

# Monitoring for HABs

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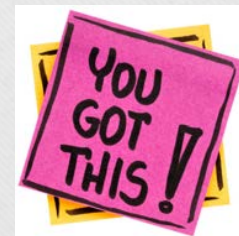
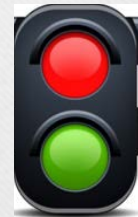
Montclair State University

| HAB Alert Levels  | Criteria   | Recommendation   |
|---|--|--|
| HAB Not Present   | HAB reported and investigated. No HAB present.   | None   |
| <p><b>WATCH</b></p> <p><i>Suspected or confirmed HAB with potential for allergenic and irritative health effects</i></p>  | <p>Suspected HAB based on field survey</p> <p><b>OR</b></p> <p>Confirmed cell counts <math>\geq 20k - \leq 80k</math> cells/mL</p> <p><b>OR</b></p> <p>No known toxins above public health thresholds</p>  | <p><b>Public Bathing Beaches Open</b></p> <p>Waterbody Accessible:</p> <p>Use caution during <b>primary contact (e.g. swimming) and secondary (e.g. non-contact boating)</b> activities</p> <p>Do not ingest water (people/pets/livestock)</p> <p>Do not consume fish</p> <p>An <i>Alert</i> is initiated at beaches if cell counts are 40K to &lt; 80K.</p> <p>An <i>Alert</i> begins actions to monitor the beach more frequently due to increasing potential for toxin production and to ensure the HAB has not elevated to a higher risk Tier.</p> |
| <p><b>ADVISORY</b></p> <p><i>Confirmed HAB with moderate risk of adverse health effects and increased potential for toxins above public health thresholds</i></p> | <p>Lab testing for toxins</p> <p>Microcystins: <math>\geq 2 \mu\text{g/L}</math></p> <p>Cylindrospermopsin: <math>\geq 5 \mu\text{g/L}</math></p> <p>Anatoxin-a <math>\geq 15 \mu\text{g/L}</math></p> <p>Saxitoxin-a <math>\geq 0.6 \mu\text{g/L}</math></p> <p><b>OR</b></p> <p>Confirmed cell counts <math>\geq 80k</math> ug/L</p> | <p><b>Public Bathing Beaches Closed</b></p> <p>Waterbody Remains Accessible:</p> <p>Avoid primary contact recreation</p> <p>Use caution for secondary contact recreation</p> <p>Do not ingest water (people/pets/livestock)</p> <p>Do not consume fish</p>   |
| <p><b>WARNING</b></p> <p><i>Confirmed HAB with high risk of adverse health effects due to high toxin levels</i></p>   | <p>Toxin (microcystin)</p> <p><math>\geq 20 - \leq 2000 \mu\text{g/L}</math></p>   | <p><b>Public Bathing Beaches Closed</b></p> <p>Cautions as above</p> <p>May recommend against secondary contact recreation</p>   |
| <p><b>DANGER</b></p> <p><i>Confirmed HAB with very high risk of adverse health effects due to very high toxin levels</i></p>                                      | <p>Toxin (microcystin)</p> <p><math>\geq 2000 \mu\text{g/L}</math></p>   | <p><b>Public Bathing Beaches Closed</b></p> <p>Cautions as above.</p> <p>Possible closure of all or portions of waterbody and possible restrictions of access to the shoreline.</p>  |

# Objectives

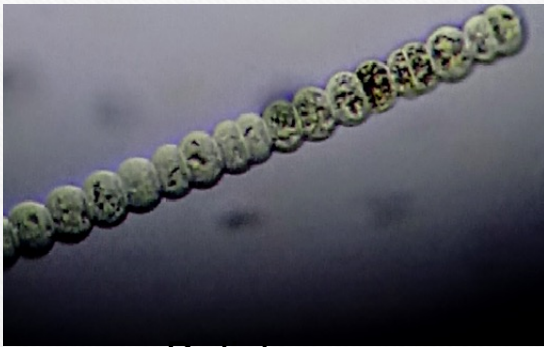
Field measurements, laboratory measurements and innovative technologies to assess the cyanobacterial cell density and cyanotoxin concentrations

- Data for HAB Management Decision
- Professional Analysis vs. Volunteers for Data Collection

