

ASCMAP WORKSHOP 15-21 APRIL 2002 AWI BREMERHAVEN

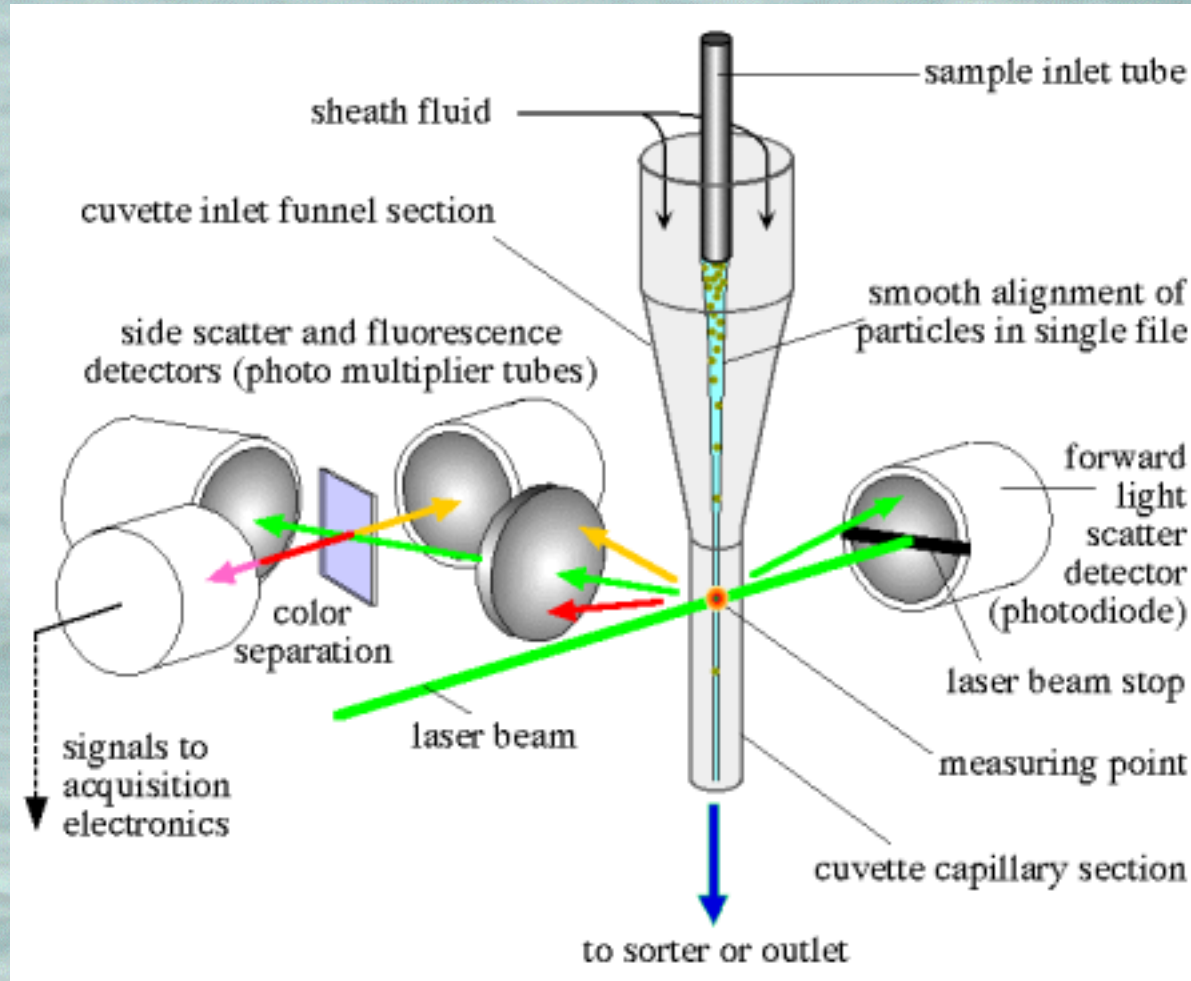
FLOW CYTOMETRY INSTRUMENTATION

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CytoBuoy
flow cytometers for the environment



