FlowCam®

Yokogawa Fluid Imaging Technologies, Inc.

# Algal Identification Using Flow Imaging Microscopy

Savannah Judge

OTCO Reservoir Management Webinar

July 12, 2022

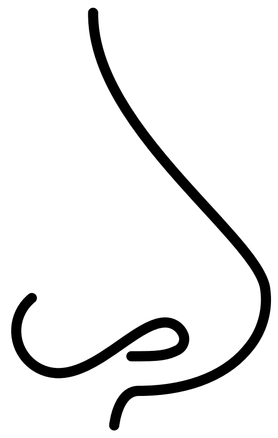
# Agenda

- Algae Monitoring Essentials: T&O, Toxin, Filter Cloggers
- Source Water Monitoring & FlowCam
- Example Images
- Case Studies
- Summary

# Algae Monitoring Essentials

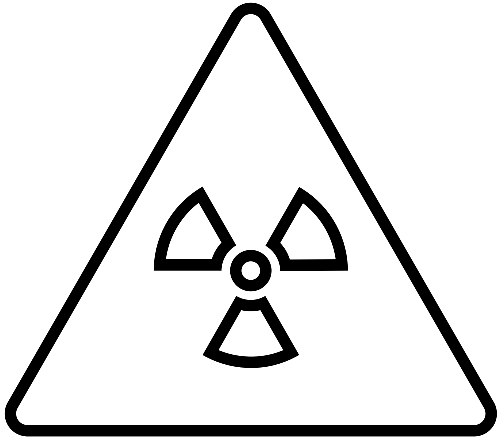
# Algae Monitoring Essentials

- Taste and Odor Producers



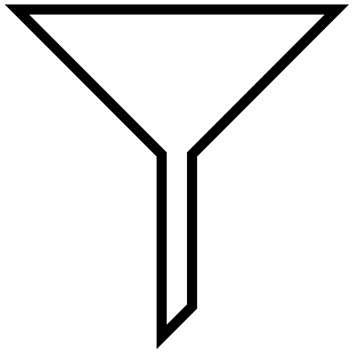
# Algae Monitoring Essentials

- Taste and Odor Producers
- Toxin Producers



# Algae Monitoring Essentials

- Taste and Odor Producers
- Toxin Producers
- Filter Cloggers



# Taste & Odor Monitoring

- Many sources of T&O complaints: natural, synthetic

# Taste & Odor Monitoring

- Many sources of T&O complaints: natural, synthetic
- Volatile organic compounds produced by actinomycetes (bacteria) & various algal species

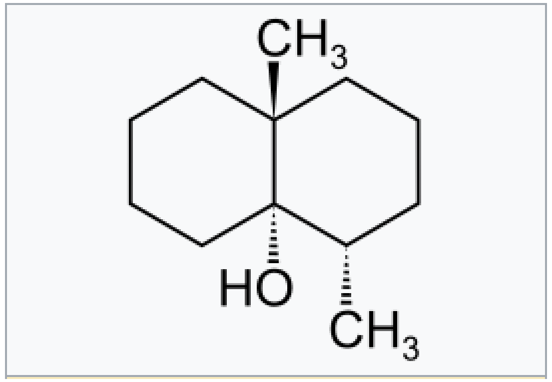


FlowCam®

# Taste & Odor Monitoring

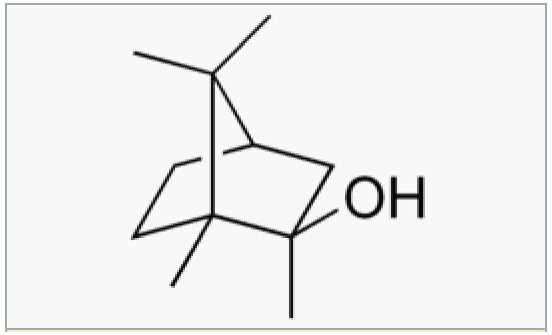
- Many sources of T&O complaints: natural, synthetic
- Volatile organic compounds produced by actinomycetes (bacteria) & various algal species
- Most commonly known T&O compounds are produced by cyanobacteria:
  - Geosmin – earthy odor
  - 2-Methylisoborneol (MIB) – musty odor

Geosmin




Low odor detection threshold: 0.006 to 0.01 ppb

2-Methylisoborneol

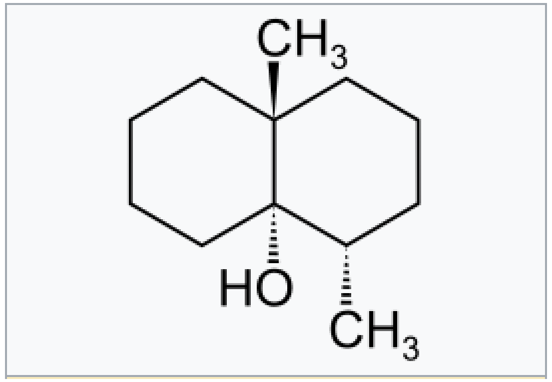



Low odor detection threshold: 0.002 to 0.02 ppb

# Taste & Odor Monitoring

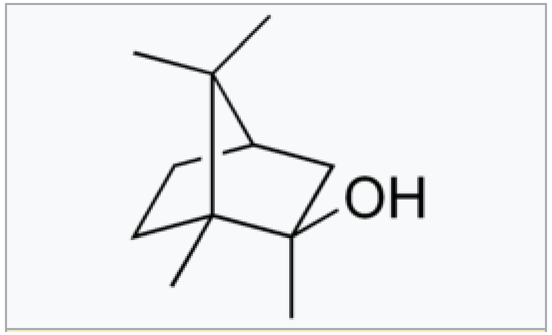
- Many sources of T&O complaints: natural, synthetic
- Volatile organic compounds produced by actinomycetes (bacteria) & various algal species
- Most commonly known T&O compounds are produced by cyanobacteria:
  - Geosmin – earthy odor
  - 2-Methylisoborneol (MIB) – musty odor
- Metals (manganese, copper, iron)

Geosmin




Low odor detection threshold: 0.006 to 0.01 ppb

2-Methylisoborneol




Low odor detection threshold: 0.002 to 0.02 ppb



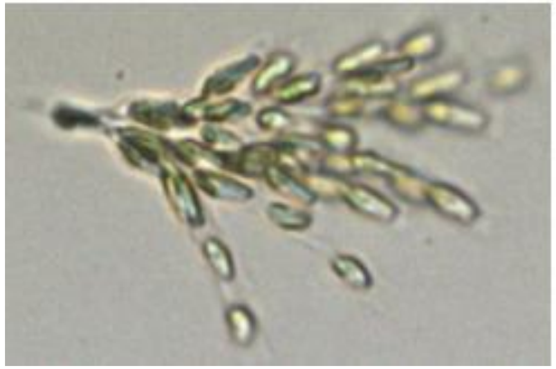
FlowCam®

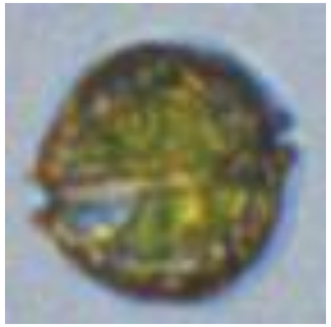
| Compound | OTC µg.L⁻¹ | Odour | Compound | OTC µg.L⁻¹ | Odour |
|---|---|---|---|---|---|
| *SULPHUROUS* | | | *AMINES* | | |
| dimethyltrisulphide | 0.01 | septic, garlic, putrid,swampy | Ethanolamine | 6.5 | Mild ammonia –fish[Y] |
| dimethyldisulphide | <4.0 | septic, garlic, putrid | isopropylamine | 210 | Ammonical, amine |
| methanethiol | 2.1 | | butylamine | 80 | Sour, ammonical, amine |
| ethanethiol | 1 | | propylamine | 90,000 | ammonia |
| propanethiol | 0.74 | | methylamine | 21 | ammonia |
| t-butythiol | 0.09 | | trimethylamine | 0.21 | pungent, fishy, ammonia |
| dimethyl sulphide | 1 | | *Dimethyl amine* | 47 | |
| hydrogen sulphide | 7.2 | | | | |
| | | | *TERPENOIDS* | | |
| *PUFA DERIVATIVES* | | | α-ionone | 0.007 | violets |
| n-heptanal | 3 | fishy, oily | β-ionone | 0.007 | violets |
| n-hexanal | 4.5 | grassy, fatty | epoxy -α-ionone | 0.007 | |
| 3-methyl-butyrate | 20 | rotten, rancid | geosmin | 0.004 | earthy/musty |
| n-pentanal | 60 | fishy | 3-methylbut-2-enal | 0.15 | rancid; putrid |
| *trans*-2-nonenal | 0.8 | cucumber | 3-methyl butanal | 0.15 | rancid/putrid |
| 1-penten-3-one | 1.25 | pungent; rancid; fishy | 2-methylisoborneol | 0.015 | earthy, musty |
| *trans*-2-hexenal | 17 | | limonene | 4 | citrus |
| *cis*-3-hexen-1-ol | 70 | grassy | linalool | 6 | grassy, floral |
| 2-methylpent-2-enal | 290 | rhum, marzipan | cieneole (1,8) | 12 | camphor, spicy, cool |
| *trans*-2, *cis*-6 -nonadienal | 0.08 | grassy; cucumber | 6-methyl-5-hepten-2-one | 50 | fruity; ester-like |
| 1,3-octadiene | 5600 | earthy/mushroom | β-cyclocitral | 19.3 | tobacco, smoky/mouldy |
| *trans, cis*-2,4 heptadienal | 5 | fishy, oily | styrene | 65 | sweet, balsamic |
| *trans, cis,cis*-2,4,7-decatrienal | 1.5 | fishy, oily | | | |
| | | | *PYRAZINES* | | |
| | | | 2,6-dimethyl pyrazine | 6 | cocoa, roasted nuts, coffee |
| | | | 3-methoxy-2-isopropyl pyrazine | 0.0002 | earthy/potato bin |
| | | | 2-isobutyl-3-methoxy pyrazine | 0.001 | earthy/potato bin |

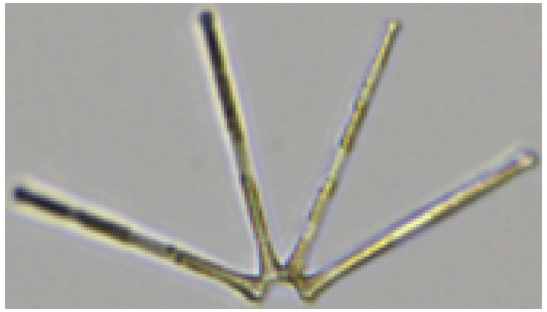
Table 1. Survey of aquatic Odour Threshold Concentrations (OTC; $\mu g.L^{-1}$) reported for selected odourous algal metabolites. Source: Maleville and Suffet 1987; Young et al. 1996; Watson 2002)

# Common T&O Producers

| | |
|---|---|
| Cyanobacteria | • *Dolichospermum*<br>• *Microcystis* |
| Diatoms | • *Synedra*<br>• *Asterionella* |
| Dinoflagellates | • *Ceratium*<br>• *Peridinium* |
| Crysophytes | • *Dinobryon*<br>• *Prymesium* |
| Synurophytes | • *Synura*<br>• *Mallomonas* |

