MoFlo Astrios^{EQ}™ Forward Scatter: Cell Sorting of Nano and Large Phytoplankton Simultaneously with High Purity

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B158

Abstract

Background: Phytoplankton conversion of light on the upper limits of the ocean consists of half of the photosynthesis on the Earth. The population densities indicate the health of not only the phytoplankton, but the entire aquatic ecosystem. The isolation and sorting of aquatic samples using flow cytometry allows for quick and effective population analysis. The forward scatter on the MoFlo Astrios allows for differentiation of small and large particles from 0.2 to 30 µm on FSC. This design provides researchers greater flexibility to isolate and sort specific phytoplankton of different sizes while utilizing the 7 laser, 42 parameter MoFlo Astrios.

Methods: Plankton species; *Chlorella, Phormidium inundatum, Phormidium persicinum, Cryptomonas, Rhodosorus, Synechococcus, Skeletonema, Fremyella*, were acquired from the UTEX: The Culture of Algae and grown in photobioreactors and cultured in specialized salt and fresh water media. Plankton species *Prochlorococcus marinus* and *Emiliana huxlei* were grown in sterile 2 L containers supplied with 0.2 um filtered air in salt water with fertilizer. Instant Ocean, ½cup per gallon of deionized water, was added to the culture with Microalgae Grow Mass Pack with Silicate. Cells were harvested by gentle centrifugation, roughly 300 x g for 5-10 minutes. The supernatant was decanted/aspirated and the pellet resuspended in a sterile saline solution to achieve $1x10^6$ cells/mL. Plankton were then stained with SYTOX Green (Invitrogen, S7020), at a maximum concentration of 5 µM for 20 minutes after vortexing. Samples were filtered with a 70 µM Partec filter and kept on ice before flow cytometric analysis.

Flow Cytometry: The MoFlo Astrios^{EQ} was configured with 7 lasers and setup with a 100 µm tip to accommodate the larger phytoplankton (*Cryptomonas*). Cells were selected on their "live" status by being highly fluorescent in the red channels (chlorophyll) and low in the green channels (Sytox -). For small particle analysis, *Prochlorococcus* and *Synechococcus* were simultaneously analysed on FSC-Log parameters. Populations were sorted based on fluorescence and size as a 6-way sort into 5 mL tubes. The plankton were sorted at 25K eps to collect at least 100,000 events per each population using sort mode Purify 1-2.

Results: Plankton populations were distinguishable using fluorescence and scatter patterns on both log and linear scales simultaneously. With the Astrios optical flexibility, the plankton fluorescence spectra were optimized for signal to noise. Isolation of the *Prochlorococcus, Synechococcus* and other plankton species using cell sorting achieved 99% purity for all populations.

Conclusions: The forward scatter and optical collection design of the MoFlo Astrios^{EQ} provide flexibility to detect large and small populations and sort them with high purity.

Introduction

Marine biology, specifically photosynthetic plankton, is another sorting application for the new MoFlo™ Astrios^{EQ} forward scatter design. Flow cytometry has previously been used with phytoplankton for rapid and objective evaluation of water quality (Trask), to analyze grazing (Cucci), cell viability (Dorsey), rapid strain identification (Simon), diversity assessment (Marie D), and to characterize plankton populations through molecular (Shi, Marie D) and genetic approaches (Shi).

Photosynthetic Pigments Found in Phytoplankton

Using the flow cytometer to analyze plankton is useful in the ability to identify plankton populations as well as sort the populations for further population identification. The high speed sort capabilities and small particle detection of the MoFlo AstriosEQ expand the researchers capabilities to identify and isolate phytoplankton populations.

Plant Pigments Absorption Spectra

In a general photosystem, as seen in the figure to the right, the chlorophyll (b) transfers energy through the antenna pigment complex to chlorophyll (a) reaction center. The accessory pigments, such as Phycocyanin, Allophycocyanin, Allophycocyanin B, and Phycoerythrin, provide a composite absorption spectrum such that a wider range of visible and infrared radiation is absorbed by plants and algae.

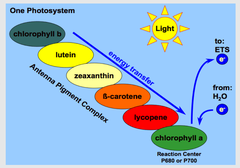


Image taken from: Koning, Ross E. 1994. Light. Plant Physiology Information Website. http://plantphys.info/plant_physiology/light.shtml. (5-9-2013).

