Flow Imaging Microscopy for Harmful Algal Bloom Monitoring

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Key Takeaways

- Flow imaging microscopy (FIM) combines a flow cytometer's fluidic architecture with a microscope's spatial resolution to allow morphometric analysis of objects in suspensions such as water.
- FIM's real-time analysis and identification of potential cyanotoxin producers help utilities understand population dynamics to maintain healthier source waters and reduce response times when a bloom is indicated.
- Case studies show FIM's advantages and limitations in harmful algal bloom monitoring programs for reservoir management and water treatment.

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Traditional flow cytometry has been used in conjunction with microscopy in harmful algal bloom (HAB) and phytoplankton research for decades, primarily to determine microscopic organism distribution and population dynamics. Flow cytometry directs particles, single file, through a light beam where their physical properties such as size and complexity determine the extent of light scatter in forward and side directions, respectively. Fluorescence emissions can be measured and provide data on additional properties such as the presence of chlorophyll pigment. However, environmental monitoring of algae using flow cytometry has been limited by its inability to process larger organisms or provide enough relevant morphological data necessary for identification beyond the broader class.

HABs pose challenges to drinking water utilities and lake managers due to the toxic compounds that can be released upon lysis of the cells (Westrick & Szlag 2018). Blooms also often produce a variety of taste and odor (T&O) compounds, and while they are not usually a health concern, they can be problematic when it comes to consumer perception and satisfaction (Carniero et al. 2020, Dietrich & Burlingame 2014, Watson 2010). Both long-term and acute exposure to the neurotoxins and hepatotoxins produced by cyanobacteria can cause sickness and death, so it is important that drinking water utilities implement monitoring programs to track and treat HABs (Chorus & Welker 2021).

Basic Flow Imaging Microscopy Schematic

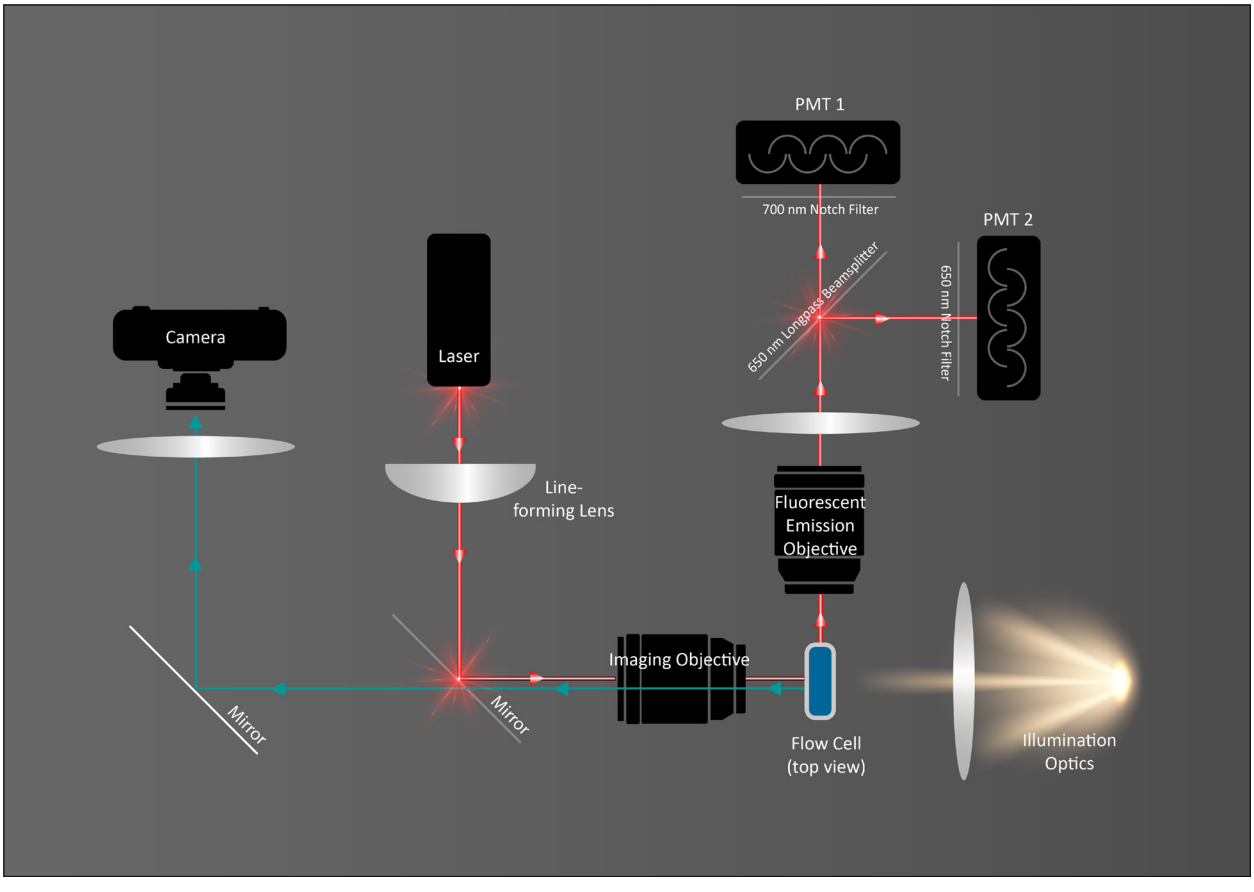


Figure 1

Flow Imaging Microscopy

Flow imaging microscopy (FIM) combines the high content data of microscopy with the high throughput and statistical power of flow cytometry to quantitatively characterize objects (or particles) in solution. FIM can measure a much wider range of particle sizes than traditional flow cytometry, with the additional benefit of high-resolution particle imaging (Sieracki et al. 1998). It rapidly produces easily quantifiable results, and data acquisition can be viewed in real time.

