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 fluorescent 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.

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

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).

$\frac{\text{Total area}}{\text{Total cells}} = \frac{\sum_{i=1}^{n} A_{i\text{cell}}}{n}$

Where $A_{i\text{cell}}$ is the area of a single cell as calculated by ViSP.

3) 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 a single colony ($A_{avg/cel}$) and then divides it by the total number of cells in the colony. This calculation is made using the following formula (Fig. 5):

$D = \frac{A_{avg/cell}}{A_{avg/cel}}$

$D = \frac{A_{avg/cell}}{A_{avg/cel}}$ = \frac{161.26 \text{ µm}^2}{49.54 \text{ µm}^2}$ = 3.25 \text{ µm}^2/cell

Results


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