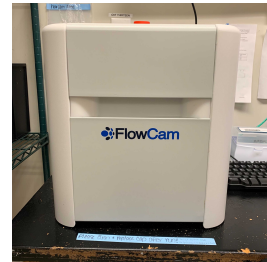


Dec 06, 2022

Use of a FlowCam for descriptive measurements of *Aureococcus anophagefferens* live culture cells

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn2336g5d/v1>



Emily Chase¹, Laura Smith¹, Alex Truchon¹, Steven W Wilhelm¹

¹The University of Tennessee, Knoxville



Emily E. Chase

University of Winnipeg

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egn2336g5d/v1>

Protocol Citation: Emily Chase, Laura Smith, Alex Truchon, Steven W Wilhelm 2022. Use of a FlowCam for descriptive measurements of *Aureococcus anophagefferens* live culture cells. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.14egn2336g5d/v1>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: November 14, 2022

Last Modified: December 06, 2022

Protocol Integer ID: 72727

Keywords: Aureococcus anophagefferens, algae, cell counts, cell size, culture, flowcam, imaging, descriptive measurements of aureococcus anophagefferen, monoculture of aureococcus anophagefferen, aureococcus anophagefferen, culture cell, cell count, study cell, cell volume, descriptive measurement, cell, cell diameter, infection

Abstract

A protocol for acquiring an array of descriptive statistics (e.g., cell count, cell diameter, cell volume) for a monoculture of *Aureococcus anophagefferens*. This procedure permits an in-depth profile of your cultures via a substantial number of viewable photos and measurements. We recommend this familiarity with your study cells, especially when applying stress treatments such as virus infection, light cycle changes, etc.

Materials

Fluid imaging technologies INC. FlowCam system

Visual Spreadsheet software loaded on the computing component of the FlowCam system

1 mL funnel (kept cell specific and regularly cleaned)

1000 mL pipette tips and pipette

Sample culture(s)

Extra media (if dilution is required)

FlowCam function, cleaning, and maintenance supplies (Contrad, designated waste disposal, Milli-Q water, etc.)

Before start

Familiarise yourself with the functions of the FlowCam equipment and Visual Spreadsheet5 software.

FLOWCAM 8000 OPERATION

- 1 An explanation of the operating practices of the Flow CAM 8000 are presented under another protocol and through the relevant software and equipment manuals. Equipment setup can be achieved through the linked protocol below.

Protocol



NAME

Flow CAM 8000 Operation Protocol/Sample

CREATED BY

Brittany N Zepernick

[Preview](#)

SAMPLE AND CONTEXT FILE PREPARATION

- 2 *Aureococcus anophagefferens* cells are retrieved from a culture for measuring. At least 500 μ L are required for each sample.

Note

Samples can be diluted with media to achieve accurate results. Test runs with different density cultures and adjusting the "context" file of the FlowCam can accomplish this. Generally, cultures were run as is if at a "low density" (under ~5 million cells per mL), or diluted 1:4 media (e.g., ASP12A) to culture. It is advisable to prepare several 1.5–2.0 mL Eppendorf tubes with culture for testing purposes, alongside readily available media for diluting. ASP12A media preparation is defined in the protocol linked below.

- 3 Open the Visual Spreadsheet5 software, and load the appropriate context file. Measuring *A. anophagefferens* can be accomplished with the following settings in the context file:
Distance to nearest neighbour: 1.0
Close hole iteration: 2.0
Collage image border padding: 2
Dark pixels: 20.0
Light pixels: 20.0
Acceptable region left: 110; right: 1115; top: 1; bottom: 1919

Note

*Adjustments can be made to the context file for optimisation if results are unsatisfactory. Situations where this may arise include physiological changes of the culture where cells may be "stickier" and adhering to one another more, where cells are notably smaller or bigger, or when the samples are not monocultures (or monostrains). In general, the above context file settings has worked for *A. anophagefferens* cells under a variety of stressors.*

SAMPLE DESCRIPTIVE STATISTICS ACQUISITION

- 4 Following set-up of the FlowCam by manufacturer's instructions (using the 20× objective, 20× flow cells, and 0.5 or 1.0 mL syringe; green set) and the full clearance of Milli-Q water through the tubing, enter the flow cell view mode (*i.e.*, "Camera View"), apply at least 500 µL of your sample, and start the flow at 1–2 mL/minute. Once cells appear within the on screen camera aim at the flow cell, halt the manual priming (close the window).

Note

The 20× objective scheme permits a maximum particle size of 50 µm, an Autolmage rate range of 20 to 84 frames per second, and an efficiency range of 33.0 to 99.7%. The suggested flow rate for measuring (Autolmage) is 0.03 mL/minute.

- 5 Cell image capture (and subsequent descriptive statistics) is processed via the Autolmage mode. Simply click the auto image mode, set the acquisition method constraints, provide a sample name/ID, and initiate the sampling.

Note

For statistically significant results set the number of cells measured accordingly. Most in-lab cultures will be dense enough to achieve at least 5000–10 000 cell photos. It is possible to reach up to 35 000 (or more) photos in highly dense cultures. Environmental samples will likely possess much fewer cells and may require several addition steps, including pre-filtering to accommodate the size limitation of a 20× objective set-up and determining the sample volume required to reach statistically significant results (i.e., enough *A. anophagefferens* images captured). Environmental studies of *A. anophagefferens* are outside the scope of this protocol.

6 Record or export relevant data.

An example of resulting data for *A. anophagefferens* are provided as **Figure 1** and **Figure 2** below.