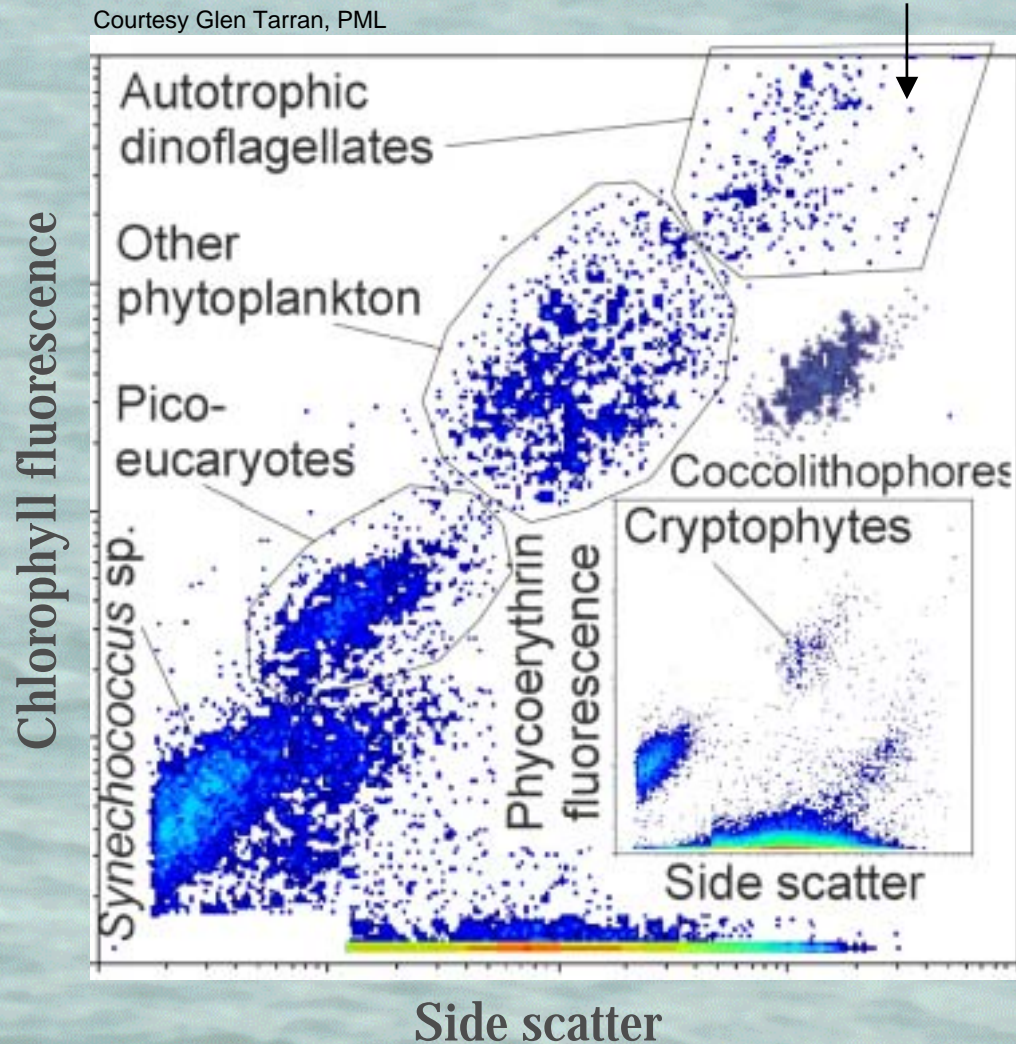
PRINCIPLE OF FLOW CYTOMETRIC ANALYSIS



Flow cytometers count and analyse individual particles in a fluid. Fast and one by one. The analysis consist of forward and sideward scattered light as well as fluorecence of the cells. The cells are funneled by a sheath fluid in single file through a laser beam focus at typically 1,000 or more per second. The detected optical signals from each passing particle are digitized and listed into correlated data. If a sorter unit is present, individual particles may be sorted physically depending on their optical signals.



Each dot represents
a single measured particle.



Data are displayed as dual-parameter plots of combinations of light scatter and fluorescence depending on the particles being analysed.

