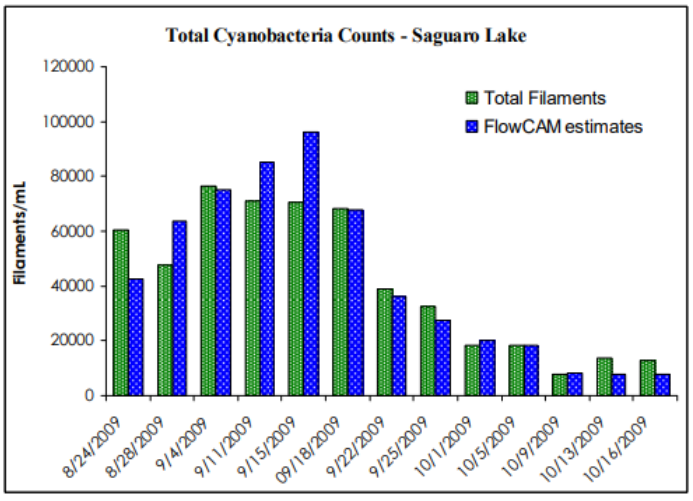
Top Studies Comparing FlowCam® to Light Microscopy

Feasibility Study for Early Warning Systems for Algae-Induced Tastes and Odors - Final Report

Arizona State University (2009)
P. Tarrant, T. Sawyer, R. Mestek, S. Neuer

INTRODUCTION "Many of the observed taste and odor problems identified in drinking water in central Arizona are attributed to cyanobacteria (blue-green algae). Several species of filamentous cyanobacteria ... are known to release the two most common compounds associated with taste and odor events, 2-methylisoborneol (MIB) and geosmin. This study investigated the link between cyanobacteria abundance and the levels of MIB and geosmin present in the water ... Cyanobacteria filaments were quantified using two methods: 1) epifluorescence microscopy of cells filtered onto membrane filters and 2) automated particle counting of water samples using a FlowCam particle analyzer ... This side-by-side comparison was intended to assess how the availability of automated technologies may contribute to simplifying and speeding up the process of monitoring water supplies by agencies and utilities."

METHOD For epifluorescence microscopy, each sample was fixed with 0.1 ml glutaraldehyde and stained with 0.2 ml DAPI. Then, 1-15 ml of sample was filtered onto black polycarbonate filters. Filters were placed on a labeled slide, embedded in immersion oil and covered with a cover slip. Slides were viewed under blue light and UV excitation. Organisms were logged by taxonomic group.



For FlowCam analysis, samples were processed using Auto Imaging mode with a 100 µm flow cell and 10X objective lens. For Salt River samples, 2 ml of sample was used for FlowCam processing, while samples from the Phoenix metropolitan area canal system - which were less dense - used 10 ml. This helped the authors "to ensure that sufficient numbers of particles were observed by the analyzer."

RESULTS & CONCLUSIONS "There are different issues associated with monitoring water supplies dependent on which method is adopted. Agencies choosing to use microscopy may expect to invest more time in sample processing on a sample by sample basis ... this technique requires 'hands on' processing at all stages ... Additionally, taxonomic identification of species requires technicians to have received the necessary training or education. Organizations choosing to adopt the automated technologies ... may be able to process samples with less experienced personnel [and] the FlowCam...can be left unattended for short periods of time ... [T]his technology, however, requires an upfront investment in the development of 'filters' capable of isolating target organisms ... [I]t is unlikely in our view that filters could operate at a species level, although this is not necessarily an issue as so many filamentous cyanobacteria species are known MIB producers. However, all images processed by the [FlowCam] particle analyzer are retained and can be further examined ... if taxonomic classification is required ... [The] cost of providing an ongoing monitoring program requires an investment in trained personnel and the necessary capital equipment, whether that is an epifluorescence microscope or a particle analyzer."

High-resolution imaging particle analysis of freshwater cyanobacterial blooms

Limnol. Oceanogr.: Methods (2018)
doi:10.1002/lom3.10274
M.D. Graham, J. Cook, J. Graydon, K. Kinniburgh, H. Nelson, S. Pilieci, R.D. Vinebrooke

INTRODUCTION "Here, we report on the development of a FlowCam-based method for providing high taxonomic resolution and accurate cell count data in a timely manner (e.g. 50 samples enumerated per week) to the Alberta

Health Services cyanobacterial monitoring network of 50-60 lake beaches across the province ... We compared total cyanobacterial and finer taxonomic cell counts generated through the use of a FlowCam vs. a standard light microscopy method."