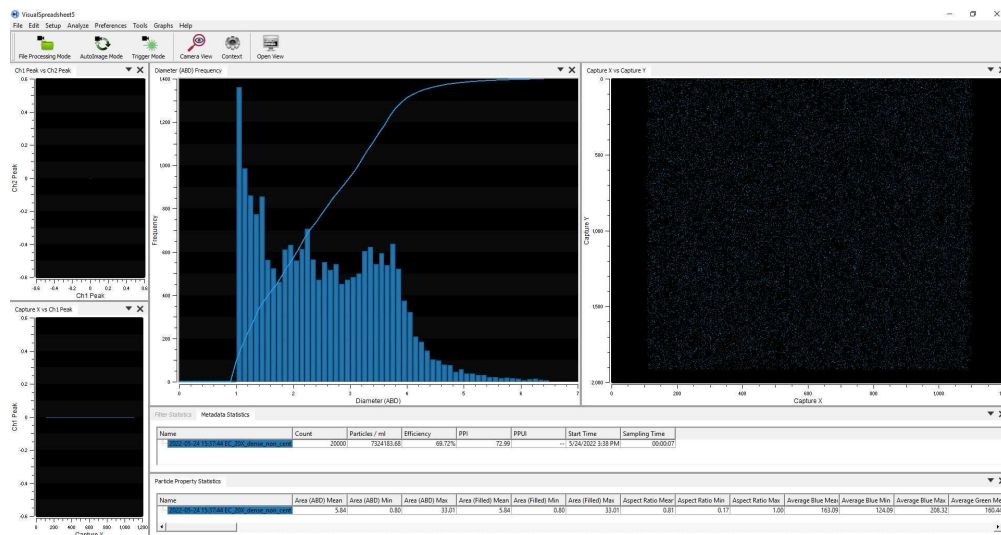


Figure 1. *Aureococcous anophagefferens* as defined through the FlowCam Autolmage mode data collection. A histogram defines counts (frequency) of different cell diameters. The Capture X versus Capture Y image explains what position datum (i.e., cells) were measured within the field of the flowcell. A uniform distribution among the Capture X versus Capture Y signifies that the flowcell is unimpeded; a uneven distrubution or "holes" in the collection space would signify a block in the flowcell and cause uneven sampling (i.e., photos).

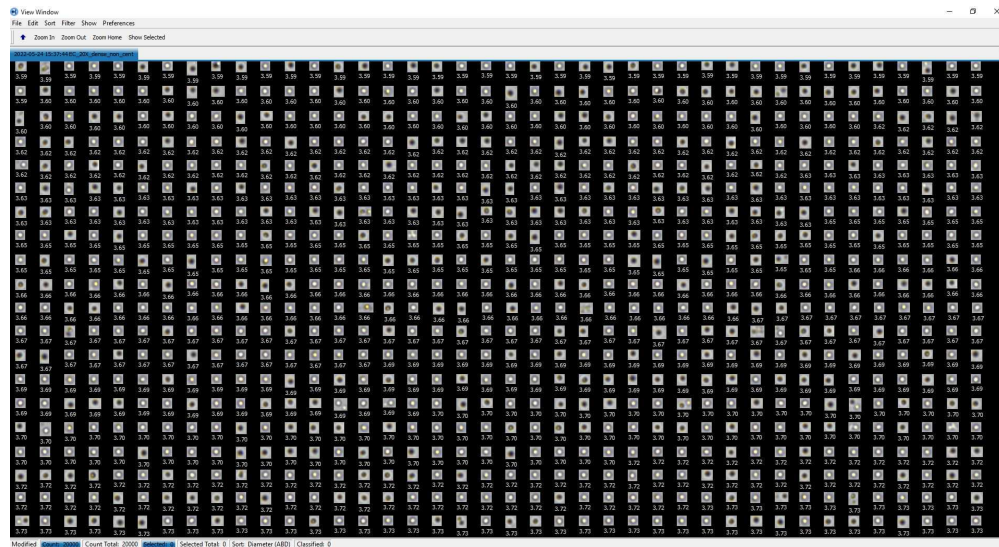


Figure 2. Example of *Aureococccous anophagefferens* photos collected through FlowCam measurements. Below each photo cell diameter is defined.

Note

Interesting measurements include not only particle counts (i.e., cell counts per mL), but also an estimate of cell volume and cell size. Ample other statistics are available. Careful attention must be paid to what the FlowCam has defined the cell shape as. Checks can be done via the "open view" section (i.e., the image viewer) while a results file is opened by clicking Show -> Binary image and particle edge trace, and observing the perimeter of the cell that has been established (signified by a blue line and red filled image). Troubleshooting with the context file may need to occur if a statistically significant number of cells are being improperly measured. Keep in mind that some duplicates and incorrectly measured cells are normal, and could be negligible with consideration to the number of cell images collected. Additionally, images can be selected and deleted from the inclusion of the descriptive statistics for a data clean up.

- 7 Clear the entire FlowCam tubing system of the previous sample, administer a Milli-Q water rinse, and apply a new sample as previously described. Repeat for all samples and then apply FlowCam cleaning as established in your lab.

Note

A low percent Contrad (2–5%) solution will function well for cleaning residual *A. anophagefferens* cells and culture debris from the FlowCam machine. This should be run after each sampling day. Milli-Q water should be applied until soap bubbles are completely cleared from the syringe. Generally, this should also be completed between drastically different samples (e.g. different algae types, or moving from (mono)culture samples to environment samples) to ensure no carry over.