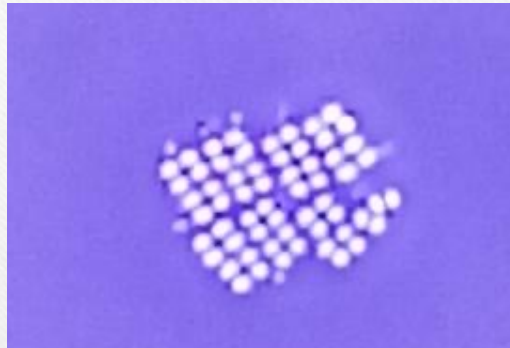


# Cyanobacteria

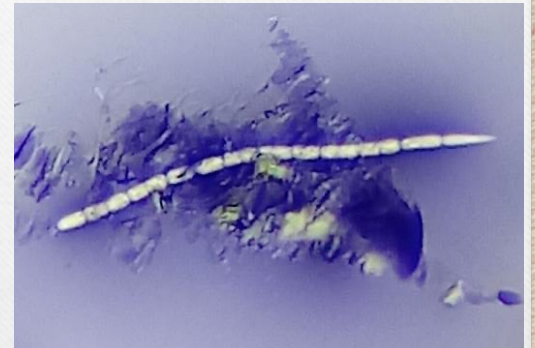
Comes in all shapes, Sizes & Colors



*Dolichospermum*



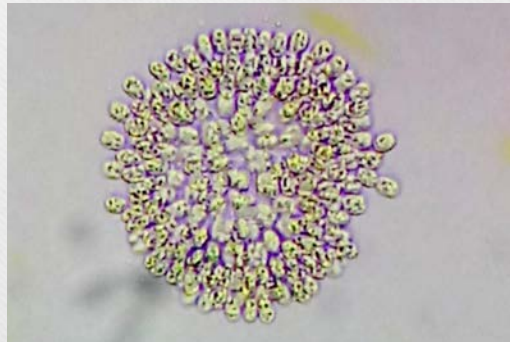
*Merismopedia*



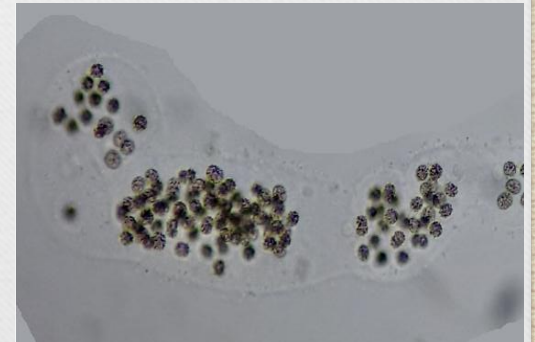
*Raphidiopsis*



*Oscillatoria*



*Woronichinia*



*Microsystis*

# Methods for Cyanobacteria

VISUAL ASSESSMENT

JAR & STICK TESTS

PIGMENTS

REMOTE SENSING

MICROSCOPY

IMAGING FLOW CYTOMETER

GENETIC ANALYSIS

Simple -  
minimal training & tools



Complex -  
specific training and equipment

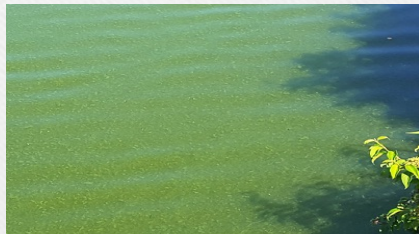
# Visual Assessment



Foam



Foam



Strike



Strike



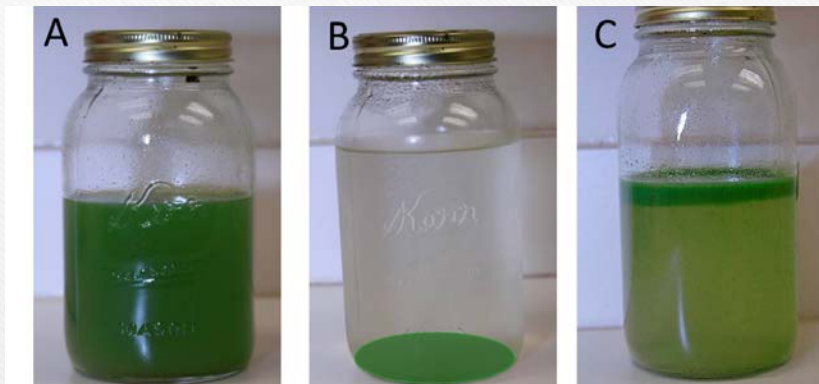
Scum

- No equipment needed
- Minimal training
- \$
- Qualitative



# Jar Test

for Planktonic cyanobacteria



(A): a well-mixed sample. Allow it to rest for 15-30 minutes.

(B): Other algae and sediments sink to the bottom; settled material not likely to be cyanobacteria.

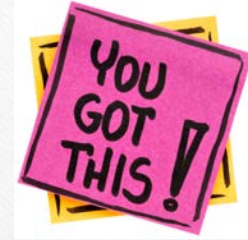
(C): Cyanobacteria typically float to the surface; floating material likely to be cyanobacteria

- No equipment needed
- Minimal training
- \$
- Qualitative



# Stick Test

Planktonic Cyanobacteria or filamentous green algae?



Filamentous green algae has hair-like appearance and can be picked up by a stick.

- No equipment needed
- Minimal training
- \$
- Qualitative

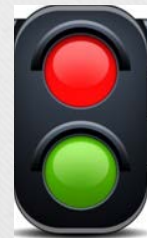


Photo credit: ITRC

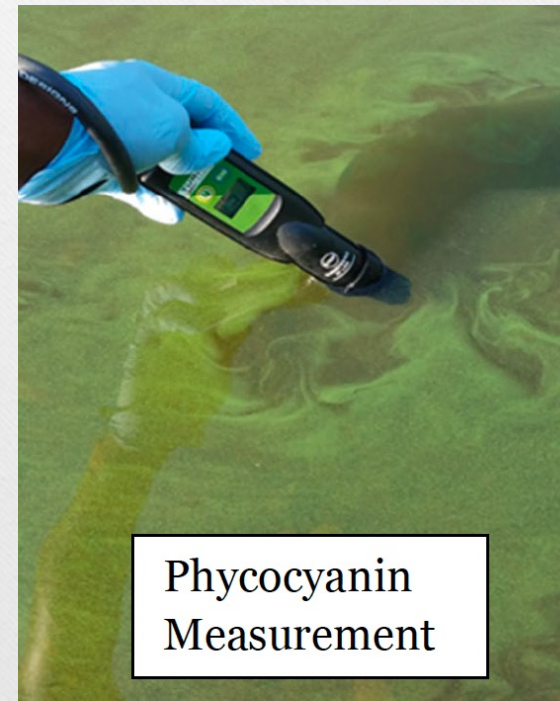
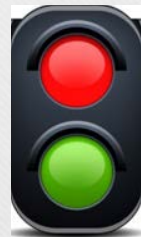