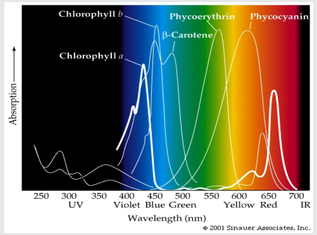


Image of the plant absorption spectra including chlorophyll a and b, phycoerythrin, phycocyanin and beta-carotene.

Quantity of Pigments in Phyoplankton

The quantity and concentration of the plant pigments found in the phytoplankton vary depending on the organism size and oceanic environment (including the habitat depth from ocean surface). Plankton populations may be distinguished by their fluorescence spectra using flow cytometry using multiple laser lines and emission filters.

Methods

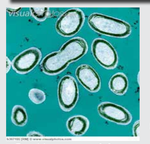
Culture Preparation: Plankton were purchased from University of Texas at Austin, UTEX, "colors of algae" and NCMC (*Prochlorococcus marinus* and *Emiliana huxlei*.) Viability was tested using the Beckman Coulter Vicell for the plankton and then less than 5x10^6 cells were stained with Sytox Green (Invitrogen, S7020) 2 ul, per 1 mL of culture, incubated for 20 min in the dark. Three mL were stained for each culture as single controls. The FSC 1 and FSC 2 detectors were designated for large and small plankton with an ND absorbing filter in front of the FSC 2 detector. A mixture of YG beads size 0.2 - 6 um (Polysciences, Fluorobrite kit 1 and 2, 21636-I and 21637-I) were tested prior to plankton to measure the instrument noise/detection threshold.

FSC 1 ND 1 absorbing filter and FSC 2 had no ND filter. The FSC was aligned to the 488 nm laser with the trigger on 640 SSC to visualize the FSC parameters clearly.

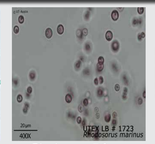
Sorting out the plankton populations required a 100 um tip at 10 K EPS sort speed. Samples were sorted into Enriched Seawater media and reanalyzed after cleaning the fluidics with bleach and DI.

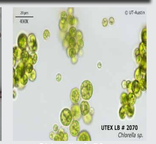
MOFLO ASTRIOS^{EQ}™
FLOW CYTOMETER SYSTEM

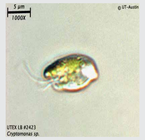
Plankton


Prochlorococcus 0.8 um


Synechococcus 0.8-1.6 um


Rhodosorus 7.6 um


Chlorella 8-50 um


Cryptomonas 10-50 um


Skeletonema 10 um

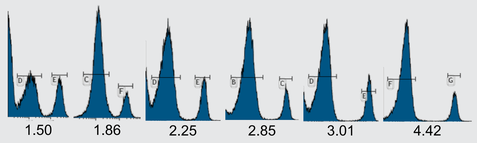

Fremyella 7-10 um


Phormidium

Fisher Distance

- Quantifies the separation between a stained and unstained population in terms of the population widths
- Requirements: Median of auto-fluorescent population needs to be above the baseline

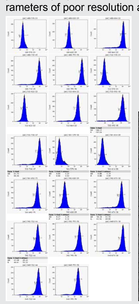
$$\text{Fisher Distance} = \frac{|Mean_1 - Mean_2|}{SD_1 + SD_2}$$



CD19 on Peripheral Blood Mononuclear Cells from an Astrios system on different parameters. The resolution index focuses on spread as well as separation.

Fluorescence

The Fisher Distance was used to determine the optimum excitation laser and emission bandpass using Kaluza 1.2 Software. The Kaluza software was used to find the initial optimum median channel and then used to calculate the Fisher Distance in the analysis package for quick data analysis. Below is an example of the fluorescence of the Cryptomonas plankton (left) and the resulting Fisher Distance of all of the plankton analyzed in Kaluza (right). The red indicates parameters of poor resolution and blue indicates parameters of high separation.

