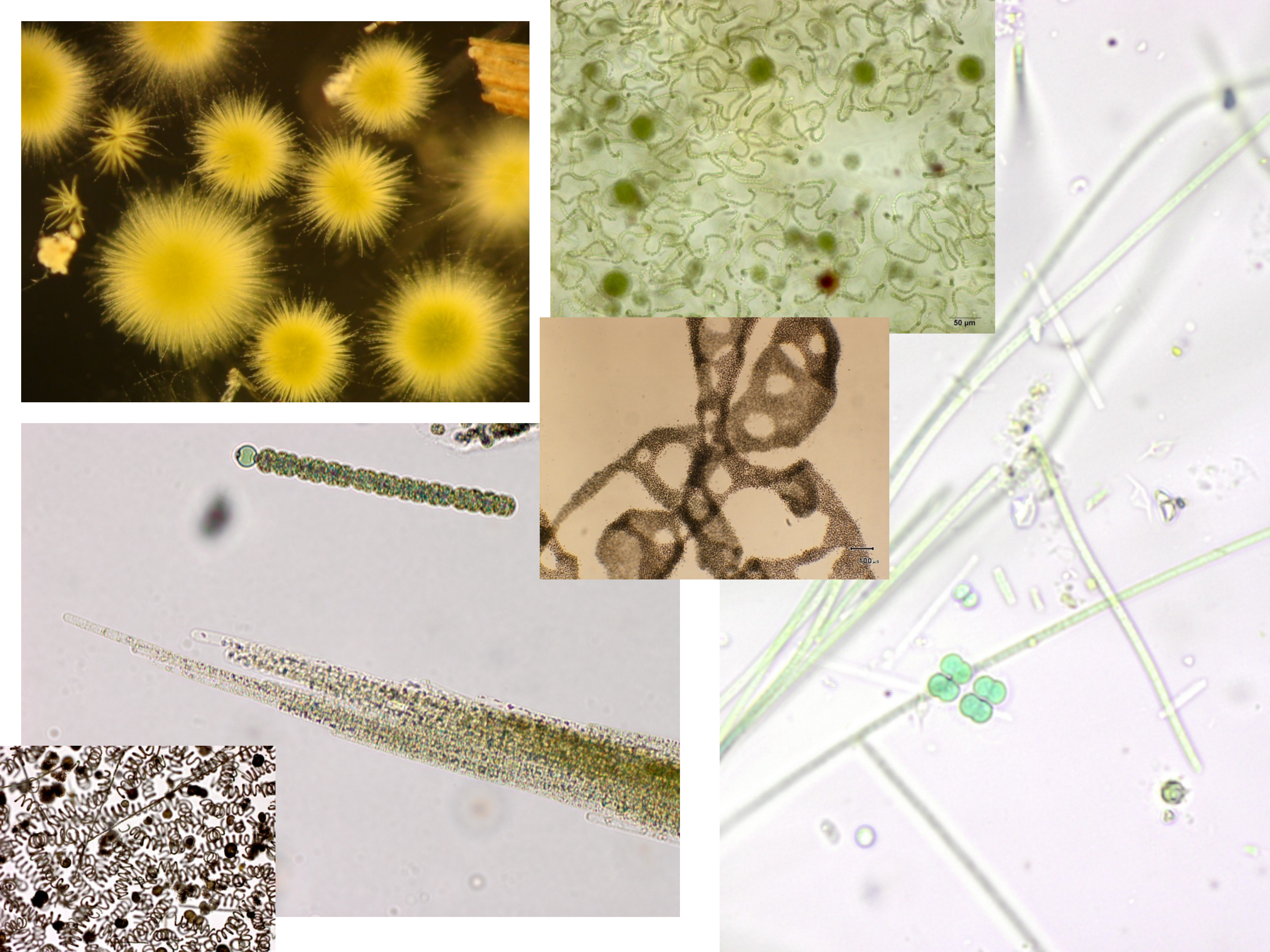
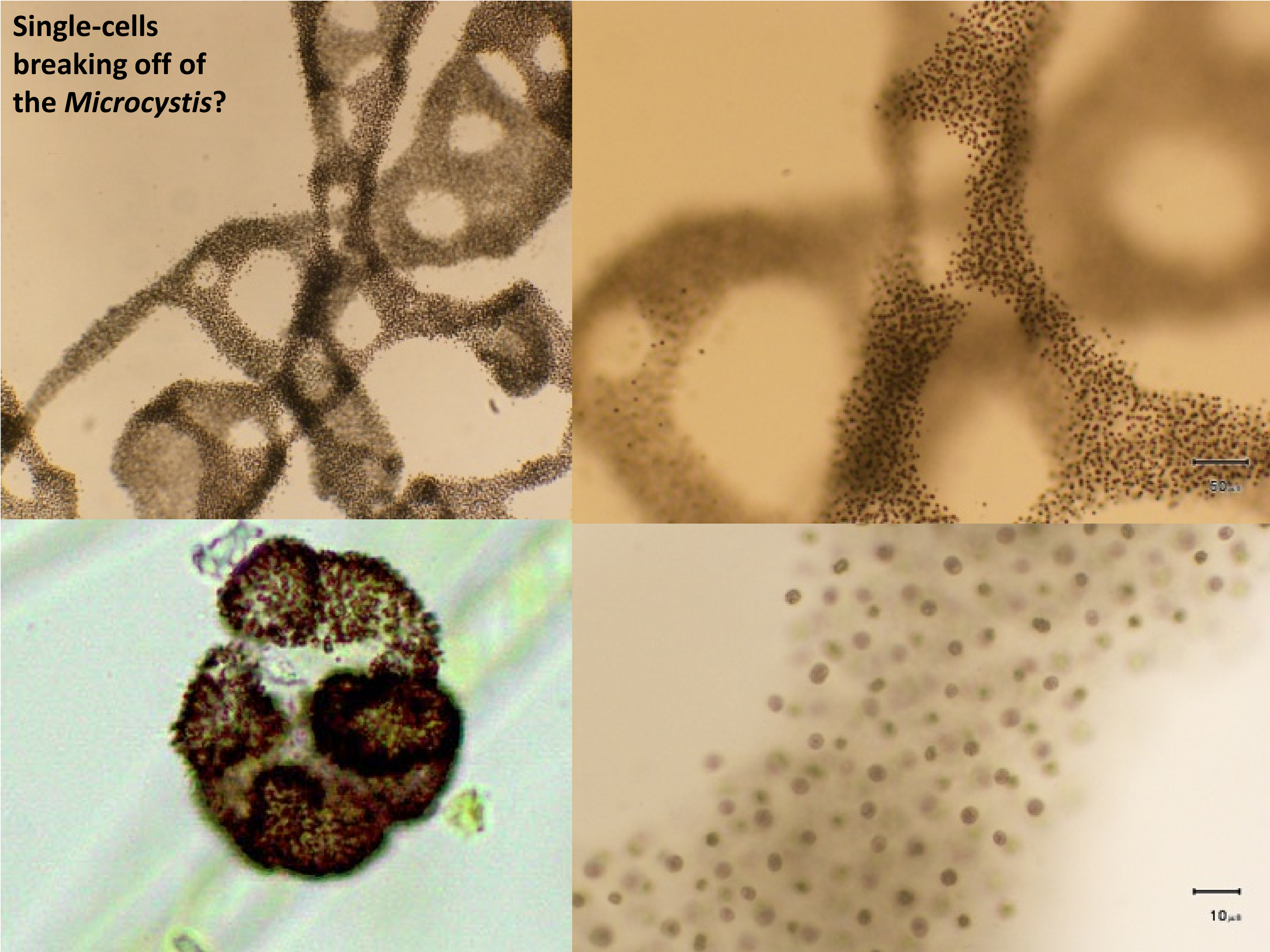
Amanda Murby
University of New Hampshire
Cyanobacteria Monitoring and Analysis Workshop
June 26, 2013