# Pigment

Phycocyanins are photosynthetic pigments found in freshwater cyanobacteria



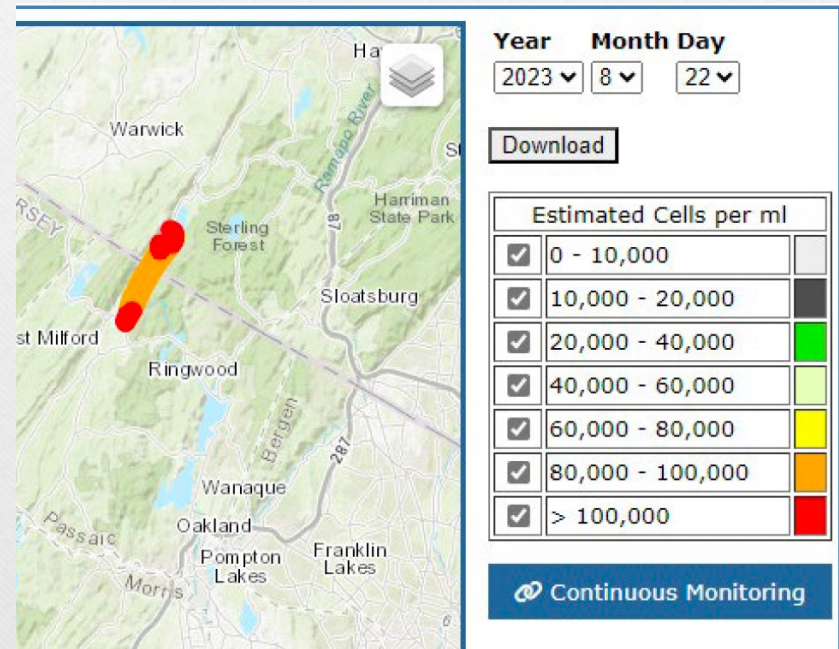
- <13
- Need a handheld phycocyanin meter
- NJDEP meter loan program
- Minimal training
- \$
- Quantitative



# Remote Sensing

measures reflected light energy

- Remote sensing of phycocyanin pigments to estimate cell density
- NJDEP's flight surveillance
- \$ \$
- Qualitative/Quantitative



# Microscopy

- A microscope & a counting chamber
- Identify cyanobacteria based on its morphological features
- Count cells to determine cyanobacterial cell density, cells/mL
- Intermediate training
- \$ \$
- Qualitative/quantitative

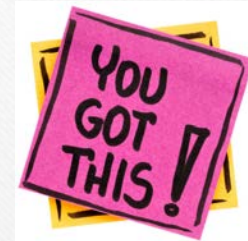
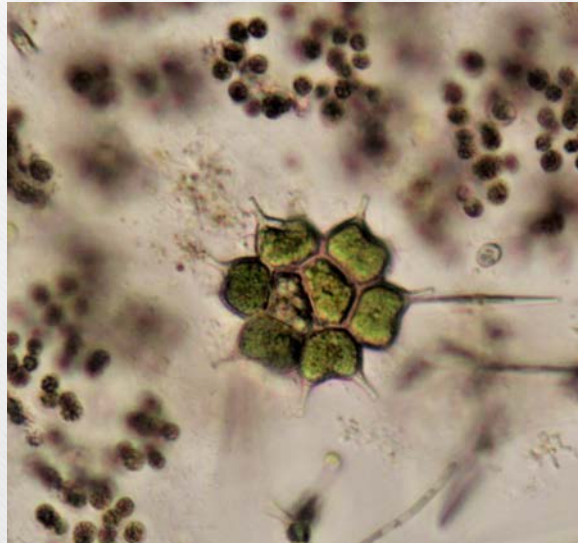


Photo credit: NJDEP

# Types of algae that are common in the Freshwater phytoplankton communities

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- Cyanobacteria
- Green algae
- Diatoms
- Golden algae
- Cryptomonads
- Dinoflagellates
- Euglenoids

