YOKOGAWA

Yokogawa Fluid Imaging Technologies, Inc.

FlowCam®

Integrated Approach to Monitor Taste and Odor Producing Cyanobacteria

Presented by Yokogawa Fluid Imaging Technologies

August 25, 2020 11:00 a.m – 12:00 p.m. MT

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Webinar Moderator

Billie Emas

Sales Associate

American Water Works Association

Billie Emas is the Sales Associate to the NE and SE territories in the Sales Department at AWWA. She has been with AWWA for six months and she has been corresponding and building relationships with the members, advertisers, exhibitors and sponsors with AWWA. She has over 20 years of experience marketing, sales, event planning and membership. Billie has a BS in Business Administration from Bowling Green State University.

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Panel of Experts

Hunter Adams
Environmental Laboratory Supervisor
Cypress Environmental Laboratory - City of Wichita Falls, TX

Frances Buerkens
Director of Sales - Water Quality Markets
Yokogawa Fluid Imaging Technologies

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Agenda

I. **Integrated Approach to Monitor Algal Blooms**
Hunter Adams, City of Wichita Falls, TX

II. **Semi-automated Method for Monitoring Taste and Odor Organisms and Cyanotoxin Producers**
Frances Buerkens, Yokogawa Fluid Imaging Technologies

Time Permitting – Q&A

Enter your **question** into the **question pane** at the lower right-hand side of the screen.

Please specify to whom you are addressing the question.

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Integrated Approach to Monitor Algal Blooms

Wichita Falls
TEXAS
Blue Skies. Golden Opportunities.

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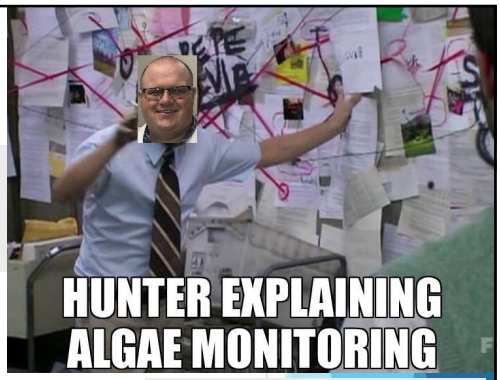
Integrated Toolkit

YSI EXO1 Sondes: Chlorophyll and Phycocyanin sensors can trigger a trip to the field

FlowCam Cyano: identify organisms, plus count, and concentration



Toxin Path

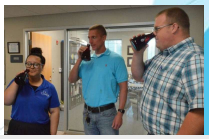
T&O Path

Thermo GC-MS/ECD: quantify MIB/geosmin and others

Thermo LC-MS/MS: quantify toxins

Abraxis Dip Sticks: Presence/Absence

Thermo qPCR: Phytoxigene to quantify toxin risk



TON/FPA: Classify odor & intensity

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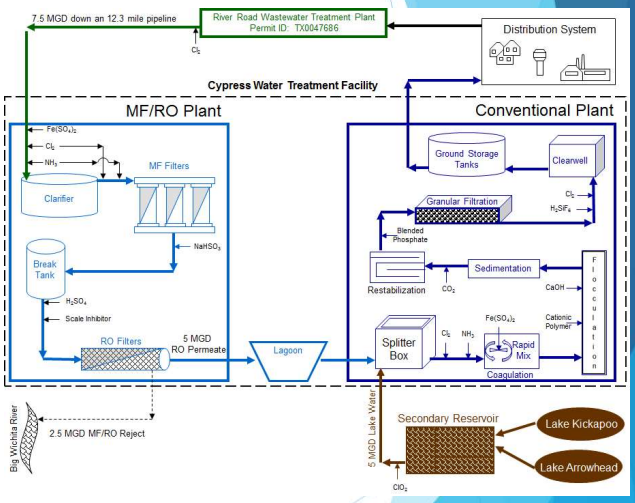
Timeline

- 1966 – July 7, 2014
  - Regular T&O cycles: > 2 per year
- July 7, 2014 – July 21, 2015
  - DPR – No T&O cycles or complaints



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Timeline

- July 21, 2015 – Fall 2016
  - Worst T&O event in memory

Is Your Water Smelly and Foul Tasting? Here's Why

by: Staff
Posted: Aug 8, 2016 / 06:26 PM CDT / Updated: Aug 8, 2016 / 06:26 PM CDT

Wichita Falls citizens express concerns over tap water

Yes, Wichita Falls' water is the yuckiest it has ever been

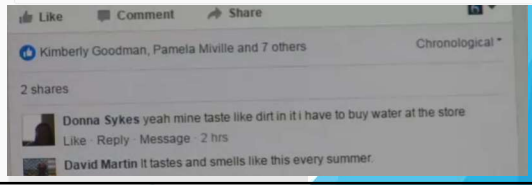
Claire Kowalick, Times Record News Published 4:00 p.m. CT Aug. 17, 2016

CONNECT TWEET LINKEDIN COMMENT EMAIL MORE

'When's the water going to taste better?' is a question on many Wichita Falls residents' lips. Maybe by the end of the week appears to be the best guess from the city. Public Works Director Russell Schreiber said a 'perfect storm' of conditions has created the worst conditions in more than 20 years for water taste and smell.

Lifeline: Severity of city water odor calls for three-chemical treatment

John Ingle, Times Record News Published 12:00 p.m. CT Aug. 18, 2016



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The Origin - 2016 T&O Events

February 2016

- Algae bloom
- Geosmin > 400 ng/L
  - Source and Tap
- Duration: 1 week
- Complaints: 5/day

October 2016

- *Anabaena* bloom
- MIB explosion
  - Source > 1700 ng/L
  - Tap > 250 ng/L
- Duration: 3 weeks
- Complaints: Hundreds

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Timeline

- Fall 2016 – Present
  - Current T&O program
  - Total complaints: ZERO
  - Total days: 1,415

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Time for a New Approach

- Investigation
  - 1. Are algae or cyanobacteria present?
  - 2. What quantity of algae or cyanobacteria are present?
  - 3. Can the organisms produce T&O compounds or cyanotoxins?
  - 4. What is the concentration of T&O compounds or cyanotoxins?
  - 5. How do we know if we have a problem and how do we mitigate the problem?

