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Preserving Phytoplankton Samples with Glutaraldehyde for FlowCam Analysis

SUMMARY

Aquatic scientists often need to store natural samples for a period of time before processing them using FlowCam. Glutaraldehyde is a popular choice of preservative in flow cytometry[1] and flow imaging microscopy because, although more toxic than Lugol's, it is able to preserve pigment autofluorescence. This is important because it allows users to delay sample processing and still be able to take advantage of Trigger mode on FlowCam Cyano and other FlowCam 8400 configurations, allowing the differentiation of cyanobacteria from other algae, and reducing images of detritus and other nonfluorescing particles. Here we provide instructions on how to use glutaraldehyde as a preservative with refrigeration storage (approx. 4 °C) until a technician has the time to run the samples on FlowCam. For those who may not be able to preserve samples in glutaraldehyde, we also tested refrigeration alone.

This technical note covers the following topics:

- How to prepare glutaraldehyde-preserved aquatic samples
- Results from experiments where samples were stored for 30 days in a refrigerator
- Additional considerations when using glutaraldehyde as a preservative to store aquatic samples and how to run them on FlowCam

Cultures tested:

- *Anabaena, Gloeocapsa* (Carolina Biological, catalog #151710, #151800)

SAMPLE PREPARATION

This technical note contains data from monitoring the total Trigger mode concentration, chlorophyll, and phycocyanin fluorescence of glutaraldehyde-preserved samples over time. (Autoimage mode can also be used). Samples preserved with glutaraldehyde were tested against samples where no glutaraldehyde was used.

1. Samples were preserved using EM Grade 25% glutaraldehyde (EMS Catalog #16200[2]). The final concentration of glutaraldehyde used was 0.25%[3].
2. Phytoplankton cultures were initially diluted to obtain a PPUI less than 1.2 and run on FlowCam Cyano using Trigger mode.
4. Samples were stored in 50mL centrifuge tubes (VWR Catalog #89079-494) and refrigerated at approximately 4 °C.
5. Triplicate samples were run using Trigger mode to measure the concentration, chlorophyll and phycocyanin fluorescence over a period of 30 days.

FLOWCAM PROCEDURE

The FlowCam instrument used in these experiments was equipped with a 633 nm red laser (FlowCam Cyano) and two PMT detectors, one tuned for chlorophyll (Channel 1, 700 nm, BP10) and the other tuned for phycocyanin (Channel 2, 650 nm, BP10). The software for data acquisition and data analysis was VisualSpreadsheet. A 10X objective, FOV 100 flow cell, and 1 mL syringe were used.

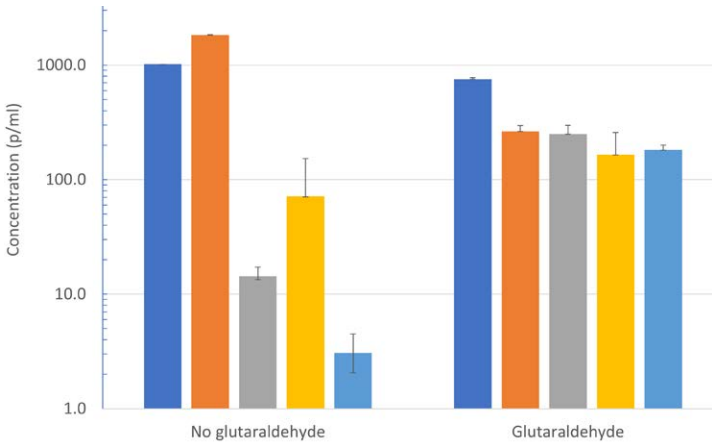
When operating FlowCam in Trigger mode, cells containing sufficient levels of chlorophyll or phycocyanin will "trigger" the camera to capture an image and the corresponding relative fluorescence is measured.

ANALYSIS

Graph 1 compares the total particle concentration of *Anabaena* preserved with glutaraldehyde. The Trigger mode concentration is displayed using the logarithmic scale. The error bars displayed are the standard deviation for triplicate runs. In *Anabaena*, the Trigger mode concentration slightly decreased, then remained stable throughout the 30-day period.

