Estimating Cell Counts for Colonial and Filamentous Cyanobacteria

SUMMARY

Determining cell counts of colonial and filamentous cyanobacteria such as *Microcystis* and *Dolichospermum* is critical for monitoring potentially toxic cyanobacteria. Traditional methods, including manual microscopy, are time consuming, tedious, prone to error and difficult to verify.

In the March 2017 issue of *Harmful Algae*, scientists from the California Department of Water Resources describe how they estimated cell abundance of colonial *Microcystis* using FlowCam. This technical note explains their methodology and also applies it to filamentous cyanobacteria.

Figure 1. Colonial algae (*Microcystis*) and filamentous algae (*Dolichospermum*) can be enumerated using FlowCam and its analysis software, VisualSpreadsheet®. Area (ABD) (μm^2) of the colony is noted below each image.

SAMPLE COLLECTION AND PREPARATION

The following excerpts are from Lehman et al. (2017), Impacts of the 2014 severe drought on the *Microcystis* bloom in San Francisco Estuary, Harmful Algae 63: 94-1208

"Net tow samples for determination of *Microcystis* biovolume (>75 µm size fraction) were preserved with Lugol's solution. The biovolume of *Microcystis* colonies was computed using Area (ABD) with a FlowCam digital imaging flow cytometer (Fluid Imaging Technologies; Sieracki et al., 1998). In order to more easily measure the biovolume of the colonies, the samples were size fractionated into <300 µm diameter size fractions and read at a magnification of 10X and 4X, and >300 µm and analyzed at a magnification of 2X.

Cell abundance estimates based on FlowCam measurements were closely correlated with those determined by microscopic analyses (r = 0.88, p < 0.01).

Whole water (unpreserved) samples collected from 0.3 m depth were used to determine phytoplankton and cyanobacteria biovolume and taxonomic composition (>10 µm size fraction) in sub-surface water. These samples were kept at 4 °C and processed live within 1 to 3 h with a FlowCam digital imaging flow cytometer. FlowCam was fitted with a fluorescence trigger to isolate live phytoplankton from detritus (Sieracki et al., 1998). Digital images of cells were obtained by passing samples through a 100 mL flow cell for 10 min at 10X magnification."
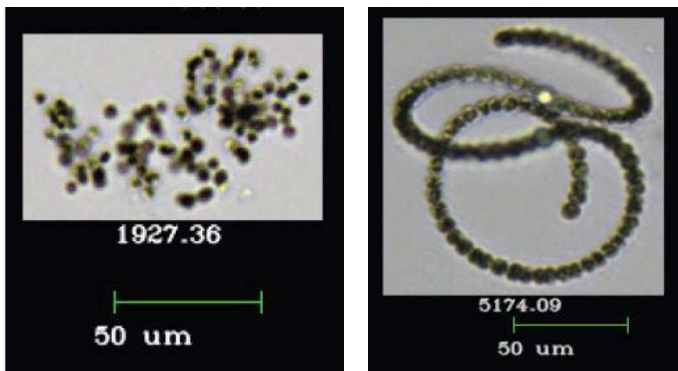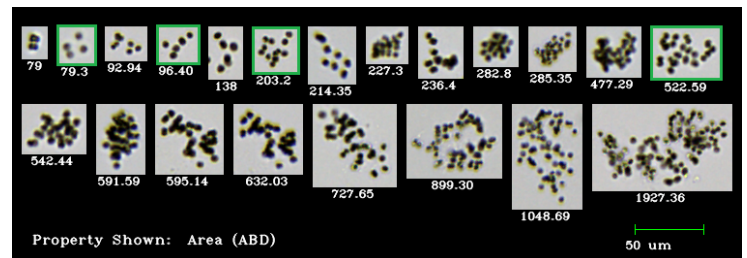
COMPUTATION OF CELL DENSITY: COLONIAL ALGAE

Lehman et al. used the following method to calculate the cell density (cells/mL) of a sample containing colonial *Microcystis*.

