

CYTOSENSE Benchtop aquatic science flow cytometer BASIC

INSTRUMENT TYPE

The CytoSense is a benchtop flow cytometer for fast analysis of individual particles in a fluid. The measurements include the detection and quantification of fluorescence and light scattering emitted by individual particles flowing in single file through a sharply focussed laser beam. The digitized signal courses are preserved in the data format representing one-dimensional scans of particle morphology.

TARGET PARTICLES

type: suspended microscopic particles; particularly phytoplankton, single cells and colonies including fragile structures

size minimum: particles of appr. 1 μm (standard version) or appr. 0.4 μm (pico version)

size maximum: diameter: 700 μm (basing on standard flow cell size of 0.9x1.1 mm), length: 10 mm

density: close to water; high sinking rate ($>10\text{ mm/s}$) particles (e.g. sand) are not pumped in.

conc. maximum: appr. 10^9 particles/liter

conc. minimum: appr. 10^3 particles/liter

medium: water with salinity between marine and fresh water can be used.

ANALYSIS CAPABILITIES

- particle numbers and concentrations per subgroup or per sample;
- optical properties per particle (including optional curvature and the number of cells in a chain), per subgroup or per whole sample:

forward scatter, side scatter and fluorescence per available emission band:

10	software & computer	computer	contemporary lap/desktop with Windows OS.
		CytoUSB	software for instrument operation and data acquisition • interactive and scheduled mode • realtime pulse and scatter plots • diagnostics functions
		CytoClus	data analysis software for flow cytometry data cluster analysis with additional functions for: • individual particle profile visualization and parameterization: up to 8 parameters from each detector output; • display of images coupled to profile data (in case of imaging-in-flow module)
11	hardware	Laser	20 mW CW high quality laser module (various wavelengths and power
		Focussing	flat light distribution to allow a high sample flow rate at undeteriorated data quality and the use of the instrument on moving platforms such as
		Flow cell	suprasil quartz cuvette with 2-stage hydrodynamic injection. Standard orifice is 0,9x1,1 mm - a different version is mounted instead if item 15 is ordered. Low fluid shear design for fragile particles and filaments.
		Sheath fluid	sheath fluid principle with clean particle free carrying fluid recirculating with high quality microgear pump with electronic speed control, fiberflo capsule filters (0.45 and 0.1 μm), automatic venting and pressure safety relief, electronic pressure sensors for system and filter diagnosis. Color
		Sample dosing	valveless, volumetric, semi-continuous tubing pump with 1 mm ID (other diameters available). Direct concentration readout, automatic speed selection option. 30 - 0,1 $\mu\text{l/s}$ dosing range with continuous velocity feed back. Movement and pressure sensing and calibration wizard.
		Main detection module	• 1) photodiode for forward scatter detection including power supply and EMC shielded preamplifier. • 2) photomultiplier tube (PMT) for side scatter or fluorescence including power supply and EMC shielded preamplifier. • 3) photomultiplier tube (PMT) for side scatter or fluorescence including power supply and EMC shielded preamplifier.
		Data acquisition	full pulse digitizing electronics (4MHz) and realtime capturing of all detector output signal courses for morphological particle analysis (nano- and microplankton). Traditional cytometry outputs for picoplankton.
		Power & control	Special interface board for multiple powering options and full computer control of electro/opto/mechanical components.
		M&M housing and packing	User operation and maintenance manual; set of user inspection and Cylindrical instrument (interchangeable with SUB / BUOY housing). Suspended in aluminium frame with high quality antivibration supports. Complete set packed in Proflite heavy duty flight case 52x52x75 cm.

Options			#REF!
12	extra channel	green/yellow fluor detector	Channel PMT for yellow/green fluorescence detection, including a high voltage power supply, a dichroic mirror/color filter combination in optical mounts, a shielded signal preamplifier and software control.
13	extra channel	orange fluor detector	Channel PMT for orange fluorescence detection, including a high voltage power supply, a dichroic mirror/color filter combination in optical mounts, a shielded signal preamplifier and software control.
14	extra channel	curvature detector	One FWS 'curvature' photodiode to detect curved and/or spiral shaped particles. Includes a low voltage power supply, beam splitting and polarization optics and optical mounts, a shielded signal preamplifier and software control.
15	different fluidics bore	wider or narrower flow cell	Special flow cell with larger square cross section (1.5 x 1.5 mm) or narrower (0.7 X 0.7 mm), adapted two-stage injector with adapted needles, adapted sample pump tubing and adjusted sheath fluid pump.
17	sampling Tpiece	perspex unit	For aseptic, sand and bubble free sampling from a (sea) water supply hose (with connectors for various hose diameters). Perspex design for visual inspection and easy cleaning.
17A	multiple sampling unit	selector system to draw samples from multiple tanks	Miniaturized sampling system with one 10 ports 1,5 mm internal orifice selection valve and tubings, pump and flush valve. Various tubing widths, valves and larger lengths possible.
18	picopl. option A	higher power laser	Instrument settings (including PMT yield, sheath and sample flow rates, etc.) optimized for small particles, and mounting of a 50 mW laser instead of a 20mW laser and specific wavelength. Note that this renders scanning
19	picopl. option B	switch unit with higher power laser	Circuitry and software that allows 'mouse click' switching (also preprogrammed) between 'pico'sensitivity and 'low' sensitivity for the analysis of bigger cells and colonies , and mounting of a 50 mW laser
20	picopl. option C	basic module + higher power laser	Main optical mount including mounts for PMT's, mirror and filter holders - additional main electronics grabber board. Also includes the installation of 50 mW instead of 20 mW laser.
Options continued			#REF!
21		SideScatter detector	includes high voltage power supply, appropriate optical filters (dichroic mirror, color filter) and optical mounts, a shielded signal preamplifier and
22		YellowFluor. detector	includes high voltage power supply, appropriate optical filters (dichroic mirror, color filter) and optical mounts, a shielded signal preamplifier and
23		OrangeFluor. detector	includes high voltage power supply, appropriate optical filters (dichroic mirror, color filter) and optical mounts, a shielded signal preamplifier and
24		RedFluor. detector	includes high voltage power supply, appropriate optical filters (dichroic mirror, color filter) and optical mounts, a shielded signal preamplifier and

