Application Note

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Advanced Analysis of Aquatic Plankton using Flow Cytometry

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Introduction

Flow cytometry has routinely been used to study phytoplankton by taking advantage of innate fluorescence molecules that distinguish different species and their physiological conditions. Advances in cytometer design and functionality have modernized certain aspects of marine biology applications, creating a more accurate, data-rich, and timely assessment of microscopic marine organisms. This application note examines the distribution and abundance of the phytoplankton, bacterial, and viral fractions of seawater determined using the two elements of the Agilent NovoCyte flow cytometer; volumetric sample delivery using precise syringe injection and extended fluorescence profiling with three excitation lasers.

Methods and materials

Sample collection

Samples were analyzed from a range of environments, including near shore marine samples (Scripps Institution of Oceanography Pier, La Jolla, CA), and University of California San Diego micro-algae raceway ponds. Field samples were collected in sterile tubes and fixed with 0.02 µm filtered glutaraldehyde (0.5% final concentration, incubated at 4 °C for 20 minutes), flash frozen in liquid nitrogen, and stored at –80 °C. Samples were thawed in small batches in a 20 °C water bath. Unstained samples were analyzed immediately for photosynthetic plankton groups. Heterotrophic bacterial and viral populations are stained using the nucleic acid stain SYBR Green I (1:10,000 final concentration, incubated at room temperature for 15 minutes).

Sample analysis

Photosynthetic pico (<2 µm) and nano (2 to 20 µm) plankton groups (eukaryotic and prokaryotic) were assessed using natural pigment fluorescence and scatter. Signal detection is triggered on chlorophyll fluorescence, using 0.2 µm filtered seawater to set the threshold. Heterotrophic bacterial and viral populations were distinguished using the nucleic acid stain SYBR Green I. Signal detection is triggered on nucleic acid fluorescence, again using 0.2 µm filtered seawater to set the threshold.

Pigment	Channel	Excitation	Emission
Chlorophyll	BL4	488	675/30
Phycoerythrin	BL2	488	585/40
Phycocyanin	RL1	640	675/30
Divinyl chlorophyll A	VL5	405	675/30
SYBR Green	BL1	488	530/30
Light scatter	FSC/SSC	488	488/10

Eukaryotes (Euk01-05 and PE+Euk)
High chlorophyll, large cells, some classes phycoerytherin-positive
Cryptophytes (Crypto)
High chlorophyll, large cells, allophycocyanin-positive
Pico Eukaryotes (PicoEuk)
Low chlorophyll, smaller cells, no phycoerytherin
Synechococcus PE+ (Syn-PE+)
Low chlorophyll, small cells, phycoerytherin-positive
Synechococcus APC+ (Syn-PC+)
Low chlorophyll, small cells, allophycocyanin-positive
Prochlorococcus (Pro)
Low chlorophyll, small cells, positive for divinyl chlorophyll A