# Cyanobacteria
## Importance of Toxins and Size

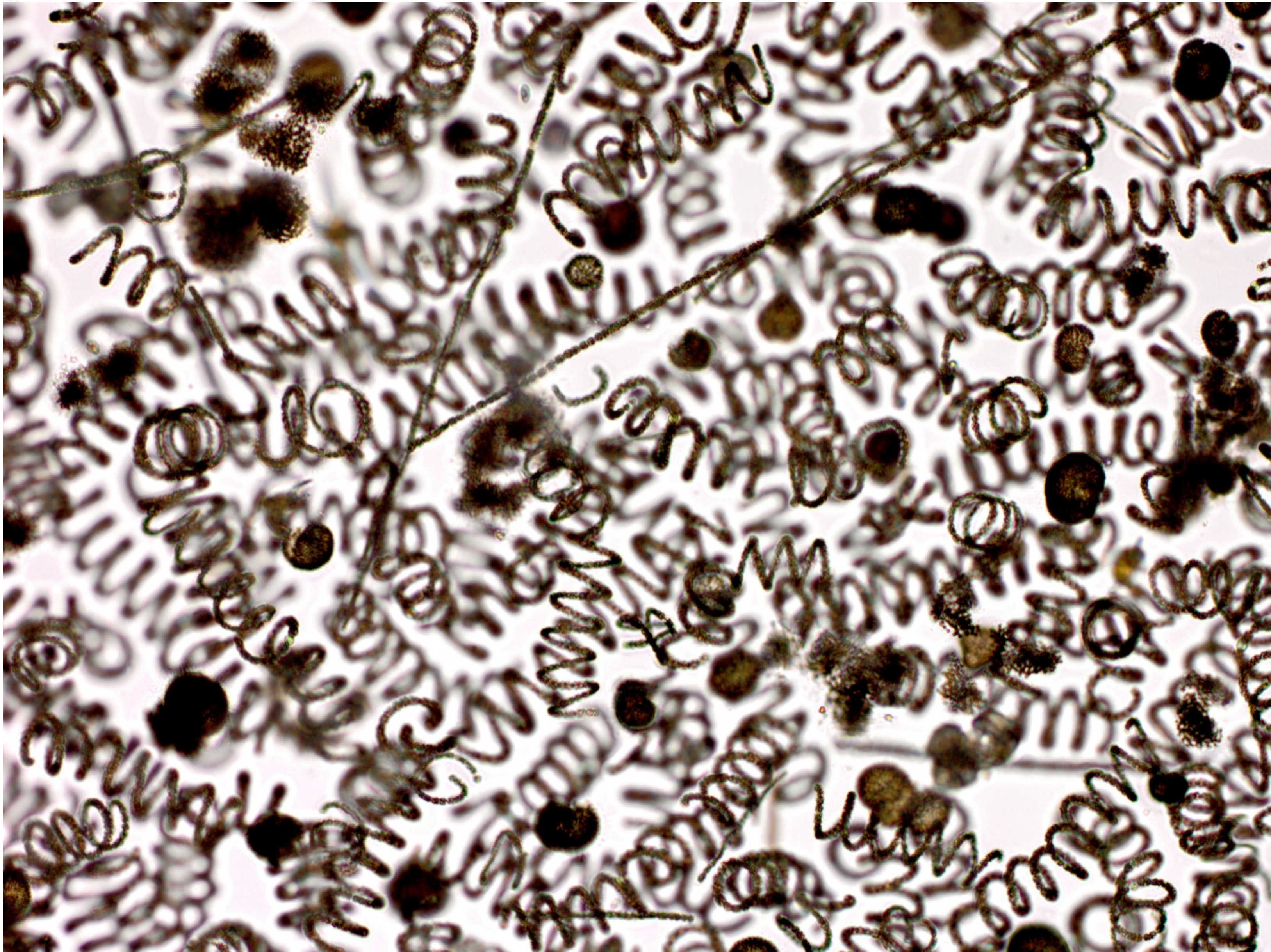

50 µm


Single-cells
breaking off of
the *Microcystis*?

_Aphanizomenon_

Cyanos to know…. "Annie, Fannie and Mike"…. What about "Pike"?

_Anabaena_ _Microcystis_ Picocyanobacteria

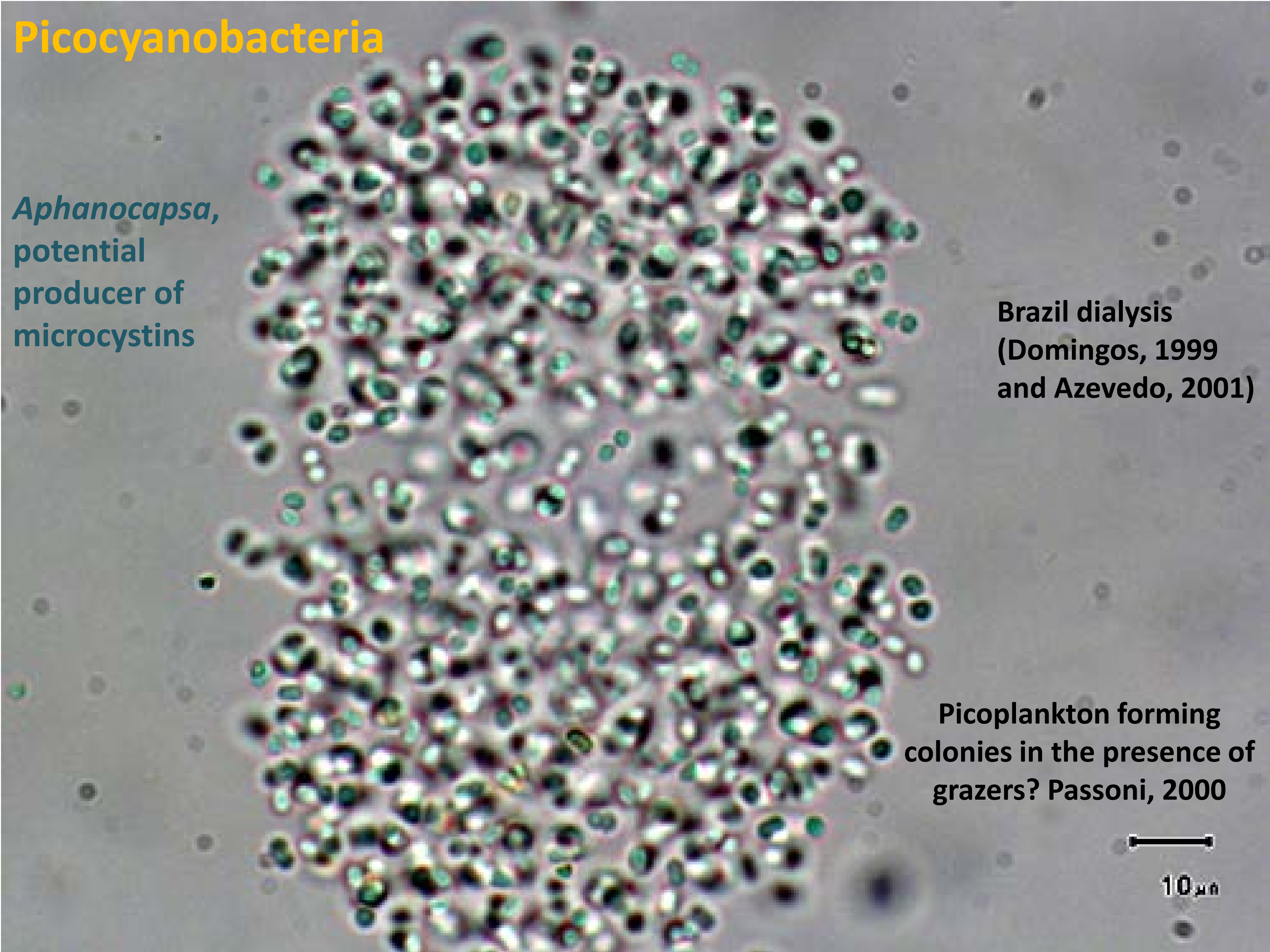


Lake Attitash

40x

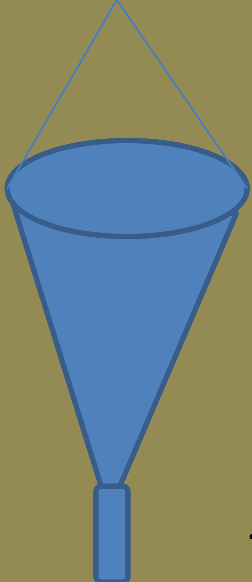
# Picocyanobacteria

*Aphanocapsa*, potential producer of microcystins




Brazil dialysis (Domingos, 1999 and Azevedo, 2001)

Picoplankton forming colonies in the presence of grazers? Passoni, 2000



# Plankton defined by size

WLW

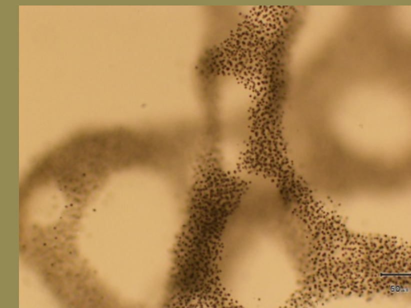
<50 um

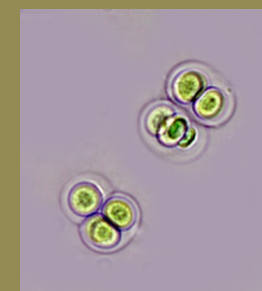
<2 um

<0.2 um

Whole lake water

- Net plankton (> 50 μm)
- Nanoplankton (2-50 μm)
- Picoplankton (0.2-2.0 μm)



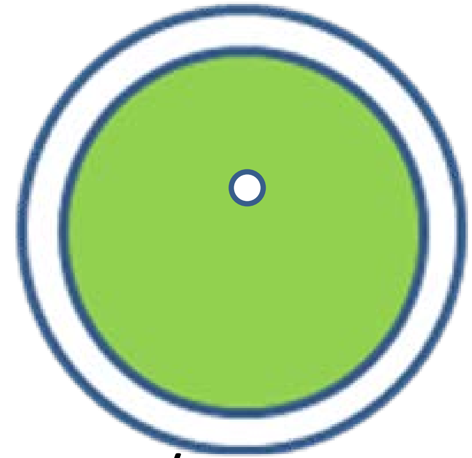


If picocyanobacteria (such as *Synechococcus* spp.) are **toxic**, then filtrates and filters from "fractionation" of whole lake water could be analyzed to target different sizes of cyanobacteria.

Filters and filtrates could be processed and analyzed for pigments and cyanotoxins.

