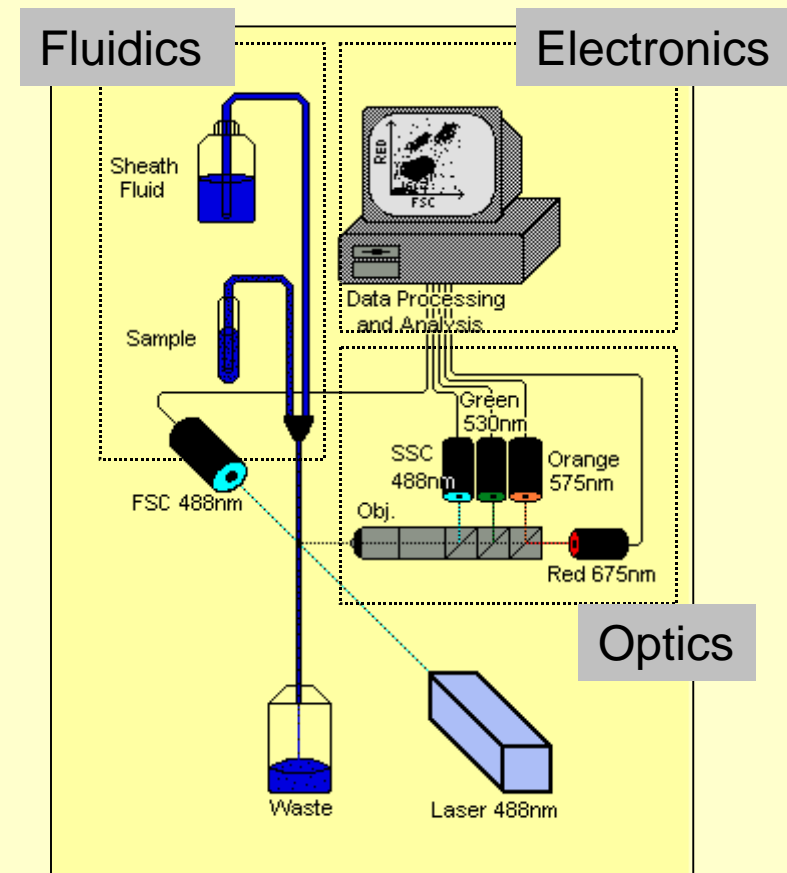


Phytoplankton Analysis and Sorting by Flow Cytometry

Marcus Reckermann
Research and Technology Centre Westcoast (FTZ)
at Büsum (Kiel University)

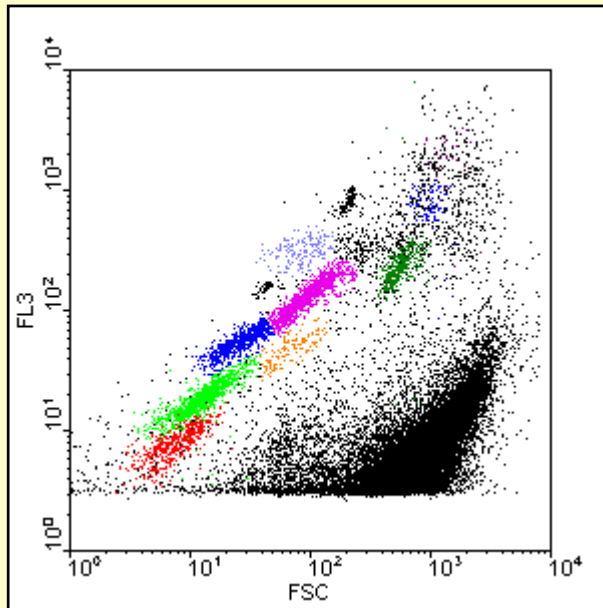
1. Short Intro to Aquatic Flow Cytometry
2. Red Laser Line 633nm
3. Sorting Principles
4. Sorting Applications

- originated in medical research (blood/tissue cell analysis)
- measures optical properties of cells (light scatter, fluorescence)
- cells are linearly aligned in a narrow fluid stream by hydrodynamic focussing so they pass the excitation laser beam one by one

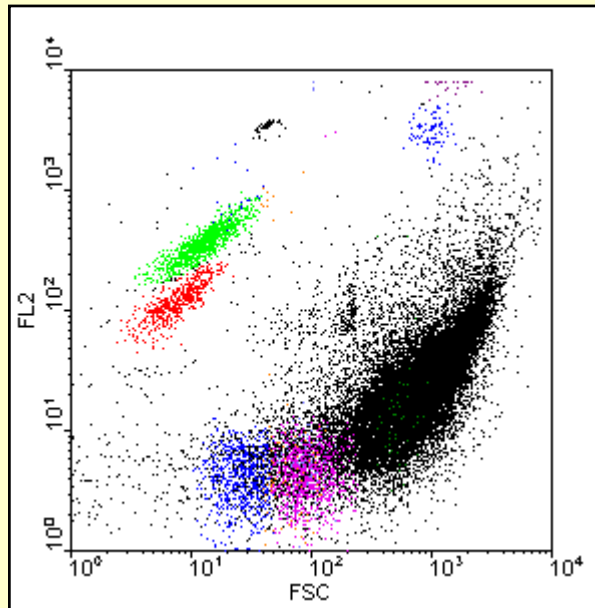


Which taxonomic classes are characterized by which fluorescence colours ?

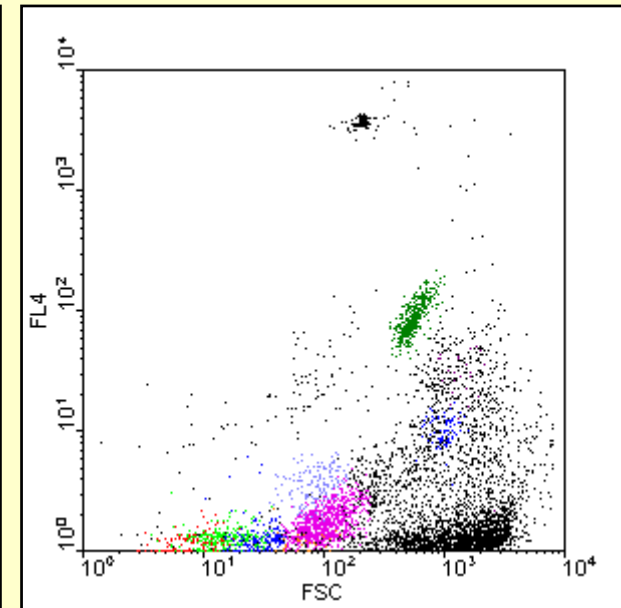
Chlorophyll
(Red Fl. at 488nm Ex.)
vs. Forward Scatter
All Phytoplankton



Phycoerythrine
(Orange Fl. at 488nm Ex.)
vs. Forward Scatter
Cyanobacteria, Cryptophytes



Phycocyanine
(Red Fl. at 633nm Ex.)
vs. Forward Scatter
Cyanobacteria, Cryptophytes



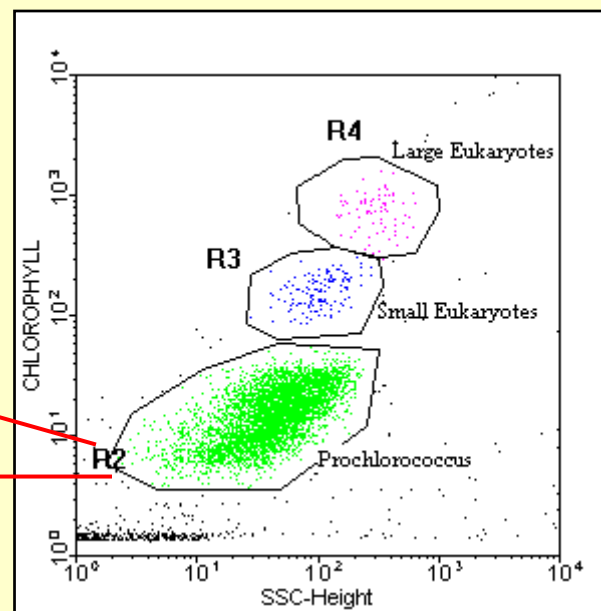
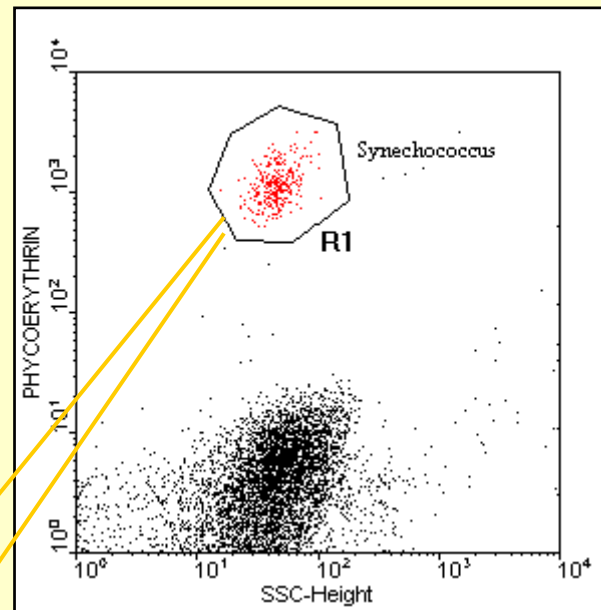
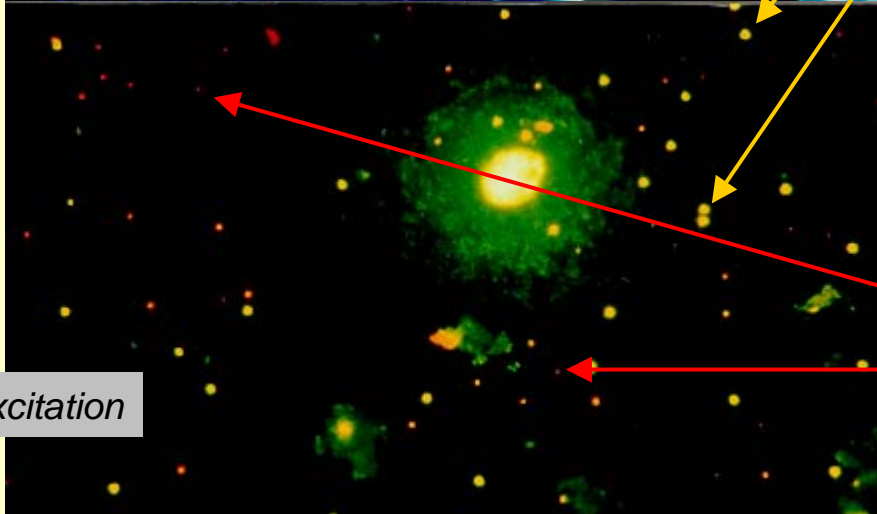
Wadden Sea phytoplankton (eutrophied coastal)

