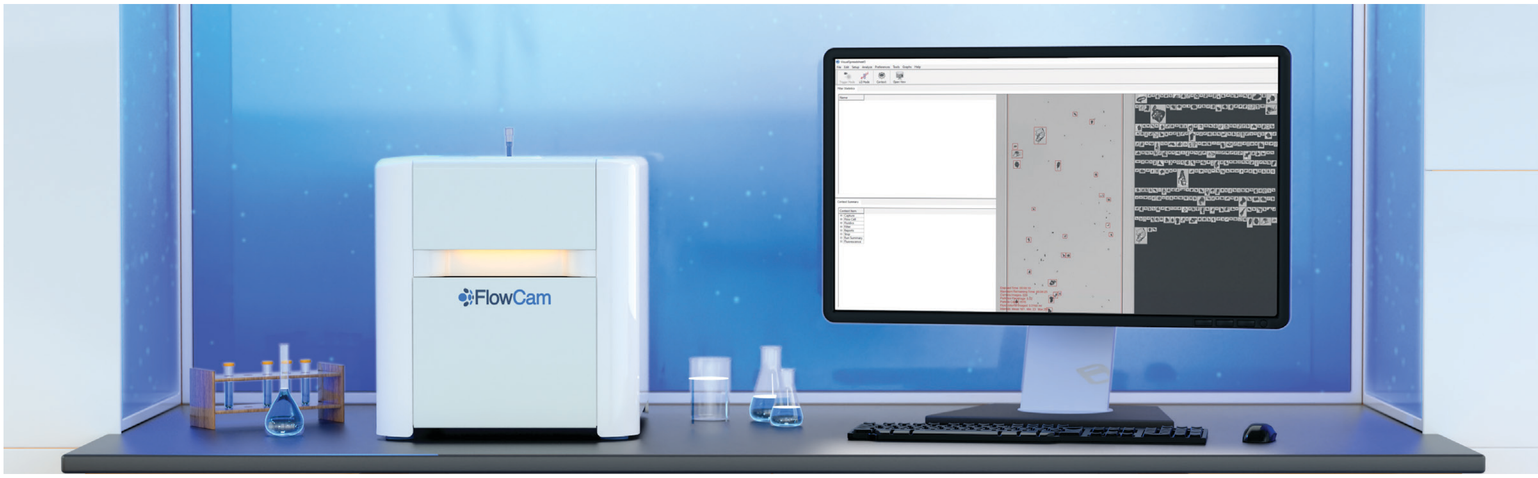
YOKOGAWA

EBOOK

The Ultimate Guide to Flow Imaging Microscopy for Aquatic Life Sciences

FlowCam®

Yokogawa Fluid Imaging Technologies, Inc.



YOKOGAWA FLUID IMAGING TECHNOLOGIES

Flow Imaging Microscopy:

A Better Way to Analyze Aquatic Microorganisms

CONTENTS

Overview

In this eBook, we'll introduce Flow Imaging Microscopy (FIM) and briefly review various methods available for particle analysis, including the pros and cons of each. Then, we'll take a deeper dive into how FIM works and how FlowCam is used at academic institutions, drinking water utilities, algae culturing facilities, and more. Lastly, we will provide an overview of the different types of FlowCam instruments available to meet a variety of needs.

FlowCam is used in both marine and freshwater habitats. Various FlowCam configurations can be used to study (clockwise from upper left): plankton community assemblages, copepods, and cultivated algae like *Haematococcus pluvialis*.

FlowCam®

CHAPTER ONE

Introducing Flow Imaging Microscopy

WHAT IS FLOW IMAGING MICROSCOPY?

Flow Imaging Microscopy (FIM) is a technique for automatically detecting, imaging, enumerating, and measuring particles in-flow, then using software to organize image data by taxa, size, and other parameters.

FlowCam is the first instrument developed using this technology, and was originally invented by biological oceanographers at the Bigelow Laboratory for Ocean Sciences (BLOS) in Boothbay, Maine, in the United States. It was developed to study marine phytoplankton.

In 1996, researchers at BLOS were able to combine the technologies of digital photography, flow cytometry, and advances in machine vision and statistical pattern recognition to develop an instrument which could automate and streamline the collection and analysis of plankton data.

After three years - and with support of a grant from the National Science Foundation - the researchers developed a prototype ‘imaging flow cytometer’ they called FlowCam. One of the researchers was Dr. Christian Sieracki, founder of Fluid Imaging Technologies, Inc.

Today, FlowCam is used for marine and freshwater research, drinking water monitoring, algae cultivation, and more, and has been cited in hundreds of papers. It is also used in the biopharmaceutical industry to aid in drug development. It provides a good solution for many applications that require rapid detection, enumeration, and imaging of microscopic particles in a fluid medium.

“Plankton-imaging-system development has been strongly influenced by the desire to reduce sample processing time”

(Mark Benfield et al, Oceanography Vol. 20, No. 2 2008)

Pictured at right: FlowCam inventor, Chris Sieracki, with the original FlowCam instrument developed at BLOS.

CHAPTER TWO

Alternative Particle Analysis Techniques

In order to better understand the benefits and tradeoffs of FIM technology, first let's explore the types of techniques that led to its development, which are still popular today.

SECTION 2.1: MICROSCOPY

The introduction of the microscope in the 1600s changed the world for scientists. For the first time, they could observe and record organisms too small to see with the naked eye.

To this day, microscopy remains the most common method for subvisible particle analysis.

