

A Novel, Transportable Flow Cytometer Facilitates Algal Quantification in Cultures and Environmental Samples

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Technical Bulletin

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Abstract

Flow cytometers are useful tools for monitoring phytoplankton populations both in cultures and environmental samples, since multiparametric measurements of particle size and autofluorescence can be collected quickly (10,000 events per second) on large samples of up to several mL. We have utilized the BD Accuri™ C6 flow cytometer in both a lab setting and onboard a small boat to assess the feasibility of real-time monitoring of *Microcystis* blooms in Michigan lakes. The BD Accuri C6 system has several advantages for this application. 1) The system has pre-optimized voltage settings and >6 logs of dynamic range on all detectors, which simplifies data collection for the novice user and reduces the chance of data loss due to improper instrument setup. 2) Unlike other small, easily portable cytometers, the BD Accuri C6 has a microprocessor-controlled, peristaltic pump driven fluidics system that allows large volumes of sample to be collected continuously, and a count per mL of any identified population to be determined without the addition of counting beads. 3) The system is light (30 lb/13.6 kg), can be operated from a laptop, and is easily connected to an onboard electric system. The flow cytometer was validated for particle size discrimination of 0.5 µm and larger using Megamix™ sizing beads (BioCytex).

Fluorescence excitations and emissions (Ex/Em) were analyzed at the following wavelengths: 488Ex/585 BP, 488Ex/670 LP, and 640Ex/675 BP, corresponding, respectively, to the following endogenous fluorescent species: phycoerythrin (PE), chlorophyll *a* and *b*, and phycocyanins. The fluorescence “signatures” (median channel fluorescence value ratios determined from the emissions at each of the wavelengths listed) of 5 different *Microcystis* cultures and 19 other phytoplankton species (diatoms, cyanobacteria, and green algae) were determined. Data collected from 9 different lake samples was compared to these signatures, and counts per mL of each putative population were determined. In future studies, gene expression analysis will be added to further confirm population identification.



The BD Accuri C6 Flow Cytometer Configuration

Flow Rates and Core Sizes:

Three fixed fluidics acquisition options

Slow: 14 $\mu\text{L}/\text{min}$, 10- μm core

Medium: 35 $\mu\text{L}/\text{min}$, 16- μm core

Fast: 66 $\mu\text{L}/\text{min}$, 22- μm core

User selectable core sizes from 5 to 40 μm

Lasers

488 nm, 640 nm

Signal Detectors

2 scatter and 4 fluorescence parameters, with pre-optimized voltage settings

Forward angle light scatter, FSC ($0^\circ \pm 13$)

Side light scatter, SSC ($90^\circ \pm 13$)

Fluorescence (standard configuration)

FL1 533 ± 15 nm (488 ex.)

FL2 585 ± 20 nm (488 ex.)

FL3 670-nm LP (488 ex.)

FL4 675 ± 12.5 nm (640 ex.)

The lasers and detectors are aligned and locked down at manufacture (Figure 1). No adjustments were made to the optical or laser alignments during end-user instrument setup. After setup, instrument performance was validated using the same SPHERO™ Rainbow Calibration Particles used to optimize performance at manufacture.