Particulate versus dissolved…

# “Hole-punch”

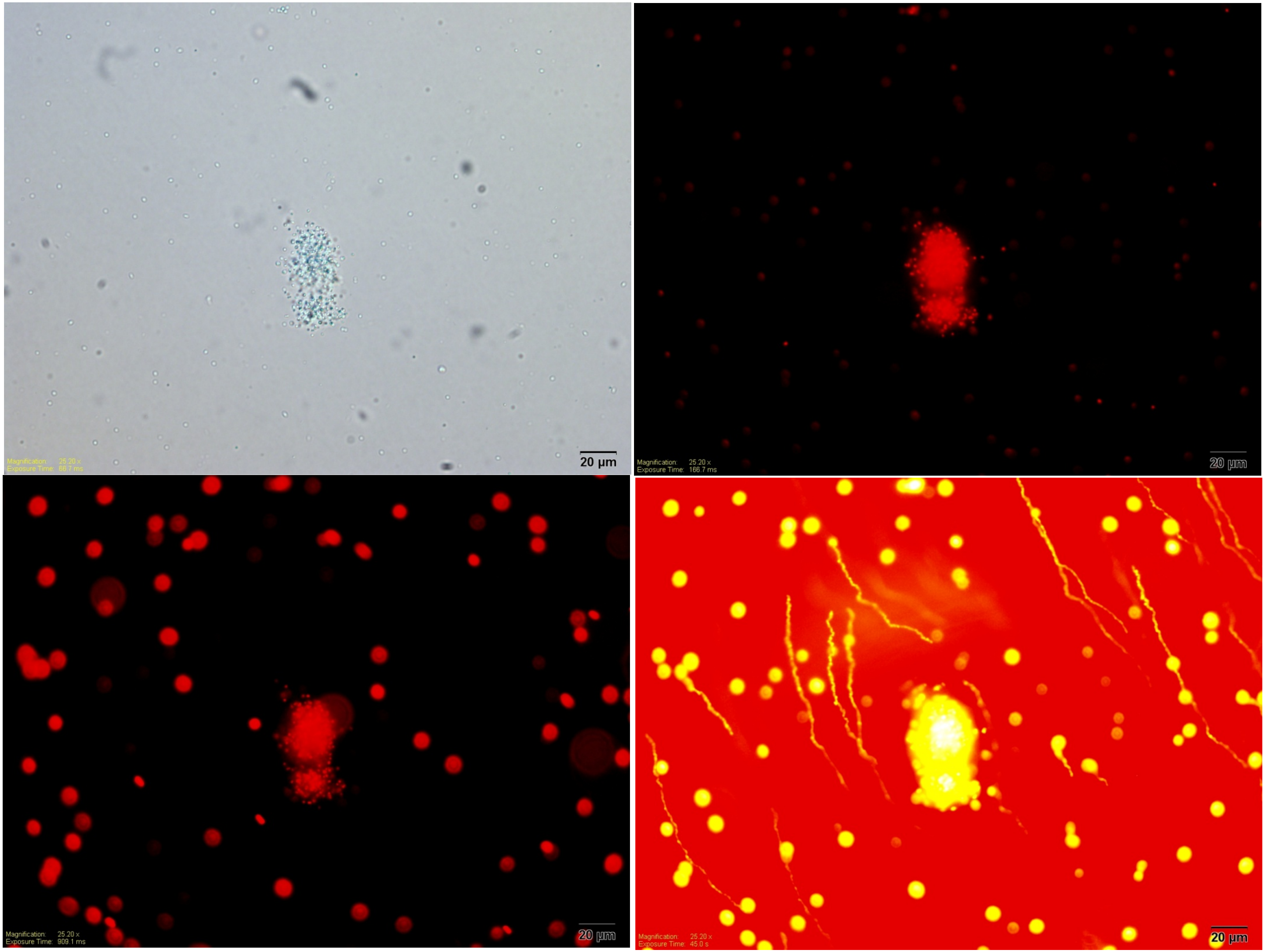


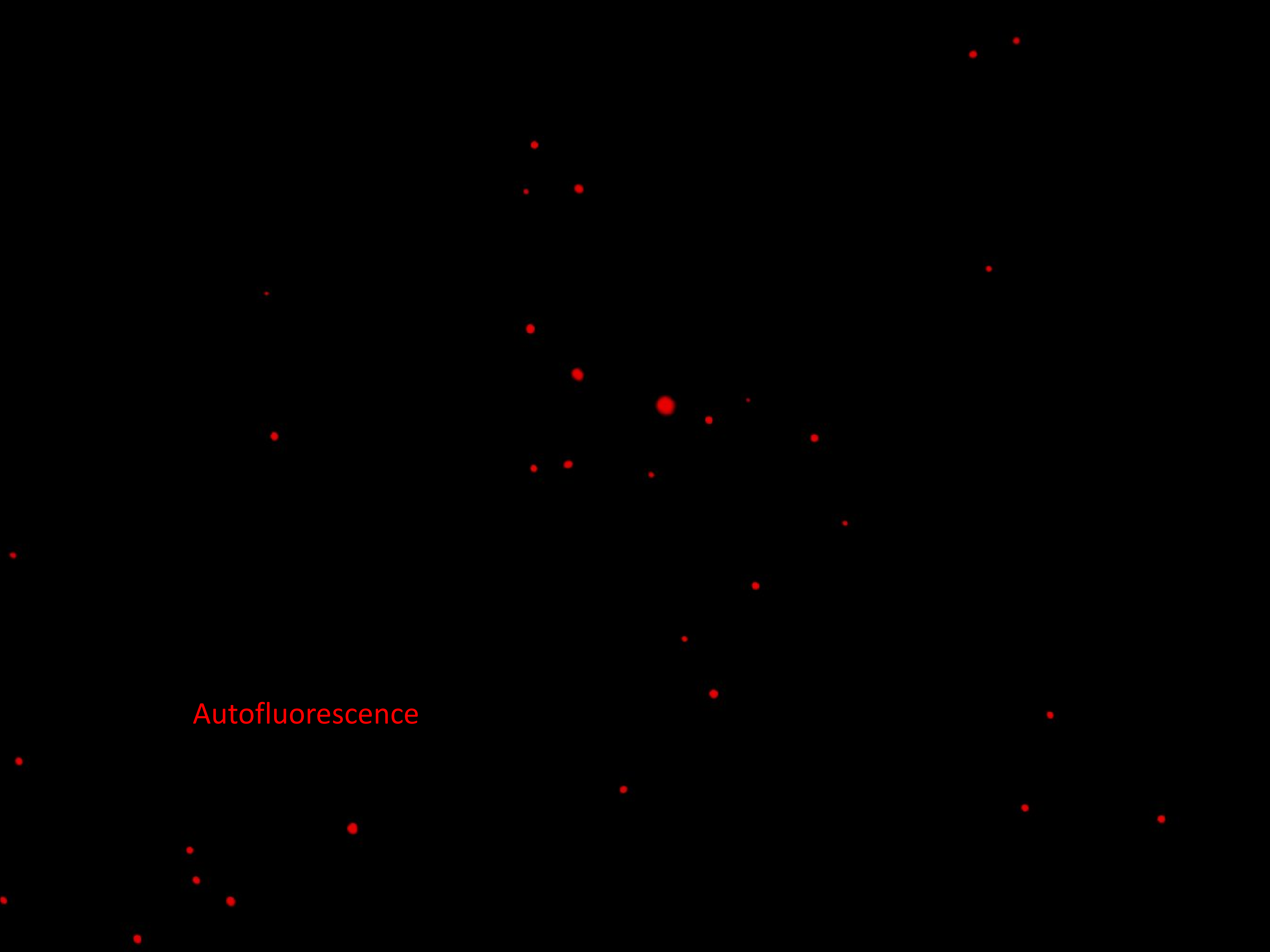
2007-2008 NELP lakes studied

- A potential method for monitoring cyanobacteria/ microcystins from already established methods (chlorophyll filtration <0.45 um).
- This study revealed that a high percentage of microcystins in New England Lake water were in the dissolved fraction (in the form of extracellular cyanotoxins).
- Extracellular toxins can occur due to natural senescence; due to light, grazers, and/or time of bloom degradation.
- Typically, extracellular MCs will persist in the water column longer than intracellular toxins

Picocyanobacteria are a more likely source of food for zooplankton than net colonies or filaments of cyanobacteria that are difficult to filter.

Are picocyanobacteria potentially toxic too?