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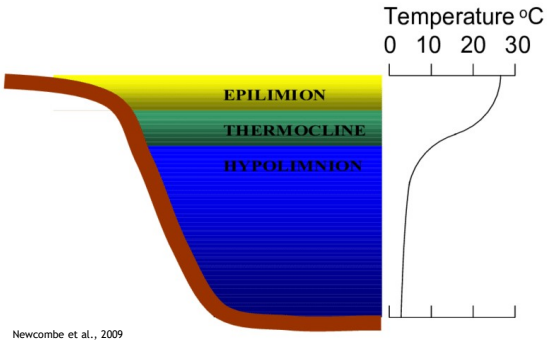
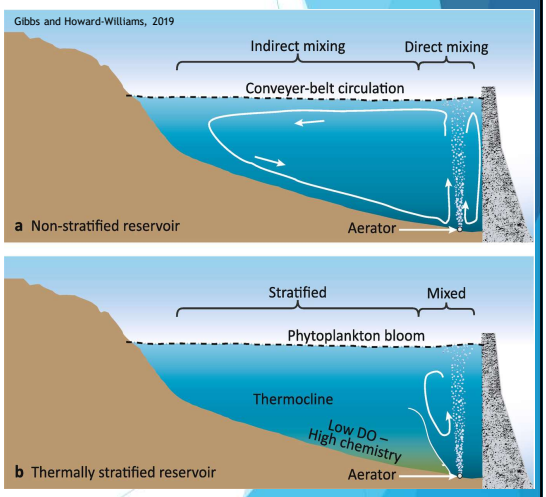
An Integrated Approach – YSI EXO1 Sonde

- Reservoir Management - Thermal Profiling





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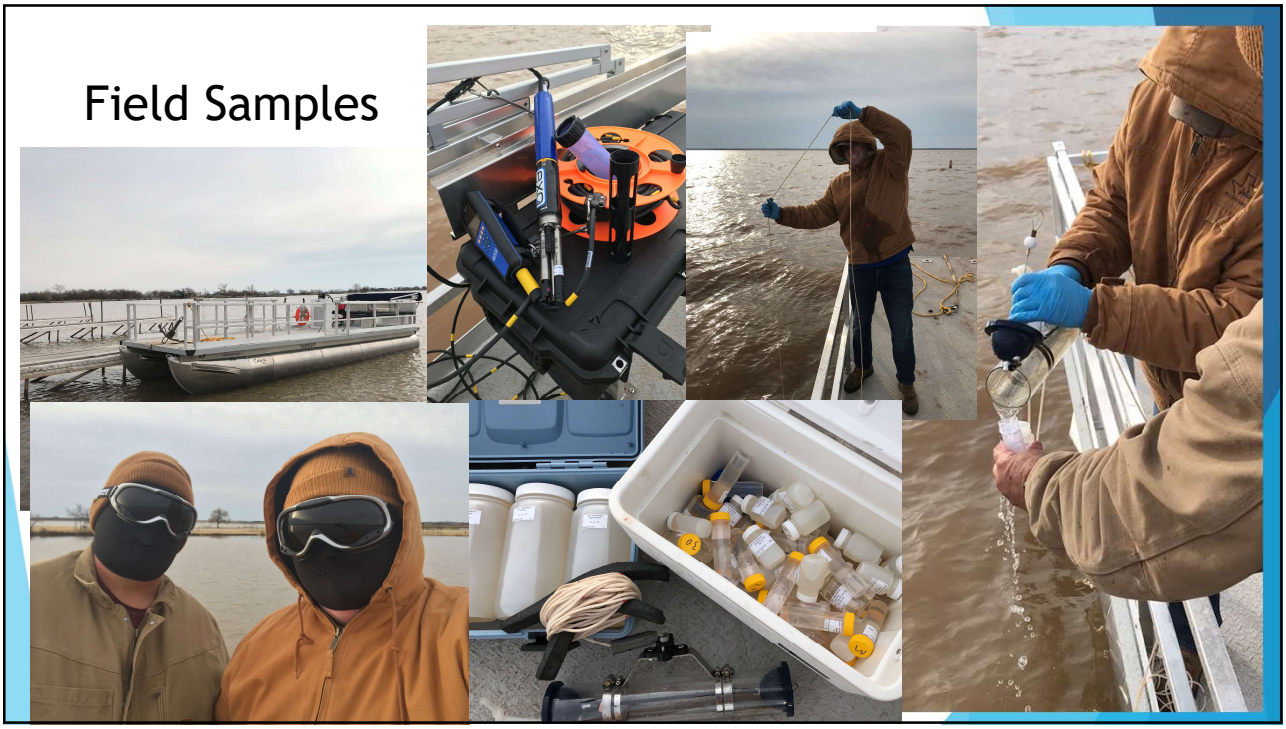
Field Samples



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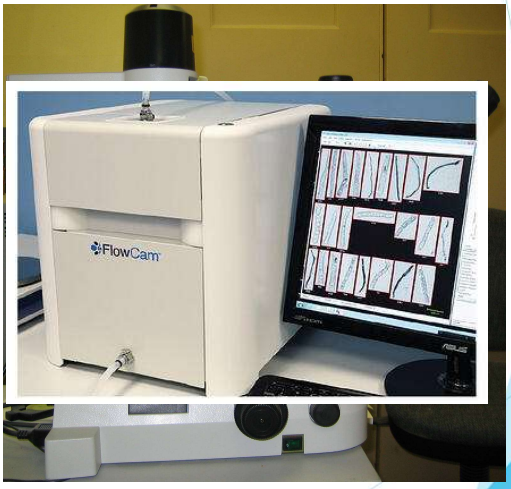
An Integrated Approach - FlowCam

FlowCam – Fluid Imaging Technologies

- Images and enumerates algae and cyanobacteria
- Classify into categories:
  - T&O algae and cyanobacteria
  - Toxin-producing cyanobacteria
  - Filter-clogging algae
  - Green algae
- 3 min per sample
- Saved run files



