FlowCam®

Flow Imaging Microscopy for Shellfish Aquaculture and Research

OVERVIEW

Flow Imaging Microscopy (FIM) is a technique for automatically detecting, imaging, enumerating, and measuring particles and microorganisms in-flow, then using software to organize image data by taxa, size, and other parameters. FIM combines the optical elements of a microscope with the speed of a particle analyzer. Some FIM instruments even utilize principles of flow cytometry by using a laser to trigger image capture when a particle containing certain pigments is detected.

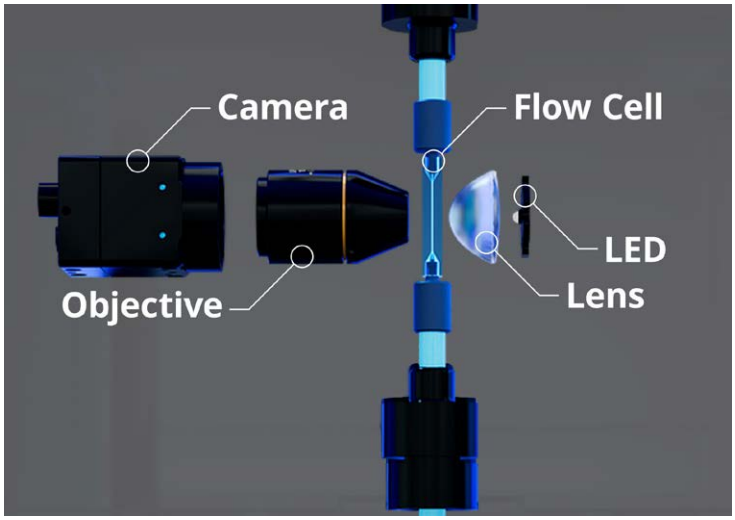
FlowCam, the first FIM instrument ever developed, is a versatile instrument with a variety of models optimized for different applications. FlowCam is ideally suited for aquaculture research thanks to its unique ability to target phytoplankton *and* shellfish larvae in a single platform. Complementary applications include phytoplankton and zooplankton research, water quality monitoring, and microalgae cultivation. It provides an excellent solution for any applications that require rapid detection, imaging, and enumeration of microscopic particles in a fluid medium.

> *"[FlowCam is] versatile for sizing a wide range of particles, including micro-animals like zooplankton, phytoplankton and unicellular organisms. A complement of microscopy lenses allow for counting different size ranges of particles."*
>
> **Mike Brueseke, Laboratory Specialist, University of Notre Dame**

FlowCam 8000 instrument

HOW FLOWCAM WORKS

The FlowCam imaging analysis system consists of a light microscopy apparatus with a sample flow cell placed in the optics path between the light source and the camera and objective. During analysis, a sample is introduced via a syringe pump, and images of particles that pass through the optics are automatically captured and recorded. The resulting microscopic images can then be analyzed to determine the sizes, concentration, morphology, and types of particles in the sample.



Schematic of the internal components of the FlowCam system

VISUALSPREADSHEET PARTICLE ANALYSIS SOFTWARE

Turning Data into Insight

VisualSpreadsheet is a powerful and flexible software program for both data acquisition and analysis of images captured with FlowCam. Some of the morphological properties determined by VisualSpreadsheet include diameter, area, aspect ratio, circularity, color, transparency, and intensity. The user can filter and sort particles according to their properties and display results in interactive scattergrams or histograms.

Sophisticated pattern recognition allows researchers to immediately find and display organisms with similar properties. Users can create image libraries and classification templates that can be applied to new samples to help determine concentrations of specific populations.