Oceanic phytoplankton (Arabian Sea, spring inter-monsoon)

DAPI stained
UV excitation



Blue excitation



The usefulness of a red laser in aquatic flow cytometry: **Phycocyanine** !

Excitation and emission bands of a flow cytometer equipped with a **488 nm laser** and a **633 nm laser**, with analyzed cell properties

Pigment	Excitation	Emission
Chlorophyll	488	> 645; 675/ 20
Phycocerythrine	488	575/ 26; 585/ 42
Phycocyanine	633	660/ 20
Light Scatter	488; 633	488/ 10; 633/ 10

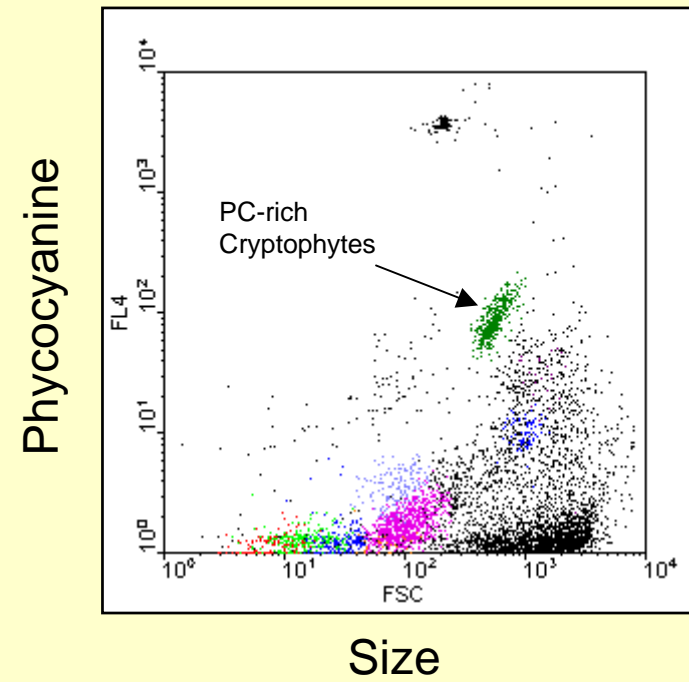
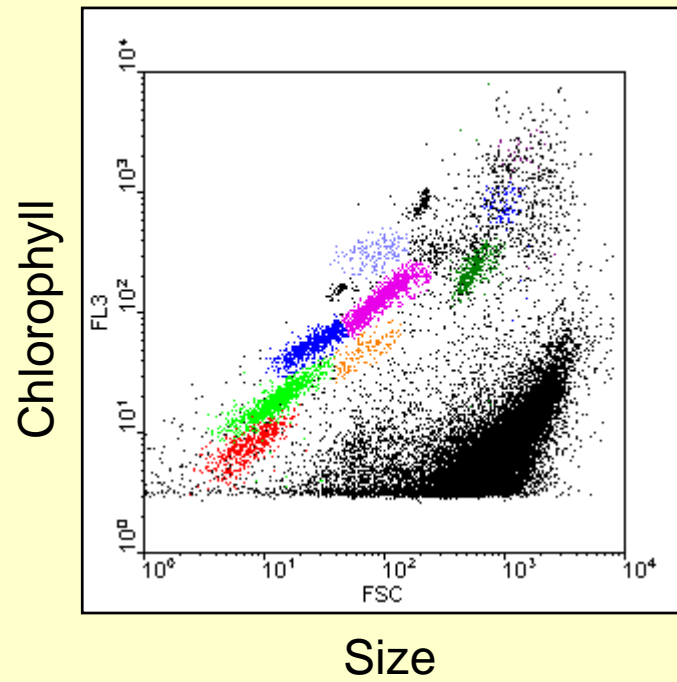
Absorption and fluorescence emission maxima of the **cryptophyte phycocyanins** (from Hill and Rowan, 1989)

Pigment	Genus/ Species	Absorption peak	Fluorescence Peak
Cr-phycocyanine 569	<i>Chroomonas daucoides</i>	569	650-656
Cr-phycocyanine 615	<i>Hemiselmis virescens</i>	612-615	634-641
Cr-phycocyanine 630	various <i>Chroomonas</i> species	625-630	648-649
Cr-phycocyanine 645	various <i>Chroomonas</i> species <i>Hemiselmis virescens</i>	641-650	654-661

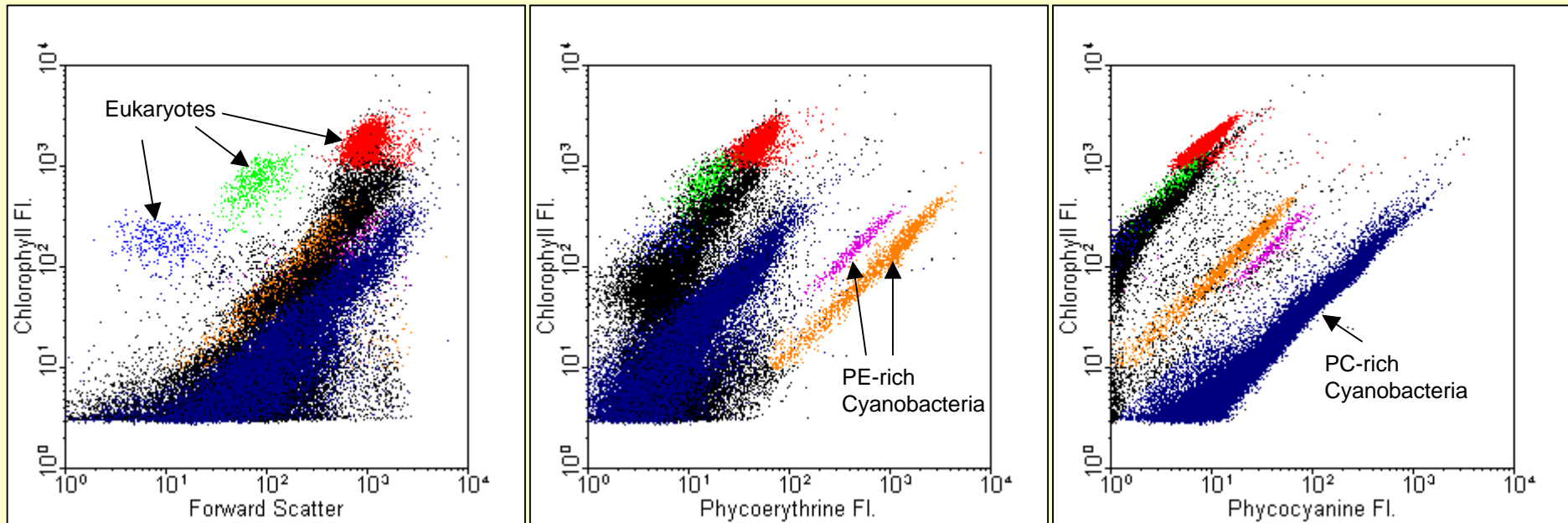
Absorption and fluorescence emission maxima of some **cyanobacterial phycocyanins** (from Lee et al., 1994).

