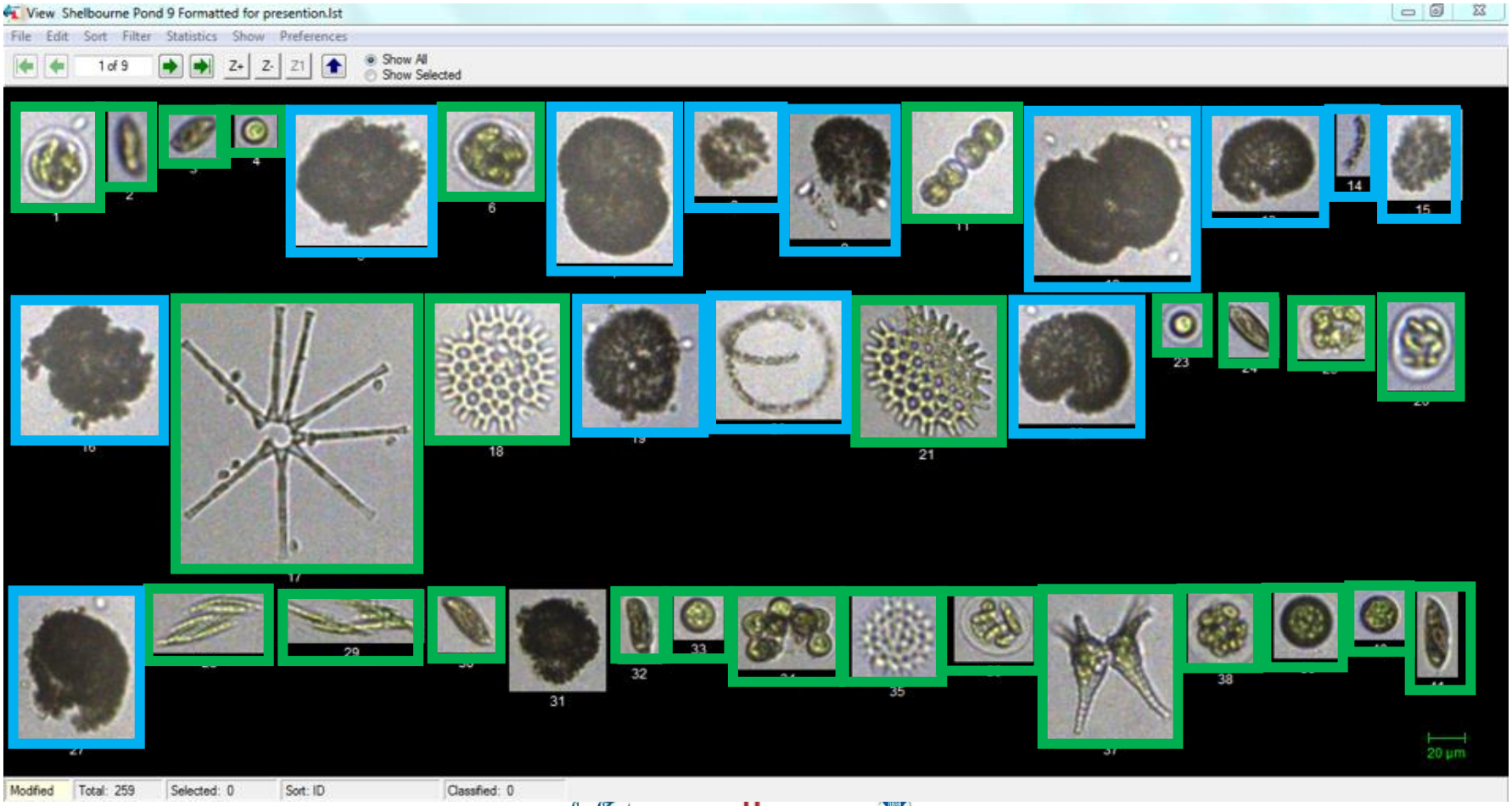
- Flow cytometer microscope with a camera and an image recognition software

