High-resolution imaging particle analysis of freshwater cyanobacterial blooms

M. D. Graham,1* J. Cook,1 J. Graydon,2 D. Kinniburgh,3 H. Nelson,4 S. Pilieci,1 R. D. Vinebrooke1

1Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada
2Alberta Health, Health Protection Branch, Edmonton, Alberta, Canada
3Alberta Centre for Toxicology, Alberta Health & Wellness, University of Calgary, Calgary, Alberta, Canada
4Fluid Imaging Technologies, Inc., Scarborough, Maine

Abstract

Effective assessment of the health risk of cyanobacterial blooms requires an early warning system, which enables rapid detection of species of concern and determination of whether their cell concentrations exceed advisory guidelines. Advanced digital flow cytometry using FlowCam® (Fluid Imaging Technologies) in combination with light microscopy is a solid prospect for tracking cyanobacterial communities in a timely manner. However, implementation of such a method poses several challenges for the user. We first address sample preparation, instrumentation, taxonomic enumeration, and trouble-shooting to facilitate high throughput of analyses of water samples for total cyanobacterial cell counts and their species composition. Preservation and initial screening of samples using light microscopy to estimate community size structure are endorsed to insure their archival quality and avoid clogging of the flow cell. We show that the highest magnification (×20 objective) is needed to achieve representative total and species-specific cell enumerations. We also report that total cyanobacterial cell counts for samples analyzed using FlowCam vs. inverted light microscopy show significant positive correlation, as do those for preserved vs. live samples. Quantification of community composition using FlowCam vs. light microscopy also shows strong concordance. Although our FlowCam method performs well in the context of the World Health Organization advisory threshold of a total cyanobacterial count of 100,000 cells mL−1, it remains a work in progress in terms of reliably automated species-level identifications.

*Correspondence: mdgraham@ualberta.ca

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closely track the potential for human, agricultural, and environmental risk.

Digital flow cytometry using FlowCam® (Fluid Imaging Technologies, Scarborough, Maine U.S.A.) has gained attention as a means of rapid cell counting of CHABs since being first used by Sieracki et al. (1998). The technology is most often applied in marine and estuarine studies (See et al. 2005; Buskey et al. 2006; Lehman et al. 2013, 2015; Álvarez et al. 2014; Cetic et al. 2014; Poulton 2016). In comparison, its use in freshwater studies is less common (Reilly-Mathews 2007; Tarrant et al. 2009; Spaudling et al. 2012). Most studies have focused on more easily enumerated single-celled or relatively large marine species of diatoms and dinoflagellates; however, FlowCam has also been used to reliably quantify the abundance of smaller cells of potentially toxic colonial cyanobacteria belonging to the genus Microcystis along with other phytoplankton taxa (Lehman et al. 2013, 2015; Wang et al. 2015).

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 (AHS) cyanobacterial monitoring network of 50–60 lake beaches across the province. First, we developed a protocol for sample preparation and instrumentation to facilitate the detection and quantification of cyanobacteria in samples collected from lakes suspected of containing either the early or advanced stages of a CHAB. Next, we assessed our method across a range of lake samples. We compared total cyanobacterial and finer taxonomic cell counts generated through the use of a FlowCam vs. a standard light microscopy method (Utermöhl 1958; Nauwerck 1963; Graham et al. 2007) using a magnification of ×40 and targeted minimum total cell count of 600 for later comparison with total cell counts obtained using the FlowCam. The bench-top FlowCAM® VS-IV (Fluid Imaging Technologies, Yarmouth, Maine, U.S.A.) was equipped with VisualSpreadsheet® Version 3.8.2 software and auto-focused for ×20, ×10, ×4, and ×2 objectives, respectively. Taxonomic references included Desikachary (1959), Dwivedi et al. (2010), Findlay and Kling (1979), Komárek and Anagnostidis (1998, 2008, 2013), Komárek (2000), Komárek and Mares (2012), Komárek and Zapomelova (2007, 2008), McGregor et al. (2007), Nguyen et al. (2007), Prescott (1982), Rott (1981), and Willame et al. (2006). All taxonomic analyses using both the FlowCam and light microscope were performed by the same taxonomist (M.D. Graham).

