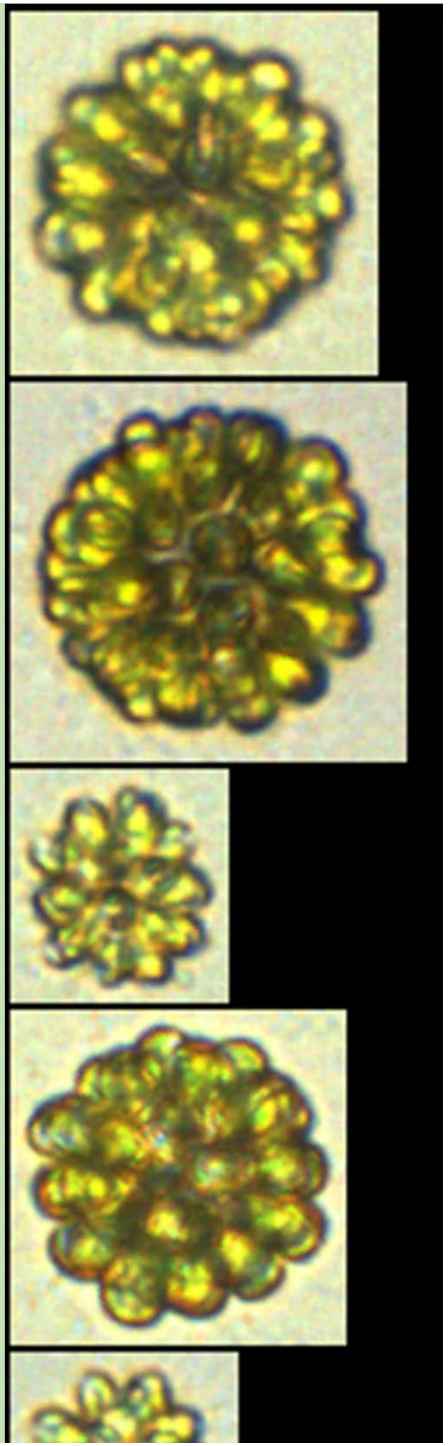
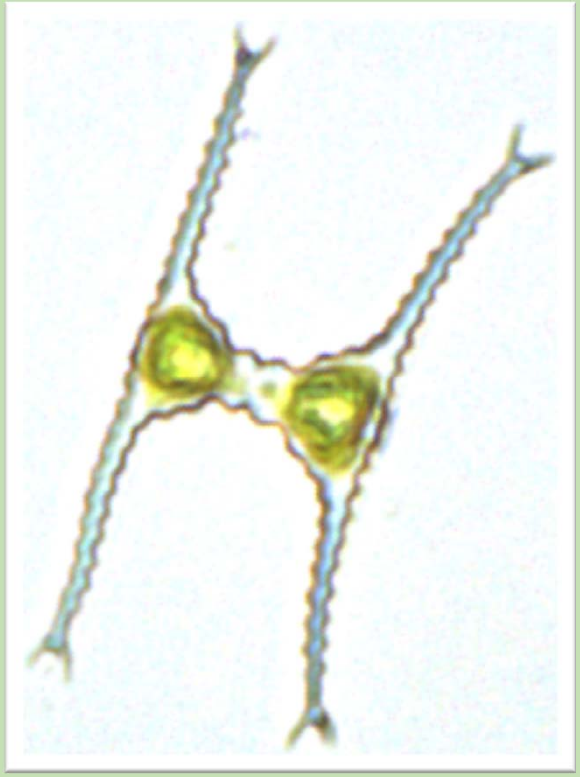
Source Water Cyanotoxin Monitoring Program at Newport News Waterworks

