Semi-Automated Method to Identify and Count Cells of Cyanobacteria Colonies and Filaments

Harry Nelson[1], Peggy Lehman[2], Frances Buerkens[1], Michelle Devoe[1]

1) Fluid Imaging Technologies, Inc., 200 Enterprise Drive, Scarborough, ME 04074
2) California Department of Fish and Wildlife, 2109 Arch Airport Road, Stockton, CA 95206

FlowCam® FLUID IMAGING TECHNOLOGIES, INC.

Abstract

Various technologies utilize fluorescence measurements to detect cyanobacteria and to estimate biovolume or cell counts. However, results from fluorometers can be significantly skewed by turbidity and the presence of other fluorescing pigments, which can limit taxonomic information.

Fluid Imaging Technologies has adapted their imaging flow cytometer, FlowCam®, to detect the presence of phycocyanin in cyanobacteria and to measure size distribution, biovolume, and more. Lehman *et al.* (2017) developed a method to enumerate cells within cyanobacteria colonies and filaments using FlowCam data and a simple formula. This method enables monitoring agencies and researchers to rapidly enumerate cells in large sample volumes. The instrument is also able to detect chlorophyll, allowing for identification and enumeration of chlorophyll-containing microalgae including diatoms.

Here we present an overview of the FlowCam technology and cell enumeration method as developed by Lehman *et al.* using data from natural samples.

FlowCam Distinguishes Cyanobacteria From Other Algae Using Phycocyanin Detection

The FlowCam Cyano is designed to differentiate cyanobacteria from green algae using a red, 633 nm laser and two photomultiplier tubes (PMTs). The 633 nm laser excites both phycocyanin and chlorophyll pigments **(Fig. 1)**. The FlowCam Cyano is configured to differentiate the emissions from each pigment **(Fig. 2)** which enables VisualSpreadsheet (ViSP), the FlowCam's analysis software, to calculate the ratio of phycocyanin fluorescence (Channel 2) to chlorophyll fluorescence (Channel 1). Ch2:Ch1 ratios between 0 and 1 are indicative of cyanobacteria. Ch2:Ch1 ratios <0 are indicative of algae **(Fig 3.)**, and in the sample shown in **Fig. 3,** Ch2:Ch1 ratios >1 are indicative of detritus.

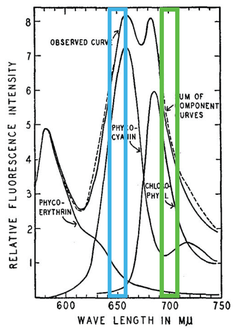

Figure 1. The FlowCam Cyano uses a red laser to excite pigments present in cyanobacteria (phycocyanin) and other algae (chlorophyll). The excitation emissions are unique for each pigment, enabling differentiation between algae and cyanobacteria (Figure 3).

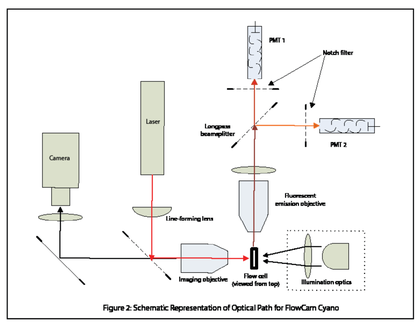

Figure 2. Emitted fluorescence is directed through a longpass beamsplitter followed by selective notch filters. The notch filters restrict emissions received by the photomultiplier tubes (PMT). PMT 1 receives fluorescence representative of chlorophyll (700±10 nm). PMT 2 receives fluorescence representative of phycocyanin (650±10 nm).

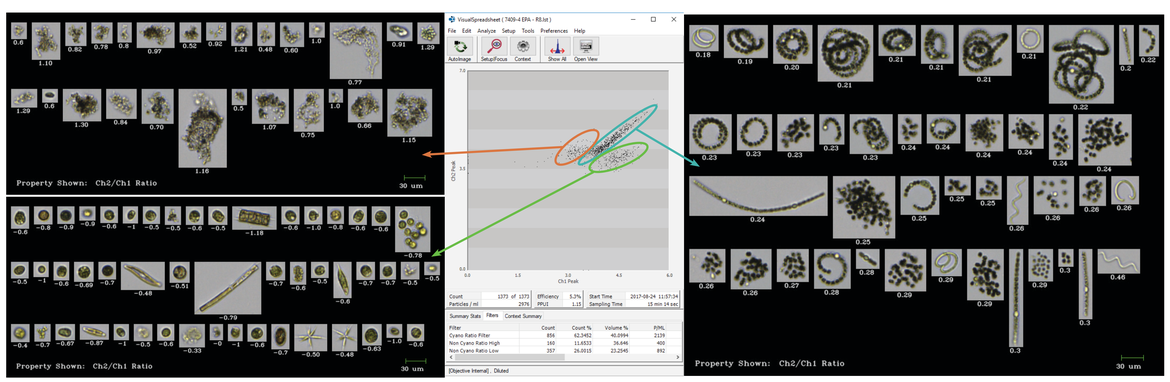

Figure 3. VisualSpreadsheet distinguished cyanobacteria (right window) from other algae and detritus (top left window) using the Ch2:Ch1 ratio detected from each image. In this sample, a Ch2:Ch1 ratio >0 is indicative of cyanobacteria, <0 is indicative of algae, and >1 is indicative of detritus.

