Practical Application of Flow Imaging Microscopy in Improving Water Quality

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Abstract

Traditional methods like microscopy, particle counter, and flow cytometry have long been utilized in aquatic research and water quality monitoring. However, these techniques present limitations: microscopy is time-consuming, particle counter and flow cytometry are not able to capture crucial morphological data. To overcome these challenges, Flow Imaging Microscopy (FIM) has emerged as a transformative tool, combining imaging and analytical capabilities for more comprehensive and efficient analysis.

Introduction

Harmful algal blooms (HABs) are one of the main challenges in global water resource management, especially in lakes and reservoirs that serve as drinking water sources. These blooms are usually caused by cyanobacteria, with toxin-producing species including Microcystis, Nodularia, Oscillatoria, Anabaena, and Aphanizomenon, among dozens of other genera. Cyanotoxins can be categorized based on their toxicity into endocrine toxins, neurotoxins, and hepatotoxins. These toxins cause serious threats to human health, leading to acute poisoning, liver damage, and neurological disorders. Numerous HAB related poisoning incidents have shown the risks that HABs cause serious threats to drinking water quality and public health. In recent years, environmental pollution and climate change have contributed to the increasing frequency and geographic spread of HABs. Therefore, developing rapid and efficient monitoring technologies is essential for environmental management and ecological protection.

Discussion

Traditionally, microscopes, particle counters, and flow cytometers have been widely used to detect HABs. However, each of these methods has its own advantages and limitations. Microscopy is one of the oldest and most common methods used for particle analysis. Operators can clearly observe plankton, algae and other microscopic particles by utilizing optical method through their eyes. Moreover, by changing the objective lens, operators can observe overall image and different developmental stages of the sample. Thus, microscopes have significant contributions to morphology and taxonomy. However, despite the high research value of microscopes, some challenges remain some potential challenges, such as time consuming and labor intensity. Time-consuming procedures

require long analysis times due to the need for sample preparation and observation. Additionally, the reliability of statistical results is relatively low, as an effective statistical analysis typically requires counting at least 400 individual organisms. This makes microscopy less efficient for large-scale HABs monitoring. Moreover, labor intensity is another reason that must be considered. Physical and mental fatigue may affect an operator's judgment. Different operators might lead to different judgment. These factors contribute to variability in accuracy, making it difficult to maintain reproducibility. Therefore, while microscopy remains an essential tool in algal taxonomy, its application in HABs monitoring is significantly constrained by time and labor requirements.

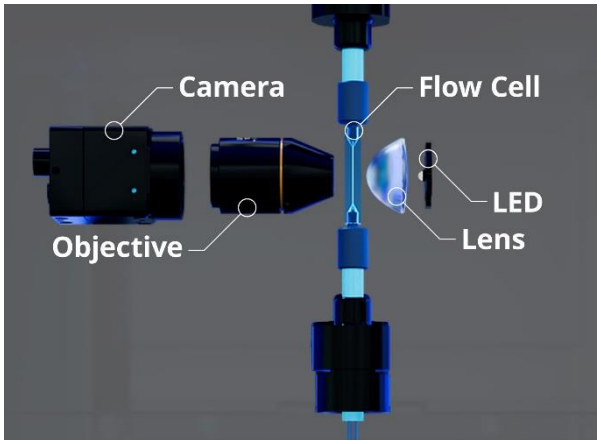
While microscopy is usually considered to provide morphologic data, there is limitation for quantitative counting or size measurements. particle counters or flow cytometers are commonly used for rapid acquisition of particle count and size data, making them complementary to microscopy.

One widely used particle counting technique is the Coulter Counter, which measures changes in electrical current to determine count, size, and concentration of particles in a sample. While particle counters efficiently measure size distribution and concentration, they cannot identify the actual shape or biological classification. Therefore, when monitoring HABs, distinguishing different phytoplankton species or analyzing morphological characteristics is crucial, additional imaging techniques must be integrated to supplement particle counting.

Compared to particle counters, flow cytometry enables both quantitative and qualitative analysis of suspended particles, cells, and microorganisms in liquid samples.

Flow cytometers utilize fluorescence signals to classify cells and assess their physiological state. Additionally, fluorescence measurements can detect intracellular pigments such as chlorophyll, providing further insights into cellular composition and function. Moreover, it provides real-time data on the physical and chemical characteristics of living organisms. Fluorescence data collected via flow cytometry can assist in identification, differentiation, and provide data about cell health.

