Flow Cytometry– Technology Review and State of the Art

TechSurge– Ocean Sensors

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Peter Lopez

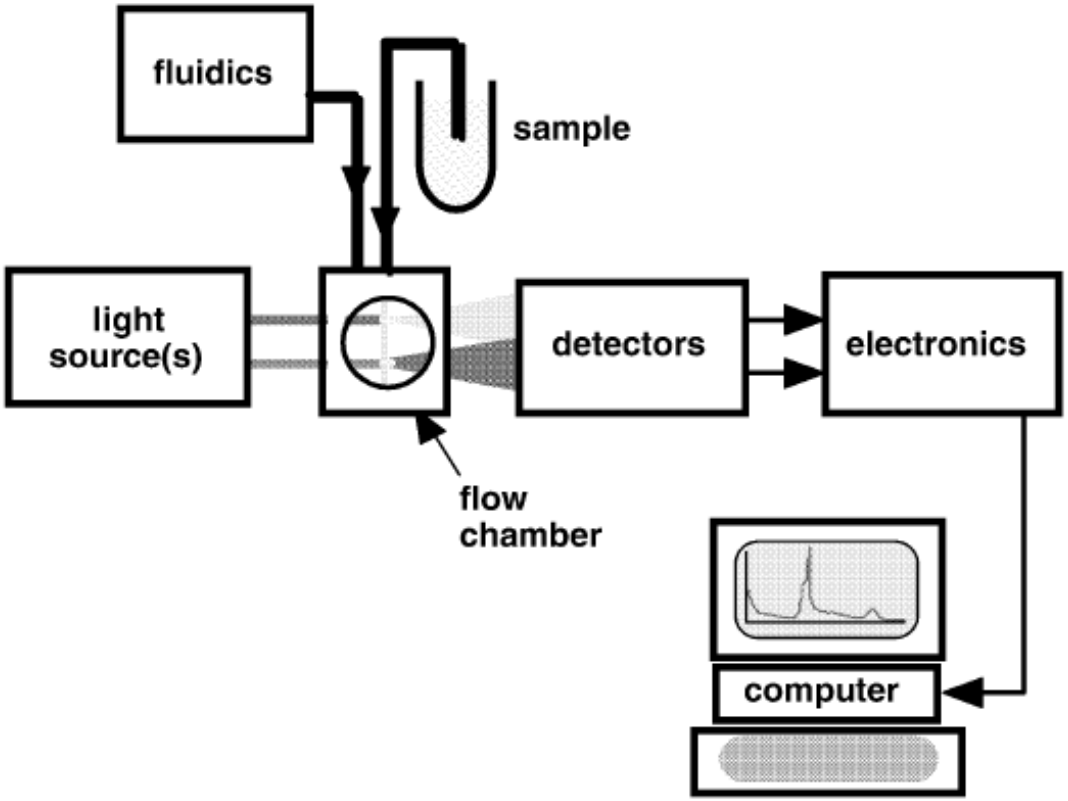
NYU School of Medicine
NYU LANGONE MEDICAL CENTER

Flow Cytometry

- A robust technology for characterizing individual particles in fluid at a rapid rate (10's of thousands per second)
- Multiple discrete parameters can be measured from each particle
- Parameters are typically derived optically– light scatter, fluorescence, although electrical impedance (Coulter volume) is available.
- Fluorescence and Coulter volume measurements are very quantitative, light scatter semi-quantitative

