WATER COLUMN TIME SERIES PROTOCOL
FLOW CYTOMETRY ANALYSIS
Moorea Coral Reef LTER

Sample collection, transport and storage is described in a separate protocol document.

Protocol follows method outlined in Nelson et al. (2011). Briefly, cryovial samples are thawed and 250 uL aliquots are distributed into two separate 96-well plates. One plate is stained with Invitrogen SYBR Green I at 1X final concentration for 5-30 minutes before counting. Samples are counted on the Carlson Lab BD LSRII flow cytometer using the HTS Autosampler with different flow settings for Stained (no mixing, 0.5 uL/sec, 45 uL total volume) and Autofluorescent Cells (mixing, 2 uL/sec, 180uL total volume). Gates and laser settings are outlined below. As of 2011 we have not added in detailed population-level analyses of Autofluorescent cells but potential gating is outlined below

Reference:

Nelson CE, Alldredge AL, McCliment EA, Amaral-Zettler LA, Carlson CA. (2011). Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. ISME J. doi:10.1038/ismej.2011.12

Parameter	Stained Cells	Autofluorescent Cells
Threshold Channel	FITC 200	PerCP-Cy5-5 200 AND SSC 200
FSC	600	550
SSC	450	500
FITC	450	580
PE	650	650
PerCP-Cy5-5	650	500
PE-Cy7	700	700
APC-Cy7	700	700
APC	700	700
Gating	Remove noise (low SSC, low FITC)	For total autofluorescent cells no gates. Populations may be defined as follows: Synechococcus: low FSC, high PE Prochlorococcus: low FSC, zero PE Phycoerythrin PicoEuks: High FSC & PE Chlorophyll PicoEuks: High FSC, Low PE Other Autofluor: Moderatee FSC & PE

Written by C. Nelson. Updated May 2011.