Detecting & Monitoring Cyanobacteria and Green Algae with Continuous Imaging Flow Cytometry

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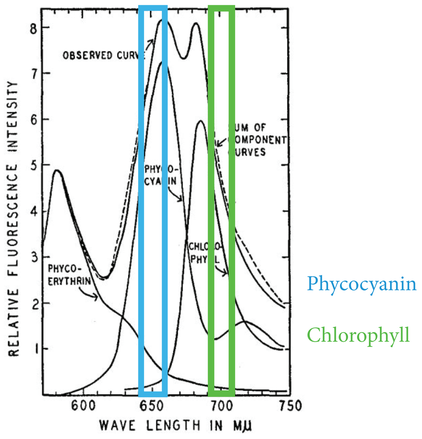
INTRODUCTION

Monitoring for harmful algae blooms is time and resource intensive. Various technologies utilize fluorescence measurements to detect cyanobacteria and estimate biovolume, concentration, and cell counts within a water system. However, results from fluorometers can be skewed by turbidity and the presence of other fluorescing pigments, and little if any taxonomic information can be obtained.

Fluid Imaging Technologies has adapted their imaging flow cytometer, FlowCam®, to simultaneously detect phycocyanin (found in cyanobacteria) and chlorophyll (found in other algae).

The FlowCam Cyano can be used for automated detection, monitoring and identification of all microalgae. Here we present an overview of the technology along with field data from various lakes.

FLUORESCENCE SPECTRA AND DETECTION



The FlowCam Cyano utilizes a red laser to excite pigments present in cyanobacteria (phycocyanin) and green algae (chlorophyll). While only one laser is used, the excitation emissions are unique for each pigment, enabling differentiation between green algae and cyanobacteria.

Figure 1. (Left) Relative Fluoresence Intensity wavelengths for photosynthetic pigments phycoerythrin, phycocyanin, and chlorophyll. The blue and green boxes on Figure 1 delineate the fluorescence range of phycocyanin (650±10nm) and chlorophyll (700±10nm), respectively, detected by the FlowCam Cyano. Modified from French and Young, 1951.

INSTRUMENTATION: HOW IT WORKS

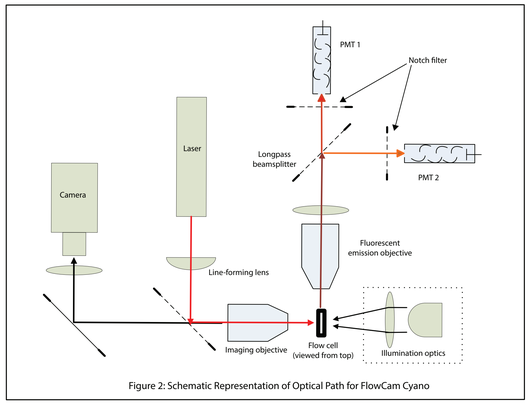


Figure 2: Schematic Representation of Optical Path for FlowCam Cyano

Figure 2 (above) is a schematic diagram of the FlowCam Cyano. The following describes fluorescence from its point of excitation to its use in analysis:

1) A red laser excites microalgae fluorescence as a sample passes through the flow cell.
2) Emitted fluorescence is sent through a longpass beamsplitter followed by selective notch filters. The notch filters restrict emissions received by the photomultiplier tubes (PMT). PMT 1 receives fluorescence representative of chlorophyll (700±10nm). PMT 2 receives fluorescence representative of phycocyanin (650±10nm).
3) VisualSpreadsheet utilizes the fluoresence signals recieved by the PMTs to generate a ratio of phycocyanin to chlorophyll fluoresence (Channel 2/Channel 1). This ratio and its function in cyanobacteria and green algae differentiation is explained in the following section: Software Analysis.