Pigment	Genus/ Species	Absorption peak	Fluorescence Peak
Phycocyanine	<i>Microcystis aeruginosa</i>	630	645
Phycocyanine	<i>Spirulina platensis</i>	630	647
Phycocyanine	<i>A nabaena cylindrica</i>	630	651
Phycocyanine	<i>Porphidium tenue</i>	630	646

633nm laser line allows separation of PE-rich cryptophytes and cyanobacteria

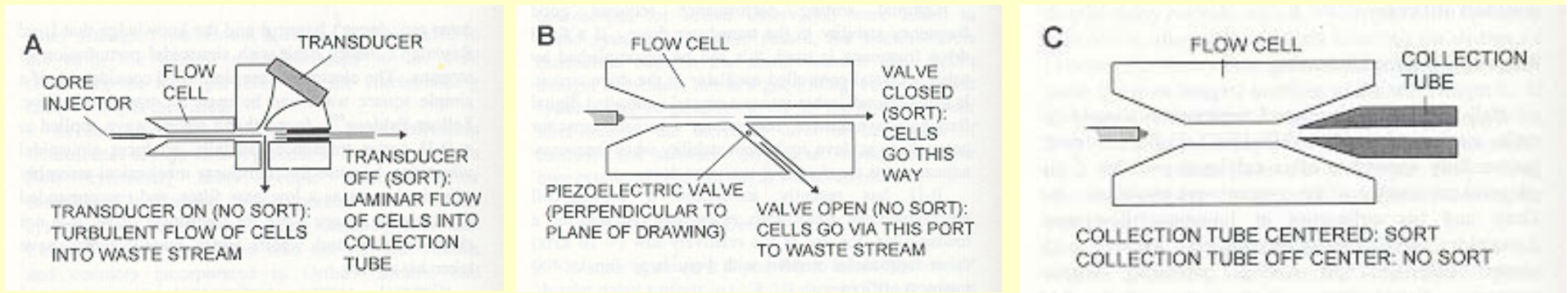


**A brackish water sample
(Hedwigenkoog, Dithmarschen, Northern Germany)**



Sorter designs

Closed Flow Cell Sorters



Friedman's acoustic sorter

Fluidic switching sorter
(e.g. Partec)

BD FACSort™ (Calibur?)

from Shapiro (1995): Practical Flow Cytometry

Droplet Sorters



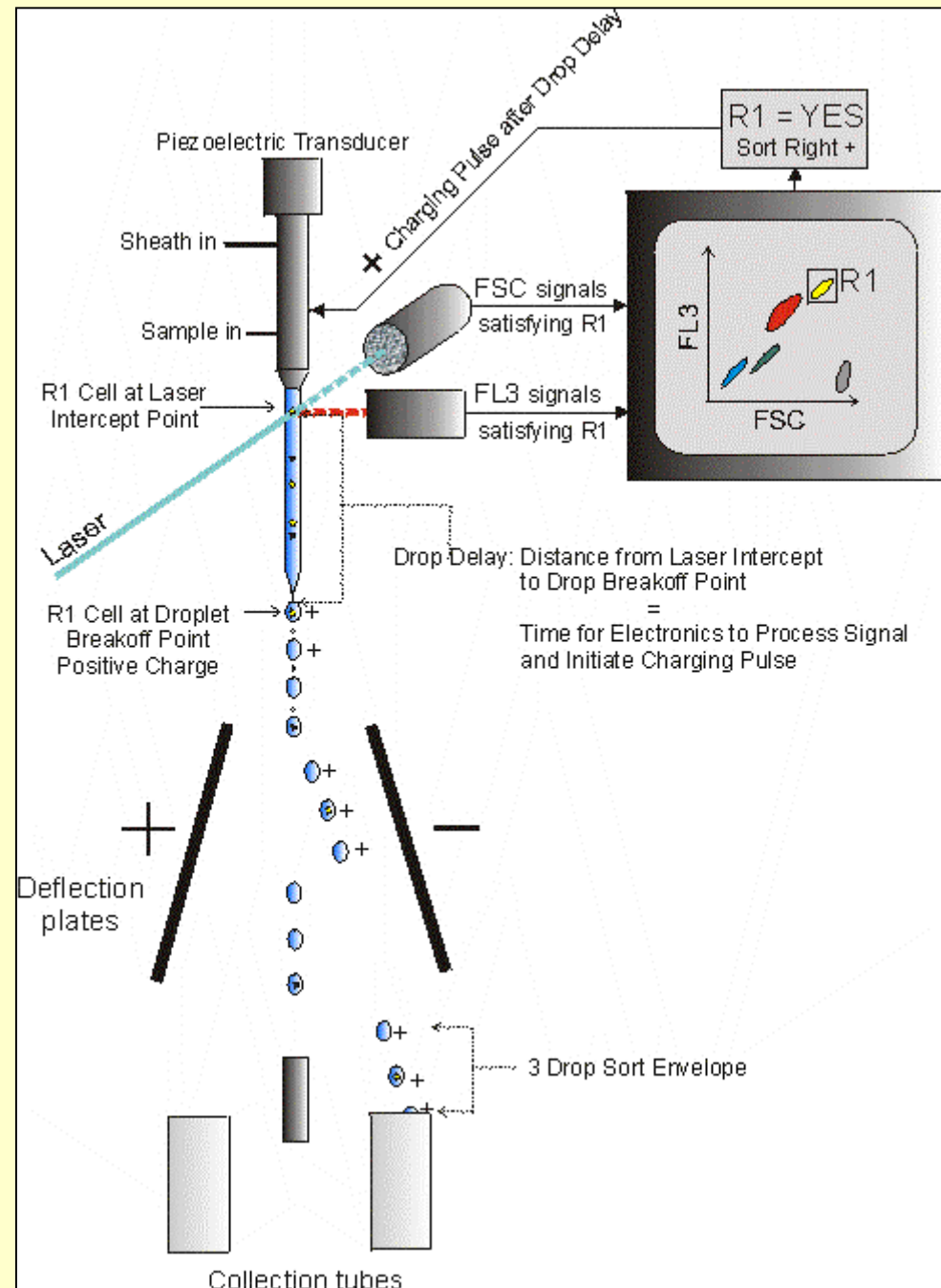
Droplet Sorting:

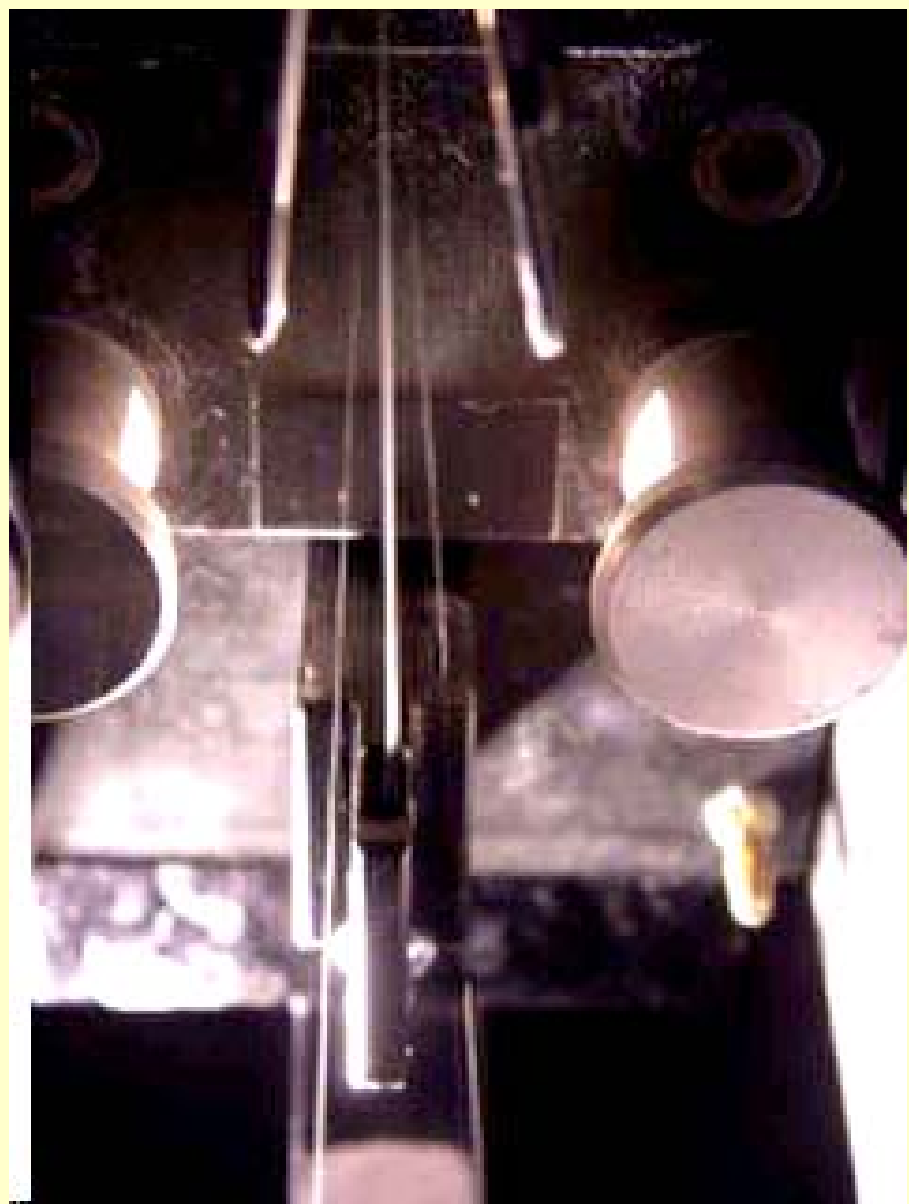
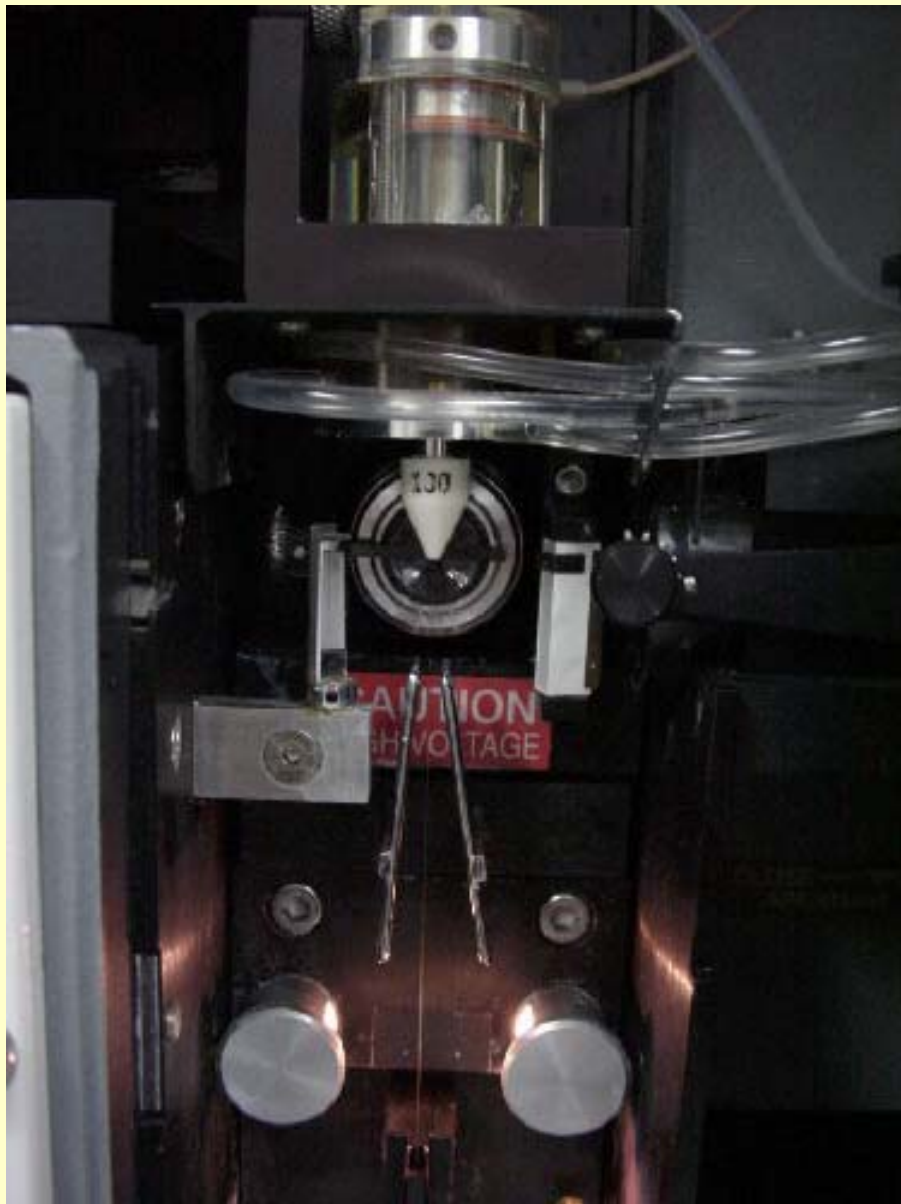
- The fluid stream is forced to vibrate at very high frequencies (15.000 - 35.000 Hz), so that at a defined distance from the laser beam intercept point, a drop breaks off ("drop delay")