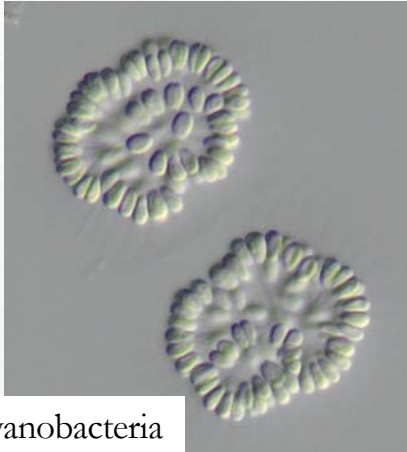


Green Algae *Pediastrum*

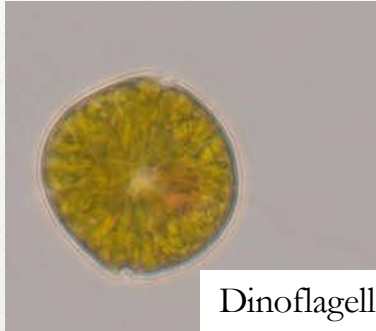


Golden Algae *Dinobryon*

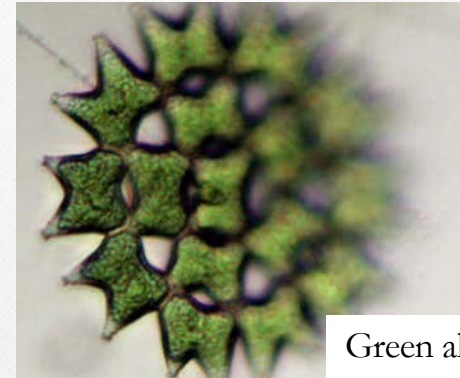
## Morphologically Very Diverse



Cyanobacteria



Dinoflagellate



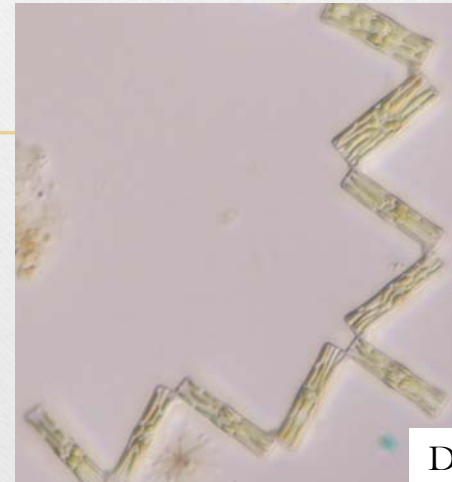
Green algae



Cryptomonad



Golden algae



Diatoms

Cyanobacteria: lack flagella; unicellular, colonial, or filamentous; photosynthetic pigments not contained in chloroplasts; cell contents blue-green, greyish-blue, dark brown, or otherwise colored, but not grass green.

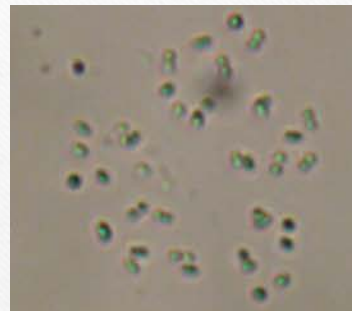
# Common Freshwater Cyanobacteria of New Jersey



*Synechococcus*



*Dolichospermum*



*Aphanocapsa*



*Microcystis*



*Chroococcus*



*Aphanizomenon*



*Raphidiopsis*



*Pseudanabaena*



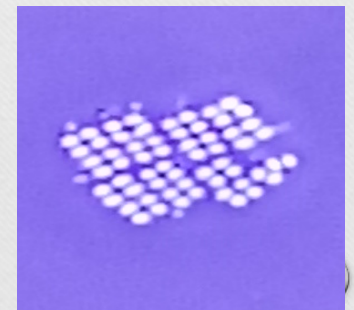
*Planktolyngbya*



*Limnoraphis*

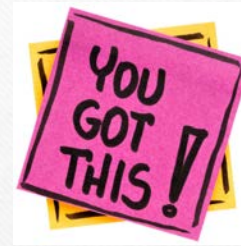


*Aphanothece*



*Merismopedia*

# Microscopy



- Recommended method to identify cyanobacteria and quantify cell density
- Identification down to the genus level whenever possible
- Interstate Technology and Regulatory Council's [A visual guide to common harmful cyanobacteria](#)
- MSU's [Guide to freshwater algae of New Jersey](#) and [Visual Guide to Cyanobacteria of New Jersey](#)

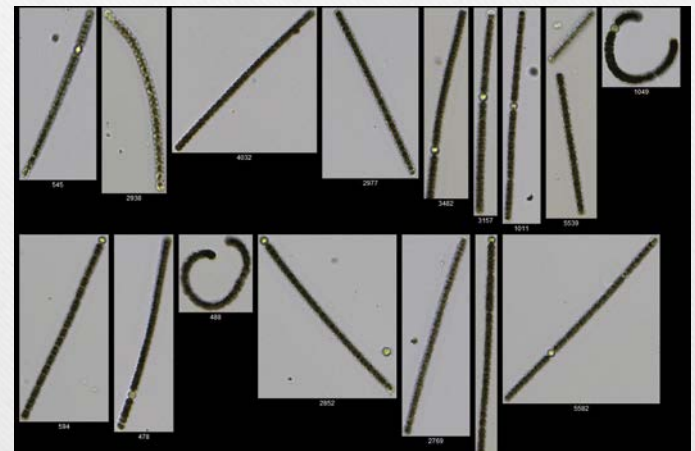


# Imaging Flow Cytometer

Photograph particles as a sample flows through



- Semi-automatic
- Identify and enumerate algae
- Identify algae based on its morphological features
- Measure cell size and estimate biovolume
- Require specific training & an IFC
- \$ \$ \$
- Quantitative