Magnification: 25.20 x
Exposure Time: 66.7 ms
20 µm
Magnification: 25.20 x
Exposure Time: 166.7 ms
20 µm
Magnification: 25.20 x
Exposure Time: 909.1 ms
20 µm
Magnification: 25.20 x
Exposure Time: 45.0 s
20 µm


Autofluorescence

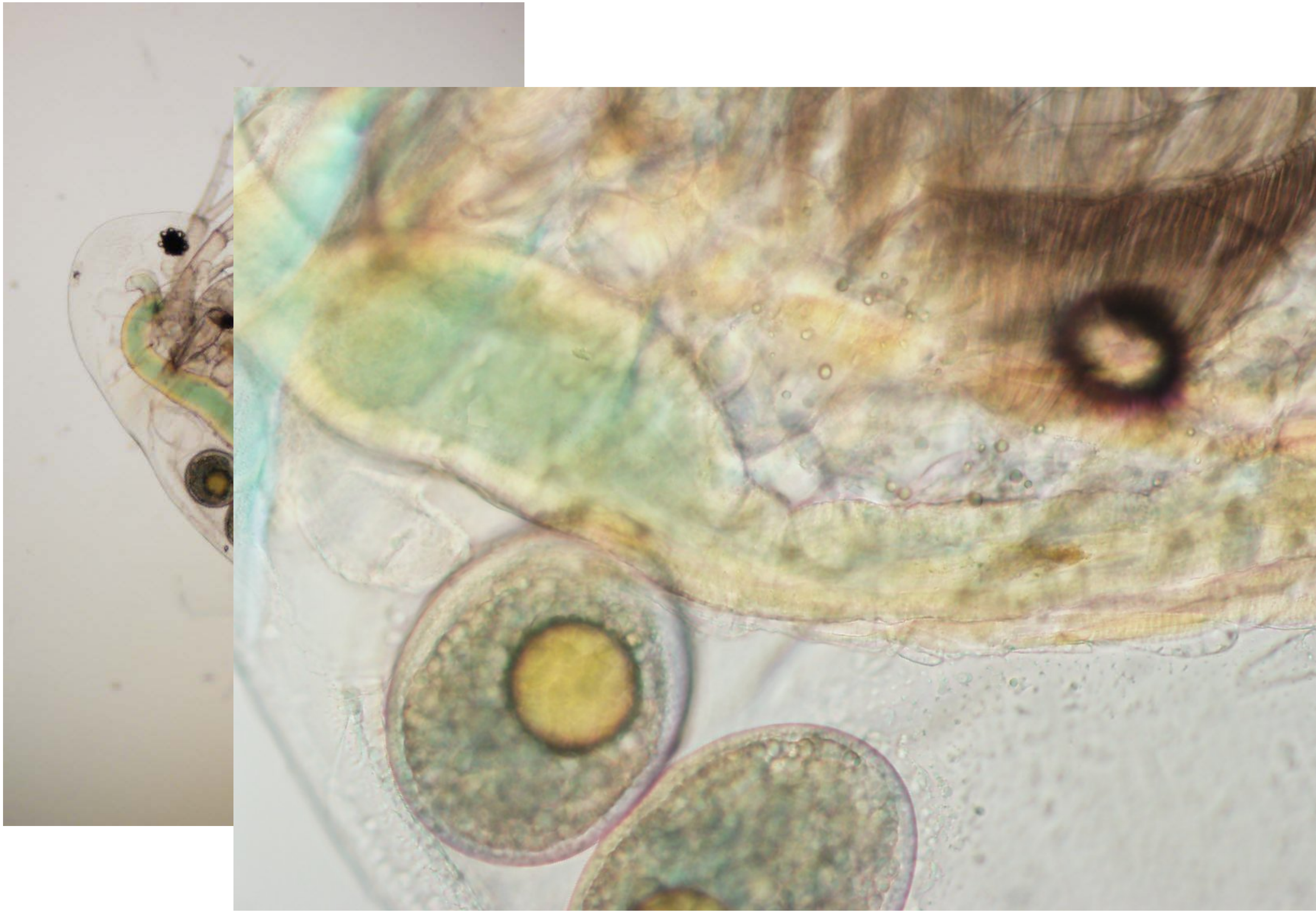
# Techniques in Epifluorescence

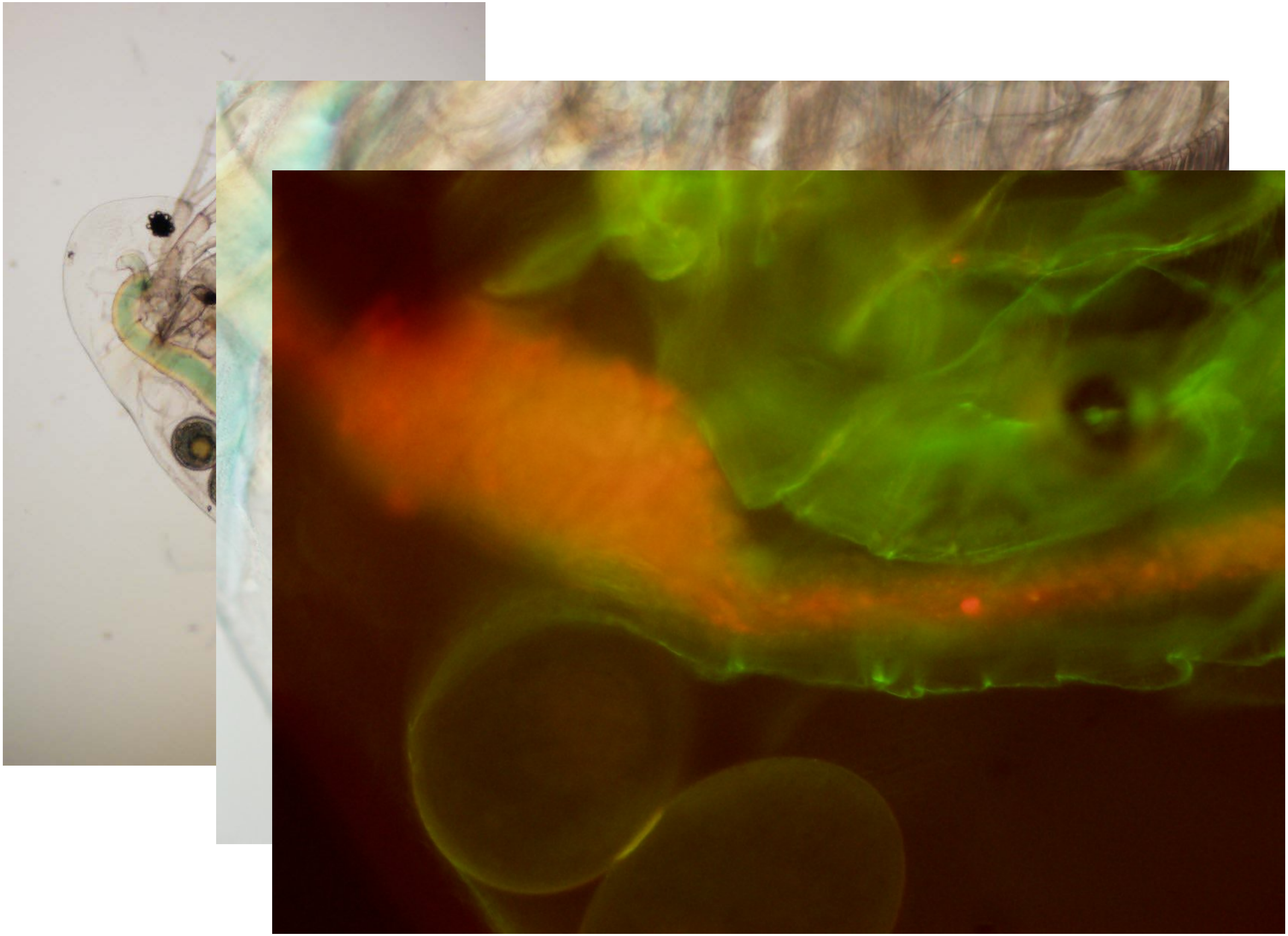
The microscope has two filter sets for epifluorescence; each includes an emitter, dichroic mirror and exciter:

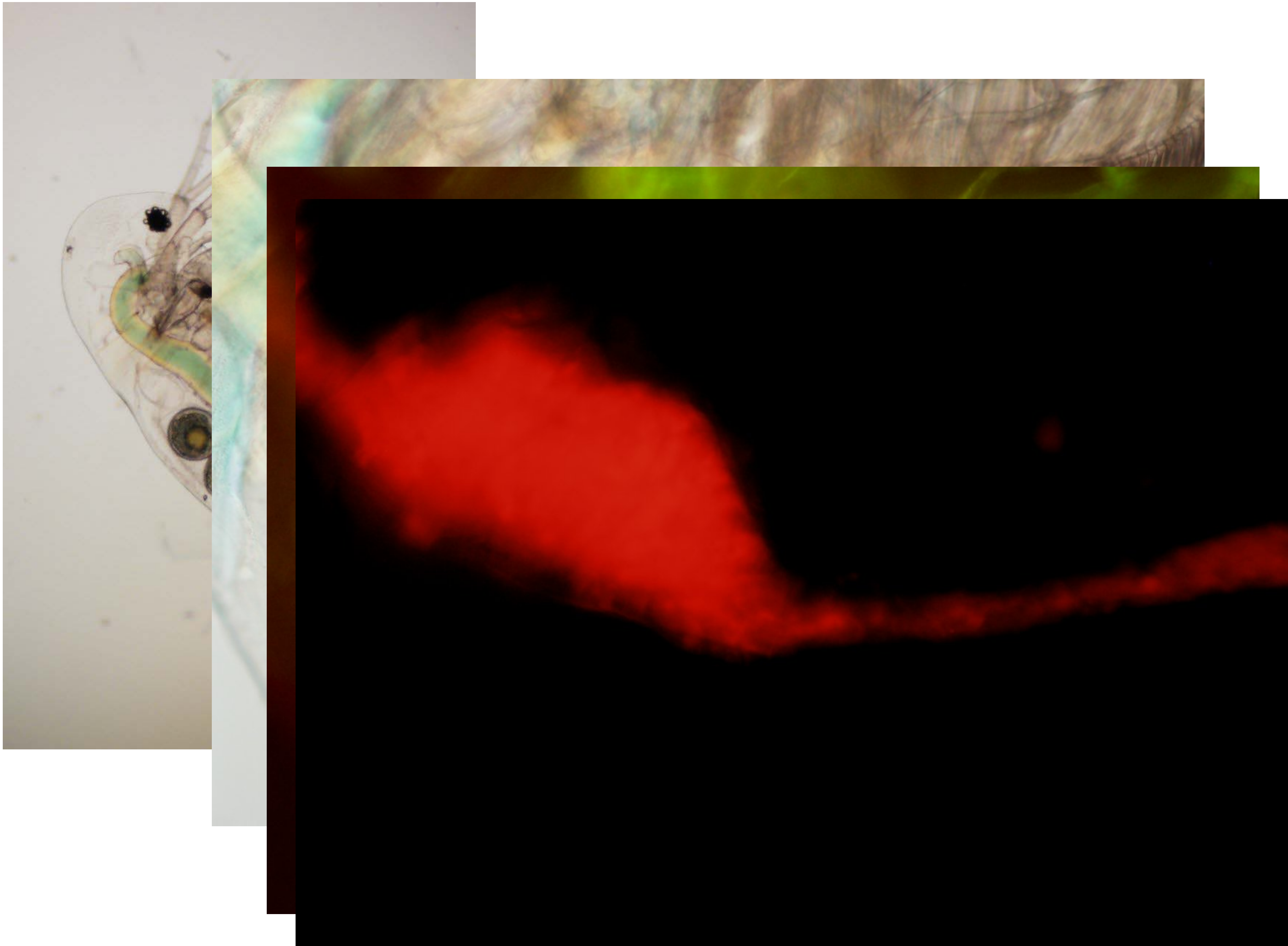
1.) The “green” cube excites at 435 nm and excites the “green window”, which includes a broader range of the emitted chlorophylls.

2.) The “PC” cube excites the cells with a wavelength of 572 and emits in the range of 605-630 nm.

These cubes were especially chosen to target the autofluorescence of photosynthetic picoplankton. Therefore, the first cube allows for viewing a wide range of chlorophyll and the second cube allows for viewing those with phycobillin pigments, phycocyanin and phycoerythrin.



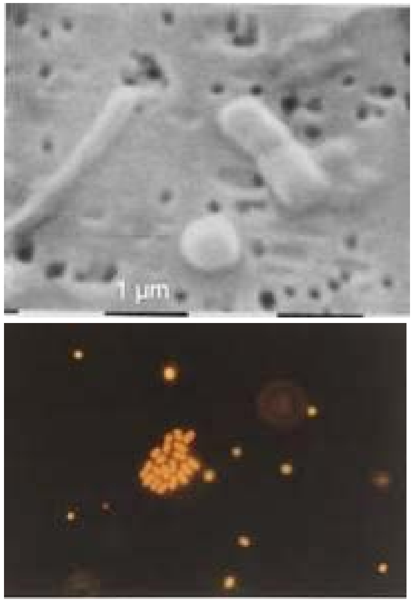


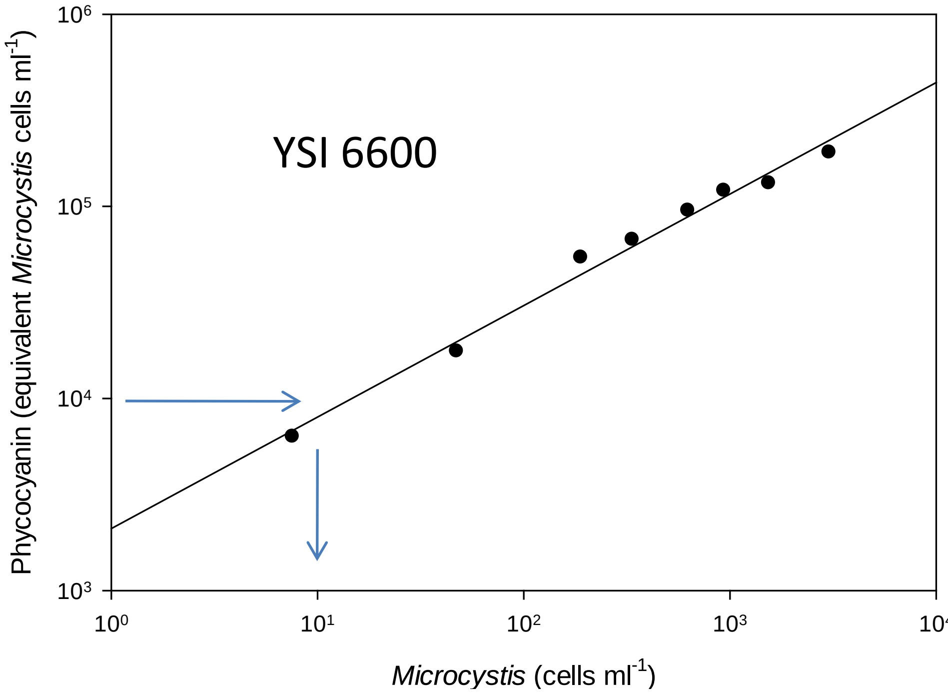


# The use of phycocyanin for determining cells of cyanobacteria

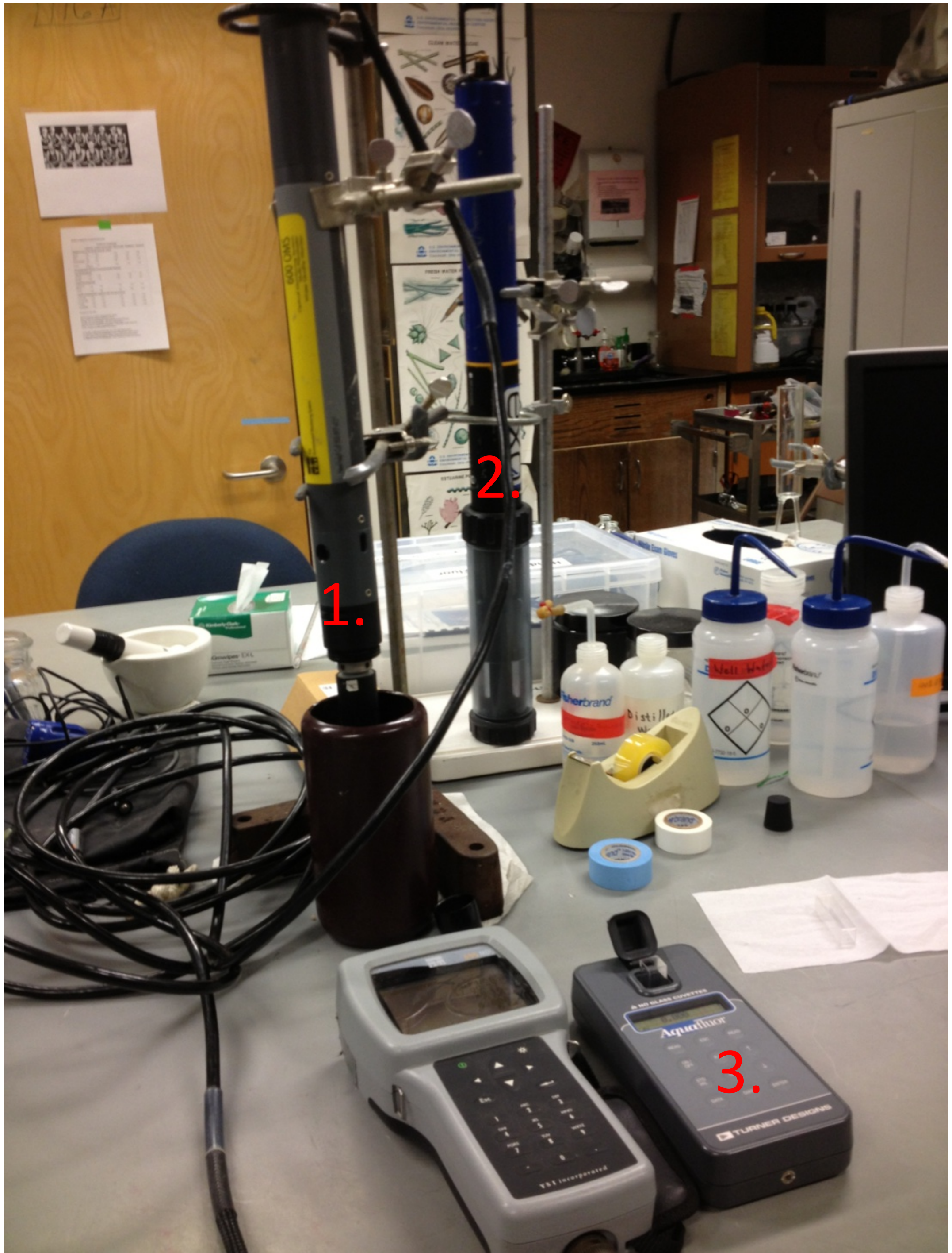
Overestimates due to contribution of single cells (not counted because went through the net (50um))?

**Comparing net counts to fluorescence signals…**




Overestimates due to CDOM?




