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Description of the instrument

Samples were analyzed on a Becton Dickinson FACScan using a 488 nm (40 mW) blue excitation laser. Emission filters used were relative green fluorescence (515 ± 30 nm Band Pass filter), right angle light scatter (side scatter – SSC), Forward scatter (FSC), relative orange fluorescence (575 ± 25 nm Band Pass filter), and relative red fluorescence (670 Long Pass filter). All detectors were PMTs (photomultiplier tubes), except FSC which was a photodiode.

Instrument calibration and maintenance

Mean cell sizes of heterotrophic and phototrophic nanoplankton were determined from flow cytometric forward light scatter (FSC). The relationship between FSC and size was determined using a set of standard microbeads (1, 2, 2.5, 4, 6, 10, 15 and 20 µm). These microbeads were run at the beginning and end of the cruise and at sea when any adjustment was made to the flow cytometer. These results were used to create calibration curves relating forward scatter to "bead diameter" (µm) - see Figure 1. After the cruise, in the laboratory a set of 14 cultures ranging in size from 2 to 30 µm (see Table 1) were analyzed along with the microbeads on both a Coulter Counter (Beckman Coulter Multisizer) and on the BD FACScan to determine mean forward light scatter. Cultures of cells too small for the Coulter Counter (*Synechococcus* and *Micromonas*) were measured by image analysis using a Zeiss epifluorescence microscope and a Diagnostic Research Instruments color camera. Bead and cell size vs. forward light scatter calibration curves were determined (Figure 2), and these results were used to establish a standard curve relating "bead diameter" to "cell diameter" (µm) using forward light scatter - see Figure 3.

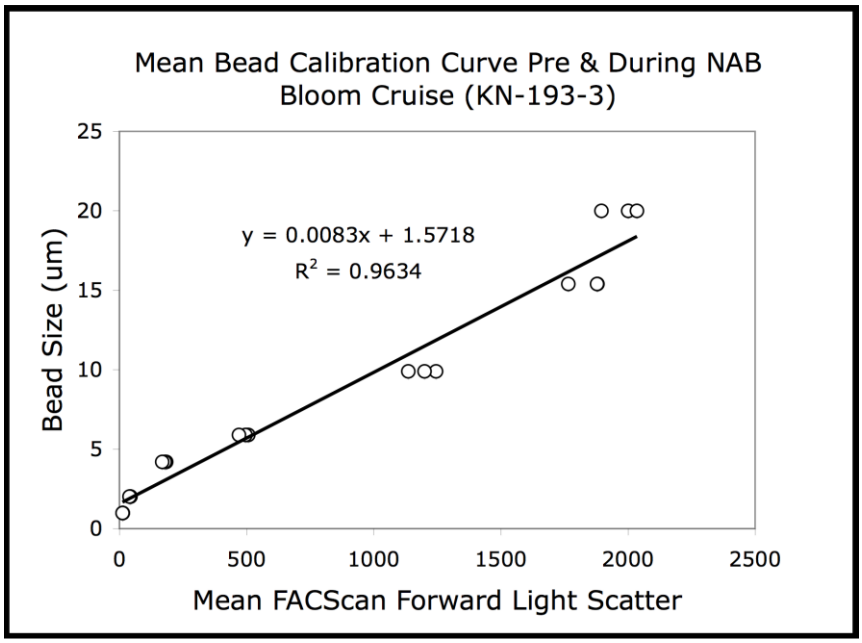

Figure 1. Relationship between Bead Size and Mean Forward Light Scatter (FSC) just prior and during the KN-193-3 2008 North Atlantic Bloom Research Cruise.

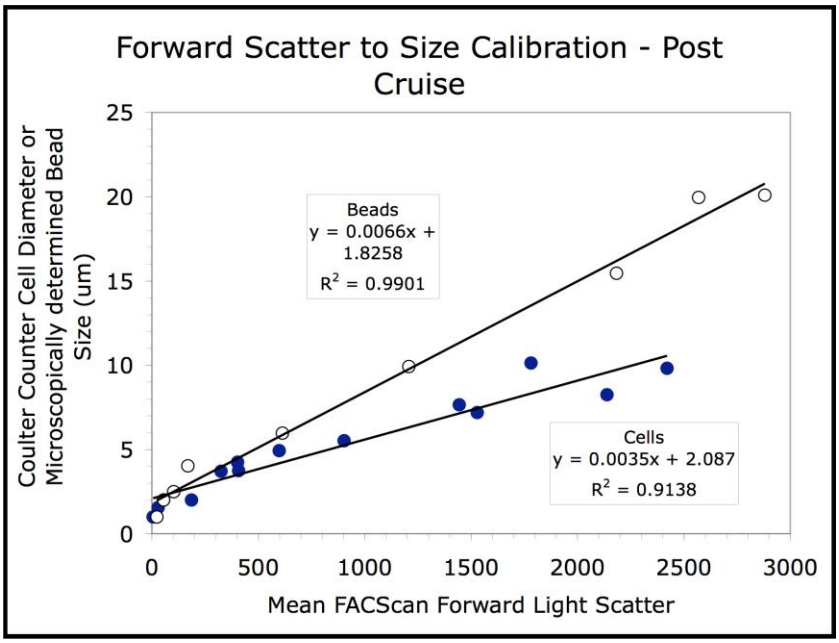

Figure 2. Bead and culture cell calibration curves relating bead or cell diameter to mean forward light scatter (FSC) – determined in the laboratory (after cruise).