Use of a particular flow cell assembly for enumeration of cyanobacteria depends on both the requirement of the user for taxonomic resolution, and also what is the largest size of a counting unit (i.e., colony or filament) initially observed in a sample using light microscopy. For example, the pairing of the 50-μm deep flow cell with the ×20 objective to maximize taxonomic resolution was only feasible if samples consisted of cells less than 50-μm in diameter, and therefore able to pass through the flow cell without clogging (see “Troubleshooting” section). Further, while the ×10 objective paired with the larger 100-μm deep flow cell provided less taxonomic resolution, it enabled analyses of samples comprised of cells spanning a much wider size spectrum (5–100 μm cell diameter). Also, use of a higher magnification often requires longer run times to enumerate a minimum number of cells since less sample volume passes through the smaller flow cell per unit time. In general, use of the FlowCam equipped with a ×20 objective enabled taxonomic identification to the genus- and often species-level, thereby equating approximately to light

**Materials and procedures**

Live cyanobacterial samples were collected biweekly to monthly at each site from a water depth of 1 m near shore during June to September in accordance with AHS (2016). At each site, a 60-cm long integrated tube fitted with a one-way valve was used to collect 10 samples of water from along a 1-m depth transect parallel to the shoreline. Samples were then pooled into a container from which a 50 mL polyethylene conical centrifuge tube was used to obtain a well-mixed, composite subsample. At the beginning of site monitoring in 2013, live samples were initially kept cold and in the dark while transported unpreserved to our lab for enumeration of live cells within 24 h of collection prior to the use of the FlowCam. Samples were later immediately preserved upon collection with Lugol’s solution to both insure their cellular integrity and reduce the potential adhesion to the flow cell (see below for “Instrumentation” and “Troubleshooting” section).

**Instrumentation**

Our taxonomic analyses involved complete counts of all images of cyanobacteria captured in a sample by the FlowCam after initial inspection using standard light microscopy. A Wild M40 (Heerbrugg, Switzerland) inverted light microscope at magnification of ×40–63 was first used to qualitatively determine the general taxonomic composition along with the largest size of cyanobacteria contained in each sample, which is a key determinant of the allowable size of flow cell and magnification setting for use with the FlowCam (see below). Then, samples were taxonomically enumerated in accordance with a modified Utermöhl technique (Utermöhl 1958; Nauwerck 1963; Graham et al. 2007) using a magnification of ×400 and targeted minimum total cell count of 600 for later comparison with total cell counts obtained using the FlowCam. The bench-top FlowCAM® VS-IV (Fluid Imaging Technologies, Yarmouth, Maine, U.S.A.) was equipped with VisualSpreadsheet® Version 3.8.2 software and auto-focused for ×20, ×10, ×4, and ×2 objectives, respectively. Taxonomic references included Desikachary (1959), Dwivedi et al. (2010), Findlay and Kling (1979), Komárek and Anagnostidis (1998, 2008, 2013), Komárek (2000), Komárek and Mares (2012), Komárek and Zapomelova (2007, 2008), McGregor et al. (2007), Nguyen et al. (2007), Prescott (1982), Rott (1981), and Willame et al. (2006). All taxonomic analyses using both the FlowCam and light microscope were performed by the same taxonomist (M.D. Graham).
microscopy performed using a ×63 objective. Use of the FlowCam with a ×20 objective and 50-μm deep flow cell also enabled detection of smaller picocyanobacteria (2–5 μm diameters). The ×20 objective could possibly be used in conjunction with the 100-μm deep flow cell to lessen the potential for clogging (e.g., Camoying and Yñiguez 2016), but image quality is then compromised so as to impair identification of smaller species. Similarly, the use of ×10 or ×4 objectives limited taxonomic resolution to the level of genus or morphotype (e.g., colonial, filamentous, unicellular), respectively (see “Assessment” section below). To ensure optimal resolution was achieved at the beginning of each day of operation, the Tools and Setup/View dropdown menus of the VisualSpreadsheet software were used to enable pre-focusing on cyanobacteria contained in a sample flowing at a rate of 0.001 mL min⁻¹.

The settings under the Setup dropdown menu also depends on the nature of the sample as determined qualitatively using light microscopy. In the Fluidics submenu, if a sample contained an anticipated high concentration of cells (e.g., >100,000 cells mL⁻¹), then the total volume to be drawn was set at the minimum allowable setting of 0.035 mL when using a ×20 objective. Otherwise, we found that a volume setting equaling 0.200 mL was adequate for detecting a minimum of 600 cells in water collected from either a meso- or eutrophic lake. In either case, use of an Eppendorf® pipette is recommended to transfer to the FlowCAM® a precise volume that equals the specified volume setting to avoid the negative consequences of sedimentation, namely overestimation of cell concentrations and risk of clogging (but see “Troubleshooting” section). We did not dilute or size fractionate via sieving dense samples to avoid both time consuming additional processing steps and overestimation of cell concentrations. Use of Lugol’s solution reduced the unwanted adhesion of cells to surfaces, the associated use of a lower magnification reduced taxonomic resolution and underestimated cell concentrations (see below).