Unlike traditional flow cytometry, FIM does not use a sheath fluid to transport particles; instead, it works by aspirating sample through a flow cell using an ultra-high-precision syringe pump. The camera, paired with an objective lens that magnifies the particles, can capture thousands of particle images per second (Figure 1). Imaged particles are extracted and stored for further analysis using the software. Saved data can be analyzed at any time, and organisms can be classified using particle properties such as size, area, biovolume, and fluorescence.

Some FIM instrument models include a laser so they can capture two channels of fluorescence data per camera frame, which enables sorting and classifying data according to fluorescence. As shown in Table 1, three lasers are currently available, each offering different algal pigment information. The use of particle properties and fluorescence data, along with morphological information, allows monitoring of a more diverse group of organisms including phytoplankton, cyanobacteria, zebra and quagga mussels, and zooplankton.

When using a laser, the beam is focused on the center of the flow cell, and when a particle passes through it, the optical system is triggered to capture an image, recording the fluorescent emission value. Particles can be sorted according to these values and automatically classified into one of three categories: cyanobacteria, other algae and diatoms, and detritus. This approach reduces analysis time by only imaging particles fluorescing at specific wavelengths.

Users can also classify all particles in their sample, which is useful when determining pre- and posttreatment particle concentrations or if the more specific approach fails to capture expected particle concentrations, especially in the case of a green lake or reservoir. As algae cells begin to degrade, their ability to fluoresce decreases, which can reduce the number of particles detected.

Following image acquisition, particle images can be sorted using different statistical parameters. Image libraries and filters built from the statistical properties of particles can be used to quickly screen samples. By performing this semi-automated analysis, laboratories can speed up their HAB analysis by hours or, if using a contract laboratory, by days.

FIM and HAB Research

Since the early 2000s, drinking water utilities and government agencies have considered using FIM to replace manual microscopy to monitor the growth of algal blooms in lakes and reservoirs (Reilley-Matthews 2007). Manual microscopic analysis is slow, the raw data from these reads cannot be saved, and results can vary depending on the analyst.

FIM Lasers With Associated Excitation/Emission Wavelength and Common Monitoring Uses

Laser Excitation Wavelength *nm*	Laser Color	Channel 1 Emission Wavelength *nm*	Channel 1 Pigment Target	Channel 2 Emission Wavelength *nm*	Channel 2 Pigment Target	Monitoring Use
488	Blue	650 long pass	Chlorophyll	525 band pass 30	Stain (FITC), green	Predominantly research
532	Green	650 long pass	Chlorophyll	575 band pass 30	Phycoerythrin orange	Cyanobacteria/ red algae
633	Red	700 band pass 10	Chlorophyll	650 band pass 10	Phycocyanin	Cyanobacteria versus other algae and diatoms

FIM—flow imaging microscopy, FITC—fluorescein isothiocyanate

Table 1

Additionally, operators relying on water quality analysis to make treatment decisions may have to wait multiple days for results, by which time conditions may have changed. Inaccurate algae concentrations can yield less effective, costlier treatment plans along with the danger of toxin release or T&O problems. FIM was developed to produce fast, quantifiable results with limited prep and analysis time.

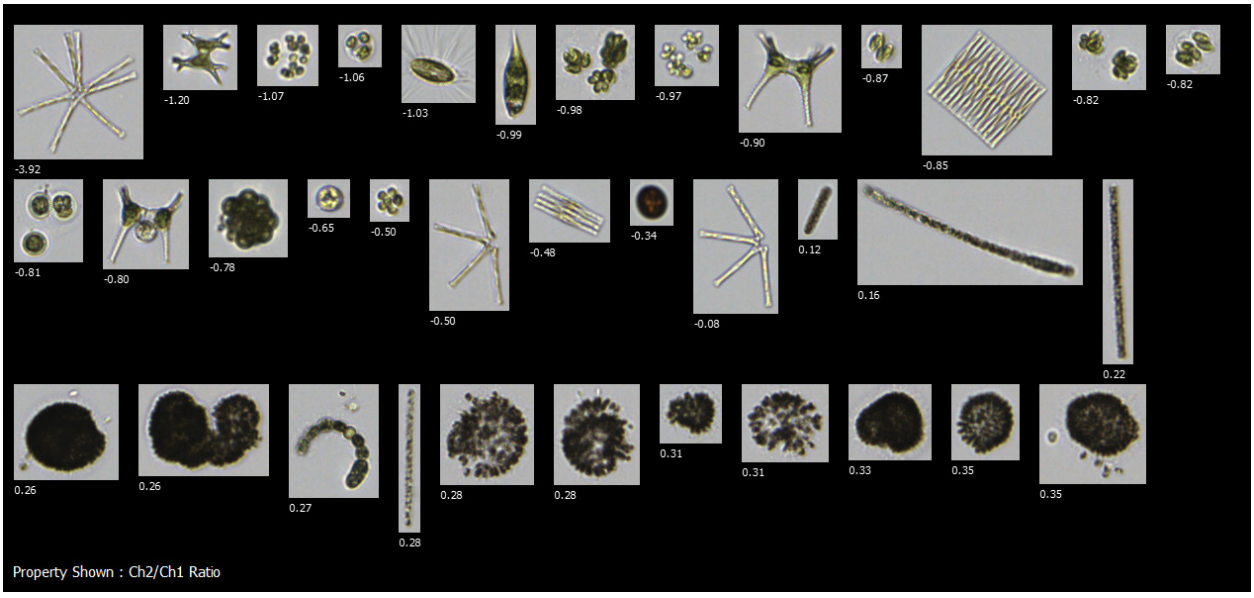
HABs are caused by an uncontrolled growth of algae and/or cyanobacteria in lakes, rivers, reservoirs, and in the marine environment. Cyanobacteria can quickly increase, and blooms occur as they exploit the overabundance of nutrients—e.g., phosphorus and nitrogen species, in the presence of warm temperatures and sunlight.

