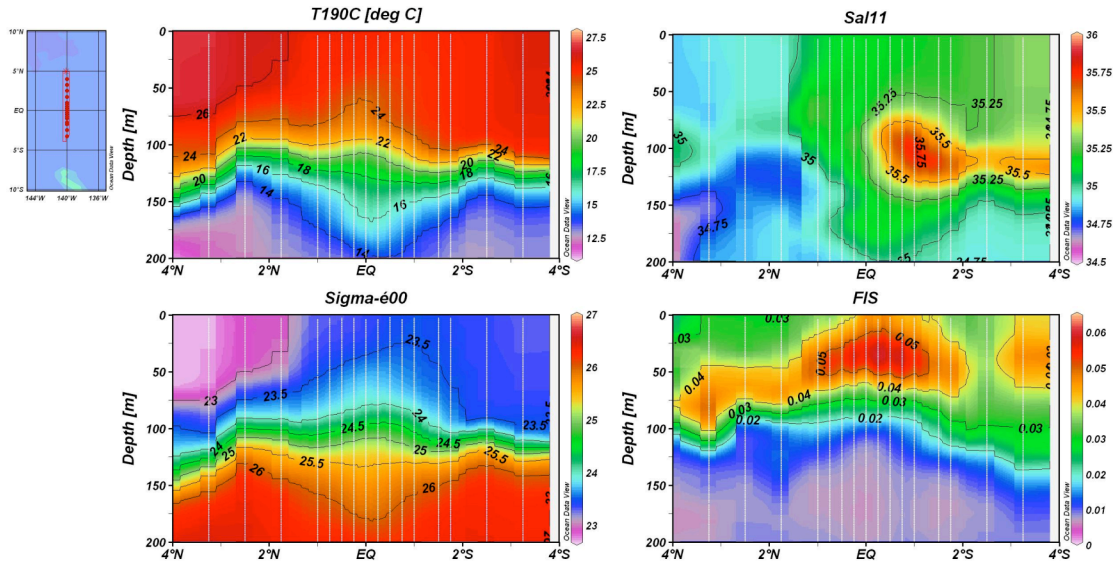
Log 16 Saturday

1. 172100Z September 2005
2. Position: Lat: 1-59.0S LONG 137-38.1W
3. Course: 062-T
4. Speed: 10.4 kts
5. Distance: 206.6 NM
6. Steaming Time: 19H 54M
7. Station Time: 4H 06M
8. Fuel: 3692 gals
9. Sky: Ptly Cldy: Cu, Sc
10. Wind: 110-T, 19 kts
11. Sea: 110-T, 3-5 ft
12. Swell: 120-T, 5-7 ft
13. Barometer: 1011.7 mb
14. Temperature: Air: 26.5 C, Sea: 26.3 C
15. Equipment Status: No change.
16. Comments: On station En route to station #17.

Alex has plotted up the CTD data for all of the stations that our group sampled (typically the 0430 rate cast and Dana's PM 500m cast).



The top left is Sea Surface temperature, the top right is Salinity
the bottom left is density as a factor of salinity and temp and the bottom right is fluorescence which is looking at chlorophyll levels (how many little phytoplankton there are in the water)

Dr. Landry's group and Dr. Dugdale's groups are using Flow Cytometry to do plankton community analyses: The abundance of the biomass and size and structure of the component populations of the microplankton community are being analyzed using flow cytometer systems with different characteristics, augmented with microscopy. Samples for epifllourescence analyses are being collected from the mixed-layer, mid and deep euphotic zone at each station, preserved and stained and mounted onto slides. Slides will be analyzed for detail for all major heterotrophic (grazer) eukaryotes and autotrophic (() groups. Results of these analyses will be integrated with other methods to yield water column distributions of all relevant populations and sizes.

They are using this data to be able to differentiate among *Synechoccus,* cryptophytes, diatoms and coccolithophores based on sized and fluorescence.