Equipment and techniques that aid in determining relative concentrations of cyanobacteria by measuring phycocyanin….

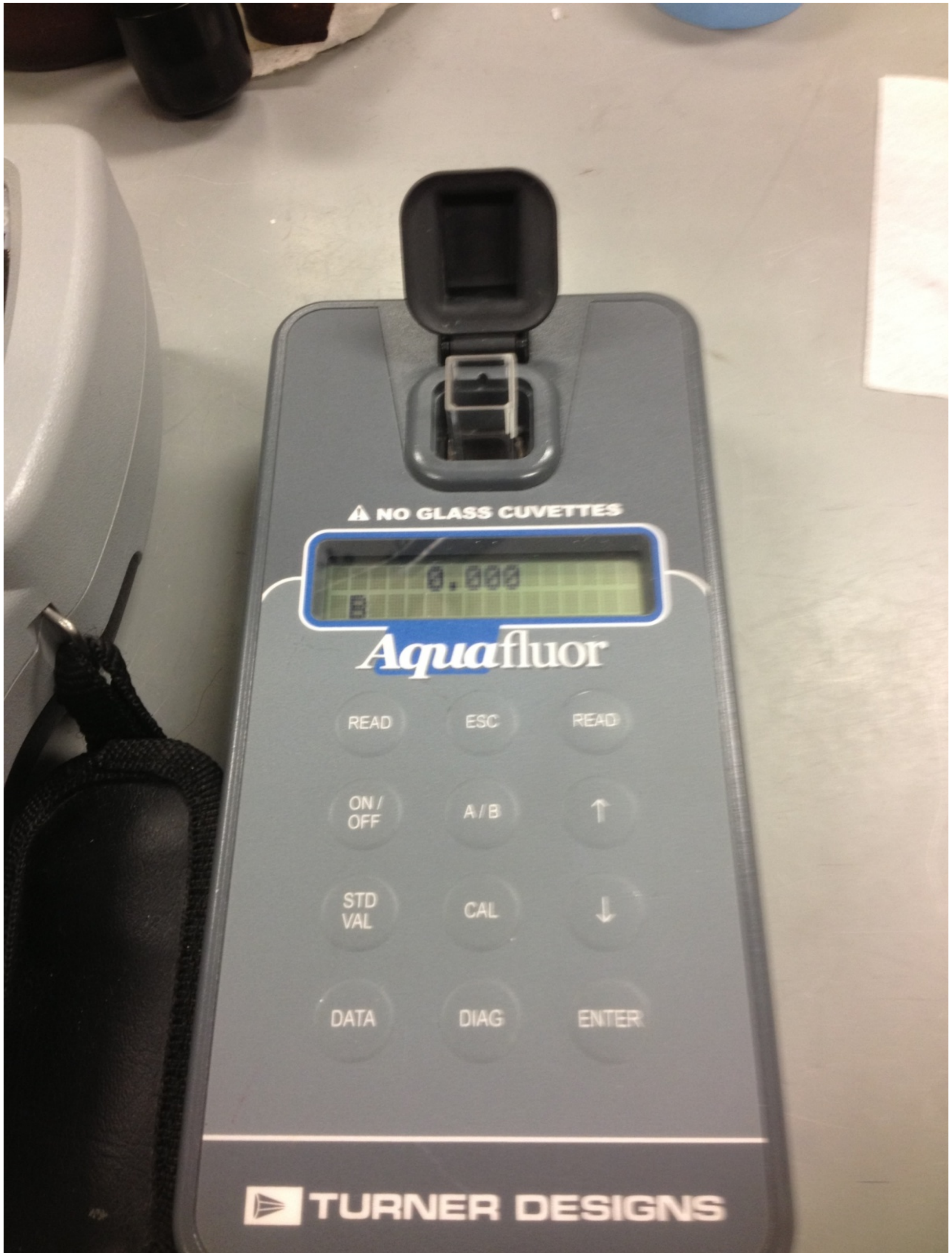
1. YSI (600 OMS) phycocyanin probe
2. YSI EXO sonde (correction for FDOM)
3. Turner hand-held Aquafluor (RFU of chlorophyll and phycocyanin)


PC probes

YSI has many design to choose from. Other companies have versions of this as well.

YSI 6600 or 600 (equipped with PC probe) does not correct for CDOM and cause an artificially high level if color is significant in the water (stained lakes will have higher levels)

Potential “quench factor” CDOM or blooms




## Hand-held Devices

Turner offers a hand-held device for PC and Chl. The small cuvette only allows for about 3 ml of water. Not much sample is needed and a Relative Fluorescent Unit (RFU) is reported.
Cheaper and convenient. Good, quick method for determining relative concentrations of cyanobacteria. Reasonable and easy to use for lay monitors.

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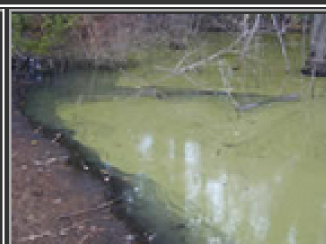
# Programs

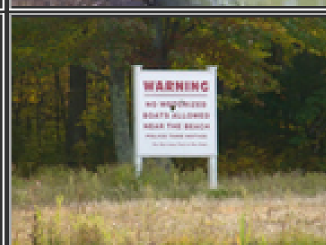
## Citizen-based Cyanobacteria Monitoring Program

Sampling protocols and services provided by the Center for Freshwater Biology (UNHCFB CCMP):

Cyanobacteria Lake Monitoring



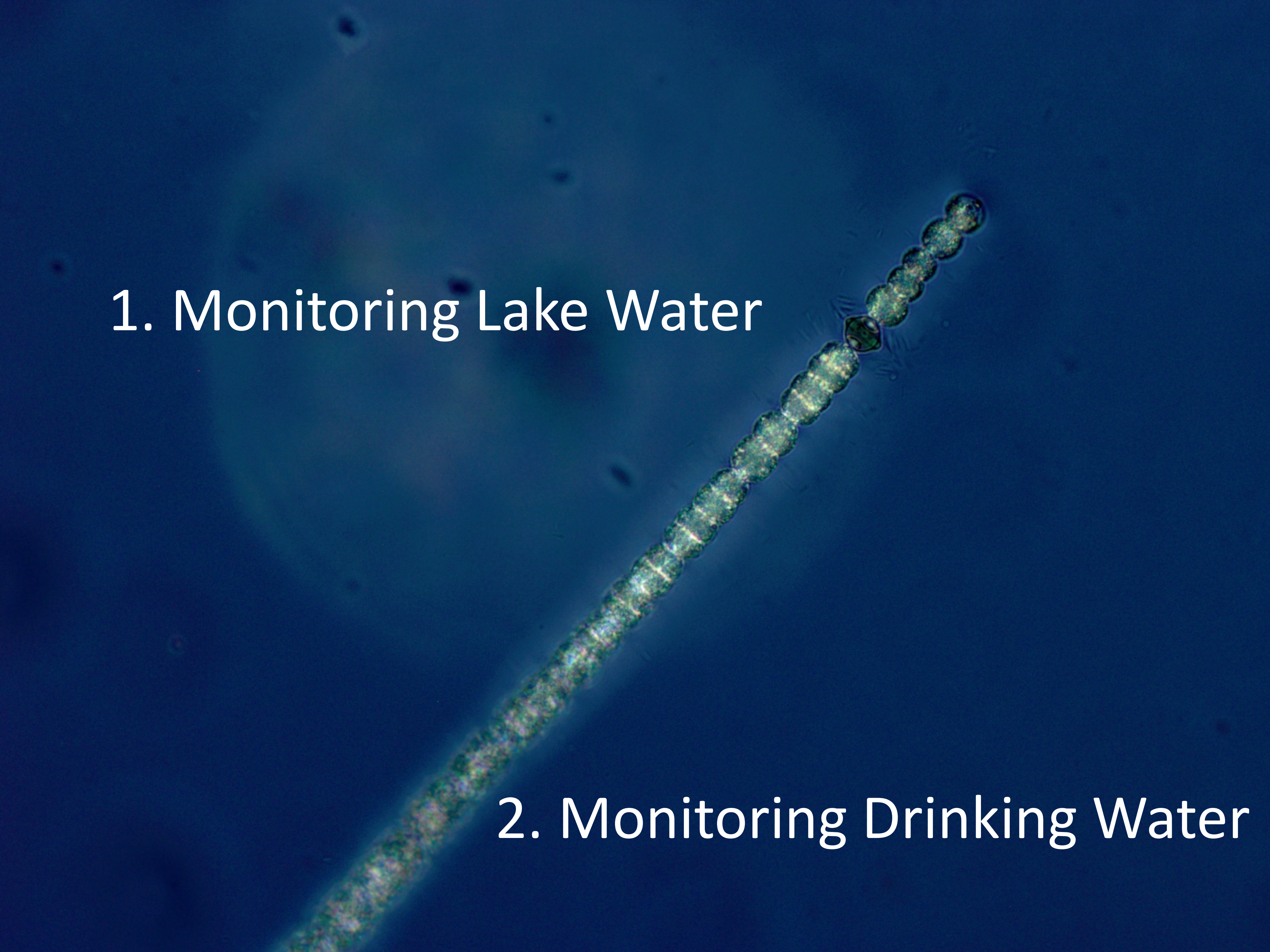
Cyanotoxins in Drinking Water




The Center for Freshwater Biology at the University of New Hampshire initiated a Citizen-based Cyanobacteria Monitoring Program (CCMP) in 2010, providing assistance to Lake Associations and Drinking Water Facilities in the tracking of cyanobacteria and microcystins in various bodies of water. Protocols and services can be accessed by clicking the links at the left of the panels.

