DEC 01, 2022

WORKS FOR ME 1

Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter)

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

Emily E. Chase[1], Alex Truchon[1], Steven W Wilhelm[1]

[1]The University of Tennessee, Knoxville

Emily E. Chase
The University of Tennessee, Knoxville

ABSTRACT

A method for obtaining relative cell size, population density, etc., of the brown tide algae *Aureococcous anophagefferens* by a Violet Side Scatter configuration on a Beckman Coulter CytoFLEX S Flow Cytometry System (CytExpert software).

DOI

dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

PROTOCOL CITATION

Emily E. Chase, Alex Truchon, Steven W Wilhelm 2022. Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

KEYWORDS

cytoflex, flow cytometry, violet side scatter, cell size, cell counts, Aureococcus anophagefferens

CREATED

Sep 22, 2022

LAST MODIFIED

Dec 01, 2022

PROTOCOL INTEGER ID

70407

GUIDELINES

To achieve the intended results of this protocol a configuration using a violet laser must be setup for a CytoFLEX S Flow Cytometer. This protocol is based on a CytoFLEX with Violet SSC on the Violet laser at the 405/10 position. See the "Setting Up Violet Side Scatter (VSSC) Channel" section of the CytoFLEX manual. While visualization of *A. anophagefferens* can be achieved without a violet laser, it will not be possible to determine relative cell size.

MATERIALS TEXT

Cytoflex S Flow Cytometer
CytExpert Software + desktop computer with appropriate specs
96 well CoStar [REF#3795] Assay Plates
1000mL pipette and tips (or alternatives based on sampling size volumes)

Shealth fluid + cleaning fluid + Mili Q water (or alternative) [Cytometer maintenance only]

BEFORE STARTING

Boot up the CytExpert Software and follow the start up protocol. Familiarity with the machine and its setting will permit necessary adjustments for your experiments and help ensure accurate results.

CytoFLEX Experiment Setup

1 Create a new experiment with **sample acquisition** settings as follows:

- FSC 200
- SSC 40
- VioSSC 75
- FITC 200
- PerCP 150
- PB450 1 *[placeholder; this could be set for a different dye than Pacific Blue shown here, this will not change the results]*
- Threshold: (manual) PerCP 5000

Set the flow rate to medium (30 µL/minute), the sampling to 60 seconds (not by events), and the display to 100,000 events.

Note

Flow rate may need to be adjusted for a high abort percentage (>10%), both adjusting the rate (custom or otherwise) and diluting dense culture samples will solve this problem. Users should also aim for a certain number of events per second, typically between 300–2000.

Settings can be adjusted in real time by selecting "run" on samples and changing the acquisition settings.

Citation: Emily E. Chase, Alex Truchon, Steven W Wilhelm Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter) https://dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

*The full CytoFLEX configuration of which this protocol is based off of is pictured below (**Figure 1**) for full repeatability.*

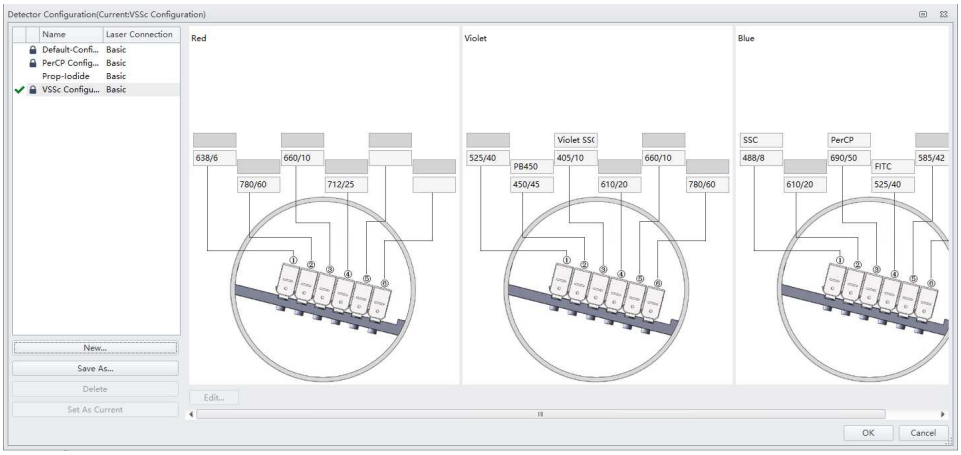

Figure 1. Detector configuration setup for the CytoFLEX Flow Cytometer used to establish this protocol.

2 *Optional* Create a combination of density plots and histograms best suited for your analyses. An example set up is provided in the image below (**Figure 2**), where *A. anophagefferens* populations are clearly achieved. It is recommended to set up a histogram with time on the x-axis (final histogram) to account for the machine's measuring consistency as sampling will often start out slower and then stabilize. This can be applied to any gated population you have selected by clicking the text at the top of the histogram and selecting the population within the dropdown menu.

