

## Microbial Observatories

### Enumerating Marine Bacteria, Prochlorococcus, Synechococcus, and Phototrophic Eukaryotes using Flow Cytometry

#### Materials

Beads: 1 $\mu$ m and 2.5  $\mu$ m green fluorescent beads (Polysciences and Molecular Probes)  
SYTO 13 nucleic acid stain (Molecular Probes)  
Filtered seawater for sheath fluid (prepared fresh daily)  
Falcon tubes with caps for sample processing  
Analytical balance

#### Samples

Natural seawater samples preserved in 2% buffered formalin (.22 $\mu$ m filtered), flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

#### Sample Processing

Thaw the samples rapidly in running tap water. Store in dark, on ice until used. Vortex and subsample 1 ml into each of 2 tubes.

Add beads to all tubes as an internal size standard.

Set aside 1 tube for staining of bacteria with SYTO13. Add 0.5 $\mu$ l ( final concentration of 2.5 $\mu$ M) of SYTO 13 to each tube. Vortex for 5 seconds. Incubate at room temperature in the dark for 15 minutes.

#### Instrument Set-up

Turn on FACSCalibur Flow Cytometer (FC) and CellQuest software and prepare for daily use. Use appropriate pre-set instrument settings and experiment documents for each category of data (heterotrophic bacteria vs. autotrophs).

Choose folder and file names for data files in CellQuest.

#### Sample acquisition-Autotrophs

1. Set sampling time to 60 seconds.
2. Set run speed to HI and press "Run" button on FC.
3. Uncheck "Set-up" box in acquisition control in CellQuest. File will be saved automatically at the end of the run..
4. Remove droplet containment tube from SIP to reduce sample aspiration.
5. Weigh sample tube, place sample tube on SIP after removing droplets from SIP. Simultaneously, move arm underneath sample tube, and press "acquire" in CellQuest.
6. At the end of the run, immediately remove tube and weigh sample again and note volume change.

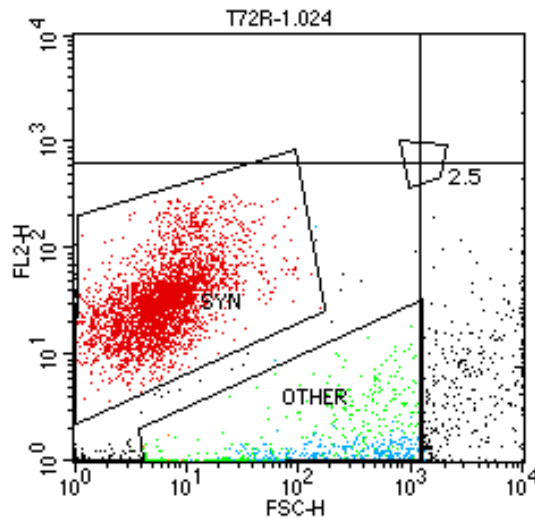
#### Sample acquisition-Heterotrophic bacteria

1. Set sampling time to 60 seconds.
2. Set run speed to LOW and press "Run" on FC. Follow steps 3-6 above.

## Sample analysis

The experiment files have pre-set gates for determining concentrations of the desired populations. These gates should be examined for their relevance to each individual sample. Once the gates are set, the data in the statistics box and the known sampling volume can be used to determine the concentration of each populations within the sample.

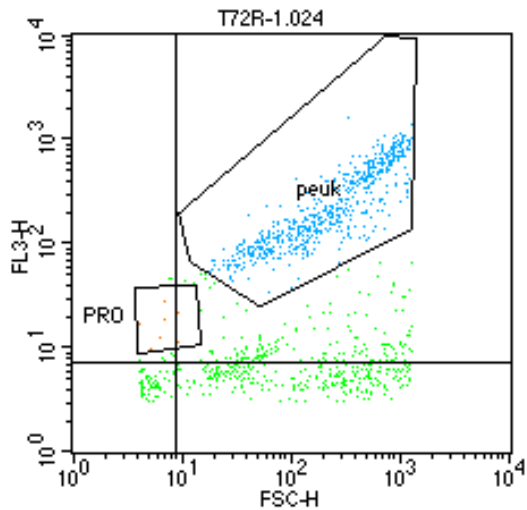
Sample cytograms are shown below:



### Region Statistics

File: T72R-1.024 Log Data Units: Linear  
 Sample ID: T72R-1 Acquisition Date: 30-Apr  
 Gate: No Gate Gated Events: 5490  
 Total Events: 5490 X Parameter: FSC-H (L)  
 Y Parameter: FL2-H (Log)

Region	Events	% Gated	% Total	X Mean	Y Mean
SYN	3447	62.79	62.79	8.20	43.23
OTHER	1101	20.05	20.05	310.07	1.64
PRO	1229	22.39	22.39	7.65	54.02
2.5	0	0.00	0.00	***	***
peuk	673	12.26	12.26	334.67	17.45



### Region Statistics

File: T72R-1.024 Log Data Units: Line  
 Sample ID: T72R-1 Tube:  
 Acquisition Date: 30-Apr-03 Gate: G2  
 Gated Events: 1101 Total Events: 5490  
 X Parameter: FSC-H (Log) Y Parameter: FL3-H

Region	Events	% Gated	% Total	X Mean	Y Mean
SYN	0	0.00	0.00	***	***
OTHER	1101	100.00	20.05	310.07	173.67
PRO	8	0.73	0.15	6.54	15.93
2.5	0	0.00	0.00	***	***
peuk	602	54.68	10.97	356.33	310.22

Top: Synechococcus cells are identified and gated based on low forward scatter (FSC) and high relative orange fluorescence due to phycoerythrin (FL2). Cultures can also be used to pre-set gates for Synechococcus. All other cells and debris less than 2.5µm are included in the 'OTHER' gate

Bottom: The 'OTHER' gate is isolated and cells are further separated into Prochlorococcus and phototrophic eukaryote populations based on chlorophyll (FL3) fluorescence. Cultures are also used to pre-set gates for Prochlorococcus.