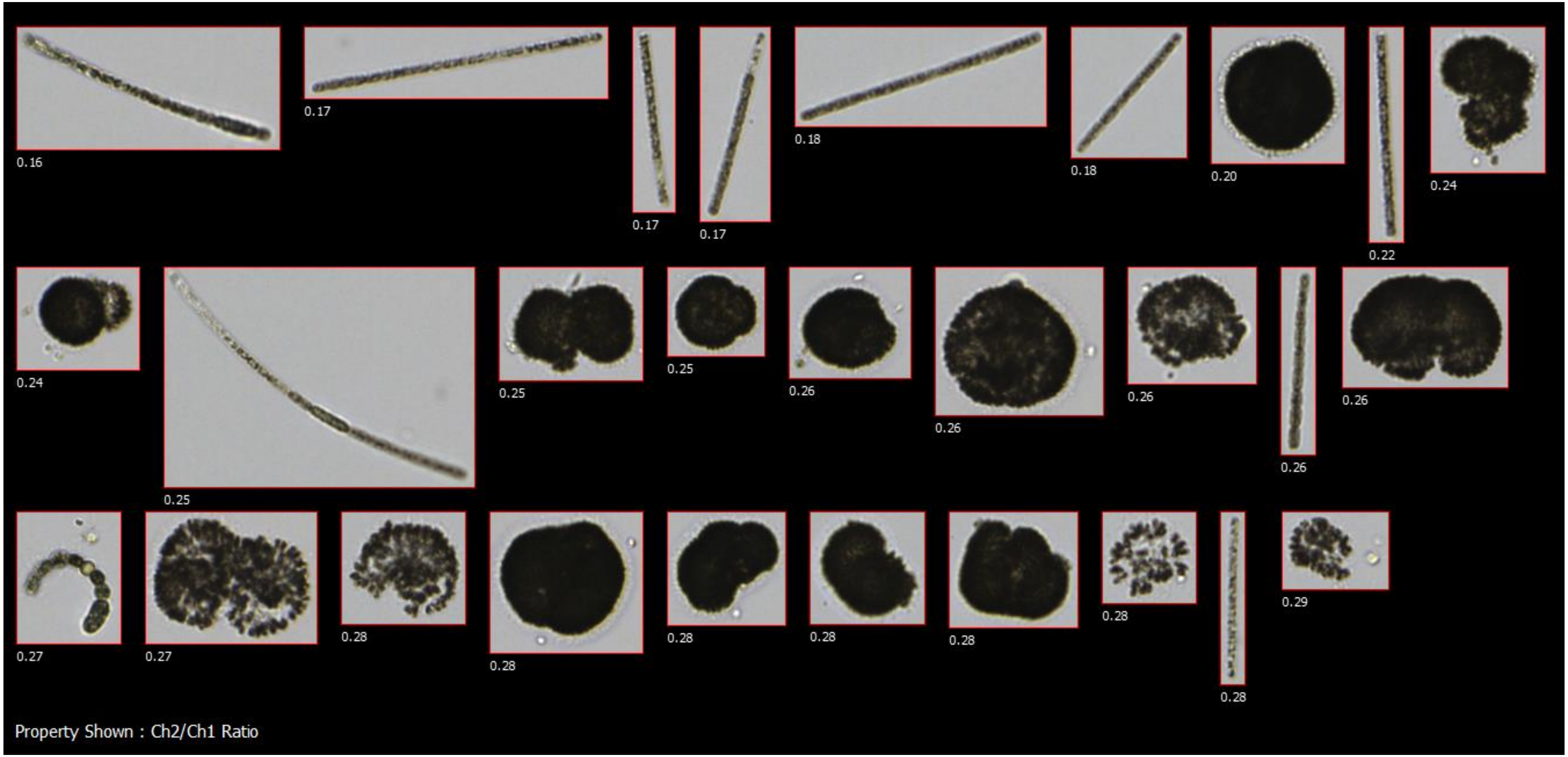






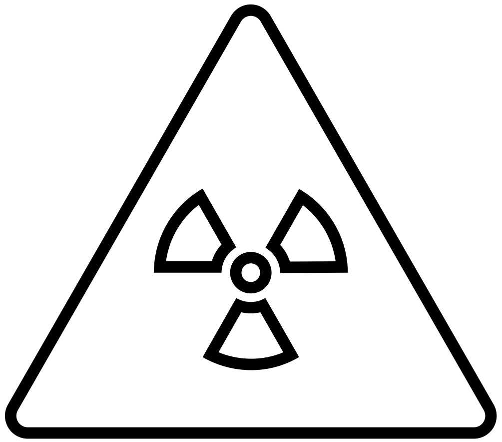


FlowCam®


0.16
0.17
0.17
0.17
0.18
0.18
0.20
0.22
0.24
0.24
0.25
0.25
0.25
0.26
0.26
0.26
0.26
0.26
0.27
0.27
0.28
0.28
0.28
0.28
0.28
0.28
0.28
0.29
Property Shown : Ch2/Ch1 Ratio

# Algae Monitoring Essentials

- Taste and Odor Producers
- Toxin Producers

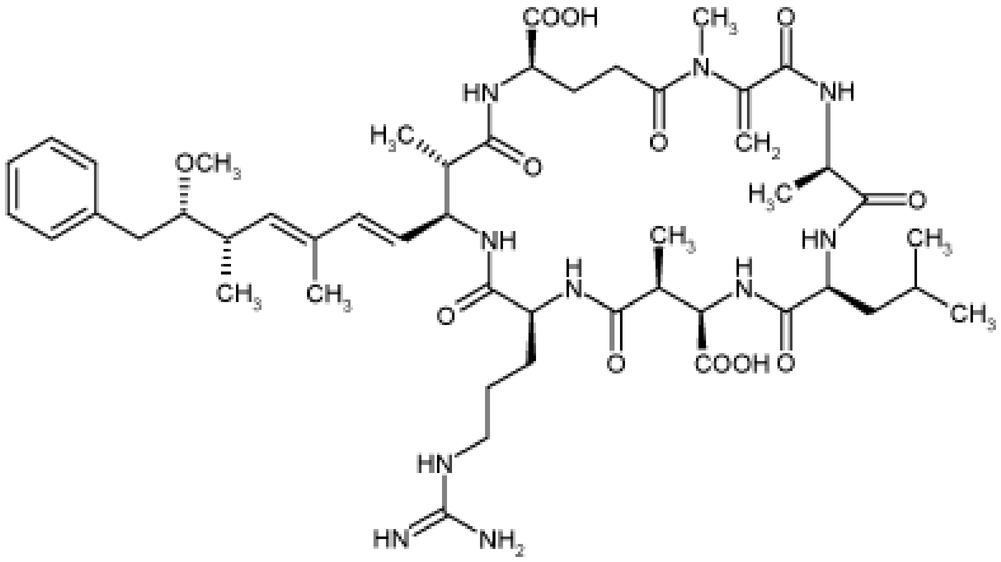




FlowCam®

# Toxin Producers

- In addition to VOCs, algae can also produce toxins
- Cyanotoxins: cyanobacteria
  - Include: neurotoxins, hepatotoxins, cytotoxins, dermatotoxins, & lipopolysaccharides
- Icthyotoxins
  - Prymnesin *(Prymnesium parvum)*
  - Euglenophycin *(Euglena sanguinea)*



| Toxin |
| --- |
| Anatoxin-A |
| Azaspiracid |
| Brevetoxin |
| Ciguatoxin |
| Cylindrospermopsin |
| Domoic acid |
| Dinophysistoxin |
| Haemolytic toxin |
| Homoanatoxin |
| Karlotoxin |
| Lyngbyatoxin |
| Maitotoxin |
| Microcystin |
| Nodularin |
| Okadaic acid |
| Pectenotoxin |
| Prymnesin |
| Saxitoxin |
| Other |
| Unknown |

# Common Toxin Producers

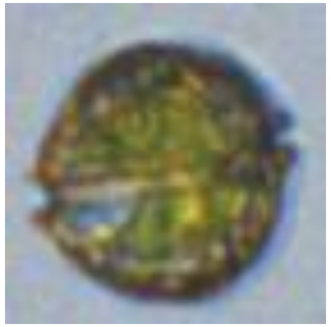
Cyanobacteria

- *Aphanizomenon*
- *Dolichospermum*
- *Microcystis*
- *Planktothrix*
- *Cylindrospermopsis*

Chrysophytes

(Golden Algae)

- *Prymnesium*

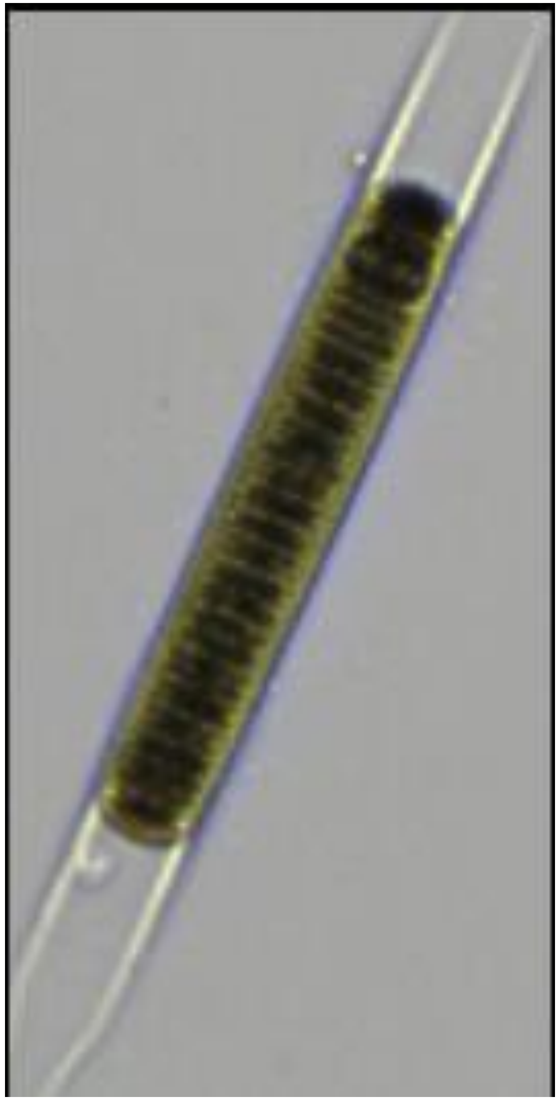




FlowCam®

# Toxin Producers

- States monitoring for common toxins: Microcystin-LR, Saxitoxin, Cylindrospermopsin
- UCMR4 monitored 10 toxins with >35,000 samples
  - Detections were low
  - Not bloom focused
- By focusing on only a few toxins over a set schedule we are failing to get the big picture
  - Is it better to monitor algae community composition which in turn can point to further toxin monitoring

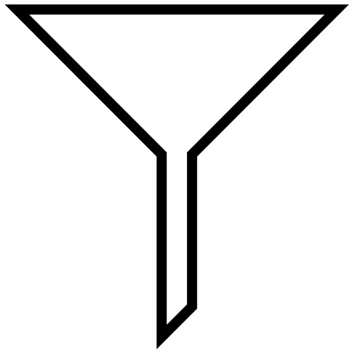




FlowCam®

# Algae Monitoring Essentials

- Taste and Odor Producers
- Toxin Producers
- Filter Cloggers



# Filter Cloggers

- Algae that do not settle and are not removed by the water treatment process
- Require increased backwashing
- Reduced production
- Economic loss

# Common Filter Cloggers

- Most common filter cloggers are generally Diatoms like:
  - Asterionella
  - Fragilaria
  - Pediastrum

# Agenda

- Algae Monitoring Essentials: T&O, Toxin, Filter Cloggers
- Source Water Monitoring & FlowCam

# Monitoring

- An algae monitoring program is essential for every water supplier.
- Routine testing of raw and finished water should be conducted to identify and treat problems before they reach the distribution system.
- Blooms caught early reduce health risks and customer complaints.
- Lakes and Reservoirs
  - Algal community composition
  - Cyanobacteria
  - Taste & Odor producers
- Water Treatment Plant
  - Filter cloggers

# Monitoring Tools

- Algae community composition
  - Microscope
  - FlowCam
- Algae Toxins
  - ELISA
  - LCMS-MS
- QPCR

# Monitoring Tools

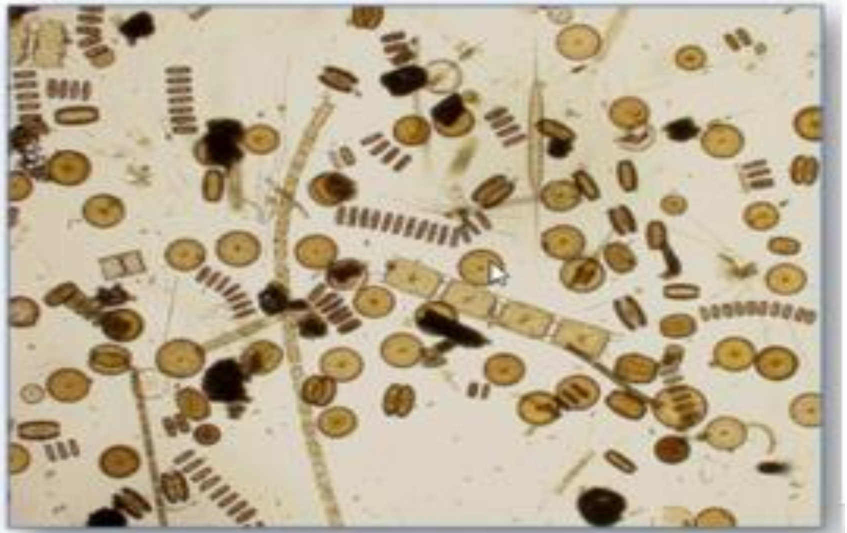
- Algae community composition
  - Microscope
  - **FlowCam**
- Algae Toxins
  - ELISA
  - LCMS-MS
- QPCR

# FlowCam

- Flow-imaging microscope
- Fluid Imaging Technologies founded in Maine in 1999
  - Became part of Yokogawa Electric in 2020
  - Combines principles of flow cytometry (fluorescence) and microscopy
- FlowCam is a tool for water managers looking to implement a T&O monitoring plan
- Developed for algae monitoring but has since been recognized as a valuable tool for the analysis of any particle types between 2µm – 1mm.