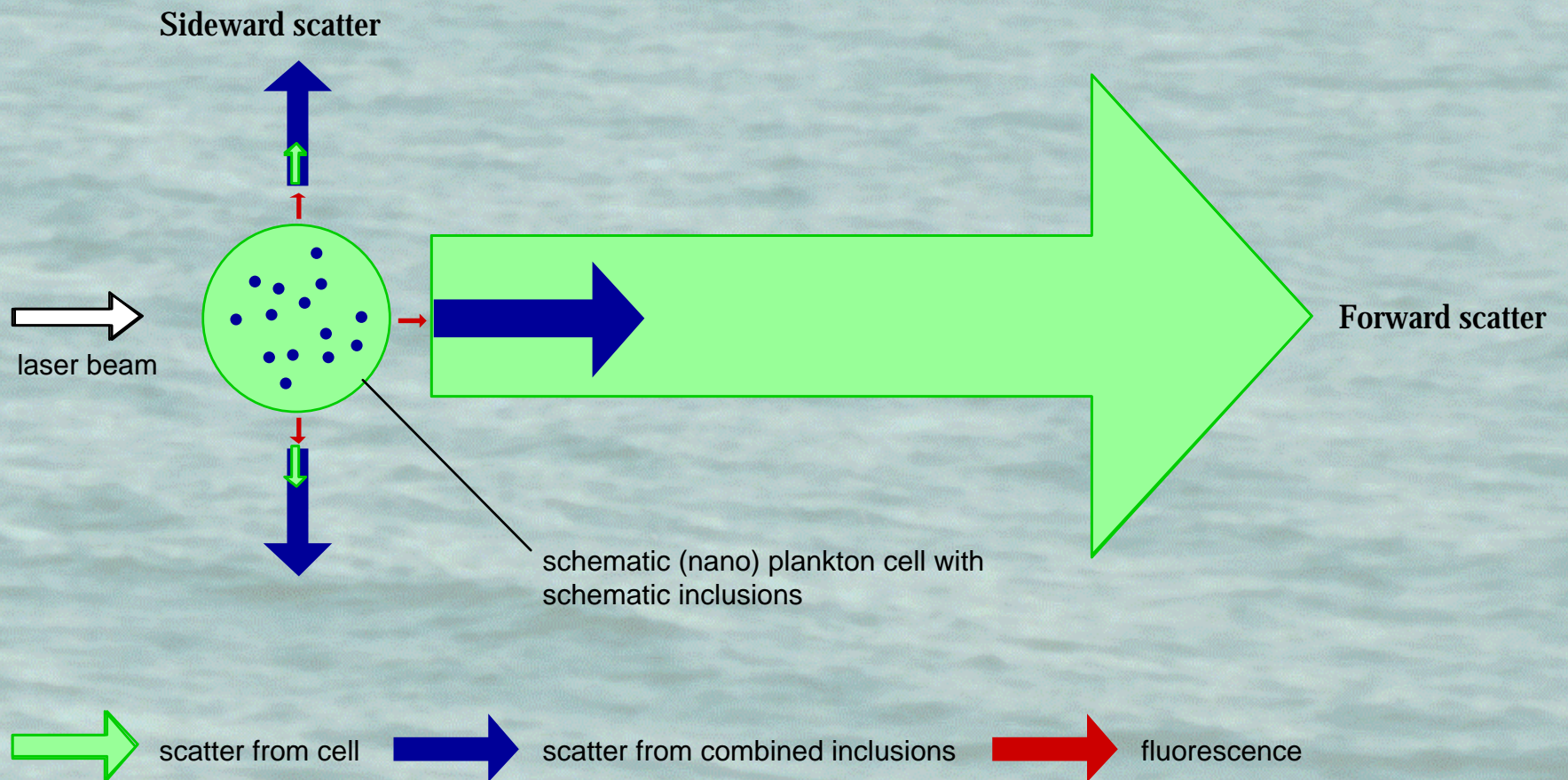
Forward light scatter is governed by particle size and sideward scatter is mostly sensitive for small cellular structures.

The fluorescence, probed in several emission bands relates to the amount of natural cellular pigment and its composition, and/or artificially introduced pigment.

Applications range from simple phytoplankton counting and sizing to more elaborate physiological analysis, and species recognition using artificial neural net algorithms.



IMPRESSION OF RELATIVE INTENSITY OF MEASURING PARAMETERS



SOME DEVELOPMENTS IN FLOW CYTOMETRY

GENERAL

MORE OF THE SAME: MORE LASERS, FLUORESCENCE BANDS, HIGHER SENSITIVITY

INTEGRATION OF OTHER ANALYSES SUCH AS ELECTRONIC RESISTANCE SIZING AND IMAGING

Fluvo flow cytometer: electronic pulse sizing and imaging (Kachel and Wietzorrek)

EurOPA: particle profiles and imaging (Rutten - this workshop)

SPECIFIC DEVELOPMENTS FOR GENERAL PURPOSES

SPATIALLY RESOLVED (FORWARD) LIGHT SCATTER MEASUREMENTS

Variable patterns (Cunningham et al.)

Flying Light Scattering Indicatrix (Maltsev et al.)

PROBING POLARIZATION EFFECTS OF FLUORESCENCE AND SCATTER SIGNAL

DEVELOPMENTS FOR AQUATIC STUDIES

LARGE PARTICLE SIZE RANGE FOR FIELD SAMPLES / EurOPA (prototype) CytoBuoy (commercial)