Anna-Maria Miller



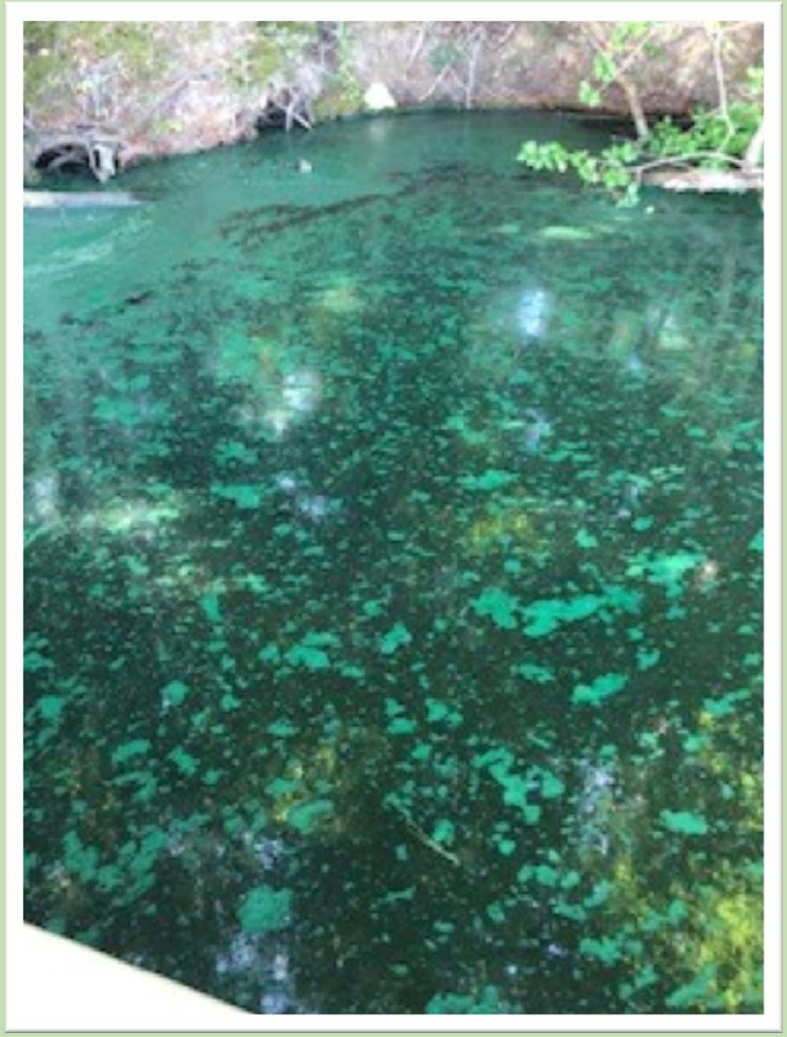
Why Monitor?

1) Getting a “Heads Up” on harmful or problematic algae and cyanobacteria
2) Compiling useful data on algal populations of source waters Changes can be related to:
    1) Seasons
    2) Water Quality
3) Operators and decision makers want information they can use to **take action**