# Why FlowCam?

- FlowCam was invented because the standard way of counting plankton using traditional microscopy can be slow and tedious
- However, it is important to remember that both methods have their strengths and weaknesses.

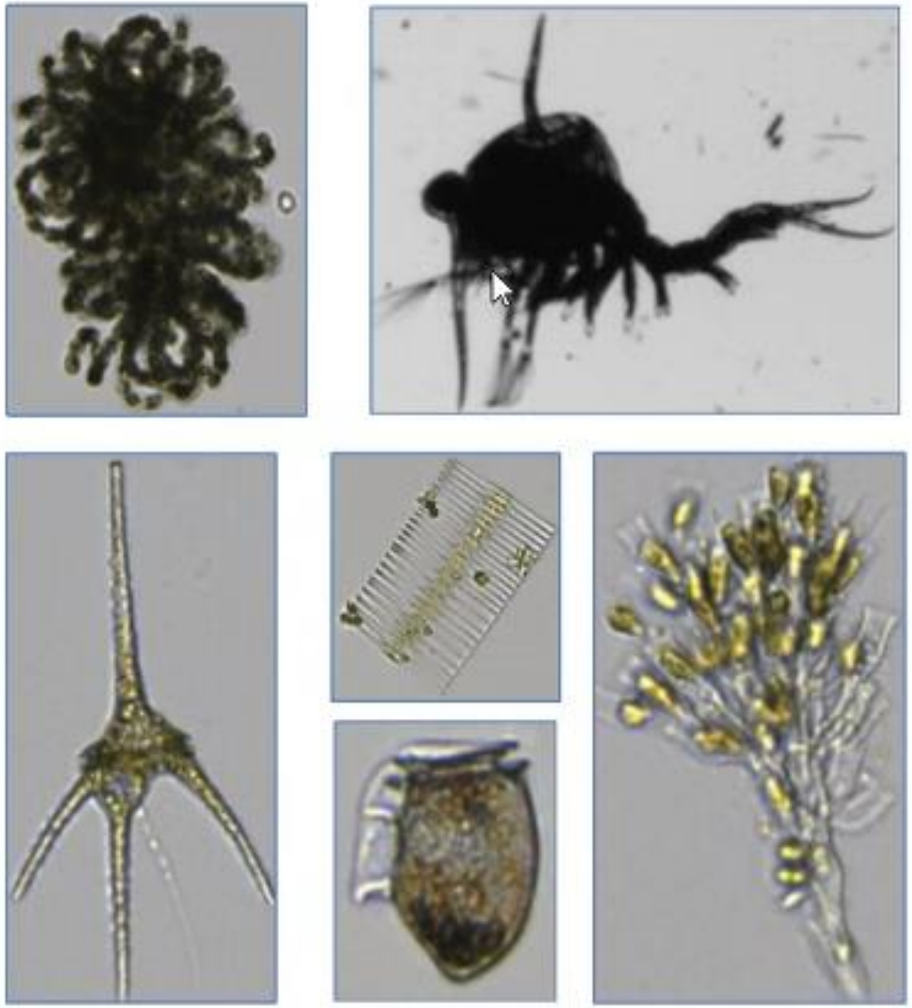




FlowCam®

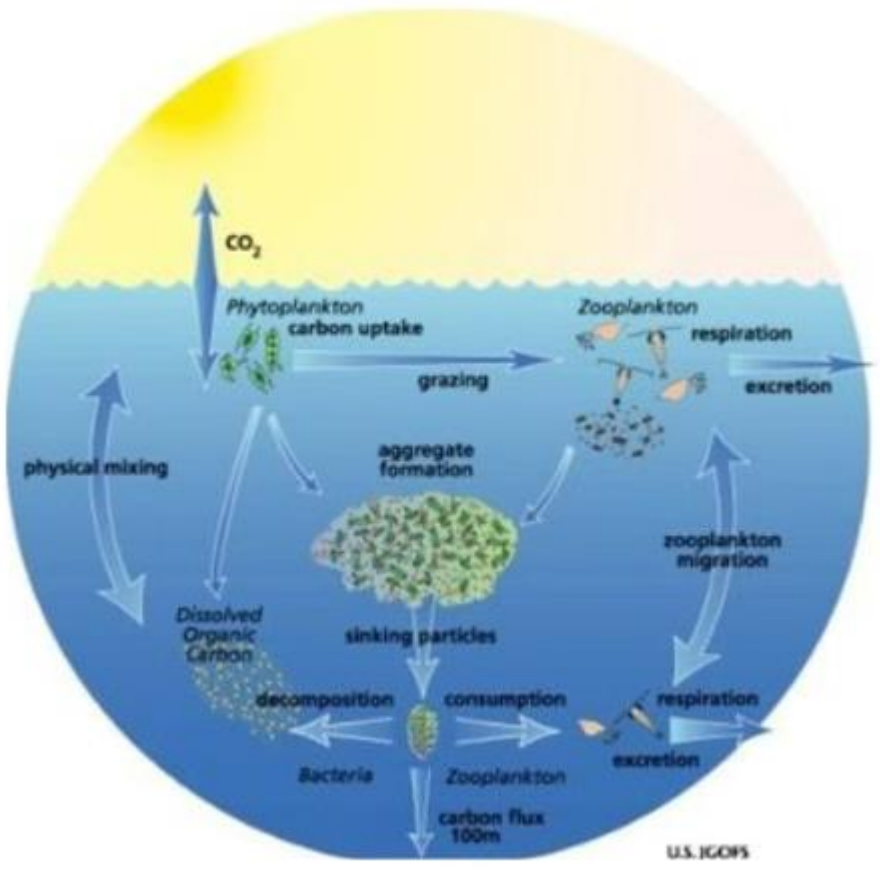
# Plankton Overview

- **Plankton** are organisms that are too small to overcome the current
  - **Phytoplankton**: tiny photosynthetic plants (aka alga)
  - **Cyanobacteria**: main culprits of freshwater harmful algal blooms (some of the oldest organisms on Earth!)
  - **Zooplankton**: slightly bigger predators that eat phytoplankton
- Marine and freshwater
- Categorized in many ways (size, taxonomy, morphology and means of obtaining energy)
- Questions users want to answer about plankton and other particles using the FlowCam
  - What kind?
  - How many?
  - How big?



# Phytoplankton

- Base of all aquatic food webs
- Sequester CO2, generate oxygen and transport nutrients (Biological Pump)
- Human influence and rising temperatures can upset the community balance and certain organisms are able to bloom.



Historically microscopes were used to monitor algae in lakes and reservoirs. Utilities are now switching to FlowCam.

- Passive, rapid data acquisition (1 mL in 6 minutes)
- Taxonomic training helpful but not required
- Automated classification of Cyanobacteria and algae helps technicians quickly ID problems
- Facilitates forecasting: statistically significant data sets
- Method consistent regardless of staff turnover
- Morphology (biovolume) automatically measured
- Import data to LIMS

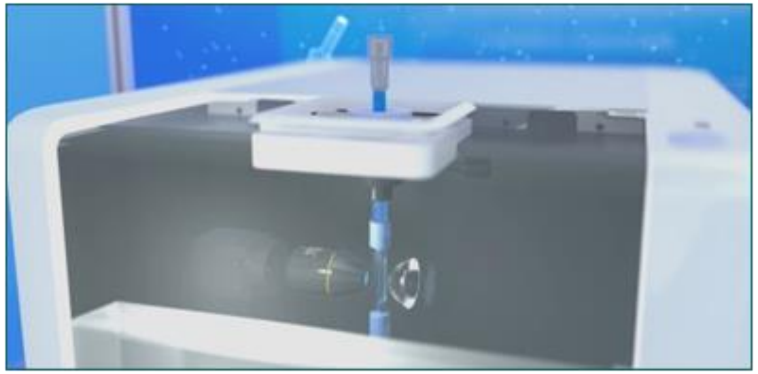
# Algae analysis considerations…

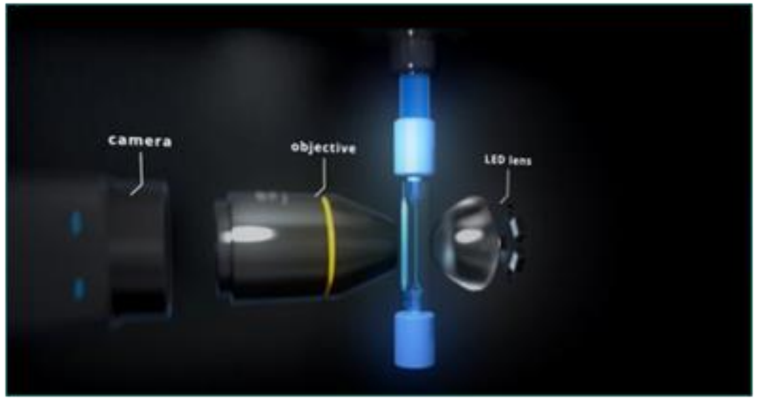
- FlowCam is NOT a tool for speciation
- Does not confirm presence/absence of toxins or toxin producing genes.
- Filtration often required, especially in bloom conditions
- Can count colonies as large as 1 mm, but requires extra sample preparation.
- FlowCam under counts cells in dense colonies

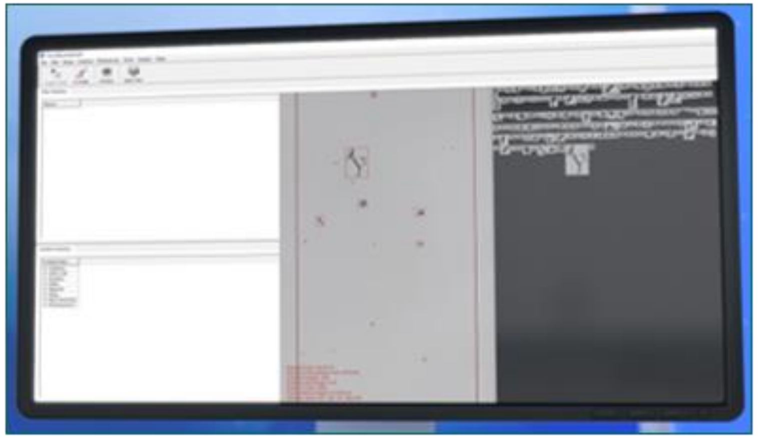
  FYI this is challenging on a microscope too

# FlowCam Cyano Operation

1. Sample is prepared & introduced to the top of the FlowCam.
2. Syringe pump draws sample into the flow cell.
3. Sample passes in front of LED light, objective lens, and digital camera.
4. Software "crops" particles (ROIs), saving them to a database and recording concentration and particle property data.
5. User post-processes the data, builds libraries, classifies particles, and exports reports.



