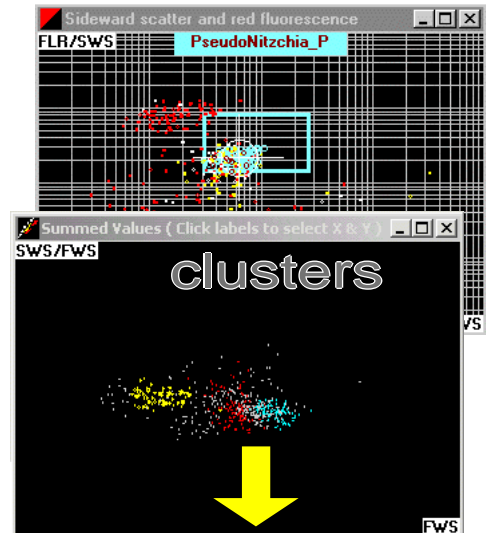
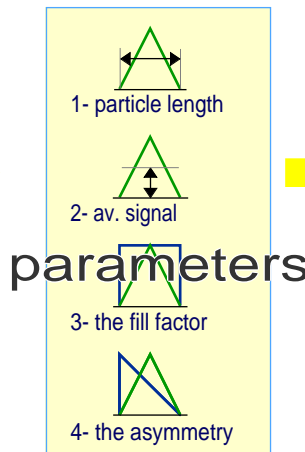
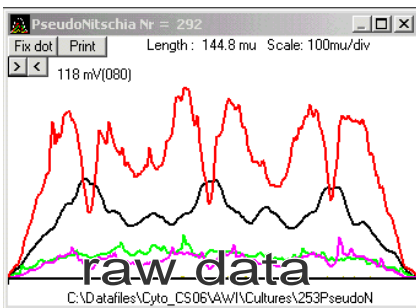


**CONCEPT** Flow cytometry and simultaneous imaging of target cells, called 'flow cytometric imaging', is an ideal procedure for plankton analysis: it combines the speed, precision and objectivity of flow cytometry with real images. The system works with 'targetting imaging' which means that instead of taking random pictures, the imaging may be targetted to specific particle types of interest. Directly after a particle has intersected the analysing laser beam of the flow cytometer a powerful realtime calculation proces starts up to analyze the fully scanned optical cytometric signature and decide whether this particle fits the preselected target group description. Within a few milliseconds the camera can be triggered to make the picture. In this way, the full species/groups discrimination power of the CytoSense particle scan data can be used seamlessly for the preselection of the target groups which is a unique and very efficient combination of imaging and high throughput particle analysis.

**DESIGN ASPECTS** Contrary to the situation in regular microscopy the sample is not fixed between a slide and a cover glass but it moves through the cuvette of the cytometer at high speed (meters per second). The optics of the imaging module therefore generate images of fast moving particles. The time of exposure of the camera target is ultra short (submicrosecond), whereas the intensity of the light source is much higher than used in standard video microscopy. The positioning of the sample in the focal plane of the camera is achieved by means of accurate hydrodynamic focusing with a very stable flow system. The camera is mounted close to the laser focus, leaving only milliseconds for the realtime decision to take an image or not. This on-the-fly comparison of the measured signals of each particle with the selected range of preset signal sizes and shapes selected in CytoClus requires a high calculating capacity which is executed by a special DSP board.

**THE IMAGE-IN-FLOW MODULE** is an add-on module for the CytoSense bench-top flow cytometer. If the CytoSense is to be used in a pressure housing or buoy for submerged or moored operation (CytoSub - CytoBuoy), the module can be used although data transfer may be limited by the chosen telemetry solutions. The targetted imaging approach allows optimum use of data transfer band width.