"By having the use of a FlowCam instrument, I will be able to collect phytoplankton data...much faster and more accurately than...by use of microscopy alone...I would [also] be able to publish much faster than I originally thought possible"

— Savannah Mapes, Virginia Institute of Marine Science (pictured here), winner of the 2020 FlowCam Student Grant Program

ADVANTAGES OF MANUAL MICROSCOPY

The benefits of a microscope are simple. It allows you to study subvisible particles in great detail , allowing the user full control over the field of view and the ability to observe the same specimen under many magnifications. Microscopes have improved over time, allowing us to look at increasingly smaller particles. Advantages of manual microscopy include:

- The ability for a trained taxonomist to speciate, i.e. identify an organism down to the species level
- The ease of learning taxonomy due to greater image resolution and control over the field of view
- The ability to view the same sample under multiple objectives
- The ability to observe the entire particle size spectrum in a single sample

DRAWBACKS OF MANUAL MICROSCOPY

Using manual microscopy for particle analysis is time-consuming. Depending on the subject, it can take hours to prepare the samples and set up the slides. Due to the time involved, samples usually require preservation before analysis, which can affect plankton color and size. And it can be difficult to get results that are statistically significant.

For example, a procedure may require the user to count a minimum number of 400 organisms to capture sufficient diversity.

Human factors must also be considered using microscopy. Tired eyes, interruptions, and time of day can all have an effect on the operator, and therefore the results. Unless the user captured images to accompany their sample, the only output will be cell counts; a second person cannot cross-check the results after sample processing without preparing another subsample. The process can be imprecise, prone to error, time consuming, and a strain on the operator.

SECTION 2.2: PARTICLE COUNTERS

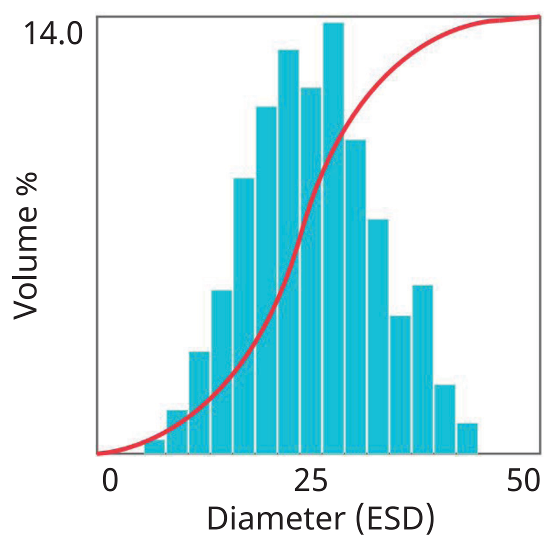
While microscopy is usually considered the gold standard for identification, a variety of volumetric particle counting techniques were born out of the need to gather particle data more quickly.

These indirect techniques lack the optical properties of a microscope and FIM. Instead, they measure a signal that is proportional to the volume of a particle and not the actual physical dimensions of the particle.

A common volumetric technique is the Coulter Counter which measures changes in electrical current to determine count, size, and concentration of particles in a sample.

The fundamental principle of indirect techniques like the Coulter Counter is that all particles are assumed to be spherical in shape, and the volume is converted to an equivalent spherical diameter (ESD). In these situations, it is not possible to know the actual shape of the particle or taxonomy of the organism, just the count and size distribution.

The Coulter Counter, shown here counting cells in solution, is an indirect volumetric particle analysis method.



While this graph shows particle size clearly, when shape and/or morphological data is needed, a more in-depth analysis is required to truly characterize a particle.

CHAPTER THREE

A Closer Look at Flow Imaging Microscopy

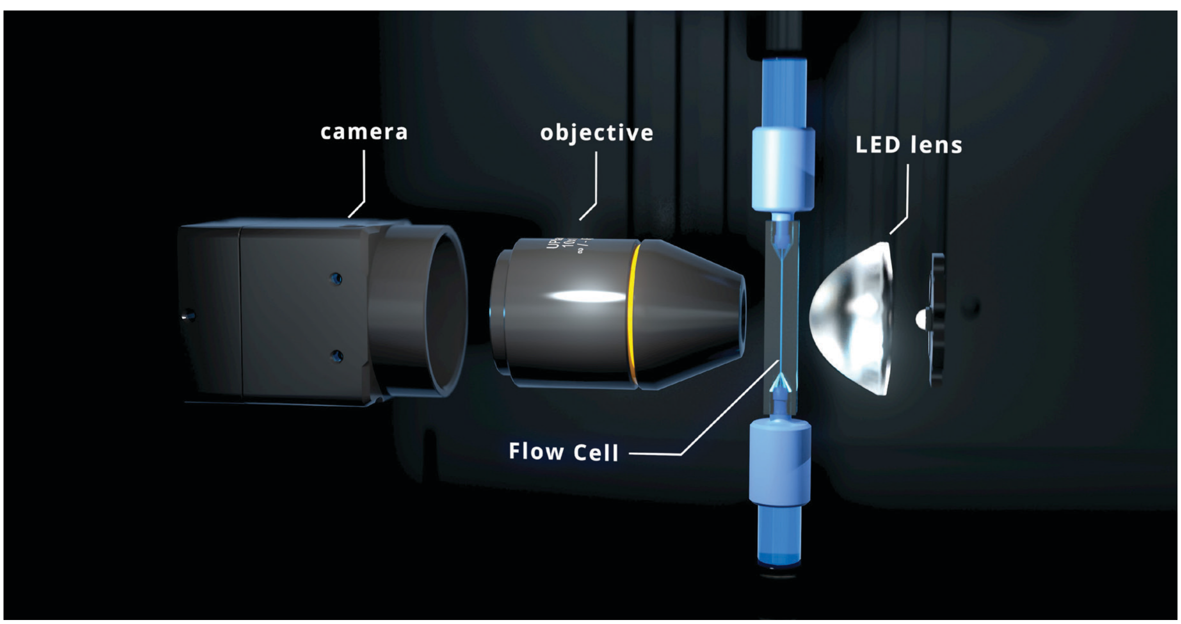
SECTION 3.1: HOW FLOW IMAGING MICROSCOPY WORKS

Flow imaging microscopy uses 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 to extract the information from the images.