Below: Schematic of the internal components of FIM

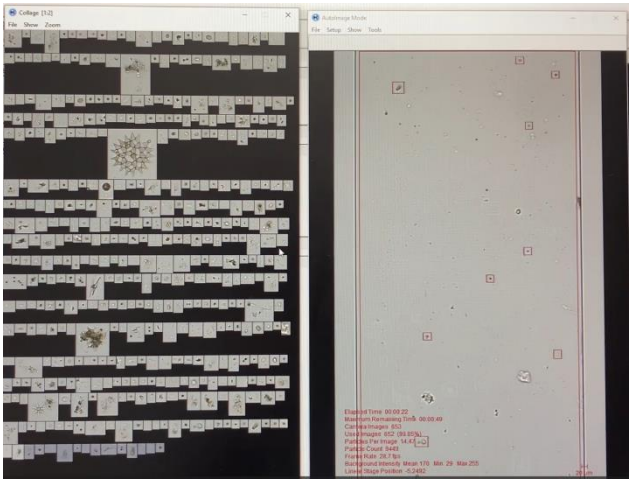


However, despite its analytical capabilities, flow cytometry is unable to provide morphologic data, which make it difficult to identify individual cells or conduct morphological classification.

Due to this lack of imaging capabilities, flow cytometry alone is insufficient for monitoring the species composition and toxicity risks of HABs. Therefore, it must be integrated with other complementary technologies such as FIM, qPCR, and ELISA to establish a comprehensive evaluation system.

Flow imaging microscopy utilizes digital images to measure the size and shape of each particle in a sample. Essentially, the operator in classical microscopy is replaced by a computer that extracts the information from the images.

Below: FIM screen during image capture, including the full field-of-view of the flow cell. Red boxes indicate particles found. Images collect in the collage window as they are saved and stored.



Step1 : Sample Flow Through the Flow Cell

A sample containing particles streams through the flow cell past the microscope optics

Step2: LED Strobe Illumination

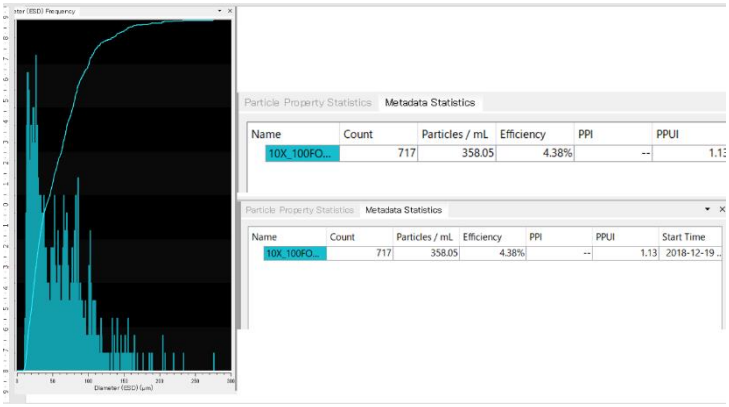
The moving particles are “frozen” in space by synchronizing the strobed LED illumination source with the very fast shutter speed of the digital camera.

Step3: High-Resolution Image Capture

Thousands of high-resolution particle images can be captured per second, ensuring comprehensive recording of all particles in the sample.

Step4: Real-Time Image Processing and Data Analysis

As each frame of the camera's field of view is captured, the software extracts the particle images from the background in real time and stores them.