Images from FIT

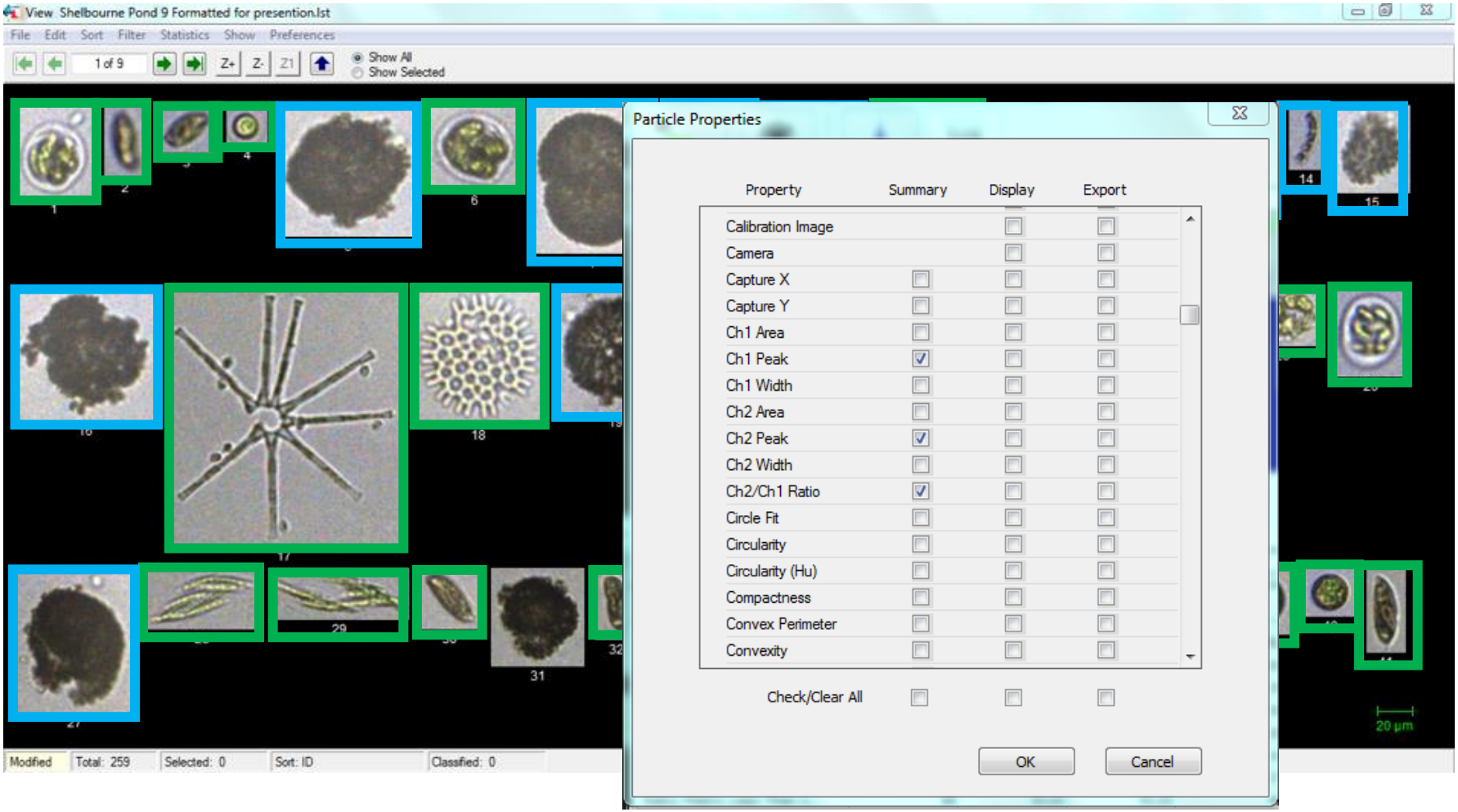
Flow Cam - Fluid Imaging Technologies



Flow Cam – Sorting plankton based on the presence of phycocyanin (cyanobacteria vs others)