Please contact Amanda Murby for questions or assistance.

UNH CFB 2010 Pilot “trial” Program


1. Monitoring Lake Water
2. Monitoring Drinking Water

*Adapted from laboratory protocols of the Center for Freshwater Biology, University of New Hampshire, Durham, N.H.* 2010

# Standard Operating Procedure for Field Sampling of Cyanobacteria in Lakes

**2010**

**Contacts:**

- cfb.unh.edu
- 603-862-2105: "Haney Lab"
- Dr. Jim Haney: Jfhaney@unh.edu
- Amanda Murby: amurby@unh.edu

1. **Materials:**
   - Integrated water sampler (see page 3 for details)
   - HDPE bottles (1 liter)
   - Cooler with ice/refrigeration/freezer
   - Labels for samples

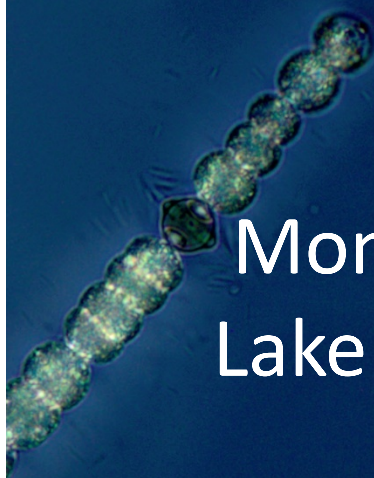
Labeling:
Please include: *date, time, body of water, sample location/site/depth, and weather conditions.*
*If bloom material is sampled, please also indicate (if possible) when the bloom was first reported and how long it persisted for.

2. **Sampling Types:**
   A. Lake Water Quality Monitoring
   B. Cyanobacteria Blooms

**A. Lake Water Quality Monitoring:**

1) Sampling should be done mid-day between the hours of 10am and 3 pm.
2) Cyanobacteria are transported by wind and water currents and thus tend to have a very patchy distribution. In order to obtain a sample that is representative of the entire lake, it is necessary to collect samples from several locations. The number of locations needed depends on the size and complexity of the lake.
3) Lake water should be sampled from at least 3-5 locations that represent the major embayments and sub basins within the lake, including the deepest site. Samples from each of the locations may be combined for a single toxicity test. They can also be stored and analyzed separately if information on the spatial variation of microcystin

1



# Monitoring Lake Water:

1. Lake Water Quality
2. Bloom Watches

Samples analyzed for Phycocyanin and Microcystins

*Adapted from laboratory protocols of the Center for Freshwater Biology,*
*University of New Hampshire, Durham, N.H.* 2010

## UNH CFB Protocol for the Monitoring of Cyanobacteria & Microcystins in Drinking Water: 2010

1. Water collections should be sampled from both treated and untreated (raw) water. You may also choose to sample water from other stages of the treatment if desired.
2. Rinse the HDPE bottle (1 liter) with a small amount of sample water before collection and clearly label each bottle.
3. The HDPE sample bottle should be filled ¾ to allow for expansion when frozen.
4. Place the samples on ice and in the dark until delivery to UNH CFB lab.
5. Freeze the sample if delivery/ drop-off time exceeds 12 hours.

**Analyses:**

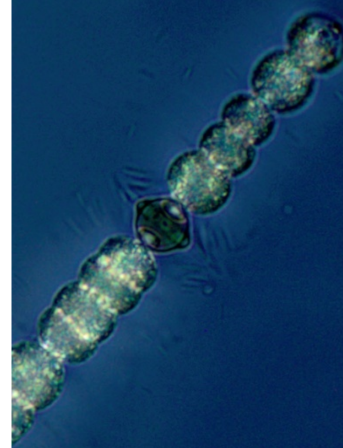
a. Samples will be analyzed for the concentration of the liver toxin, microcystin, using the Envirologix, Quantiplate-ELISA Kit, (Portland, Me) with increased sensitivity (UNH, CFB). Results will be reported as ng microcystins per liter.
b. Phycocyanin fluorescence (a pigment characteristic of cyanobacteria) will be determined and converted to equivalent *Microcystis aeruginosa* cells $ml^{-1}$.

**Deliver to:**

Dr. Jim Haney, Center for Freshwater Biology
38 Academic Way, Spaulding G28 (mail) or Spaulding 116 (in person)
Durham, NH 03824

**Contacts:**

- cfb.unh.edu
- 603-862-2105: "Haney Lab"
- Dr. Jim Haney: jfhaney@unh.edu
- Amanda Murby: amurby@unh.edu

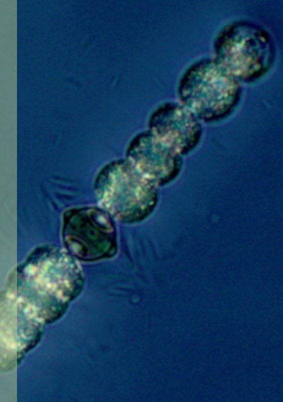


# Monitoring Drinking Water:

1. Untreated (raw)
2. Treated (specific to facility)

Samples analyzed for Phycocyanin and Microcystins

## CCMP 2010



**General Procedure:**

1. Samples were received in 250 ml containers
2. Samples were integrated in the lab and mixed thoroughly
3. Fluorescence of phycocyanin was measured using a Turner Design hand-held Fluorometer (fluorescence was to determine a relative concentration of cyanobacteria)
4. Samples were frozen and thawed in triplicate to lyse cells
5. Water samples were concentrated 10-fold by lyophillization
6. Microcystin concentrations were determined using the Envirologix Quantiplate Kit for Microcystins, Portland, ME (tests results are equivalent to all variants of microcystin and nodularins*)
7. Detection range (with standards) between 25 and 2500 ng MC L-1

(lower limit of detection for concentrated water (x10) is therefore 2.5 ng MC L-1)

What about other cyanotoxins?

# CCMP Reports: Detailed reports to help explain the results

1. Summary
2. Specific results
3. Figures and tables to show data
4. References to guidelines on cyanobacteria and toxins (MCs)

Citizen Cyanobacteria Monitoring Program

Lake Waukewan 2010 Report

University of New Hampshire
**Center for Freshwater Biology**
CFB.UNH.EDU
603-862-2105

Center for UNH CFB Freshwater Biology

Summary:

Lake Waukewan was surveyed for cyanobacteria and microcystin on August 12, 2010. The lake

# UNH Center for Freshwater Biology

## LAKES LAY MONITORING PROGRAM

## CYANOBACTERIA MONITORING DATA SHEET (2013)

**MONITOR NAME:**__________________________

**DATE:**_________________________________

**LAKE NAME:**___________________________

**SITE NAME:**___________________________

**SAMPLE DEPTH:**______________________

**SAMPLE VOLUME:**____________________

**SAMPLE METHOD:**____________________

________________________________________

________________________________________

**Pigment analyses for relative concentrations of primary productivity (cyanobacteria and other phytoplankton):**

**YSI Multi-parameter probe (6600 M V2/ 600 OMS)**
**Last Calibration:**__________________

| Replicates | Phycocyanin (cells/mL) | Chl *a* (µg/L) |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| Average | | |

*Phycocyanin Fluorescence (equivalent to Utex #2385, *Microcystis aeruginosa* cells ml$^{-1}$)

**Turner Design™ Aquafluor Hand-held Device**

| Replicates | Sample | Phycocyanin (RFU) | Chl *a* (RFU) |
|---|---|---|---|
| | | | |
| | H20 Blank | | |
| | | | |
| | | | |
| | Correction Factor | | |
| | Sample 1.) | | |
| | 2.) | | |
| | 3.) | | |
| | Corrected Sample 1.) | | |
| | 2.) | | |
| | 3.) | | |
| | Average | | |

## *Potential* Toxicity…… Is it good enough for now??? Good to know???

| Genus | Microcystin (Hepatotoxin) | Anatoxin-A (Neurotoxin) | BMAA (Neurotoxin) | Dermatotoxin | Cyanobacteria in sample (Yes/No) | Abundance (#/mL) |
|---|---|---|---|---|---|---|
| *Anabaena* | X | X | * | | | |
| *Aphanocapsa* | | | * | | | |
| *Aphanonizomen* | | | * | | | |
| *Coelospherium* | | | * | | | |
| *Microcystis* | X | X | * | | | |
| *Oscillitoria* | X | X | * | X | | |
| *Woronichinia* | X | | * | | | |
| *Merismopedia* | | | * | | | |
| *Gloeocapsa* | | | * | | | |
| *Gloeotrichia* | X | | * | | | |
| *Lyngbya* | | | * | X | | |
| *Phormidium* | | | * | | | |
| Picocyanobacteria | * | * | * | * | | |

****This is not a complete list of cyanobacteria and their potential toxins****

* Indicates unknown

Steps taken for water samples: CFB group 2013

1. Receive complaint and water sample
2. Record location and methods for sampling
3. Measure the phycocyanin and chlorophyll levels
 (YSI probes, Turner Hand-held, Algae Torch)
4. Microscopic identifications
5. Photos of cyanobacteria
6. Notes on other plankton or parameters
7. Addresses to potential toxicity