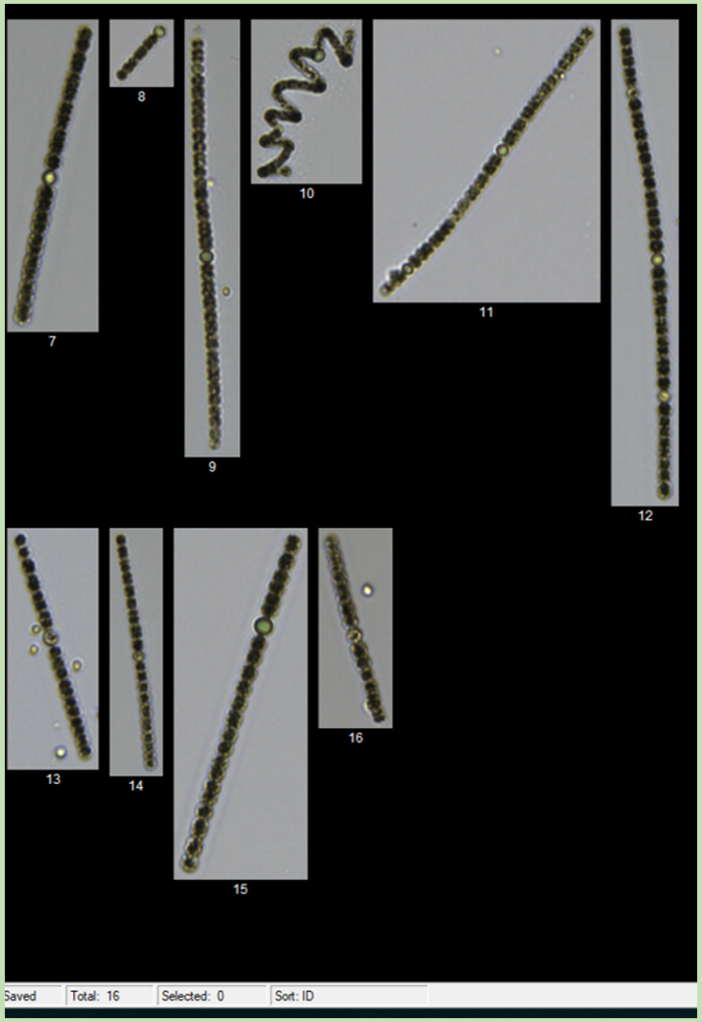


Monitoring Program at a Glance

- Monthly Field Monitoring
  - Probes for chlorophyll-a (all photosynthesizing organisms) and chlorophyll-b (accessory pigment, common in cyanobacteria) provide in situ cells/mL counts
  - Includes visual observations
- Daily On-Line monitors at intakes to treatment plants
- Weekly Enumeration/Classification in the Lab
  - Using FlowCam
  - Provides taxonomic information, particles/mL, and particle area
- Weekly ELISA (Method 546)
  - Detection of microsystins and nodularins
  - Can be used for raw and finished waters



Alvin Copeland

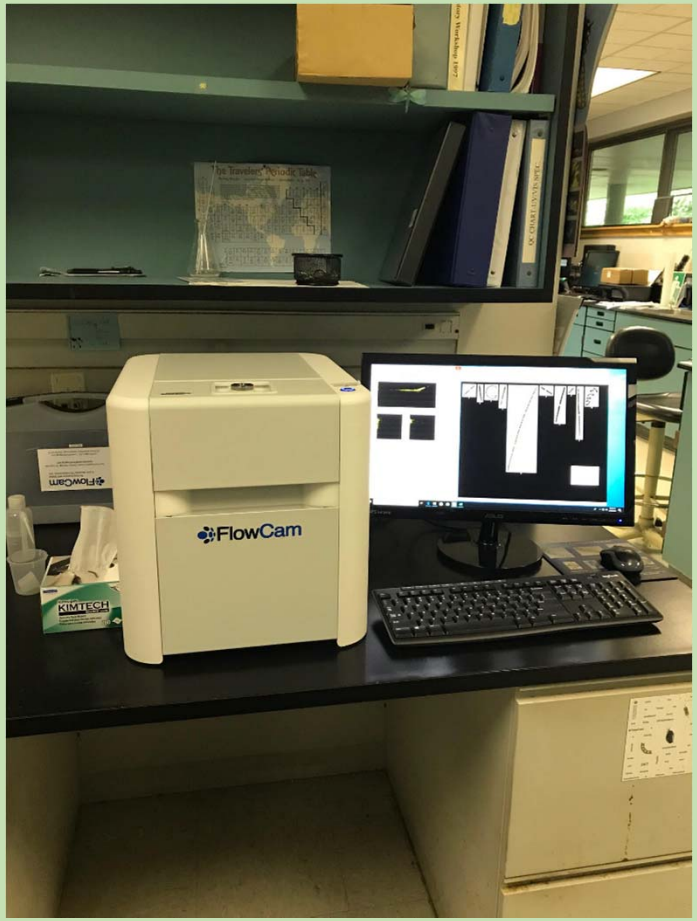




Classification/Enumeration of Algae

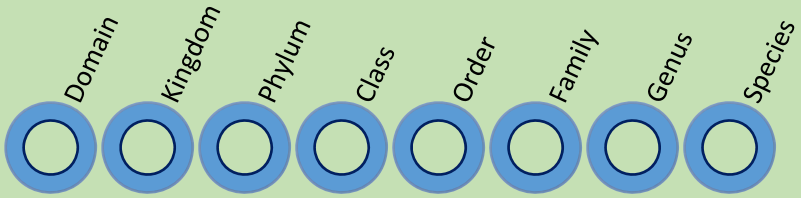
FlowCam Cyano

- Detection, Enumeration and Classification of Algae
  - Photographs particles as they flow past the objective at a set rate
  - Or: photographs particles that trigger excitation wavelength (633 nm)
  - Software can be used to further categorize particles, calculate parameters



Algal Classification

- Not phylogenetically unified
  - More related by characteristics than by genes
  - Lacking complex reproductive systems and vascular systems
- Very small and often lack distinguishing features
  - Visual inspection is often the only method available for identification

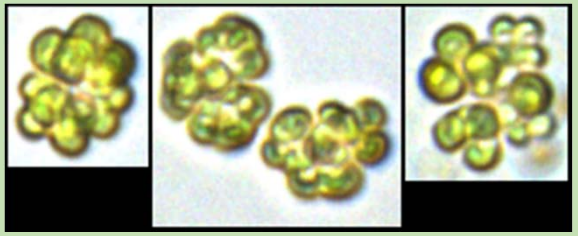


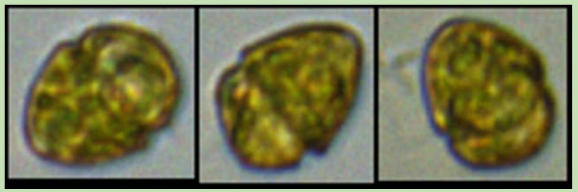
- Practice increases confidence in identifying taxa with common features
  - What are the focus groups?
    - Cyanobacteria
    - Filter cloggers
    - Taste and odor
  - No one manual or method – one size does not fit all