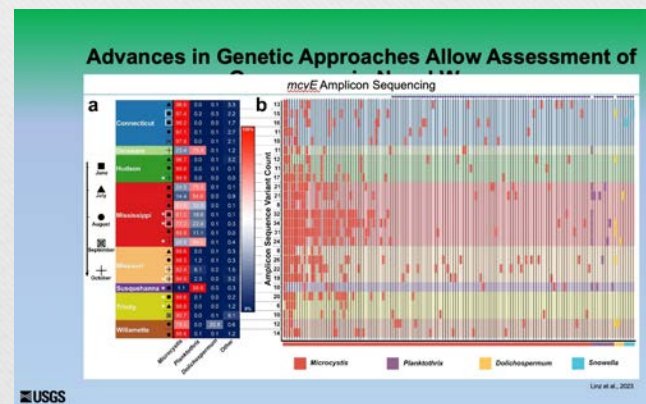
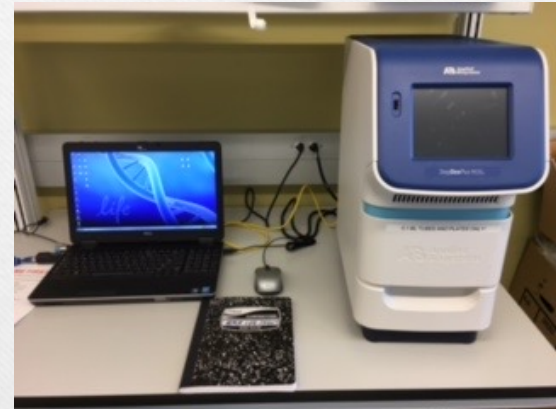
| Summary Stats      | Mean     | Min   | Max       | StdDev   |
|--------------------|----------|-------|-----------|----------|
| Area (ABD)         | 328.93   | 2.03  | 12231.73  | 787.09   |
| Biovolume (Sphere) | 10064.02 | 2.17  | 1.018e+06 | 42435.90 |
| Diameter (ABD)     | 15.13    | 1.61  | 124.80    | 13.78    |
| Length             | 43.24    | 10.66 | 856.64    | 67.07    |
| Volume (ABD)       | 10064.02 | 2.17  | 1.018e+06 | 42435.90 |
| Width              | 11.44    | 1.10  | 208.37    | 10.30    |



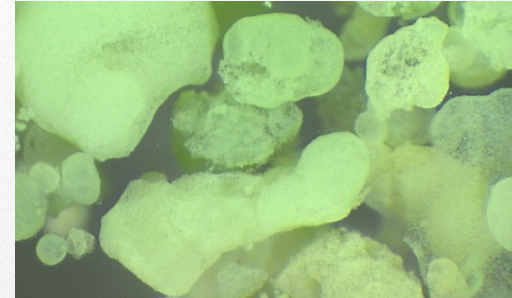


# Genetic Analysis

- PCR, qPCR, shotgun sequencing, metabarcoding
- Require sending samples to a lab
- Expert training & specialized equipment
- \$\$\$
- Quantitative



# Cyanotoxins



- Cyanobacteria can produce cyanotoxins and other irritants that cause serious health effects in people and animals.
- Multiple toxin types may be produced by single species
- Toxins generally held within the cyanobacterial cell.
- Toxins are released to water as the cell dies/lyses
  - when the bloom naturally decays
  - when a chemical treatment is applied
  - when cells are ingested

# Methods for Cyanotoxins

STRIP TEST

ENZYME-LINKED IMMUNOSORBENT ASSAY

CHROMATOGRAPHY

GENETIC ANALYSIS

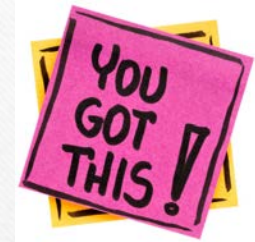
Simple -  
minimal training & tools



Complex –  
specific training and equipment

# Strip Test

Use a test kit to rapidly screen



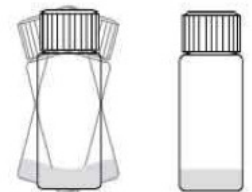
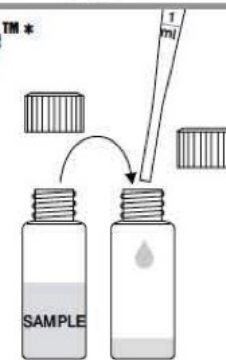
## 1. Collect Sample



Collect 1 to 2 mL of sample.

## 2. Transfer/QuikLyse™\*

Using the graduated pipette provided, transfer 1 mL of SAMPLE to the lysis vial containing the dried lysis reagent.



2 min.

8 min.

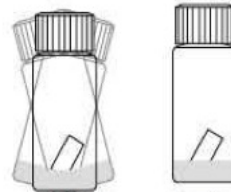
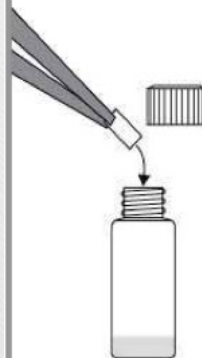
Cap and shake for 2 minutes.

Let rest for 8 minutes.

\*Patent Pending

## 3. Add Reagent Paper/QuikLyse™\*

Using the forceps provided, add 1 reagent paper to the lysis vial.



2 min.

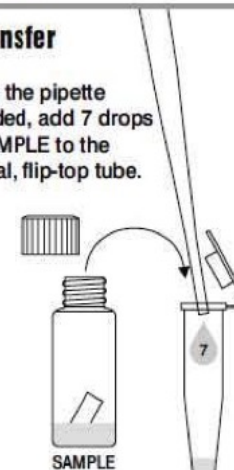
8 min.

Cap and shake for 2 minutes.

Let rest for 8 minutes.

## 4. Transfer

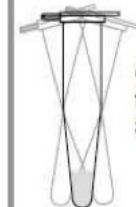
Using the pipette provided, add 7 drops of SAMPLE to the conical, flip-top tube.



SAMPLE

(The conical, flip-top tube contains dried reagents.)

## 5. Shake and incubate

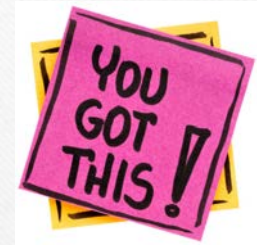


Close the conical, flip-top tube and shake for 30 seconds.