Rapid analysis and identification of changing population dynamics and potential bloom indicators such as pH and chlorophyll concentration are key to maintaining healthy source waters. FIM yields fast results, typically taking only five minutes to run a 1-mL sample. Additional processing time is required to classify the sample; however, semi-automated software can significantly reduce analysis time.

Using the fluorescence data gathered by FIM, organisms causing excitation at 700 nm (channel 1 [CH1]/chlorophyll) can be separated from those causing excitation at 650 nm (channel 2 [CH2]/phycocyanin). By analyzing CH2/CH1 ratios, the user can sort and quickly obtain semi-automated concentrations of organisms according to their pigment. Detection of high levels of phycocyanin production (with the exception of cryptomonads and red algae) indicates the presence of cyanobacteria, while chlorophyll-only production indicates that other algae and/or diatoms are present (Figure 2).

If an in-depth analysis is needed, the user can also sort images taxonomically to the genus level. It is important to note that FIM's two-dimensional imaging with a maximum of 20× magnification does not provide the image resolution necessary for speciation. There is a trade-off between sample analysis processing time and the more detailed cellular information that can be gathered using microscopic analysis. In most circumstances, managers have determined that classification to the genus level is sufficient for lake and reservoir management, and that going the extra step to speciate does not bring enough relevant

Example Images Sorted Using CH2/CH1 Ratio



CH—channel

Figure 2

information to influence rapid decision-making in treatment plant operations and/or reservoir management.

FIM and Water Treatment

For drinking water utilities, FIM has become a reliable monitoring tool for HAB and T&O challenges, and it can be used to observe changes in population dynamics and correlate these results to cyanotoxin testing and T&O results. Monitoring programs must be able to sample regularly to track increases in potential problem organisms. Receiving data as quickly as possible is sometimes the key to avoiding problems.

When blooms occur, FIM can be used to identify the organisms responsible and determine if they have the potential to produce toxins or T&O compounds. Monitoring is essential to catching blooms in their nascent stages (Almuhtaram et al. 2021, Kibuye et al. 2021). Over time, baseline algae concentrations for each source can be developed, and these can be used to establish trigger levels for additional monitoring and treatment. Prompt treatment in the reservoir is most effective and less costly than treating cyanotoxins and T&O compounds at the treatment plant (Adams et al. 2022).

At the time of this writing, no FIM methods are included in *Standard Methods for the Examination of Water and Wastewater* (currently in its 24th edition), so utilities must develop their own methods and quality assurance/quality control (QA/QC) practices. Even so, it's important for the water industry to develop this guidance. Standardization is important to ensure every laboratory follows the same procedures, making data legally defensible and comparable across laboratories and analysts. QA/QC and method validation are important practices, and future standardization of these methods is a key step in the process of making data comparable across laboratories (Owen et al. 2022).

Utility Case Studies

Utilities have been implementing early warning systems for HABs, T&O, and cyanotoxins for decades (Means & McGuire 1986). However, it has not been until the last 10 years that utilities have widely used FIM in their monitoring programs. The following four case studies from US utilities show how FIM can be used in a HAB monitoring program for reservoir management and water treatment.

City of Wichita Falls, Texas

The City of Wichita Falls has a surface water system with adjudicated rights to five reservoirs. This public water system (PWS) includes two treatment facilities and serves around 150,000 residents in the North Central Texas region. Land use around its reservoirs is affected by agricultural land to the south and ranch land to the west. One of the reservoirs, Lake Arrowhead, is also affected by an indirect potable reuse system that directs Wichita Falls wastewater effluent to the lake.

Wichita Falls has experienced HAB issues for decades. In response, the PWS's municipal laboratory, the Cypress Environmental Laboratory (CEL), used a monitoring program that consisted of sensory analysis and organism identification/enumeration by light microscopy. This proved to be time-consuming work, so in 2016, an enhanced monitoring program was implemented in response to an extreme bloom that caused hundreds of T&O complaints (Adams et al. 2018).