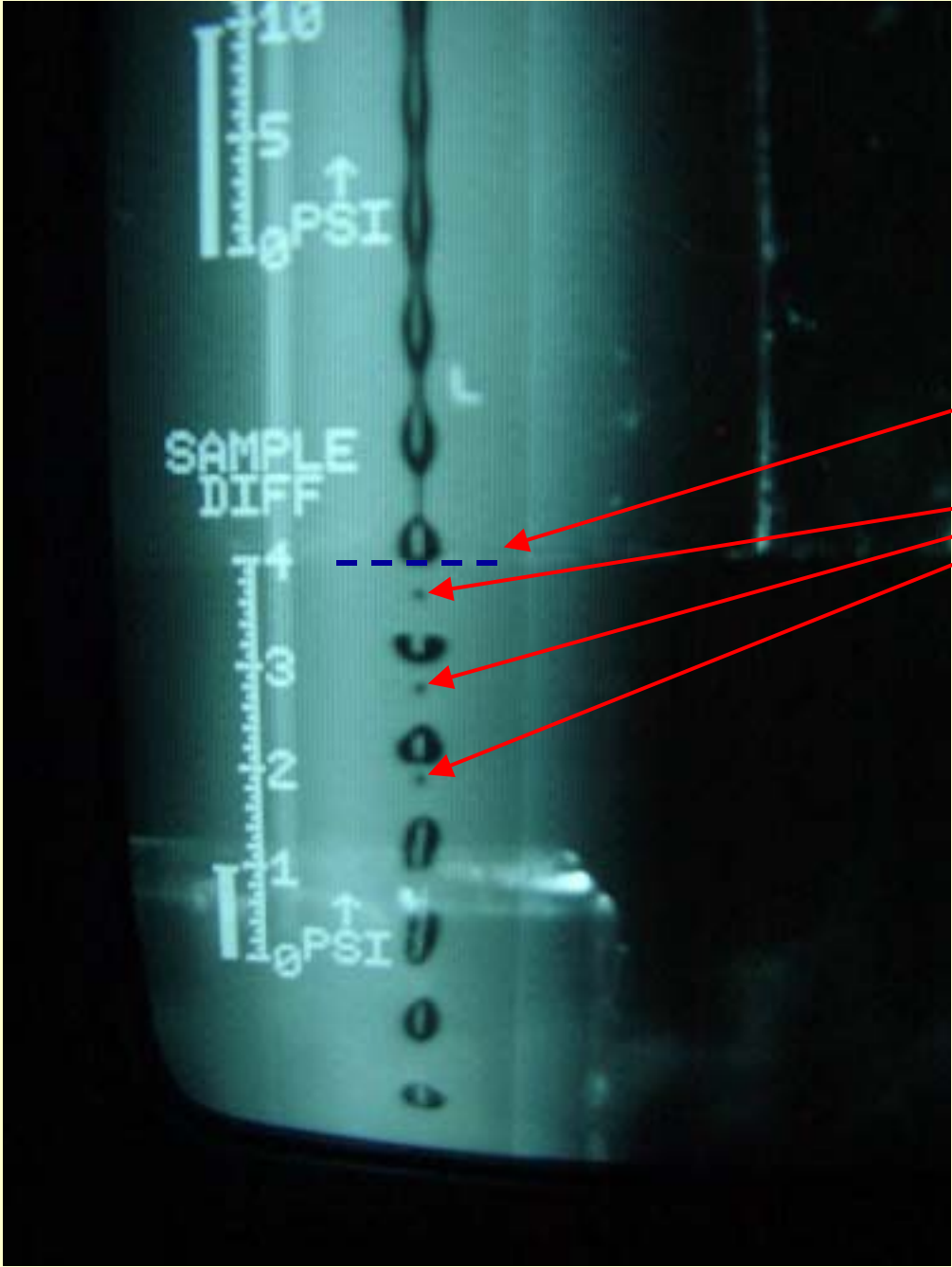
- if desired cell is in the the forming drop (as defined by the drop delay), the entire stream is charged until the drop has just detached from the stream

- charged drop is deflected by deflection plates according to their charge

- the fluid stream is de-charged after droplet break-off, following drops will not be charged and deflected