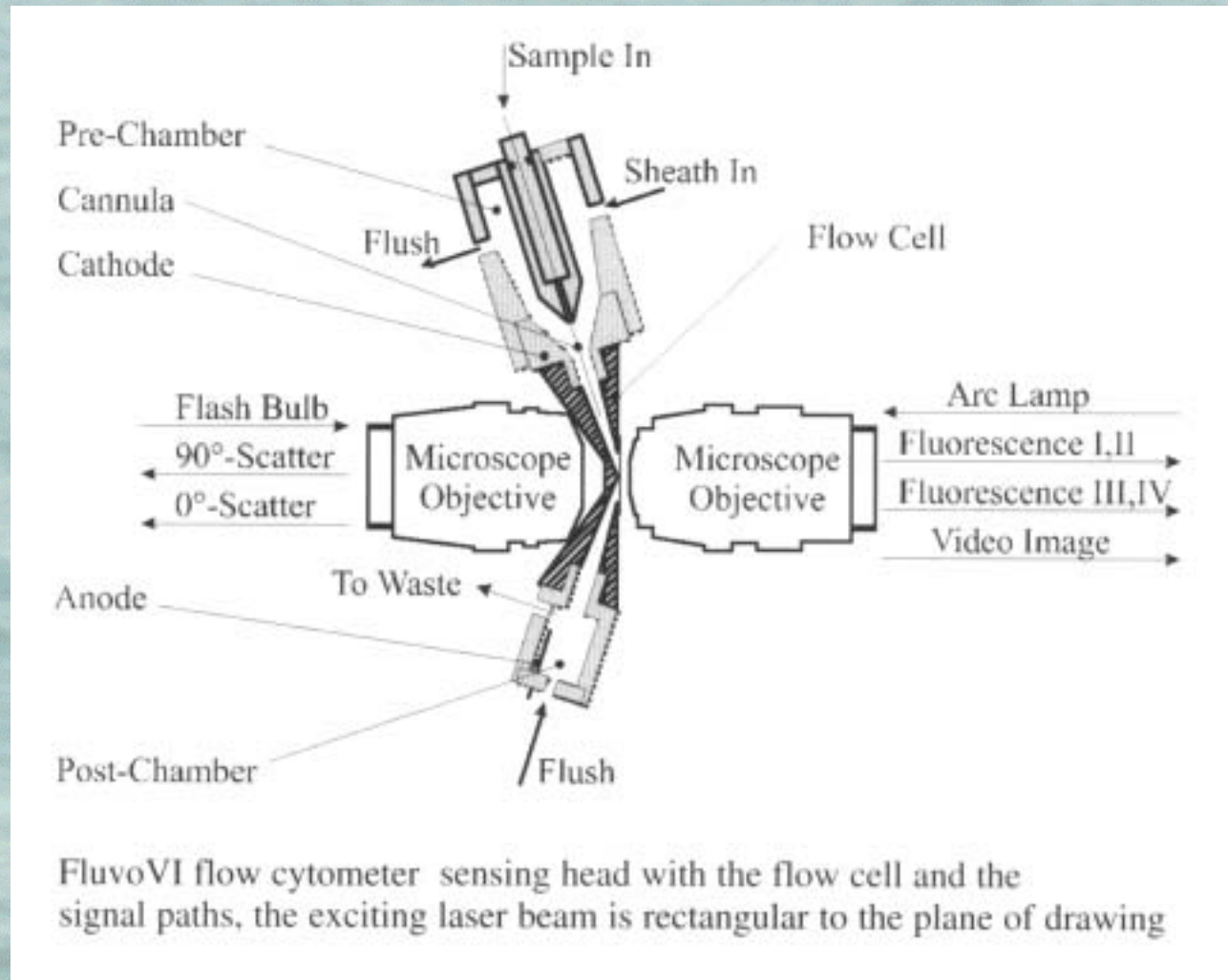
PARTICLE PROFILE FLOW CYTOMETRY / EurOPA (prototype), CytoBuoy (commercial)

PUMP-DURING-PROBE FLOW CYTOMETRY OF PHYTOPLANKTON / WHOI (prototype)

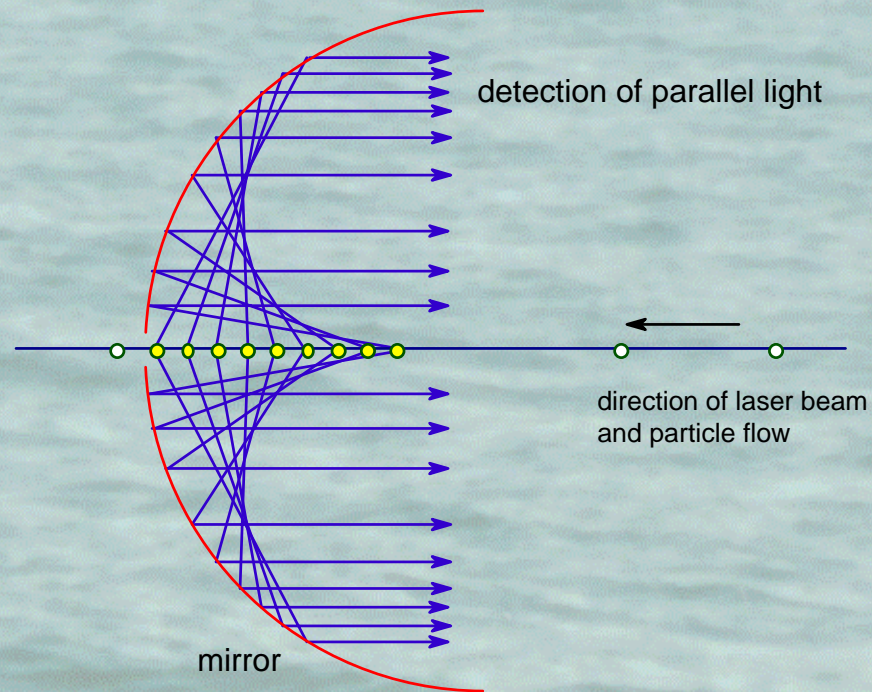
IN-SITU FLOW CYTOMETRY / WHOI (prototype), CytoBuoy (commercial)