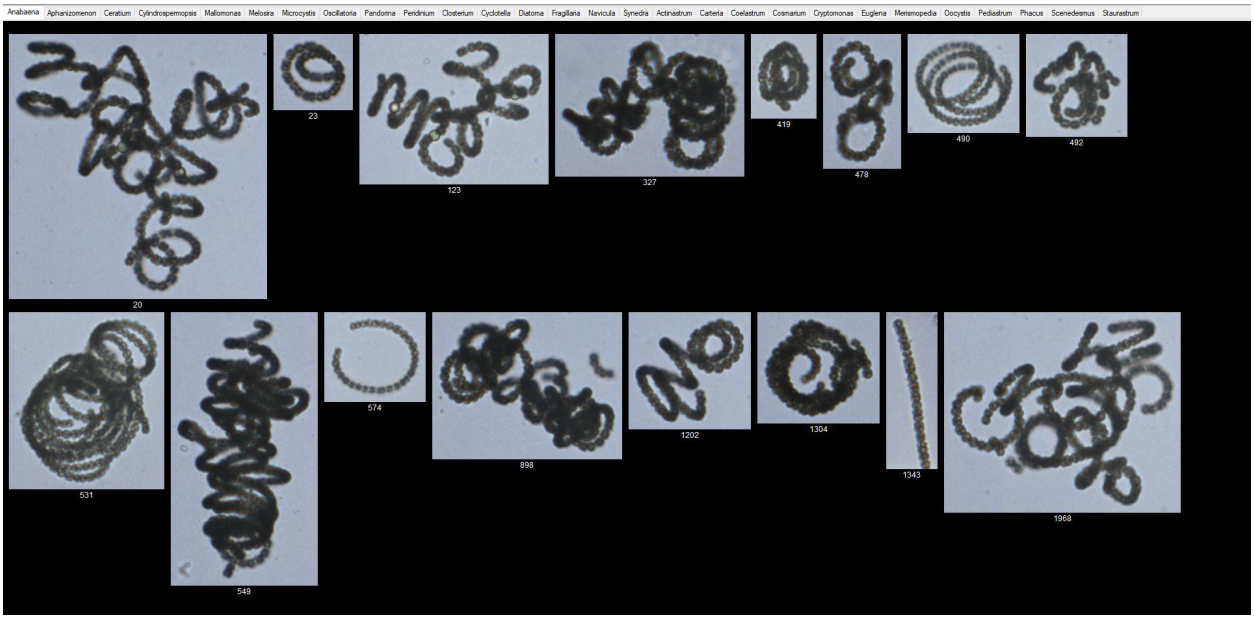
Wichita Falls' updated monitoring strategy includes monitoring source reservoirs using sondes; sensory analysis for T&O according to method 2150 (Threshold Odor Number) and method 2170 (Flavor Profile Analysis) (*Standard Methods* 2023); algae/cyanobacteria identification and enumeration by FIM; T&O compound detection and quantification by gas chromatography–mass spectrometry; and cyanotoxin-producing gene detection by quantitative polymerase chain reaction (qPCR) and fluorometric planar wavelength assay (Adams et al. 2023, 2022, 2021).

At the program's inception, the CEL understood that to make fast and informed data-driven decisions, it had to know what type of organisms were in the source water, how many were present, and if they were likely to cause a T&O or cyanotoxin problem. By replacing traditional light microscopy with FIM, the CEL has been able to quickly screen samples for normal nuisance organisms—e.g., *Microcystis, Dolichospermum* (formerly *Anabaena*), *Peridinium,* and *Aphanizomenon*—and make decisions within the hour on the need for treatment at the reservoirs or switching sources to another reservoir (Figure 3). Once samples are processed, they are sorted into genus-level bins and evaluated on the basis of functional group totals—e.g., T&O producers, cyanotoxin producers, green algae, detritus.

During the program's first year, the CEL ran samples five days each week to establish a baseline for its reservoirs. Analyses were then scaled back to seasonal frequencies—specifically, once per week during winter, twice per week during spring and fall, and three to five times per week during summer. This approach to monitoring has integrated field, microbiological, sensory, molecular, and analytical chemistry testing. Using this strategy, Wichita Falls has detected and mitigated more than 18 blooms in the past seven years, reducing customer complaints by the timely addition of powdered activated carbon (PAC) and potassium permanganate ($KMnO_4$).

FIM has become a focal point of the monitoring plan for this system, and this proactive approach to HAB detection and mitigation has proved highly effective. For the CEL,

***Dolichospermum* (Formerly *Anabaena*) Imaged by CEL Staff, Wichita Falls, Texas**



Source: Adams et al. 2021; Figure 5, part B

CEL—Cypress Environmental Laboratory

Figure 3

the greatest advantages to using FIM have been the ease of use, instrument reliability, and saved run files that can be referenced at a later date. Limitations are a large, one-time capital investment and the inability of FIM to identify organisms at the species level; however, this level of identification is rarely needed in normal operations.

New Jersey Water System

The water system described in this section, located in New Jersey, serves approximately 80,000 customers. Surface water is derived from river sources and pumped into off-stream raw water storage reservoirs that are susceptible to cyanobacteria growth throughout the year.

In the reservoir that serves as the raw water supply to the treatment plant, floating circulators, aerators, and aspirators help address cyanobacteria challenges.

FIM and other efforts assist with proactive monitoring and management of cyanobacteria and associated water quality (e.g., cyanotoxins, T&O issues) and treatment (e.g., coagulation) challenges.

The treatment process of this water system consists of pre-oxidation and dissolved air flotation, followed by granular activated carbon (GAC) and disinfection. Prior to distribution, the water mixes in a clearwell with treated groundwater. While the treatment process successfully addresses cyanobacteria challenges, routine monitoring to evaluate changes in cell counts and population dominance is utilized and contributes to this multibarrier approach addressing HABs.

The cyanotoxin monitoring plan provides guidance for managing cyanobacteria and cyanotoxins in the source water. Environmental indicators that serve as warnings include

- decreased Secchi depth;
- increased water temperature;
- diurnal pH variations;
- increased surface dissolved-oxygen levels, along with diurnal variation of the water column profile; and
- increased odor and color.

If these indicators are occurring and cell counts exceed a threshold level, the monitoring frequency is increased. In addition, weekly samples for cyanotoxin analysis are collected from the reservoir and the finished water and analyzed in-house using

enzyme-linked immunosorbent assay (ELISA) methods for microcystins, cylindrospermopsin, and anatoxin-a. If cyanotoxins are present above the US Environmental Protection Agency (EPA) health advisory level (HAL), confirmation samples are sent to certified laboratories for additional analyses following EPA methods 544, 545, and 546 (EPA 2019).

FIM has been used since 2015 to monitor phytoplankton throughout the year. Between April and November, phytoplankton analysis using FIM is conducted weekly to assess the population dominance and cell counts of algae/cyanobacteria species present in the reservoir. While diatoms and golden algae are dominant in the colder months, cyanobacteria are generally dominant from April/May through October (Figure 4).

The most common cyanobacteria detected are *Dolichospermum*, *Planktothrix*, *Microcystis*, and *Woronichinia*. During summer months, HABs have been found to produce microcystin levels up to 6 µg/L and anatoxin-a levels up to 2.7 µg/L. The treatment at this water system has been efficient in the removal of cyanotoxins, and they have not been above the detection limit in finished water samples during HAB events.