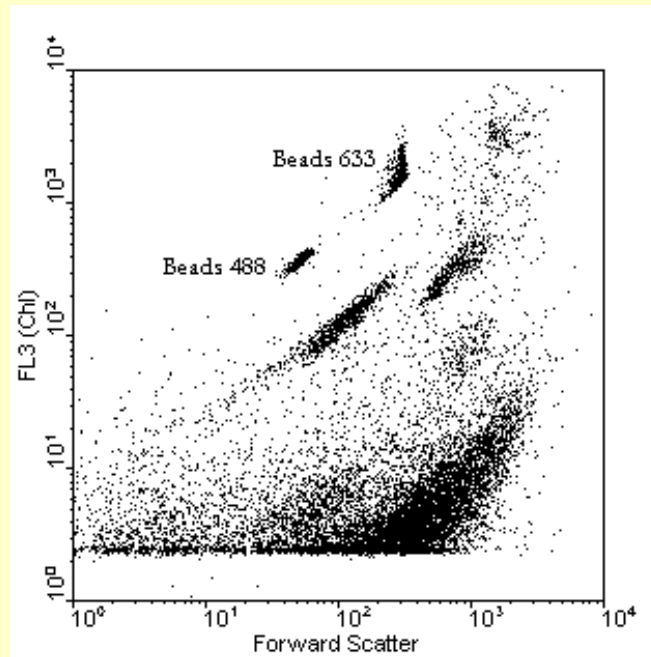


Droplet breakoff point

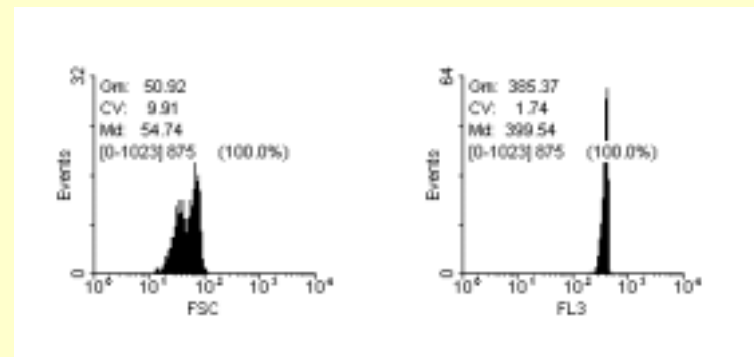
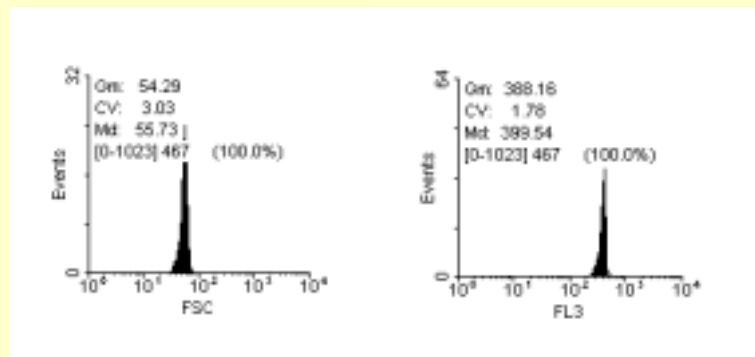
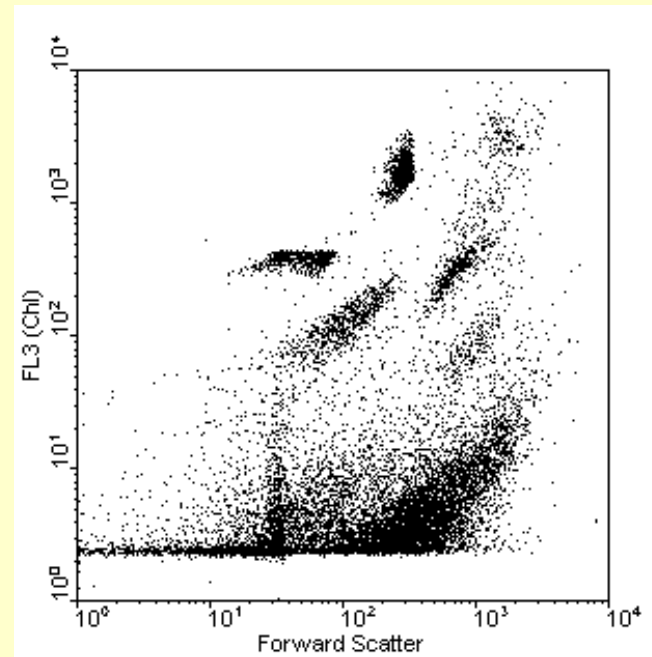
Satellite drops

**Most important: a well aligned system and stable fluid stream
(droplet generation causes "wobbling" of signals)**

Drop Drive OFF



Drop Drive ON



Sorting Applications

- the identification and characterization of unknown clusters
 - microscopy, molecular techniques, immunological methods
- the production of clonal cultures
- post - incubation separation to account for group-specific physiological parameters
 - ^{14}C -uptake (i.e. Rivkin et al. 1986, Li 1994)
 - ^{15}N -uptake (i.e. Lipschultz 1995)
 - any other physiological feature or cell property

Sorting procedures

For identification purposes:

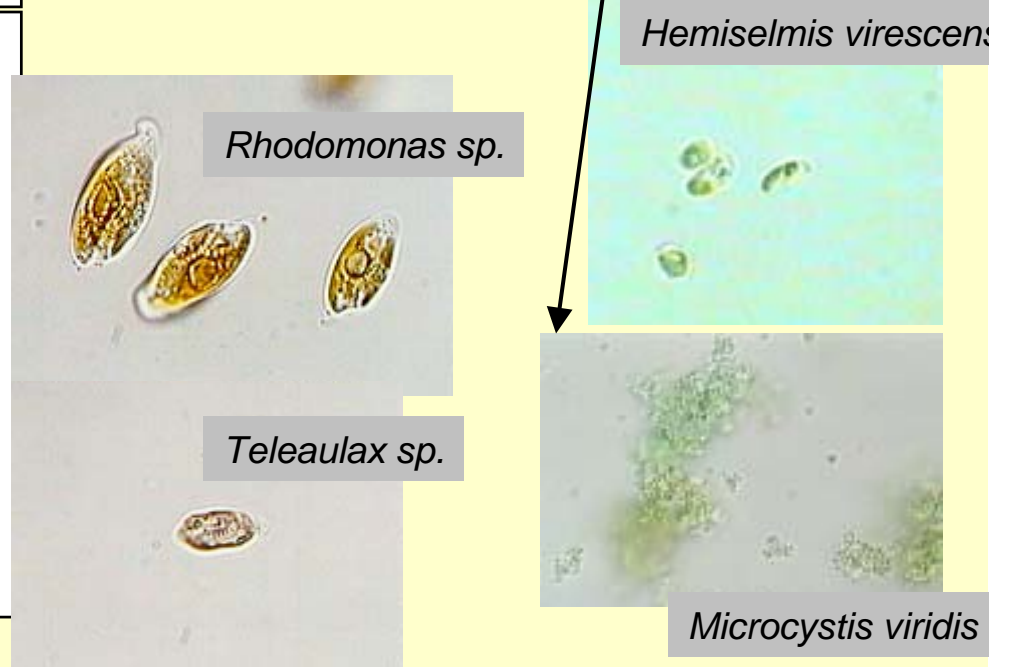
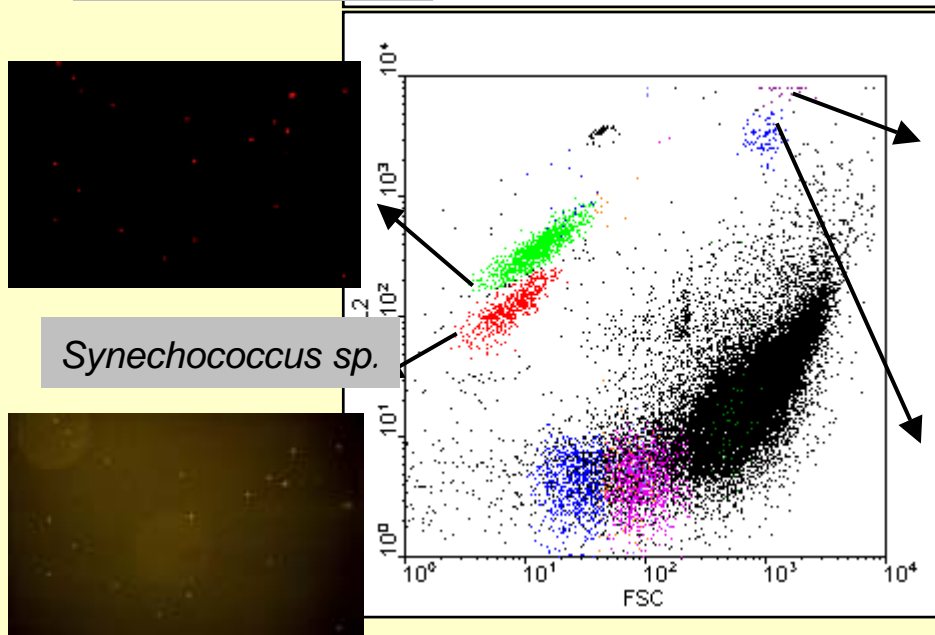
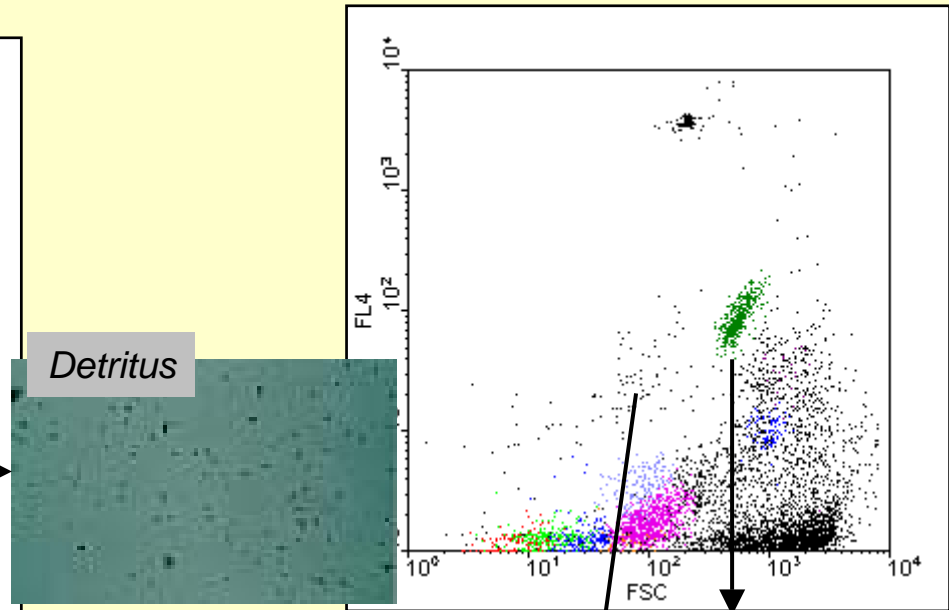
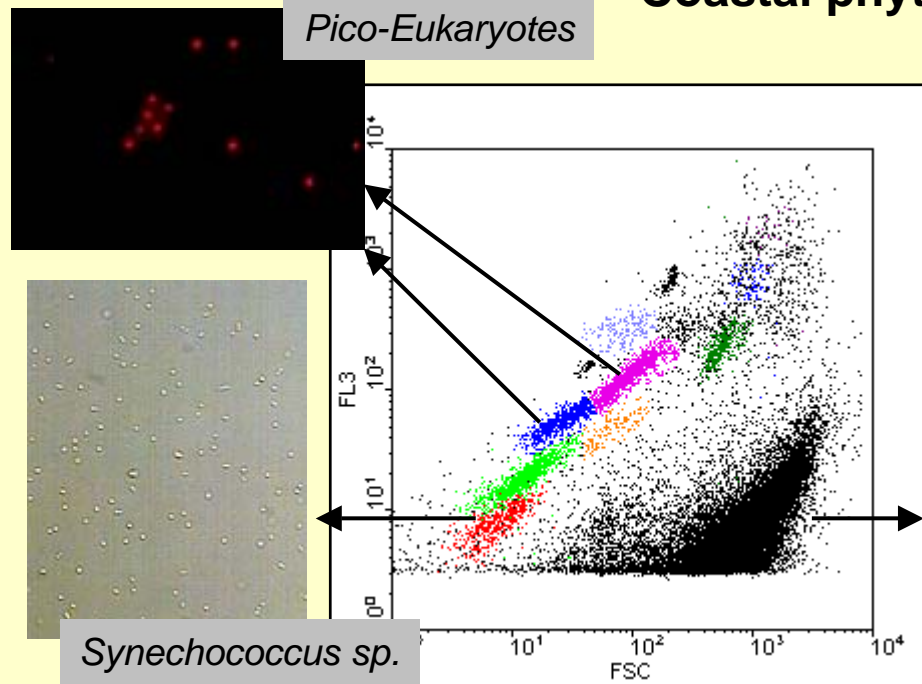
- Sort onto a microscopic slide, view immediately under microscope (drop on slide dries off very quickly!!)