Modules			#REF!
30	imaging-in-flow		Upgrades a CytoSense flow cytometer with TARGETED IMAGING capabilities. It allows the imaging of particles belonging to a preselected type out of a mixed particle flow basing on real time analysis of the measured signals from the cytometer (set of pulse profiles). • illumination: Osram SPL-LL85 laser diode, 850 nm, flash 50-100ns; directly mounted on flow cell • camera: triggerable cmos camera (Pixelink PL-A741-BL), shutterspeed $\leq 40 \mu s$; frame rate 25 fps. • frame size: 1280x1024 pixels $6.7 \mu m$ square • objective: custom 10x magnification, focus focal depth ca. 17 μm @ 2 μm spot diameter; • data storage: raw photos to disc. display in CytoClus • triggering: random or selected (CytoClus input). • targeting: the measured pulse data of each particle is evaluated before photographing to decide to make a photo or not. • matching: for each photo the corresponding measured laser pulse scan of the particle is shown • software integrated in the CytoUSB and CytoClus applications. • geometry: ultra-compact design to fit inside the CytoSense housing (also fits in CytoSub and CytoBuoy).
40	2-laser option	mounting of 635nm laser + focusing optics	Upgrade providing an extra laser (high quality red 635 nm laser) to allow the detection of fluorescence originating from an excitation wavelength different from the main laser in the CytoSense, while using the existing PMT. Mounting of optical beam steering to place the focus of the extra laser just below the existing focus of the main laser (or superposed in case of item 41). Mounting of dichroics/notch filters for required spectral separation in the detection unit. The output signal scans of each particle show 2 peaks for particles < 10μm - the first peak originates from the 488 laser excitation and the other from the 635 nm laser. These peaks can be separated in CytoClus. For larger particles there is less separation (viz. item 41).
41		HF modulation + extra data channel	Separate channel for 635 nm excited fluorescence. The second laser is set to high frequency 4MHz modulation mode (125 ns ON 125 ns OFF repeating sequence). The detector output is fed into a frequency deconvolution board which splits the signals into a low frequency component (existing red emission excited by main laser) and a high frequency component (red emission excited by the extra laser). Extra detection channel on the grabber board. This extra option adds some noise to the signal making it less suited for weakly fluorescent particles.
42		other wavelength 2nd laser	mounting of a 405 nm laser or 532 nm laser (etc) instead of a 635 nm laser.
50	sorter module		Very large orifice device coupled to the 1 mm² CytoSense flow cell with a piézo-actuator driven fluid switch. The maximum actuator frequency is ca. hundred sorting events per second. A large part of the sheath fluid runs continuously into the sort flow channel over a polycarbonate filter to let the water pass through and retain the sorted particles. The module consists of the sorting hardware, a filter holder, fast particle data comparison and triggering circuitry & software (DSP board with preselection of target particles based on realtime scanned shape calculations from CytoClus). Please note that the sorter is too large to fit in the CytoSense / CytoSub housings and is a lab based instrument. The sorter can not be combined with an imaging-in-flow module in the same instrument.

