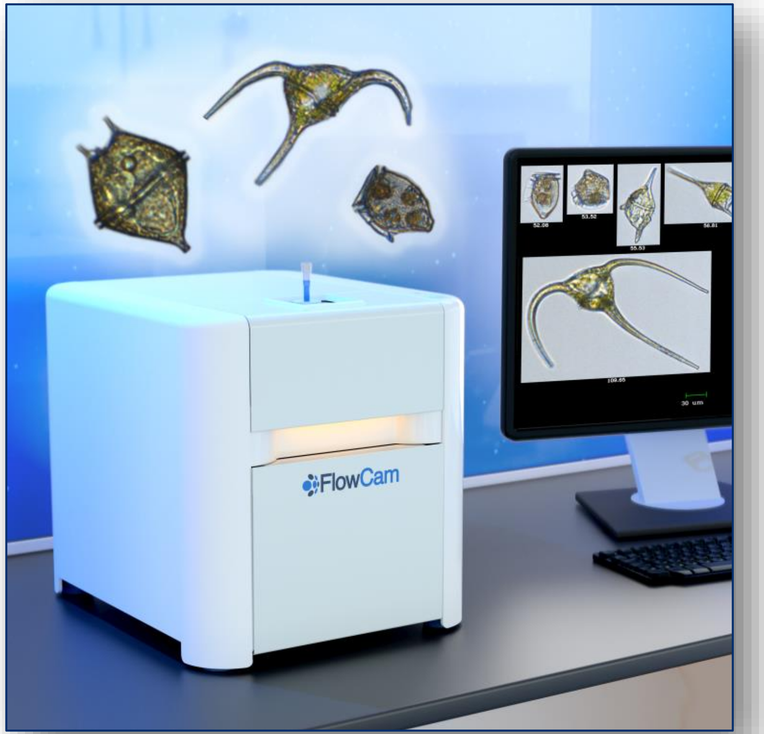
FlowCam for Freshwater Applications

Bhushan Pandit

Yokogawa India Limited

Outline

- FlowCam Overview
- What regulations say
- Applications in freshwater
- Instrument Types
- Example Data



FlowCam 8000 with marine dinoflagellates

FlowCam Overview

- Flow Imaging Microscopy (FIM)
- Invented by biological oceanographers to:
  - Provide a faster alternative to manual microscopy, and
  - Add a visual component to flow cytometry
- Used today in a variety of fields, including marine research, Freshwater, aquaculture, microalgae cultivation, biopharmaceutical research, and more.

Click here to watch a 3-minute FlowCam video

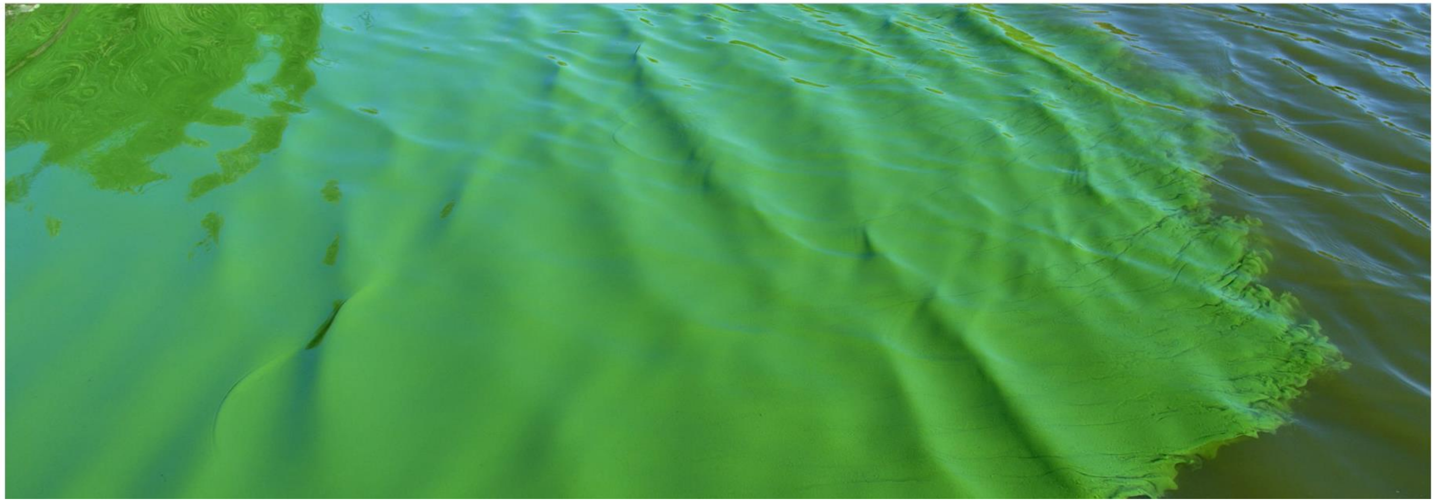
FlowCam

HABs

Freshwater and marine algal blooms can be called **harmful** because they lower dissolved oxygen concentrations, alter aquatic food webs, leave ugly scums along shorelines, produce taste-and-odor compounds that cause drinking water and fish flesh to taste bad, or produce toxins so potent they poison organisms in the water and on the land.

- Cyanobacteria
  - Toxin producers
  - Taste and odor producers
- Dinoflagellates
  - Taste and Odor producers
  - Toxin producers

Golden Algae
  - Taste and odor producers
  - Toxin producer
- Diatoms
  - Filter cloggers
  - Taste and Odor producers
  - Toxin producers
- Euglenoids, cryptomonads…



FlowCam

- Why are customers choosing FlowCam vs. Microscope

- 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

FlowCam

HAB Monitoring Criteria- Regulations

- No Federal Standard
  - Most toxin based
- WHO 2003
  - Broad guidelines for total cyanobacteria (cells/mL)
- EPA 2015
  - **Cylindrospermopsin** 0.7 ug/L
  - **Microcystin** 0.3 ug/L
- State Specific Guidelines
  - OH: Microcystin action levels 0.3 ug/L for at risk individuals and 1.6 ug/L for all individuals. Increased monitoring required when detected followed by QPCR
- Utilities must develop their own plan

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll a (µg/L)	Estimated Microcystin Levels (µg/L)
Low	< 20,000	< 10	< 10
Moderate	20,000 – 100,000	10 – 50	10 – 20
High	> 100,000 – 10,000,000	50 – 5,000	20 – 2,000
Very High	> 10,000,000	> 5,000	> 2,000

FlowCam

BIS Regulations- IS 10500-2012

ANNEX C

(*Clause* 4.3.10)

ILLUSTRATIVE LIST OF MICROSCOPIC ORGANISMS PRESENT IN WATER

Sl No.	*Classification of Microscopic Organism*	*Group and Name of the Organism*	*Habitat*	*Effect of the Organisms and Significance*
(1)	(2)	(3)	(4)	(5)
i)	Algae	a) Chlorophyceae:		
		1) *Species of* Coelastrum, Gomphospherium, Micractinium, Mougeotia, Oocystis, Euastrum, Scenedesmus, Actinastrum, Gonium, Eudorina Pandorina, Pediastrum, Zygnema, Chlamydomonas, Careteria, Chlorella, Chroococcus, Spirogyra, Tetraedron, Chlorogonium, Stigeoclonium	Polluted water, impounded sources	Impart colouration
		2) *Species of* Pandorina, Volvox, Gomphospherium, Staurastrum, Hydrodictyon, Nitella	Polluted waters	Produce taste and odour
		3) *Species of* Rhizoclonium, Cladothrix, Ankistrodesmus, Ulothrix, Micrasterias, Chromulina	Clean water	Indicate clean condition
		4) *Species of* Chlorella, Tribonema, Clostrium, Spirogyra, Palmella	Polluted waters, impounded sources	Clog filters and create impounded difficulties
		b) Cyanophyceae:		
		1) *Species of* Anacystis and Cylindrospermum	Polluted waters	Cause water bloom and impart colour
		2) *Species of* Anabena, Phormidium, Lyngbya, Arthrospira, Oscillatona	Polluted waters	Impart colour
		3) *Species of* Anabena, Anacystis, Aphanizomenon	Polluted waters, impounded sources	Produce taste and odour
		4) *Species of* Anacystis, Anabena, Coelospherium, Cleotrichina, Aphanizomenon	Polluted waters	Toxin producing
		5) *Species of* Anacystis, Rivularia, Oscillatoria, Anabena	Polluted waters	Clog filters

			temperate waters	
ii)	Zooplankton	a) Protozoa:		
		1) Amoeba, Giardia Lamblia Arcella, Difflugia, Actinophrys	Polluted waters	Pollution indicators
		2) Endamoeba, Histolytica	Sewage and activated sludge	Parasitic and pathogenic
		b) Ciliates: Paramoecium, Vorticella, Carchesium, Stentor, Colpidium, Coleps, Euplotes, Colopoda, Bodo	Highly polluted waters, sewage and activated sludge	Bacteria eaters
		c) Crustacea:		
		1) Bosmina, Daphnia	Stagnant pollu-ted waters	Indicators of pollution
		2) Cyclops	Step wells in tropical climate	Carrier host of guinea worm
iii)	Rotifers	a) Rotifers: Anurea, Rotaria, Philodina	Polluted and Algae laden waters	Feed on algae
		b) Flagellates:		
		1) Ceratium, Glenodinium, Peridinium Dinobryon	Rocky strata, iron bearing and acidic waters	Impart colour and fishy taste
		2) Euglena, Phacus	Polluted waters	Impart colour

10

IS 10500 : 2012

Sl No.	*Classification of Microscopic Organism*	*Group and Name of the Organism*	*Habitat*	*Effect of the Organisms and Significance*
(1)	(2)	(3)	(4)	(5)
iv)	Miscellaneous Organisms	a) Sponges, Hydra	Fresh water	Clog filters and affect purification systems
		b) Tubifex, Eristalls, Chironomids	Highly polluted waters, sewage and activated sludge and bottom deposits	Clog filters and render water unaesthetic
		c) Plumatella	Polluted waters	Produces biological slimes and causes filter operational difficulties
		c) Dreissena, Asellus	Polluted waters	Harbour pathogenic organisms

FlowCam

South African National Standard (SANS) 241

j	Microcystin only needs to be measured where algal bloom (>20000 cyanobacteria cells per millilitre) is present in a raw water source. In the absence of algal monitoring, an algal bloom is deemed to occur where the surface water is visibly green in the vicinity of the abstraction, or samples taken have a strong musty odour.

- Microcystin producing organisms include but are not limited to:
  - Microcystis
  - Anabaenopsis
  - Aphanizomenon
  - Dolichospermum
  - Merismopedia
  - Nodularia
  - Nostoc
  - Oscillatoria
  - Planktothrix
  - Woronichinia
  - Gleotrachia

Yokogawa Fluid Imaging Technologies

FlowCam

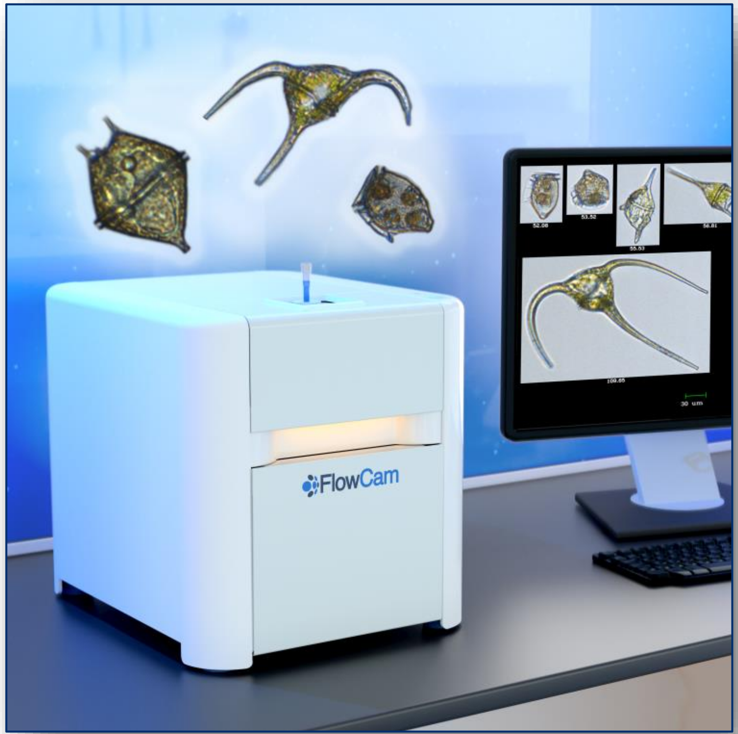
Outline

FlowCam Overview

Applications in Fresh water

Instrument Types

Example Data



FlowCam 8000 with
marine dinoflagellates

Monitoring Programs

Why Monitor

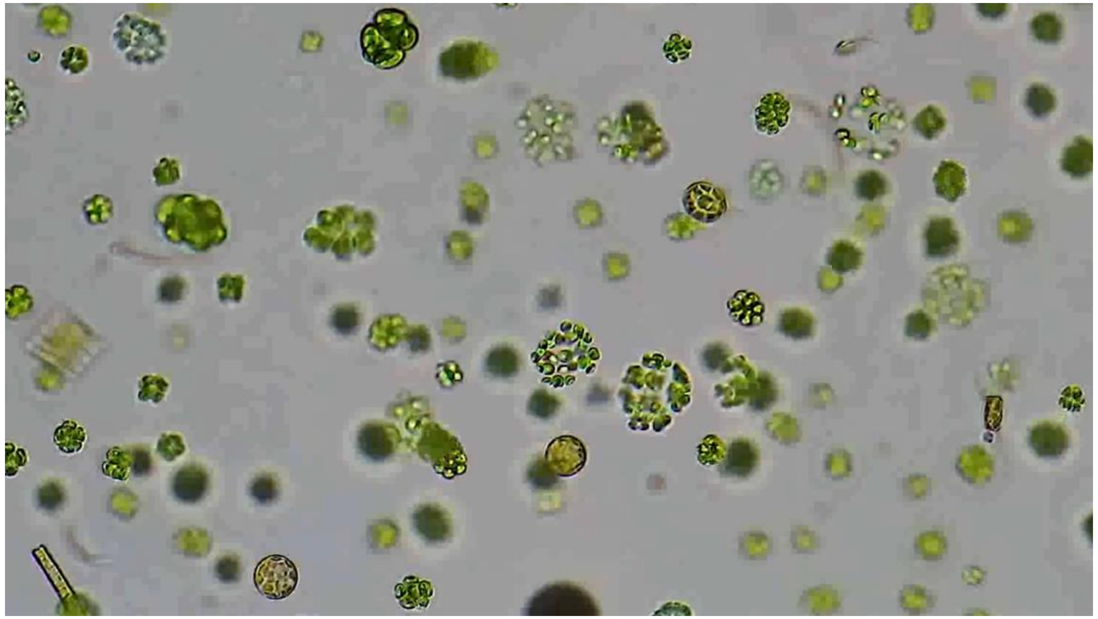
- Drinking Water Aesthetics (Taste and Odor)
- Public Health
- Bloom Mitigation Research

What to Monitor

- Full community composition
- Cyanobacteria
- Taste and odor producers
- Filter cloggers
- Zooplankton
- Other particles

Baseline Levels

- Understand algal diversity and abundance
- Unique to each waterbody
- Challenging
  - Population changes over time
    - Seasonally
    - Diurnally
    - Lake stratification
    - Nutrient availability



FlowCam

Trigger Levels

- The point at which the concentration of one genus of phytoplankton reaches a number that prompts further sampling and/or treatment.
- Developed from Baseline levels
  - Use monitoring goals to determine organisms of interest

The City of Wichita Falls Cypress Environmental Laboratory (CEL) set trigger levels low to minimize taste and odor (T&O) issues.