First, the average area of a single cell in a *Microcystis* colony was determined. A selection of colonies were chosen from the VisualSpreadsheet list file (Fig. 2). The Area (ABD) of the colony, as calculated by VisualSpreadsheet, was divided by number of cells manually counted in the image (Fig. 2).

Figure 2. VisualSpreadsheet images of *Microcystis* colonies. The Area (ABD) of each colony (μm^2) is noted beneath the image. Colony images highlighted in green were used in the calculations below. A manual count reveals that each highlighted colony has 4, 6, 13, and 28 *Microcystis* cells, from left to right, respectively.

Note that the Area (ABD) is calculated from the number of pixels in the image converted to μm^2 using a calibration factor. The average Area (ABD) for any large and/or dense colony tends to be underestimated as cells tend to overlap in the colony's image.

Below is an example of how to calculate the average area per cell, based on the highlighted colonies in Figure 2.

Total Area (ABD) of the 4 highlighted colonies:
79.3 + 96.4 + 203.2 + 522.59 = 901.49 µm^2

Total number of cells: 4 + 6 + 13 + 28 = 51 cells

Average Area (ABD) of 1 cell: 901.49 µm2 / 51 cells = 17.5 µm^2

After *Microcystis* had been isolated in the list (LST) file, the file was exported to Excel as a Classification Summary report. The average cell density of the sample was calculated by populating the following formula in an Excel cell:

=[(Mean Area (ABD)) * (Particles per mL)] / (average cell Area (ABD))

=(**161.26** * **18,005**)/17.5 = 170793 cells/mL

Table 1. Abbreviated Classification Summary report of a *Microcystis*-containing list file

List Name	Lake XX				Cells/mL	170793
Count	631					
Class	Microcystis					
Particles/ mL	**18,005**					
Summary Stats	Mean	Min	Max	Std Dev	%CV	Total
Area (ABD)	**161.26**	78.89	1927.36	136.41	84.59	101753.8
Diameter (ABD)	13.67	10.02	49.54	4.29	31.34	8627.8
Volume (ABD)	1845.95	527.08	63651.56	3457.49	187.3	1164793

COMPUTATION OF CELL DENSITY: FILAMENTOUS ALGAE

The method used to calculate the cell density of colonial algae can also be applied to calculate the cell density of filamentous cyanobacteria, including *Dolichospermum*.

To determine the Area (ABD) for a single cell, the Area (ABD) of a *Dolichospermum* filament was divided by the number of cells in the image.

Using a selection of images from the VisualSpreadsheet file (Fig. 3), the average Area (ABD) of a *Dolichospermum* cell was determined to be 48 µm^2.

Figure 3. VisualSpreadsheet images of *Dolichospermum*. The Area (ABD) of the whole filamentous algae (µm^2) is noted beneath each image. A selection of these images were used to calculate the average Area (ABD) of a single cell.

After the *Dolichospermum* had been isolated in the VisualSpreadsheet list (LST) file, the data was exported to Excel as a Classification Summary report. The following formula was populated into an Excel cell to calculate the cell density of the filamentous algae sample:

=[(Mean Area (ABD)) * (Particles per mL)] / (average cell Area (ABD))

=(**1,168.29** * **13,814**)/48 = 336224 cells/mL

Table 2. Abbreviated Classification Summary report of a *Dolichospermum* library

Count	293				Cells/mL	336224
Class	Dolicho-spermum					
Particles/ mL	**13,814**					
Summary Stats	Mean	Min	Max	Std Dev	%CV	Total
Area (ABD)	**1168.29**	88.35	5863.68	945.54	80.93	342308.7
Diameter (ABD)	35.47	10.61	86.41	15.16	42.74	10391.4
Volume (ABD)	36931.59	624.75	3.38E+05	44160.23	119.57	1.00E+07

CONCLUSION

Cell abundance is used to track bloom growth. Using the method described above, FlowCam can be used to estimate the cell concentration of colonial or filamentous cyanobacteria.

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