Citation: Emily E. Chase, Alex Truchon, Steven W Wilhelm Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter) https://dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

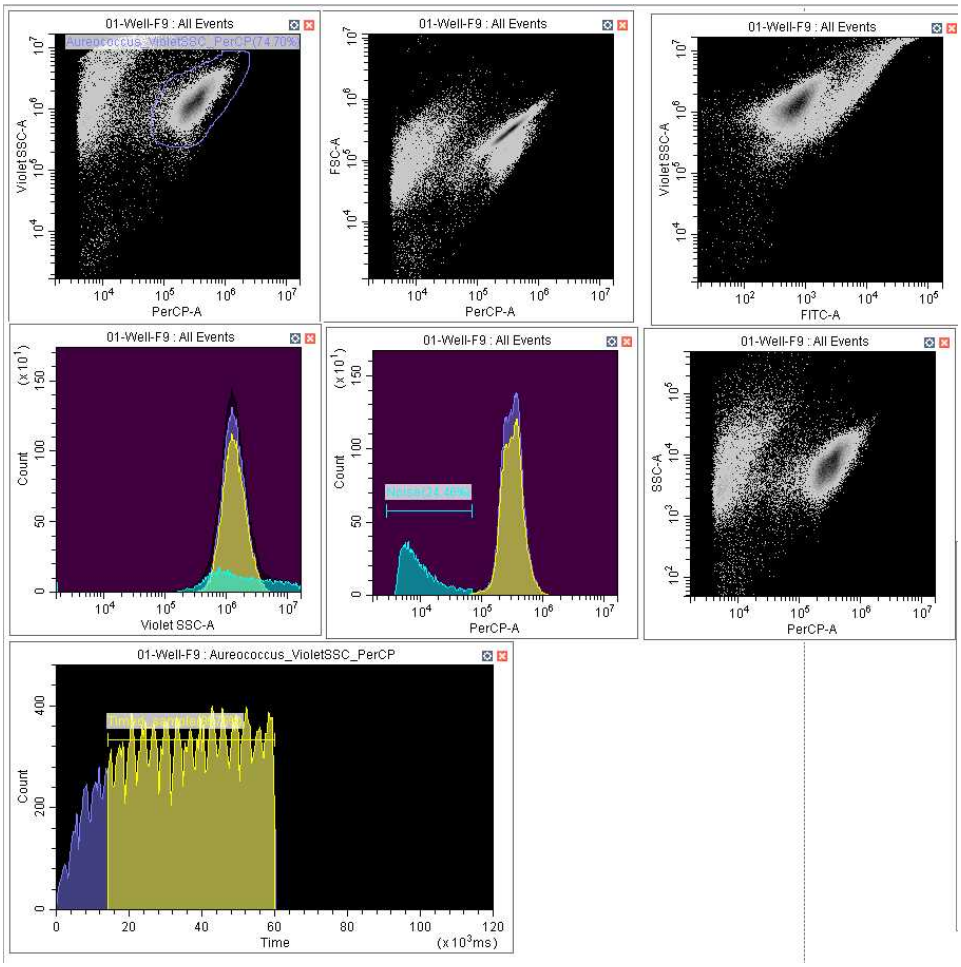

Figure 2. CytoFLEX S Flow Cytometer discovery of *Aureococcus anophagefferens* cell populations. Lassoed (*i.e.,* gated) populations represent *A. anophagefferens* classified events.

Sampling Process

3 250 µL of each sample to be measured (*e.g., A. anophagefferens* culture) are pipetted into a 96 well CoStar [REF#3795] Assay Plate round bottom plate (can be substituted) and loaded into the CytoFLEX. After opening the "Plate" window and clicking "Add Plate", samples can then be labelled.

Note

Volumes can be reduced after taking into account the flow rate and sample timing.

4 Labelled samples can then be run sequentially using the "Auto Record". Upon completion data can be either exported (most conveniently as a .csv) or reviewed using the "Statistics" window according to all events and

Citation: Emily E. Chase, Alex Truchon, Steven W Wilhelm Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter) https://dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1

events within user defined gated populations. Population counts are achieved by number of events within the *A. anophagefferens* gates, and subsequent relative size of events (cells) can be achieved through Violet Side Scatter channel results.

Note

Relative size by Violet SSC cannot be achieved for every cell type (normally "larger" microalgae cannot), a priori comparisons with other cell size calculation methods (e.g., FlowCam) must be conducted.

Citation: Emily E. Chase, Alex Truchon, Steven W Wilhelm Aureococcus anophagefferens population count, and relative size (Violet SSC) by flow cytometry (CytoFLEX S Flow Cytometer Beckman Coulter) https://dx.doi.org/10.17504/protocols.io.q26g7yby9gwz/v1