A syringe pump draws a precise volume of sample through the flow cell past the microscope optics. Thousands of particle images are captured per second.

To capture sharp images of moving particles, they are frozen in space using a strobed illumination source combined synchronously with a very short shutter speed.

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



Internal components of the FlowCam imaging system

SECTION 3.2: DIRECT PARTICLE MEASUREMENTS

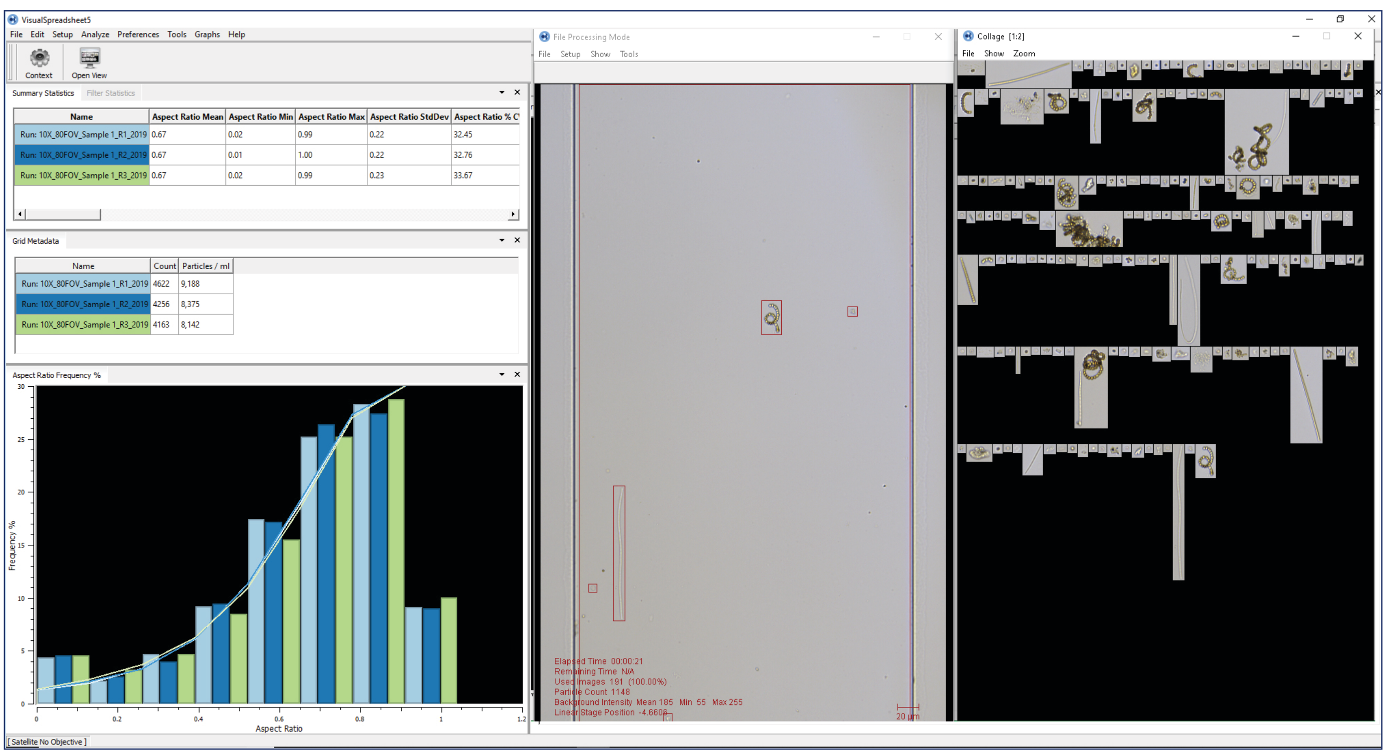
In an imaging-based system, particle measurements are made directly from the image of the particle. Since the system's optics are fixed and the magnification is known, distance measurements on the image can be directly converted to real distance measurements on the object.

No generalizations are made about a particle's shape. The user can also view the image to ensure that the data is being properly interpreted.

> ***"For the first time, we were able to see how the kind of phytoplankton influenced the optical properties of the seawater and could immediately examine whether an unusual community was related to the seawater or to the satellite images, thanks to FlowCam. FlowCam technology allows us to achieve high-resolution maps of phytoplankton that can be related to satellite data in more meaningful ways than possible before."***
>
> -Prof. Joaquim Goes, a biological oceanographer from Columbia University, on FlowCam's use in NASA's open ocean phytoplankton ground truthing study

Common measurements include:

- Equivalent spherical diameter (ESD)
- Length, width, and aspect ratio
- Area and volume
- Circularity and elongation
- Edge gradient
- Intensity, average intensity, and sigma intensity
- Transparency
- and more (40+ morphological characteristics)



FlowCam screen during image capture. The main window (left) shows metadata, like total concentration, particle property statistics, and customizable histograms and scatterplots that populate at the end of the sample run. The middle window displays the full field-of-view, showing what is happening in front of the camera. Red boxes indicate particles found. The window on the right is the collage of particle images that are saved and stored to the database.