SOFTWARE ANALYSIS: DIFFERENTIATION

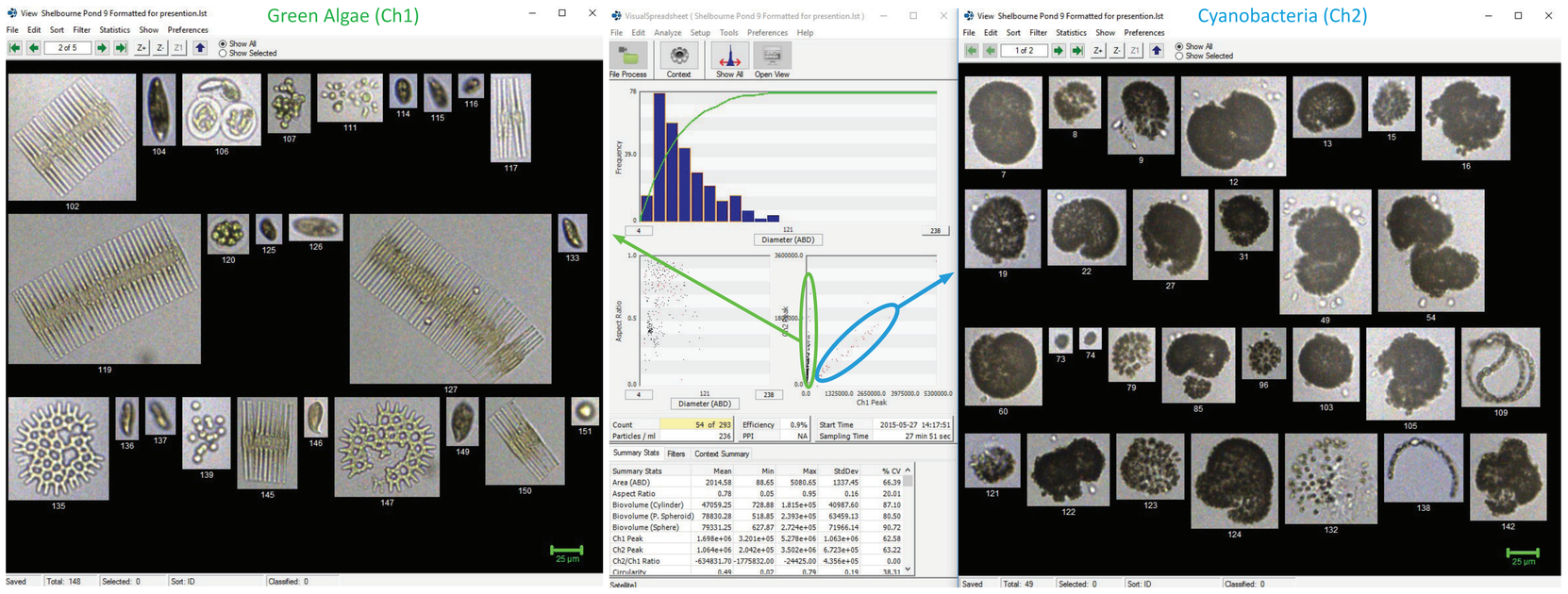


Figure 3 (above) shows algae collected from a lake sorted into two windows. The window on the left displays algae with Ch2/Ch1 ratio >1 (green algae). The window on the right shows algae with Ch2/Ch1 ratio <1 (cyanobacteria).

VisualSpreadsheet, the analysis software associated with the FlowCam, calculates the Channel 2/Channel 1 ratio for each imaged cell, a ratio calculated from relative fluorescence intensities received by PMT 2 (Channel 2=chlorophyll) and PMT 1 (Channel 1=phycocyanin). Ch2/Ch1 ratios >1 are indicative of green algae. Ch2/Ch1 ratios <1 are indicative of cyanobacteria.

SOFTWARE ANALYSIS: CLASSIFICATION

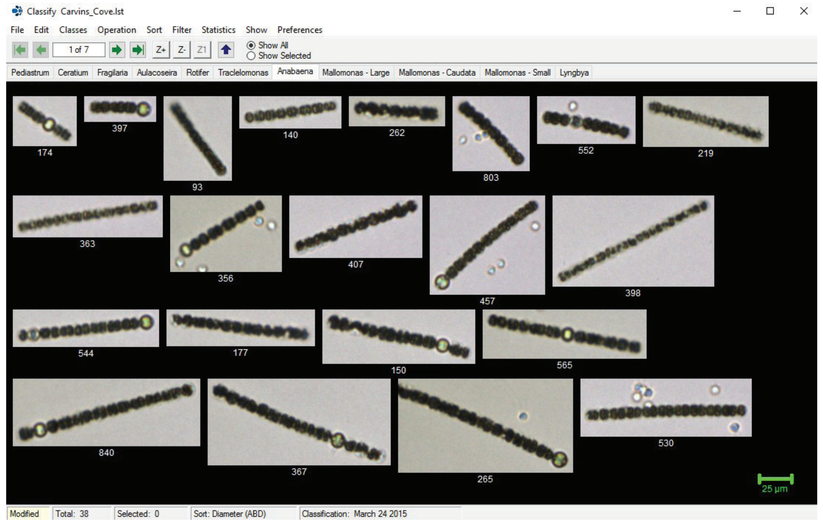


Figure 4 (above). A classifiction library showing user-set classifications of algae shown on the tabs at the top of the window. The current window display shows one "type" of algae, *Anabaena.* Libraries and classification specifications can be saved and applied to any sample data set.

ADVANTAGES

- Detect and distinguish between cyanobacteria and green algae
- Automate detection and analysis of sparse populations
- Automate population analyses, including cell density, biovolume, population growth, and morphological characteristics
- Reduce hands-on time in the lab—analyze 1 mL in ~5 minutes

APPLICATIONS

- Marine and freshwater Harmful Algal Bloom (HAB) monitoring
- Drinking water analysis: taste and odor algae & cyanobacteria monitoring
- Waste water analysis
- Ballast water analysis

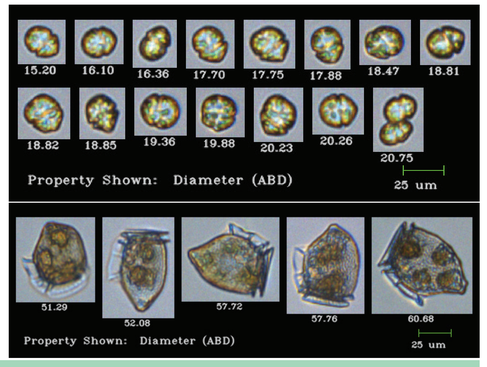


Figure 6 (left). *Karenia brevis* (top) and *Dinophysis norvegica* (bottom) as captured by the FlowCam Cyano.

ABOUT FLUID IMAGING TECHNOLOGIES, INC.

Fluid Imaging Technologies, Inc. was founded in 1999 as the manufacturer of the FlowCam. The original FlowCam was developed at Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, ME for studying plankton in the ocean. The FlowCam was designed to combine the benefits of microscopy, flow cytometry, and digital imaging into a single instrument. Since its foundation, Fluid Imaging Technologies has produced four generations of the FlowCam and the instrument has found application in other markets. Over 600 FlowCams are now used in 52 countries for plankton research, biopharmaceutical development, and other applications.

Citations:
French, C.S., and V.K. Young, 1951, The fluorescence spectra of red algae and the transfer fo energy from phycoerythrin to phycocyanin and chlorophyll, Journal of General Physiology. 35:873-890.