HAB occurrence also contributes to T&O challenges in the water. In this system, 2-methylisoborneol (MIB) and geosmin are monitored regularly throughout the year. In 2020, maximum levels of MIB and geosmin at the intake were 8 ng/L and 21 ng/L, respectively, but in August/September of 2021 at their peak, they increased to 36 ng/L of MIB and 38 ng/L of geosmin.

Using this system's monitoring and treatment strategies, MIB and geosmin have remained undetected in the plant effluent. In addition to cyanotoxins and T&O, the increase of cyanobacteria can affect coagulation, so continuous monitoring is important to catch any changes to plant performance. FIM is now a key part of this system's proactive monitoring program.

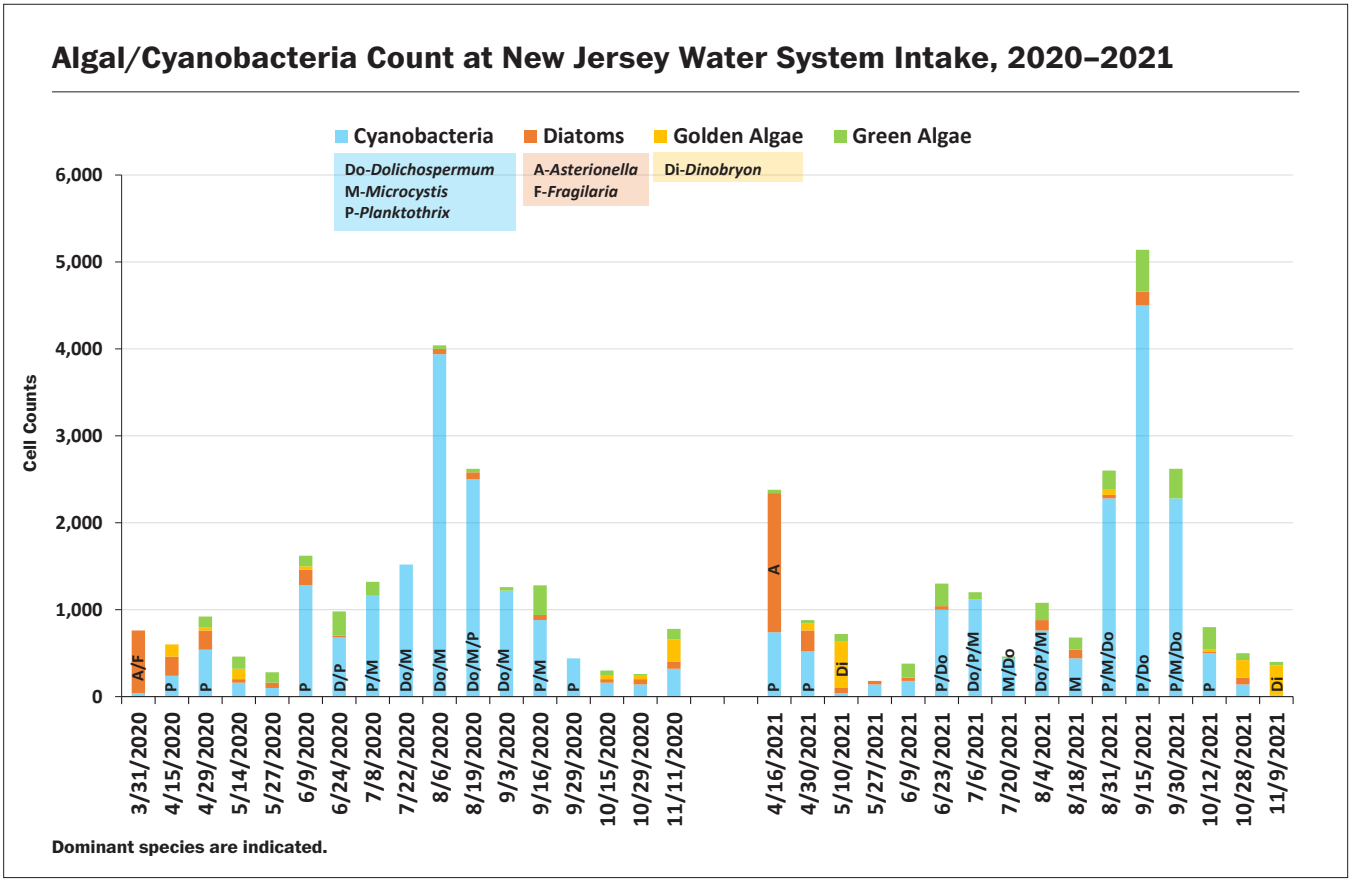


Figure 4

Passaic Valley Water Commission, Totowa, N.J.
The Passaic Valley Water Commission (PVWC) Little Falls Water Treatment Plant (LFWTP), located in Totowa, N.J., services approximately 800,000 residents across five counties in northeastern New Jersey. LFWTP treats water from the Passaic River using a four-part disinfection treatment process. The treatment facility uses sand-ballasted high-rate clarification system to remove much of the organic matter, phytoplankton, and bacteria from the raw water, followed by ozonation, filtration, and chlorination.