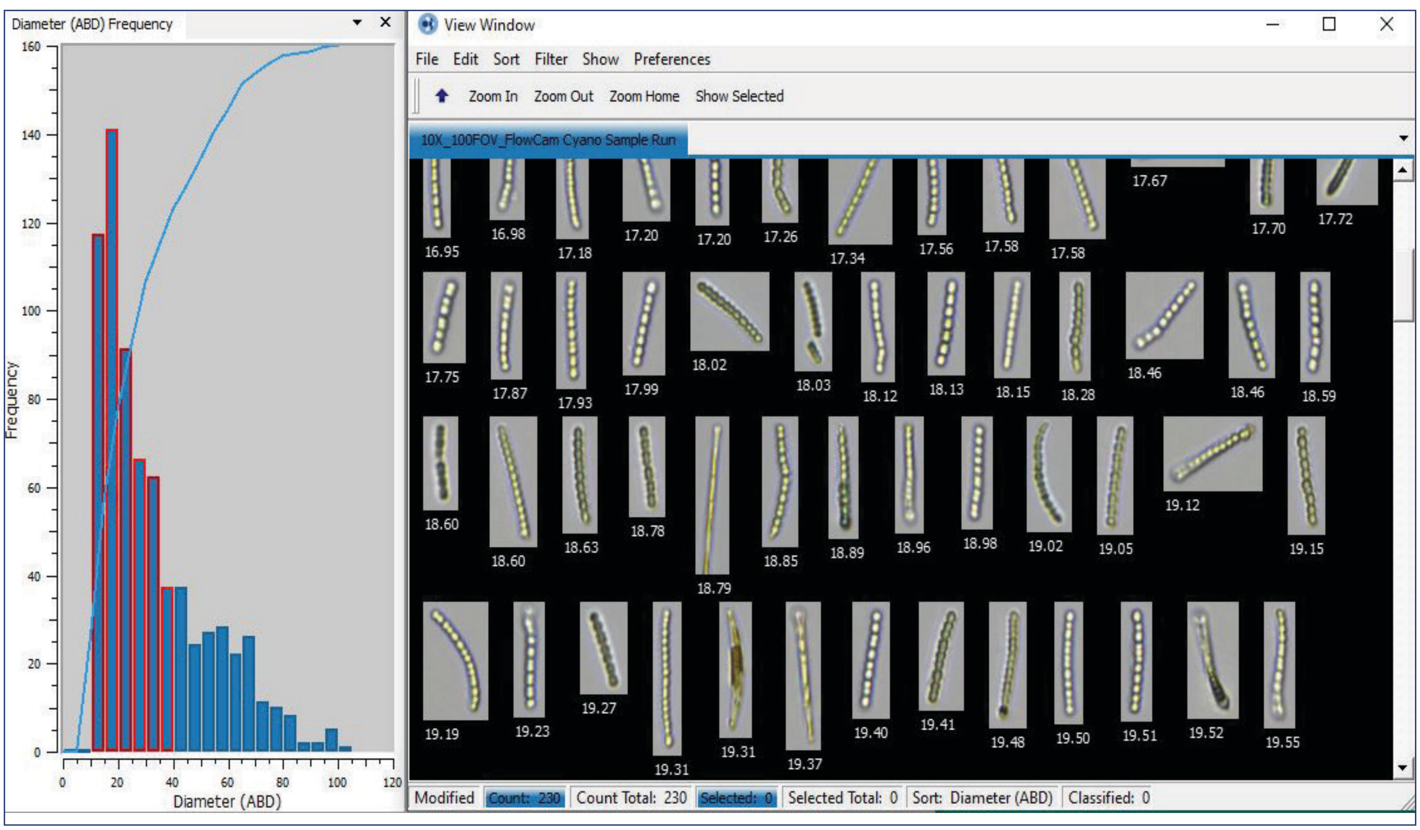
SECTION 3.3: SORTING AND FILTERING DATA

SORTING

The FlowCam system includes VisualSpreadsheet® software, a single program for both acquisition and analysis of data. VisualSpreadsheet provides the ability to sort and filter your data based on any of the measurements (or combination of measurements) acquired for the particles. The results are displayed as particle images as well as in a tabular format, and can also be exported to Excel.

The user can interact with the auto-generated scattergram to quickly select particles of interest from any of the configurable graphs.

> ***"You can get more information from FlowCam than from any other type of instrument. Going to FlowCam with a particle problem is just the best feeling in the world because it turns data into useful information that you can use to solve a real problem."***
>
> -Dan Berdovich, Owner
> Micromeasurement Laboratories, Inc.



VisualSpreadsheet automatically generates a number of interactive, customizable scatterplots and histograms that allow you to call up the images associated with different portions of your data for quick screening. In this example, we selected a portion of the Diameter Frequency histogram (left), which pulled up the images associated with the size bins we selected (right).