Genus	Trigger Count–*organism/mL*	Type	Reason
Dolichospermum	100*	Cyanobacteria	T&O- and cyanotoxin-producer
Microcystis	150	Cyanobacteria	T&O- and cyanotoxin-producer
Aphanizomenon	150	Cyanobacteria	T&O- and cyanotoxin-producer
Peridinium	400	Dinoflagellate	T&O-producer
Melosira	200	Diatom	T&O-producer
Cyclotella	No limit	Diatom	Abundant but has not been shown to cause an issue in CEL's source water
Pediastrum	No limit	Green algae	Abundant but has not been shown to cause an issue in CEL's source water

*chains/mL

Massachusetts Water Resources Authority increases monitoring if early warning levels are exceeded for algal abundance.

Genus	Early Warning Trigger Count–*ASU/mL*	Treatment Consideration Trigger Count–*ASU/mL*	Type	Reason
Dolichospermum	>15	>25	Cyanobacteria	T&O- and cyanotoxin-producer
Chrysosphaerella	>100	>500	Chrysophyte	T&O-producer
Synura	>10	>12	Chrysophyte	T&O-producer
Dinobryon	>200	>500	Chrysophyte	T&O-producer
Uroglenopsis	>200	>750	Chrysophyte	T&O-producer

ASU–areal standard unit, T&O–taste and odor

Monitoring Criteria Plan

ROUTINE MONITORING				
Sampling Frequency	Alternate plants weekly			
Sampling Location	Raw Plant Influent		Finished	
Cyanobacteria Counts	Cells/mL		µm³/mL	
	MIN	MAX	MIN	MAX
	0	20,000	0	8,000,000
Monitor for changes in dissolved oxygen levels, turbidity, chlorophyll-a, total phosphorous, surface scums or blooms.				

ALERT LEVEL I				
Sampling Frequency	Weekly for elevated source			
Cyanobacteria Counts	Cells/mL		µm³/mL	
	MIN	MAX	MIN	MAX
	20,001	50,000	8,000,001	20,000,000
Monitor for elevated nutrients, pigment, or any other visual observations.				

ALERT LEVEL II				
Sampling Frequency	Weekly for elevated source			
Cyanobacteria Counts	Cells/mL		µm³/mL	
	MIN	MAX	MIN	MAX
	50,001	100,000	20,000,001	40,000,000
Toxin Screening	Microcystins	Saxitoxin	Cylindrospermopsin	Anatoxin-a
Toxins Present?	Yes		No	
	All positive toxin results must be confirmed by a contract lab.		Request genetic testing from a contract lab to determine if the cyanobacteria have toxin producing genes.	
	Implement Cyanotoxin Treatment Response Plan			
	Continue weekly toxin screening at Raw Plant Influent and Finished Water sample sites.			

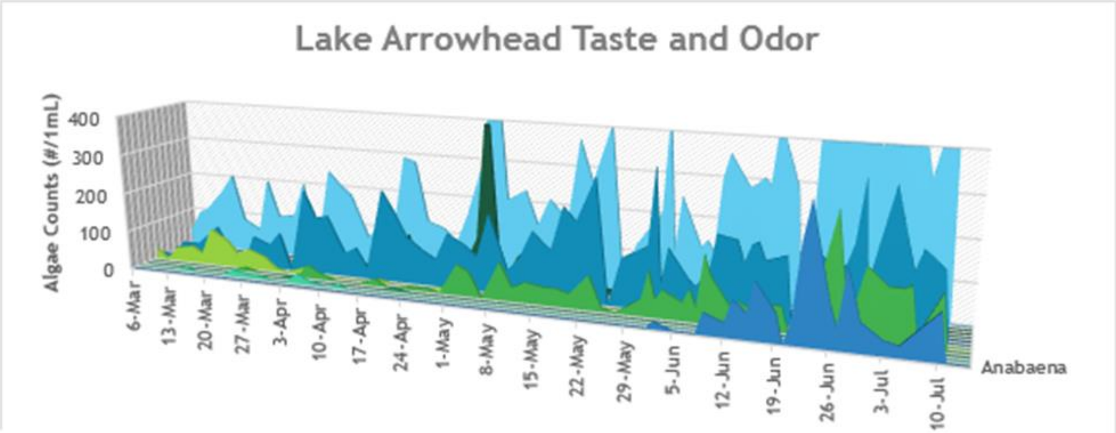
ALERT LEVEL III				
Sampling Frequency	Weekly for elevated source			
Cyanobacteria Counts	Cells/mL		µm³/mL	
	MIN	MAX	MIN	MAX
	100,001	undefined	40,000,001	undefined
Toxin Screening	Microcystins	Saxitoxin	Cylindrospermopsin	Anatoxin-a
Toxins Present?	Yes		No	
	All positive toxin results must be confirmed by a contract		Request genetic testing from a contract lab to determine if the cyanobacteria have toxin producing genes	
	Implement Cyanotoxin Treatment Response Plan			
	Enumerate toxin-specific cyanobacteria			
	Determine intracellular/extracellular ratios			
	Continue daily toxin screening			
	Notify Oklahoma Tourism and Recreation Department if toxins exceed threshold limit in Lake Eucha or Lake Spavinaw			

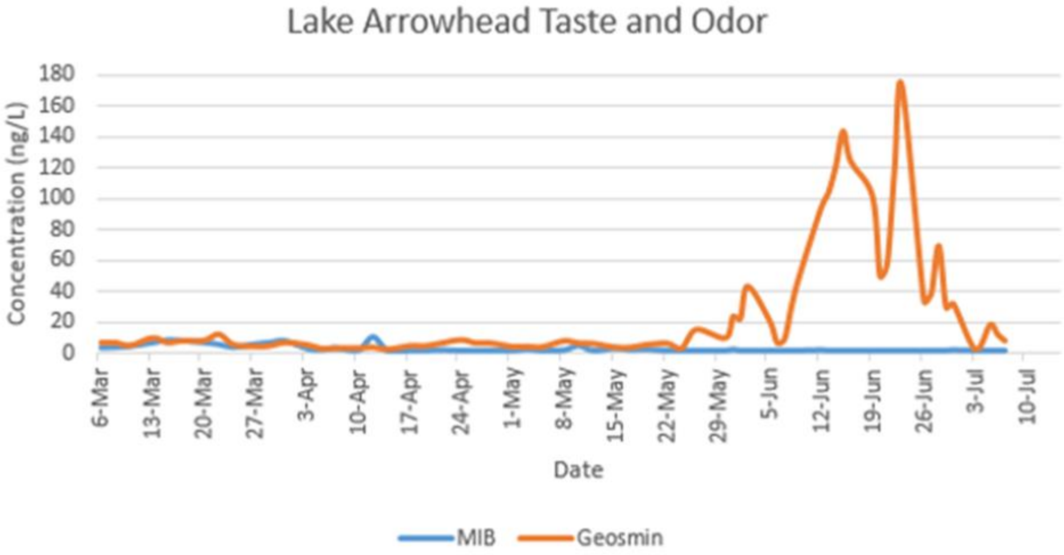
Table 1. Current alert levels and subsequent actions included in the City of Tulsa's Cyanobacteria Early Warning Plan.

FlowCam

Develop Trends

- Dolichospermum
  - Spike in organisms in June
  - MIB/Geosmin levels also spike
- CUSO applied in response
- Levels of cyanobacteria drop back to normal





FlowCam

Phytoplankton Monitoring Goals

Common
Taste & Odor Algae

Common
Filter Clogging Algae

Common
Toxin producing Algae

Taste & Odor Algae

	Sampling Site		
	1	2	3
Species	**Count per mL**	**Count per mL**	**Count per mL**
Anabaena			
Aphanizomenon			
Ceratium			
Cylindrospermopsis			
Mallomonas			
Melosira			
Microcystis			
Oscillatoria			
Pandorina			
Peridinium			
Total			

Filter Clogging Algae

Species	**Count per mL**	**Count per mL**	**Count per mL**
Closterium			
Cyclotella			
Diatoma			
Fragillaria			
Navicula			
Synedra			
Total			

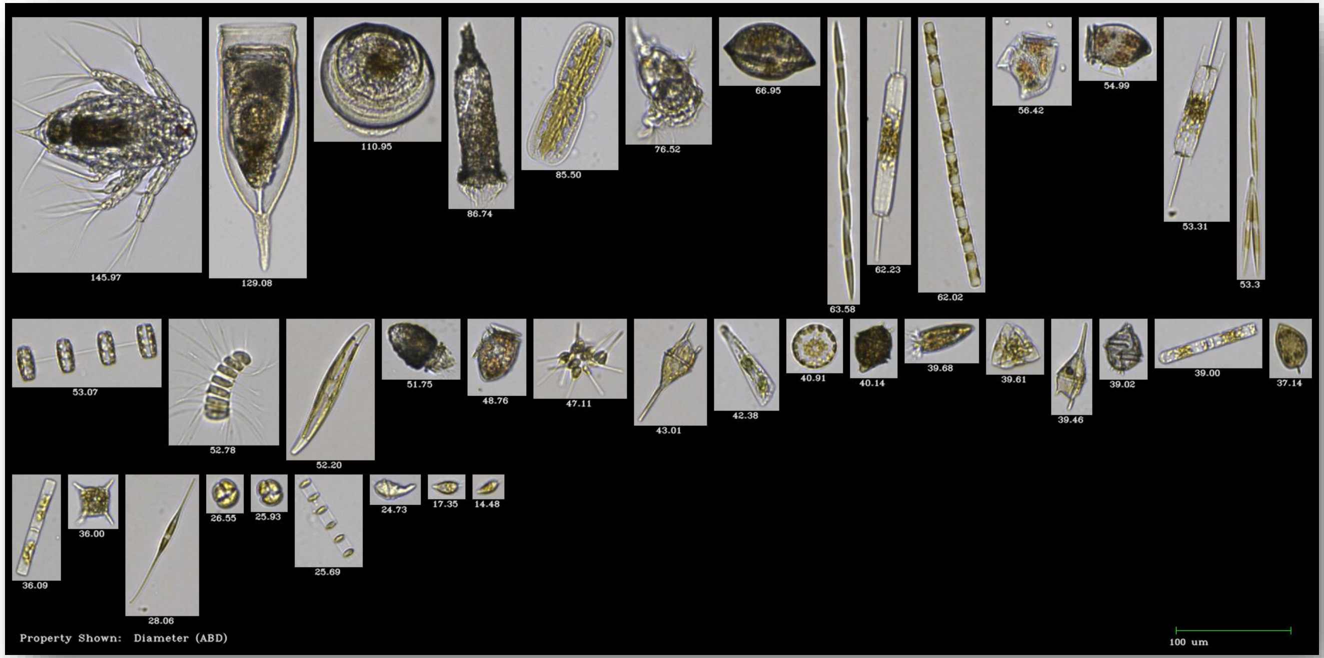
Toxin producing algae

Species	**Count per mL**	**Count per mL**	**Count per mL**
Microcystis			
Dolichospermum			
Planktothrix			
Lyngbya			
Phormidium			
Aphanizomenon			
Total			