METHOD "All taxonomic analyses using both the FlowCam and light microscope were performed by the same taxonomist (M.D. Graham)." First, Graham performed an "initial [sample] inspection using standard light microscopy ... to qualitatively determine the general taxonomic composition [and maximum particle size to inform flow cell and objective selection] ... Then, samples were taxonomically enumerated [using] a modified Utermöhl technique ... [The FlowCam's] 50-µm deep flow cell with the x20 objective maximize[d] taxonomic resolution [but] was only feasible [for] cells less than 50 µm in diameter [and] require[d] longer run times ... We did not dilute or size fractionate via sieving dense samples to avoid both time consuming additional processing of samples and potential introduction of handling error... [W]e occasionally observed cyanobacterial cells adhering to ... the flow cell [which we remedied using a Lugol's solution] ... Thereafter, each Lugol's preserved sample passed through a 50-µm deep flow cell at a flow rate of 0.02 mL min^{-1} and analyzed using the x20 objective... Concordance between light microscopy- and FlowCam-based cyanobacterial cell counts was determined using correlation and correspondence analyses."

RESULTS & CONCLUSIONS "In general, use of the FlowCam equipped with a x20 objective [and 50 µm flow cell] enabled taxonomic identification to the genus- and often species-level, thereby equating approximately to light microscopy performed using a x63 objective...[The FlowCam with a x20 objective] also enabled detection of smaller picocyanobacteria (2-5 µm diameters)".

"Total [FlowCam] cyanobacterial cell counts for live and preserved water samples … showed significant positive correlation …. Lugol's preserved samples tended to produce higher estimates of cyanobacterial abundance [relative to live samples]".

The x20 FlowCam objective "resulted in cell density estimates … four times higher than the ×10 objective ... Better image resolution … helped maximize both detection and taxonomical identification of cyanobacteria ... [H]igher total cell counts and greater species richness were mainly attributable to detection of … smaller genera measuring less than 5 µm in cell diameter, such as *Cyanodictyon*, *Merismopedia*, and *Synechococcus*."

"Total cyanobacterial cell counts based on light micrscopy vs. the FlowCam showed significant positive correlation … [as did] total cell counts for major cyanobacterial taxa (*Anabaena spp.*, *Microcystis spp.*, and *Aphanizomenon spp.*) … [and] *Microcystis*." By comparison, "total cell counts for *Aphanizomenon* were often underestimated [with the] FlowCam [Figure 5] ... [T]otal cyanobacterial and species-level cell counts performed using either FlowCam or light microscopy showed strong agreement, attesting to the reliability of cyanobacterial enumerations derived from the relatively less time-consuming digital flow cytometry approach."

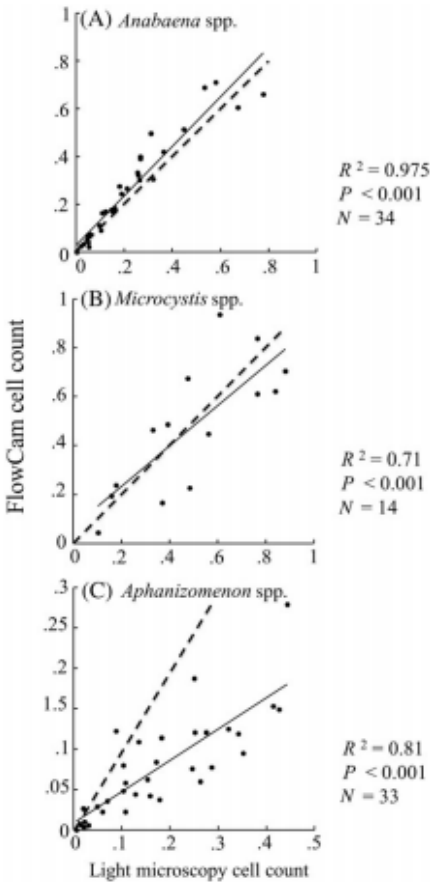


Fig. 5. Comparisons of total *Anabaena* spp. (**A**), *Microcystis* spp. (**B**), and *Aphanizomenon* spp. (**C**) densities (X 105 cells/mL) obtained with light microscopy (*x*-axis) vs. the density obtained with FlowCam (*y*-axis). All samples were preserved with 5% Lugol's solution. Solid line represents the best-line line while the dashed line depicts the 1 : 1 relationship.

"Unfortunately, accurate species-level identification … using AutoClassification in VisualSpreadsheet … remains a work in progress because of several challenging factors, including their relatively small cell sizes, polymorphism, and lack of taxonomically diagnostic sexual reproductive structures ... [I] nterference from multiple co-occurring cyanobacteria within a sample can also hamper detection and enumeration of certain taxa … [W]e strongly believe that complementary use of light microscopy is essential to QA/QC assurance when using a FlowCam for … enumeration of cyanobacterial cells within a water sample ... Nevertheless, … FlowCam greatly relieves operator fatigue, … facilitating turnaround times and greater throughput of sample enumerations [e.g. 50 samples per week vs. 15] … Most importantly, our comparative investigation … highlights that use of a FlowCam can facilitate intensive lake monitoring by providing the data to end users in an equally reliable, yet more timely manner."