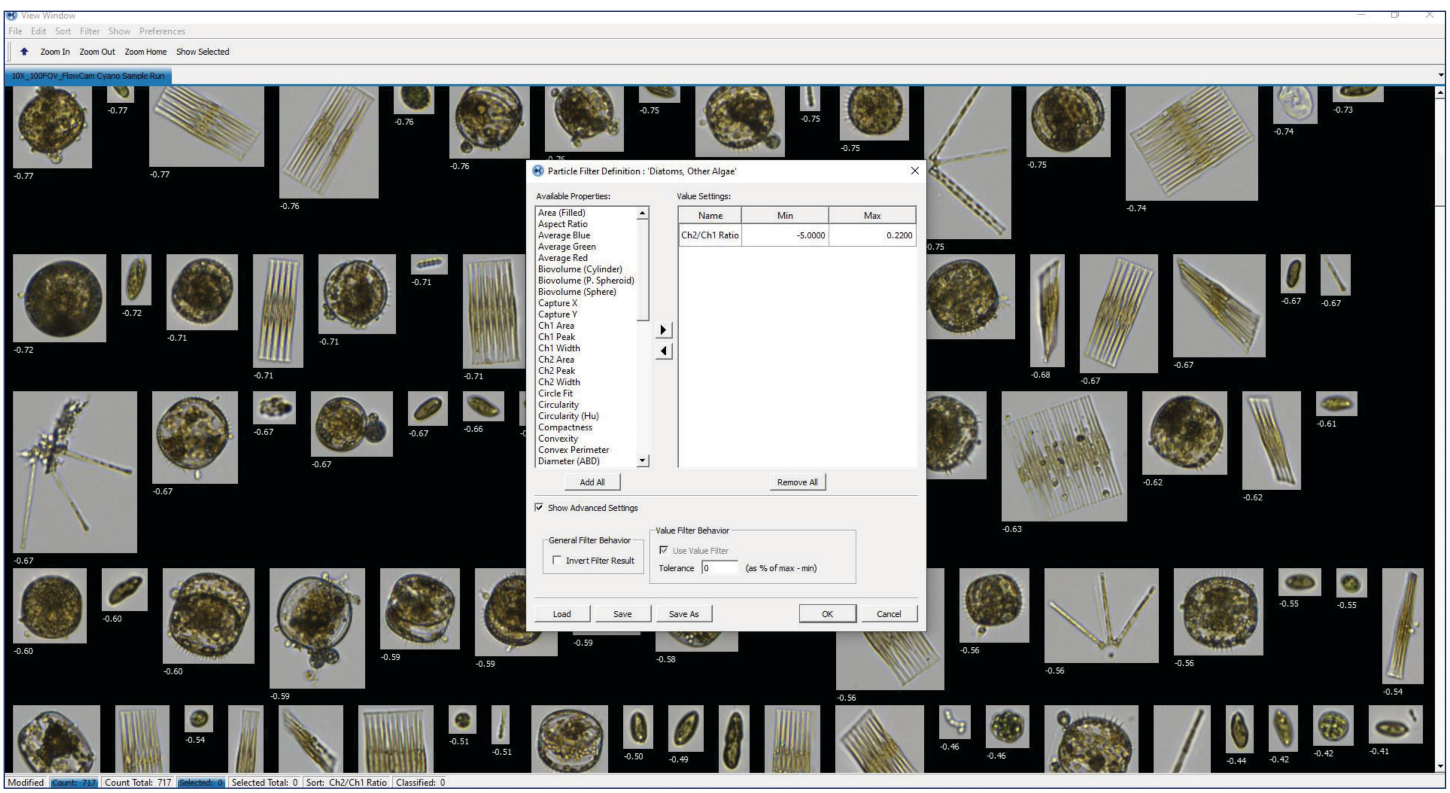
FILTERING

You can also build filters based on particle properties with VisualSpreadsheet to automatically isolate particles of a particular type.

Filters can be created, saved, and applied to future runs, or in post-processing mode of past runs.

Value filters can be used to isolate particles within a specified range of any particle property. Statistical filters can be used to identify images similar to a population of user-selected images.

> ***" It used to take us 3-4 hours to do algae counts in the summer. Now it takes 15 minutes. We use FlowCam because it's quick and easy.... Using FlowCam as part of our integrated strategy has prevented larger outbreaks, compared to previous years...."***
>
> \- Hunter Adams, Water Laboratory Supervisor
> for the City of Wichita Falls, TX



A VisualSpreadsheet value filter is used to separate cyanobacteria from diatoms, other algae, and detritus based on the ratio of chlorophyll to phycocyanin emissions using FlowCam Cyano. FlowCam Cyano employs these filters to help users quickly gauge the proportion of cyanobacteria in a sample based on fluorescence.

CHAPTER FOUR

Who Uses Flow Imaging Microscopy?

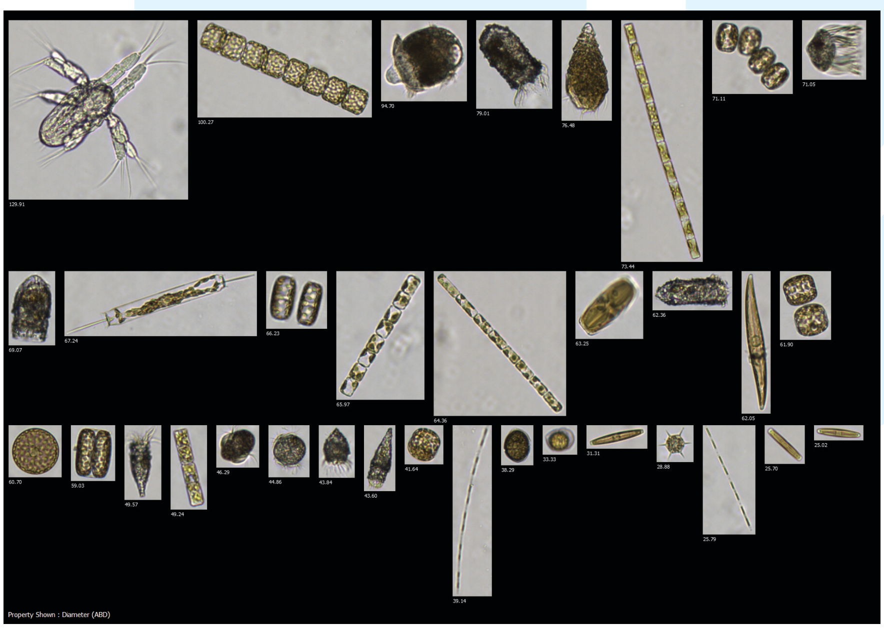
SECTION 4.1: Public & Private Research Institutions

SECTION 4.2: Drinking Water Utilities & Government Monitoring Agencies

SECTION 4.3: Aquaculture Companies

SECTION 4.4: Microalgae Production Companies

SECTION 4.5: Other Applications



A VisualSpreadsheet collage of marine phytoplankton

SECTION 4.1: PUBLIC & PRIVATE RESEARCH INSTITUTIONS

FlowCam is used by researchers at universities and institutions around the globe to study phytoplankton and zooplankton, and monitor for harmful bacteria in marine and freshwater environments.