As a caveat, enumeration of Lugol preserved cyanobacterial samples comes at a cost when using a FlowCam equipped with a fluorescence detector. Specifically, sample preservation with Lugol’s solution negates the ability to discriminate cyanobacterial cells from detrital material through detection of chlorophyll autofluorescence using the Trigger Mode in VisualSpreadsheet. Alternatively, alkaline hydrolysis or heating/boiling can also reduce adhesion of live cyanobacterial cells (Wang et al. 2015). Ultra-sonication is another means of the homogenization of colonial and filamentous cyanobacteria, although its effectiveness is highly variable, likely reflecting the differential sensitivities of species to this technique (e.g., Zhou et al. 2012). Elsewhere, aldehydes are often recommended to preserve algal cells because they do not affect pigmentation (Poulton 2016). For example, fixation of algal cells using a dilute 0.6% glutaraldehyde solution still enables measurement of chlorophyll autofluorescence (Mayers et al. 2016). If samples can be analyzed using a FlowCam within a few hours of collection, then they may be kept live and transported at 4°C (e.g., Lehman et al. 2017).

Although all cyanobacterial cells captured from a sample using the FlowCam were taxonomically enumerated during the development of our method, we can recommend a stopping rule as a time saving measure. Whereas only a portion of a settled algal sample totaling 300–600 cells is typically enumerated when using a light microscope (WHO 2011), FlowCam analysis can often generate thousands of captured images of cells in a sample taken during a cyanobacterial bloom. Here, these images appear as a homogenous distribution plotted along the capture x- and capture y-axes of the detection window in VisualSpreadsheet. A transect can then be drawn across any portion of the distribution, providing the user with a precise and unbiased subset of images for taxonomic enumeration. For example, a random transect can be
selected by clicking and dragging the mouse across a portion of an entire detection window containing 10,000 cyanobacterial cells, resulting in a collage containing a specific fraction (e.g., 1000 cells or 10% of the total sample). These images can then be sorted (see below), saved as a library, and later enumerations converted to a total cell count by applying a conversion factor of x10.

To further help facilitate taxonomic enumeration of a sample once run through the FlowCam, the captured images can thereafter be organized using the image parameter sorting function in VisualSpreadsheet. We found that the particle length trait in most cases best grouped the images into major cyanobacterial morphotypes, which could then be further identified to the genus or species level (see Supporting Information). The latter frames of the sorted images were then first analyzed taxonomically as they contained the more readily identifiable larger colonial and filamentous forms. Alternatively, a FlowCam can potentially be used to automatically classify certain phytoplankton into taxonomic groups by constructing statistical filters based on the particle properties of selected images of various taxa (e.g., Sosik and Olson 2007; Camoying and Yniguez 2016). However, we observed that the accuracy of classification of relatively smaller cyanobacteria to the species-level rather than simply morphotype was poor. Therefore, we consider optimization of the taxonomic Auto-Classification capacity of the FlowCam for freshwater cyanobacteria to be a challenging work in progress.

Using the VisualSpreadsheet software, estimates of total cyanobacterial cell count and biomass can be computed for a sample. The total surface (ABD) of all captured particles belonging to a particular colonial or filamentous species is first determined by sorting and saving them in a list (LST) file. Then, manual cell counts of a few of the captured images are performed to estimate the average ABD per cell. Next, the total ABD is divided by the average ABD per cell to compute a total cell count per unit sample volume (e.g., Lehman et al. 2017). Further, total cell counts for each taxon can then be converted into biomass by matching morphologies with best fitting geometric shapes and their volumetric formula (Hillebrand et al. 1999; Sun and Liu 2003). Here, cell dimensions necessary to each formula can also be measured using VisualSpreadsheet.

Troubleshooting

Clogging at the top of the flow cell may occur, especially when using the smallest flow cell (50-μm deep) to maximize taxonomic resolution. Diagnosis of clogging of a sample can involve detection of (1) destabilization of the capture rate of images, and/or (2) cavitation (i.e., formation of air spaces) immediately below the flow cell. Further, failure to detect certain large colonial or filamentous taxa (Gloeotrichia, Lyngbya) observed during routine initial sample screening using light microscopy also attests to the occurrence of clogging immediately above the flow cell.