Fluorescence measurements: Microscopy

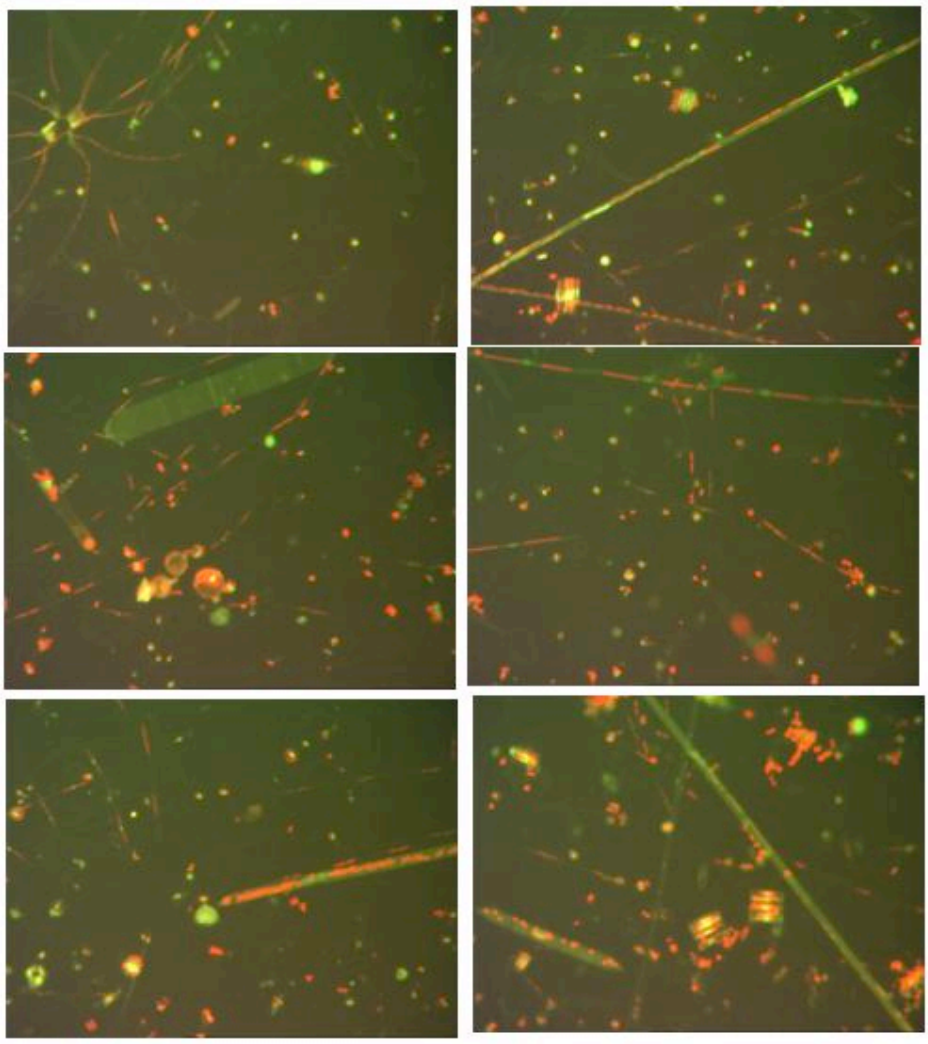
●Sample is illuminated, and light that is transmitted through or scattered, reflected, or emitted from specimen (depending on type of microscope) is sent to the eye. The eye obtains a real image of the specimen, and the sample can be moved manually.

●Response time for human observer: milli-seconds

●Main disadvantage: slow sample processing time

Examples of EpiFluorescent Microscopy

Southern Patch IN (JD 46)



Flow Cytometry

What is a Flow Cytometer?

✲*It characterizes particles in suspension*, based on their light scatter properties and their auto- and induced fluorescence properties

✲*It consists of*

laser light source(s)
fluid controls for sample and sheath streams
light scatter and fluorescence detectors
software to analyze particle signatures

Fluorescence measurements: Flow Cytometry

●Sample is illuminated and light is collected by detectors selectively (i.e., certain wavelengths), which then produce electronic signals. These signals are quantified in various ways. The sample moves in a fluid stream, past the detectors.
●Response time: <10 µseconds.
●Main disadvantage: limited resolution -- can only classify plankton on basis of optical characteristics, not ultrastructure details.