Flow Cam – Sorting plankton based on pigments



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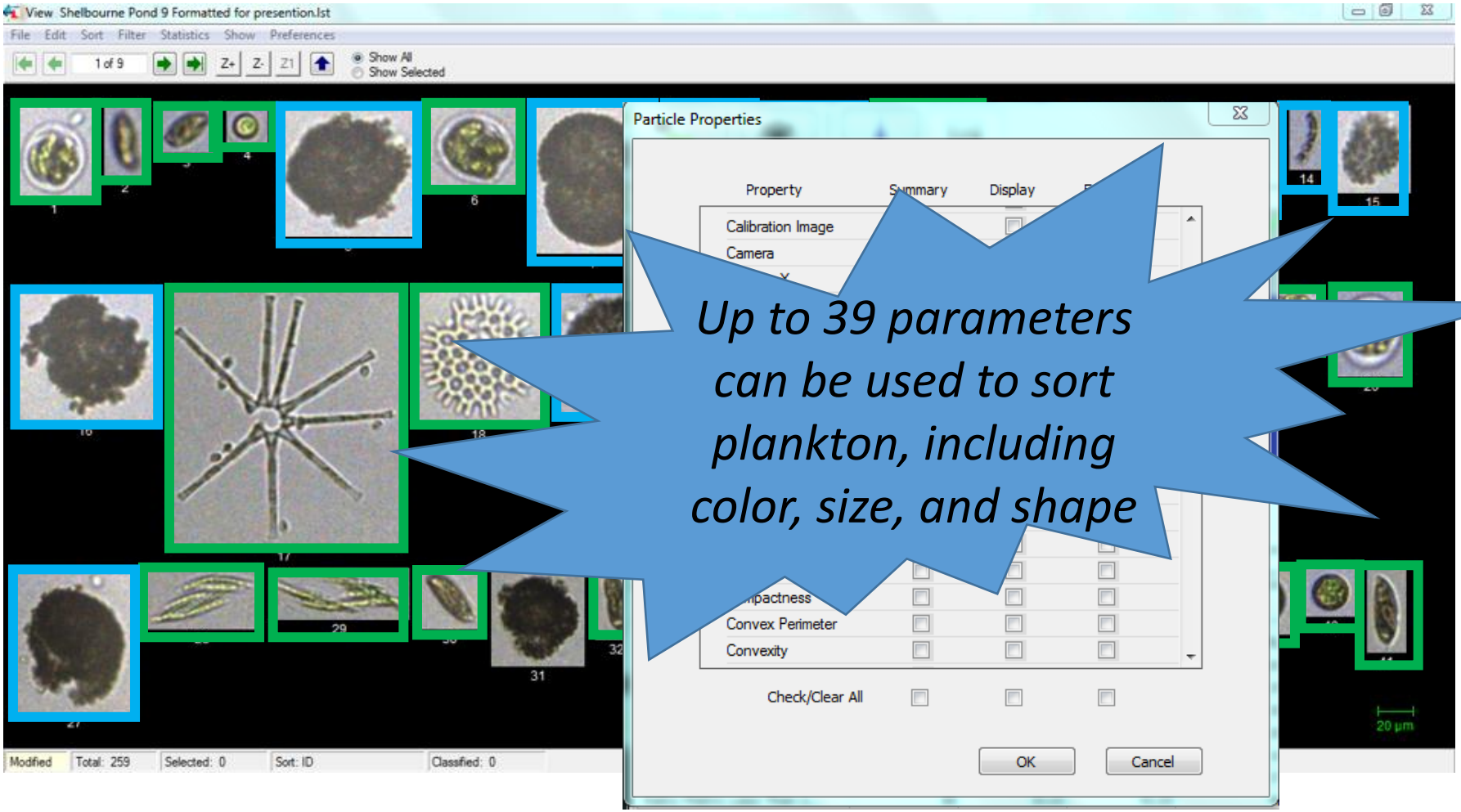
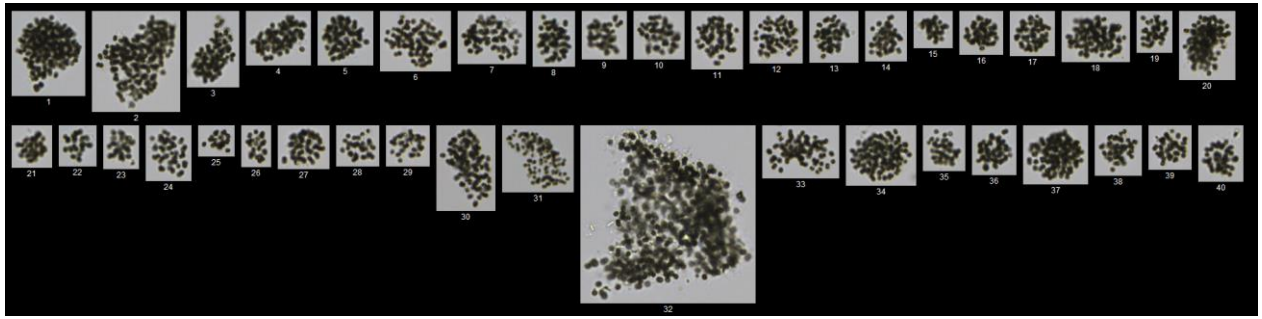
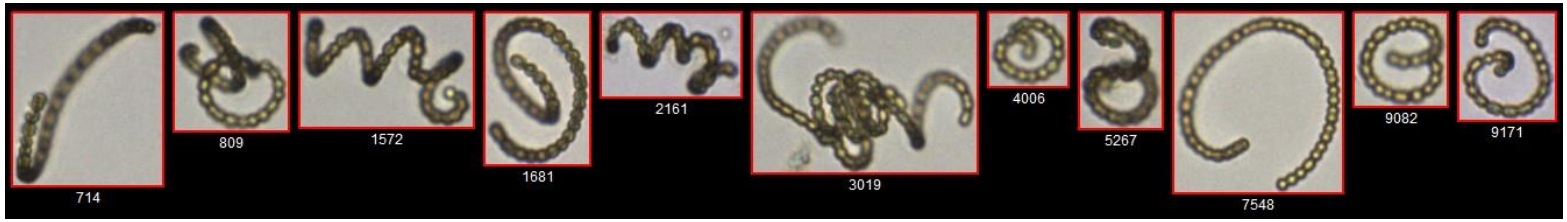