→ *best suitable for larger cells (>5 μ m)*

- Sort onto black polycarbonate membrane filter membrane filter for epifluorescence microscopy or any suitable filter for subsequent analysis
- Sort into vial, fix and stain, then concentrate onto black polycarbonate membrane filter for epifluorescence microscopy or any suitable filter for subsequent analysis

→ *best suitable for smaller cells (<5 μ m)*

Coastal phytoplankton (German Wadden Sea, summer)

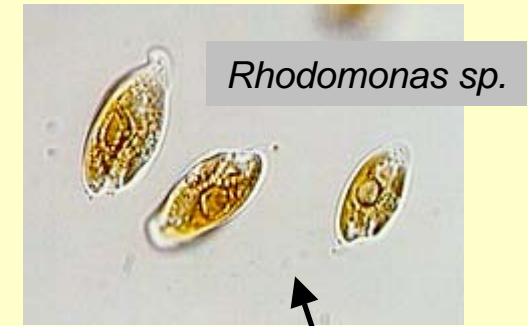


Coastal phytoplankton (German Wadden Sea, summer)

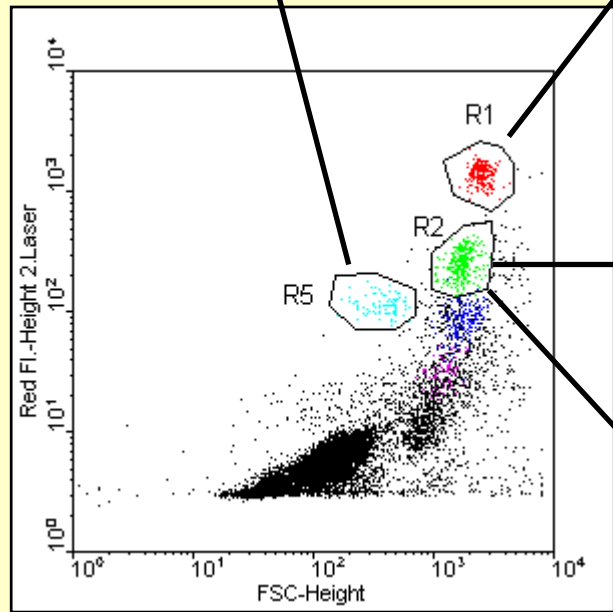
Leptocylindrus danicus



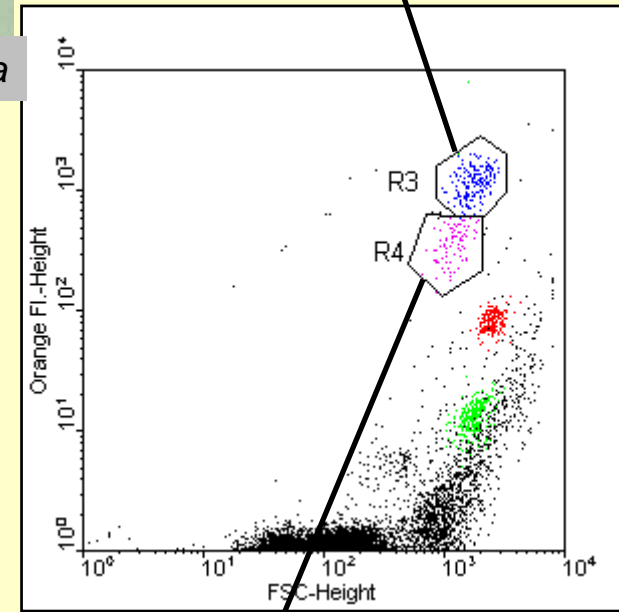
Fibrocapsa japonica



Rhodomonas sp.



Thalassiosira minima



Teleaulax sp.

Sorting procedures

For cultivation purposes:

- Sort into 24-well plates
 - Sort in „Counter mode“ (highest purity, highest accuracy)
 - Use „Auto-Stop feature“; stop after one sorted cell, then move to the next well
- only one sorted cell per well, need many plates; results approximately after 2 - 12 weeks

Sorting procedures

Problems:

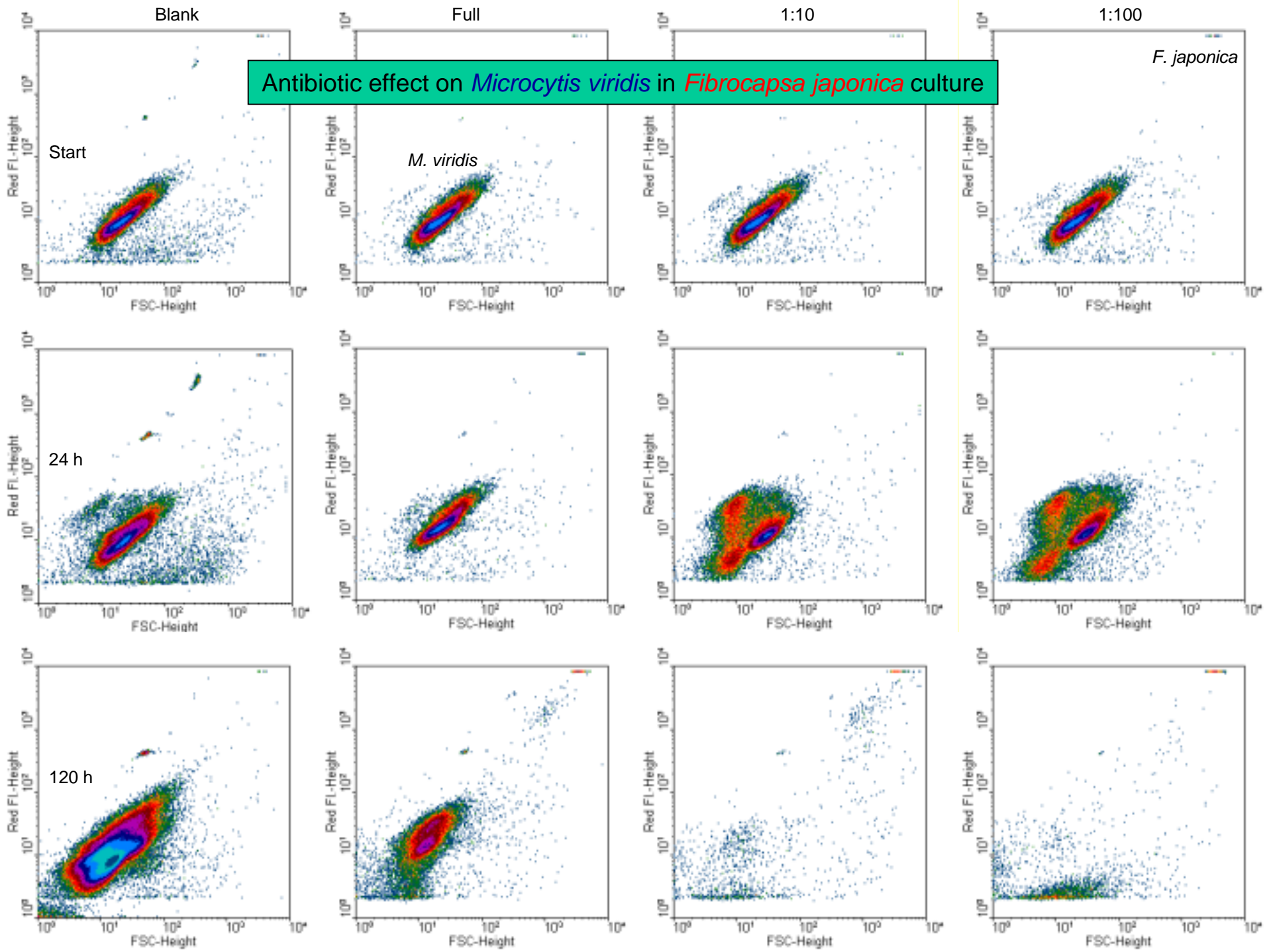
- mechanical and optical stress (high impact velocities!)