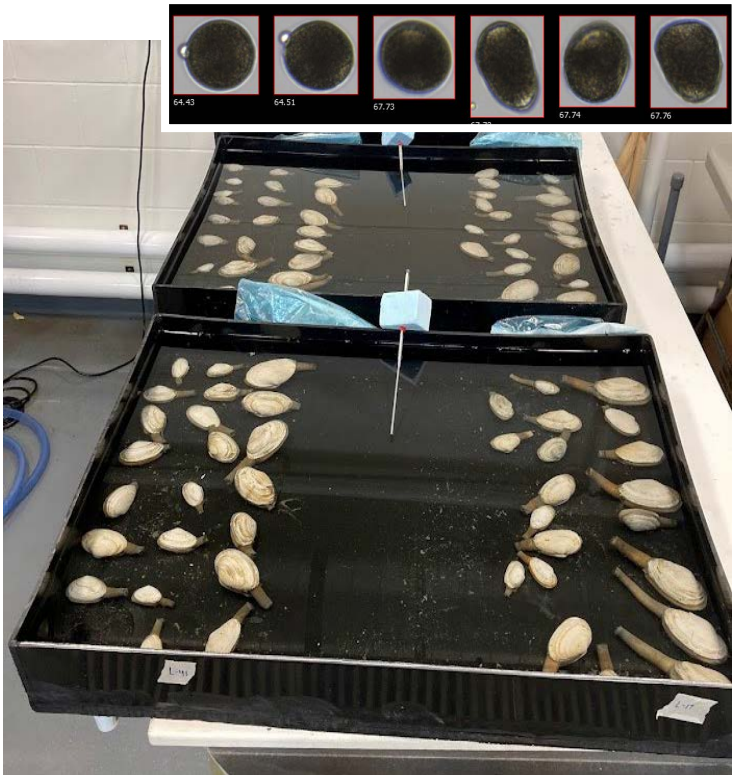
APPLICATIONS

Shellfish Reproduction

Quickly and reliably evaluate fertilization success rate, egg growth, and abnormalities

Egg density and viability is critical to the success of shellfish spawning and breeding. Oysters, mussels, and clams can produce millions of eggs per spawning event. FlowCam is ideally suited to automatically image, count, measure, and characterize these shellfish eggs both pre- and post-fertilization.

Egg size is a critical data point, and egg shape can be used to detect abnormalities that indicate underdevelopment or poor conditioning. Having images to differentiate fertilized from unfertilized eggs based on the presence of polar bodies can help maximize the data from a single sample. Flow imaging microscopy is used by labs like the Downeast Institute (Maine, USA) to accelerate particle counting and measurement during each spawn.



Clams spawning at DEI's lab in Maine. Inset photo: Mussel eggs with and without polar bodies imaged with FlowCam 8100 (10X, FOV100), ranging in size from 64 to 67 µm in diameter.

"The post-spawning analysis was so much easier with FlowCam. We used to spend hours looking at the microscope, analyzing upwards of 30 samples (10-15 minutes per sample). With FlowCam, this process took us 4 minutes per sample."

Tessa Houston, Research Assistant
Downeast Institute

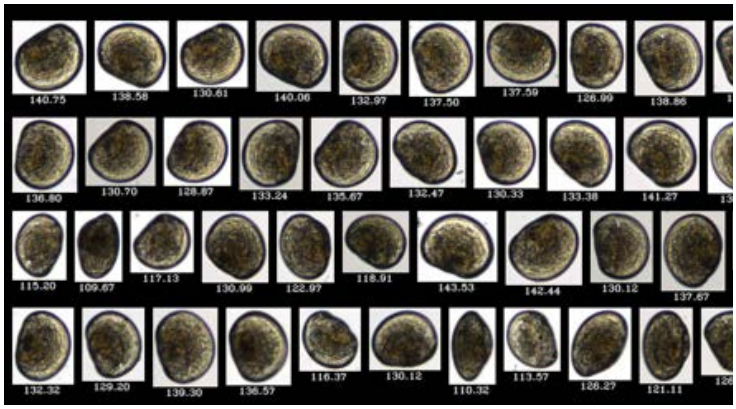
Shellfish Larvae

Rapidly count and measure larvae at several developmental stages, assess their viability, and monitor larval transport