Flow Cam images of cyanobacteria

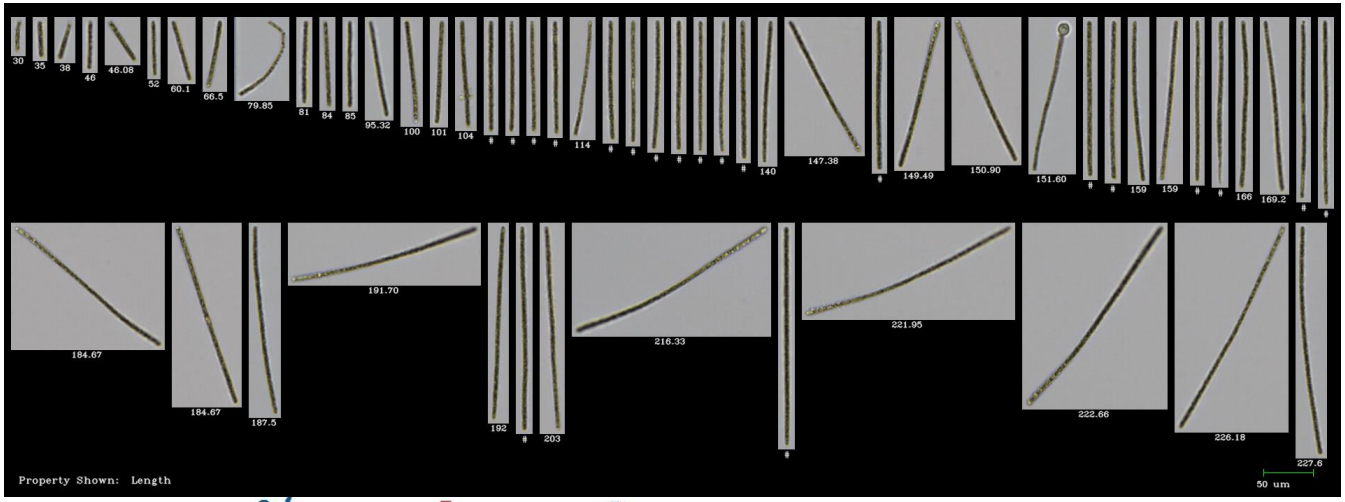
Microcystis



Dolichospermum

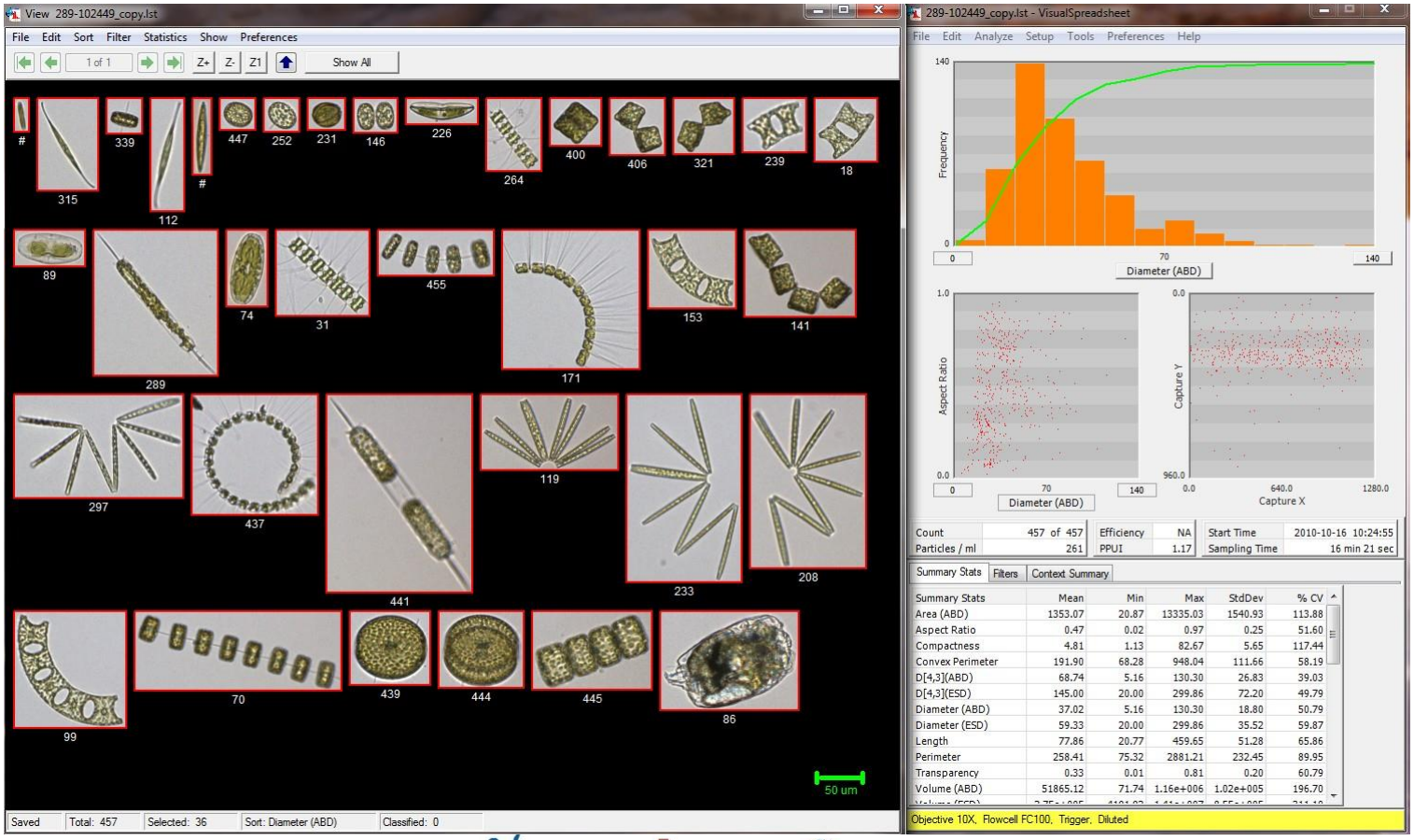


Planktothrix



Flow Cam can enumerate plankton

Count/mL, Biovolume/mL



Flow Cam pros and cons

PROS

- ID and enumerate to genus level
- Method consistent regardless of staffing changes. Quantification will not change.
- Speed of analysis (1 mL in 6 minutes)
- High throughput
- Cost per sample: 1 pipette tip.
- Morphology (ex: biovolume) automatically measured
- Semi-automated taxonomic classification capabilities
- Import data to LIMS: Ditigal record of data!
- Helpful staff!

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CONS

- Similar looking taxa: *Planktothrix* & single-stranded *Aphanizomenon*
- Filtration often required
- Clogging more likely with filamentous cyanobacteria and diatoms
- Limited ability to visually identify organisms <15 µm
  - Need higher power objective, which is slower and samples smaller volume
- Does not measure toxicity (like all methods discussed today).
- Operator must be able to ID plankton, but 15-20 taxa are most common and libraries can be built to help classification.

FluoroProbe – bbe Moldaenke

- Fluorescence-based instrument to differenate chlorophyll *a* among green algae, cyanobacteria, diatoms, and cryptophytes

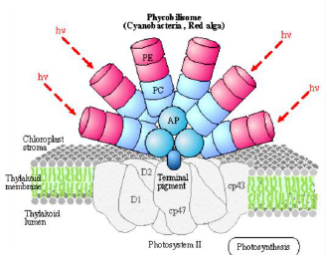
Algae groups measured by the FluoroProbe

- *Green group*: Green algae, euglenoids
- *Blue group*: Phycocyanin-containing cyanobacteria
- *Brown group*: Diatom, Dinoflagellates, Chrysophytes
- *Mixed group*: Cryptophytes, Red algae, Phycoerythrin-containing cyanobacteria

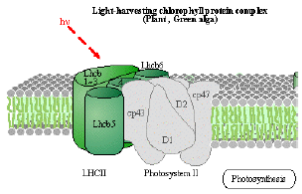
Images from Tim Doyle, bbe Moldaenke

FluoroProbe uses 7 LED lights and measure fluorescence from chl-a and accessory pigments

Spectral Group	Green	Blue	Brown	Red	Mixed
Peripheral Antenna	Chlorophyll a/b-Xanthophyll	Phycobilisomes (Phycocyanin)	Chlorophyll a/c-Xanthophyll	Phycobilisomes (Phycoerythrin)	Chlorophyll a/c-Phycobiliprotein
Division	Chlorophyta	Cyanophyta Glaucophyta	Heterokonto-phyta Haptophyta Dinophyta	Rhodophyta	Cryptophyta