Savannah Mapes, a graduate student at the Virginia Institute of Marine Science (VIMS), studies harmful algal blooming (HAB) species in the lower Chesapeake Bay and has set out to better understand two dinoflagellates in particular: *Alexandrium monilatum* and *Margalefidinium polykrikoides*.

Mapes conducted extensive field work during late summer to collect samples from blooms as they occurred. She observed *M. polykrikoides* blooms around the area in late summer 2020, including one in the York River, followed by an *A. monilatum* bloom.

VIMS previously documented daily species concentrations and cell counts using light microscopy and quantitative PCR (qPCR).

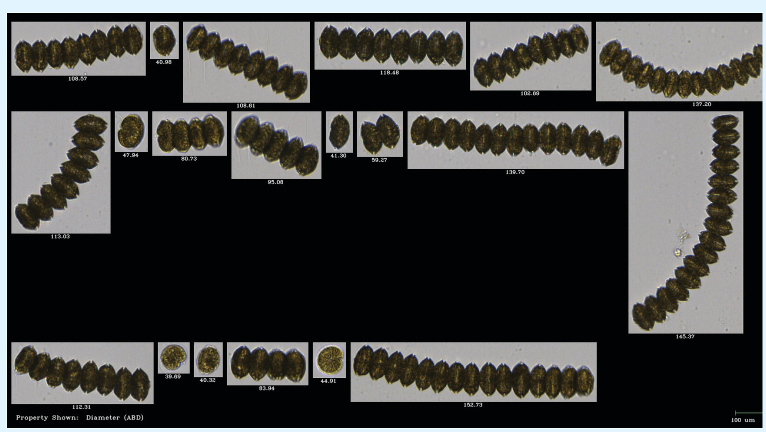
One of the benefits of FlowCam for supplementing this type of work, Mapes noted, was the amount of data that can be collected from a single milliliter of water, including a "visual representation of the microscopic community, classification and enumeration of cells, and the dimensions of every particle captured."

"*Alexandrium monilatum* is a tricky species to cell count due to its long-chain-forming behavior," Mapes wrote. "With FlowCam, I was able to filter different sized chains into corresponding categories with a defined number of cells, which was an efficient method for doing cell counts."

Researchers like Mapes are working towards advancing our understanding of the complicated life cycle of HAB species like *A. monilatum*.

According to Mapes, the single biggest advantage of FlowCam is its "visual representation of the microscopic community, classification and enumeration of cells, and dimensions of every particle captured."

Pictured left: A Lower Chesapeake Bay water sample. A bloom can sometimes be so dense that individual chains are visible to the naked eye.



Pictured above: Examples of one of Mapes' *A. monilatum* classifications in VisualSpreadsheet

SECTION 4.2: DRINKING WATER UTILITIES AND GOVERNMENT MONITORING AGENCIES

Climate conditions are conducive to both harmful algae blooms (HABs) as well as taste and odor events with increasing frequency and intensity. As a result, EPA regulations are moving toward requiring cyanobacteria monitoring. Proactive drinking water agencies are seeking a streamlined approach to monitor cyanobacteria and nuisance algae.

When Hunter Adams, Water Laboratory Supervisor for the City of Wichita Falls, TX, learned that there was an impending water crisis, he responded as if it were a crime scene - asking who, what, where, how and when - to identify the source of the problem. Those questions are answered through their monitoring strategies.

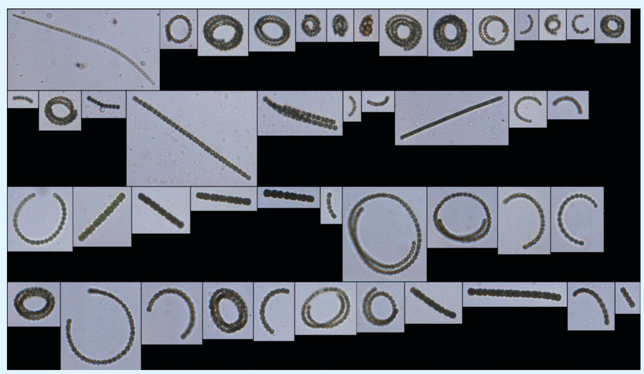
Adams incorporated FlowCam with three other methods - qPCR, LC-MS/MS & GC/MS - in an integrated strategy to monitor two lakes and one holding reservoir. These methods build upon one another. Some are used multiple times per week for triage, while other methods, such as LC-MS/MS are used only on an as-needed basis due to the high cost per sample.

FlowCam is used to run samples three times per week in the summer and once each week in the winter in order to identify and enumerate filter-clogging algae, taste and odor algae, and cyanobacteria. FlowCam requires an upfront capital investment, but there is no cost per sample and no limit to the number of samples that can be analyzed.

“It used to take us 3-4 hours to do algae counts in the summer. Now it takes 15 minutes. We use FlowCam because it’s quick and easy and shows you what’s there.

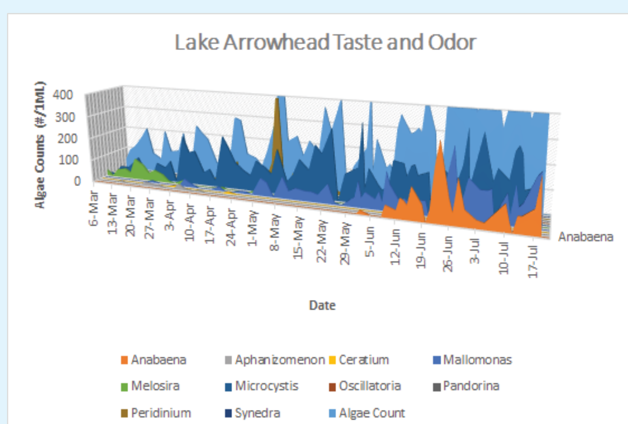
If you can see what you have in a 3-minute test, then you have what you need. You’re wasting time and money if you test for toxins in the winter with no toxin-producing organisms in the sample.” says Adams.