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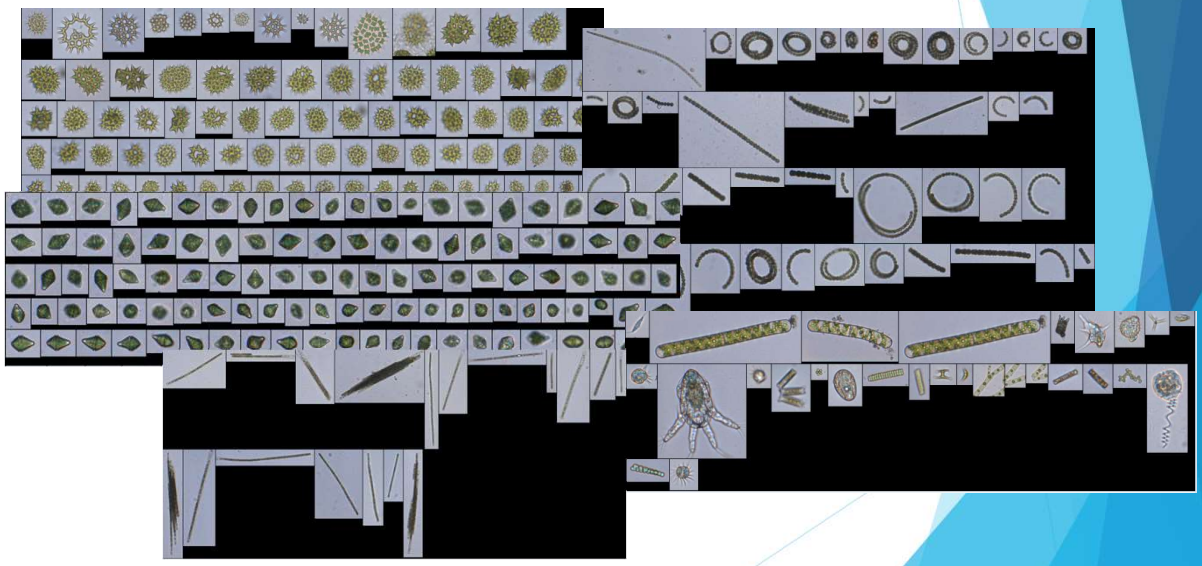
An Integrated Approach – FlowCam



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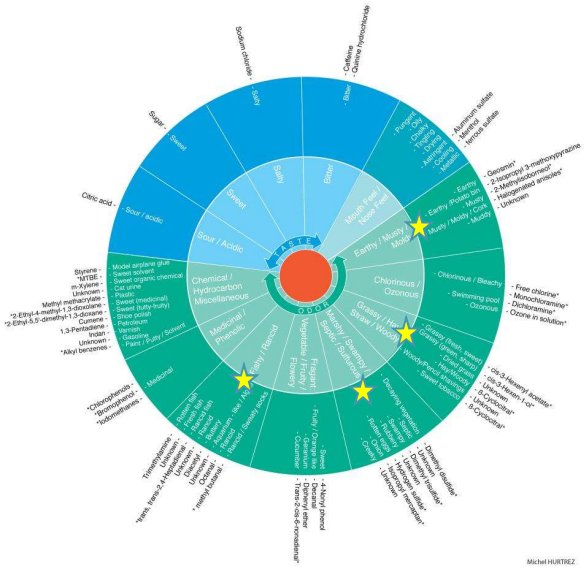
An Integrated Approach – GC-MS/ECD



GC-MS/ECD – Trace 1310 & ISQ QD

- Earthy/Musty/Moldy - SPME
  - IBMP, IPMP, Geosmin, MIB, 2,4,6-TCA
- Grassy/Hay/Straw/Woody - SPME
  - cis-3-Hexenyl acetate, cis-3-Hexen-1-ol, ß-cyclocitral
- Fishy/Rancid - SPME
  - Octanal, Isovaleraldehyde, Trimethylamine hydrochloride, trans, trans-2-4-Heptadienal
- Moldy/Swampy/Septic/Sulfurous - HS
  - Dimethyl disulfide, Dimethyl trisulfide, Isopropyl mercaptan

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An Integrated Approach – GC-MS/ECD

T&O Events Since Fall 2016

- May 2017 – Geosmin 40 ng/L
- June 2017 – Geosmin 175 ng/L
- January 2018 – MIB 17 ng/L
- June 2018 – Geosmin 37 ng/L
- July 2018 – Geosmin 65 ng/L
  MIB 21 ng/L
- December 2018 – MIB 19 ng/L
- March 2019 – Geosmin 24 ng/L
- April 2019 – Geosmin 1,268 ng/L
- June 2019 – Geosmin 150 ng/L
- July 2019 – Geosmin 67 ng/L
- February 2020 – Geosmin 304 ng/L
- March 2020 – Geosmin 3,387 ng/L
- April 2020 – Geosmin 14,897 ng/L

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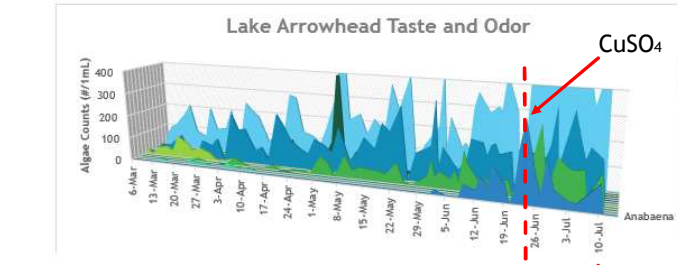
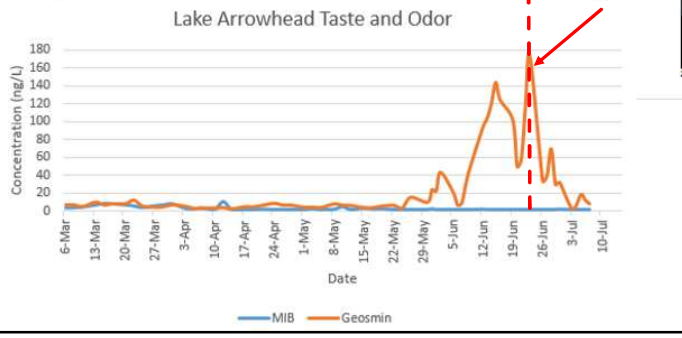
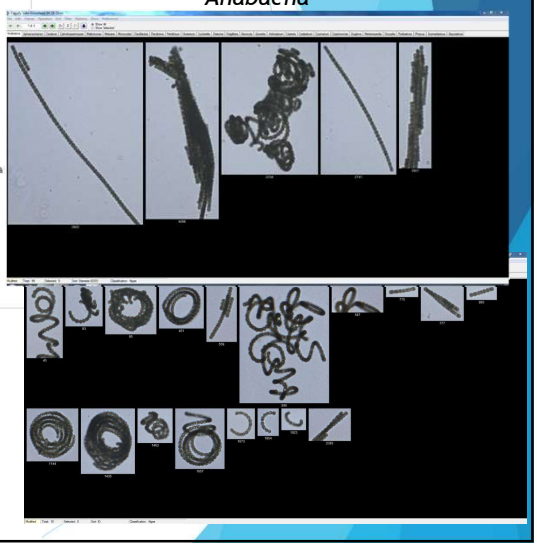
An Integrated Approach – FlowCam & GC-MS/ECD