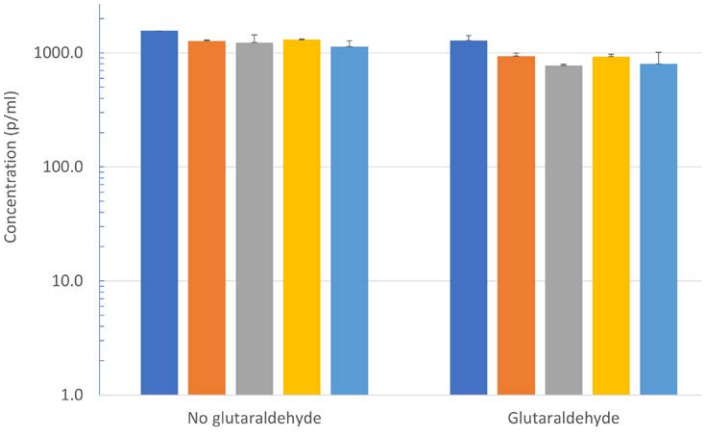
When no glutaraldehyde was used, there was a noticeable drop in concentration over the 30-day period, with the exception of a slight increase from day four to day eight, likely due to some of the *Anabaena* cells breaking apart. In contrast, the glutaraldehyde-preserved *Anabeana* samples maintained a consistent concentration throughout the 30-day period.

Graph 1 - *Anabaena*, data acquired using Trigger Mode



Graph 2 compares the particle concentration for glutaraldehyde-preserved *Gloeocapsa* over a 30-day period. The Trigger mode concentration is displayed with a logarithmic scale and the error bars represent the standard deviation of triplicate runs. *Gloeocapsa* did not show a noticeable difference in concentration over time whether glutaraldehyde was used or not.

Graph - *Gloeocapsa*, data acquired using Trigger Mode



IMPORTANT CONSIDERATIONS

- Using Trigger mode on FlowCam Cyano or FlowCam 8400 is the best option for running glutaraldehyde-preserved samples, but Autoimage mode may also be used.
- Flow cytometric analyses of oceanic phytoplankton with glutaraldehyde preservation is commonly used with liquid Nitrogen followed by storage at -70 °C[1]. It is likely these types of samples will work well on the FlowCam 8400 and Cyano.
- Glutaraldehyde is considered hazardous waste, so waste should be separated and disposed of according to local regulations.
- Data presented here suggests that if glutaraldehyde cannot be used, phytoplankton samples can be refrigerated for approximately one week without experiencing any noticeable decreases in Trigger mode concentration, chlorophyll or phycocyanin fluorescence.
- Glutaraldehyde has been used to study diatoms with fluorescence microscopes[4]. The approach described in this technical note may transfer well using diatoms and other algae.

CONCLUSIONS

The main takeaway of this technical note is that freshwater samples preserved in glutaraldehyde in a standard refrigerator show some decline in fluorescence peaks after Day 1, but remain relatively consistent after that for 30 days or more. If you have samples to send to our Laboratory for analysis and you cannot send fresh samples, you may consider using this method for analysis to take place one month after shipping.

REFERENCES

1. Vaulot D, Courties C, and Partensky F (1989) A Simple Method to Preserve Oceanic Phytoplankton for Flow Cytometric Analysis. Cytometry 10: 629-635. doi: 10.1002/cyto.990100519
2. EMS Catalog #16200 can be purchased here: https://www.emsdiasum.com/microscopy/products/chemicals/glutaraldehyde.aspx
3. Sample Preservation & Shipment Instructions | PhycoTech, Inc. https://www.phycotech.com/Resources/Sample-Preservation-Shipment. Accessed 10 Aug 2021
4. Jayakumar S, Bhuyar P, Pugazhendhi A, Rahim MHAb, Maniam GP, Govindan N (2021) Effects of light intensity and nutrients on the lipid content of marine microalga (diatom) Amphiprora sp. for promising biodiesel production. Sci Total Environ 768:145471. doi: 10.1016/j.scitotenv.2021.14571

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