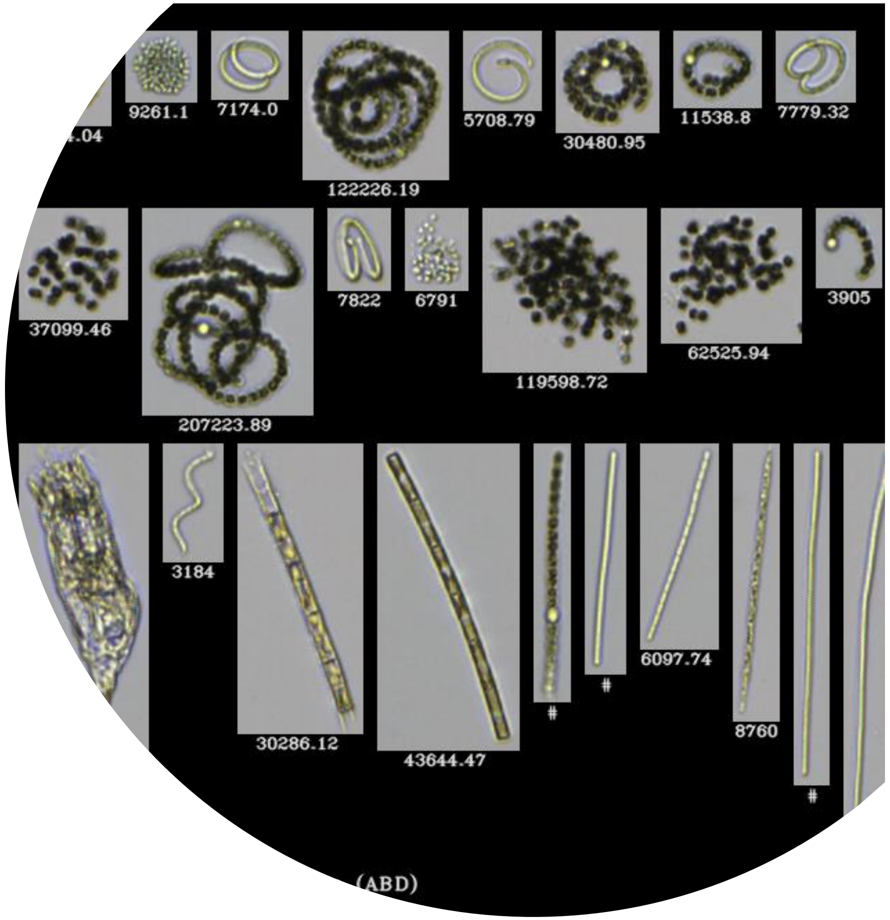


# Data Classification

Organize data using morphological features:

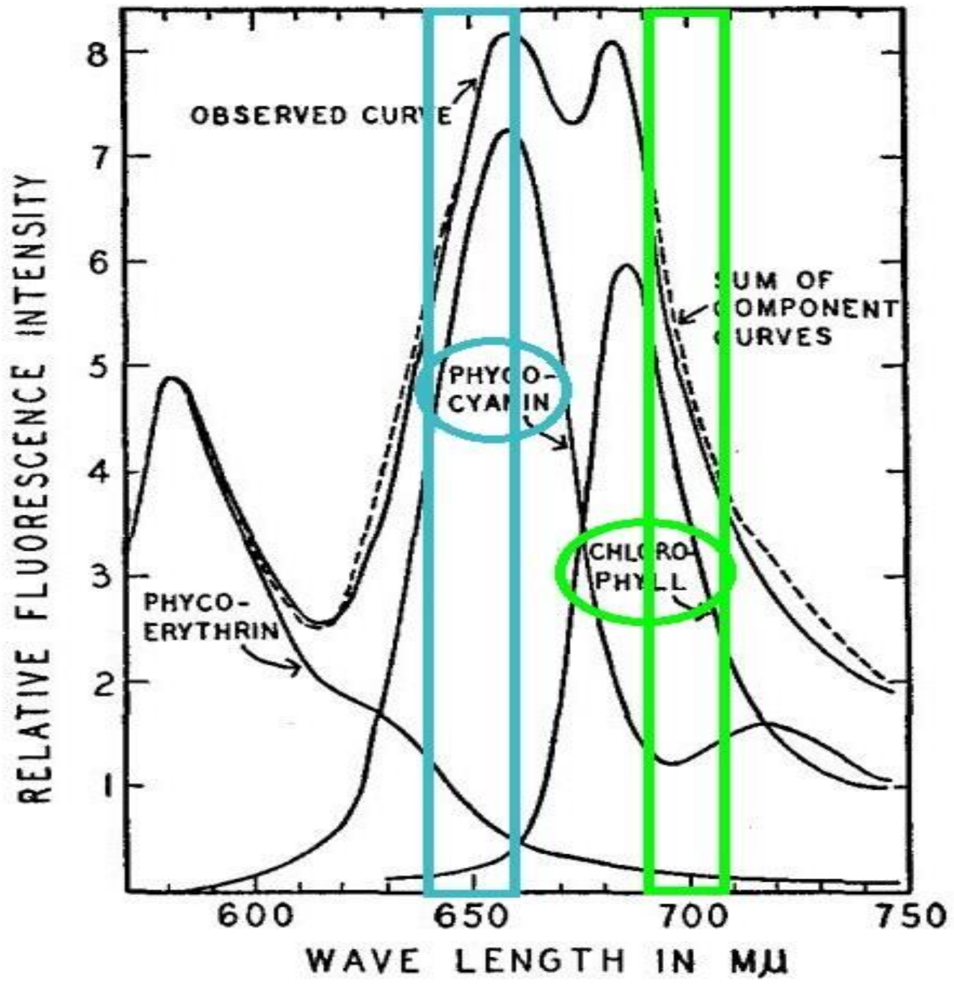
- Size, shape, color
- Semi-automated
- Pigment (FlowCam Cyano):
  - Chlorophyll (Algae)
  - Phycocyanin (Cyanobacteria)






FlowCam®

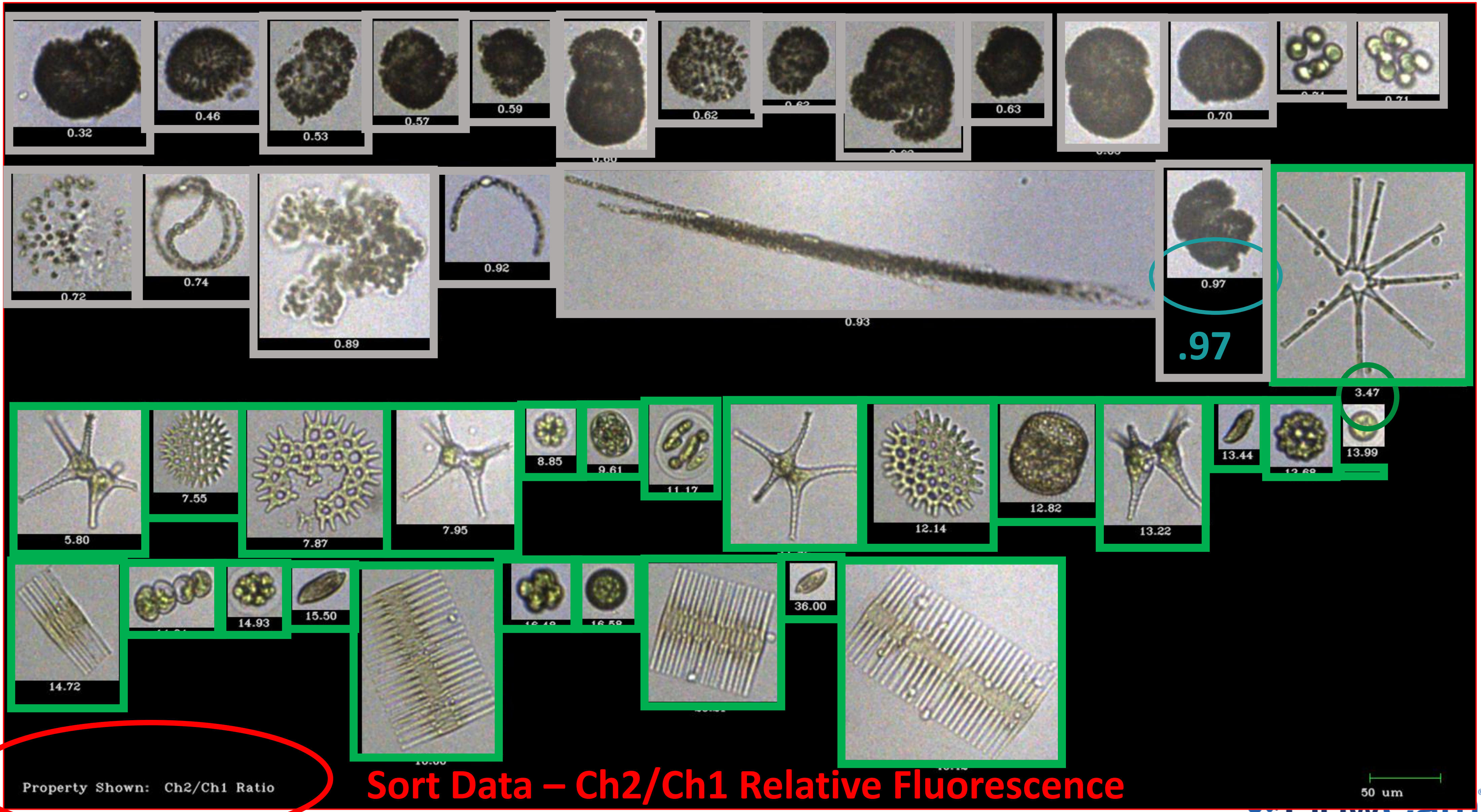
# FlowCam Cyano for HAB Monitoring




- Differentiate and quantify cyanobacteria from other algae (pigment analysis)
- 633 nm laser
- 2 fluorescence channels:
  - Ch 1: 700nm ± 10nm (detects Chlorophyll)
  - Ch 2: 650nm ± 10nm (detects Phycocyanin)