Algal Enumeration

- Algae are irregular in shape, so calculating volume is difficult almost to the point of impossible
- Colonies or filaments – do you choose total cell count vs. natural unit count
- What about biomass?

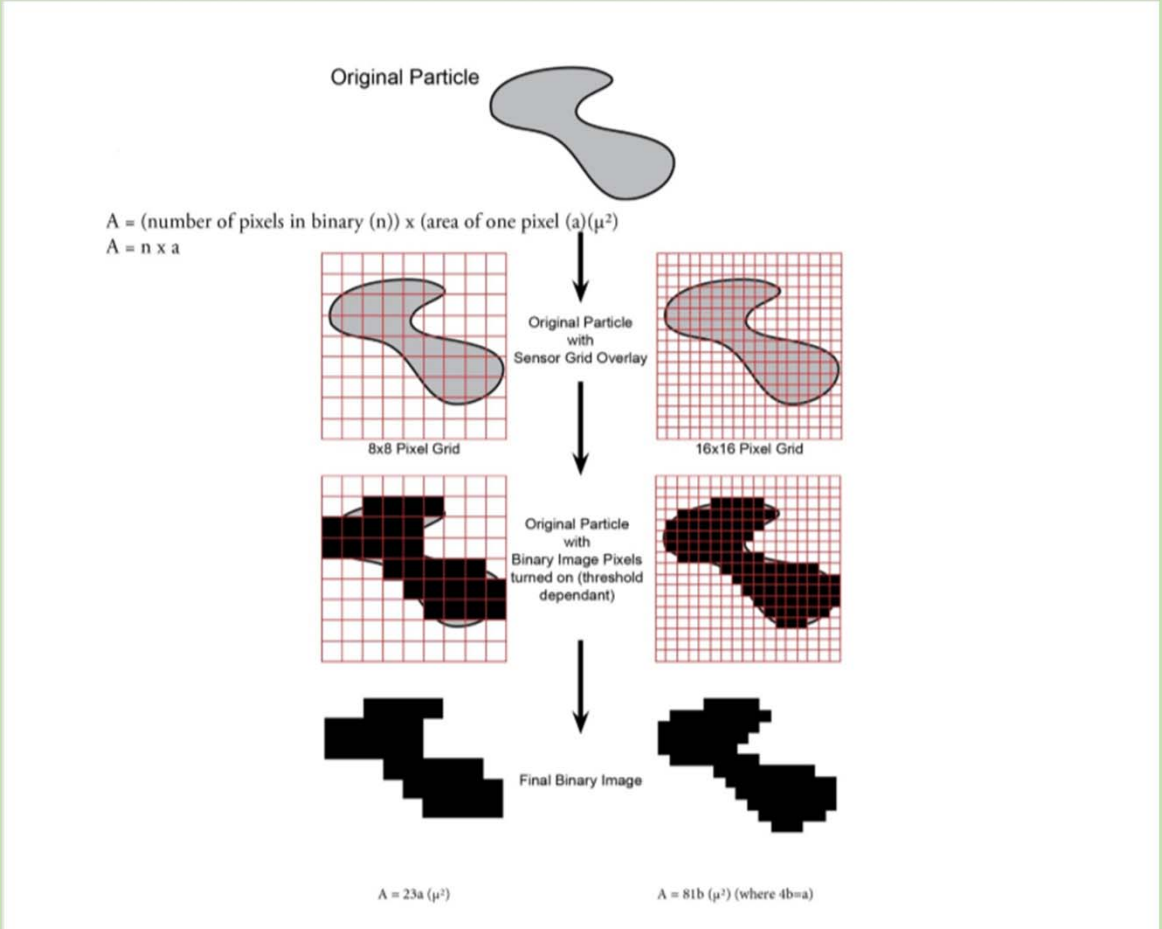
- Report particle area rather than particle volume