Comparison of FlowCam and microscope biovolume measurements for a diverse freshwater phytoplankton community

Journal of Plankton Research (2019)
doi:10.1093/plankt/fbz056
A.R. Hrycik, A. Shambaugh, J.D. Stockwell

INTRODUCTION "FlowCam combines flow cytometry and imaging to rapidly enumerate, classify and measure particles. The instrument potentially increases processing speed of phytoplankton samples. FlowCam, however, requires extensive comparison to microscopy before incorporation into monitoring and research … We compared phytoplankton biovolume, density and taxonomic classifications between FlowCam and microscopy for 113 samples from Lake Champlain, USA".

METHOD The researchers preserved samples in 1% acid Lugol's solution or more, and first enumerated and measured phytoplankton using a microscope. "Additional aliquots from the same samples … were then analyzed using a Benchtop B3 Series FlowCam Model VS-IV ... Samples were diluted [and] each sample was run at x4 and x10 objectives ... Samples were filtered through 100-µm mesh for the x10 objective and 300-µm mesh for the x4 objective to prevent flow cell clogging. FlowCam data were processed to match microscope methods as closely as possible."

RESULTS & CONCLUSIONS "With careful calibration to the phytoplankton community in question, FlowCam can be a powerful tool for rapid phytoplankton processing and may be valuable for research programs limited by personnel or time ... Although FlowCam and microscope biovolumes do not follow a one-to-one ratio, we found strong positive relationships between … the two methods for total sample biovolumes and for most group-specific biovolumes, excluding coccoid cyanobacteria and groups that were sparse in our samples. Close relationships between microscope and FlowCam biovolumes demonstrate that results can be calibrated from one method to the other if a similar comparison is completed for the system in question. Both methods were highly reproducible … [and] FlowCam has the added reproducibility benefit that classified images are automatically saved and can be revisited later. The main drawback to FlowCam is that it necessitates coarser taxonomic resolution than is typically available with microscopy."

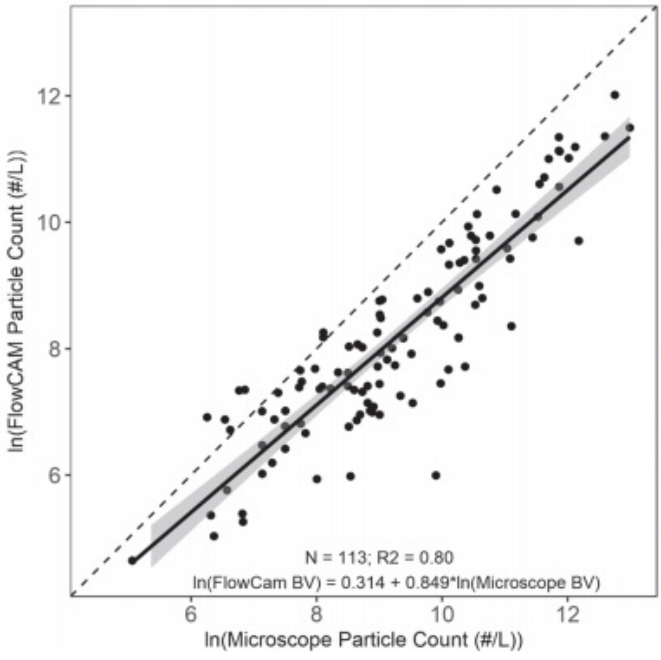


Fig. 4. Comparison of total densities calculated from FlowCAM and microscope methods. Each point represents one sample. Solid lines indicate linear regressions, gray shading represents 95% confidence intervals, and dotted lines represent 1:1 lines.

Comparison of microscopy to a semiautomated method (FlowCam) for characterization of individual-, population-, and community-level measurements of zooplankton

Hydrobiologia (2019)
doi:10.1007/s10750-019-03980-w
T. Detmer, K. Broadway, C. Potter, S. Collins, J. Parkos, D. Wahl

INTRODUCTION "We evaluated the reliability of a semiautomated method by comparing it to traditional microscopy. Our goals were to (1) characterize detection rates and body sizes, (2) assess the accuracy of population and community analyses, and (3) determine processing efficiency in terms of processing time per sample."

METHOD The researchers used the FlowCam to "enumerate and measure body characteristics of individuals … Once the sample was photographed, images were auto-identified by the VisualSpreadsheet software and categorized by taxonomic group … manual postprocessing validation was included … Once images were correctly sorted, the total number of images per taxa was recorded and densities derived ... [and] particle lengths were then estimated for each identified organism."