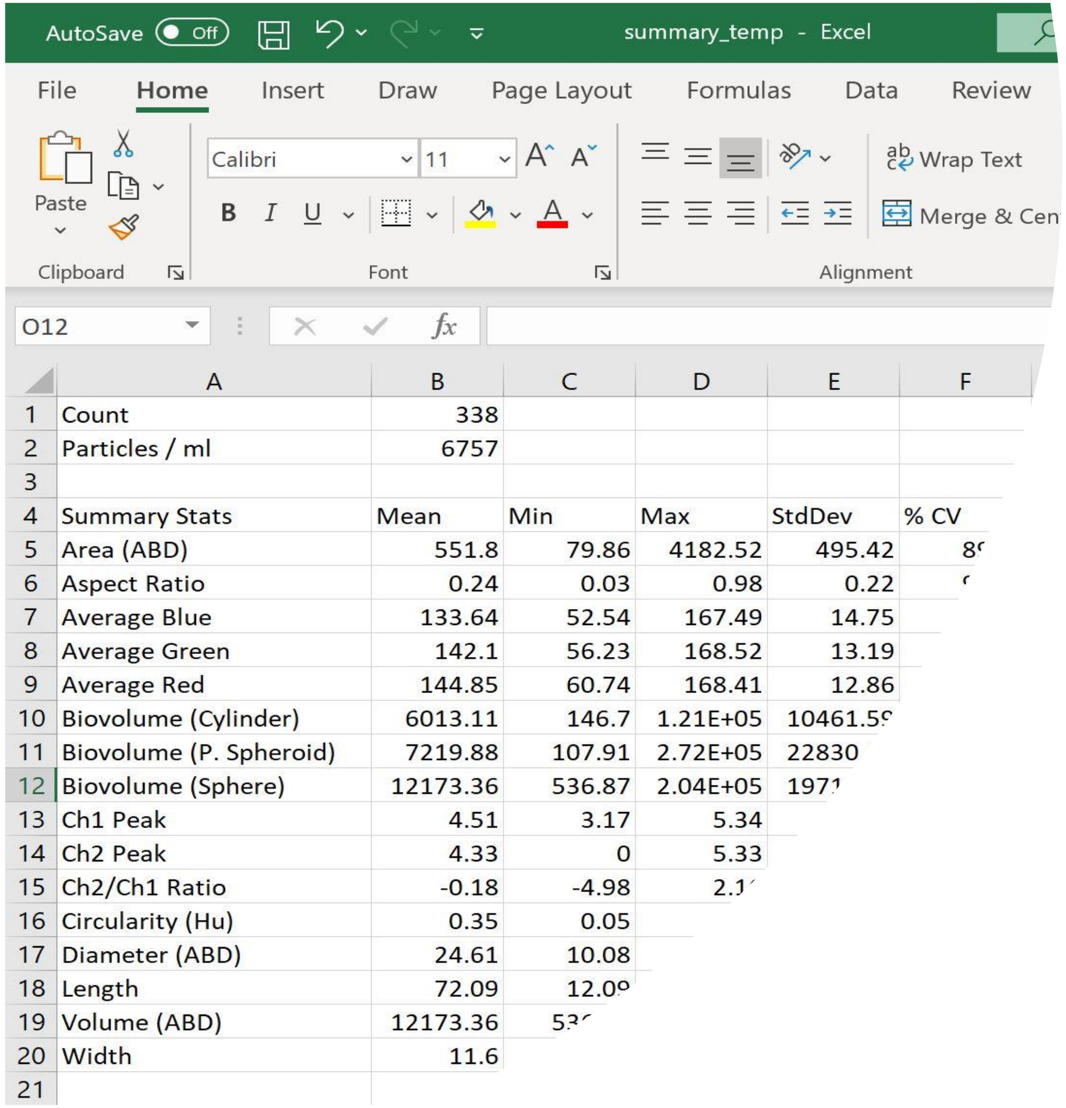
***Ratio of Ch2/Ch1 enables classification (since cyanobacteria can contain chlorophyll).***

# Sort Data – Ch2/Ch1 Relative Fluorescence




3.47

# Reporting: CSV Output




| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Count | 338 | | | | |
| 2 | Particles / ml | 6757 | | | | |
| 3 | | | | | | |
| 4 | Summary Stats | Mean | Min | Max | StdDev | % CV |
| 5 | Area (ABD) | 551.8 | 79.86 | 4182.52 | 495.42 | 8 |
| 6 | Aspect Ratio | 0.24 | 0.03 | 0.98 | 0.22 | |
| 7 | Average Blue | 133.64 | 52.54 | 167.49 | 14.75 | |
| 8 | Average Green | 142.1 | 56.23 | 168.52 | 13.19 | |
| 9 | Average Red | 144.85 | 60.74 | 168.41 | 12.86 | |
| 10 | Biovolume (Cylinder) | 6013.11 | 146.7 | 1.21E+05 | 10461.5 | |
| 11 | Biovolume (P. Spheroid) | 7219.88 | 107.91 | 2.72E+05 | 22830 | |
| 12 | Biovolume (Sphere) | 12173.36 | 536.87 | 2.04E+05 | 1971 | |
| 13 | Ch1 Peak | 4.51 | 3.17 | 5.34 | | |
| 14 | Ch2 Peak | 4.33 | 0 | 5.33 | | |
| 15 | Ch2/Ch1 Ratio | -0.18 | -4.98 | 2.1 | | |
| 16 | Circularity (Hu) | 0.35 | 0.05 | | | |
| 17 | Diameter (ABD) | 24.61 | 10.08 | | | |
| 18 | Length | 72.09 | 12.0 | | | |
| 19 | Volume (ABD) | 12173.36 | 53 | | | |
| 20 | Width | 11.6 | | | | |
| 21 | | | | | | |



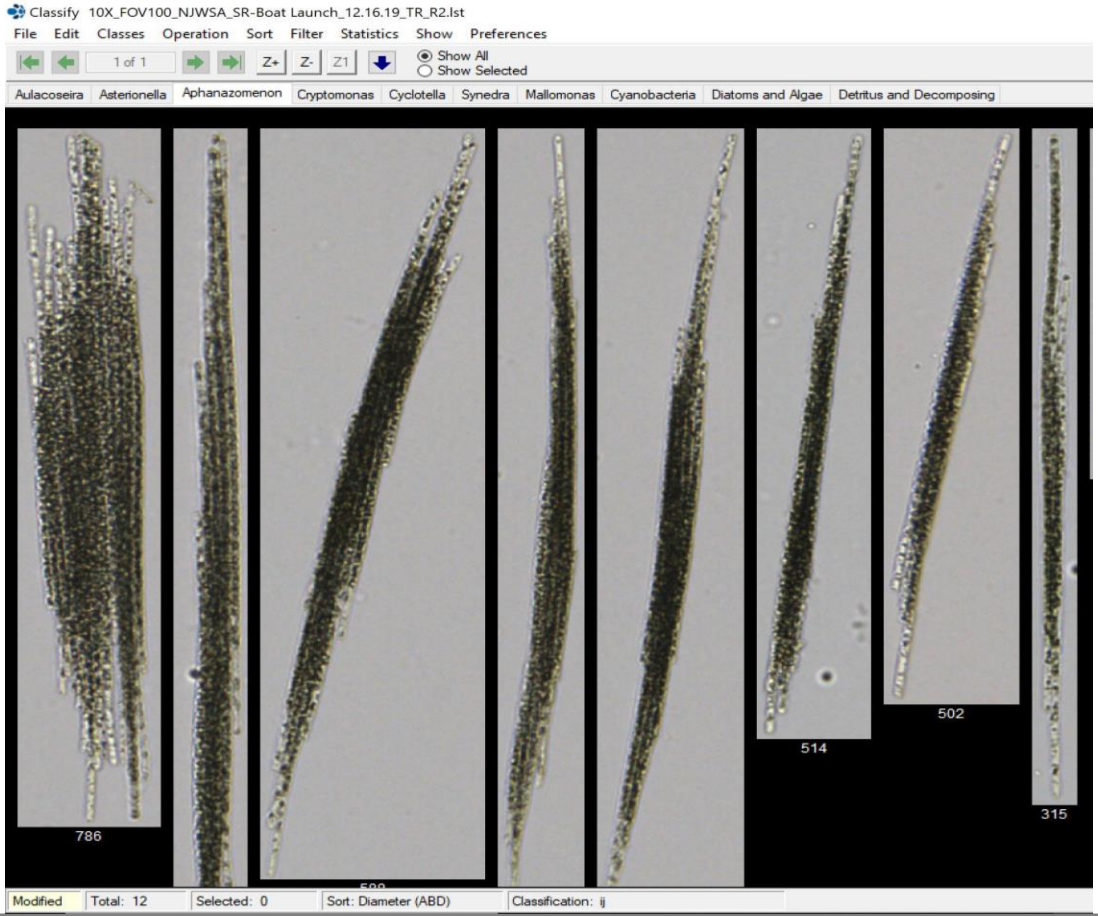
FlowCam®

# Libraries and Filters

- Over time develop libraries of images
  - Semi-automate analysis using filters built from libraries
  - Determination of organisms based on image statistics
  - Libraries and filters function best with an appropriate number of representative images
  - Separate organisms within the same genus that have different morphological characteristics.

# Classification

- Feature dominant nuisance organisms
- FlowCam automatically classifies remaining organisms by functional group:
  - Cyanobacteria
  - Diatoms and Algae
  - Detritus and Decomposing Organisms
  - Genus




Aphanazomenon: Cyanobacteria capable of producing toxins, as well as T&O compounds.



FlowCam®

# Source Water Monitoring

## How does FlowCam Impact Monitoring?

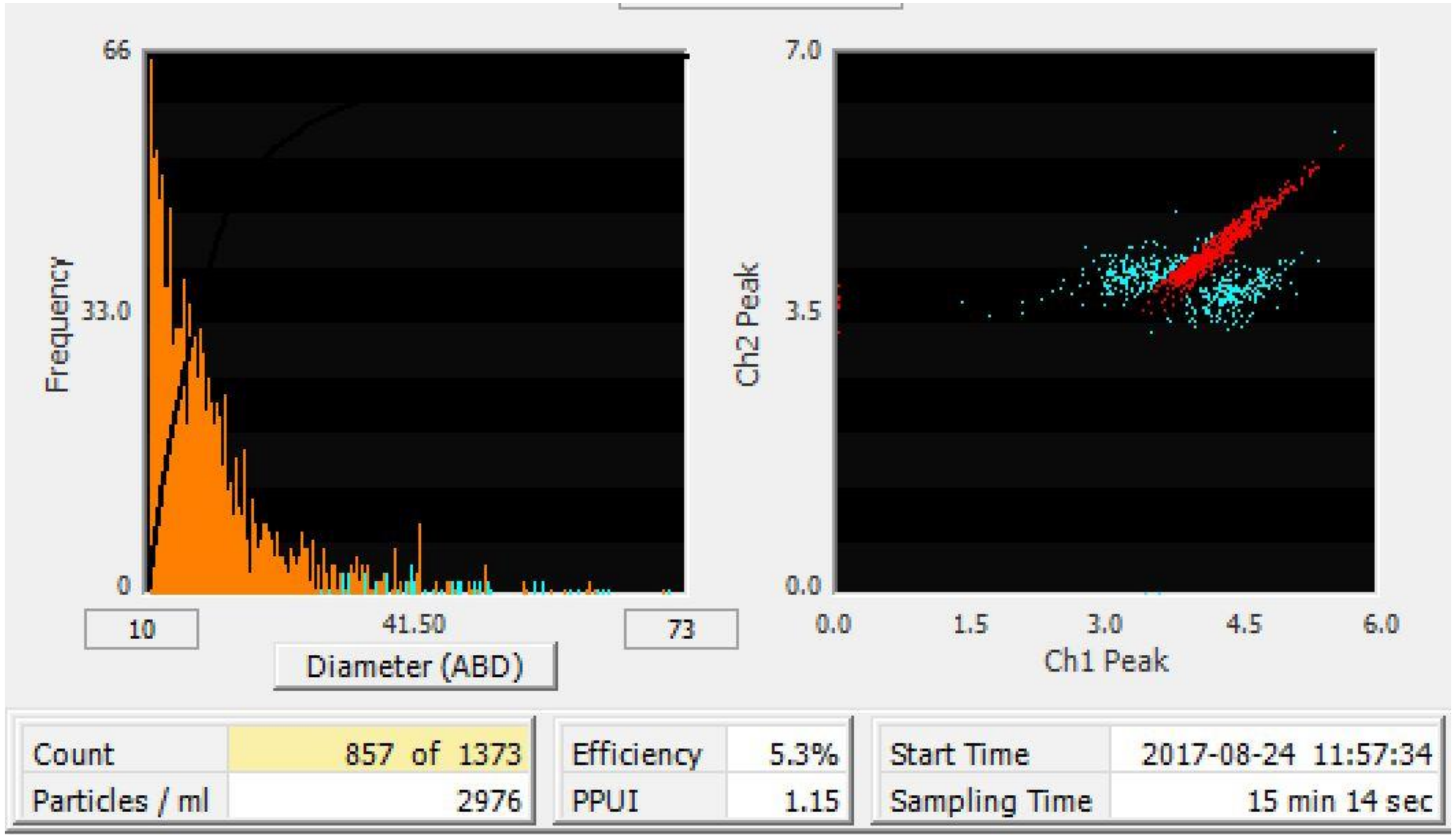
- Utilities can increase sampling frequency, location and depth
  - Cyanobacteria move in the water column to outcompete algae for light. They are simple but evolved, so be strategic!
  - Treatment strategies evolve with data collected at an increased frequency rather than relying solely on large sample volumes.
  - Treat small problems before they become blooms. Small problems are easier to identify with increased sampling frequency

# Freshwater Monitoring of Lakes and Reservoirs for HABs and T&O Compounds

Pose simple questions that were not possible to ask with a microscope:

*"Are we at risk for a significant problem?"*

*Yes/No*




62% of this utility's sample contained cyanobacteria. Could they have a problem?