FlowCam

General FlowCam Use Cases

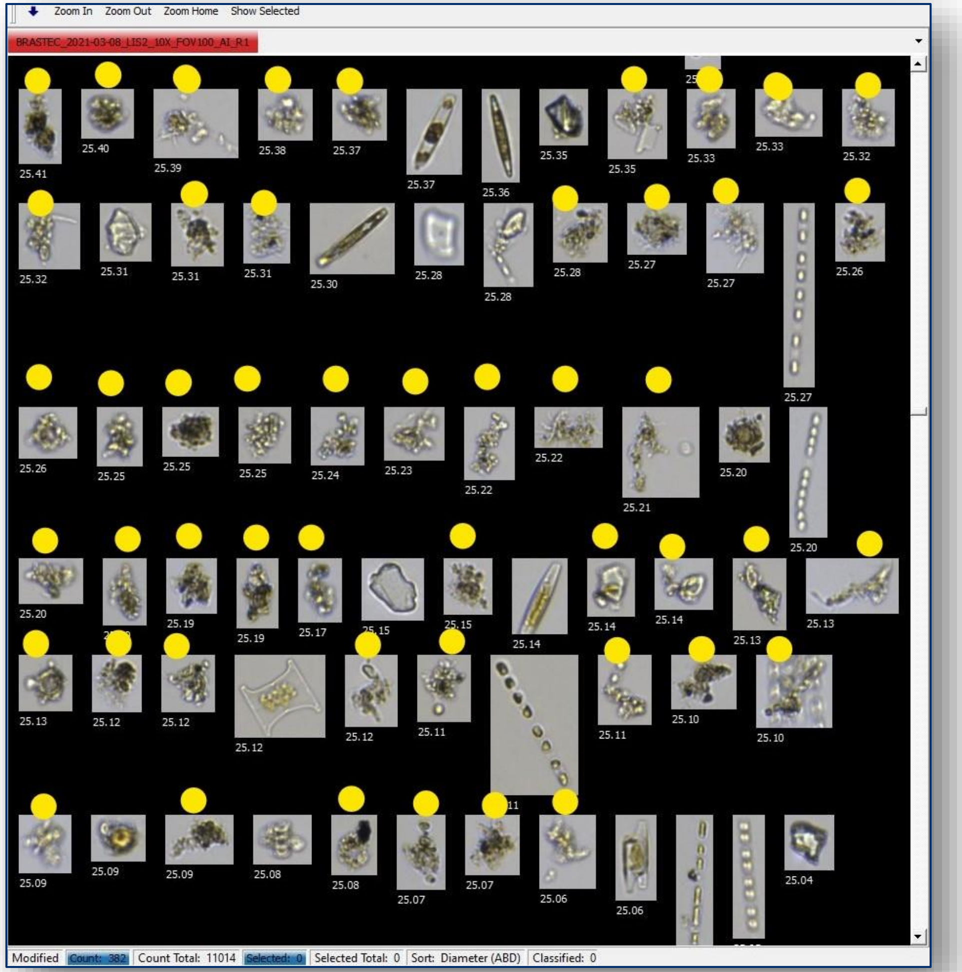
- Concentration (particles/mL)
- Size distribution
- Morphology
- Classification
- Biovolume



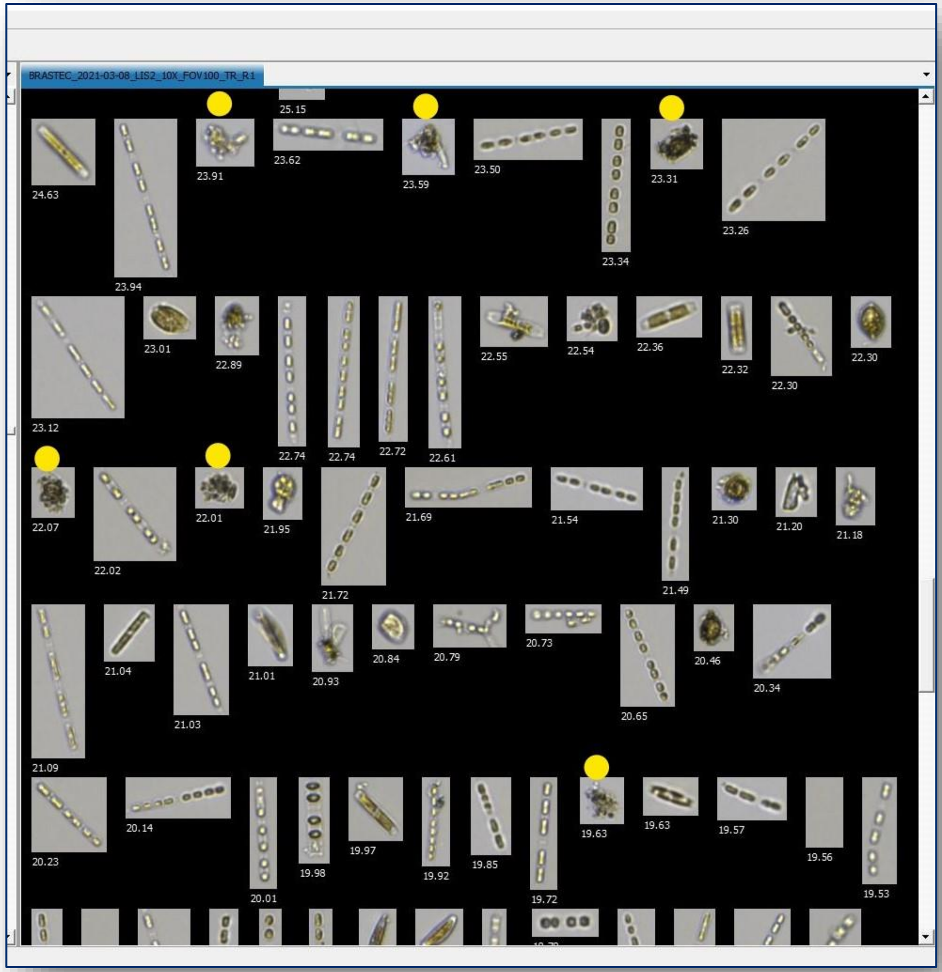
Plankton assemblage from the Gulf of Maine imaged with FlowCam at 10X in Autoimage Mode.

= Detritus

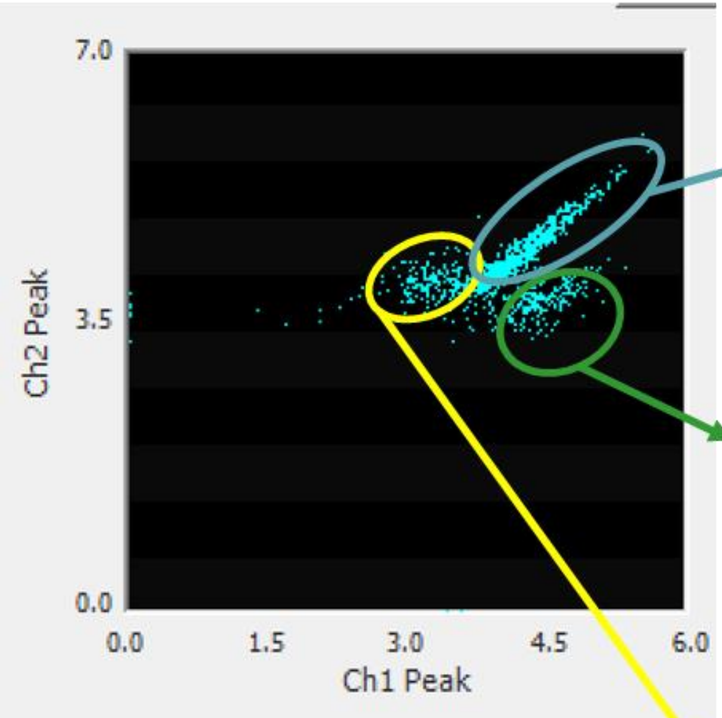
Autoimage Mode



Trigger Mode



FlowCam

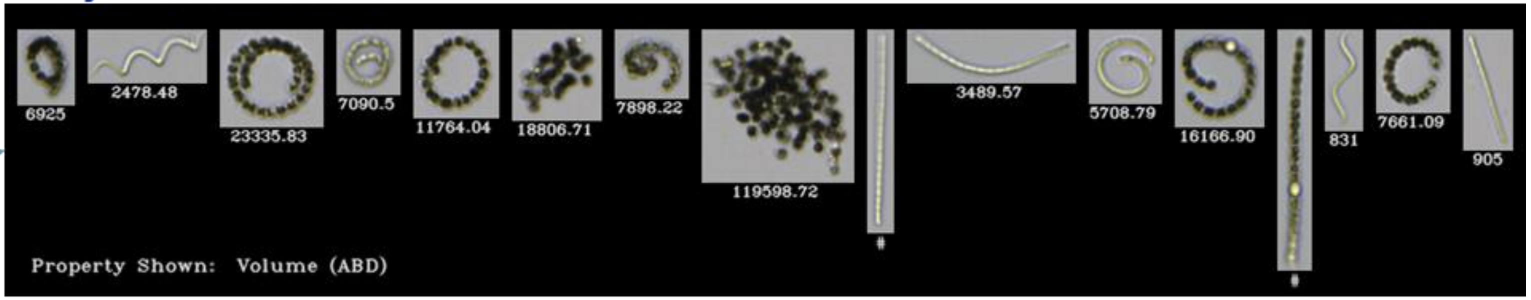


Count	1372 of 1373	Efficiency	
Particles / ml	2976	PPUI	

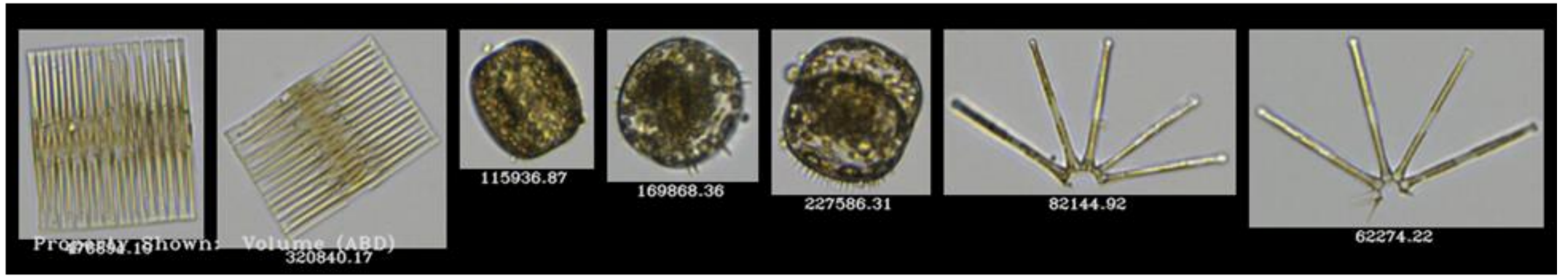
Summary Stats | Filters | Context Summary

Filter	Count
Cyanobacteria	754
Detritus, Decomposing	159
Diatoms, Other Algae	462