INTEGRATION OF OTHER ANALYSES SUCH AS ELECTRONIC RESISTANCE SIZING AND IMAGING



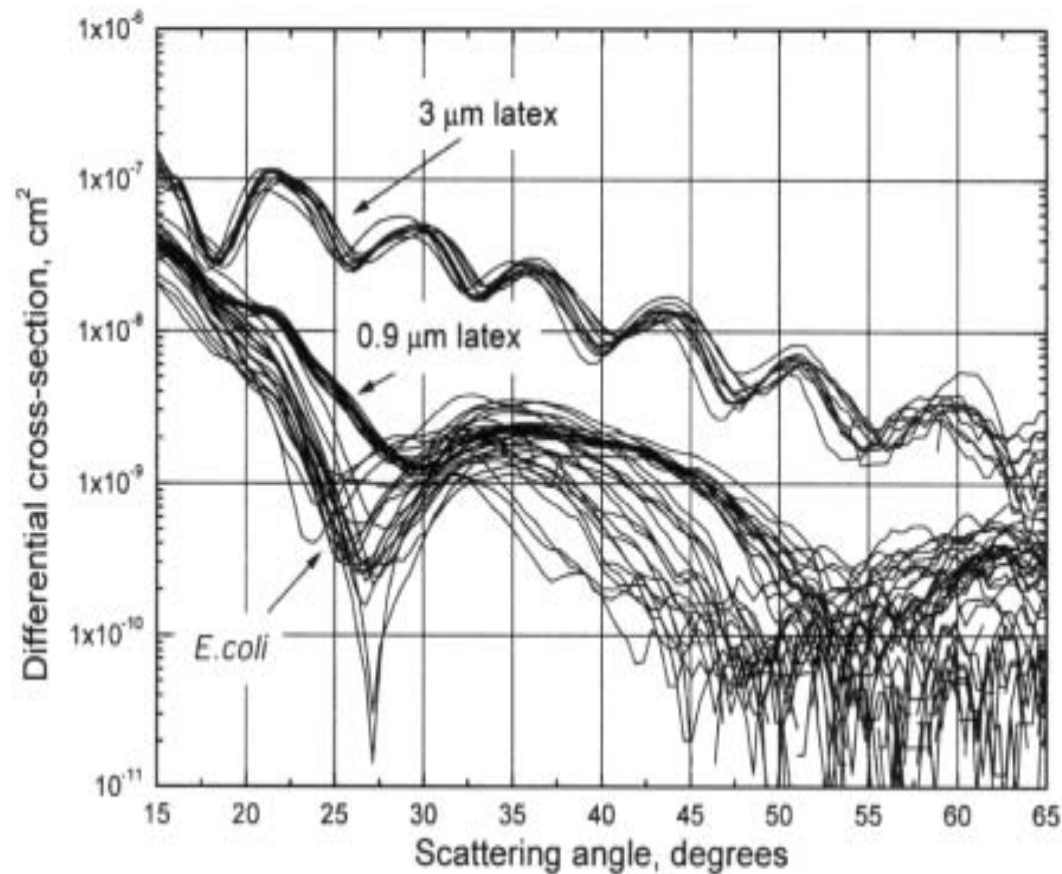
SPATIALLY RESOLVED (FORWARD) LIGHT SCATTER MEASUREMENTS



redrawn from:
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The Scanning Flow Cytometer (SFC) permits measurement of the angular dependency of the scattered light from individual moving particles (Flying Light Scattering Indicatrix, FLSI).
Angles :10 deg to 120 deg with integration over full azimuthal angle).