Anabaena



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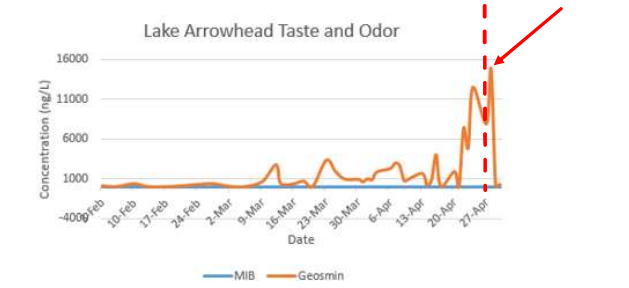
An Integrated Approach – FlowCam & GC-MS/ECD



Anabaena soup

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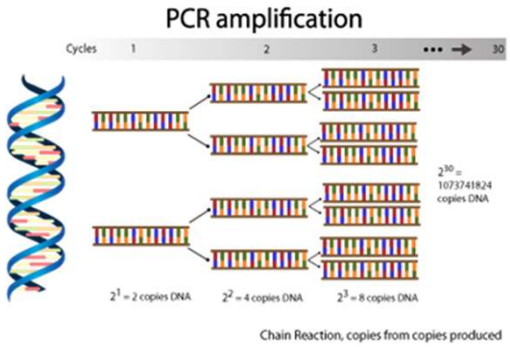
An Integrated Approach – qPCR

qPCR – QuantStudio 5

- Method: EPA 705.0
- Kit: Phytoxigene CyanoDTec
- Detection and quantification of cyanotoxin-producing genes
  - 2 surface reservoirs
  - 1 secondary reservoir
- 60 min of sample prep
- 70 min per sample run



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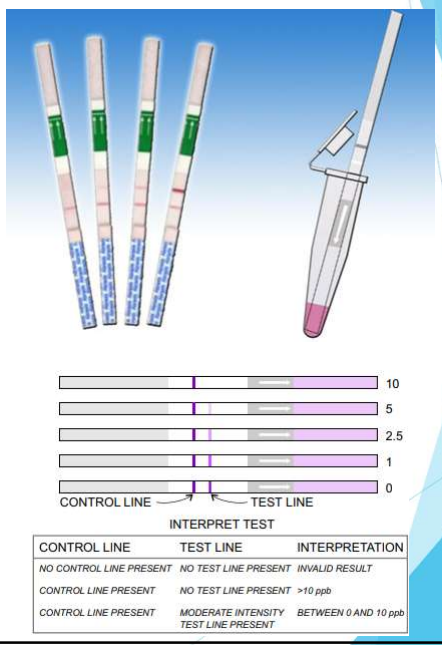
qPCR & LC-MS/MS Intermediary

Abraxis Test Strips

- Semi-quantitative/Qualitative
- Rapid screening
  - Results < 1 hr
- Field-friendly
- Kits
  - Anatoxin-a
  - Cylindrospermopsin
  - Microcystins/Nodularins
- Cost effective
  - $20-$40/test



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An Integrated Approach – qPCR & LC-MS/MS

LC-MS/MS

- Eurofins Eaton Analytical- EPA 545 & 546
- As needed based on qPCR results
- July 11, 2018
  - Lake Arrowhead
    - Microcystin-LA: 0.015 µg/L
  - Cypress WTP
    - Non-detect
  - Jasper WTP
    - Microcystin-RR: 0.007 µg/L
- July 9, 2019
  - Lake Kickapoo
    - Microcystin-LR: 0.100 µg/L
    - Microcystin-RR: 0.177 µg/L
    - Microcystin-YR: 0.034 µg/L
  - Cypress & Jasper WTPs
    - Non-detect

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Monitoring Plan – An Integrated Approach

Analyses

- Algae Counts – FlowCam
- Modified TON & FPA – SM 2150B & 2170
- Actinomycetes – SM 9250B*
- GC-MS/ECD SIM
  - Earthy/Musty/Moldy Category
    - Geosmin, MIB, IPMP, 2,4,6-TCA
- GC-MS/ECD Scans*
  - Grassy/Hay/Straw/Woody Category
  - Marshy/Swampy/Septic/Sulfurous
  - Fishy/Rancid
- qPCR – EPA 705.0*
- Abraxis Dip Sticks**

Frequency

- December – February: 1x/week
- March – May: 2x/week
- June – August: 3x/week
- September – November: 2x/week

- *Performed weekly
- **Performed as needed

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Monitoring Plan – An Integrated Approach

Triggers

- Algae Counts >200-500/mL
  - Copper Sulfate/Citric Acid treatment
- TON/FPA
  - Additional GC-MS/ECD scans
- Geosmin/MIB >10 ng/L
  - Daily testing
- Customer Complaints >3/day
  - Additional TON/FPA and GC-MS/ECD scans

Distribution System

- Triggered by Customer Complaints
- RTCR BacT sites & NAP sites
  - TON/FPA, Actinomycetes
- Flushing as needed

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Monitoring Plan – An Integrated Approach