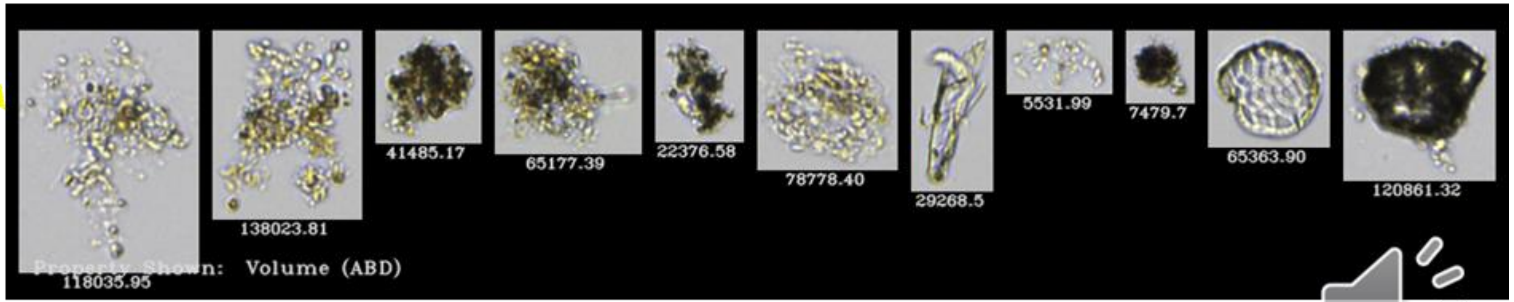
Cyanobacteria



Diatoms & Other Algae



Detritus & Decomposing Algae



FlowCam

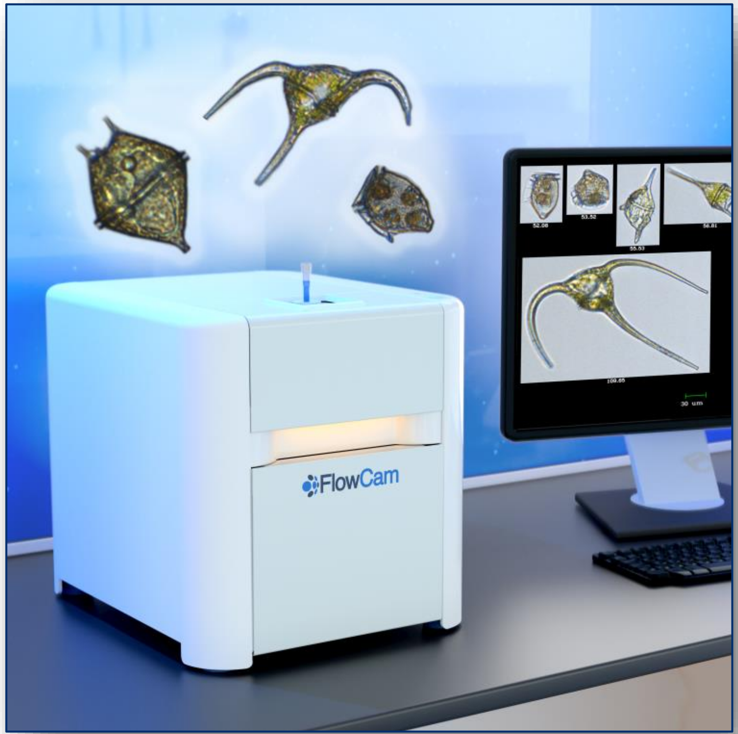
Outline

FlowCam Overview

Applications in Marine Science

Instrument Types

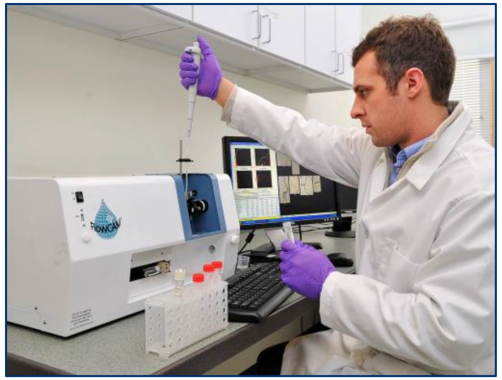
Example Data

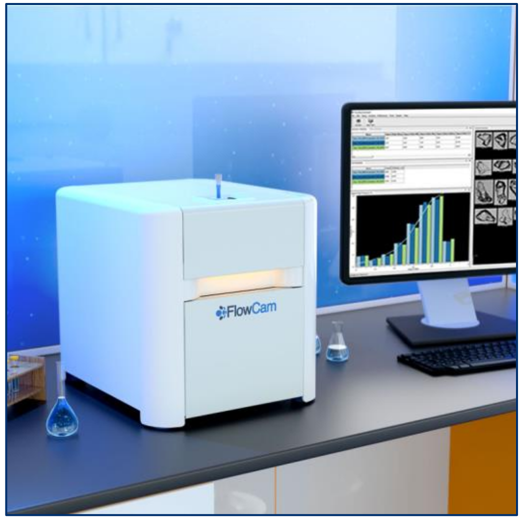


FlowCam 8000 with marine dinoflagellates

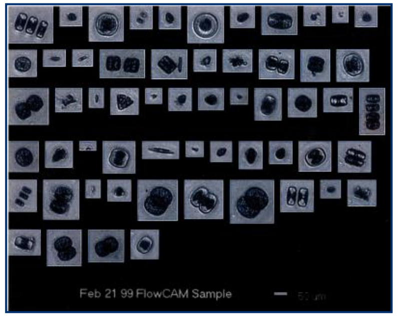
FlowCam

FlowCam Through the Years





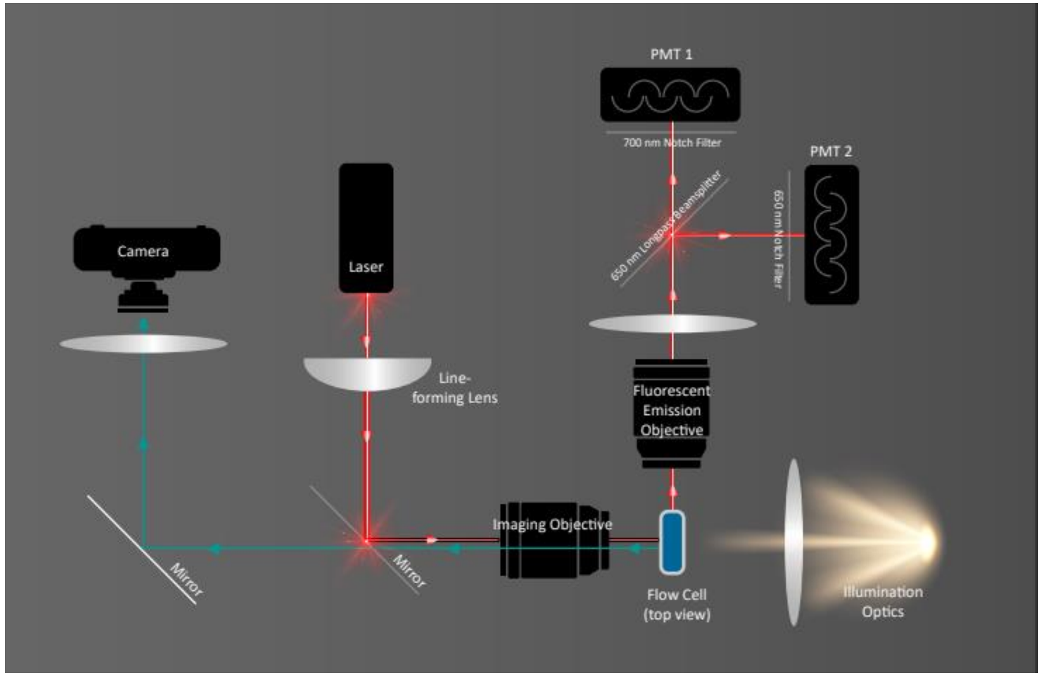
1999 | 2006 to 2010 | 2010 to 2016 | 2016 to 2024



FlowCam

Trigger Mode- FlowCam

- Instead of the LED light flashing at a pre-defined rate, a **laser** triggers the camera to take a picture.
- When a particle gets excited, it emits light at a higher wavelength.
- 2 channels of detection.
  - Channel 1 is always for chlorophyll
- Best for turbid environments.



	Laser Detector Bands (Photomultiplier Tube Filters, PMTs)	
Laser Excitation Wavelength	**CH1** Emission Wavelength	**CH2** Emission Wavelength
488 nm (Blue)	650 LP (Chlorophyll)	525±30 (Green, FITC)
532 nm (Green)	650 LP (Chlorophyll)	575±30 (Orange, Phycoerythrin)
633 nm (Red)	700±10 (Chlorophyll)	650±10 (Red, Phycocyanin)

FlowCam

FlowCam® Product Portfolio for Aquatic Applications

Subvisible Imaging Particle Analysis

FlowCam 8100

Size, concentration, and morphology from 2 µm - 1 mm

FlowCam Cyano

For quick cyanobacteria detection and enumeration using pigment fluorescence.
Particle size range: 2 µm - 1 mm

FlowCam 8400

Isolate phytoplankton images from other particles using fluorescence
Particle size range: 2 µm - 1 mm

FlowCam Macro

Analysis of large particles and zooplankton from 300 µm - 5 mm

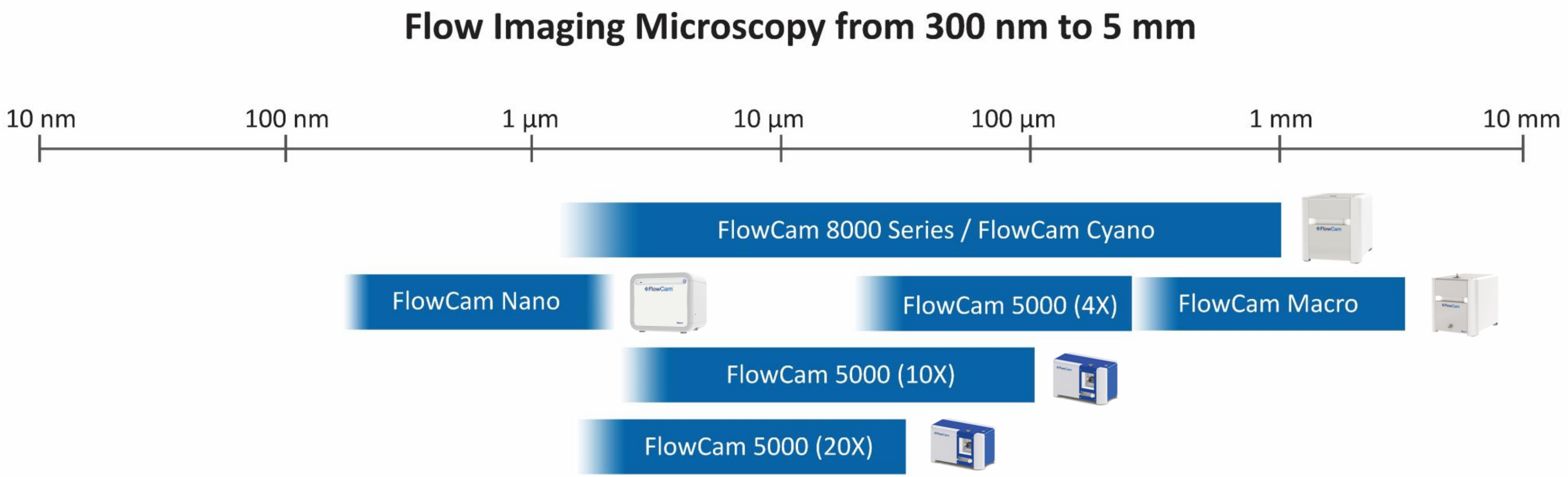
FlowCam 5000

Optimized for your application; our most affordable instrument.
Particle size range: 2 µm - 300 µm

VisualSpreadsheet®

Powerful software to analyze images and visualize your results

YOKOGAWA | Yokogawa Fluid Imaging Technologies | www.flowcam.com | info@fluidimaging.com



Flow Imaging Microscopy from 300 nm to 5 mm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
10 mm
FlowCam 8000 Series / FlowCam Cyano
FlowCam Nano
FlowCam 5000 (4X)
FlowCam Macro
FlowCam 5000 (10X)
FlowCam 5000 (20X)

FlowCam

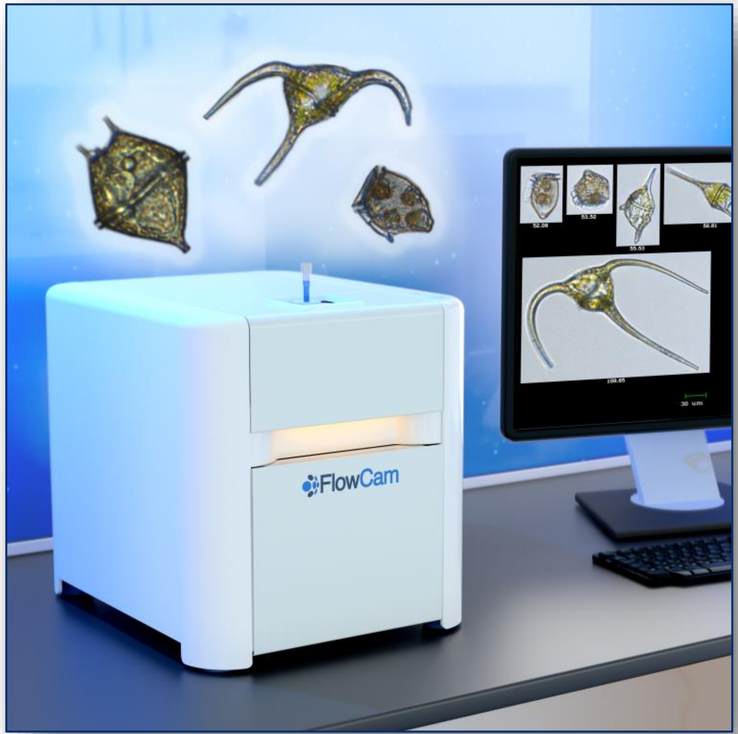
Outline

FlowCam Overview

Applications in Marine Science

Instrument Types

Example Data



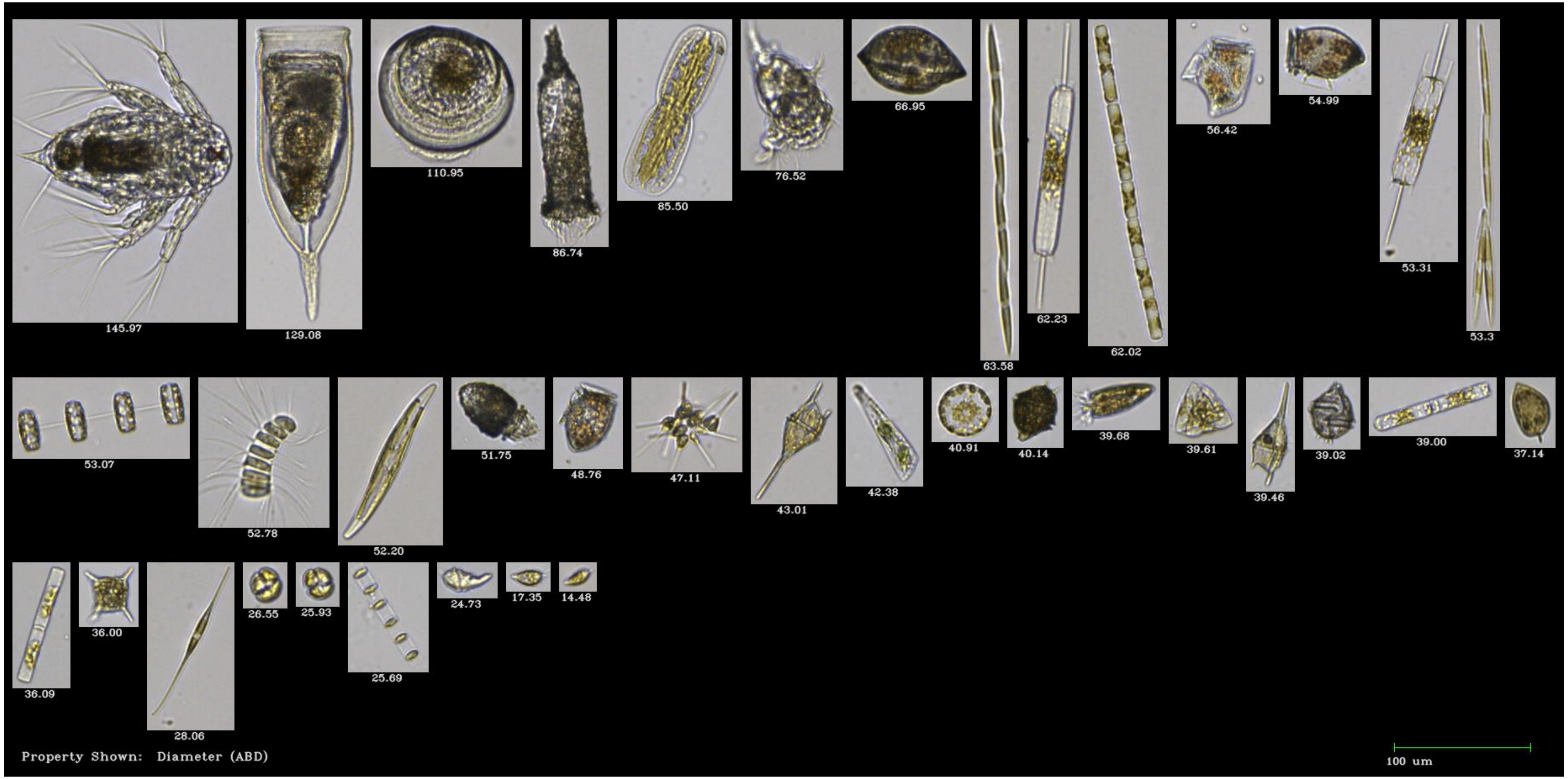
FlowCam 8000 with
marine dinoflagellates