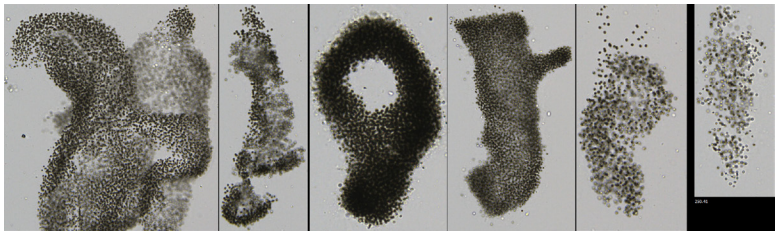
The Passaic River flows through many counties and is influenced by several rivers and creeks in its watershed. Before reaching the intake of LFWTP, the river receives runoff from residential areas, major highways, golf courses, and malls. In addition, a wastewater treatment facility, located about 4 miles upstream from LFWTP, discharges into the Pompton River just before its confluence with the Passaic River. PVWC also owns and operates three open finished drinking water reservoirs (OFDWRs), located in Woodland Park and Paterson, N.J.: Great Notch, New Street, and Stanley Levine Reservoirs. These OFDWRs store finished drinking water that is subsequently exposed the effects of weather, plants, and wildlife.

In 2009, PVWC was ordered by the New Jersey Department of Environmental Protection (NJDEP) to cover its open reservoirs (New Jersey 2009). Projects are in development to cover Stanley Levine Reservoir first, but until all three OFDWRs are covered or replaced with water storage tanks, they are closely monitored for a variety of water quality parameters, especially microorganisms.

The OFDWRs also limit PVWC's corrosion control program. Less than a decade ago, PVWC began testing to find a suitable chemical for corrosion control in its distribution system. The study concluded an orthophosphate blend yielded the best results in reducing leaching and conducting a cost analysis. The ideal dosing location for corrosion control is at the entry point to the distribution system at LFWTP. Yet, with the three OFDWRs in operation,

Documented Cyanobacteria

A



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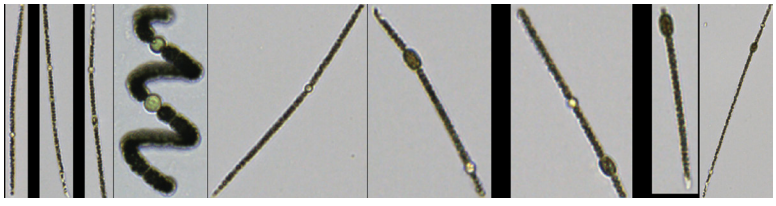

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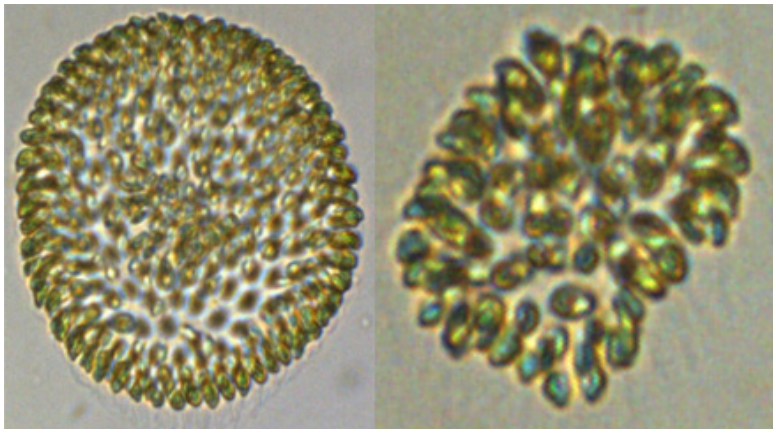

A: *Microcystis* documented at watershed sampling site 612A–Pompton River, 8/26/2020 and 9/16/2020

B: *Dolichospermum* (formerly *Anabaena*) documented at watershed sampling sites 612A–Pompton River, and 110–Passaic River, 8/25/2021

C: *Gomphosphaeria* documented at watershed sampling site 612A–Pompton River, 5/6/2020