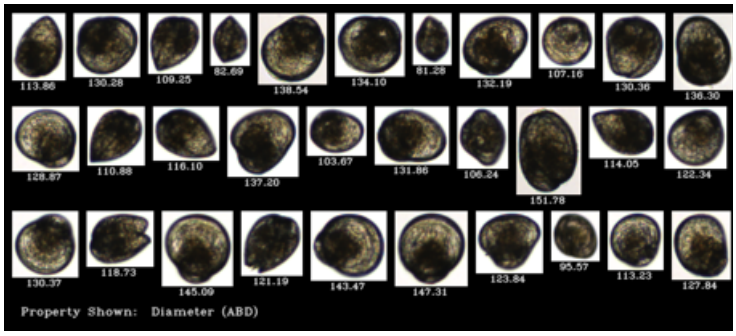
Tracking the growth and condition of shellfish larvae is a core function of any hatchery or shellfish research program, but counting and measuring larvae on a microscope is time consuming and prone to variability between operators: even a minor adjustment in the fine focus between operators can produce a 5+ µm difference in size.

FlowCam automatically generates data on length, width, and area in a consistent and reproducible way while simultaneously determining larval concentration. Additionally, FlowCam's color camera may be used to differentiate live larvae from dead larvae using a Neutral Red stain[1]. A FlowCam instrument equipped with a 488 nm laser can even be used for mark-recapture studies to track calcein-stained larval dispersal in the field; understanding larval transport is critical for shellfish management and restoration[2]. FlowCam is uniquely suited to handle the larger volumes of high-density samples in the time efficient manner that a mark-recapture study demands.

FlowCam is used by labs like NOAA's Milford Lab (Connecticut, USA) to automatically enumerate, measure, and assess shellfish larvae, producing trustworthy data faster than was possible with manual microscopy.



Mussel larvae imaged with FlowCam 8100 on Day 10 of growth
(Source: Dave Veilleux, NOAA Milford Lab)



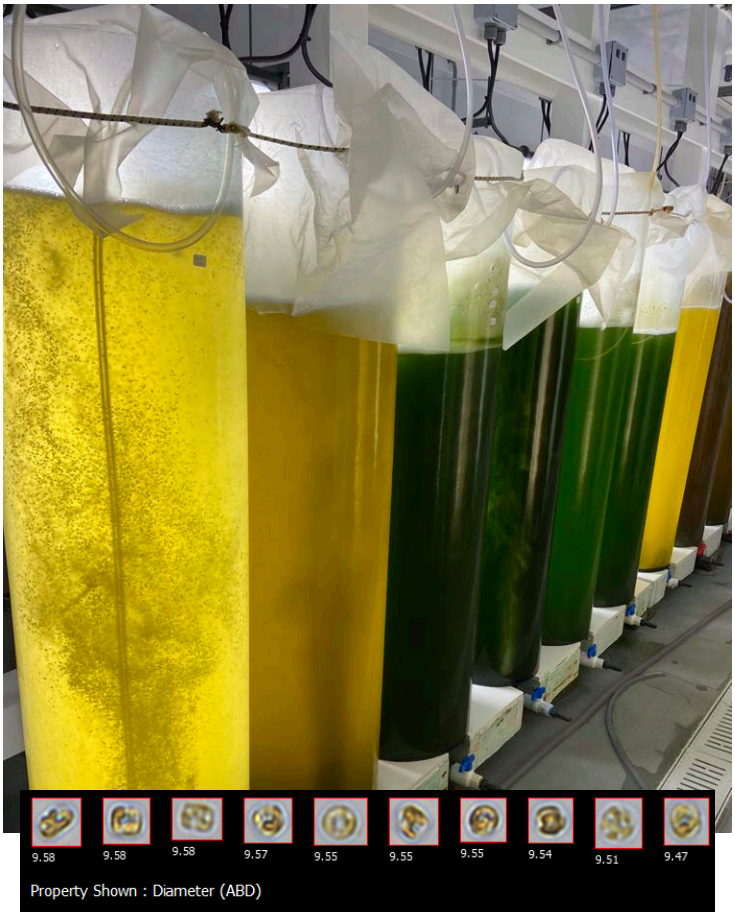
Oyster larvae imaged with FlowCam 8100 (Source: Dave Veilleux, NOAA Milford Lab)

Phytoplankton (Microalgae)

Understand the fundamental building blocks of shellfish growth

One of FlowCam's core uses is the analysis of phytoplankton communities and their size distribution. This application extends to shellfish aquaculture, where FlowCam can be used not only for evaluating shellfish eggs and larvae, but also the phytoplankton they feed on throughout their life cycle - both in the hatchery and in the field.