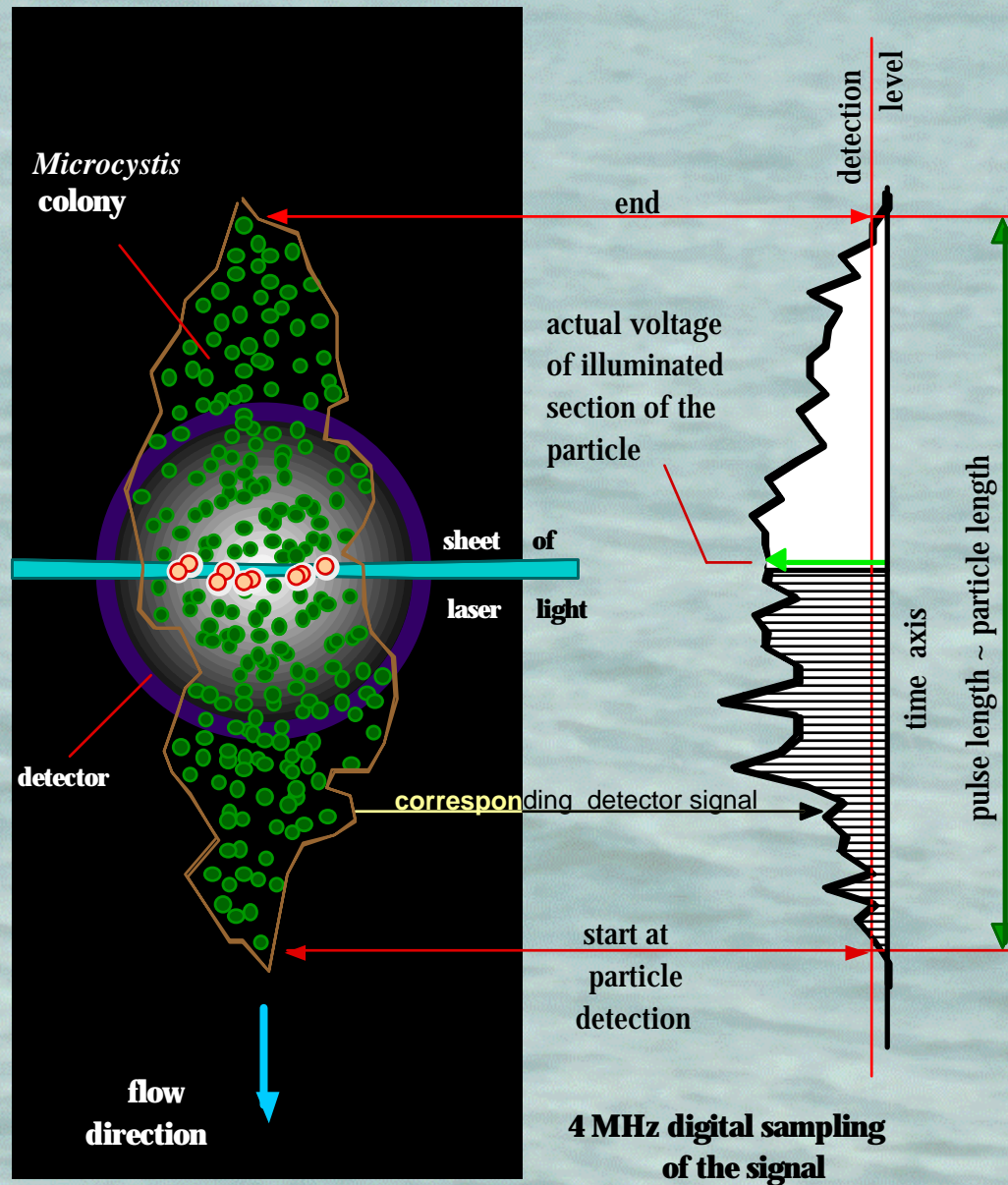
The differential cross-section of *E. coli* cells and polystyrene particles measured with scanning flow cytometer.

Recording the entire light scattering pattern of individual biological particles, may provide absolute real-time determination of size and refractive index of individual spherical particles.