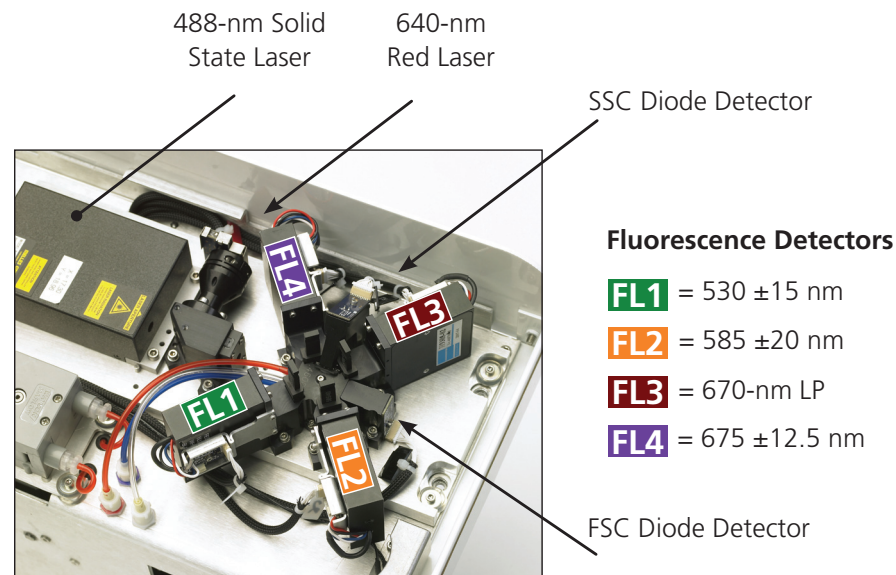


Figure 1. Layout of optics for the BD Accuri C6.

Detector Optimization with SPHERO 6- and 8-Peak Rainbow Calibration Particles

Figure 2 shows the results of detector optimization using the 6- and 8-peak particles.

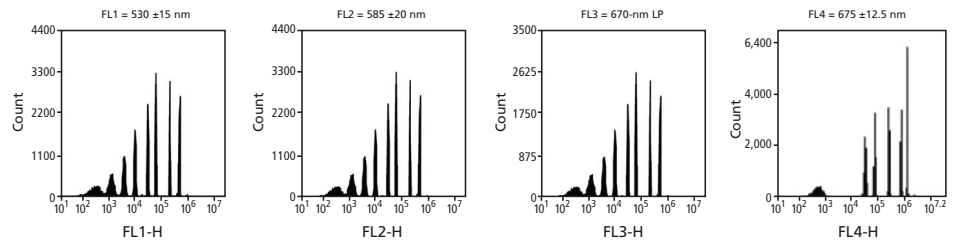


Figure 2. Representative plots of SPHERO Rainbow Particles (Cat. Nos. 556286 and 653144) distributions for four detectors of the BD Accuri C6 after each detector has been optimized for sensitivity and dynamic range. The target channels for the brightest fluorescent peak in each fluorescence detector are: FL1 = 320,400; FL2 = 275,500; FL3 = 320,400; FL4 = 630,000. Actual mean channel value for individual instruments can vary by no more than 5%.

Intrinsic Fluorochromes Detected by the BD Accuri C6

Table 1 shows the detectors, lasers, and filters used to detect the pigments.

Detector	Exciting Laser	Optical Filter	Pigments Detected
FL2	488	585 ±20 nm	phycoerythrin
FL3	488	> 670-nm LP	chlorophyll <i>a</i> and <i>b</i> , carotenoids, xanthophyll, peridinin
FL4	640	675 ±12.5 nm	phycocyanins

Table 1. Fluorochromes detected.

Data Collection: Triggering on Forward Scatter vs Fluorescence

In most flow cytometric applications, it is customary to use forward scatter (FSC) as a trigger signal. However, there are some cases in which the background noise level, due to the sample or electronics, is too high, and the desired population(s) become indistinguishable from noise. In such cases, it is possible to trigger on a fluorescence parameter. Using a fluorescence trigger allows elimination of spurious data and improves the clear separation of distinct populations.

Figures 3A–D show surface water samples collected from Lake Erie and run on the BD Accuri C6 flow cytometer. The same samples were run using a forward scatter trigger and then a fluorescence trigger (FL3). Panels A and C illustrate a field water sample, with high background noise, acquired using a forward scatter trigger. In Panels B and D, the fluorescence trigger allows gating out the background particles to clearly visualize the multiple populations in the sample.