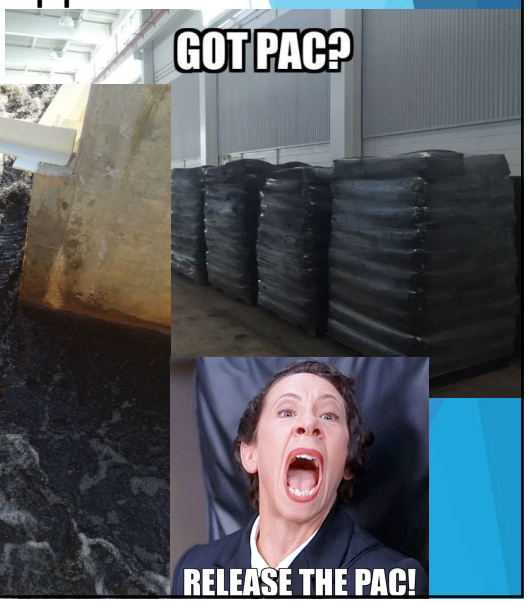
Water Treatment Plants

- Potassium Permanganate - 0.4 mg/L
  - Recovery Well and Clarifier Weirs
- Powdered Activated Carbon – 10 mg/L
  - Prior to coagulation – Raw Feed

Press Release

- Public Works Director
- Public Utilities Operations Manager
- Water Purification Superintendent

Clarifier Effluent…



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Contact Info

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- Mark Southard, City of Wichita Falls – Water Source/Purification Superintendent
- Sam Reeder, City of Wichita Falls – Senior Lab Tech
- Cali Barton, City of Wichita Falls – Lab Tech
- Daniel Nix, City of Wichita Falls – Public Utilities Operations Manager

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semi-automated method for monitoring taste and odor organisms and cyanotoxin producers

FlowCam®

YOKOGAWA
Yokogawa Fluid Imaging Technologies, Inc.

Frances.Buerkens@fluidimaging.com
www.fluidimaging.com

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Sample Multiple Locations in Reservoir

Chlorophyll and Phycocyanin sondes help justify when a trip to the field is required.

FlowCam Cyano: identify organisms, plus count, and concentration.

T&O and Toxin Monitoring Pathways

Toxin Path

T&O Path

Thermo LC-MS/MS

Abraxis Dip Sticks

qPCR: Phytoxigene to quantify toxin risk

Thermo GC-MS/ECD to quantify MIB/Geosmin

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What is the FlowCam Cyano?
Semi-automated microscope used to identify and quantify cyanobacteria, diatoms and nuisance algae
FlowCam invented in 1999 to research phytoplankton in Maine
FlowCam Cyano developed 3 years ago as a rapid response tool for utilities - and to address flaws in previous models

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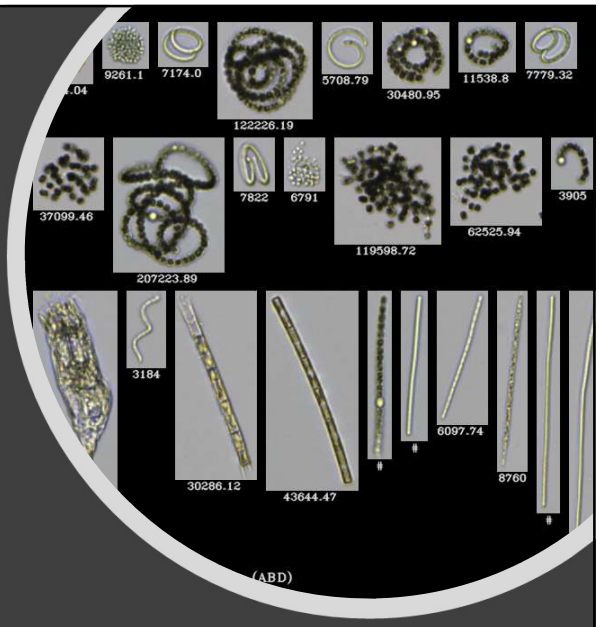
Organize data using morphological features:

- Size, shape, color
- Pigmentation:
  - chlorophyll (Algae)
  - phycocyanin (Cyanobacteria)



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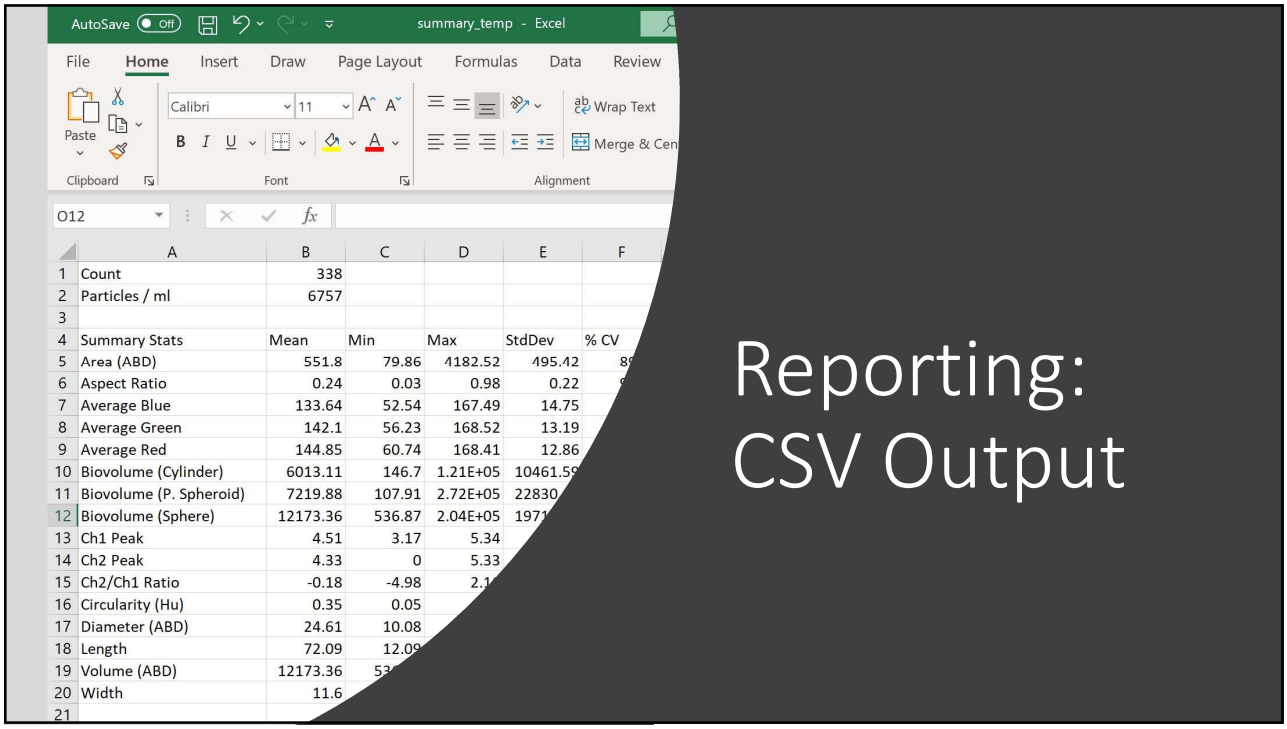