A series of steps can be used to resolve clogging of a flow cell. Gentle agitation of the tubing above the flow cell can often eliminate a minor clog, enabling the run to proceed. If unsuccessful, then the analysis would be terminated, and the clog flushed from the flow cell. The initial flush involved adjusting the Prime System settings to reverse the direction of flow, aspirating with 95% ethanol instead of water to shrink and dislodge the clog through dehydration. If the clog persisted, then we simply increased the flow rate to achieve a more forceful backward flushing of the system. Thereafter, the clog would be expelled into, and removed from the sample reservoir above the flow cell. Any persistence of cavitation following removal of a clog can indicate that the sealant of the tubing to the flow cell has been compromised, necessitating replacement of the entire flow cell assemblage.

Size-selective sieving of a sample prior to analysis with a FlowCam can obviously reduce its clogging potential. Filtration techniques have been used at either the point of the collection of the samples to minimize clogging, and concentrate larger cyanobacterial colonies (Álvarez et al. 2011, 2012; Lehman et al. 2013, 2015; Wang et al. 2015; Camoying and Yniguez 2016). We did not implement a size-fractionation step into our protocol to avoid confounding quantitative comparisons of entire cyanobacterial communities as achieved using a FlowCam vs. light microscopy, which traditionally involves enumeration of unfiltered water samples (see below). As a note, our samples contained ~ 80% cyanobacterial taxa that flowed easily through the instrument with a low probability of clogging. Only a few larger colonial and rigid filamentous genera proved to be problematic (see Supporting Information Table S1).

Data analysis

Concordance between light microscopy- and FlowCam-based cyanobacterial cell counts was determined using correlation and correspondence analyses. In addition, simple linear correlation analyses were performed to determine how well FlowCam estimates of total live and preserved cyanobacterial cell counts agreed with those derived from light microscopy enumeration. To assess the level of agreement between the two techniques at the community level, correspondence analysis (CA) was used to ordinate species abundances (cell counts and biovolume estimates as μm³ mL⁻¹) and lakes based on preserved samples obtained during the ice-free season of 2013. All cell counts and biovolume estimates were natural log-transformed prior to ordination analysis. Ordinations were performed using the computer program CANOCO version 4.5 (Microcomputer Power, Ithaca, New York, U.S.A.; ter Braak and Similauer 2002).

Assessment

Total cyanobacterial cell counts for live and preserved water samples based on analyses using FlowCam showed significant positive correlation (Fig. 1). However, taxonomic enumeration
of Lugol’s preserved samples tended to produce higher estimates of cyanobacterial abundance relative to those based on live samples. The degree of variance about the live-preserved cell count relationship also increased in proportion to the concentration of cyanobacterial cells in a sample (Fig. 1A). Most importantly, a strong positive relationship between preserved and live cell counts for a sample was observed along the range of cell concentrations that is directly relevant to assessment of health risk as based on the WHO guideline of 100,000 cells mL$^{-1}$ (Fig. 1B).

An explanation for relative higher total cell counts for Lugol’s vs. live samples based on FlowCam analysis (Fig. 1) involves the preservative improving cellular image capture efficiency. First, the preservative reduces adhesion of cells, thereby improving dispersion of cells across the plane of focus within the detection window of the flow cell. Second, application of Lugol’s solution enhances the visual contrast of individual algae through brownification (Lawton et al. 1999) and concentration of cell contents by greater than 30% (Hawkins et al. 2005; Churro et al. 2016), which therefore should also increase the likelihood of particle detection and visual differentiation of small cells. In addition, use of Lugol’s solution minimizes the loss of cells (e.g., cell lysis, grazing) in a sample following its collection and prior to analysis by rendering it biologically inert.

The magnification of the objective used with the FlowCam affected estimates of total cyanobacterial cell counts and the number of detected species (Fig. 2). Use of the ×20 objective resulted in cell density estimates that were 14 times higher compared with that obtained by analyzing the same sample volume at ×4 (paired t-test, $p = 0.003$), and four times higher than those obtained using the ×10 objective ($p = 0.005$). Use of the ×10 objective resulted in cell density values that were three times greater than those obtained using a ×4 objective ($p = 0.027$). Further, estimation of cyanobacterial species richness based on use of a ×20 objective was two to four times greater than those based on use of a ×10 ($p = 0.023$) or ×4 ($p = 0.002$) objective, respectively.