Algae pigments and their relation to taxomical algae classes

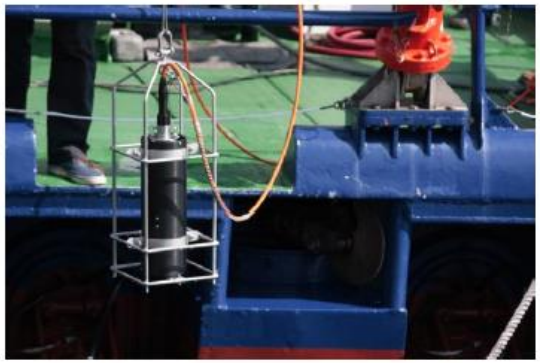


Green algae, Cyanobacteria, Diatoms, Cryptophytes

FluoroProbe Accessories

Workstation 25 with Stirrer Unit
(for laboratory work)

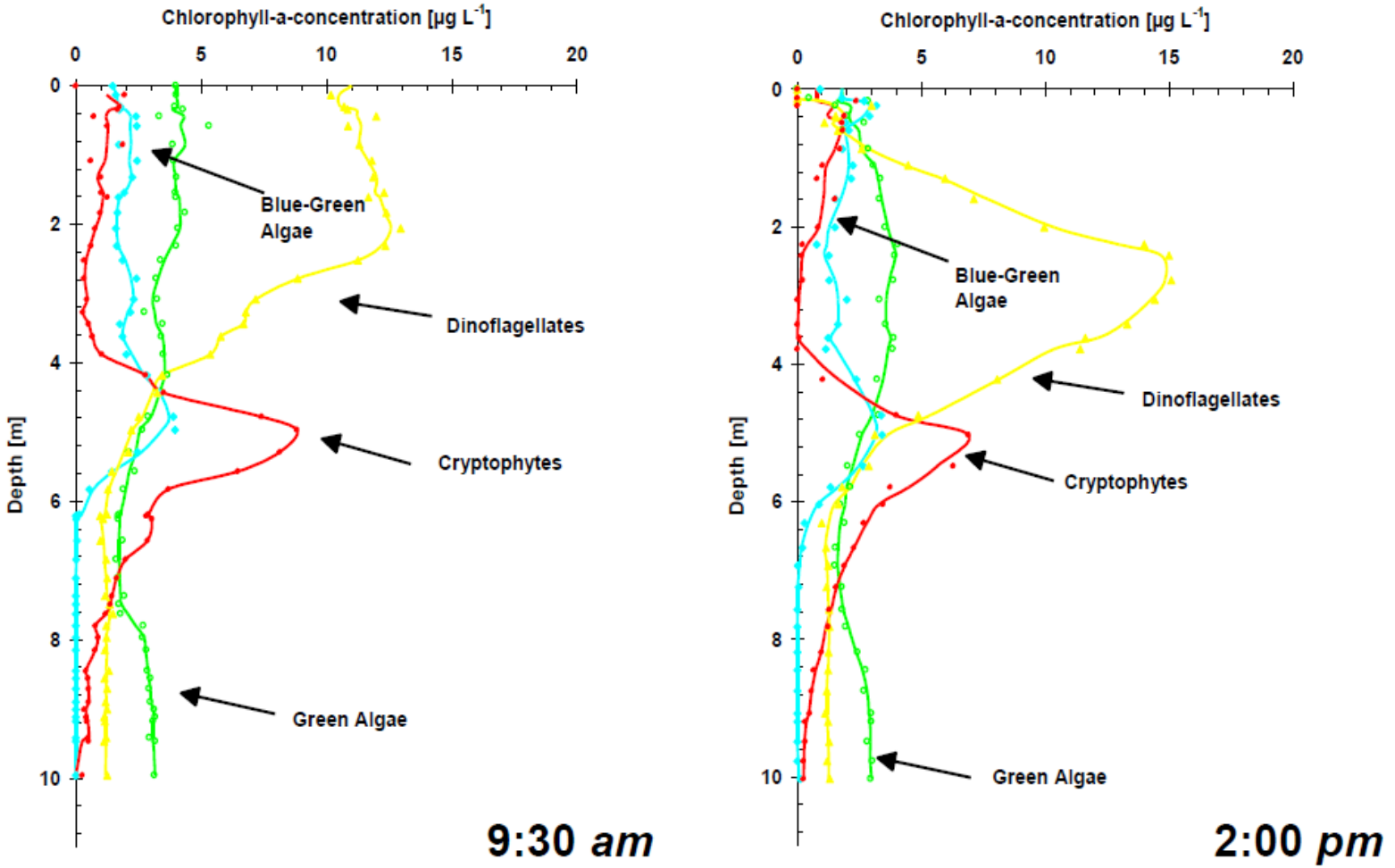
Protective Steel Cage for rough weather



HydroWiper for long-term deployment

Handheld (Bluetooth)
for field work

Migration of Dinoflagellates in Eutrophic Plussee



FluoroProbe pros and cons

PROS

- Quick data (seconds) with no processing
- Easy to operate software
- Custom classes is available, but most users use default
- Lake (profiles) and lab (cuvette) modes use same software
- Other similar instruments include benthic algae (Bentho Torch) and in situ (Algae Online Analyzer)