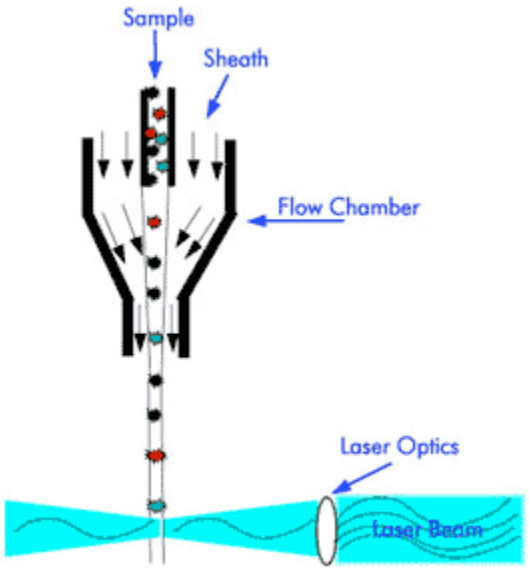


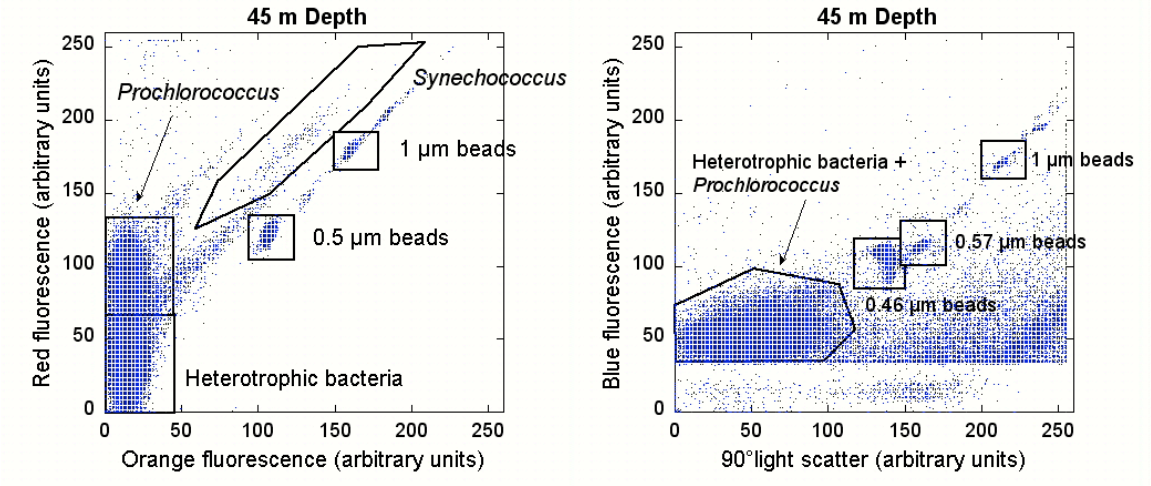
Figure 1.1 Flow cytometers use the principle of hydrodynamic focusing for presenting cells to a laser (or any other light excitation source). The sample is injected into the center of a sheath flow. The combined flow is reduced in diameter , forcing the cell into the center of the stream. This the laser one cell at a time. This schematic of the flow chamber in relation to the laser beam in the sensing area.(From Current Protocols In Cytometry, Unit 1.2 , p1.2.2).

As your cells or particles of interest intercept the light source they scatter light and fluorochromes are excited to a higher energy state. This energy is released as a photon of light with specific spectral properties unique to different fluorochromes(see Table 1.1 for a listing of commonly used fluorescent dyes and their excitation and emission spectra. This table also includes the most common laser light sources with their multiple lines of emission).

One unique feature of flow cytometry is that it measures fluorescence per cell or particle. This contrasts with spectrophotometry in which the percent absorption and transmission of specific wavelenths of light is measured for a bulk volume of sample.

Two-Parameter Histograms
A graph representing two measurement parameters, on the x- and y-axes, and cell count height on a density gradient. This is similar to a topographical map. You can select 64 or 256 channels on each axis of two-parameter histograms. Particle counts are shown by dot density or by contour plot.

Synechococcus, *Prochlorococcus* and heterotrophic bacteria from the Hawaii Ocean Time Series Station ALOHA



Two parameter histogram Dot Plot displaying FL1-FITC on the x axis and FL2-PE on the y axis.