RESULTS & CONCLUSIONS "We found congruence between microscopy and FlowCam methods across several tests and levels of biological organization ... The reliability of semi-automated methods requires validation before usage. After a thorough vetting, semiautomated methods can increase processing efficiency of zooplankton samples by reducing processing times without sacrificing accuracy … Similar to previous studies, however, FlowCam length estimates for several zooplankton taxa were slightly greater than microscopy-based estimates … The present study provides evidence that there can be a reduction in processing effort associated with the use of the semi-automated approach. We found that the identification software was fairly accurate at identifying zooplankton correctly and it rarely misclassified zooplankton as non-zooplankton particles."

Impacts of the 2014 severe drought on the *Microcystis* bloom in San Francisco Estuary

Harmful Algae 63 (2017)
P.W. Lehman, T. Kurobe, S. Lesmeister, D. Baxa, A. Tung, S.J. Teh

INTRODUCTION "The purpose of this study was to characterize the amplitude, species composition and toxin concentration of the *Microcystis* bloom in SFE [San Francisco Estuary] and its association with environmental conditions during the severe drought of 2014."

METHOD "The biovolume of *Microcystis* colonies was computed using area-based diameter with a FlowCam … Whole water samples collected from 0.3m depth were used to determine phytoplankton and cyanobacteria biovolume and taxonomic composition (>10 mm size fraction) … These samples were kept at 4°C and processed live within 1 to 3h with a FlowCam. The FlowCam was fitted with a fluorescence trigger to isolate live phytoplankton from detritus."

RESULTS & CONCLUSIONS "Cell abundance estimates based on FlowCam measurements were closely correlated with those determined by microscopic analyses ($r = 0.88$, $p < 0.01$) … *Microcystis* biomass in 2014 reached record levels and greatly expanded the range of bloom conditions in SFE … The 2014 severe drought study suggested anticipated future increases in the frequency and intensity of drought in SFE will lead to an increase in the magnitude, duration, diversity and toxic potential of *Microcystis* blooms in SFE."

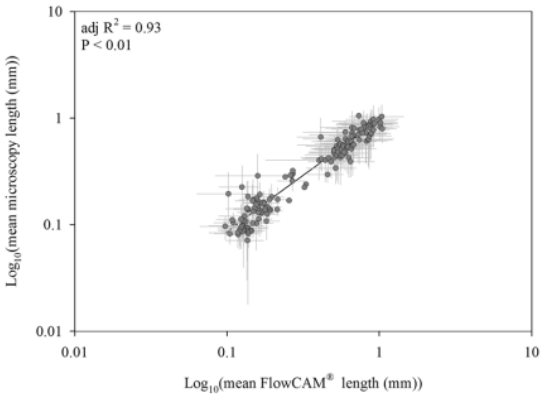


Fig. 3 The relationship between zooplankton length as determined by microscopy and FlowCAM® (log_{10}(mean microscopy length) = 0.02 + 0.95 × log_{10}(mean FlowCAM® length))

Zooplankton biodiversity monitoring in polluted freshwater ecosystems: A technical review

Indian Journal of Geo Marine Sciences, Vol. 46 (07), July 2017
C. Karnan, R. Jyothibabu, T.M. Manoj Kumar, L. Jagadeesan & N. Arunpandi

INTRODUCTION "In this paper, based on the biovolume estimation of different genera of copepods, we present the fact that only the Area-based Diameter (ABD) algorithm of FlowCam has the efficiency to measure the bio-volume of copepods better than traditional microscopy … These observations have special implications in aquatic environmental monitoring as many of the modern researchers prefer FlowCam as a better tool to accurately quantify plankton biomass."

METHOD "Copepods belonging to different taxonomic orders were selected for the present analysis using traditional microscopy and FlowCam … [T]he body dimensions of each specimen were measured using an Olympus BX53 microscope … Then the specimens were analysed through FlowCam with image processing software (Visual Spreadsheet IV). In FlowCam, 1mm field of view (FOV) flow chamber was fixed with the combination of a 2X objective lens and specimen images were captured in autoimage mode … the ABD and ESD algorithm based biovolume data were generated."

RESULTS & CONCLUSIONS "In most cases, the biovolume of copepods measured using ABD and ESD algorithms of the FlowCam varied significantly from the value obtained through traditional microscopy … Microscopy is considered to be the most accurate method accepted worldwide to measure the size and biovolume of individual copepods. In traditional microscopy, however, the protruded body parts of copepods such as appendages cannot be accounted easily in biovolume estimation … Having accepted the fact that the time required for imaging estimation of bio-volume of plankton is remarkably lower in FlowCam analysis (~3000 specimen/5min) as compared to the manual microscopy method (1 specimen/5min), the present study showed that … the ABD algorithm of FlowCam provides a better estimation of copepod biovolume compared to traditional microscopy as this method also considers the appendages and other extended portions of the copepod for biomass estimation.