FlowCam 8000

2 µm to 1 mm

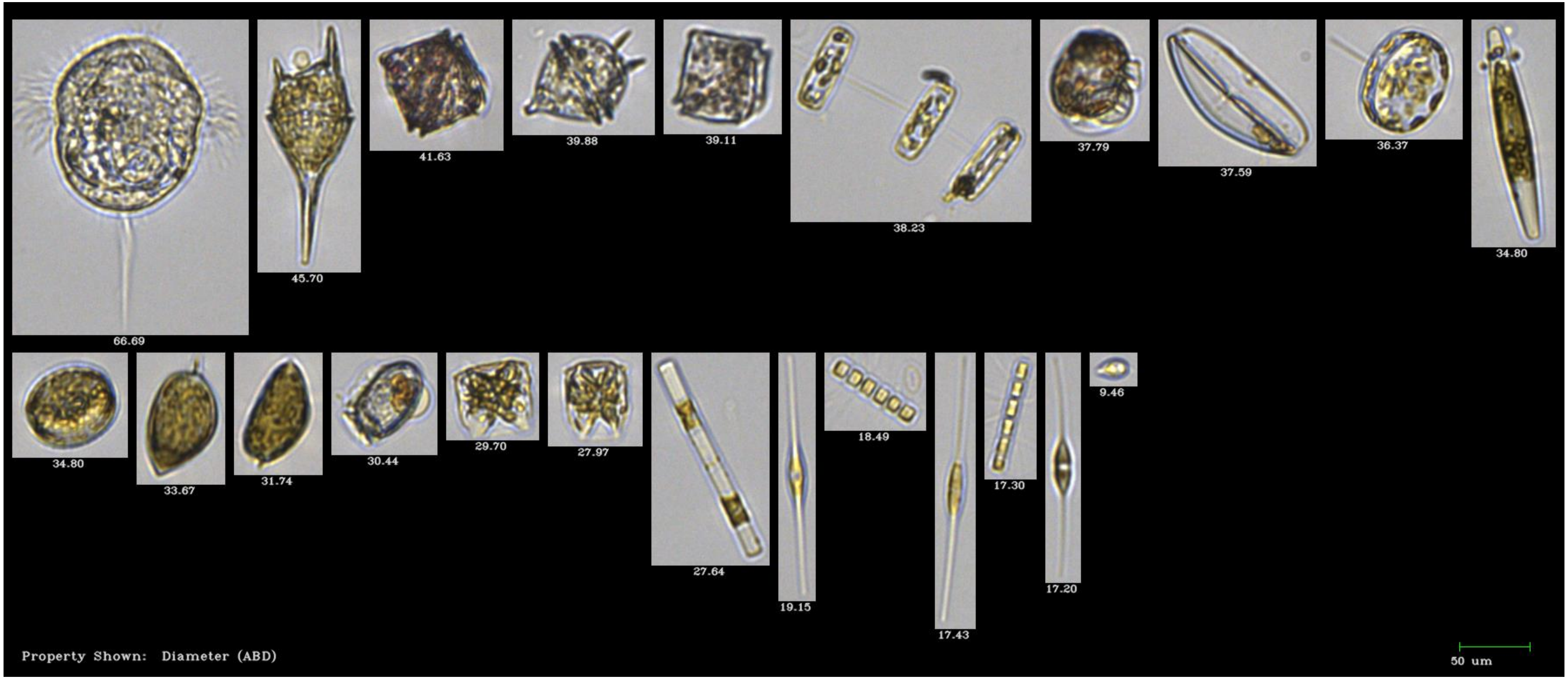
10X, FOV100

Casco Bay (Maine USA), Live sample imaged with FlowCam 8100 (Autoimage Mode)



20X, FOV50

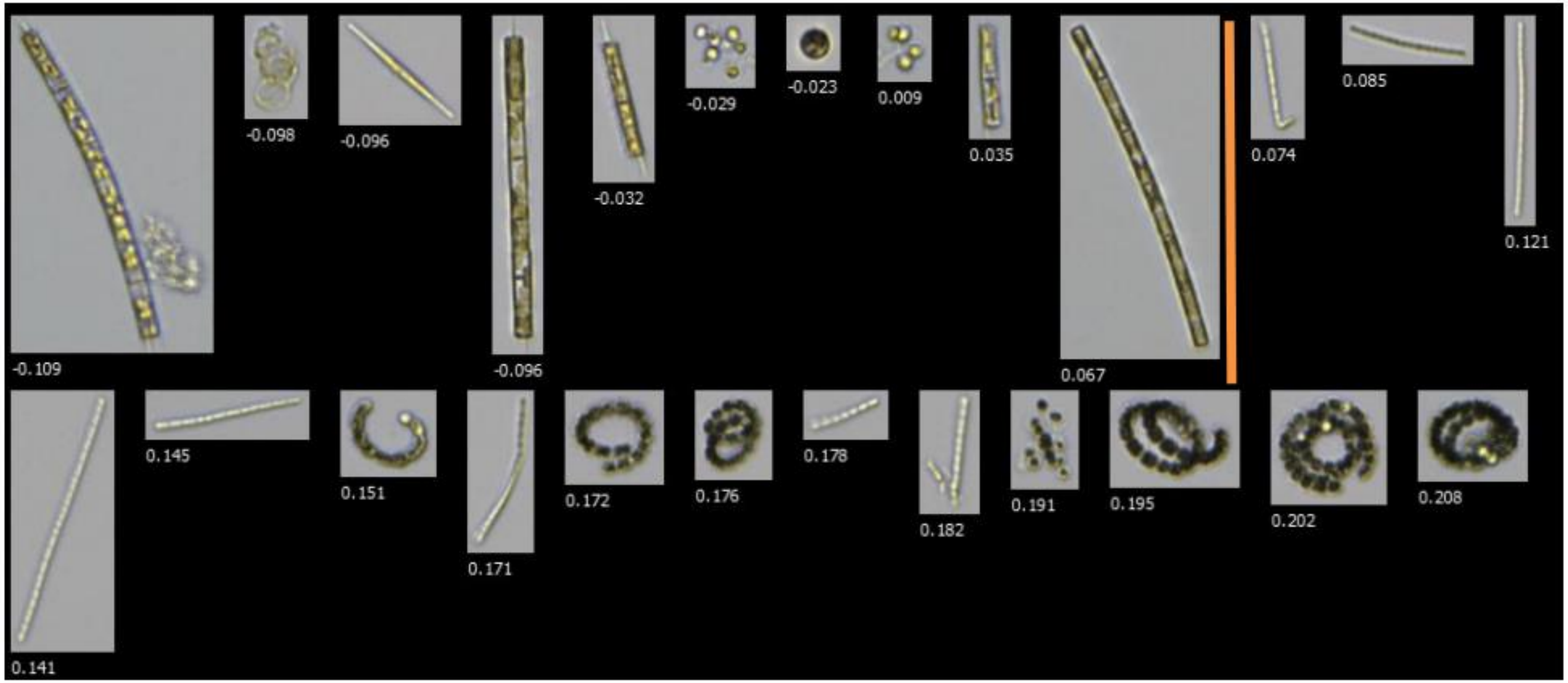
Casco Bay (Maine USA), Live sample imaged with FlowCam 8100 (Autoimage Mode)



FlowCam

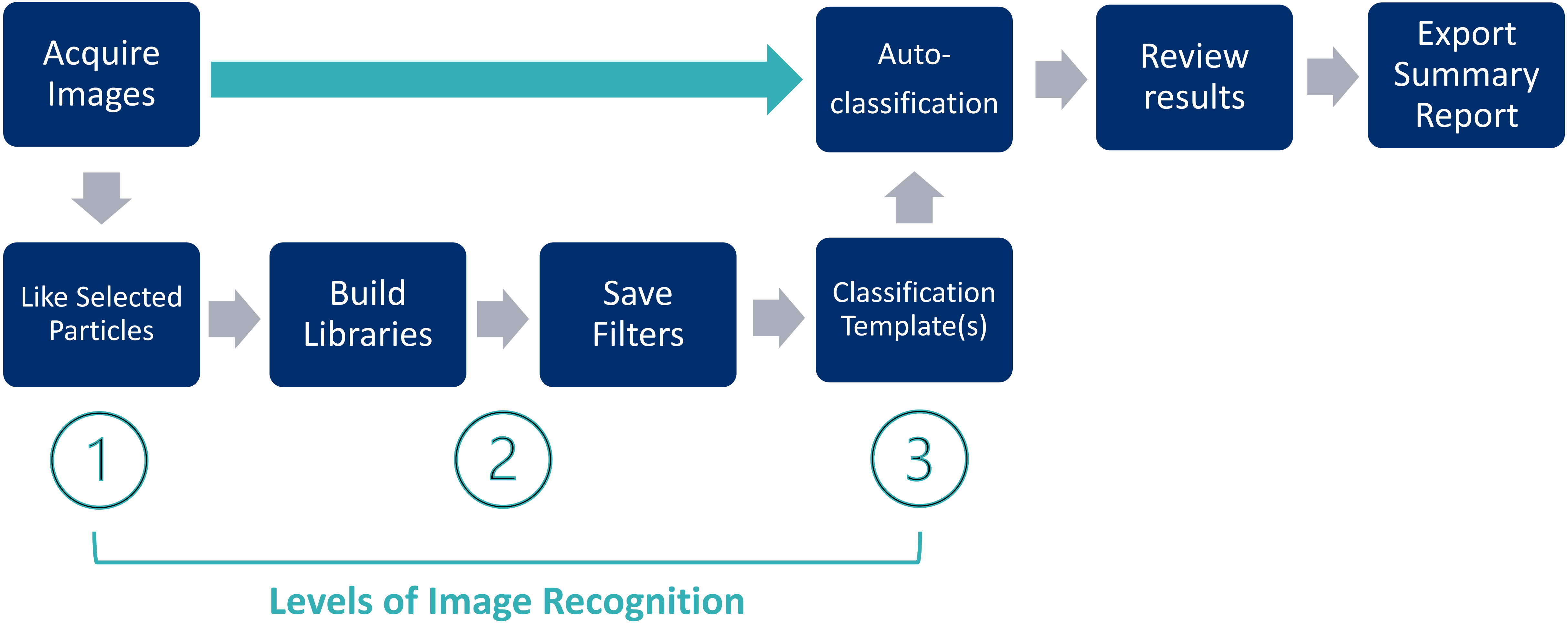
Trigger Mode: FlowCam Cyano

Primarily Diatoms & Other Algae



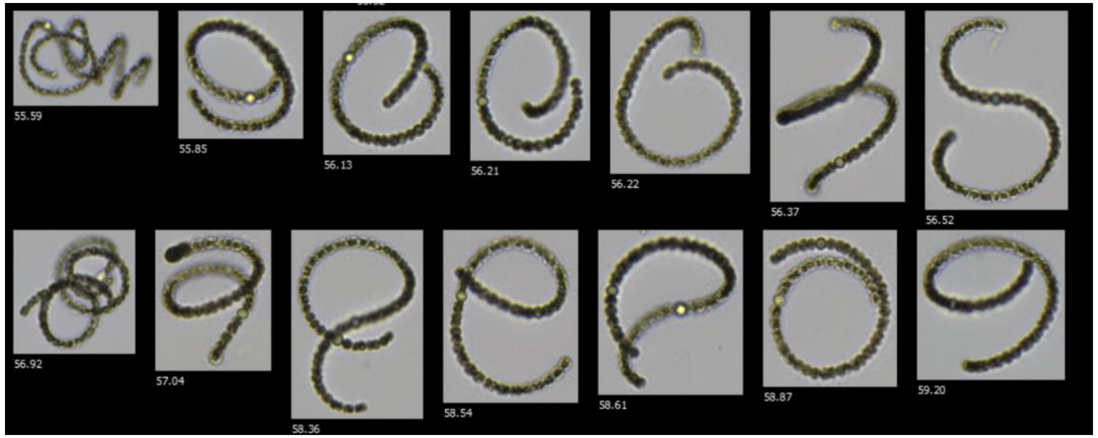
Primarily Cyanobacteria

Example Classification Workflow

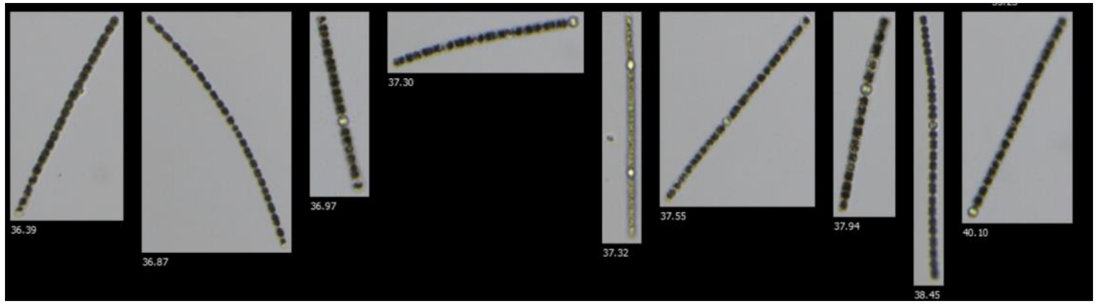


FlowCam

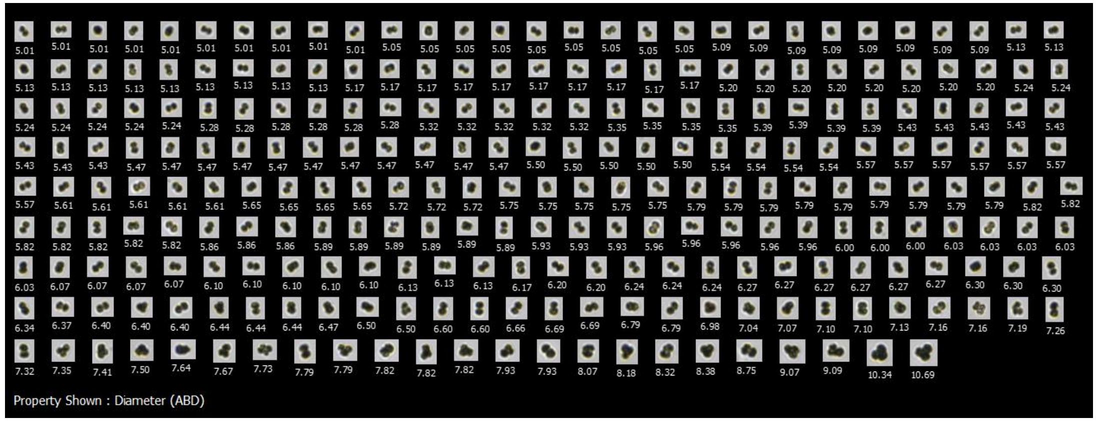
Dolichospermum (coiled)