FlowCam®

# Freshwater Monitoring of Source Water

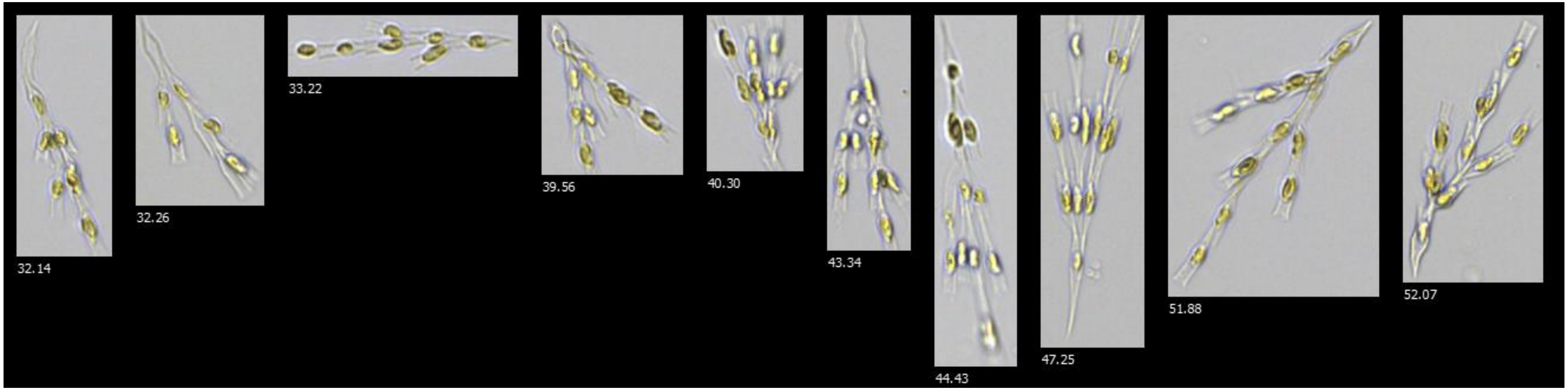
Use FlowCam to determine:

- Which organisms are dominant and likely to cause trouble?
- Produce reports featuring the "troublemakers" for faster turnaround to treatment.
- If more detail is desired, re-analysis can be done later (ex: in the slower winter months).

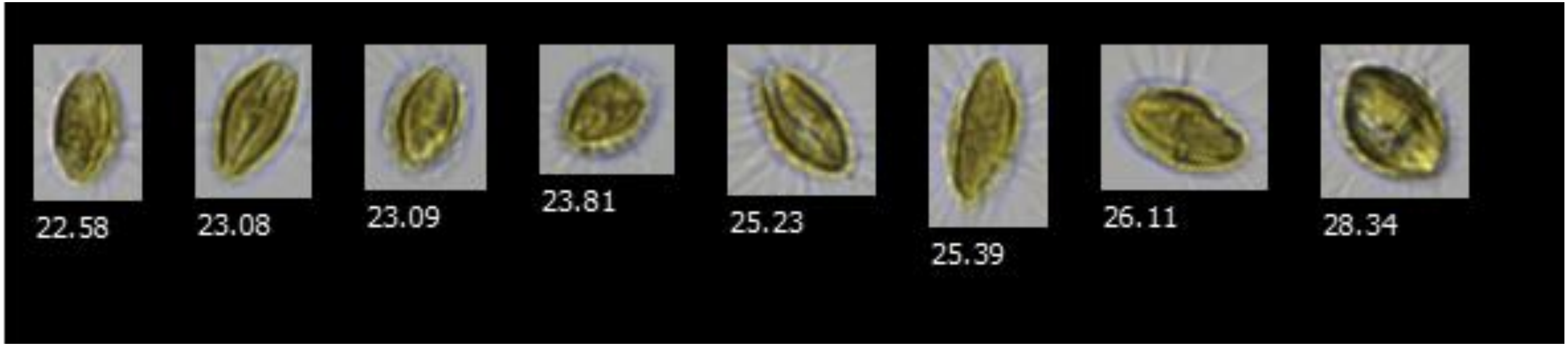
# Agenda

- Algae Monitoring Essentials: T&O, Toxin, Filter Cloggers
- Source Water Monitoring & FlowCam
- Example Images