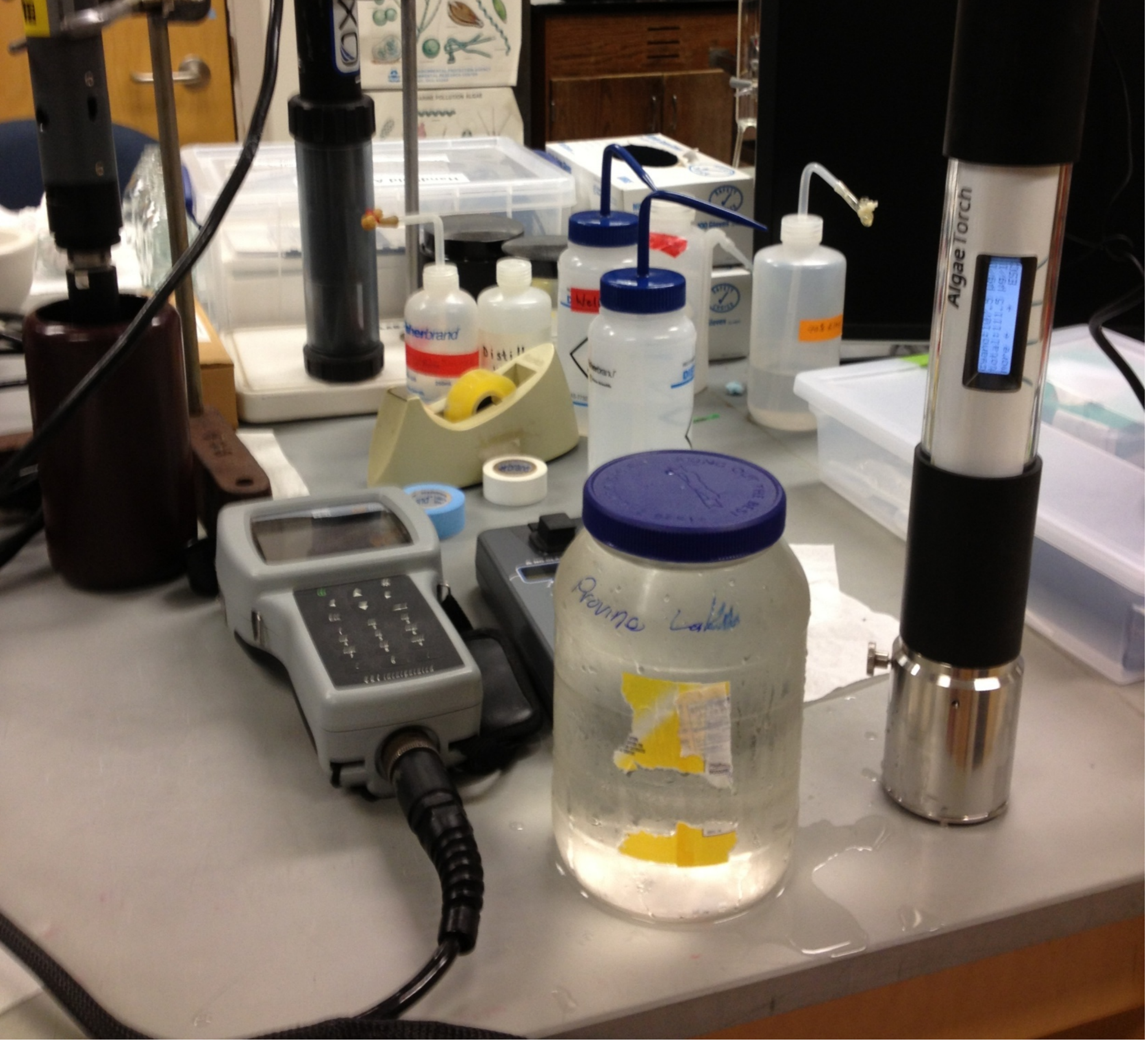
# Center for Freshwater Biology
# Lakes Lay Monitoring Program
# Cyanobacteria Analyses 2013

## Services and Education

- Responding to concerned lake users
- Training students to identify and analyze cyanobacteria
- Integration of cyanobacteria abundance and diversity with lake water quality monitoring
- Outreach and education on the potential problems associated with cyanobacteria

## Goals

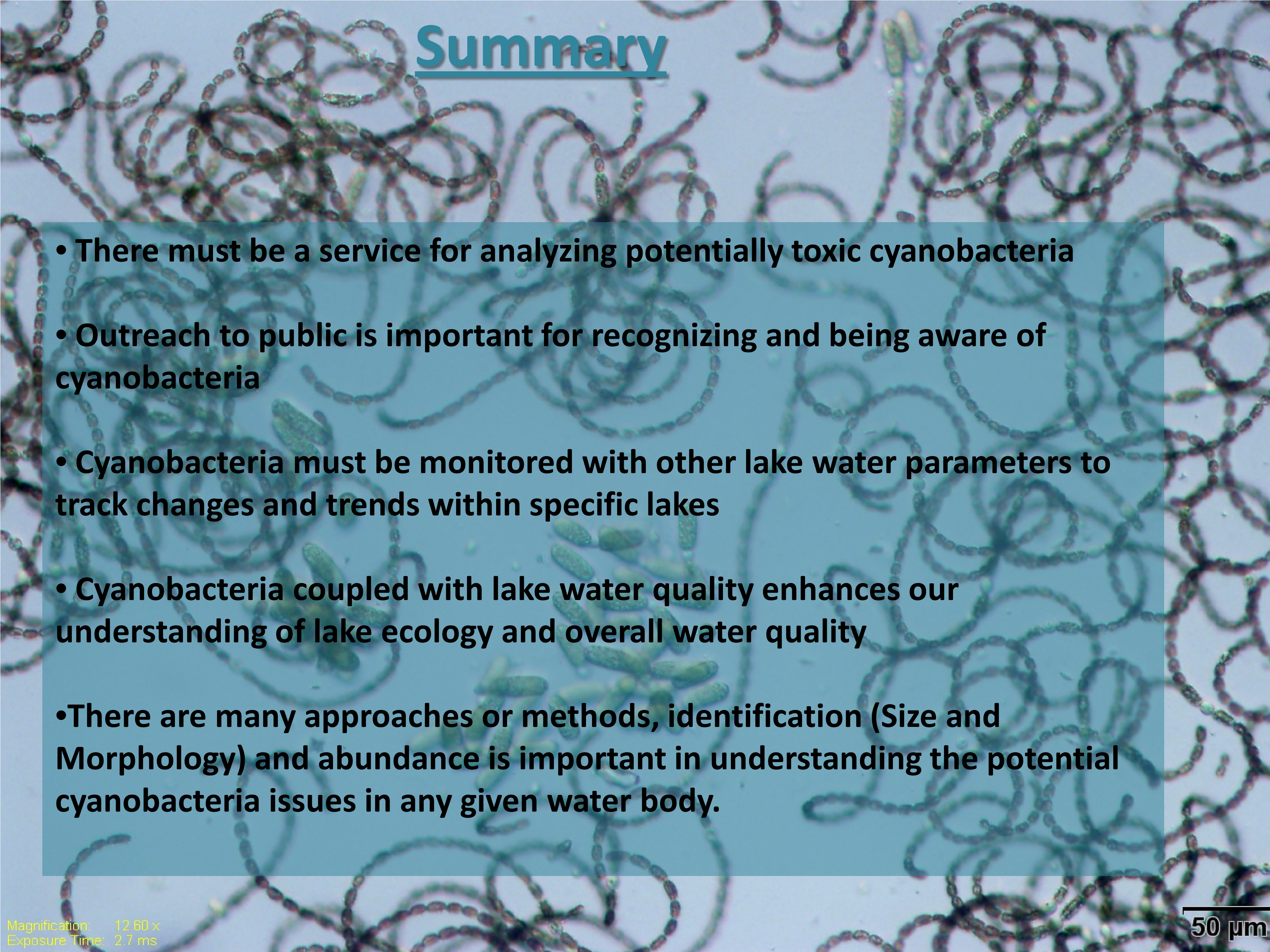
- Address public concerns on cyanobacteria and lake water quality
- Determine long term trends of cyanobacteria for specific lakes to better understand the ecology of the system
- Future advances in technology that allow for rapid *in situ* determination of cyanotoxins on a variety of scales.


AlgaeTorch


Magnification: 12.60 x
Exposure Time: 2.7 ms
50 µm

Algae Torch (Moldaenke)


Best feature of the Algae Torch is that it gives rapid, relative percentages of cyanobacteria in the water. It can be used in the field along the shore or in the lab with a water sample.




# Summary

- There must be a service for analyzing potentially toxic cyanobacteria

- Outreach to public is important for recognizing and being aware of cyanobacteria

- Cyanobacteria must be monitored with other lake water parameters to track changes and trends within specific lakes

- Cyanobacteria coupled with lake water quality enhances our understanding of lake ecology and overall water quality

- There are many approaches or methods, identification (Size and Morphology) and abundance is important in understanding the potential cyanobacteria issues in any given water body.