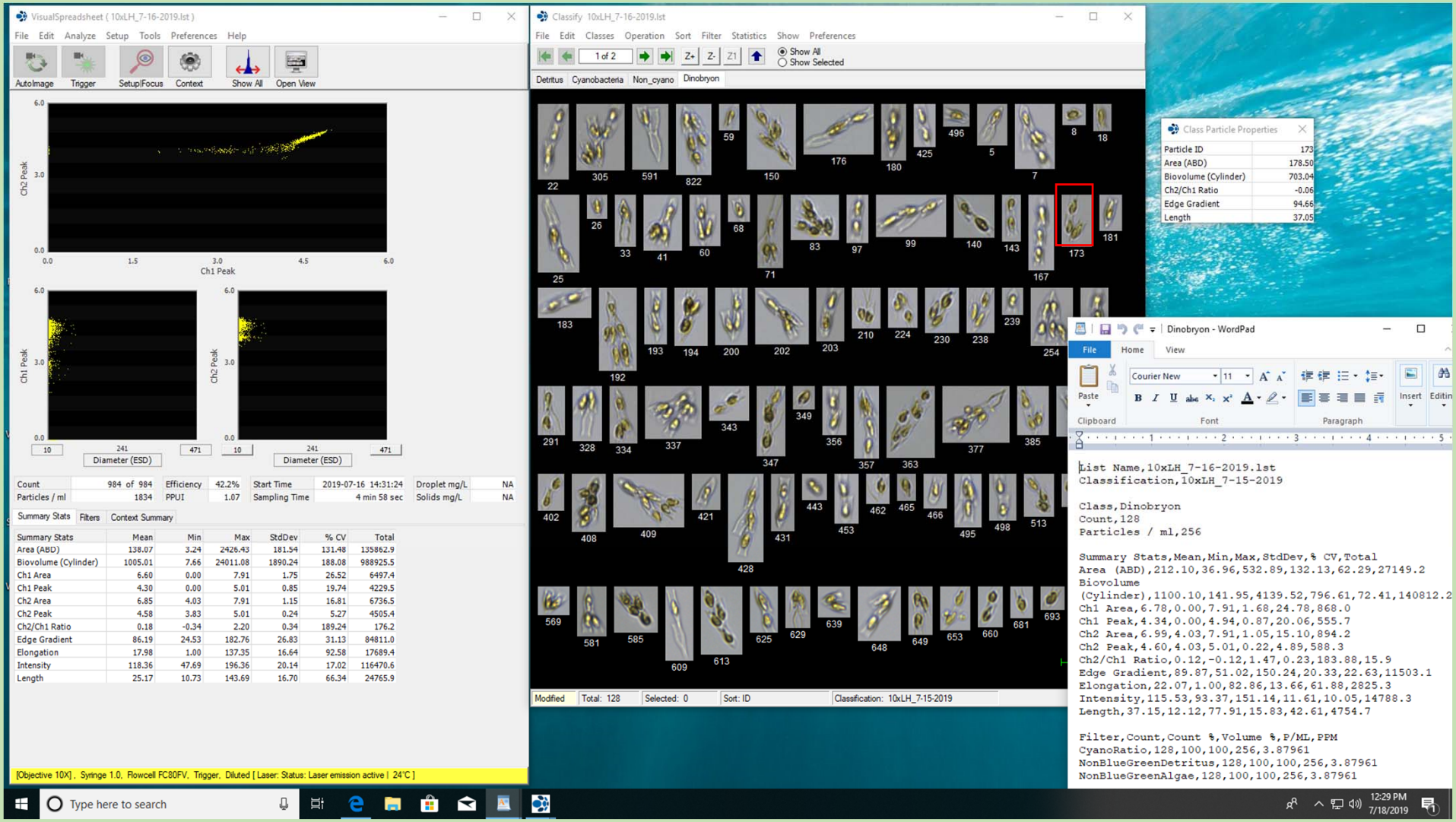


Particles and Particle Area

- Software calculates the number of particles per mL through sample volume, flow rate and frames per second – all can be adjusted for optimum efficiency
- The area is calculated by pixelating the image