Elevated FlowCam counts for filter-clogging algae or taste and odor producers are used to trigger immediate treatment. *Anabaena* concentrations of 100-200 chains/mL indicate an oncoming taste and odor event. *Microcystis* concentrations of 500 colonies/mL trigger immediate qPCR tests. Other agencies calculate cells/mL rather than colonies/mL with FlowCam. Adams’ multi-year history of FlowCam data and understanding of his reservoir’s ecology enables him to accurately leverage a simplified data set.



Above: a FlowCam collage of *Anabaena* chains

Below: Algae counts measured by FlowCam saw elevated levels of *Anabaena* in June. A spike in *Anabaena* is used as a predictive indicator of an impending taste and odor event, as it often correlates with a spike in geosmin.



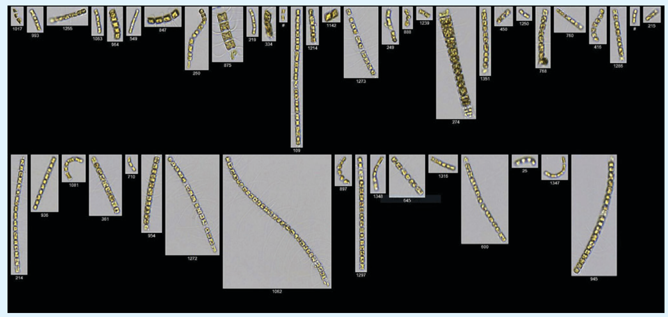
SECTION 4.3: AQUACULTURE COMPANIES

Grieg Seafood ASA, one of the world's leading fish-farming companies, used manual microscopy to identify and count algae to determine if there was a need to employ mitigation strategies. On any given day, there could be upwards of 100 species of algae, and using manual microscopy was like trying to find a needle in a haystack. Their manual process was prone to error and not supportive of their goals to actively manage large fish stocks.

There are approximately 15 species of algae that are harmful to salmon in Shetland, and harmful at different concentrations. When Grieg first purchased a FlowCam instrument for the Shetland facility, they wanted to develop an early warning system for staff to study water quality and improve their ability to monitor and mitigate harmful algae populations before they bloom. To execute this plan, they needed to collect huge amounts of field data and information.

"We've been building a big database with FlowCam because we process so many samples and produce so much data." says Rhanna Turberville, Grieg Water Quality Technician. "All the daily samples go into the database, including weather and oxygen levels. This makes it easier to track patterns."

"With over a year of daily data samples, we have a full picture of population trends to help with forecasting. We can anticipate and predict blooms with confidence. Now that the spring bloom is approaching, we can compare populations to the same time last year and make better forecasts." says Turberville.



Pictured above: Chaetoceros cells as imaged by the FlowCam

The early warning system Grieg had envisioned is well-developed and provides staff with reliable data that can be compared to the same period last year. They have been able to develop a traffic light system (green, yellow, red) based on the abundance and severity of different plankton species. This removes the subjective component from the decision process and provides more confidence behind the feed/no feed decisions.

Overall, Grieg has been able to reduce fish mortality, proactively monitor their fish pens and water quality, and reduce the effect of HABs on the health of their fish stocks by incorporating FlowCam into their daily processes.

SECTION 4.4: MICROALGAE PRODUCTION COMPANIES

Cyanotech Corporation produces the BioAstin and Spirulina Pacifica lines of high-quality microalgae products for health and human nutrition. The microalgae is grown in a sustainable, reliable, and environmentally sensitive operation based out of Kailua Kona, HI.

Cyanotech uses the power of FlowCam to count microalgae, image the organisms present in the sample, and automatically classify the organisms. These methods are leveraged as part of the routine monitoring of production cultures, as well as research to identify and implement productivity improvements.

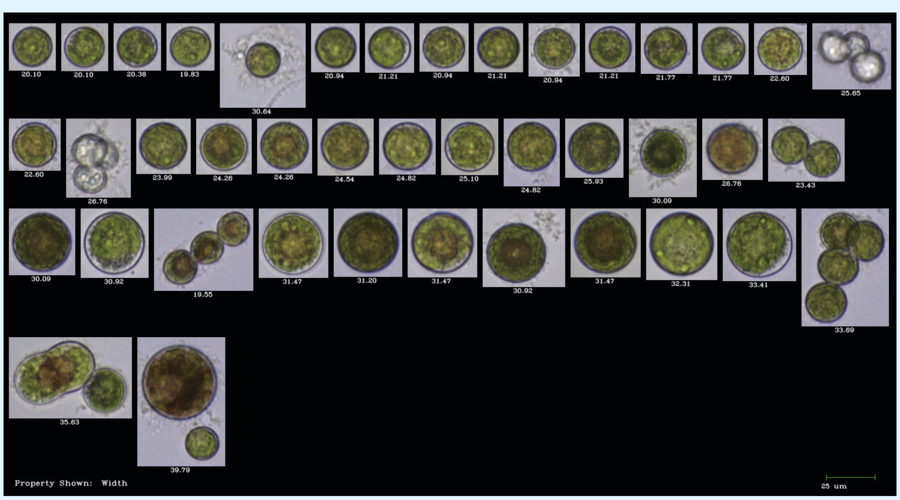
In production, FlowCam is now an essential component of daily routine monitoring. It is a key source of information on:

- How fast the algae are growing
- Whether the shape and size of the algal cells are within normal parameters
- Whether the quality of the culture is within normal parameters

"Data, especially on algal cell shape and size, and on culture quality, that were too labor-intensive to collect are now routinely acquired and shared. We have found that the data obtained by a single person using FlowCam would have required three people prior to the FlowCam acquisition" says Charley O'Kelly, Director of Applied Research.