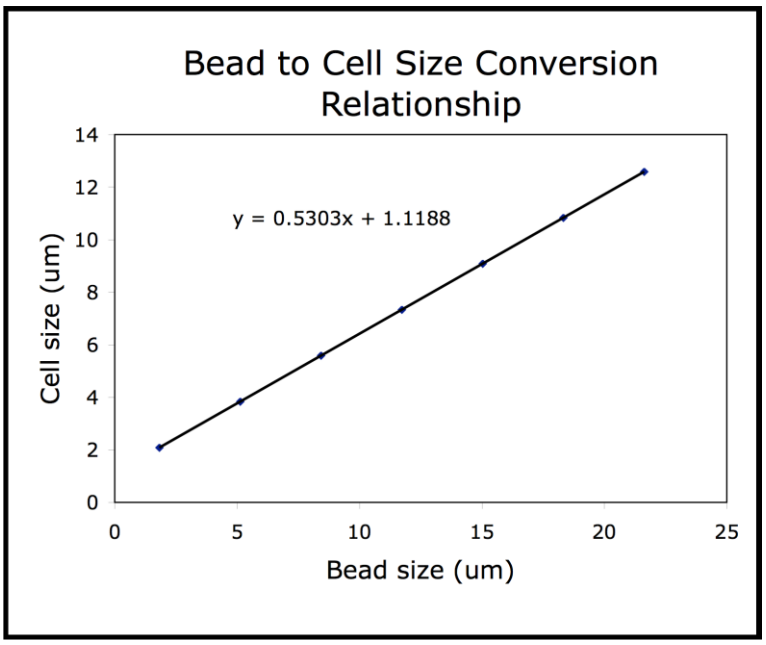

Figure 3. Standard Curve relating Bead Size to Cell Size.

Table 1. Cultures used for size calibration of forward light scatter from the BD FACScan and Coulter Counter. CCMP=Culture Center for Marine Phytoplankton.

Species Name	Stain ID – if available	Approximate Size (microns)
Alexandrium fundyense	CB-501	30
Pycnococcus provasolii	CCMP 1203	2-4
Brachiomonas		10x18
Dunaliella tertiolecta	CCMP-1302	8-13
Isochrysis galbana	CCMP 1324	4-8
Emiliana huxleyi		3-5
Thalassiosira pseudonana	CCMP 1335	4-6
Amphidinium carterae	CCMP 1314	12-18
Pleurochrysis carterae	CCMP 645	8-12
Thalassiosira weissflogii	TW	14-18
Phaeocystis globosa	CCMP 2754	5-7
Synechococcus sp. (cyanin)	8c-1k	1-2
Micromonoas pusilla	CCMP 494	1-2
Rhodomonas salina	3C / CCMP1319	9-15

Sample collection method:

Water subsamples from CTD Niskin bottles were collected for analysis. Samples were collected at depths aligning with the surface, mixed layer and chlorophyll maximum. Samples were prefiltered with 200 µm nylon mesh screen prior to measurement.

Sample analysis method:

The volume of sample analyzed was determined by a "time" method. The flow rate of the instrument was monitored twice a day using volume standards. These were tubes containing deionized water that had been weighed and sealed ashore and stored refrigerated. Approximately twice a day a volume standard tube was opened and run on the instrument for about 5 min at high flow rate. Tubes were then resealed, stored in the refrigerator, and weighed upon return to the lab. From these results it was determined that the flow rate was constant over the first 2 days of the cruise, then declined over the middle portion of the cruise, and then was higher again and constant for the last 2 days. The trend was fit to a linear regression and the resulting flow rate was used to calculate cell abundances for samples run on those days, while a constant rate was used for the first and last periods. The high flow rate averaged 51.0 µL/min^{1} (2.4 sd, 4.6% cv, range: 46.8 – 55.7), over the whole cruise.

Samples for heterotrophic bacteria were preserved with 10% paraformaldehyde (0.5% final), 100 uL of preserved sample was stained with 10 µL PicoGreen (Invitrogen - 1:10 dilution in deionized water) for 24 hours, diluted with 900 µL of filtered seawater and analyzed by flow cytometry (Veldhuis et al. 1997).

Live samples for phototrophic picoplankton and nanoplankton were run undiluted at a high flow rate as per standard flow cytometric procedures (Marie et al. 2005). Live samples for heterotrophic nanoplankton were stained with LysoTracker Green (Invitrogen). A 1mM stock of LysoTracker Green was diluted 1:10 in 0.2 µm-filtered seawater added to live seawater samples (75 nM final concentration) and incubated at in-situ temperatures for 10 minutes and enumerated by flow cytometry (Rose et al. 2004).

Notes on data post processing:

Data were analyzed using FlowJo 9.8 Software (Becton Dickinson, San Jose, CA).

Cell diameters from the cruise were determined using the bead to cell standard curve relationship in Figure 3, all heterotrophic and phototrophic nanoplankton biovolumes (µm^3) were then calculated.

Key method references:

Marie, D. N. Simon and D. Vaulot. 2005. Phytoplankton cell counting by flow cytometry. In: Algal Culture Techniques. Ed. R. A. Andersen. Elsevier. pp. 253-267.

Rose, J. M., Caron, D. A., Michael E. Sieracki, M. E., Poulton, N. 2004. Counting heterotrophic nanoplanktonic protists in cultures and aquatic communities by flow cytometry. Aquat. Microb. Ecol. 34: 263–277.

Veldhuis M. J. W., Cucci T. L., Sieracki M.E. 1997. Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. J. Phycol. 33:527-541