# Example Library: T&O Producers (Dinobryan)

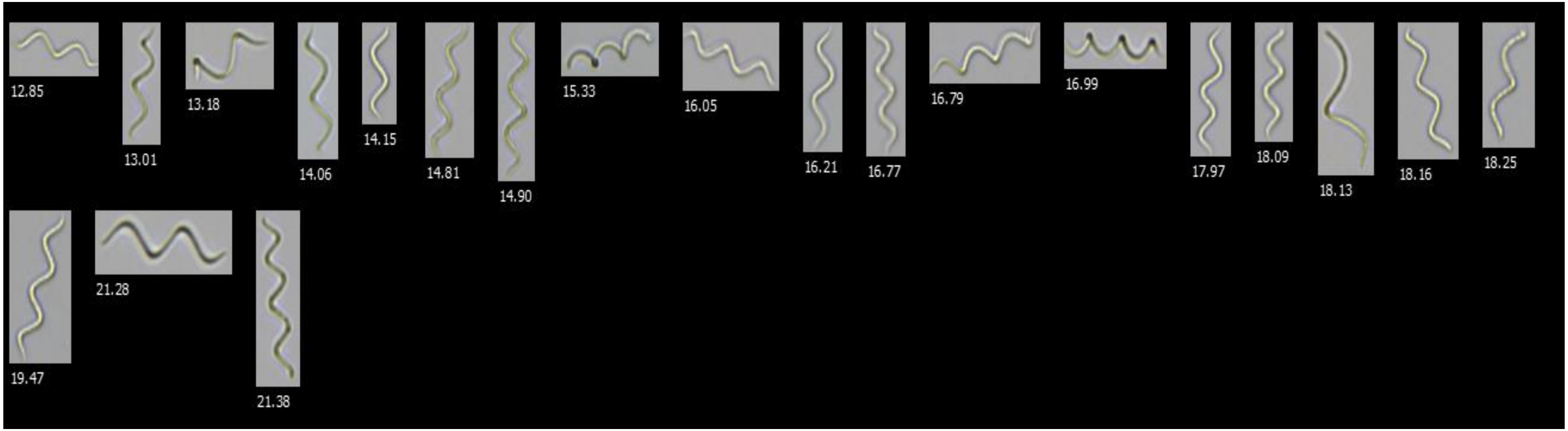


# Example Library: T&O Producers (Mallomonas)

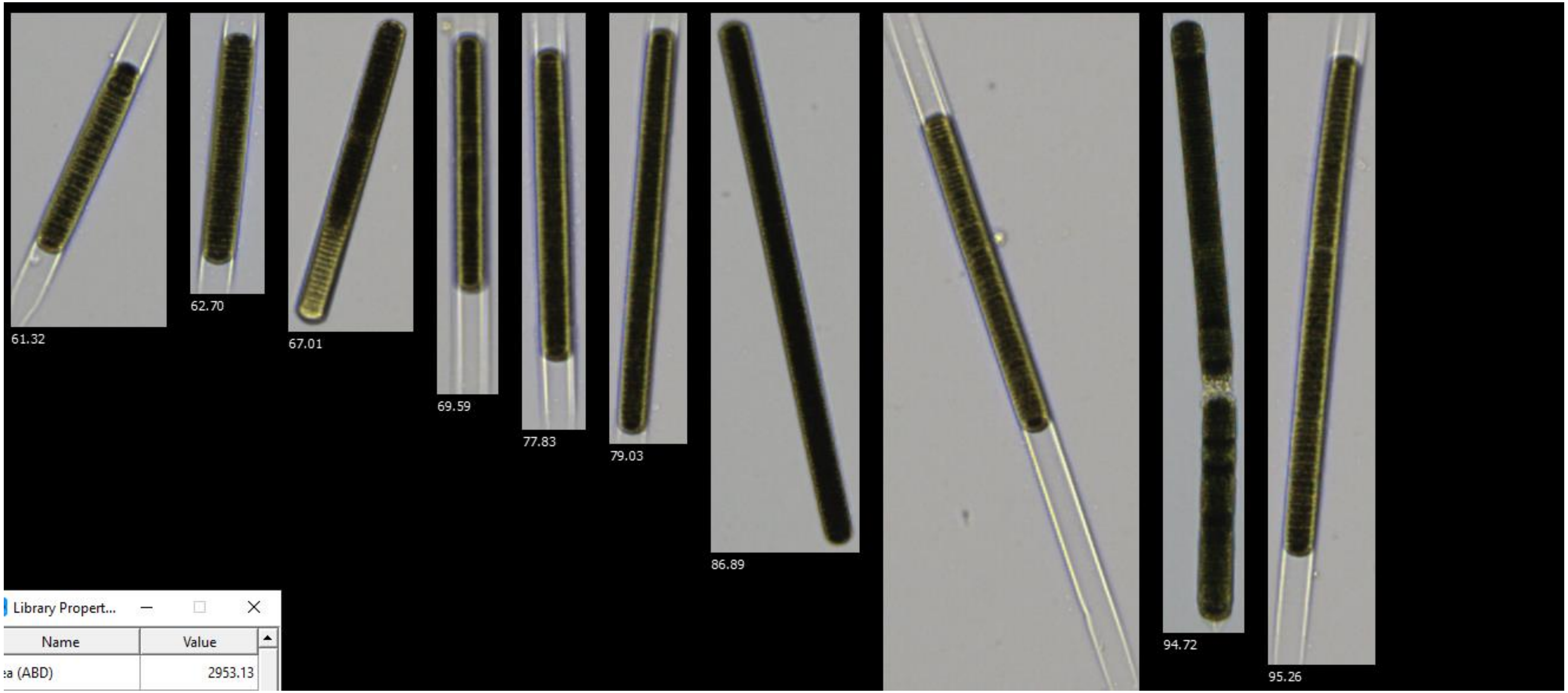


# Example Library: Toxin Producers (Dolichospermum)

# Example Library: Toxin Producers (Cylindrospermopsis)



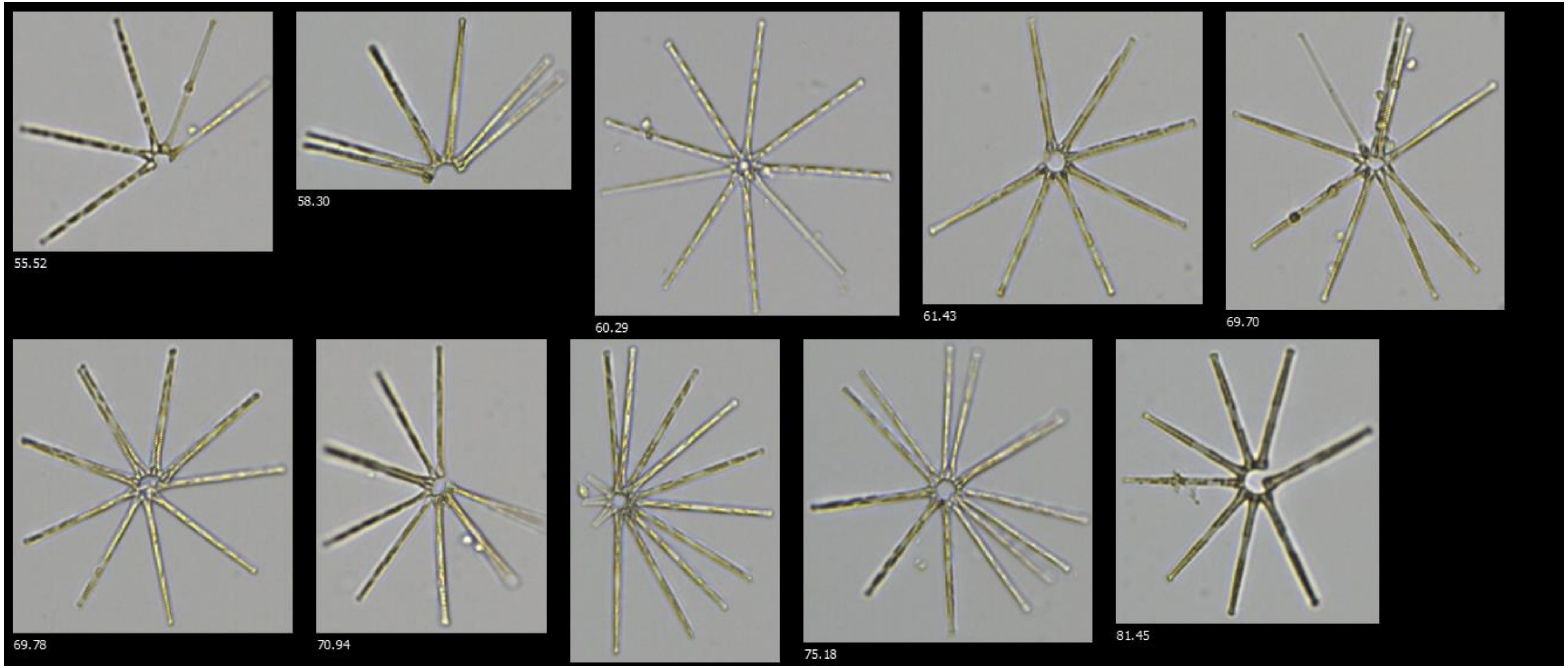
# Example Library Toxin Producers (Lyngbya)





FlowCam®

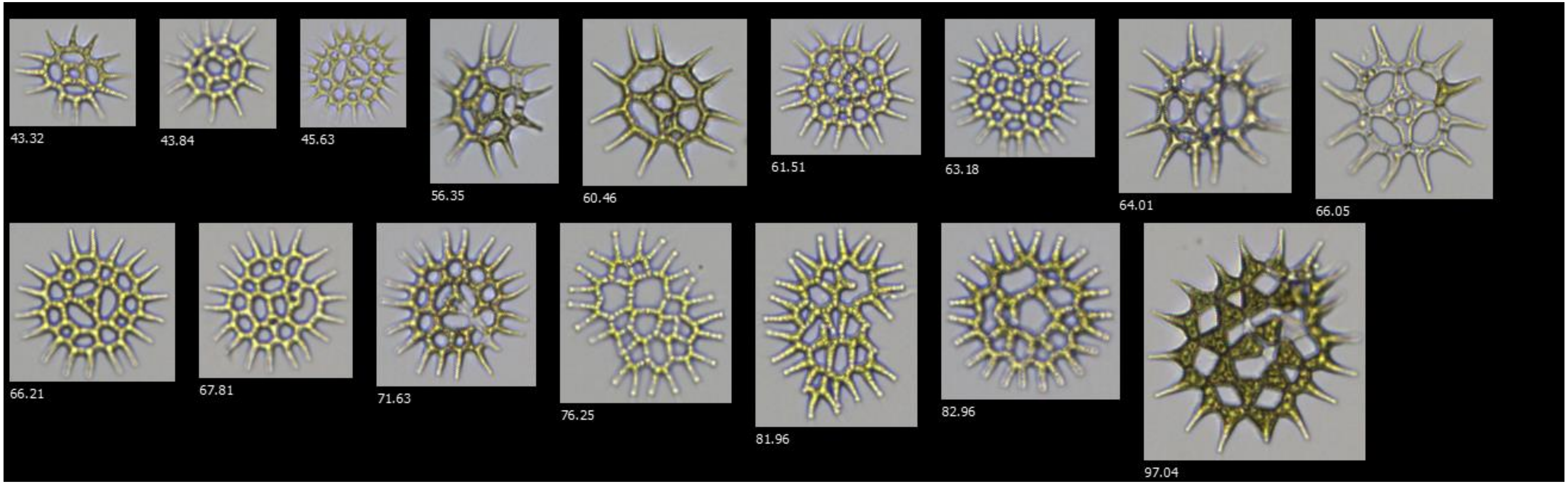
# Example Library: Filter Cloggers (Asterionella)






FlowCam®

# Example Library: Filter Cloggers (Pediastrum)





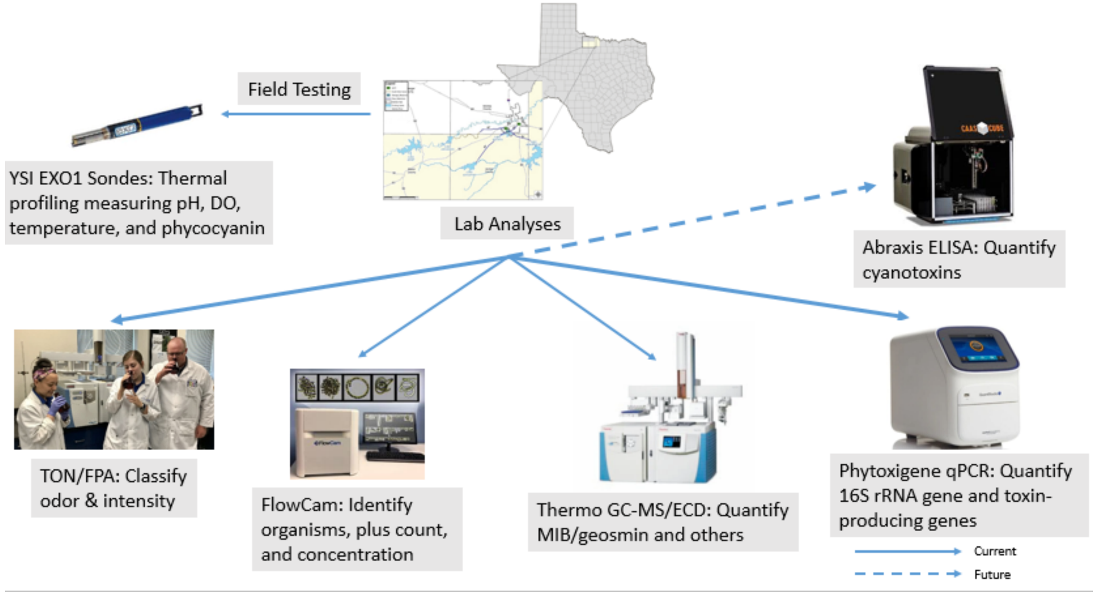
FlowCam®

# Agenda

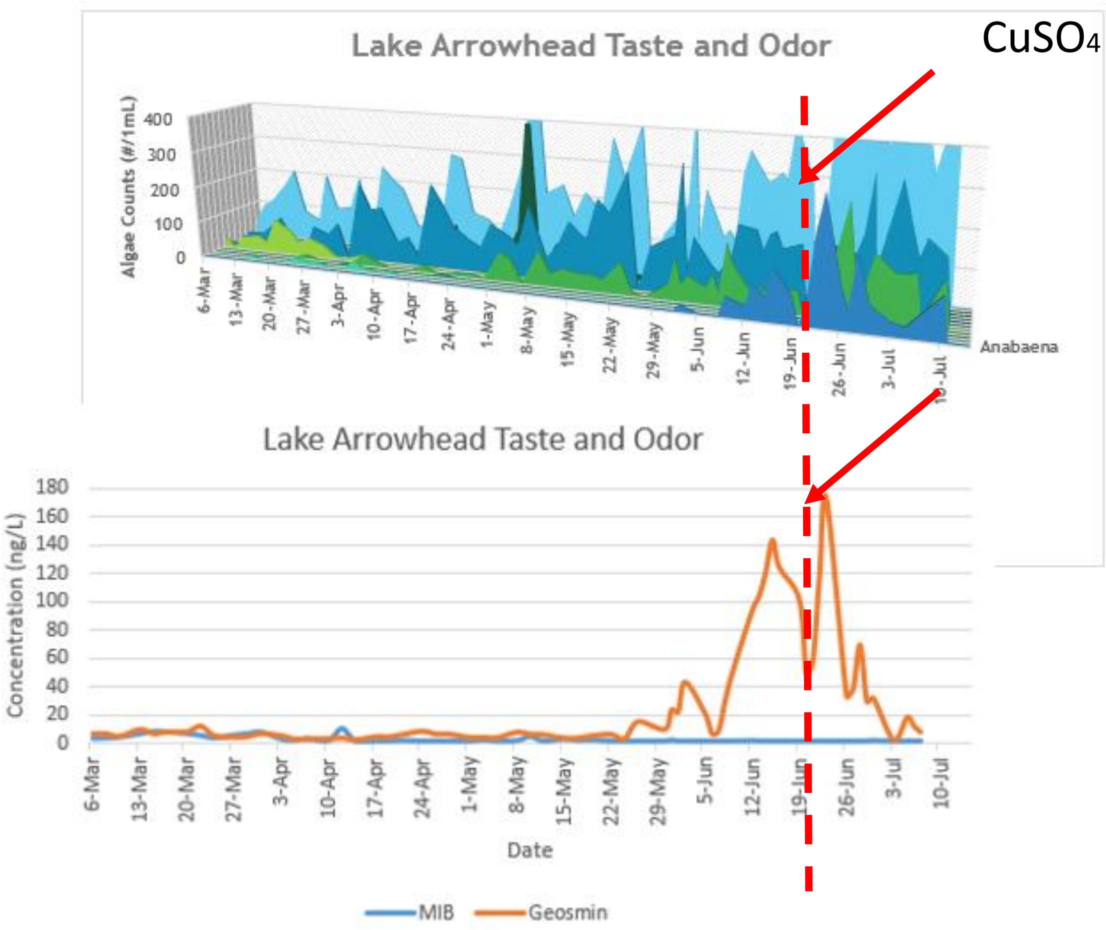
- Algae Monitoring Essentials: T&O, Toxin, Filter Cloggers
- Source Water Monitoring & FlowCam
- Example Images
- Case Studies
- Summary

# Case Study: City of Wichita Falls, TX

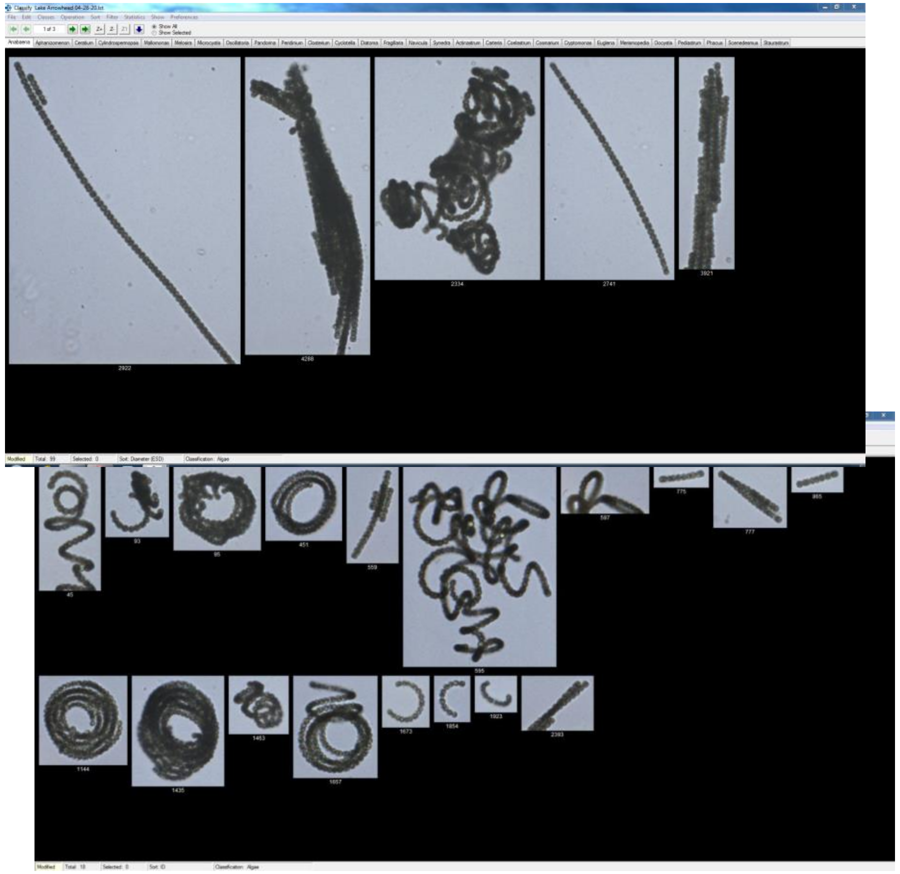
- Sondes measure pH, DO and temp.  Can trigger a trip to the field
- FlowCam used to monitor changes in algae baseline concentrations and create trigger levels
- When trigger levels are tripped, additional testing is performed.