FlowCam Manual


VisualSpreadsheet (10xLH_7-16-2019.lst)
File Edit Analyze Setup Tools Preferences Help
AutoImage Trigger Setup|Focus Context Show All Open View
Ch2 Peak
Ch1 Peak
Diameter (ESD)
Count 984 of 984 Efficiency 42.2% Start Time 2019-07-16 14:31:24 Droplet mg/L NA
Particles / ml 1834 PPUI 1.07 Sampling Time 4 min 58 sec Solids mg/L NA
Summary Stats Filters Context Summary
Summary Stats Mean Min Max StdDev % CV Total
Area (ABD) 138.07 3.24 2426.43 181.54 131.48 135862.9
Biovolume (Cylinder) 1005.01 7.66 24011.08 1890.24 188.08 988925.5
Ch1 Area 6.60 0.00 7.91 1.75 26.52 6497.4
Ch1 Peak 4.30 0.00 5.01 0.85 19.74 4229.5
Ch2 Area 6.85 4.03 7.91 1.15 16.81 6736.5
Ch2 Peak 4.58 3.83 5.01 0.24 5.27 4505.4
Ch2/Ch1 Ratio 0.18 -0.34 2.20 0.34 189.24 176.2
Edge Gradient 86.19 24.53 182.76 26.83 31.13 84811.0
Elongation 17.98 1.00 137.35 16.64 92.58 17689.4
Intensity 118.36 47.69 196.36 20.14 17.02 116470.6
Length 25.17 10.73 143.69 16.70 66.34 24765.9
[Objective 10X] , Syringe 1.0, Flowcell FC80FV, Trigger, Diluted [Laser: Status: Laser emission active | 24°C]
Classify 10xLH_7-16-2019.lst
File Edit Classes Operation Sort Filter Statistics Show Preferences
1 of 2
Show All
Show Selected
Detritus Cyanobacteria Non_cyano Dinobryon
Modified Total: 128 Selected: 0 Sort: ID Classification: 10xLH_7-15-2019
Class Particle Properties
Particle ID 173
Area (ABD) 178.50
Biovolume (Cylinder) 703.04
Ch2/Ch1 Ratio -0.06
Edge Gradient 94.66
Length 37.05
Dinobryon - WordPad
File Home View
Paste Clipboard
Courier New 11 Font
Paragraph
Insert
List Name,10xLH_7-16-2019.lst
Classification,10xLH_7-15-2019
Class,Dinobryon
Count,128
Particles / ml,256
Summary Stats,Mean,Min,Max,StdDev,% CV,Total
Area (ABD),212.10,36.96,532.89,132.13,62.29,27149.2
Biovolume (Cylinder),1100.10,141.95,4139.52,796.61,72.41,140812.2
Ch1 Area,6.78,0.00,7.91,1.68,24.78,868.0
Ch1 Peak,4.34,0.00,4.94,0.87,20.06,555.7
Ch2 Area,6.99,4.03,7.91,1.05,15.10,894.2
Ch2 Peak,4.60,4.03,5.01,0.22,4.89,588.3
Ch2/Ch1 Ratio,0.12,-0.12,1.47,0.23,183.88,15.9
Edge Gradient,89.87,51.02,150.24,20.33,22.63,11503.1
Elongation,22.07,1.00,82.86,13.66,61.88,2825.3
Intensity,115.53,93.37,151.14,11.61,10.05,14788.3
Length,37.15,12.12,77.91,15.83,42.61,4754.7
Filter,Count,Count %,Volume %,P/ML,PPM
CyanoRatio,128,100,100,256,3.87961
NonBlueGreenDetritus,128,100,100,256,3.87961
NonBlueGreenAlgae,128,100,100,256,3.87961
Type here to search
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7/18/2019

Cyanotoxin Analysis

- EPA 546
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Microcystins/Nodularins (Adda)
- Abraxis CAAS (Cyanotoxin Automated Assay System)



ELISA

Enzyme-Linked Immunosorbent Assay

-- Microcystins/Nodularins

Congener-independent, intracellular and extracellular

Tests for characteristics that are common in all congeners (structural variants) – the Adda amino acid side chain