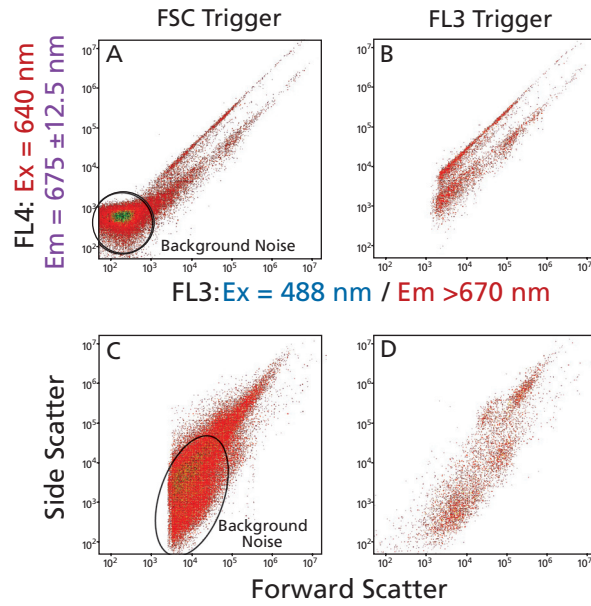


Figure 3. Plot of surface water samples from Lake Erie.

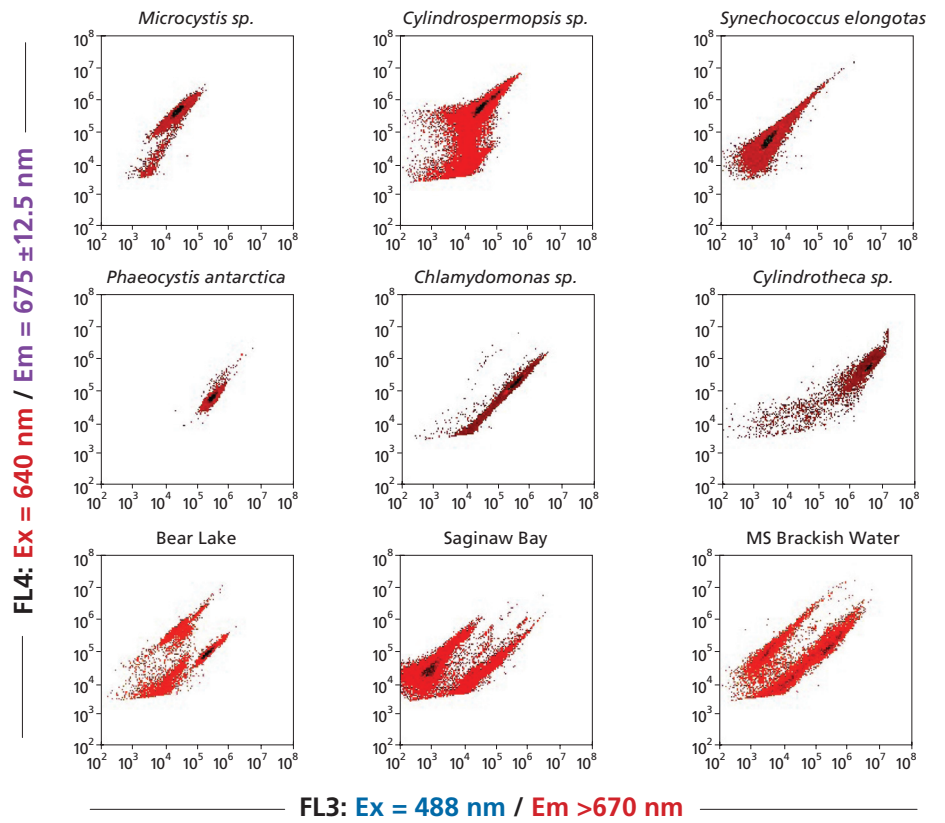


Figure 4. Examples of original fluorescence-emission data collected with the BD Accuri C6 flow cytometer. Top row: Cyanobacteria cultures, dominated by phycocyanin signal in FL4 (y-axis). Middle row: Other algal species, dominated by chlorophyll a,b fluorescence signal in FL3 (x-axis). Bottom row: Environmental samples, exhibiting a mix of FL3 and FL4 fluorescence.