Better image resolution at the highest possible magnification when using a FlowCam (i.e., ×20) clearly helped maximize both detection and taxonomically identification of cyanobacteria to the genus- and/or species-level. Here, higher total cell counts and greater species richness were mainly attributable to detection of several relatively smaller genera measuring less than 5 μm in cell diameter, such as *Cyanodictyon*, *Merismopedia*, and *Synechococcus* (see Supporting Information Table S1). These findings agree with other phycological investigations recommending use of a FlowCam at its highest magnification to achieve the image qualities that are necessary for finer taxonomic investigations (Alvarez et al. 2011; Spaulding et al. 2012; Camoying and Yniguez 2016).

In an attempt to lower the amount of run time required for particle imaging of each sample using the FlowCam, we also compared species detection as a function of run time (i.e., total sample volume analyzed) using the ×20 and ×10 objectives (Fig. 3). The experiment employed a series of...
replicated \( (N = 3) \) 2-min incremental sample runs to a maximum duration of 16 min. Altering the run times changed the volume being introduced into the FlowCam; thus, we could determine if sample volume was an important factor that could influence the number of observed species. If a \( \times 20 \) objective was used, then maximum cumulative species richness was achieved with approximately a total run time of 10 min (i.e., 0.20 mL sample volume). In comparison, maximum species richness was lower, but also achieved after a run time of 10 min using a \( \times 10 \) objective.

Total cyanobacterial cell counts based on light microscopy conducted using a \( \times 63 \) objective vs. the FlowCam equipped with a \( \times 20 \) objective showed significant positive correlation (Fig. 4). Significant agreements between both techniques were observed within both years as most points fell near the 1 : 1 line. However, deviations from the 1 : 1 line were observed when cell count abundances were greater than \( 1 \times 10^6 \) (i.e., over or underestimation of cell counts from both techniques). We found that when cyanobacterial cell densities do not exceed 100,000 mL\(^{-1} \), both the FlowCam and microscopy are closely related (Fig. 4B). This finding is important since the WHO considers a level of 100,000 of cyanobacterial cells mL\(^{-1} \) to be a working guideline for moderate health alerts in recreational waters (WHO 2011). This value also indicates an increased probability of relatively common cyanobacterial species, such as \textit{Microcystis} spp., \textit{Anabaena} spp., and \textit{Aphanizomenon} spp. forming surface scums (WHO 2011). However, we strongly believe that complementary use of light microscopy is essential to QA/QC assurance when using a FlowCam for the purpose of enumeration of cyanobacterial cells within a water sample.

Similarly, total cell counts for major cyanobacterial taxa (\textit{Anabaena} spp., \textit{Microcystis} spp., and \textit{Aphanizomenon} spp.) based on light microscopy vs. FlowCam showed significant positive correlations (Fig. 5). These genera occurred under bloom conditions across our study sites (i.e., > 90% of total cyanobacterial cell counts). \textit{Anabaena} spp. displayed the most significant correlation, and the best fit with the 1 : 1 line (Fig. 5A) as they appeared as solitary fragments of straight or coiled trichomes (Supporting Information Table S1) that were equally well enumerated using either method. Loosening of the coiled forms was routinely observed in the particle detection window during sample analysis, which promoted passage through the flowcell. FlowCam- and light microscopy-based total cell counts of \textit{Microcystis} also agreed well with each other (Fig. 5B). Here, the volume of the colony was computed based on the cross-sectional area of an assumed sphere to determine the cell count for a colony of \textit{Microcystis} (Joung et al. 2006). Other investigations have similarly shown that FlowCam-based \textit{Microcystis} spp. cell abundance estimates based on these volumetric measurements were closely correlated with those determined by microscopic analyses (Lehman et al. 2013, 2015, 2017; Wang et al. 2015). In comparison, light microscopy-based total cell counts for \textit{Aphanizomenon} were
often underestimated when using FlowCam (Fig. 5C). In particular, *Aphanizomenon flos-aquae* (Ralfs ex Bornet et Flahault) often appeared as fascicles (i.e., bundles or clusters), aggregating into parallel to form band-like colonies (Komárek and Kováčik 1989; Komárek and Anagnostidis 2013) in which individual trichomes are difficult to detect using FlowCam. More senescent colonies of *Aphanizomenon* appear easier to enumerate because they readily dissociate into individual trichomes (Almesjö and Rolff 2007). Thus, we found the FlowCam image analysis system to be best suited for enumeration of cyanobacterial taxa having more planar morphologies. As a caveat, visual interference from multiply co-occurring cyanobacteria within a sample can also hamper detection and enumeration of certain taxa when using FlowCam. More detailed taxonomic information of the 110 cyanobacterial taxa detected using our method is provided in the Supporting Information Section (Table S1).