Submersible platform			#REF!
60	SUB pack deep	SUB instrument modification "deep"	This package transforms a CytoSense bench top flow cytometer into a 'CytoSub' instrument for submerged operation down to 200 meter. It should be ordered together with a CytoSense instrument or as an upgrade for an existing CytoSense instrument. Includes: • a 200 meter rated aluminium pressure housing 320x500 mm • a high pressure sample loop section with 1.5 mm ID PEEK tubing with a circulation pump and a 6-port/2-position fluid switch valve • electronic controls and adapted operation software • embedded computer with hard disc integrated in CytoSub, • an external pressure sensor to monitor water depth • SubConn underwater electrical connectors and high pressure fluid
61		250 m cable solution	Cable solution for remote powering and control/data retrieval of the submersed CytoSub instrument. • 250 meter underwater cable (MacArtney) • a moulded Subconn connector on the instrument side • an extended ethernet converter set, integrated in the CytoSub and in the connector box, • a short (4 m) replacement cable for laboratory use, • a cable connection box for easy connection of the computer and net power.
62		battery pack solution	The battery pack consists of a 200 meter rated pressure housing of 320x500 mm (light weight aluminum or stainless steel), filled with leakage-free lead-acid batteries for powering of a CytoSub. Including a 4 m under water cable with mating plugs, and a recharging device and connector.
Submersible platform continued			#REF!
65	SUB pack shallow		This package transforms a CytoSense bench top flow cytometer into a 'CytoSub' instrument for submerged operation in shallow depths: down to 20 meter. Includes: • Pressure housing for outdoors use and submerged use of CytoSense (maximum 20 meter). It should be ordered together with a CytoSense instrument or as an upgrade for an existing CytoSense instrument. Consists of cylinder, top and bottom cap and O-rings, and bulkhead connectors for cables and fluids, safety moisture sensor. Stainless steel or light weight aluminum versions available. • Cable solution for remote powering and control/data retrieval of the submersed CytoSub instrument. Includes a 40 meter underwater cable
69		mounting rack	A stainless steel mounting frame with stainless steel wire rope shock/vibration isolators for safe mounting of the CytoSub and submersible use. Dimensions: 410 x 410 x 730 mm (w x d x h)
Buoy platform			#REF!
70	BUOY	buoy	This package transforms a CytoSense bench top flow cytometer into a 'CytoBuoy' for moored operation. It should be ordered together with a CytoSense instrument or as an upgrade for an existing CytoSense instrument. The module includes: • a 90 cm buoy hull (modified Oceanor SeaWatch mini 'old style' hull) • 8 solar panels system with rechargeable batteries, • flash light, • electronics including timer clock and embedded computer • telemetry (standard: radiomodems over line of sight, other options such as WiFi depending on local situation); • sample line bulkhead connector. NOTE: the instrument can be taken out and inserted easily for switching between benchtop and moored use.
71		argos transponder	mounted Argos beacon for positioning (the customer needs to register with CLS-Argos)

SERVICES		#REF!
0	1 YR Warranty	CytoBuoy b.v. warrants the equipment as being free from defects. The warranty period is 1 year from the date of delivery and covers free of charge parts and labour. Repairs are done at our works. Shipping to our works is paid by the customer, the return shipment same way is paid by CytoBuoy b.v. For on-site repair 50% of the travel and subsistence is paid by the customer. Notes: 1- customer performs at least one calibration during the 6th month after delivery, using microspheres & protocol provided by us; 2- failure of electronic and/or moving parts can not be excluded totally. After the warranty period CytoBuoy b.v. will repair a malfunctioning instrument at cost price. 3- please note that a new laser may cost up to 10,000.- euro depending on the type.
100	Delivery	Shipping typically air cargo (in case of a buoy sea transport is more economical)
101		transport insurance insurance through CytoBuoy b.v. policy
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103		Training at our works we advise a 3-5 day visit to our works for multidisciplinary training - flow cytometry fundamentals - instrument operation; - maintenance and basic servicing; - data analysis.
104		Installation & demo/training onsite: TBD
110	Servicing call	Servicing is best done at our works where we have all expertise and tools. We recommend at least 1 servicing annually. Standard servicing includes inspection and checkup/cleaning of optics, pumps and electronics, the exchange of disposables such as filters and sampling tubing, cuvette cleaning and calibration. Excluding shipping. CytoSub and CytoBuoy platform instruments can be shipped in their labhousing (the buoy itself is not included in this servicing offer). For on-site servicing the travel and subsistence are paid by the customer.
120	1-YR S&W Extension	One (1) year extended warranty. Includes one full servicing at the beginning of the year and warranty to cover extra maintenance and replacements/repairs whenever necessary during the remainder of the year. The customer performs at least one calibration during the 6th month, using microspheres & protocol provided by us; Includes free software upgrades of all our relevant programs whenever they come available. The total will be invoiced after the servicing call. Excluding shipping. For on-site repairs or servicing the travel and subsistence are paid by the customer. This item can be ordered as single item (total warranty 2 year) but also in twofold (total warranty 3 year) or threefold (total warranty 4 year).
150	EasyClus basic application calibration module fractioning module	Easyclus© v1.14 basic program: Manual and automatic clustering on the basis of scatter plots, automatically processed selection sets and database clustering and recognition. Calibration Controll chart v1.6: The automatic short and long term registering, visualizing and warning according to warning limits on the basis of reference material (e.g. beads) in order to controll and validate the instrument to perform between predefined limits. Fractioning module v1.0 Total concentrations of FCM analysis such as chlorophyl fluorescence (biomass indicator) or light scatter (biovolume) is expressed as a function of particle size fractions in cumulated sizefractions barplots.