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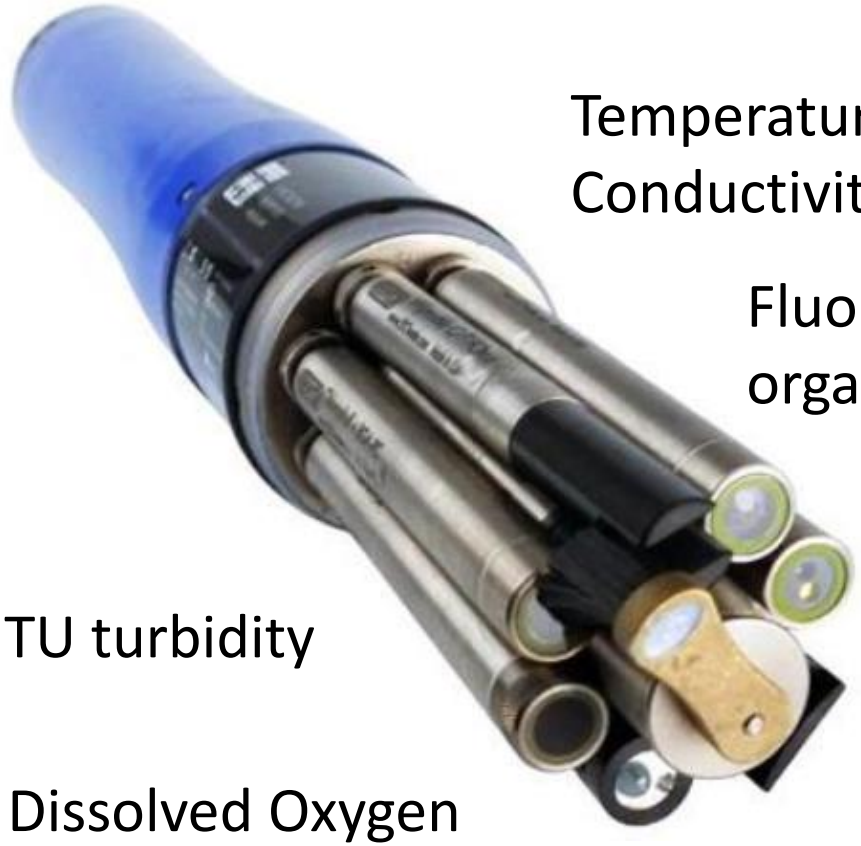
CONS

- Cyanobacteria general – not genus or species
- Non-photochemical quenching (light stress) can alter data
- Blooms swamp detection of other groups
- Chlorophyll concentration is not proportional to phytoplankton biomass
- Does not measure toxicity (like all methods discussed today).