Quality monitoring of algae production requires information on the quantity of algae and the health of the cultures. FlowCam can provide both types of data simultaneously and with a high level of reproducibility, allowing both algae cultivated in the hatchery and marine phytoplankton in shellfish growout locations to be analyzed quickly, easily, and economically.



Thalassiosira weissflogii cells imaged by FlowCam 8100 (20x) from DEI's mass culture room.

"FlowCam is like a new sleuth that helps us solve mysteries in our hatchery, sometimes before we even know we had a problem!"

Dr. Brian Beal, Director of Research
Downeast Institute

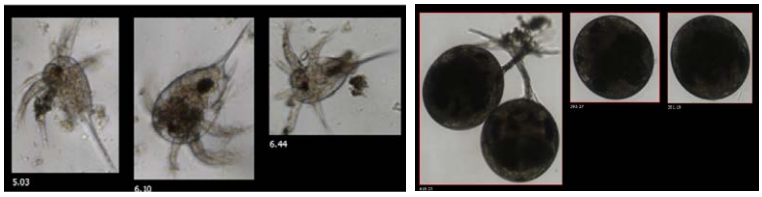
Predators

Capture top-down pressure from environmental threats to adult shellfish

FlowCam 8000's suite of magnifications enables users to target not only small particles like eggs, larvae, and phytoplankton, but even larger organisms too. Predators like invasive crabs can threaten adult shellfish during growout. These crabs start their life cycle as eggs and larvae that FlowCam can also detect and measure. Combined with genetic techniques, FlowCam offers a powerful tool to visualize and differentiate predators like the European Green Crab to better characterize when and where they spawn. FlowCam has been used by PhD students like Kelsey Meyer at the University of New Hampshire to help better understand and quantify this threat to the shellfish aquaculture industry in key growing areas thanks to FlowCam's ability to target oyster larvae and crab larvae with a single instrument.

"FlowCam offered us a reliable and objective means of counting cells and particles in our water samples. It was great to be able to pinpoint both oyster and crab larvae. We created libraries and set up a classification scheme to automatically identify and count oyster and crab larvae, and having FlowCam provide this data objectively was fantastic."

Kelsey Meyer, PhD Student
University of New Hampshire



Left: Crustacean larvae imaged with FlowCam 8100 (4X, FOV600)
(Source: Kelsey Meyer, University of New Hampshire)
Right: Asian shorecrab eggs imaged with FlowCam 8100 (4X, FOV600)
(Source: Renee Montanaro, University of Massachusetts Dartmouth)

REFERENCES

1. Veilleux, D. Rapid Assessment of Larval Bivalve Cultures Using Dynamic Imaging Particle Analysis [PowerPoint presentation]. American Fisheries Society Annual Meeting. 2018.

2. Gancel H.N., et al. Field Mark-Recapture of Calcein-Stained Larval Oysters (*Crassostrea virginica*) in a Freshwater-Dominated Estuary. Estuaries and Coasts. 2019. doi:10.1007/s12237-019-00582-6

FlowCam® Product Portfolio

FlowCam 8100

Highly versatile instrument for size, enumeration, and morphology from 2 µm - 1 mm.

FlowCam 8400

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

FlowCam 5000

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

FlowCam Macro

Rapid flow imaging microscopy for large particles and zooplankton. Particle size range: 300 µm - 5 mm.

VisualSpreadsheet®

Powerful software to analyze images and visualize your results.