Method for Cell Enumeration of Colonial and Filamentous Cyanobacteria with the FlowCam

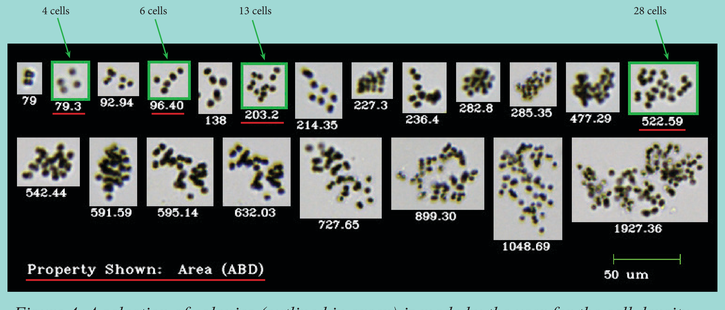

Figure 4. A selection of colonies (outlined in green) is made by the user for the cell density calculation. For colonial cyanobacteria, Area (ABD) is used for the calculation. ViSP calculates Area for each imaged colony (underlined in red), and the user manually counts the number of cells in each colony.

Class	*Microcystis*		
Count	631		
Concentration (particles/mL)	18,005		
Summary Statistics	**Mean (µm²)**	**Minimum (µm²)**	**Maximum (µm²)**
Area (ABD)	161.26	78.89	1927.36
Diameter (ABD)	13.67	10.02	49.54
Volume (ABD)	1845.95	527.08	63651.56
$D = [A_m * C] / A_{avg/cell}$			
$D = (161.26 * 18005) / 17.5 =$ 165913 cells/mL			

Figure 5. Example of a Classification Summary Report export from ViSP to Excel. The formula for D *is entered into a cell and populated with values for* A_m *, and* C *as provided by ViSP, and* $A_{avg/cell}$ *as calculated by the user.*

1) Cyanobacteria data (in this case, *Microcystis*) is isolated from other algae based on Ch2:Ch1 ratio detected by the FlowCam.

2) The average area of a single cell ($A_{avg/cell}$) is calculated. To begin, the user selects a few colonies from the ViSP list file **(Fig. 4)**. The Area (ABD) of selected colonies, calculated by ViSP, is totaled and divided by the total number of cells in the colonies, as counted by the user **(Fig. 4).**

Total area = 79.3 + 96.4 + 203.2 + 522.59 = 901.49 µm²
cells = 4 + 6 + 13 + 28 = 51 cells
$A_{avg/cell}$ = 901.49 µm² / 51 cells = 17.5 µm²

3) The cell density (D) in cells/mL is calculated. The *Microcystis* data is exported from ViSP to Excel as a Classification Summary Report. From there, D is calculated using the following formula **(Fig. 5)**:

$$D = \frac{A_m * C}{A_{avg/cell}} = \frac{161.26\ \mu m^2 * 18005\ p/mL}{17.5\ \mu m^2} = 165{,}913\ cells/mL$$

Where A_m is mean area of a cell in µm² as calculated by ViSP,
C is cell density in particles/mL as calculated by ViSP, and
$A_{avg/cell}$ is the average area of a cell in µm² as calculated by ViSP.

Conclusions

The cell density of colonial cyanobacteria, such as *Microcystis*, can be determined using the method developed by Lehman *et al*. (2017). The user calculates the average area of one cell by dividing the average area of a selection of colonies (provided by ViSP) by the total number of cells in the colonies, as counted by the user. Then, the product of the ViSP-generated mean area and cell density is divided by the average area of one cell.

This method can also be applied to other chain organisms, such as chain diatoms and *Anabaena* **(Fig. 6),** by replacing Area with the best-fit property, such as Length or Geodesic Length, depending on the shape of the cell colony/ chain.

References

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

Lehman, P.W., Kurobe, T., Lesmeister, S., Baxa, D., Tung, A., and S.J. Teh, 2017, Impacts of the 2014 severe drought on the Microcystis bloom in San Francisco Estuary, *Harmful Algae*, 63:94-108.

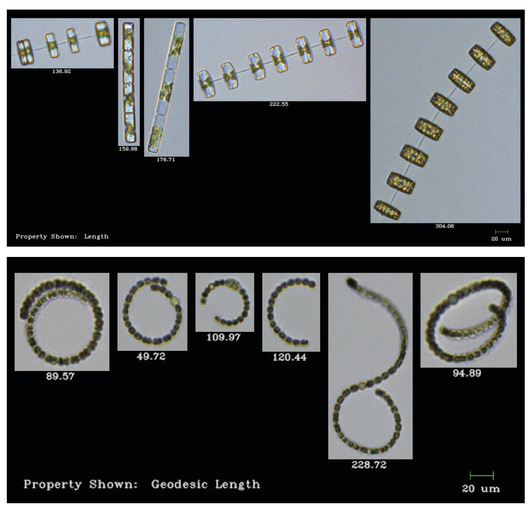

Figure 6. Lehman's method can also be applied to chain diatoms (top) and filamentous cyanobacteria (bottom). Instead of Area (ABD), the most accurate measure of colony size should be used in the calculation of cell density, in this case, Length (top) and Geodesic Length (bottom). This depends on colony shape.