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All plots were collected either by triggering or gating on fluorescence to reduce the amount of debris and non-fluorescent organisms collected in each data file. Data was collected on 10 different BD Accuri C6 instruments, all validated with the same 6- and 8-peak SPHERO Rainbow Calibration Standard (Cat. Nos. 556286 and 653144).

Algal Group	Cyanobacteria	Chlorophytes (Green Algae)	Haptophytes	Cryptophytes	Chrysophyte	Diatoms	Dinoflagellates
Organisms analyzed	<i>Microcystis aeruginosa</i>	<i>Chlamydomonas sp.</i>	<i>Chrysochromulina sp.</i>	<i>Rhodomonas salina</i>	<i>Chromulina</i>	<i>Pediastrum simplex</i>	<i>Alexandrium sp.</i>
	<i>Cylindrospermopsis racibor</i>	<i>Selenastrum sp.</i>	<i>Phaeocystis antarctica</i>	unknown		<i>Cylindrotheca sp.</i>	<i>Prorocentrum sp.</i>
	<i>Synechococcus elongatus</i>	<i>Chlorella sp.</i>	<i>Prymnesium sp.</i>				<i>Karenia sp.</i>
	<i>Anabaena flos-aquae</i>	<i>Tetraedron sp.</i>					
	<i>Aphanizomen flos-aquae</i>	<i>Tetraselmis suecica</i>					

Table 2. Algal cultures analyzed with the BD Accuri C6 flow cytometer.

Event Counts of Three Fluorescence-Signature Defined Populations in Fresh Water Samples

Striking differences in the number of organisms with particular fluorescence signatures were observed, even at various locations sampled within the same body of water (Saginaw Bay). The brackish water sample from Mississippi appeared to contain 10-fold higher concentrations of phycocyanin-containing organisms than samples from fresh water lakes.

Source	Site	Population Concentration (per mL)		
		phycocyanin	chlorophyll <i>a,b</i> *	phycoerythrin*
Saginaw Bay (MI)	1	19,075	19,120	0
Saginaw Bay (MI)	2	20,045	53,275	0
Saginaw Bay (MI)**	4	3,570	17,255	11,180
Saginaw Bay (MI)	23	3,080	5,975	0
Saginaw Bay (MI)**	BCW-B	2,993	22,207	8,807
Saginaw Bay (MI)	SB5-S	3,307	30,233	0
Bear Lake (MI)		12,640	23,240	0
Lake Mead (MI)		33,462	8,216	0
Waughop Lake (WA)		6,137	9,788	0
Brackish Water (MS)		450,563	154,354	0

Table 3. Algal cultures analyzed with the BD Accuri C6 flow cytometer.

*Note that the PE-positive population is always a subset of the chlorophyll *a,b* population.