Detection limit: 0.15ppb

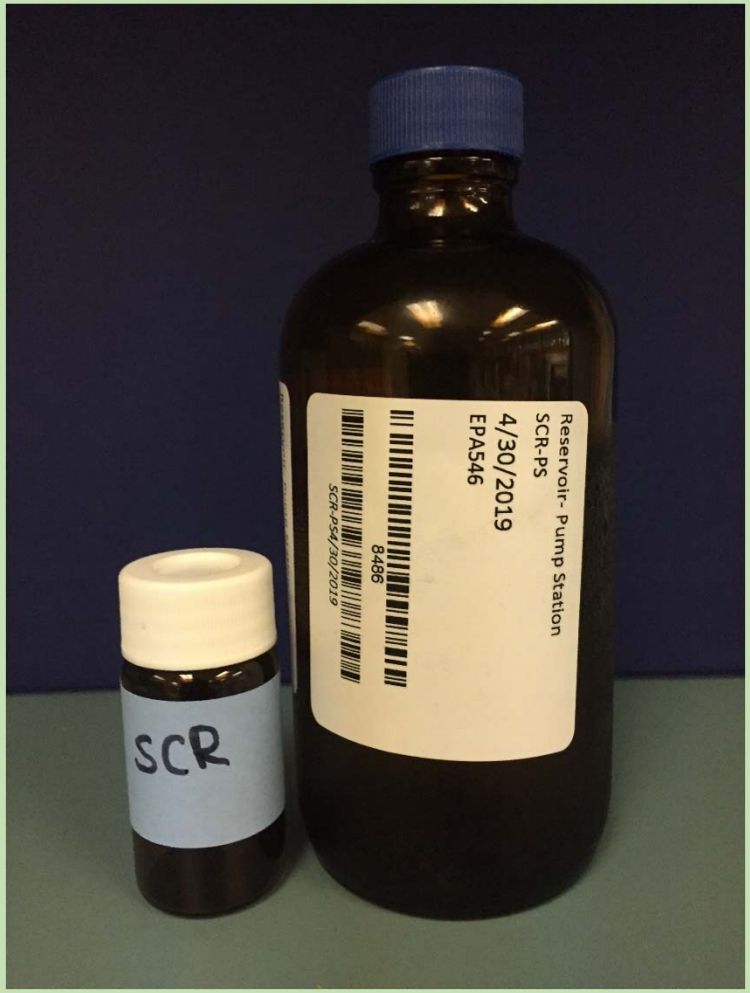
MRL/Health Advisory: 0.30ppb



Abraxis CAAS

Sample Collection, Preservation, and Storage

- Preservative
  - Sodium thiosulfate, but only for finished water
- Temperature
  - <10°C for up to 48hrs after collection. Then frozen
- Container
  - Amber borosilicate glass with PTFE-lined septa. Bottle capacity to accommodate freezing. Single use *recommended*
- Holding time
  - ELISA: 14 days
  - (FlowCam: same day analysis from same container)



Lysing, Filtering and Analysis

- Each 10mL sample (including lab blanks, fortified blanks and matrix spikes) is lysed by 3 freeze/thaw cycles
- Each sample is filtered through 0.45µm filter into 4mL vial
- Each sample will be analyzed in duplicate, including standards and QC



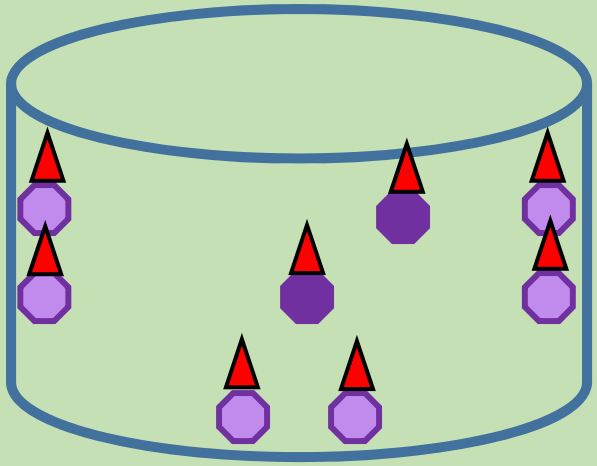
How Does The Kit Work?

Method inflexible and time-dependent

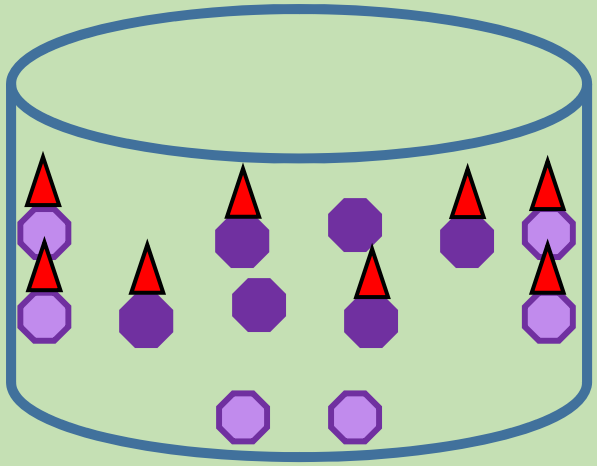
96-well microtiter plate format

Microcystins and nodularins in the samples and a microcystin-protein analogue immobilized in the wells compete for the binding sites of a primary detection antibody in solution.