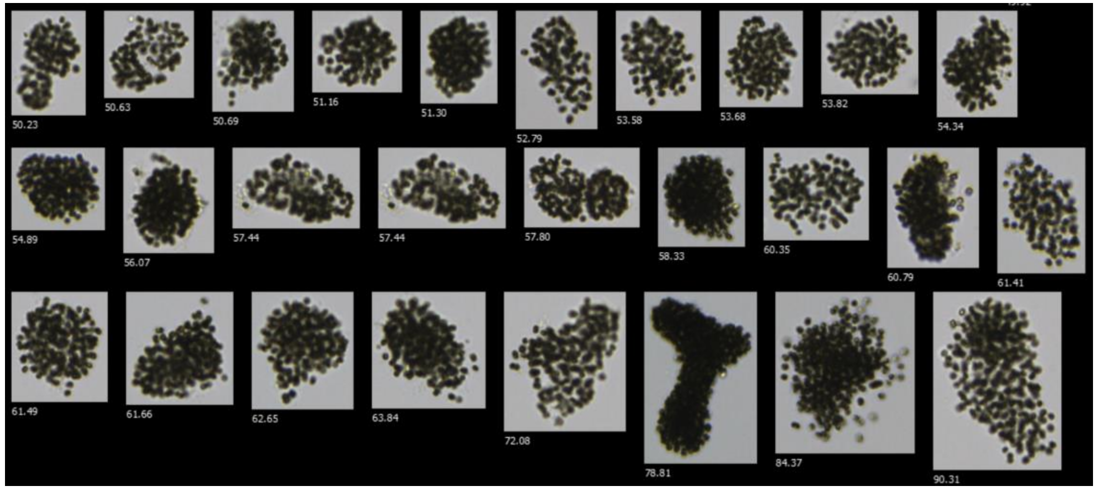
Dolichospermum (straight)



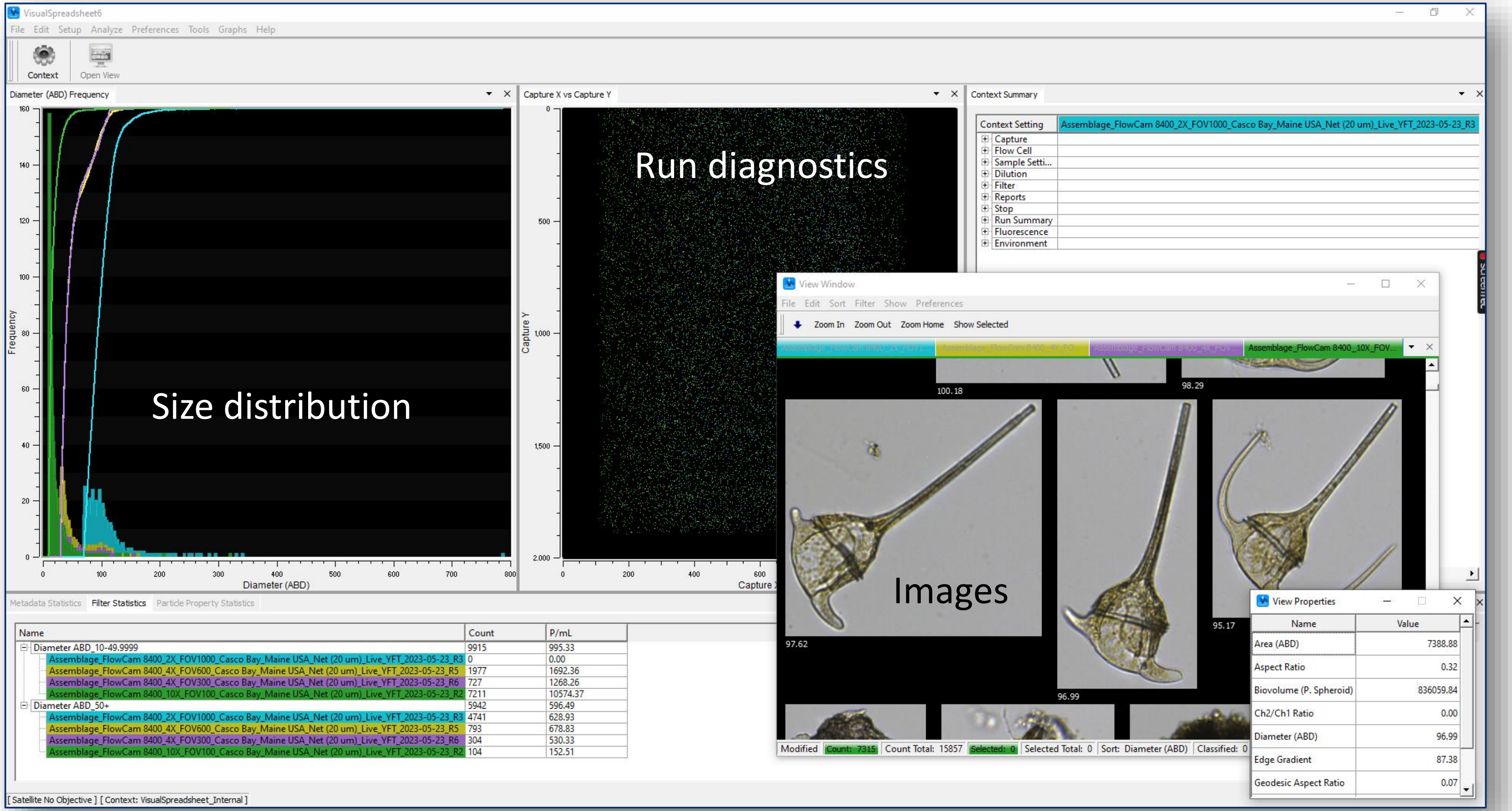
Microcystis (single)



Microcystis (colonial)