From: Shvalov et al. Cytometry 41:41, 2000



LARGE SIZE RANGE FLOW CYTOMETRY



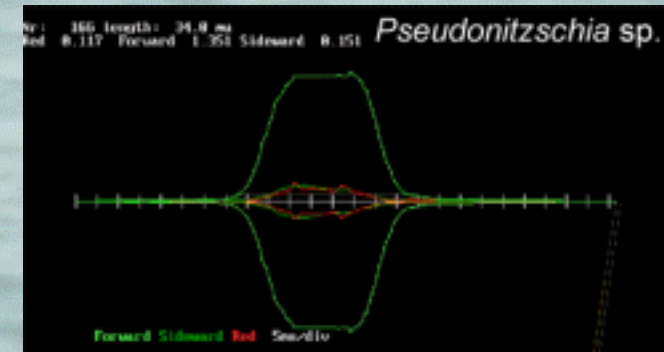
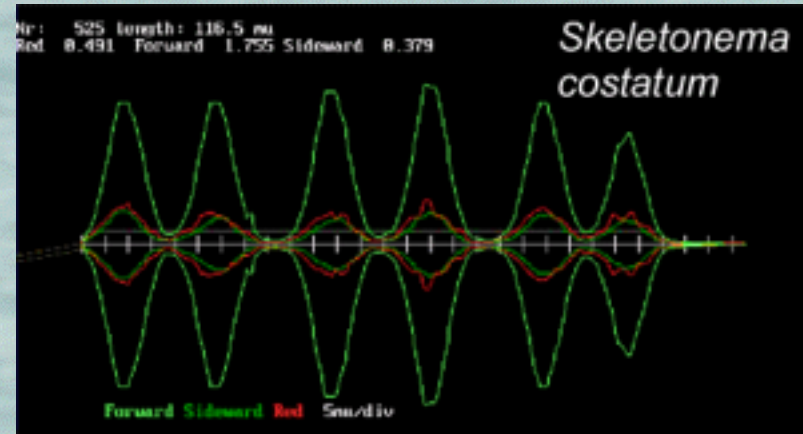
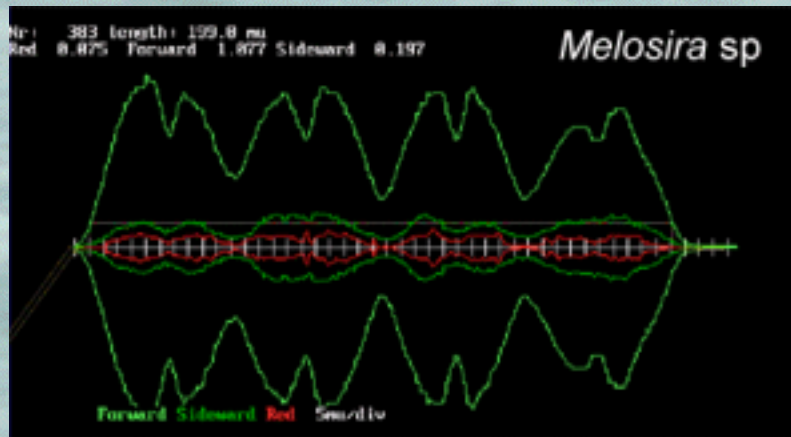
The capability to quantitatively measure a wide particle size range was introduced in the OPA instruments in 1987, involving specially tuned optics, wide bore fluidics and digital electronics.

This allows the analysis of field samples with single algal cells and colonies, including realistic size distributions of for instance the natural fresh water alga *Microcystis aeruginosa*.

Feature of EurOPA and CytoBuoy instruments.



PARTICLE PROFILE FLOW CYTOMETRY



These particle profiles expand from each dot (measured particle) in a standard flow cytometry dotplot when selected.

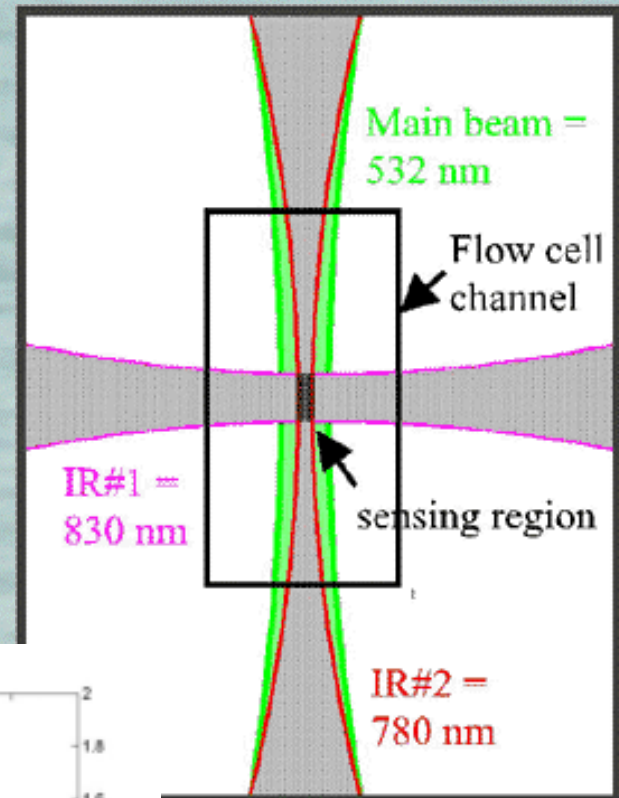
CytoBuoy raw pulse data.