+++	Cryptophytes, Diatoms
+	Pico-eukaryotes, Cyanobacteria
-	Synechococcus
- - -	Raphidophytes, Prochlorophytes

- many weeks until clean culture is established
- „clean“ cultures difficult to obtain

Antibiotic effect on *Microcytis viridis* in *Fibrocapsa japonica* culture

F. japonica



***Fibrocapsa japonica*: Crashed Cells**

Pre-sort



Post-sort

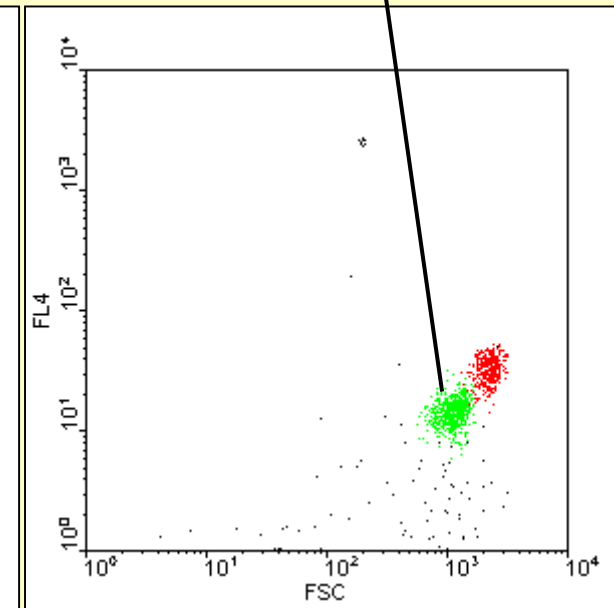
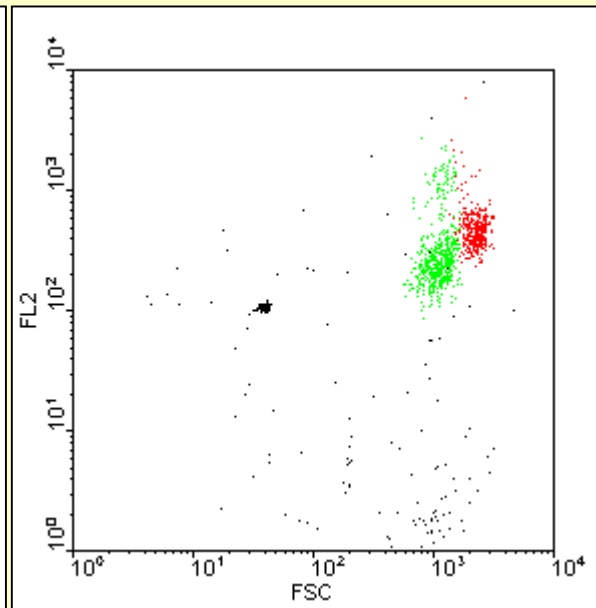
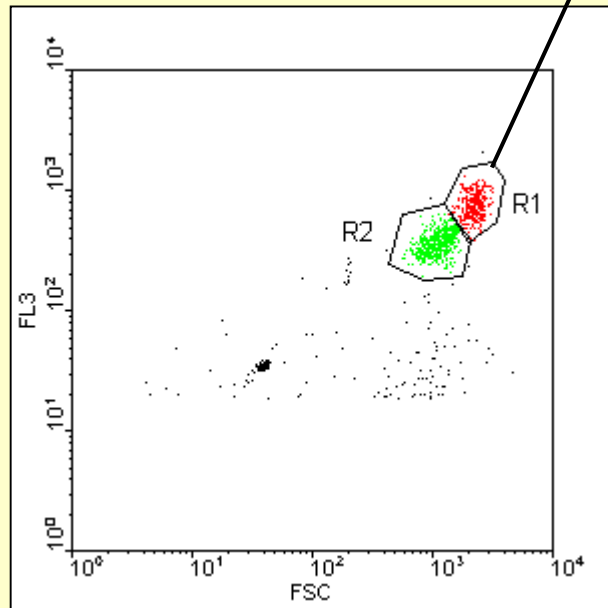
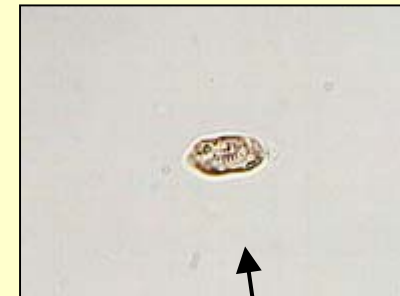


Phycocerythrine- rich Cryptophytes

R1: *Rhodomonas* sp.

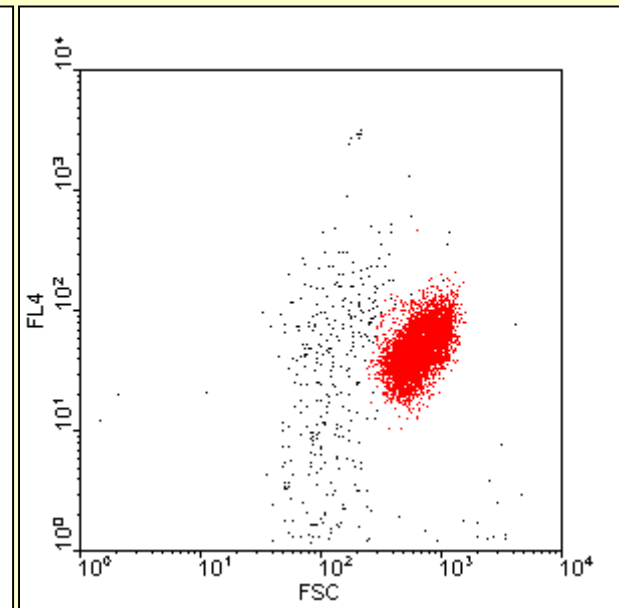
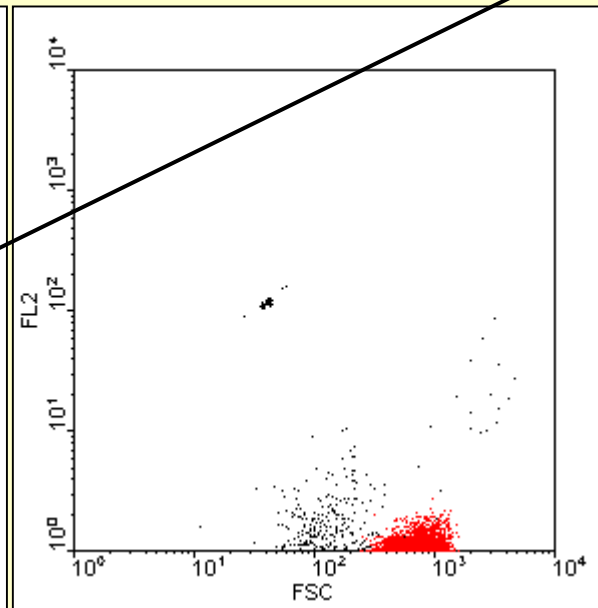
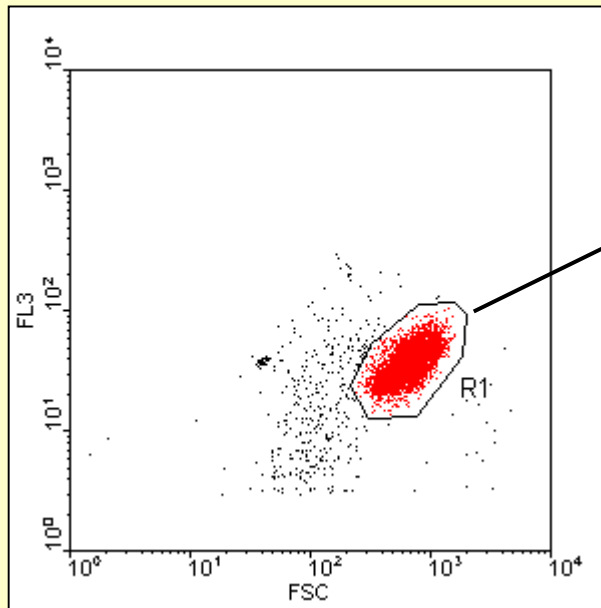
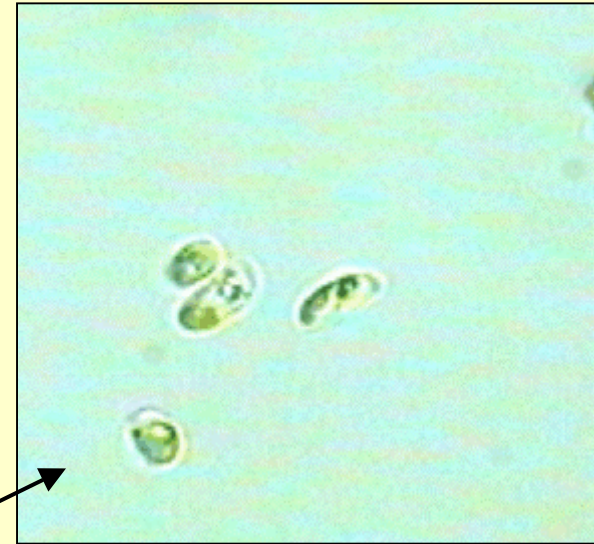


R2: *Teleaulax* sp.