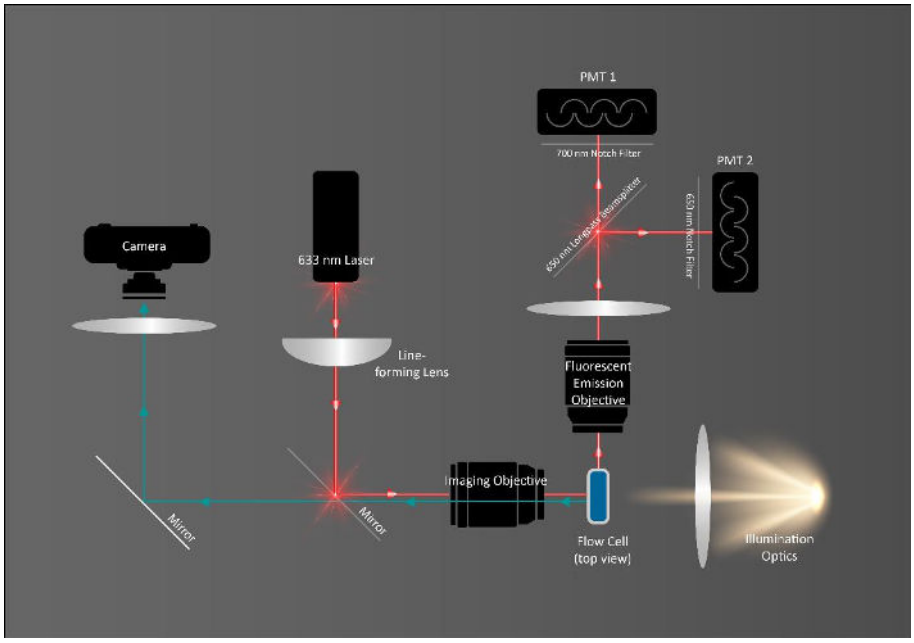


Right: Particle measurement summary graph and statistics that populate at the end of a sample run.

FIM offers high-speed, automated imaging, providing particle size distribution and real-time analysis. This technology is widely applicable in water quality monitoring, plankton research, and microbial classification, playing a particularly critical role in HABs monitoring.

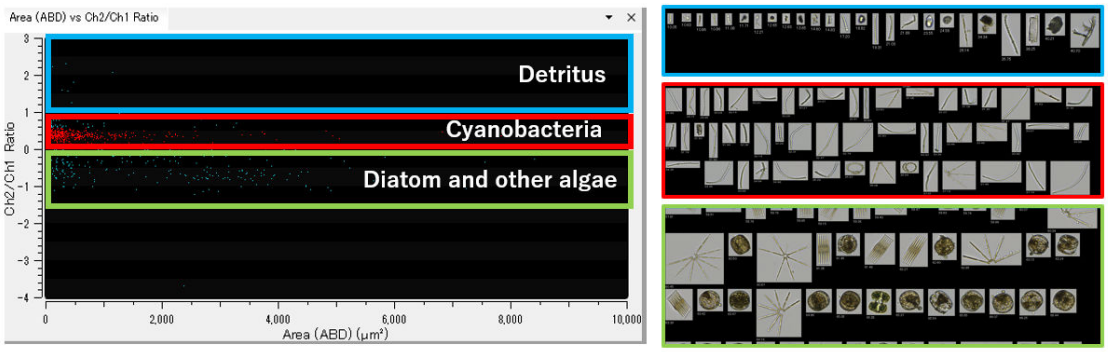
Additionally, some FIM instruments are equipped with lasers that use fluorescence excitation to trigger particle image capture whenever a fluorescing object is detected in the flow cell. When a fluorescent particle is identified, the instrument automatically captures an image of the sample, enhancing the accuracy of particle classification.

Below: The optical configuration in FIM with laser instruments



When FIM instruments utilize laser excitation, the laser beam is focused at the center of the flow cell. As particles pass through the flow cell, the optical system is triggered to capture images and record fluorescence emission values. Based on these values, particles can be classified into one of three categories: cyanobacteria, other algae and diatoms, or detritus. This method only captures images of particles emitting fluorescence at specific wavelengths, effectively reducing analysis time by eliminating irrelevant particles from imaging. Subsequently, these particles can be further classified based on various statistical parameters or morphological characteristics.

Below: Pre-built filters sort images into three categories: Cyanobacteria, Diatoms and other Algae, and Detritus.



Conclusion

FIM provides an efficient, automated, and highly accurate solution for HABs monitoring, overcoming the limitations of traditional monitoring techniques. Compared to microscopy, FIM offers greater automation and higher throughput, enabling the rapid analysis of large water samples. By utilizing image-based classification, FIM accurately differentiates cyanobacteria from other phytoplankton.

In FIM instruments equipped with lasers, laser fluorescence excitation enables the precise detection of cyanobacteria, automatically distinguishing them from other algae and non-living particles. This technology significantly enhances the sensitivity and accuracy of HABs monitoring, allowing water treatment facilities to respond promptly to potential water quality crises.

Furthermore, when FIM is integrated with qPCR and ELISA, it forms a comprehensive water monitoring system. This multi-layered monitoring strategy ensures the safety of both drinking water and aquatic ecosystems, while also providing more precise decision-making support for water treatment facilities.

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