We also compared how well light microscopy and FlowCam platforms provided for similar estimates of whole cyanobacterial community composition across a set of lake samples (Fig. 6, Supporting Information Fig. S1). First, separate ordinations of the two data sets captured similar amounts of explained taxonomic variation with their two major axes. Second, the ordinations of the samples based on their respective species compositions as determined using either method showed strong concordance, appearing as closely corresponding inverted triangular arrangements (e.g., Gull, Thunder, Calling; Fig. 6A,B).

**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.

**Fig. 6.** Ordinations showing the association of lake scores based on correspondence analysis of cyanobacterial taxa abundances as determined using FlowCam (A) vs. light microscopy (B). (Refer to Supporting Information for corresponding ordinations of taxa; Supporting Information Fig. S1; Table S1).
deep). As a consequence, flow rate was restricted to being relatively slow, which did however optimize image quality as also recommended by Camoying and Yniguez (2016). Nevertheless, the slower flow rate also increased the potential for clogging of the flow cell and adhesion of cells. The most efficient method of sample delivery to the FlowCam involved preservation with Lugol’s solution, preliminary screening for maximum cell size using light microscopy, and introduction of a precise sample volume (0.035 mL) equaling the amount entered into VisualSpreadsheet. Thereafter, total 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. Use a cell-counting stopping rule and particle image sorting using VisualSpreadsheet was also recommended to expedite sample analysis.

Although species-level data are often essential to health risk assessment of cyanobacterial blooms as only certain species are toxin-producers, there exists a mounting taxonomic impediment to the timely delivery of such information (Drew 2011). Most importantly, expert phycological taxonomists who can identify cyanobacterial species are declining in number (Irfanullah 2006). The VisualSpreadsheet software used with the FlowCam facilitates taxonomic recognition of detected cyanobacteria through allowing the user to group and better compare captured images on the basis of major morphotypes. Thus, its use can also be considered a useful study media and teaching tool for the training of new taxonomists. Unfortunately, accurate species-level identification of the cyanobacteria using Auto-Classification 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 (Dvorák 2015). Unlike in the case of automated FlowCam counting of larger and more morphologically distinct phytoplankton using a ×10 objective (Camoying and Yniguez 2016), a much higher resolution capability (i.e., > ×20 objective) and image quality would be required to insure the accuracy of automated taxonomic enumeration of cyanobacteria. Nevertheless, rapid detection of cyanobacteria and automated sorting of morphotypes based on select particle traits when using FlowCam greatly relieves operator fatigue compared with that experienced using the standard light microscopy method, thereby facilitating turnaround times and greater throughput of sample enumerations. For instance, use of our FlowCam-based method enabled us to generate on average taxonomic data for 50 samples per week as opposed to only 15 when enumerations were derived solely using standard light microscopy.

Comments and recommendations

Certain modifications to our FlowCam-based method can help adapt it to the varying needs of other users. We would recommend that investigations more focused on cyanobacterial species diversity in less productive lakes employ our method using large sample volumes and longer run times to ensure complete species inventories. Alternatively, if only total cyanobacterial rather than species-level cell counts are needed, then use of a lower magnification than we recommend may be warranted. As a caveat, we show however that use of the ×10 objective likely underestimates total cell concentrations for samples containing relatively smaller species. Nevertheless, use of a ×10 objective in combination with the larger flow cell would also facilitate use of a faster flow rate, thereby reducing the likelihood of adhesion of cyanobacterial cells. Thus, the need for sample preservation with Lugol’s solution may also be reduced if using a larger flow cell, thereby enabling detection of cyanobacteria using fluorescence of chlorophyll a (Chl a) and phycocyanin (e.g., the new FlowCam® Cyano model is equipped with a 633 nm laser that detects both Chl a and phycocyanin, allowing for discrimination between cyanobacteria and non-cyanobacteria algae). However, sample preservation is essential to extensive lake monitoring networks where substantial delays in cell-count analysis are common because of slow delivery samples from collection sites. Most importantly, our comparative investigation of imaging particle analysis and light microscopy-based methods for taxonomic enumeration of cyanobacterial samples 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.

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Graham et al. FlowCam analysis of cyanobacteria

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Conflict of Interest
None declared.