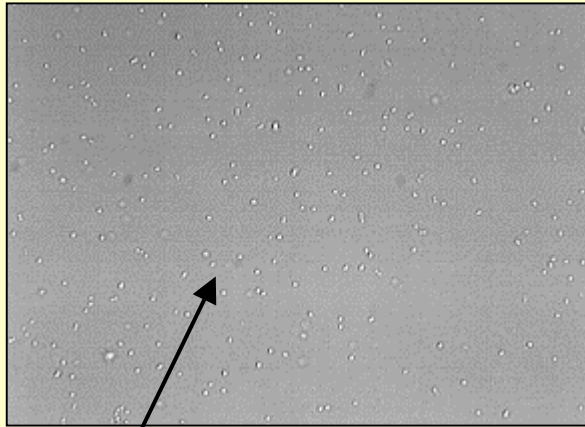
Phycocyanine - rich Cryptophyte

Hemiselmis virescens

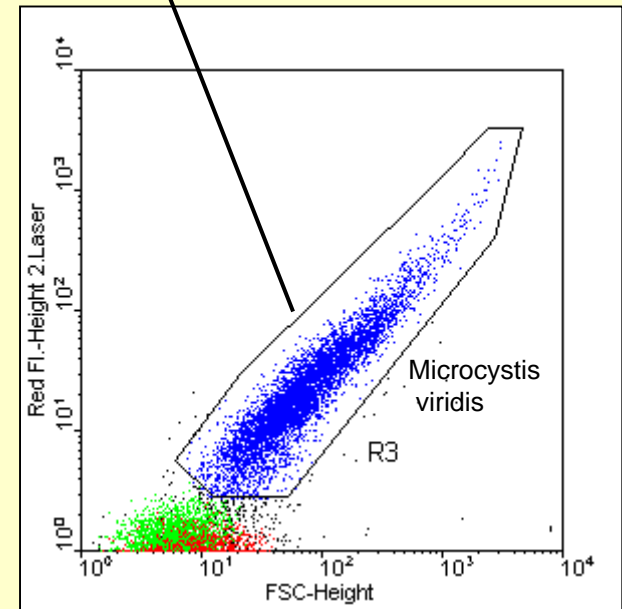
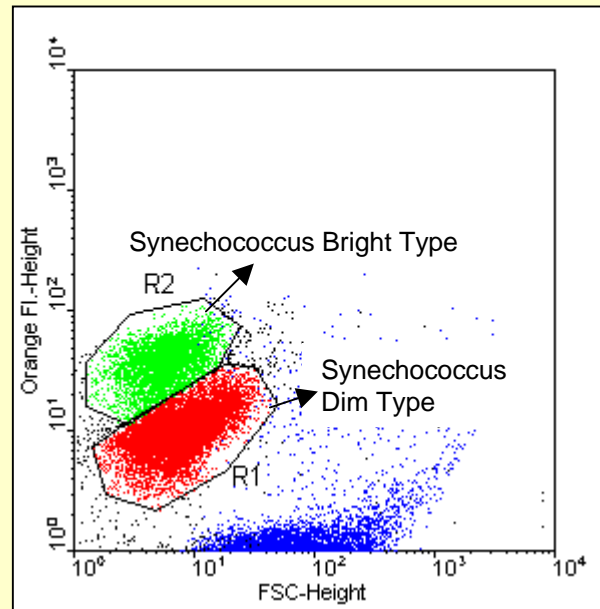
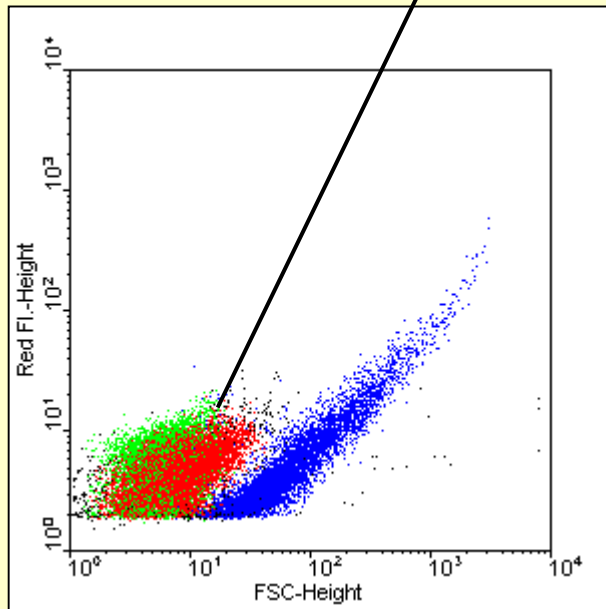


Cyanobacteria

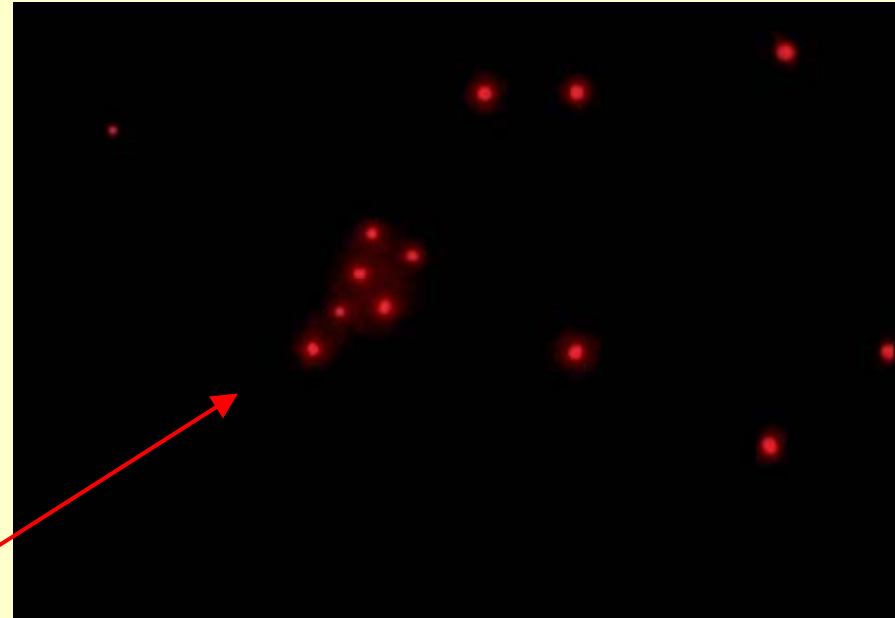
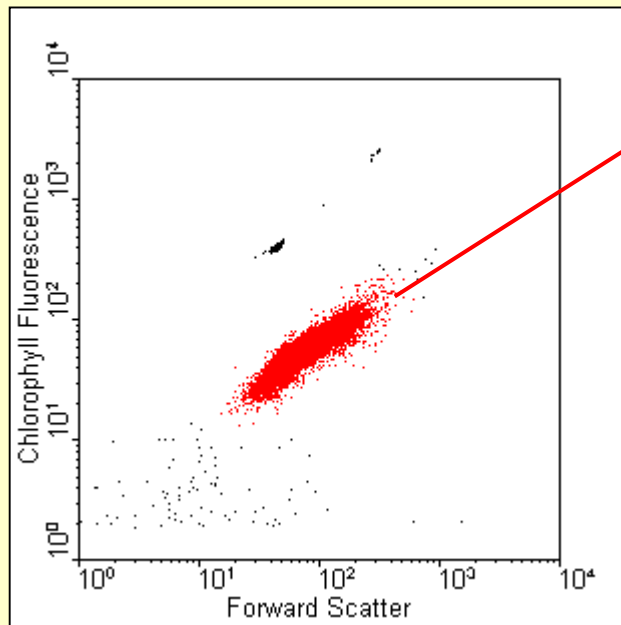
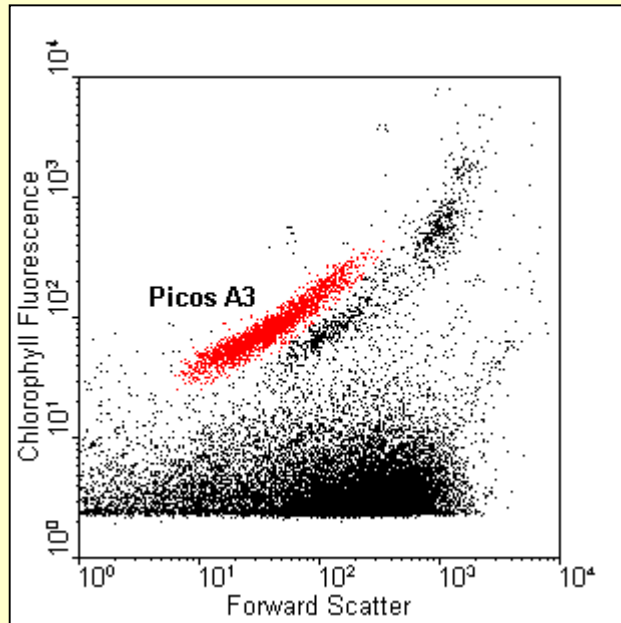
R1+ R2: *Synechococcus* ssp.
(Phycoerythrine - rich)



R3: *Microcystis viridis*
(Phycocyanine - rich)



Pico-Eukaryotes



- Eukaryotic rRNA probe positive (R. Groben, AWI Bremerhaven)
- presumably *Chlorophytes/Prasinophytes* as characterized by the presence of Chlorophyll b and Lutein (HPLC pigment analysis)