(Dried reagents will dissolve, turning the sample purple.)

# Strip Test/Dip Sticks



## 6. Test

Insert test strip into conical, flip-top tube with arrow pointing down. (sample pad down).

Incubate for 10 minutes.

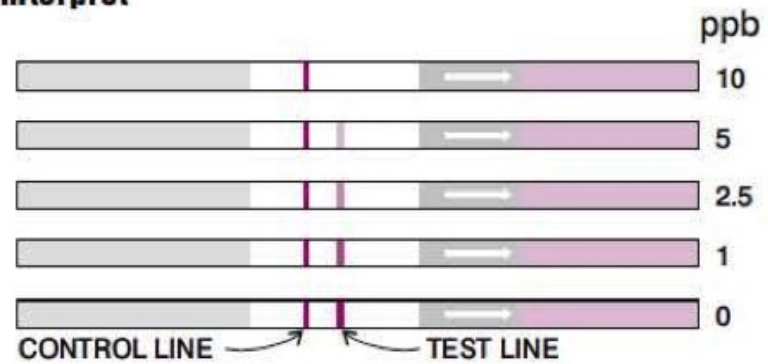


## 7. Dry

Remove test strip. Lay flat and allow to continue developing for 5 minutes.



## 8. Interpret



### INTERPRET TEST

| CONTROL LINE            | TEST LINE                            | INTERPRETATION       |
|-------------------------|--------------------------------------|----------------------|
| NO CONTROL LINE PRESENT | NO TEST LINE PRESENT                 | INVALID RESULT       |
| CONTROL LINE PRESENT    | NO TEST LINE PRESENT                 | >10 ppb              |
| CONTROL LINE PRESENT    | MODERATE INTENSITY TEST LINE PRESENT | BETWEEN 0 AND 10 ppb |

# Strip Test/Dip Sticks



- Minimal training
- Strips can be difficult to interpret.
- Possible human errors
- Possible interference from unknown compounds/contaminants
- \$ \$
- Semi-quantitative



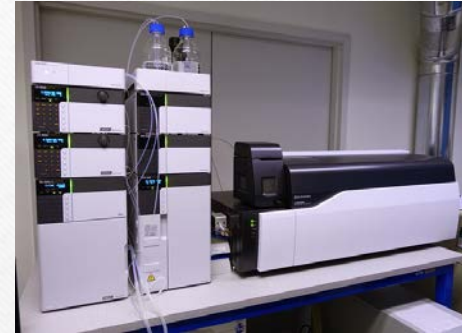
# Enzyme-linked Immunosorbent Assay

ELISA is a type of biological assay that uses reactive proteins (antibodies) to detect and quantify cyanotoxins.

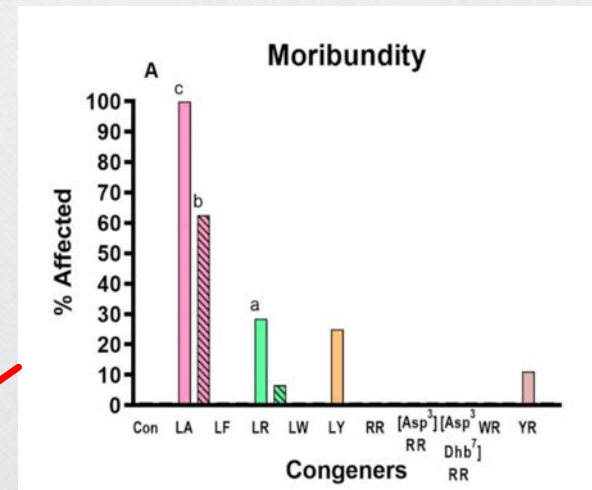
- Specific training & equipment
- \$ \$
- Quantitative
- Certified analysis (Microcystins; EPA 546)
- Recommended method to quantify cyanotoxin



# Chromatography



- Liquid Chromatography-Mass spectrometry (LC-MS/MS) separates compounds and compares it to known standards.
- Quantify individual congeners, i.e. MC-LR, MC-RR, MC-LA
- Each congener has its unique health impacts
- Expert training & specialized equipment
- \$ \$ \$
- Quantitative
- Certified analysis (EPA 544 & 545)



Chernoff et.al., 2020

doi: [10.3390/toxins12060403](https://doi.org/10.3390/toxins12060403)



# Genetic Analysis

- qPCR
- Target genes that encode the cyanotoxin
- Testing kit are also available
- Expert training & specialized equipment
- \$ \$ \$
- Quantitative