Buoys and *in situ* sensors

Data available sensors



Temperature & Conductivity

Fluorescence dissolved organic matter

Algae & Cyanobacteria

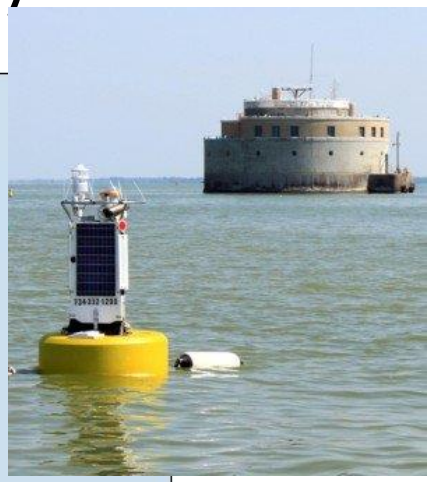
NTU turbidity

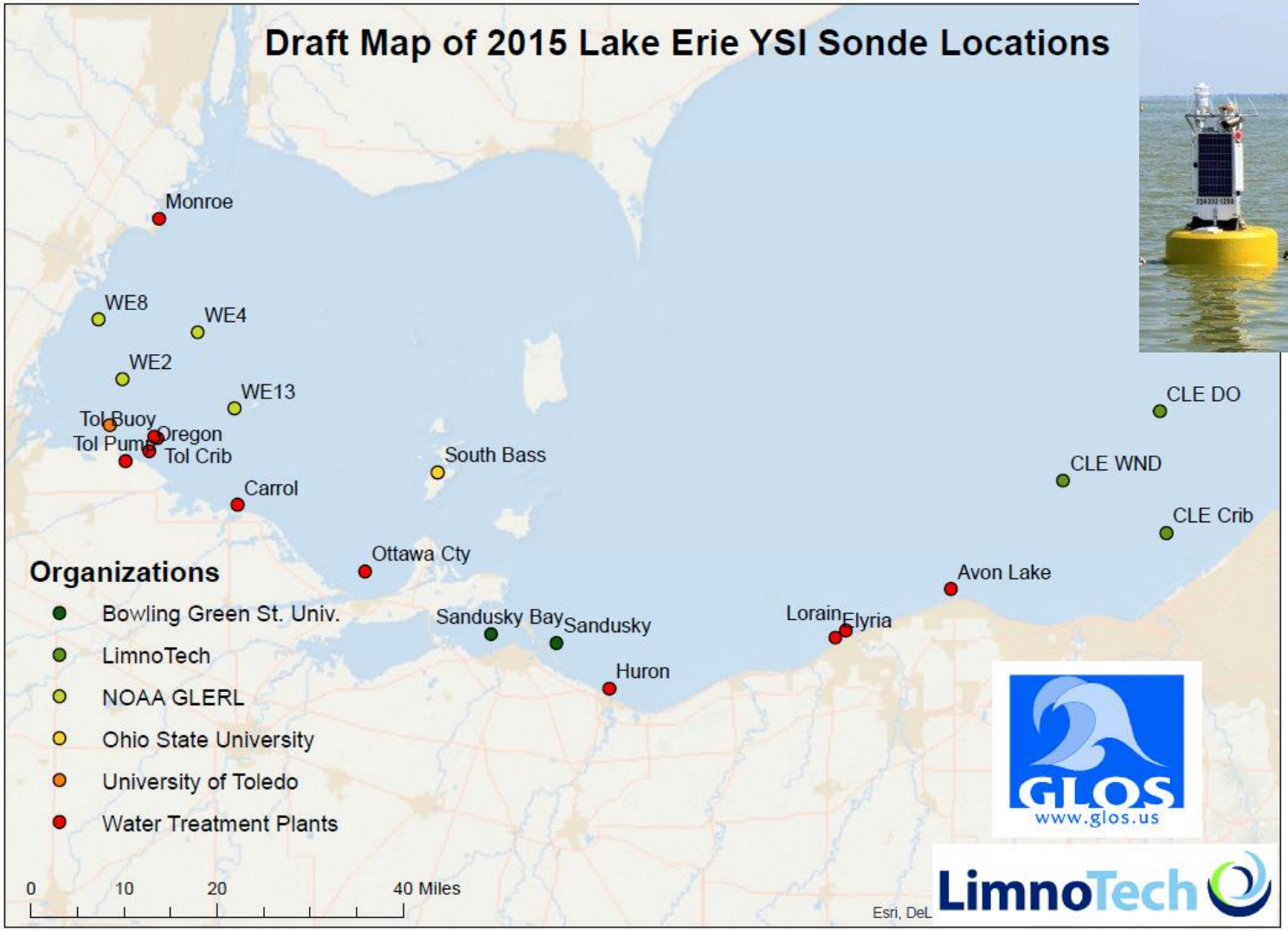
Dissolved Oxygen

pH

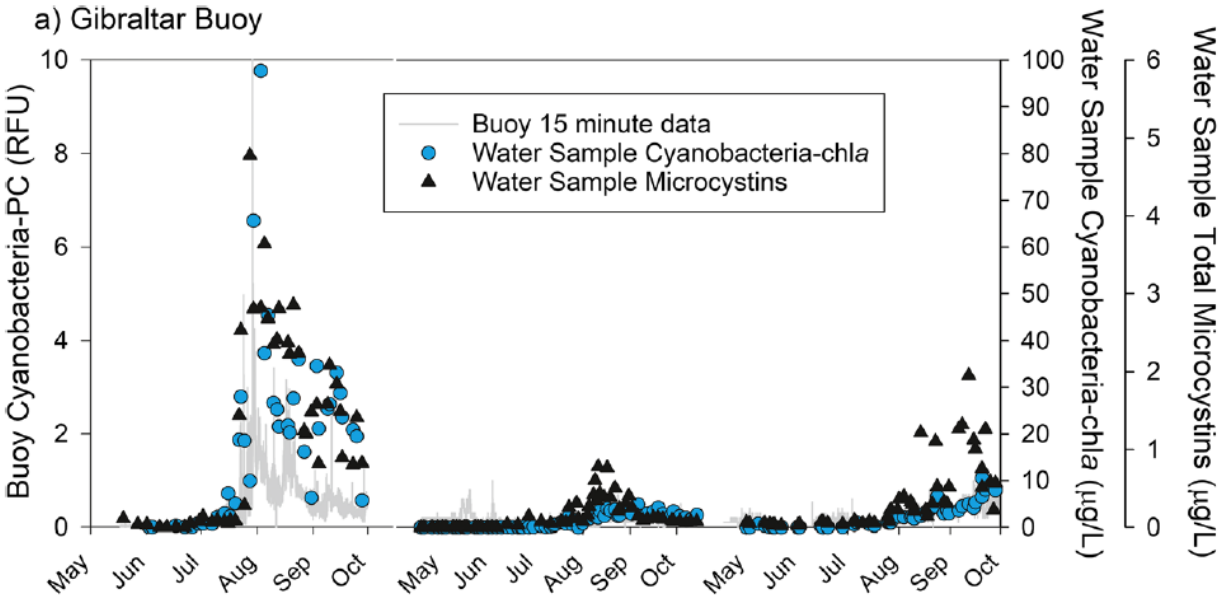
YSI.com

Real-time early HAB warning system





Buoy data accurately tracks HABs



In situ sensor pros and cons

PROS

- Real-time data
- Frequent data (minutes)
- Alerts
- Trends and peaks in sensor data align with water samples
- New sensors hold calibration
- Network of data

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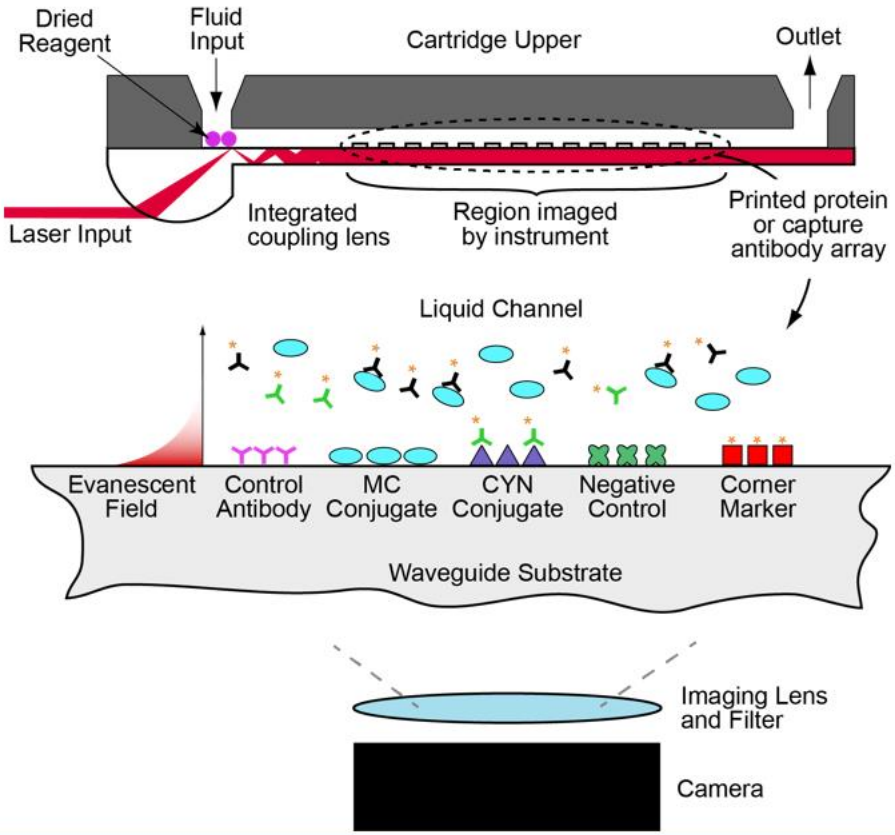
CONS

- Bio foiling
- Fixed location, fixed depth
- Potential differences between sensors/sites due to taxa present
- Daily fluorescence patterns affect data
- Old sensors lose calibration
- Does not measure toxicity (like all methods discussed today).