Reporting: CSV Output

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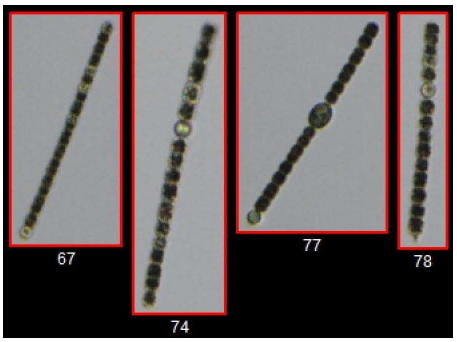
Biology 101 for Source Water Managers

- Cyanobacteria and "blue green algae" are synonymous.
- Many common taste and odor producers are cyanobacteria. (*We're looking at you, Anabaena!*)
- The only method to determine whether cyanobacteria are producing toxins *at that moment* is to test for toxins.



Anabaena: Capable of producing MIB and Geosmin, cyanotoxins: anatoxin, microcystin, saxitoxin, and cylindrospermopsin.

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FlowCam Cyano vs. Microscope: *Limitations*

- 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

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FlowCam Cyano vs. Microscope: *Benefits*

- 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

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How does FlowCam Cyano impact monitoring?

Utilities can increase sampling frequency, location, and depth:

- Cyanobacteria move in the water column to outcompete algae for light and nutrients. They are simple but evolved, so we must be strategic too!
- 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 by increasing sample frequency.

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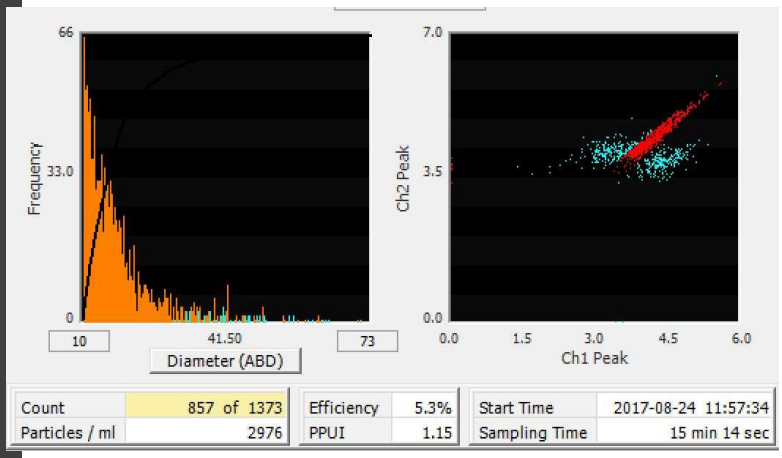
How does FlowCam Cyano impact monitoring?

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 (red dots on plot). Could they have a problem?

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How does FlowCam Cyano impact monitoring?

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).

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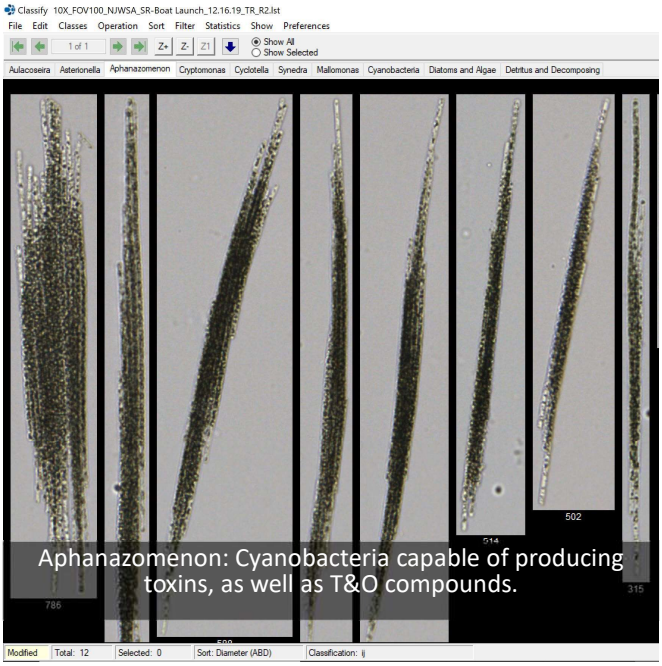
Management Report

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



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Cyanotoxin Monitoring

- Quantity of cells (or a bloom) does not indicate toxicity.
- Toxin producing genes are not always active but can "turn on". These biological mechanisms are not understood by the scientific community.
  - Quantitative analysis to predict toxicity can be done with qPCR kits by Phytoxigene
- ELISA or LC/MS for toxin testing

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Manage Risk: Cyanotoxin Monitoring

- If there are no toxin producers, the risk of toxicity is low. (Dilution can mitigate small scale toxin events.)
- Identify and quantify cyanobacteria with FlowCam.
  - No cost per sample on the FlowCam – affordable tool to quantify risk
  - If there is an abundance of cyanobacteria, then do a toxin test.
  - If there is little/no cyanobacteria, a toxin test may not be relevant.
  - $75-200+ per toxin test adds up to thousands spent each season….