**OPERATION**



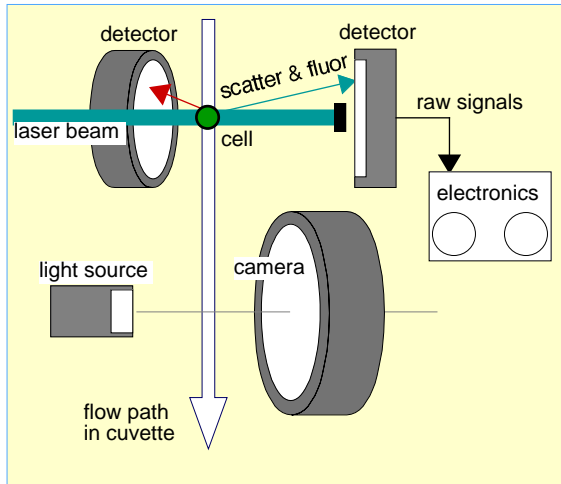
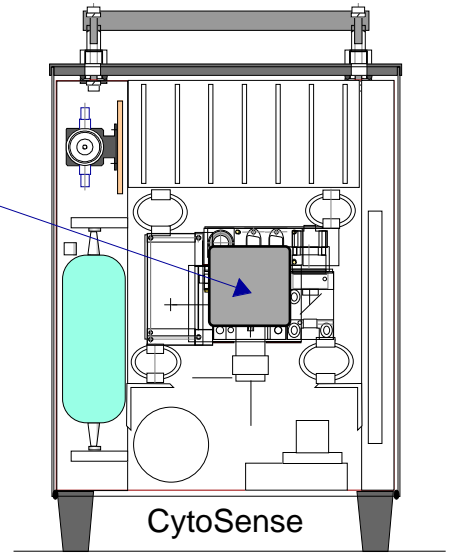
**STEP 1:** after measuring a sample with the CytoSense, use CytoClus to convert the raw data into clusters of data points representing distinct groups of particles present in the sample. Each cluster or 'Selection set' is basically a set of boundaries on the parameterized values of the measured signals.

Parameter	Minimum	Maximum	Color
Length	37.564	771.592	127.822
FWS	48.255	618.215	184.092
SWS	30.867	126.503	61.281
FLR/FWS	0.705	4.687	2.102
FLO/FLR	0.073	0.277	0.156
FLY/FLR	0.001	0.01	0.003
Fw/S Cell nr	1.462	33.653	3.758
Fw/S fill factor	0.776	1.009	0.875
Fw/S size	16.452	70.282	35.902
rel FLR size	0.388	1.244	0.651
Sw/S fill factor	0.709	1.044	0.878
FLR fill factor	0.455	0.967	0.795
Asymmetry	-0.018	0.121	0.028
Inertia	0.625	0.919	0.757

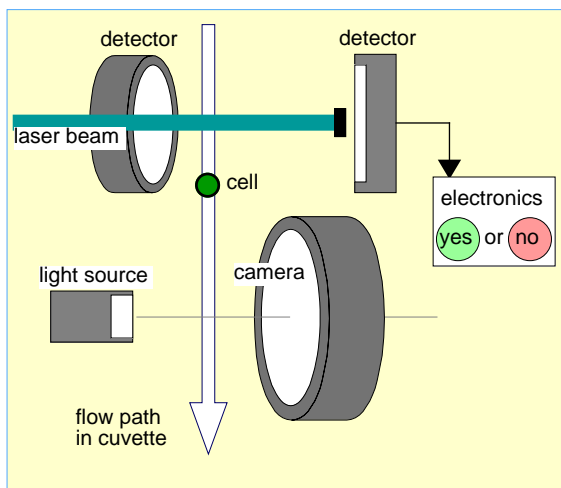
**STEP 2:** To prepare for imaging of particles belonging to a target cluster, download the selection set to the CytoSense electronics.

**STEP 3:** measure the same or a new sample with the CytoSense, and switch-on the Image-in-flow module.

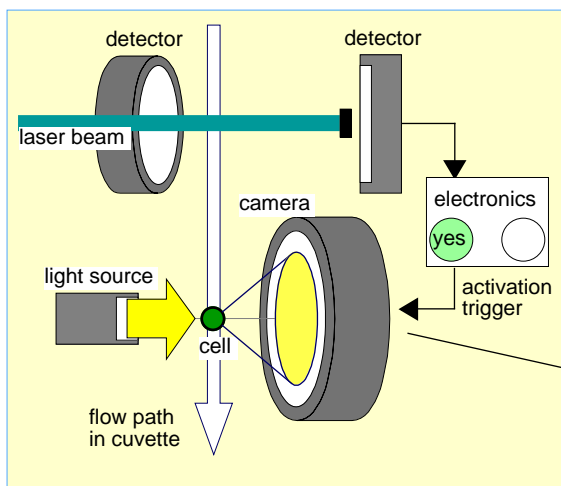
camera module



When a particle passes the laser beam the detectors are generating the output signals in the normal way. When the particle leaves the laser beam, the just acquired raw signals are parameterized directly in the CytoSense electronics.



The calculated values of the particle that just left the laser beam are being compared to the boundaries of the downloaded selection set (viz. the data analysis brochure for more information about the 'selection' of particle groups with the CytoSense scanning data format). If the parameters of the particle give a good match, 'yes' is selected - else a 'no' is selected.



If a 'yes' is selected, the electronics generate an activation trigger for the light source flash and the camera, just at the moment that the particle passes through the field of view of the camera. The image is now being stored.

IMAGES