# An Integrated Approach – FlowCam & GC-MS/ECD

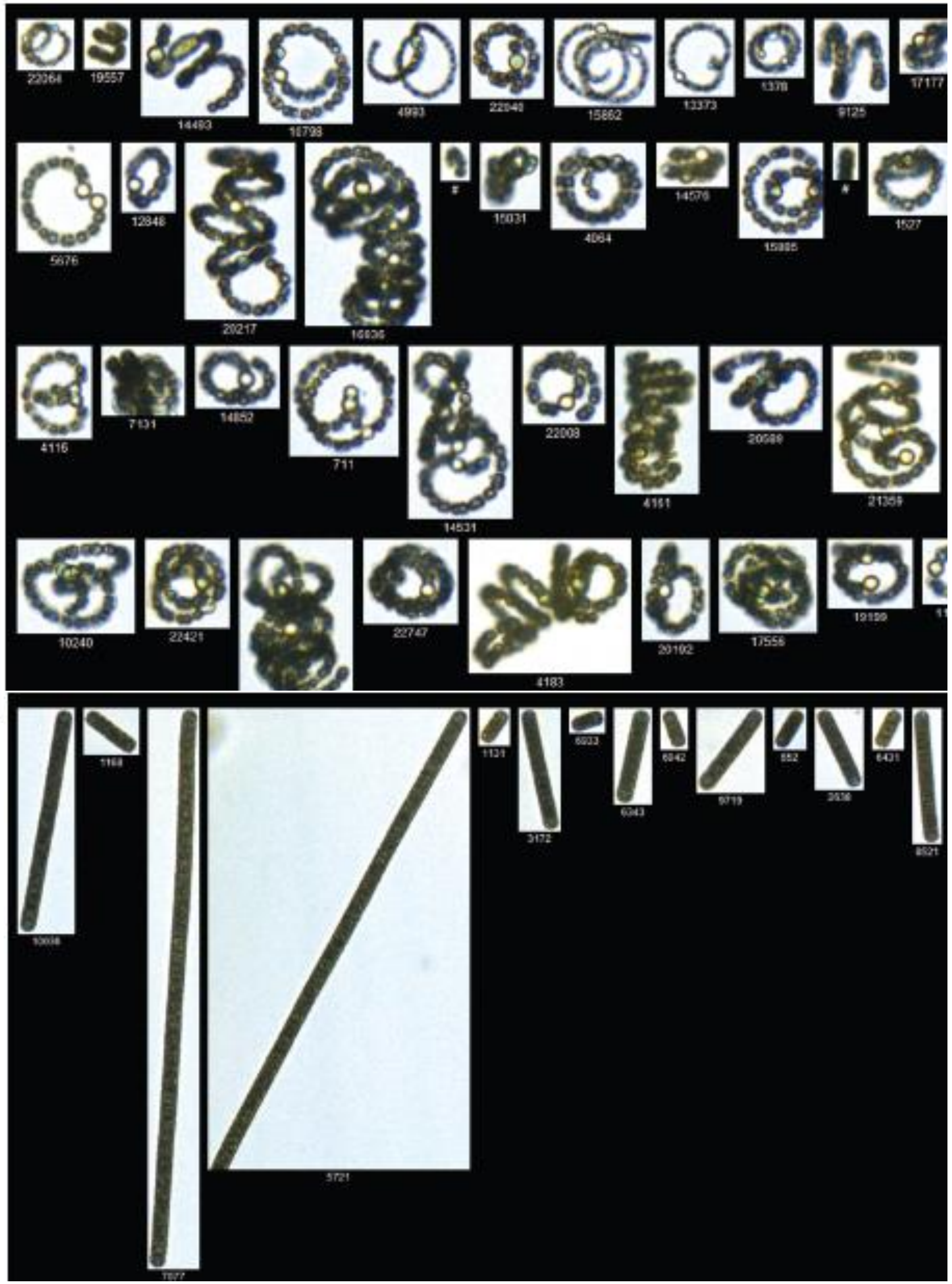



*Anabaena*

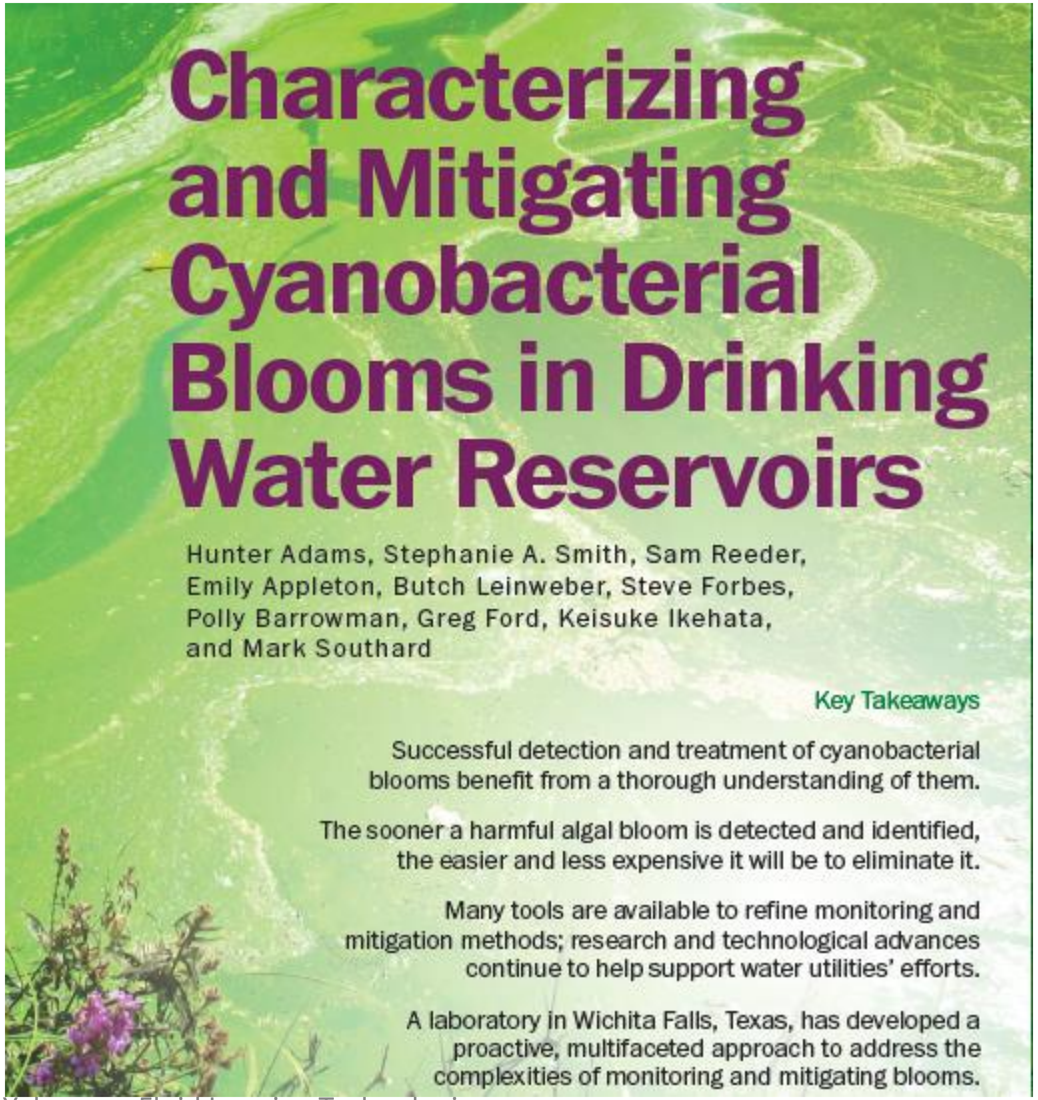


# Case Study: Newport News Waterworks

- Previously using a microscope to monitor algae
  - 1-2 samples/week
- FlowCam enabled them to increase sampling to 7-8 samples/week
  - Increased efficiency
  - Better sample visibility
- Instrument upgrade to a FlowCam Cyano has enabled them to confidently determine Cyanobacteria from other algae and diatoms



# Publications


Characterizing and Mitigating Cyanobacterial Blooms in Drinking Water Reservoirs

Hunter Adams, Stephanie A. Smith, Sam Reeder, Emily Appleton, Butch Leinweber, Steve Forbes, Polly Barrowman, Greg Ford, Keisuke Ikehata, and Mark Southard

Key Takeaways

Successful detection and treatment of cyanobacterial blooms benefit from a thorough understanding of them.

The sooner a harmful algal bloom is detected and identified, the easier and less expensive it will be to eliminate it.

Many tools are available to refine monitoring and mitigation methods; research and technological advances continue to help support water utilities' efforts.

A laboratory in Wichita Falls, Texas, has developed a proactive, multifaceted approach to address the complexities of monitoring and mitigating blooms.

Opflow

Water Quality

hhttps://doi.org/10.1002/opfl.tktk

Frances Buerkens is sales director for water quality markets, Yokogawa Fluid Imaging Technologies (www.fluidimaging.com), Scarborough, Maine. Stephanie A. Smith is product segment manager for YSI Xylem (www.ysi.com), Yellow Springs, Ohio. Greg Ford is director of development for Phytoxigene (www.phytoxigene.com), Akron, Ohio. Hunter Adams is environmental laboratory supervisor for the city of Wichita Falls, Texas (www.wichitafallstx.gov).

Microscopes can do only so much when it comes to monitoring raw water and managing potential hazards such as algal blooms. Technological advances are opening new frontiers in understanding cyanobacteria, leading to improved monitoring and forecasting at levels of efficiency and effectiveness unheard of until now.

BY FRANCES BUERKENS, STEPHANIE A. SMITH, GREG FORD, AND HUNTER ADAMS

## TACKLE TASTE AND ODOR WITH PROACTIVE WATER QUALITY MONITORING

Cyanobacteria

https://doi.org/10.1002/opfl.1113

Hunter Adams, Ashley Cottrell, Sam Reeder, and Mark Southard are with the city of Wichita Falls, Texas (www.wichitafallstx.gov). Frances Buerkens is with Fluid Imaging Technologies (www.fluidimaging.com), Scarborough, Maine.

## Use an Integrated Approach to Monitor Algal Blooms

One drinking water utility found a comprehensive, cost-effective strategy for monitoring toxin-producing cyanobacteria that can harm water quality.

BY HUNTER ADAMS, FRANCES BUERKENS, ASHLEY COTTRELL, SAM REEDER, AND MARK SOUTHARD

# Summary

- FlowCam is a powerful tool for particle analysis
- It can be used to monitor taste and odor and toxin producing organisms as well as filter cloggers in lakes and reservoirs
- It can also be used in the water treatment plant to measure particle size distribution at various points.
- FlowCam can reduce TAT from sample to results, meaning faster response time and reduced treatment cost while offering a visual of the organisms being counted.
- Particle analysis in the treatment plant is an accurate way of monitoring filter performance and adjusting protocols as necessary

# THANK YOU

www.fluidimaging.com
savannah.judge@fluidimaging.com