LOW



HIGH



How Does The Kit Work?

Wash Step

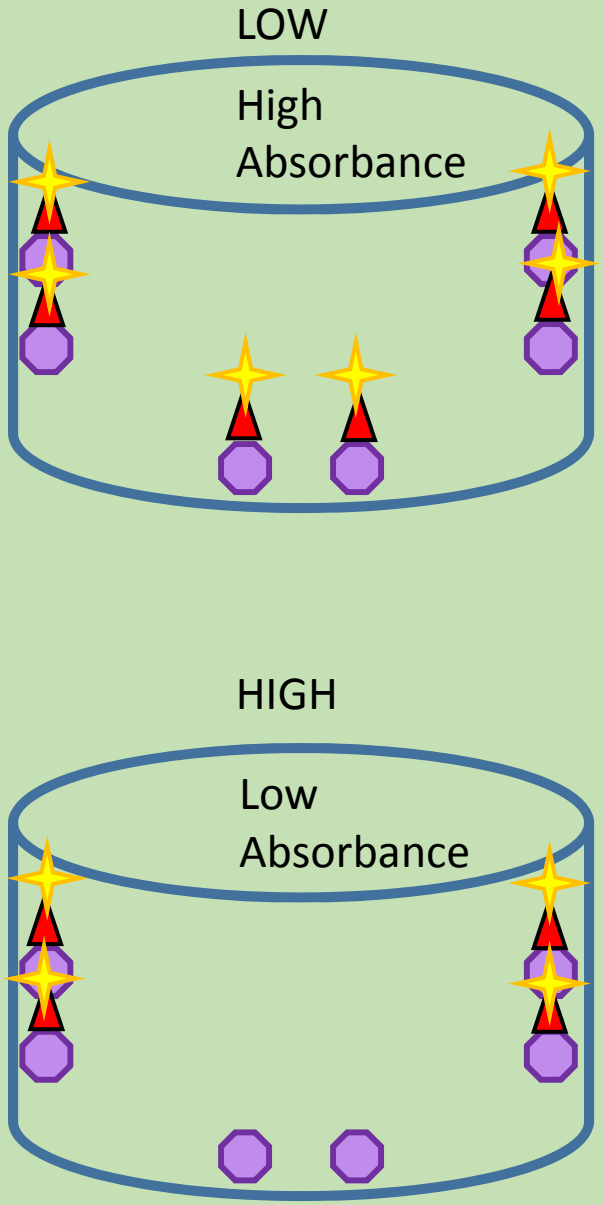
After a wash step, an enzyme-conjugate is added to the wells and binds to the primary antibody in an inverse relationship to the original concentration of the microcystins and nodularins in the sample

Second Wash Step

Tetramethylbenzidine to develop color

Then acidic solution to stop color generation

Absorbance is measured



- Percent Coefficient of Variation (%CV) of absorbance
  - Should be no more than 15% for samples. 1 standard %CV may be ≥ 10% and ≤ 15%, but only if all other %CVs are ≤ 10%
- EC_{50} is the concentration that is in the center of the most reliable measurement range
  - Average: 0.56ppb, min: 0.34, max: 0.95
- R^2 value ≥ 0.980

	0.15	**%CV**	**0.30 (0.15-0.45)**	**%CV**	**0.60 (0.36-0.84)**	**%CV**	**0.75 (0.57-0.94)**	**%CV**
average	0.14	1.95	0.26	3.09	0.56	4.08	0.74	2.69
% in range (≤ 10% for CV)		85%	90.9%	96.8%	88.1%	91.3%	88.8%	97.5%
min	0.06	0.00	0.09	0.00	0.15	0.10	0.43	0.00
max	0.25	11.40	0.79	16.40	0.95	18.90	1.02	12.50

N = 85

In Review:

1. Upon receiving sample, analyze with FlowCam
    1. Almost immediate results that alerts other staff and opens communication
    2. Field or plant data is shared

2. Set up ELISA
    1. By the time results are available, visual inspections have been made and field chlorophyll a and b data is often available
    2. Microcystin/Nodularin results may result in changes in treatment – additional ozone

3. By conclusion of a potential HAB, many parameters have been assessed and data have been collected:
    1. Photographs in the field
    2. Field chlorophyll measurements
    3. Observations of taste and odor
    4. Filter run times
    5. Particle enumeration and particle area
    6. Organism Identification
    7. Cyanotoxin levels

4. Learn From the Past – Prepare for the Future

Thank you

Questions?