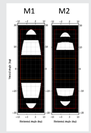


Fluorescence Parameter	Chlorella	Cryptomonas	E. huxley	Fremyella	Phormidium fragile	Phormidium inundatum	Phormidium persicinum	Prochlorococcus	Rhodosorus	Skeletonema	Synechococcus
488-576/21			0.89	0.01	1.18	0.01	0.01	0.01	2.64		
488-620/29		0.01	1.96	-0.01	1.18	0.02			2.89		0.09
488-664/22	2.53	2.07	3.23	1.32	1.83			0.08	3.11	2.97	2.01
488-710/45	2.12	1.80	3.18	0.68	1.69	0.40	1.01	0.09	2.66	2.64	1.69
488-795/70	1.84	1.77	3.07	0.97	1.71		0.13		2.60	2.42	
532-576/21				0.01	1.73	0.02	-0.05		3.24		1.79
532-622/22			1.56	0.01	1.63	0.01	0.03		2.96		
532-664/22	2.90	1.98	3.03	1.04	2.02	0.51	0.69	1.56	3.07	3.14	2.99
532-692/18	2.29	1.41	3.11	1.07	1.95	1.00	1.06	1.34	2.99	2.72	1.50
532-736/47	1.87	1.51	2.88	1.00	1.94	0.56	0.89	1.37	2.71	2.55	
561-579/16					1.75		0.01	0.03	2.98		1.82
561-614/20		0.09	0.05	0.58	1.66	0.13	0.39	1.23	2.83		3.15
561-692/75	3.25	1.53	2.90	1.07	1.98	0.54	0.64	1.92	3.10	2.89	2.36
592-620/29		0.22	2.02	3.35	1.63	1.72	3.18	1.71	2.74		0.01
592-671/30	4.42	1.72	2.95	1.17	1.94	0.54	0.74	2.36	3.14	2.99	
592-722/44	4.42	1.59	2.81	1.20	2.06	1.11	1.18	2.20	2.84	2.63	0.15
592-795/70	2.58	1.88	3.13	1.46	2.01	0.45	0.72	2.26	3.17	2.96	0.26
640-671/30	5.21	1.88	3.03	0.99	1.89	0.25	0.59	2.26	3.51	3.10	2.61
640-722/44	4.42	1.73	2.86	1.10	1.84	0.47	0.95	1.99	3.13	2.77	0.15
640-795/70	2.76	1.95	2.98	1.18	1.81	0.93	1.30	2.17	2.97	2.59	0.26

Optimizing FSC

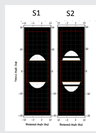
Masks

M - Material



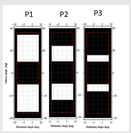
- Better relation to particle size over large ranges of particles
- Designed to pull apart particles of different material types

S - Separation

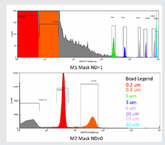


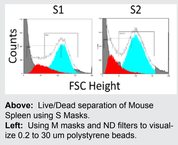
- Reduce the sensitivity to different particle types
- Better for pulling out small differences in similar particles

P - All-Purpose

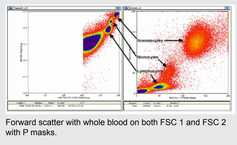


- Overall general performance
- Best when matched against "M" or "S" masks to pull out details





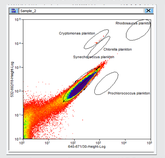
Above: Live/Dead separation of Mouse Spleen using S Masks.
Left: Using M masks and ND filters to visualize 0.2 to 30 um polystyrene beads.



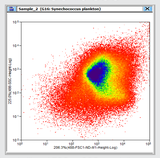
Forward scatter with whole blood on both FSC 1 and FSC 2 with P masks.

Dual FSC Used to find Unique Populations

Fluorescence



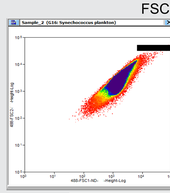
FSC vs SSC



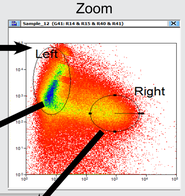
Synechococcus fluorescence (APC vs Chlorophyll) and FSC vs SSC populations.

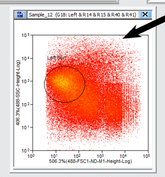
The Astrios^{EQ} forward scatter provides two scatter parameters to analyze populations. With the conventional FSC vs SSC populations, the *Synechococcus* population appears as a "blob" with the FSC 1 with a material (M) mask. With the dual FSC parameters, the *Synechococcus* population demonstrates two specific and unique populations only visible on FSC vs FSC on a zoomed population. Those two populations have distinct SSC signatures that are not visible on the fluorescence population seen on the left.