Light Deck Diagnostics HAB Toxin System

- Microcystins and Cylindrospermopsins data in 10 minutes (compared to ~4-5 hours with ELISA)

MBio LightDeck® Technology Overview



m·bio® DIAGNOSTICS

Images from Sarah Bickman

Light Deck Toxin System

Cell lysis tower

m·bio DIAGNOSTICS

MBio HAB System
MC/CYN Gen 2

Sample Information

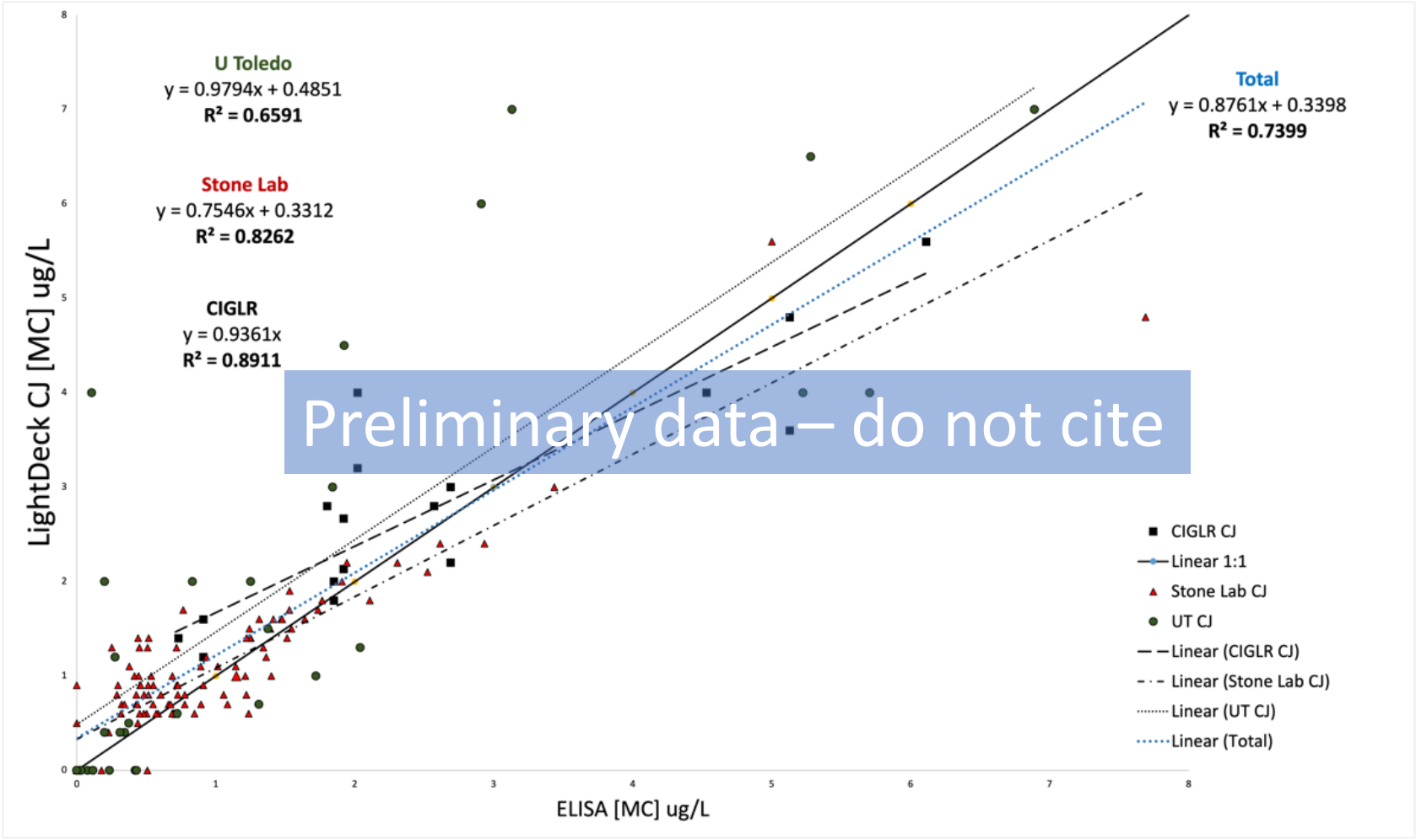
Sample ID:	Lake4_drag2
Sampler ID:	MJL
Test Type:	MC/CYN
Collection Date:	03/25/2018
Collection Time:	01:45 PM
Tester ID:	SRB
Test Type:	MC/CYN
Test Date:	04/13/2018
Test Time:	03:00 PM
Time:	03:00 PM
Cartridge Lot ID:	001056
Cartridge ID:	ZZ-02005-136
Expiration Date:	12/31/2018
Reader ID:	98708740872
Software Version:	SnapEsi-LS 3.2.0.3

Control	VALID
Microcystin	2 μg/L
Cylindrosperm.	Less than 0.3 μg/L

Sea Grant

The Ohio State University

ELISA vs LightDeck cartridges



Light Deck HAB toxin system pros and cons

PROS

- Microcystins and Cylindrospermopsins concentration data within 10 minutes
- Cell lyse in 10 minutes
- Portable; in the field measurements
- Standards and QC included on the cartridge
- We are working on saxitoxin and anatoxin

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CONS

- Limited working range (smaller range than ELISA)
- Cell lyse system not yet available (still need freeze/thaw)
- Developed towards MC-LR, less reactivity with other MCs

Conclusions about advanced technologies

- Quicker data to inform decisions
- From the researcher view point: Advanced technologies do not replace traditional methods, but supplement traditional data.
- From the applied view point: Advanced technologies increase the information available
- Start up is expensive
  - Thousands of dollars
  - Maintenance costs tend to be overlooked or underestimated

Questions & Discussion

MODIS. October 2011