Staff have developed daily dashboards and other software tools to organize FlowCam data, assess its significance, and present the results across the company. These tools present critical information to decision makers in a timely and accessible form. Any issues with the cultures are quickly spotted, are better understood, and are more expeditiously addressed.

Cyanotech constantly seeks to improve its cultivation practices. In applied research, FlowCam is an essential tool for monitoring laboratory and mid-scale experiments dedicated to boost the growth rate, productivity, and quality of the algae being cultivated. Since FlowCam can quickly and effectively collect information on algal shape and size, the Applied Research department can more easily design and conduct ongoing experiments. The department also seeks ways to streamline the data from FlowCam so that it is even better aligned with the needs of the company.



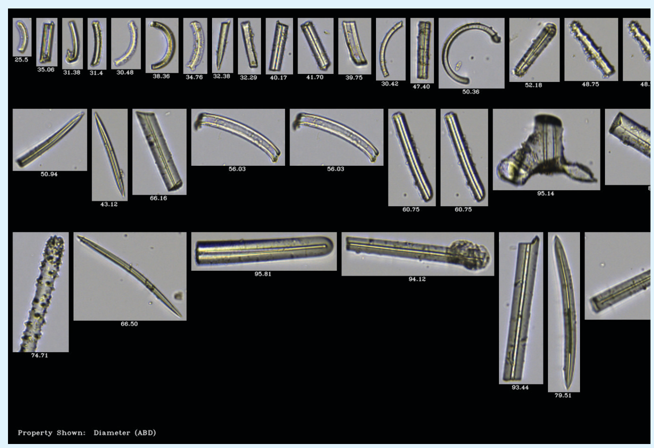
Haematococcus pluvialis culture imaged by FlowCam 8400 at 20X. This species is well known for its high anti-oxidant content, and is used in aquaculture and cosmetics applications.

SECTION 4.5: OTHER APPLICATIONS

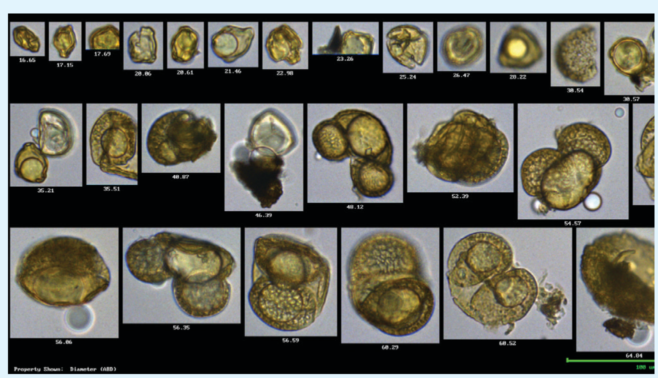
FlowCam is a useful tool for many professionals who need to image, enumerate and/or measure just about any particle suspended in a liquid medium. This means that FlowCam is not limited to analyzing plankton in water samples. It can be used for many other types of ecological research, including, but not limited to: paleolimnology, entymology, and botany. FlowCam has also been used for wastewater analysis, ballast water testing, microplastic analysis, shellfish farming, and invasive species detection.

To discuss a research question and feasibility of FlowCam for your unique application, contact us today at contact@fluidimaging.com.

Parasitoid wasps
FlowCam 8100, 4X



Sponge spicules
FlowCam 8100, 10X

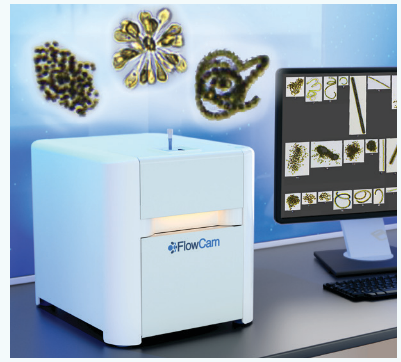


Pollen particles found in a freshwater Maine pond.
FlowCam VS4, 20X

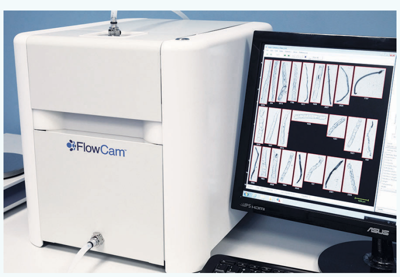
Covering the Full Spectrum of Flow Imaging Technology

FlowCam 8000 Series
Particles 2 µm to 1 mm
multiple objectives

FlowCam 5000
Particles 2 µm to 300 µm
single objective



FlowCam Cyano
Distinguish between
cyanobacteria and other algae



FlowCam Macro
Particles 300 µm to 5 mm

ALH for FlowCam™
Automated Liquid Handling
Integrates with 8000 series for
high-throughput processing

VisualSpreadsheet Software
FlowCam's image analysis
software

Don't see your specific application? Have additional questions? Wondering if the FlowCam will work for you?

Send an email to contact@fluidimaging.com with your questions, or to arrange to submit a sample.

ABOUT YOKOGAWA FLUID IMAGING TECHNOLOGIES

Yokogawa Fluid Imaging Technologies, Inc., manufactures industry-leading particle analysis instrumentation based on digital imaging technology. Our flagship product, FlowCam, is the first automated particle analysis instrument to use digital imaging for measuring size and shape of microscopic particles in a fluid medium.

With applications in marine & freshwater research, biopharmaceutical research & development, municipal water, industrial manufacturing, and many other markets, Yokogawa Fluid Imaging Technologies leads the way in imaging particle analysis.

FlowCam®

Yokogawa Fluid Imaging Technologies, Inc.

kenelec scientific

Distributed by:
Kenelec Scientific Pty Ltd
1300 73 22 33
sales@kenelec.com.au
www.kenelec.com.au