Phytoxigene CyanD'Tec



# Methods for Cyanobacteria

VISUAL ASSESSMENT ✨

JAR & STICK TESTS ✨

PIGMENTS ✨

REMOTE SENSING

MICROSCOPY ✨

IMAGING FLOW CYTOMETER

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# Methods for Cyanotoxins

STRIP TEST ✨

ENZYME-LINKED IMMUNOSORBENT ASSAY

CHROMATOGRAPHY

GENETIC ANALYSIS

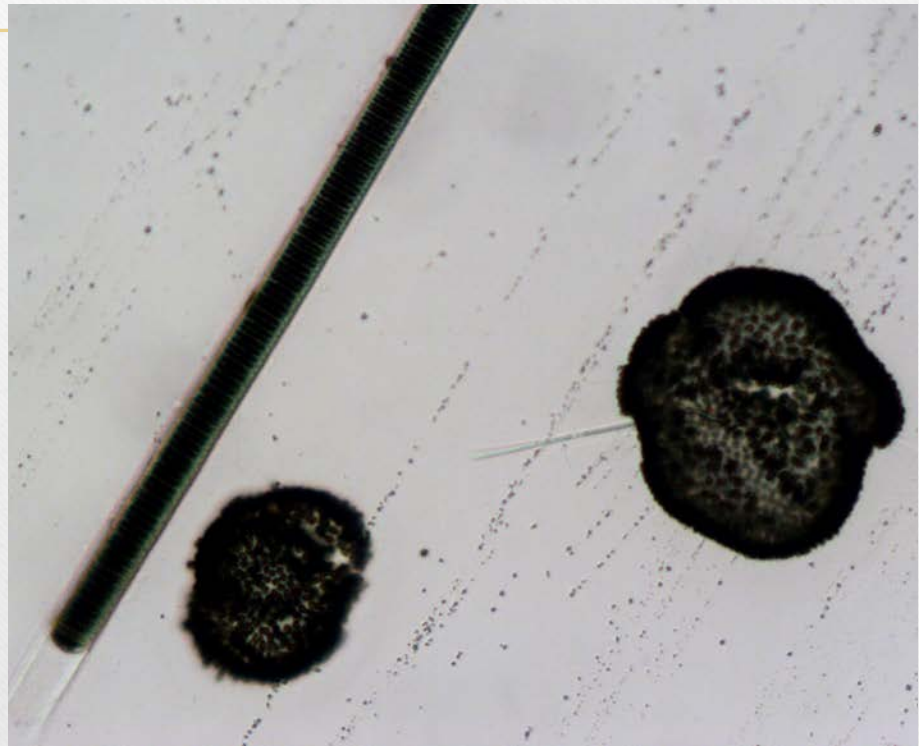
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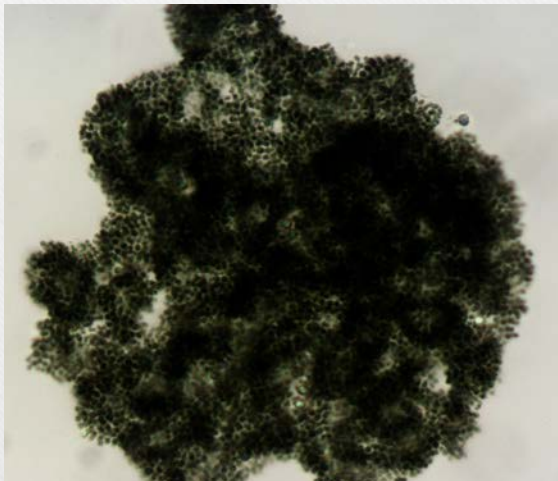
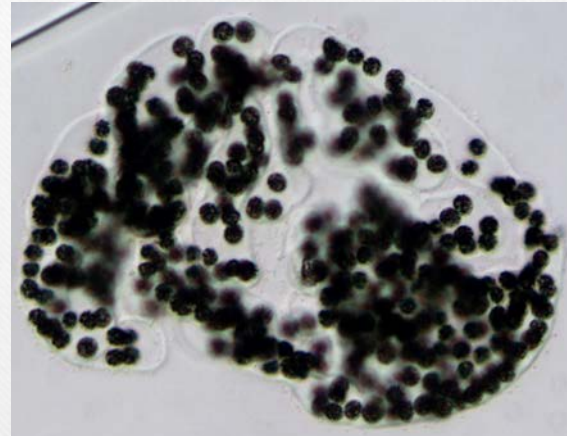
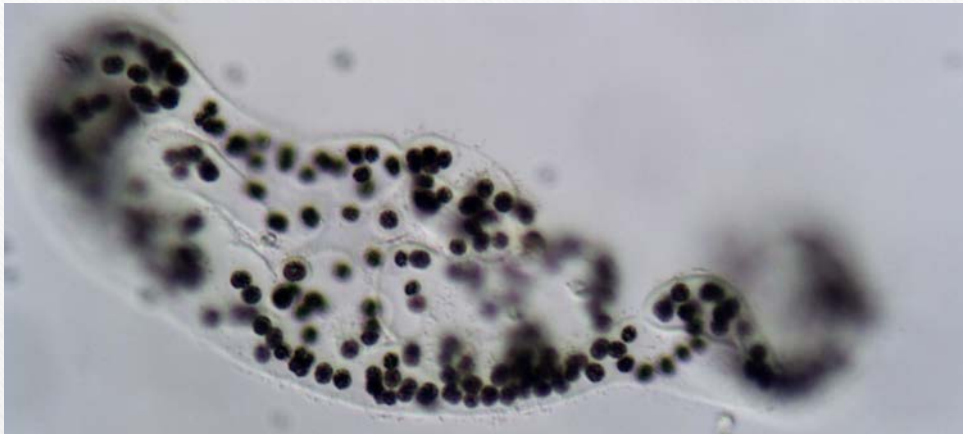
Complex –  
specific training and equipment

# Common Freshwater HAB-forming Cyanobacteria

- *Microcystis*
- *Dolichospermum*
- *Woronichinia*
- *Aphanizomenon*
- *Limnoraphis*
- *Cuspidothrix*
- *Planktothrix*
- *Raphidiopsis*