Figure 5

Dinobryon Imaged by Water Quality Laboratory at Austin Water

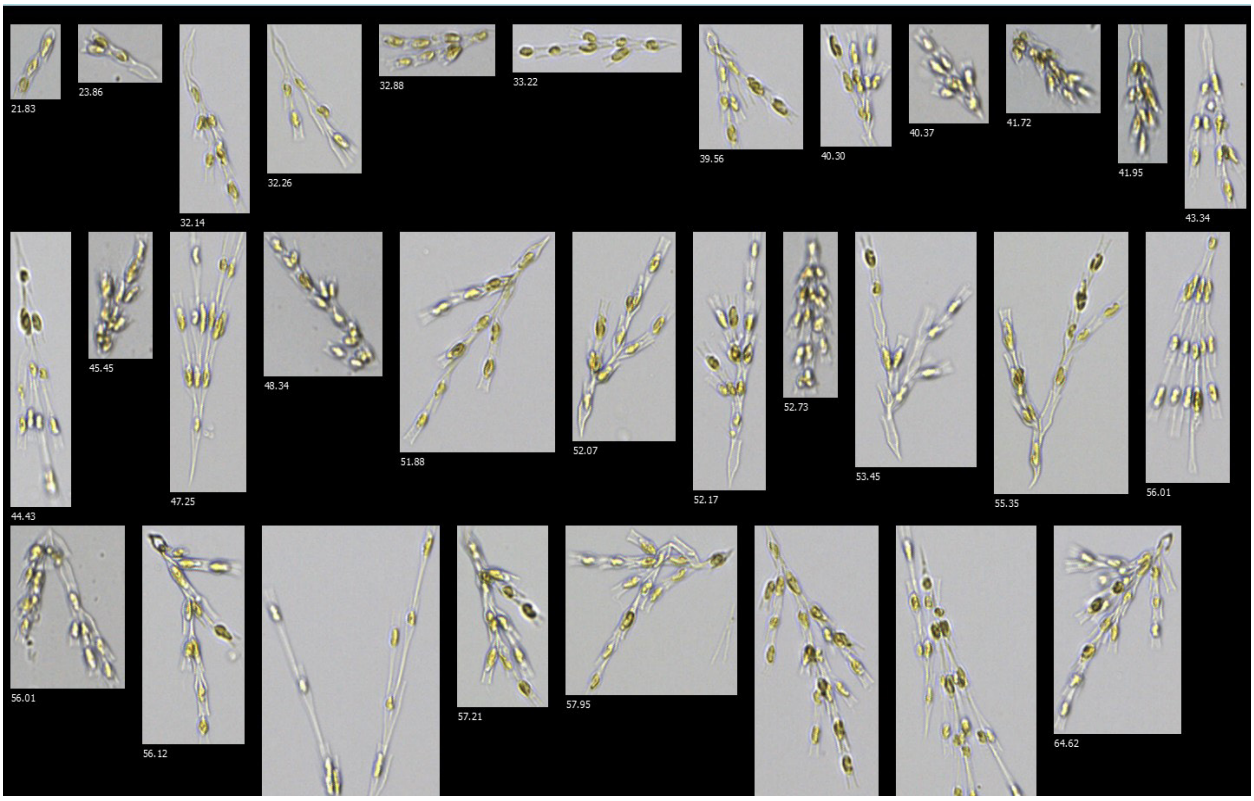

Figure 6

addition of the orthophosphate was not allowed as it would add a phosphorus into the reservoirs that could act as a nutrient and promote algal blooms or increase T&O complaints. Parts of the distribution system receive corrosion control with chemical addition via satellite pump stations after exiting the reservoir where the water is rechlorinated before entering the distribution system.

Once the OFDWRs are fully covered, corrosion control can be streamlined by dosing at the distribution system entry point. In the meantime, PVWC closely monitors field phosphate and orthophosphate levels in the distribution system, OFDWRs, and watershed. These analytes are monitored weekly in the distribution system and watershed year-round, while in the reservoirs they are monitored weekly during peak algal growth months (May–September) and monthly during the winter. Tracking the analyte data assists in troubleshooting and provides earlier awareness of conditions that could lead to problems.

Although a severe algal bloom has not affected the treatment process in decades, the watershed and OFDWRs are monitored for phytoplankton that could cause problems. Prior to implementing FIM, phytoplankton had been monitored using a compound light microscope. In 2011, PVWC's laboratory implemented FIM, providing a much faster and more accurate method of monitoring phytoplankton. Although not a regulatory analysis, a continuous electronic record of phytoplankton analyzed from the watershed and OFDWR sites has been documented since 2014.

With FIM, diatoms and other green algae have been the most documented phytoplankton, but cyanobacteria have started to become more frequent at some sites. The most documented cyanobacteria using FIM are

NJDEP DSR (Proposed) for Cyanotoxin Concentration in Finished Water

	Cyanotoxin			
Guideline	Anatoxin-a *µg/L*	Cylindrospermopsin *µg/L*	Microcystin-LR *µg/L*	Saxitoxin *µg/L*
NJDEP DSR —Children ≤6 Years	0.7	0.2	0.07	NA
NJDEP DSR —Populations >6 Years	3.3	1.0	0.3	NA

NA—not applicable, NJDEP DSR—New Jersey Department of Environmental Protection Division of Science and Research

Table 2

Microcystis (Figure 5, part A), with *Dolichospermum* and *Gomphosphaeria* also present (Figure 5, parts B and C). Analysis shows that the Pompton River has experienced the most cyanobacteria out of all the sampling sites.

In the past few years, *Microcystis* growth has become more frequent, with FIM documenting that it appears in August and lasts through September. However, *Dolichospermum* also tends to appear, though less regularly, during those same months. *Gomphosphaeria* primarily occurs in May at one of the sampling sites at Great Notch Reservoir.

In 2021, PVWC further enhanced its program by establishing a cyanotoxin monitoring protocol. Although still in development, this approach is composed of a fluid three-step monitoring plan using FIM, qPCR, and ELISA. The monitoring program covers five sampling locations within the three OFDWRs (Great Notch Dam, Great Notch Cove, New Street Cove, Stanley Levine Inlet, and Stanley Levine Dam) and the river sources that feed LFWTP. The river sources include sampling at the plant's intake as well as two upstream watershed sampling locations: one on the Passaic River (110) and another on the Pompton River (612A).

From November to March, the reservoirs and watershed are monitored once a month for phytoplankton and the toxin-producing genes associated with certain taxonomic groups. From April to October, qPCR and phytoplankton analyses are conducted weekly.

Samples are analyzed using FIM as the initial start to monitoring. Algae detected through FIM are identified and recorded as diatoms, flagellates, or cyanobacteria. If cyanobacteria are found in high concentrations, qPCR is used to determine whether any toxin-producing genes are present. If genes are detected at more than 100 gene copies per microliter while the monitoring program is being finalized, samples are collected for ELISA analysis.

If cyanotoxins are detected in the watershed or the intake, treatment may be adjusted depending on whether the toxin is intracellular or extracellular. Additional treatment may include using GAC, PAC, or increasing chlorine or ozone dosing. Samples will be collected daily from the finished drinking water output and analyzed using ELISA for any toxins that might have made it through the treatment process. If toxins are found in one of the OFDWRs, samples will be collected and analyzed daily at the affected reservoir and downstream sites.

Cyanotoxin analysis is currently not a regulatory parameter for finished drinking water in New Jersey. However, the NJDEP has drafted guidance level values for HAL concentrations that PVWC is using in reference for the toxin monitoring program, as shown in Table 2 (NJDEP 2022, EPA 2015). PVWC is required to report toxins in any surface water systems if they are detected, and it must begin a consultation process with the NJDEP. A public Do Not Drink notice will follow upon confirmation of any cyanotoxins in the distribution system. The notice depends on the type and concentration of toxin detected.