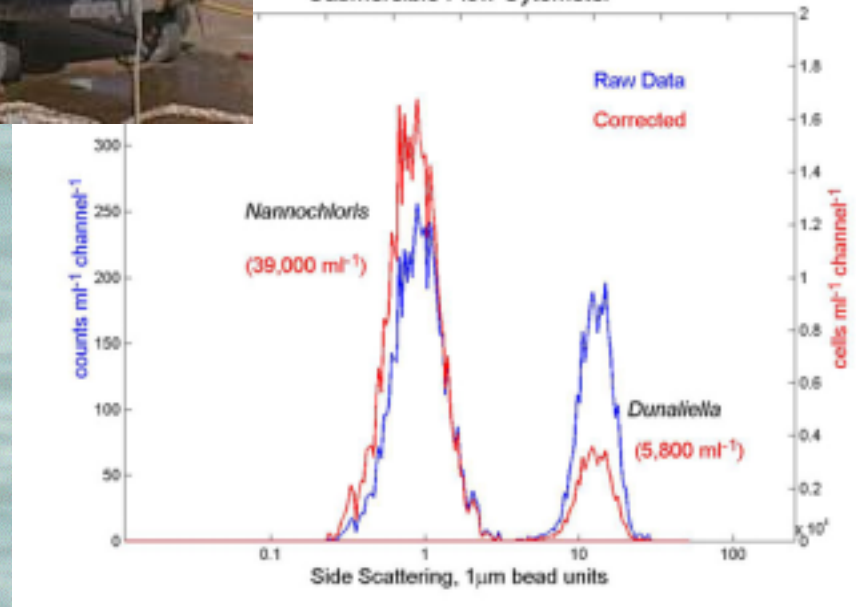
IN-SITU FLOW CYTOMETRY



WHOI prototype



Submersible Flow Cytometer



The instrument uses no sheath fluid, and uses infrared beams to determine if a particle is well centered in the sensing zone.



INSTRUMENT REQUIREMENTS FOR ROUTINE, AUTONOMOUS ANALYSIS

1	No fixation, transportation and storage of samples: in-situ analysis of live samples;
2	No division of phytoplankton samples in size fractions by filtration or other technique;
3	No preconcentration or dilution of phytoplankton samples;
4	No operator interference with instrument functioning;
1	100% computer control of operation (input of sampling regime and data acquisition)
2	Fully automated instrument functions (laser startup, flushing, sample intake etc.);
3	All properties are factory chosen for a wide range of conditions:
4	No electronic and/or gain adjustments;
5	No optical alignment;
5	No sheath fluid supply;
6	No air supply;
7	No system clogging by phytoplankton;
8	Resistant to biofouling (sheath fluid is good system);
9	Compact, easy transportable, water tight or splash proof instrument;
10	Shock, vibration and movement resistant.



AutoSub mission



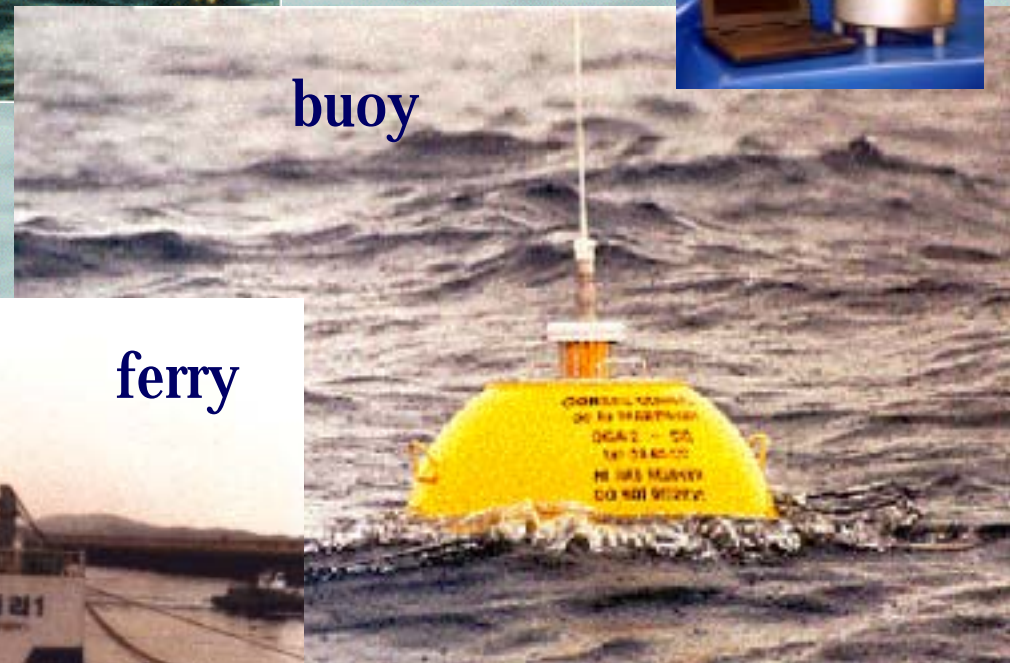
submarine

IN-SITU FLOW CYTOMETRY

CytoBuoy instrument platforms



lab



buoy



wire

To 250 m depth



ferry