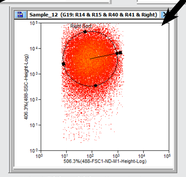
FSC 1 vs FSC 2



Zoom







The FSC vs FSC parameter, when zoomed, helped distinguish unique populations on fluorescence vs SSC. These populations would not be visible with one FSC detector.

The Left and Right populations from the FSC 1 and FSC 2 histogram have distinct FSC vs SSC patterns that are distinguishable and were sorted on the right.

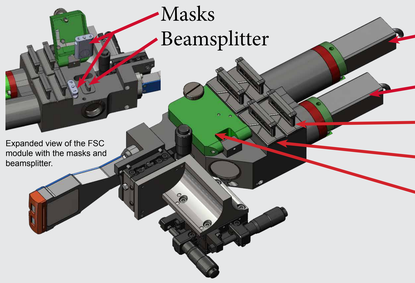
FSC Enhanced Particle Detection System

Astrios Filters for Small Particle Detection

0.04 um In-line Sheath Filter

0.1 um 3-Stage Air Filter

Two key components to the small particle detection are the air and sheath filters which eliminate the small particle debris. It is important to be aware of the sample media, tubes and other plastic components that may cause submicron background and blur the small particle resolution.



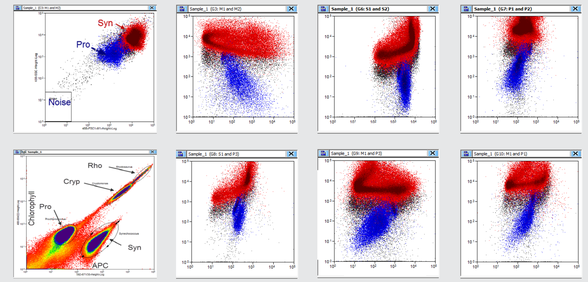
Expanded view of the FSC module with the masks and beamsplitter.

The new Astrios^{EQ} has a new FSC small particle detection system that allows users to customize the mask and laser FSC onto two FSC PMTs. The dual FSC parameter analysis gives the unique opportunity to view populations and isolate populations based on those scatter patterns as seen on the right with the *Synechococcus* sort.

The Astrios^{EQ} forward scatter particle detection system is comprised of 2 forward scatter PMTs, laser and ND filters, a mask and beam splitter. FSC can be detected off of the 405 through 640 nm lasers, split into two equal signals and detected through one of seven masks.

Results

Mask Selection of Pro and Syn

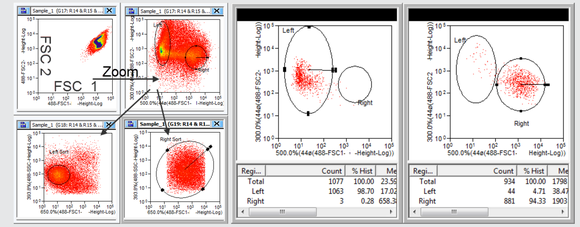


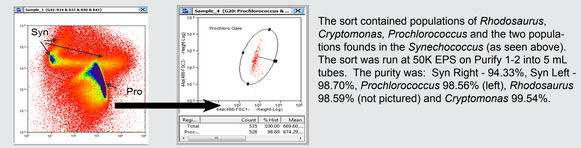
The *Synechococcus* and *Prochlorococcus* cyanobacteria have distinct fluorescence characteristics and they were used to color gate to find unique populations of each plankton. Note the "Noise" gate was very far from the submicron plankton populations.

5-way High Speed Sort

Syn Left and Right Pre-Sort

Syn Post-Sort





The sort contained populations of *Rhodosaurus*, *Cryptomonas, Prochlorococcus* and the two populations founds in the *Synechococcus* (as seen above). The sort was run at 50K EPS on Purify 1-2 into 5 mL tubes. The purity was: Syn Right - 94.33%, Syn Left - 98.70%, *Prochlorococcus* 98.56% (left), *Rhodosaurus* 98.59% (not pictured) and *Cryptomonas* 99.54%.

Conclusions

- The MoFlo Astrios^{EQ} new FSC module provided the ability to sort unique FSC populations for the *Synechococcus* population with high purity and high speed.
- The FSC module was able to distinguish from noise two submicron populations (*Prochlorococcus* and *Synechococcus*).
- The plankton populations were sorted with high purity and speed, indicating the robust nature of the MoFlo Astrios^{EQ} sorting system.

References

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