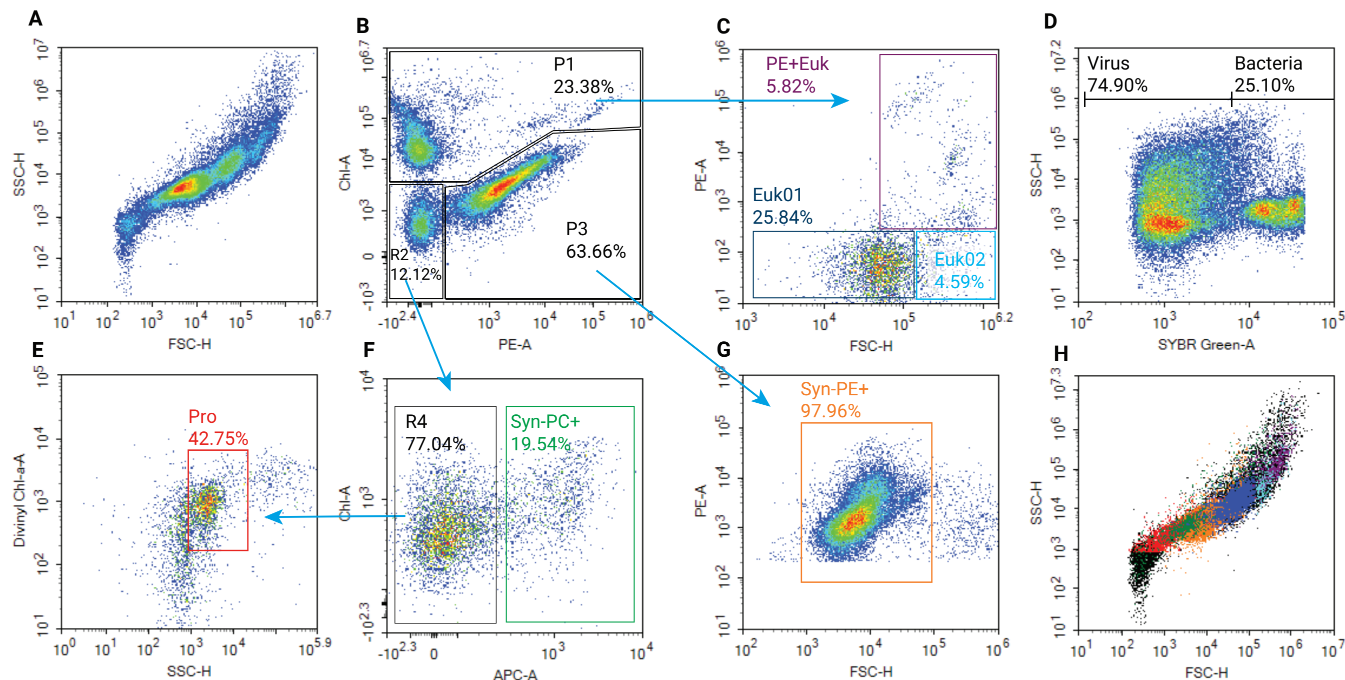

Figure 1. Flow cytometry analysis of plankton from the Scripps Institute of Oceanography pier. (A-G) Density plots with gating strategies. Six photosynthetic groups were identified, three eukaryotic (C) and three cyanobacteria (E,F,G). Synechococcus PE+ dominate group (B,G). (D) Bacteria were distinguished from viral populations using SYBR Green. (H) Dot plot of forward and side scatter, identifying phytoplankton groups by color.

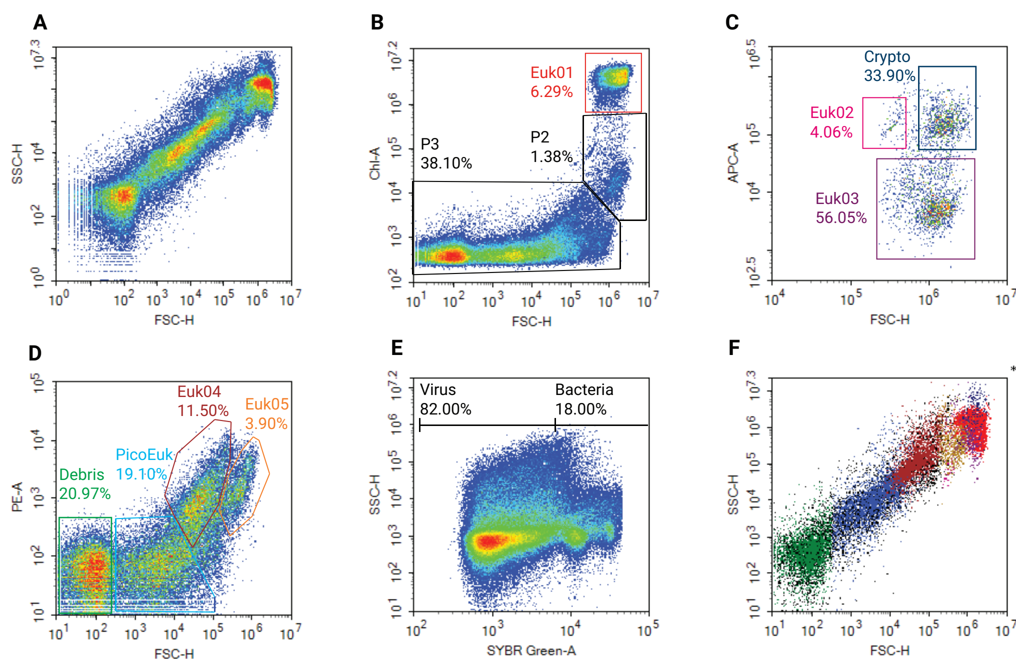

Figure 2. Flow cytometry analysis of plankton from the UCSD micro-algae raceway ponds. (A-E) Density plots with gating strategies. Seven photosynthetic groups were identified (B,C,D), all eukaryotes. Target species Scenedesmus dimorphus (Euk01) identified using FSC and Chl-A (B). (E) Bacteria were distinguished from viral populations using SYBR Green. (F) Dot plot of forward and side scatter, identifying phytoplankton groups by color.

Table 1. Absolute counting using the Agilent NovoCyte flow cytometer. Each of the groups identified in these water samples from SIO pier or the raceway ponds at UCSD and analyzed at absolute counts were obtained using the highly accurate syringe pump-driven fluidics on the NovoCyte flow cytometer. Dominant populations from each water sample are easily identified.

Sio Pier, La Jolla, CA		
Color	Gate	Cells/mL
•	Euk01	2.78E+04
•	Euk02	5.29E+03
•	PE+Euk	5.41E+03
•	Syn-PE+	2.85E+05
•	Syn-PC+	1.08E+04
•	Pro	1.82E+04
	Bacteria	1.29E+06
	Virus	3.85E+06

UCSD Micro-Algae Raceway Ponds		
Color	Gate	Cells/mL
•	Euk01	5.39E+05
•	Euk02	4.78E+03
•	Euk03	6.61E+04
•	Euk04	3.76E+05
•	Euk05	1.27E+05
•	Crypto	4.00E+04
•	PicoEuk	6.24E+05
	Bacteria	3.26E+06
	Virus	1.48E+07

Results and discussion

The use of the multilaser format (405, 488, and 640 nm excitations) allows for reliable discrimination of phototrophic eukaryotes, *Synechococcus* spp., *Prochlorococcus* spp., and heterotrophic populations in aquatic samples. Precision syringe-driven fluidics enable direct enumeration of populations, allowing reliable comparisons when assessing population dynamics. The integrated NovoSampler permits timely processing of samples in various formats that can be configured by the user. Fixed voltages facilitate setup time and experimental repeatability, and require less user training. The system's innovative engineering allows for a compact design with a small benchtop footprint and low power demand, making it ideal for shipboard analysis of marine samples. This also eliminates the need for research samples to be stored and analyzed later.

References

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