**Sample with significantly bright PE signal.

Compiled Fluorescence-Signal Data for Algal Cultures and Fresh Water Samples

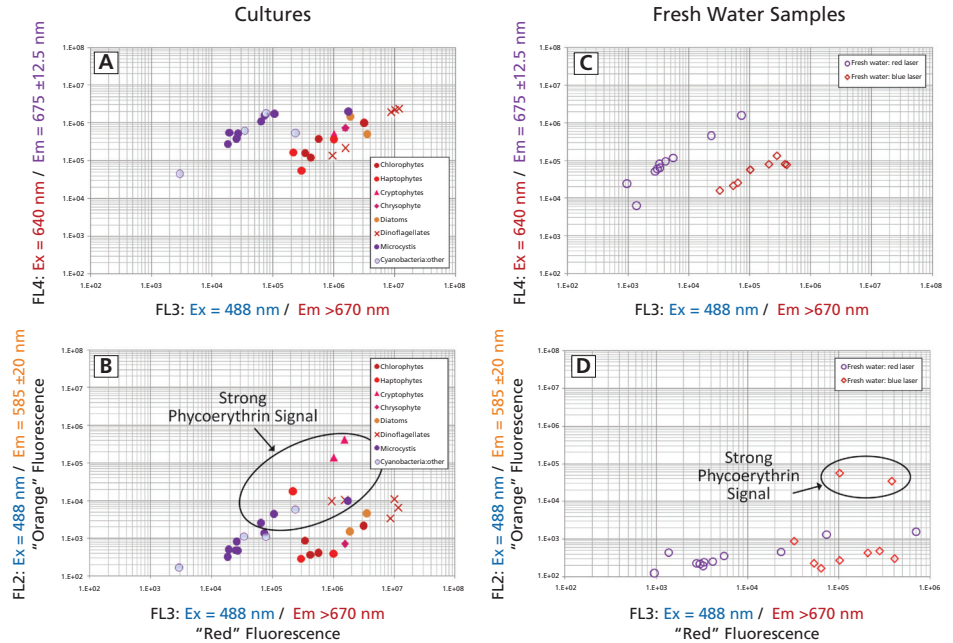


Figure 5.

A) Algal cultures could be divided into two groups based on the relationship between red fluorescence signal due to 488-nm excitation (red or orange symbols) or 640-nm excitation (purple symbols). All cyanobacteria tested were dominated by fluorescence typical of phycocyanins (purple symbols), such that the median fluorescence ratio in FL4 to FL3 equaled 16.77. All other algal types were dominated by chlorophyll *a,b* accessory pigment fluorescence, so that the median FL4 to FL3 ratio was 0.32.

B) Several cultures analyzed appeared to have strong PE signals (y-axis, FL2) as well as chlorophyll *a* accessory pigment signal (x-axis, FL3: see circled populations).

C) All fresh water samples analyzed contained a population dominated by red fluorescence due to 640-nm excitation (purple circles). The median ratio of fluorescence in FL4 to FL3 equaled 19.57, very similar to the ratio of 16.77 seen with cyanobacteria cultures. All fresh water samples also contained at least one population with fluorescence dominated by chlorophyll *a,b* accessory pigment (red diamonds). The median FL4 to FL3 ratio for that population equaled 0.40. In cultures, the FL4/FL3 value equaled 0.32.

D) Two of the fresh water samples contained a subpopulation having a strong PE signal (y-axis, FL2: see circled populations).

Summary

- The BD Accuri C6 flow cytometer is a pre-aligned, pre-optimized system with fixed-voltage detectors.
- The system is readily transported, maintains alignment post-transport, and can be easily set up for onboard data collection.
- The BD Accuri C6 system's microprocessor-controlled, peristaltic pump fluidic system allows for continuous sample collection at user selectable flow rates.
- Data file size is limited to 96×10^6 events, but is not limited by total volume collected.
- The ability to trigger on a fluorescence signal reduced data file size and improved population resolution.
- The standard laser and filter configuration of the BD Accuri C6 allowed detection of the three major pigment groups of greatest interest in the aquatic sciences.
- The fixed-voltage system, combined with >6 logs of fluorescence detection range, allowed detection of a wide range of fluorescence signals, from the dimmer fluorescence of environmental samples to the brighter fluorescence of cultures.
- The fixed-voltage system also allowed easy compilation of data collected on 10 different BD Accuri C6 instruments without the need for complicated cross-calibration to account for variations in voltage settings.
- All 10 instruments used in data collection were validated for fluorescence detection to the same bead standard on site, which allowed for easy data compilation.
- Cyanobacteria cultures could be separated from all other cultures based on the ratio of fluorescence signal intensity in FL4 to FL3.
- Populations were identified in all fresh water samples that correlated to these broad groupings based on the FL4/FL3 ratio.
- The actual number of each population per mL sample was directly calculated using the volume measurement capability of the BD Accuri C6 system.