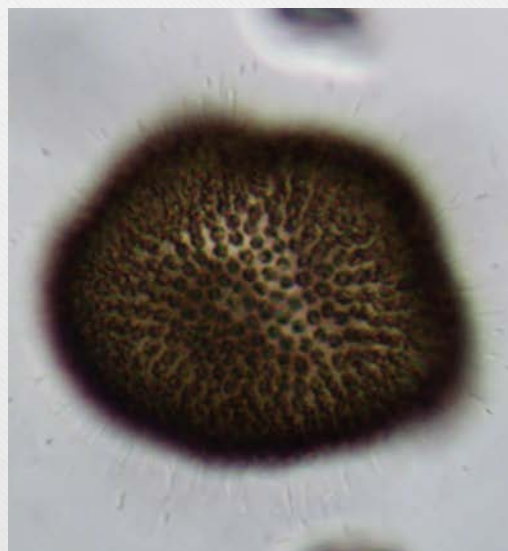
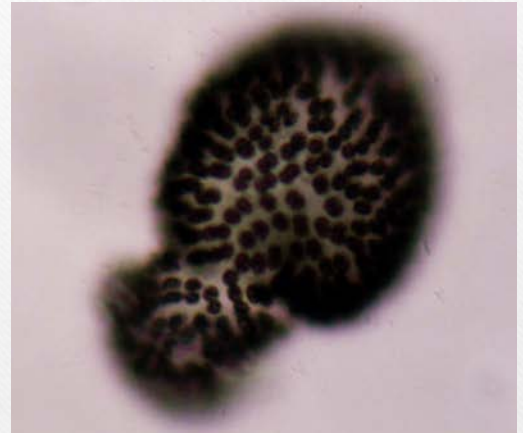
# *Microcystis*



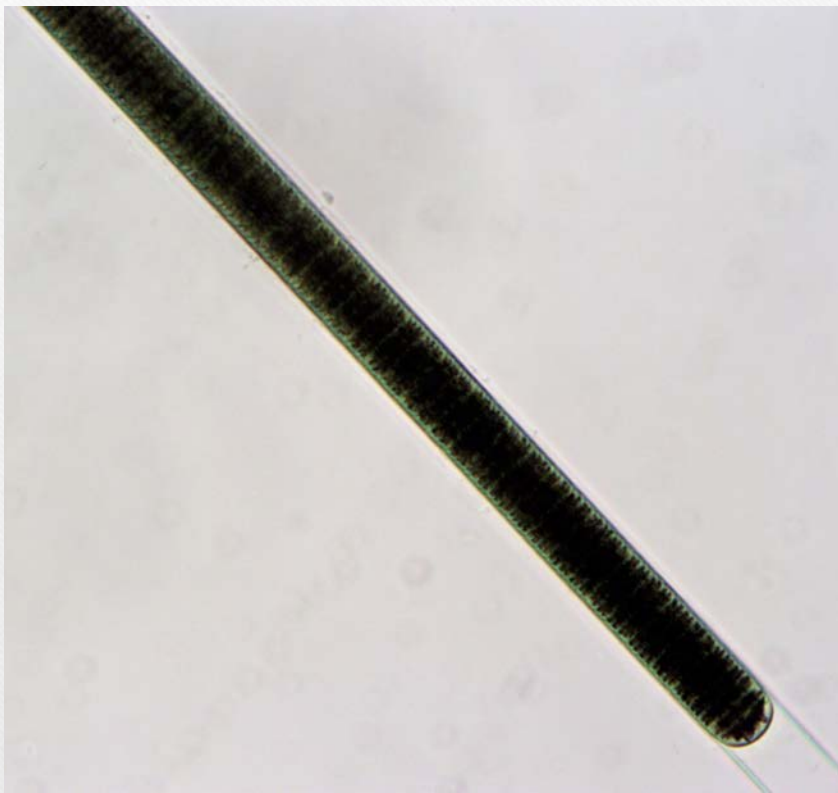
# *Dolichospermum*



*Woronichinia*



*Limnographis*

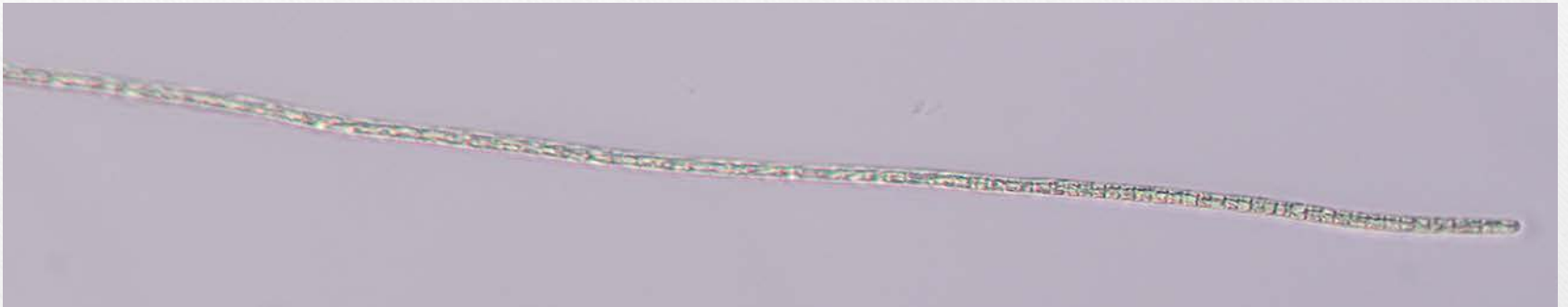


# *Aphanizomenon*





# *Planktothrix*





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