VisualSpreadsheet



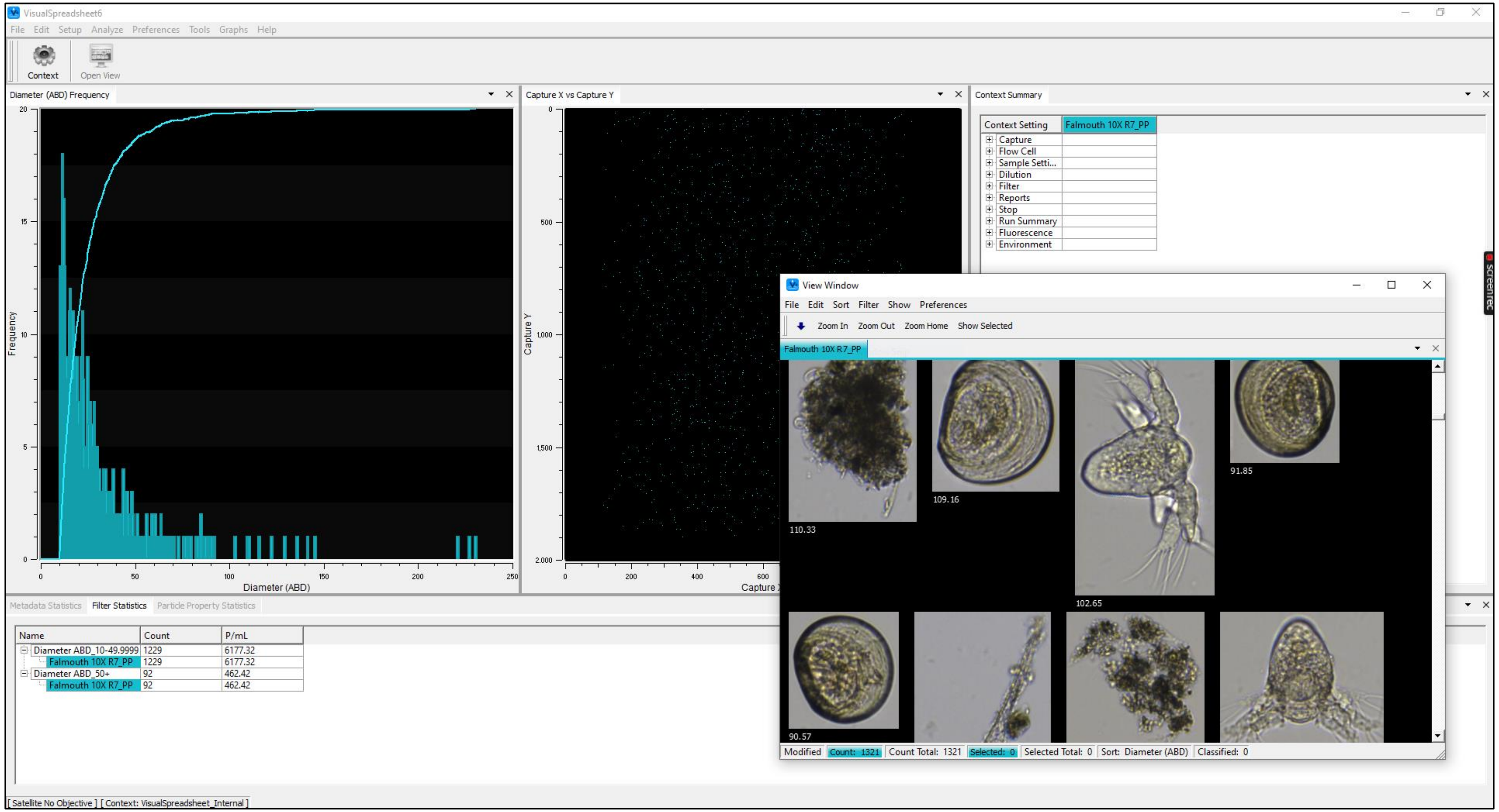
Metadata

Customizable filters

Particle properties for every image

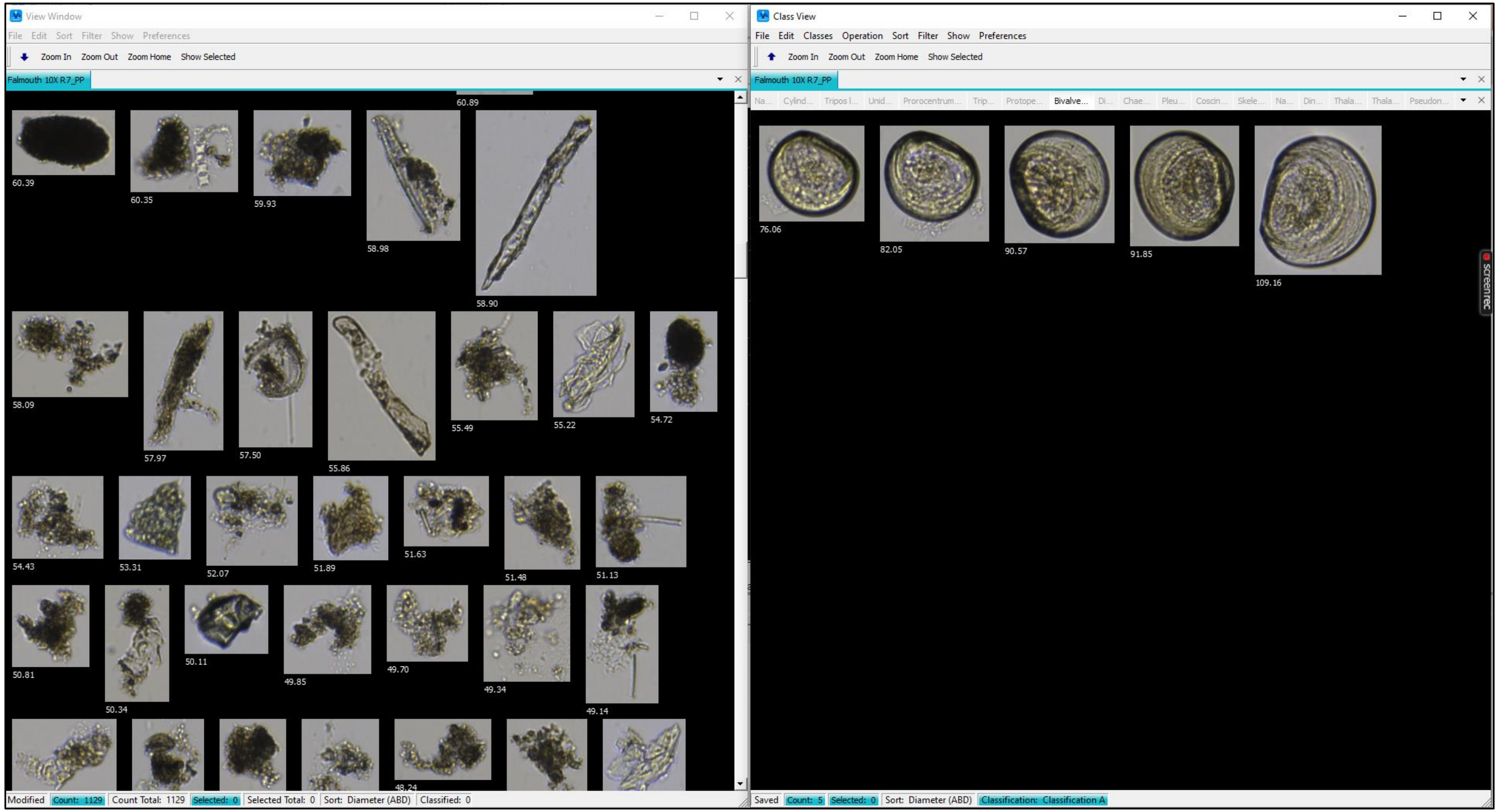
FlowCam

Acquire Images



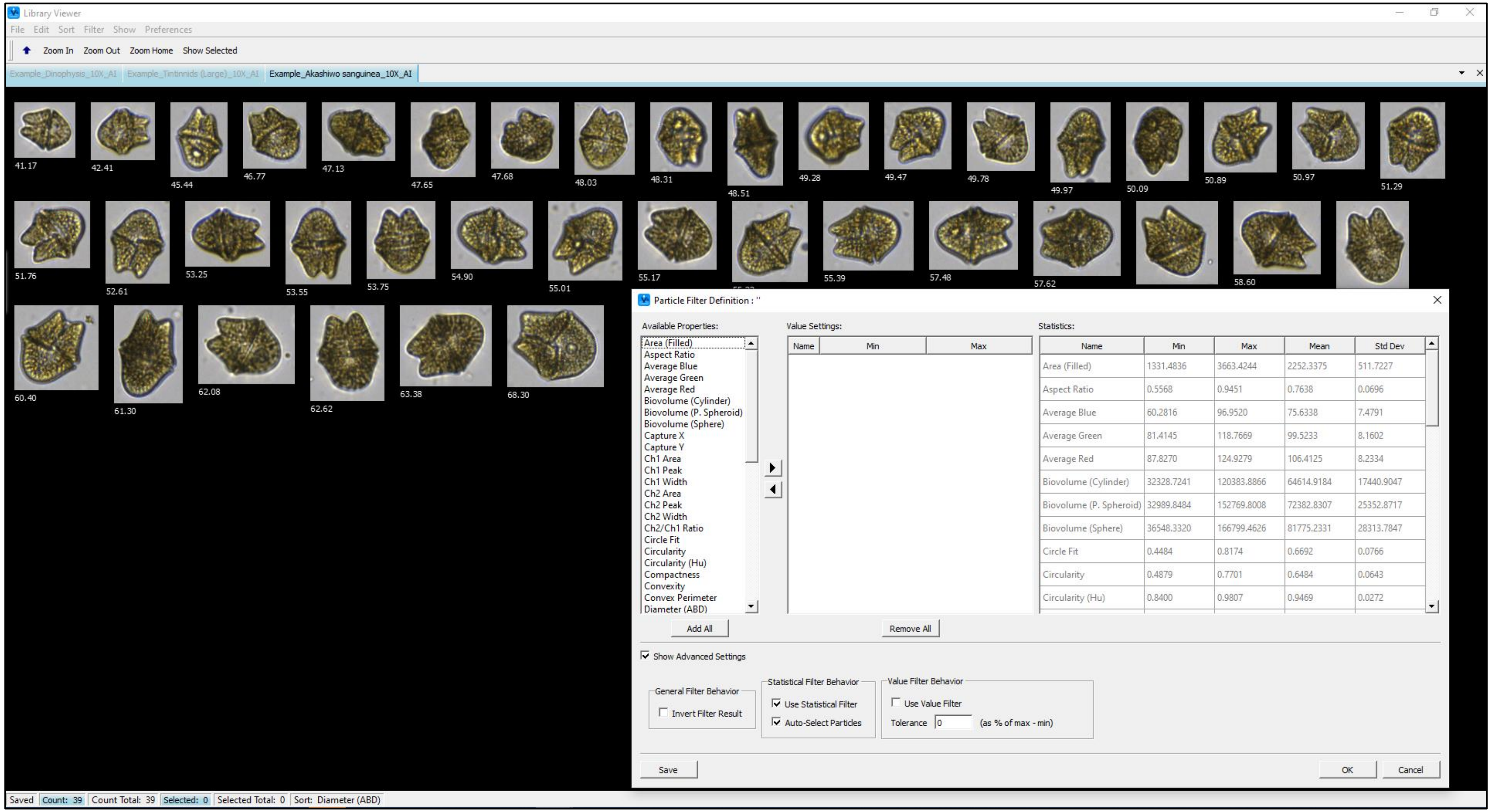
FlowCam

Manual Classification

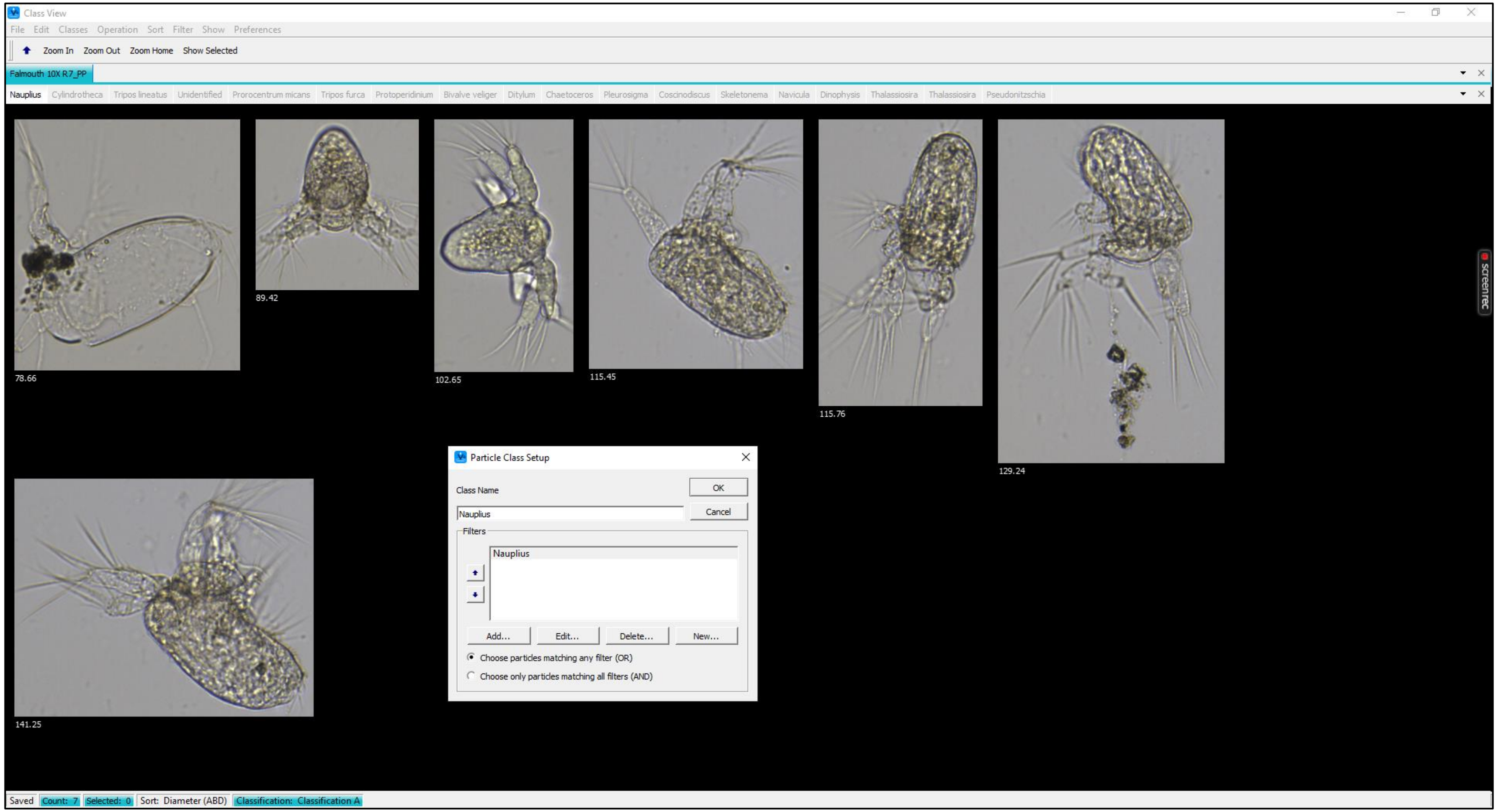


FlowCam

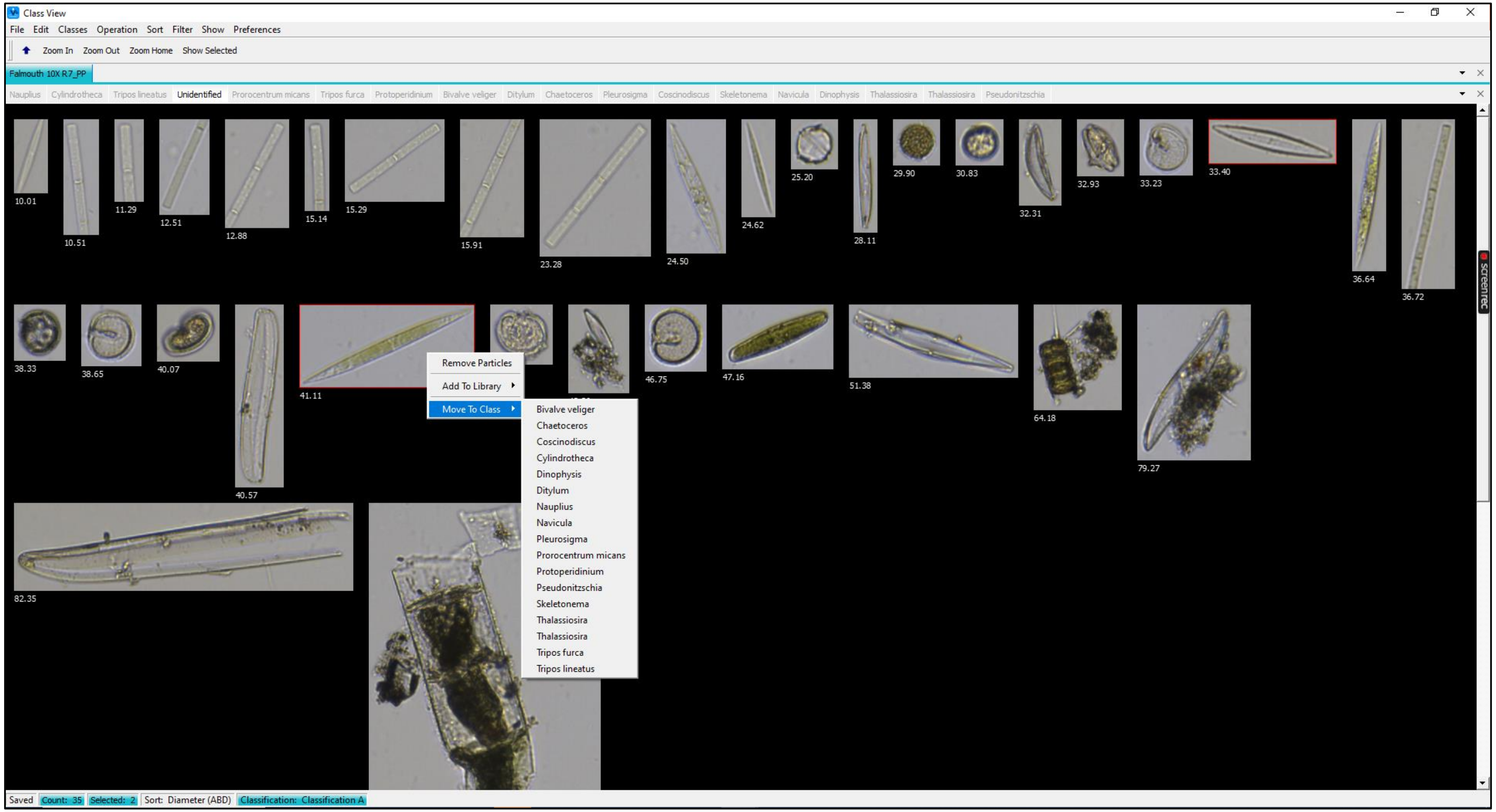
Build Libraries & Filters



Autoclassification



Review Results



Export Summary Report

- Concentration (particles per mL)
- Particle Properties
- Cumulative Statistics

======== Metadata Statistics ========

Name	Count	Particles / mL
Nauplius	7	35.1841
Cylindrotheca	0	0
Tripos lineatus	13	65.3419
Unidentified	35	175.9204
Prorocentrum micans	2	10.0526
Tripos furca	1	5.0263
Protoperidinium	2	10.0526
Bivalve veliger	5	25.1315
Ditylum	3	15.0789
Chaetoceros	30	150.7889
Pleurosigma	10	50.263
Coscinodiscus	5	25.1315
Skeletonema	71	356.8671
Navicula	0	0
Dinophysis	0	0
Thalassiosira	4	20.1052
Thalassiosira	2	10.0526
Pseudonitzschia	2	10.0526

======== End Metadata Statistics ========

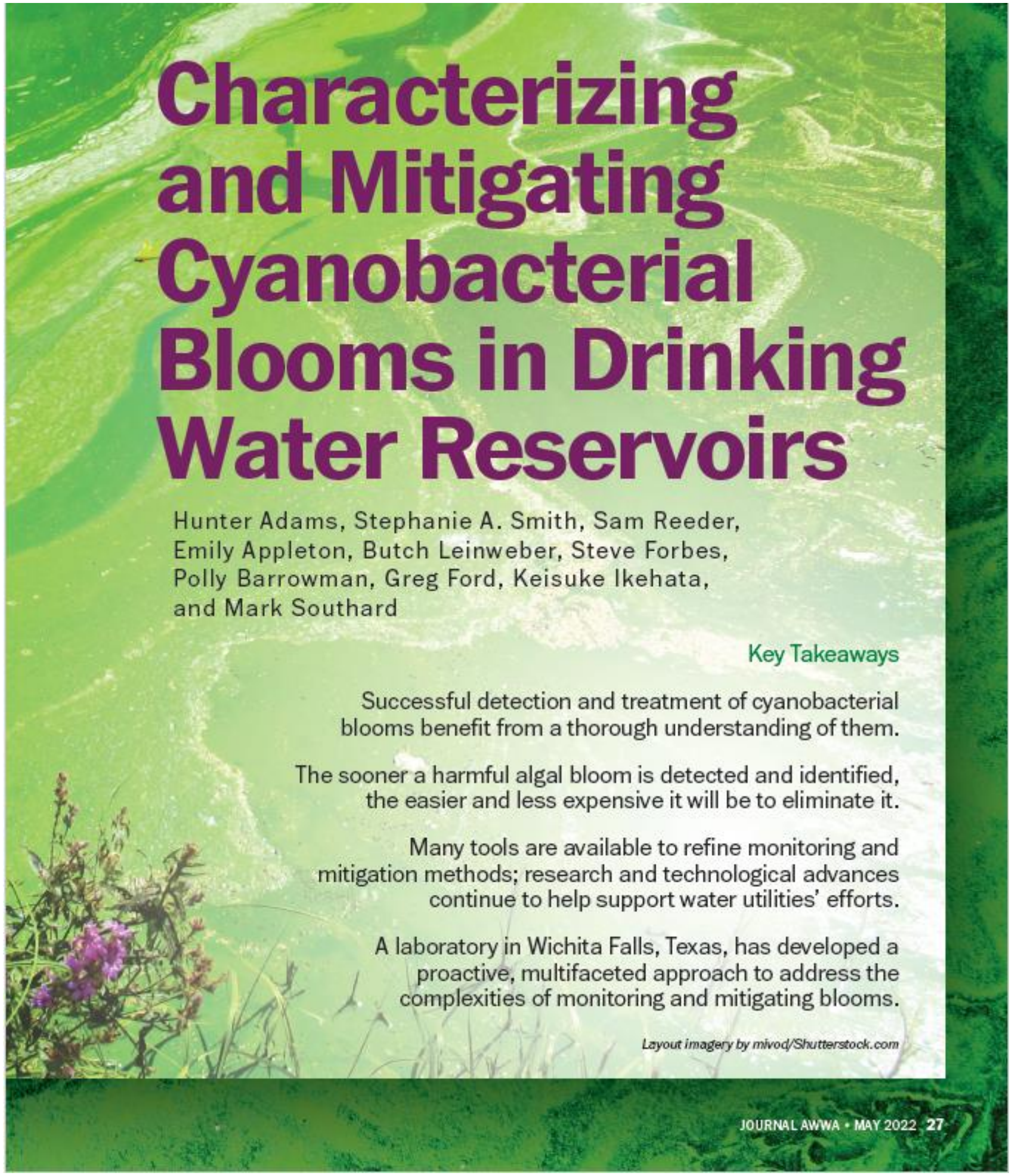
======== Particle Property Statistics ========