Austin Water, Austin, Texas

Austin Water provides drinking water to more than one million people in the Austin, Texas, community. This PWS consists of three surface water treatment plants (WTPs) on two reservoirs—Lake Austin and Lake Travis—on the Lower Colorado River, which runs through Austin. The state and local river authority implemented requirements in the 1980s and 1990s to protect the river water quality against urbanization.

Due to warm, nutrient-rich waters and recurring drought conditions, Austin Water's reservoirs are vulnerable to HABs. PAC was incorporated into Austin Water's treatment process early on to address T&O issues, and monitoring for cyanobacteria by light microscopy began in the 1990s. Using historical trends of phytoplankton data, Austin Water developed trigger levels to begin or increase PAC feeds during HABs.

With issuance of the EPA's HALs for cyanotoxins in 2015, Austin Water began monitoring for cyanotoxins as a baseline during peak season for algae growth, and in turn it created a monitoring plan on the basis of EPA's recommendations and AWWA and The Water Research Foundation publications. This initial plan relied heavily on algae and cyanobacteria identification and enumeration by traditional microscopy. Cyanotoxin monitoring was triggered if the number of organisms exceeded 15,000 org/mL.

Austin Water's initial plan proved to be laborious in September 2015—high plankton counts in the source water resulted in daily microscopic analysis from all three WTP intakes. The increase in microscopic analyses took a toll, as the examination typically required approximately 4 hours to complete. Cyanobacteria counts declined as the end of the peak algal bloom season approached in November. Similarly, high plankton counts in August 2016 triggered an increase in microscopic analyses to several times weekly.

In 2019, Austin Water monitored for cyanotoxins in accordance with the Fourth Unregulated Contaminant Monitoring Rule, commonly known as UCMR 4 (EPA 2022). Public awareness of cyanotoxins increased in Austin following the deaths of several dogs that had played in Lady Bird Lake, and cyanotoxins were detected in samples of benthic algae collected during that time. Although Lady Bird Lake was no longer a source for drinking water since the decommissioning of the Green WTP in 2008, Austin Water regularly monitored cyanobacteria and tested for cyanotoxins during that year's peak season because of its source water quality. Plankton counts remained low, and no cyanotoxins were detected again in 2019.

In February 2021, two dogs died after playing in Lake Travis in the area known as Hudson Bend, and the events triggered Austin Water to increase algae and cyanotoxin monitoring earlier than the typical peak season of May–October. The Water Quality Laboratory (WQL) increased monitoring capabilities to include FIM semi-automated algae and cyanobacteria identification and enumeration, along with biochemical detection and quantification of cyanotoxins by ELISA.

The cyanotoxin monitoring plan was further developed into a response plan by evaluating existing treatment options using the Hazen-Adams CyanoTOX Tool, available through AWWA. Austin Water's treatment plants retrofitted chemical feed points for the capability to feed $KMnO_4$ to broaden treatment options.

Since implementing FIM, WQL has increased algae/cyanobacteria monitoring to twice weekly; this is possible because the analysis time has been reduced to approximately 20 minutes from the 4 hours required using light microscopy. WQL is also able to quickly analyze additional samples on demand as issues arise.

While WQL could analyze samples using FIM immediately after initial setup and training, the data analysis portion had a steeper learning curve. Users must put in a significant amount of time and effort to initially capture images of various plankton/cyanobacteria to build up the instrument libraries and then create automated filters from them. This investment has paid off as the automated filters become more reliable and data analysis becomes less burdensome.

Phytoplankton counts from traditional microscopy do not necessarily correlate to FIM counts, so internal trigger levels must be reevaluated using FIM data (Barrowman et al. 2022). For example, in March 2022 an influx of customer odor complaints was received when no plankton trigger levels were exceeded on the basis of traditional microscopy data. WQL increased plankton analysis frequency using FIM and determined that a *Dinobryon* bloom was the source of the odor (Figure 6). With this information, the WTP was able to make a data-driven decision and quickly mitigated the problem.

In the end, Austin Water found that traditional microscopy was more labor intensive when an increase in analysis frequency was triggered. A minimum time investment of six months was required to train analysts to become proficient in the procedure. However, FIM significantly reduced the labor and time required for algae and cyanobacteria monitoring in the long run, and Austin Water continues to further refine its cyanotoxin monitoring and response plan and evaluate existing treatment capabilities against cell removal and extracellular cyanotoxins. As a result of implementing FIM, Austin Water is better prepared to quickly respond to algae blooms and prevent Do Not Drink orders being issued as a result of cyanotoxins and T&O events.

Proactive HAB Monitoring and Response

FIM has been proved effective in real-world utility management scenarios, and as shown by these case studies, it can easily become the foundation of an early warning system for reservoir monitoring. Samples can be screened quickly for the presence of toxin-producing cyanobacteria and T&O-producing phytoplankton. This allows utilities to make fast, informed decisions if treatment changes are necessary.

These case studies also highlight some limitations of FIM. However, the capital equipment cost, initial time

commitment required for building local image libraries, and the challenges of species level identification are usually outweighed by the value of objective and quantitative data analysis. In the future, method standardization through *Standard Methods* should guide utilities on the QA/QC practices necessary to produce defensible FIM results. While FIM will never replace the expert taxonomist, it enhances the ability for a utility to proactively monitor and respond to HAB challenges, especially when they are just emerging. With this approach, utilities can reduce operating costs and provide additional protection of public health.

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- **Stanton B, Little A, Miller L, et al. 2023. Microcystins at the Tap: A Closer Look at Unregulated Drinking Water Contaminants. *AWWA Water Science.* 5:3:e1337. https://doi.org/10.1002/aws2.1337**
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