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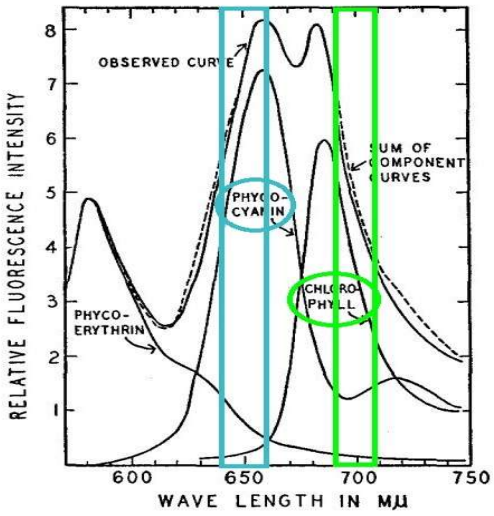

FlowCam Cyano

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

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

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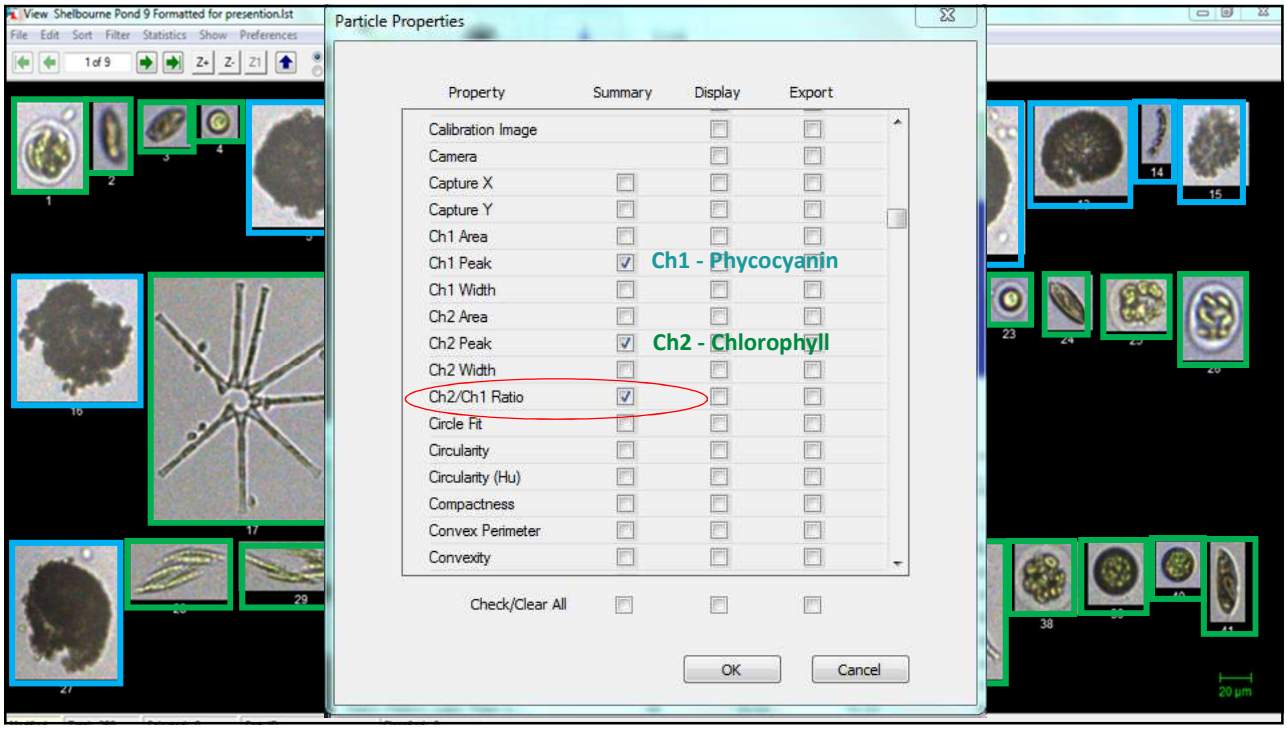
Particle Properties
Property
Summary
Display
Export
Calibration Image
Camera
Capture X
Capture Y
Ch1 Area
Ch1 Peak
Ch1 Width
Ch2 Area
Ch2 Peak
Ch2 Width
Ch2/Ch1 Ratio
Circle Fit
Circularity
Circularity (Hu)
Compactness
Convex Perimeter
Convexity
Check/Clear All
OK
Cancel
Ch1 - Phycocyanin
Ch2 - Chlorophyll

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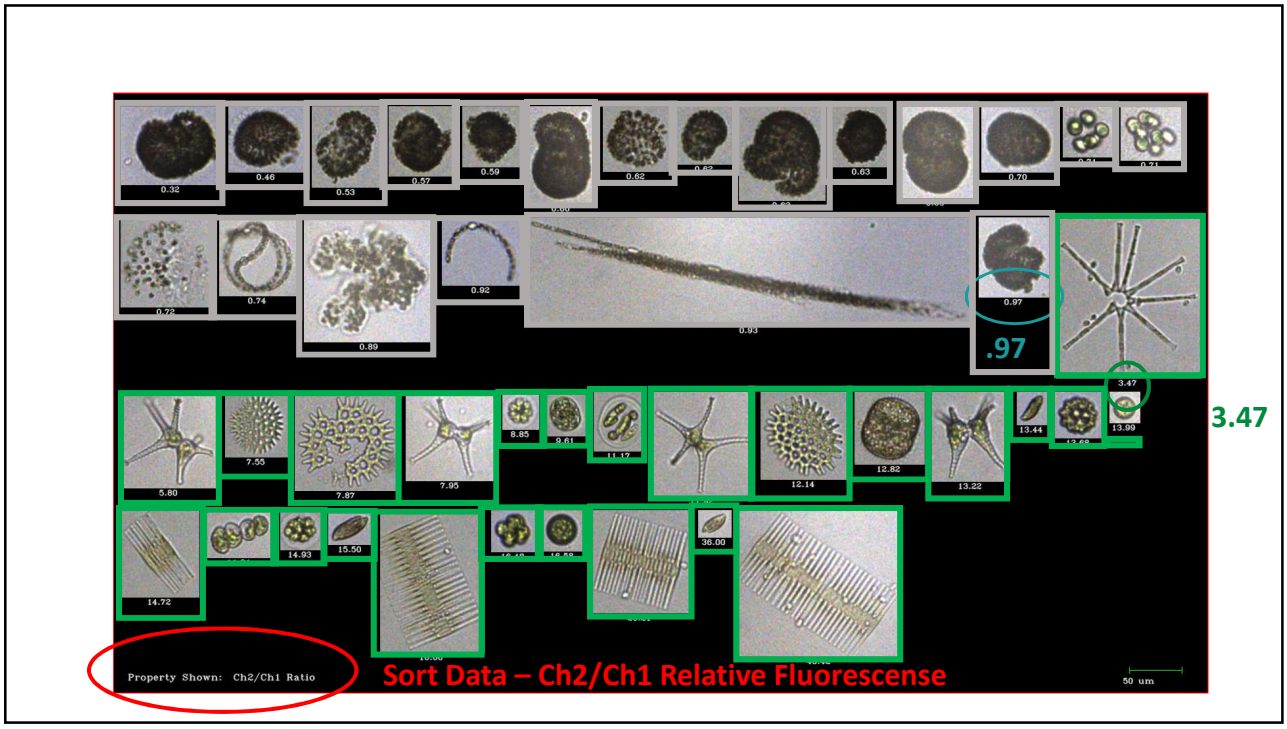
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3.47
Property Shown: Ch2/Ch1 Ratio
Sort Data – Ch2/Ch1 Relative Fluorescense