Name	Area (ABD) Mean	Aspect Ratio Mean	Biovolume (P. Spheroid) Mean	Ch2/Ch1 Ratio Mean	Diameter (ABD) Mean	Edge Gradient Mean	Geodesic Aspect Ratio Mean	Length Mean	Width Mean
Nauplius	9885.3118	0.6436	1423726.034	0	110.3474	60.2737	0.02	228.8713	144.1884
Cylindrotheca	0	0	0	0	0	0	0	0	0
Tripos lineatus	936.4887	0.3527	20592.9812	0	34.1273	86.9849	0.1045	86.4826	27.0874
Unidentified	2260.5758	0.3932	157954.5963	0	39.3559	66.3953	0.2849	108.5939	28.6398
Prorocentrum micans	582.7098	0.4777	7001.8964	0	27.1367	103.385	0.4092	43.2813	18.6975
Tripos furca	6410.2004	0.1392	181306.2907	0	90.3422	71.8224	0.0369	282.8863	49.5138
Protoperidinium	1375.1412	0.8026	47788.0997	0	41.6433	87.1947	0.3309	60.94	41.55
Bivalve veliger	6451.5999	0.8681	384911.8677	0	89.9378	152.7038	0.6832	101.8667	85.3853
Ditylum	1467.0366	0.1948	41062.6018	0	39.443	65.444	0.0297	186.1671	23.8912
Chaetoceros	617.7108	0.2887	31036.586	0	26.1976	56.6074	0.0322	83.4924	24.653
Pleurosigma	2630.1559	0.2303	99090.2202	0	56.8839	97.4703	0.1103	156.9898	33.6555
Coscinodiscus	1694.3512	0.8154	48440.4771	0	46.2932	111.5285	0.8898	53.8072	41.4808

export classification summary

FlowCam

Treatment measures and efforts-Costs



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.

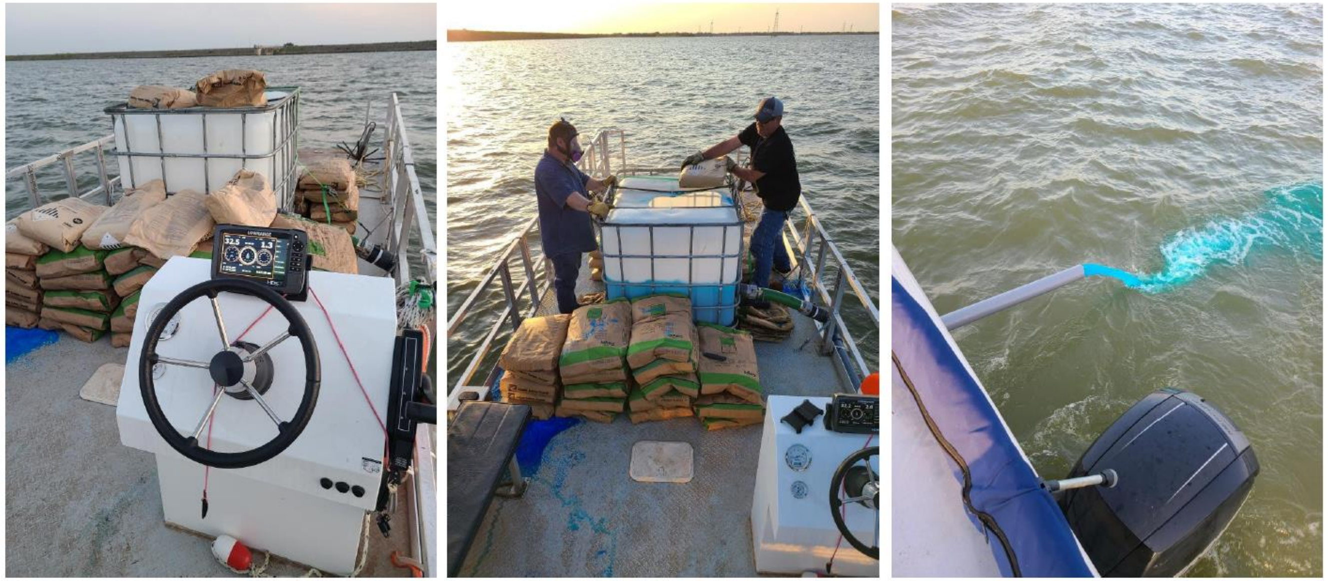
The sooner a harmful algal bloom is detected and identified, the easier and less expensive it will be to eliminate it.

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.

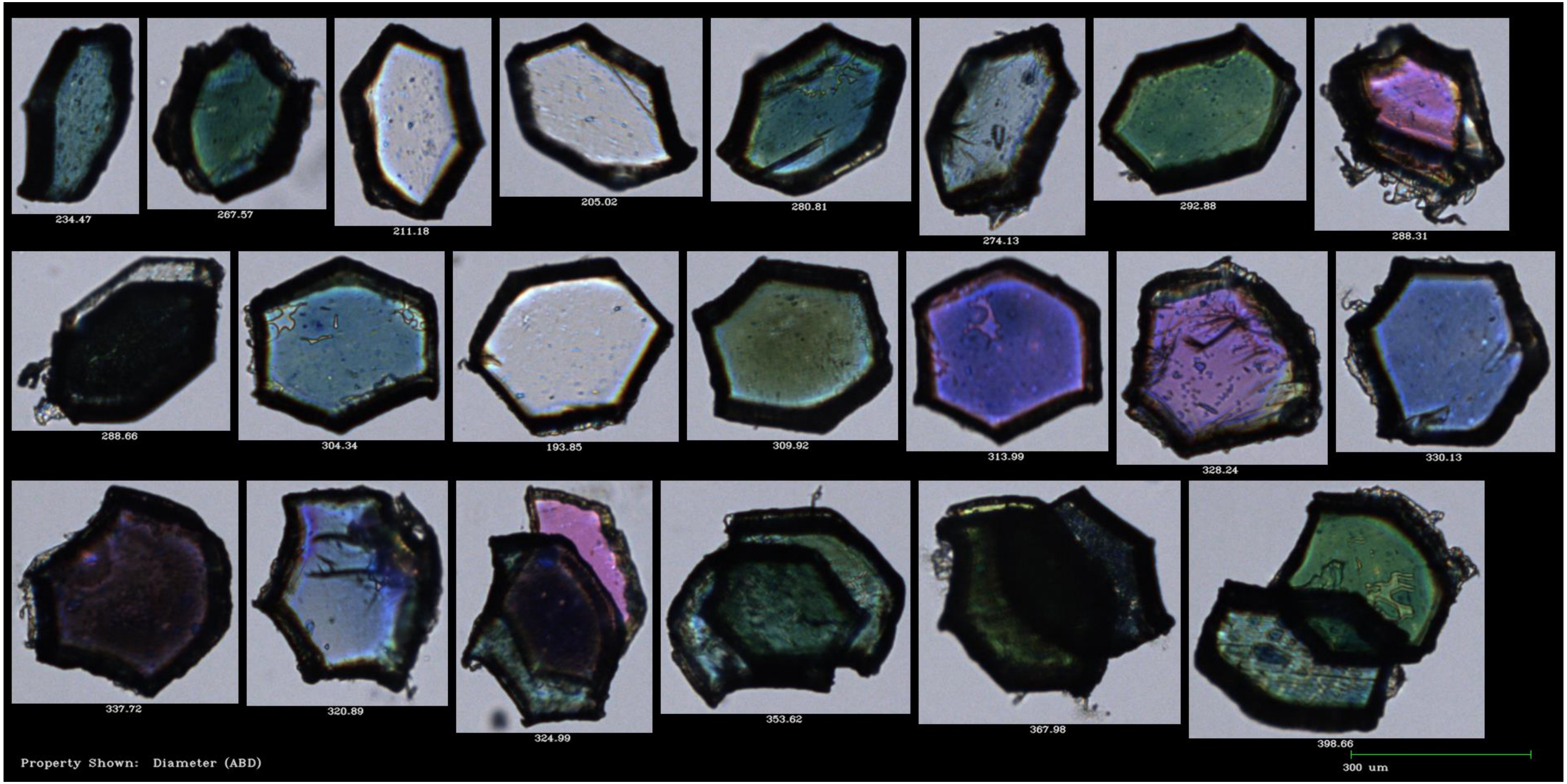
Layout imagery by mivod/Shutterstock.com

Lake Arrowhead Bloom Treated With Copper Sulfate and Citric Acid, June 2021



FlowCam

"Plastic Glitter-Microplastics"



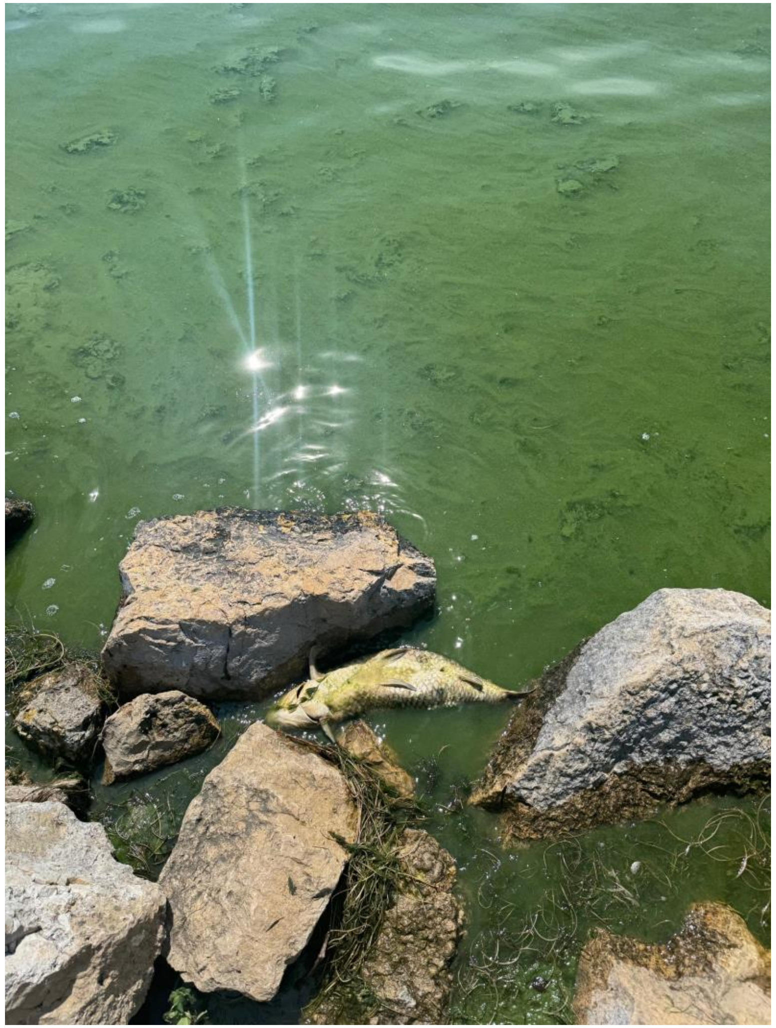
Yokogawa Fluid Imaging Technologies

Some of the installations across India and Globe

Annamalai University	Aquatic	India
APL Research Center-2 (A Division of Aurobindo Pharma Ltd)	Industrial	India
Syngene Ltd	Industrial	India
Centre for Marine Living Resources & Ecology (CMLRE)	Aquatic	India
CSIR Central Salt & Marine Chemicals Research Institute (CSMCRI)	Aquatic	India
CSIR National Institute of Oceanography (NIO) - India (Goa)	Aquatic	India
Indira Gandhi Center for Atomic Research (IGCAR)	Aquatic	India
National Centre for Antarctic & Ocean Research (NCAOR)	Aquatic	India
National Centre for Sustainable Coastal Management (NCSCM)	Aquatic	India
Reliance Industries, LTD.	Aquatic	India
National institute of oceanography (Goa)	Aquatic	India
National institute of oceanography (Goa)	Aquatic	India
National institute of oceanography (Kochi)	Aquatic	India
National institute of oceanography (Vizag)	Aquatic	India
National institute of oceanography (Goa)	Aquatic	India
National institute of oceanography (Goa)	Aquatic	India

FlowCam

Summary



- Proactive monitoring means that programs are put in place before a lake experiences a problem.
- FIM is a powerful tool for HAB monitoring programs
  - Taste and Odor
  - Filter Cloggers
  - Toxin Producers
- FIM can reduce TAT from sample to results, meaning faster response time and reduced treatment cost.
- Standardization of FIM methodology
  - JTG has been formed
  - Inclusion in Standard Methods (AWWA) within 1-2 years

FlowCam

Thank you!

Mr.Bhushan Pandit

Email: Bhushan.pandit@yokogawa.com

FlowCam