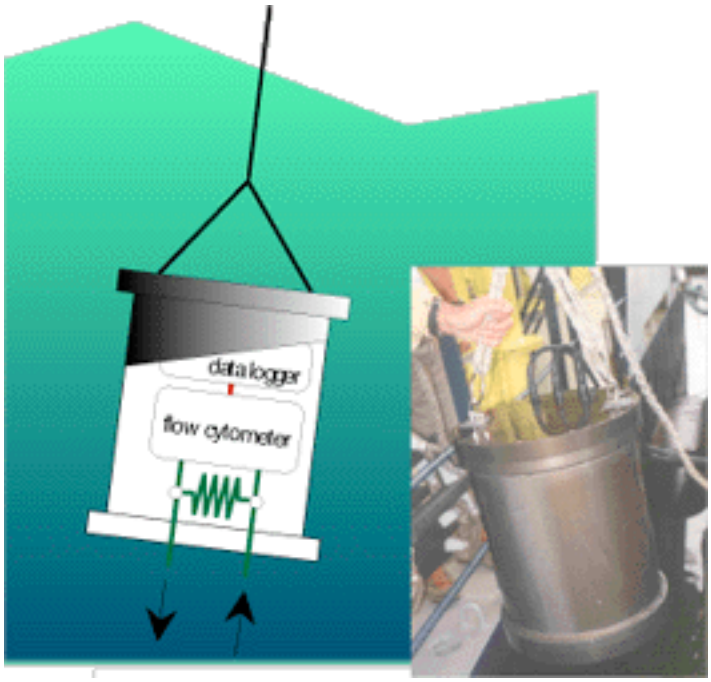
Flow Cytometry Analysis and Sorting

Flow cytometry analysis of a single cell suspension yields multiparameter data corresponding to Forward Light Scatter (FLS), 90° Light Scatter (90LS), and FL1-FL4.

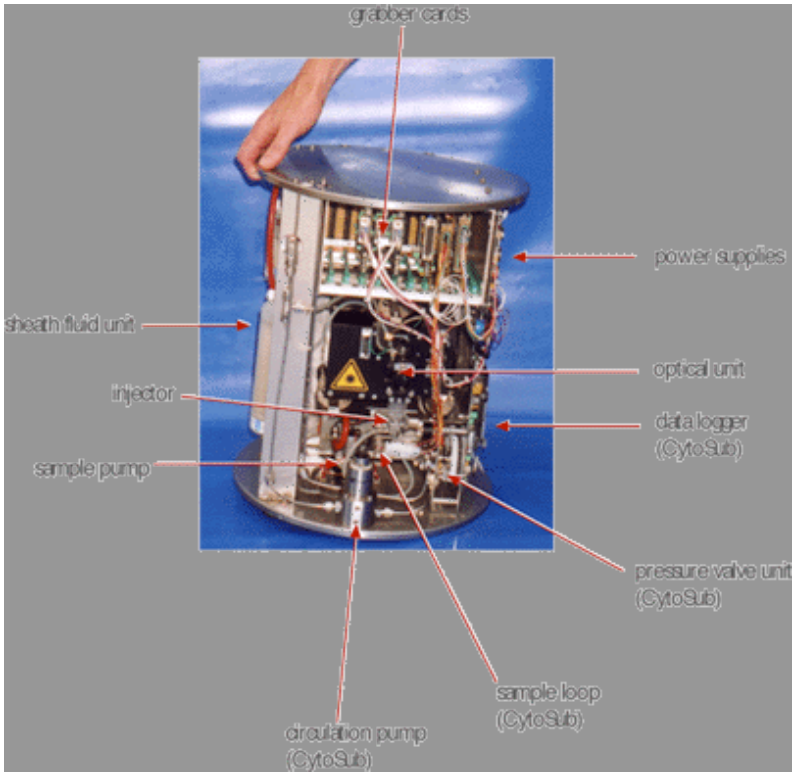
This information allows researchers to identify and characterize various subpopulations of cells. The process of separating cells using flow cytometry multiparameter data, is referred to as sorting.

Some Applications of Flow Cytometry

- Natural population analyses
- Cell cycle analyses
- Grazing rate estimates
- Ploidy assessment
- Microbial physiology
- Antibody-based probes
- Nucleic acid probes
- Sorting for subsequent culturing/isolation



CYTOBUOY



Dr. Dugdales group is using the Cytobuoy

Images from www.cytobuoy.com

Information from:

Dr. Karen Selph,

Information from: http://biology.berkeley.edu/crl/flow_cytometry_basic.html

Flow Cytometer in lab used by Dr. Karen Selph

Dr. Landry’s Lab on the Revelle