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Harmful Algae 63 (2017) 94–108

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Impacts of the 2014 severe drought on the *Microcystis* bloom in San Francisco Estuary

P.W. Lehman[a,*], T. Kurobe[b], S. Lesmeister[c], D. Baxa[b], A. Tung[c], S.J. Teh[b]

[a] Interagency Ecological Program, California Department of Fish and Wildlife, 2109 Arch Airport Road, Stockton, CA, 95206, USA
[b] Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, 1089 Veterinary Medicine Dr., Vet Med 3B, University of California, Davis, CA, 95616, USA
[c] Division of Environmental Services, California Department of Water Resources, 3500 Industrial Blvd., West Sacramento, CA, 95691, USA

diameter (ABD) with a FlowCAM digital imaging flow cytometer (Fluid Imaging Technologies; Sieracki et al., 1998). In order to more easily measure the biovolume of the colonies, the samples were size fractionated into <300 μm and >300 μm diameter size fractions and read at a magnification of 10× and 4×, respectively.

Cell abundance estimates based on FlowCAM measurements were closely correlated with those determined by microscopic analyses ($r = 0.88$, $p < 0.01$).

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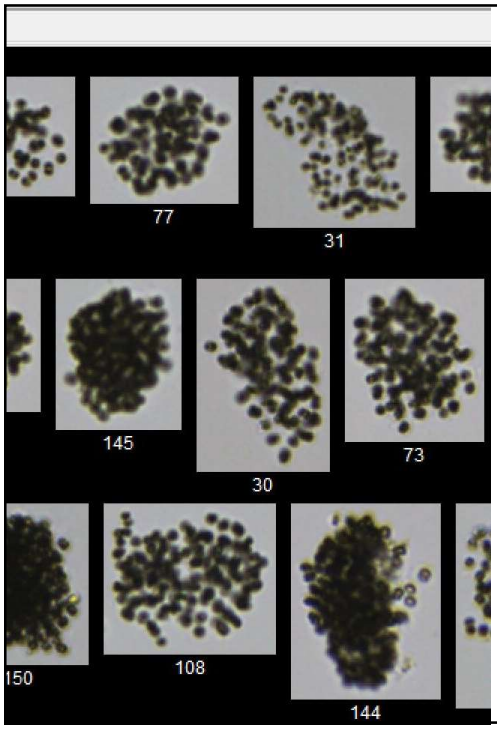

FlowCam Resources

www.fluidimaging.com

- Free Cyanobacteria and Nuisance Algae Guide
- FlowCam Cyano Information
- Send us a sample! No charge for analysis.

frances.buerkens@fluidimaging.com

Thank you!

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Ask the Experts

Hunter Adams
City of Wichita Falls, TX

Frances Buerkens
Yokogawa Fluid Imaging Technologies

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Please specify to whom you are addressing the question.

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- Until next time, keep the water safe and secure.

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Presenter Biography Information

Hunter Adams - Hunter is the Laboratory Supervisor, Deputy Quality Control Manager, and Technical Manager of Microbiology and Inorganic Chemistry for the Cypress Environmental Laboratory – City of Wichita Falls, TX. He holds a BS and MS in Biology from Midwestern State University. He is a licensed Class A Water Operator and Class C Wastewater Treatment Operator by the Texas Commission on Environmental Quality. He is also a Certified Water Professional and certified in Infrastructure Protection and Infrastructure Disaster Management by the Texas A&M Engineering Extension Service of Texas A&M University, and certified in Utility Risk and Resiliency by AWWA. He has worked in the planning and implementation of microbiological and analytical testing for Direct Potable Reuse and Indirect Potable Reuse systems for the City of Wichita Falls, TX. He has also successfully implemented a Taste and Odor Monitoring Program that has completely eliminated customer complaints for over 3.5 years. Hunter received the WEF Laboratory Analyst Excellence Award in 2020. He has authored and contributed to many publications, including manuals, Opflow, Texas H2O, Bridges, and LCGC North America. Hunter serves as a TCEQ EnviroMentor, a TNI Microbiology Expert Committee Member, an APHL Environmental Laboratory Sciences Committee Member, and on committees with AWWA and WEF.

Frances Buerkens - Frances Buerkens, Director of Sales for Water Quality Markets, holds an MBA in Operations Management and a BS in Agriculture and Natural Resource Management. Thanks to her agricultural background, she understands how water quality problems are created. She collaborates with customers around the world to monitor and manage source water, addressing concerns about the cyanobacteria and algae populations that proliferate largely because of agricultural and industrial runoff. Strategic management of toxins, and taste and odor issues form the core of her work. Water has always been central to Frances' life. She was raised on a Virginia farm where the spring ran dry during summer droughts and her family hauled drinking water from mountain springs an hour away to provide sufficient water to drink, cook, clean, and support livestock. She knows firsthand that quality water should never be taken for granted.

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