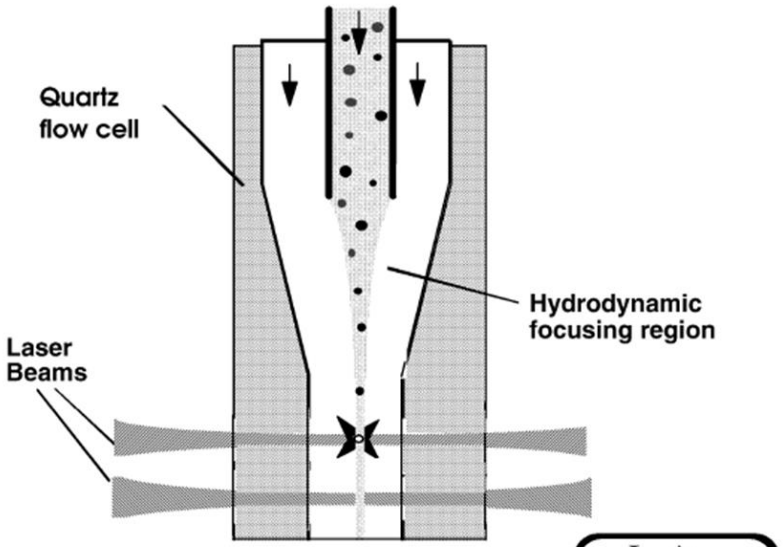
Flow Cytometer Flavors

- Analytical flow cytometer ("analyzers")
- Cell sorters are analyzers that can purify rare subpopulations (<1%) viably to high purity (99%)

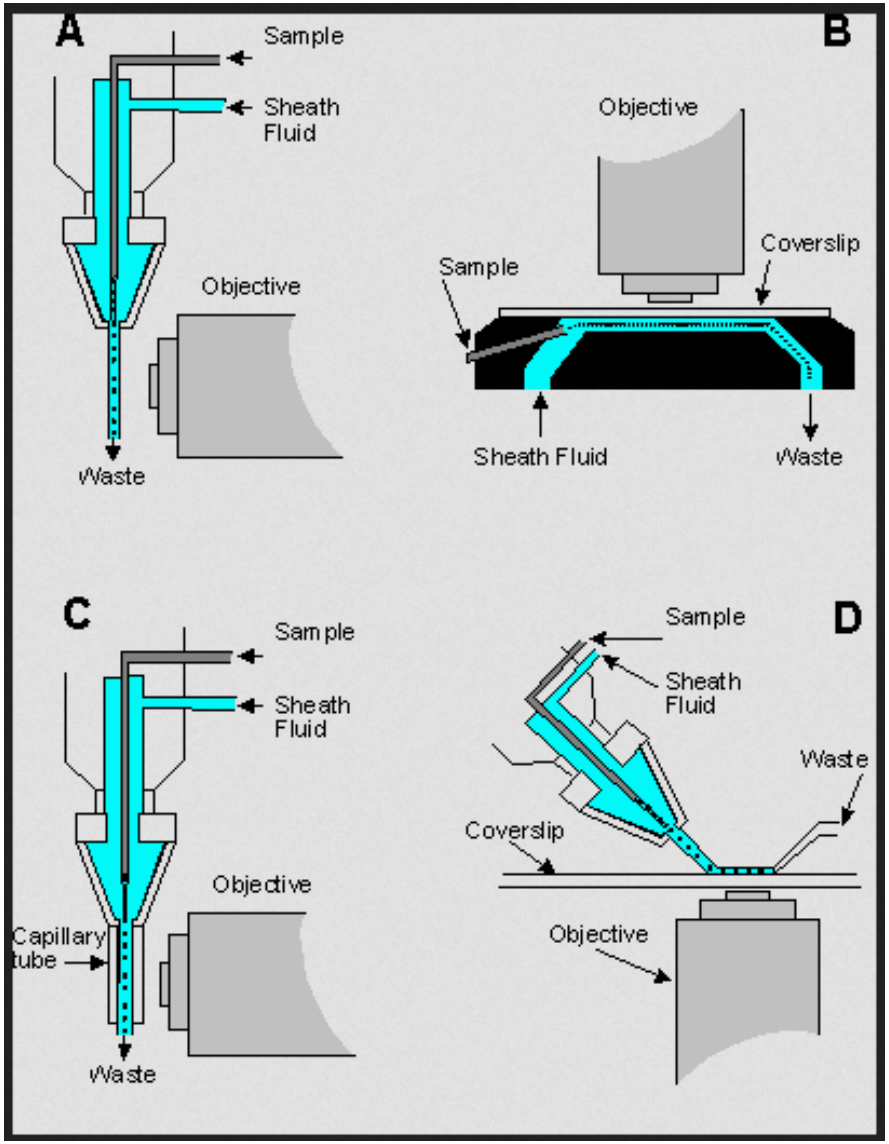


Flow Cytometer Block Diagram

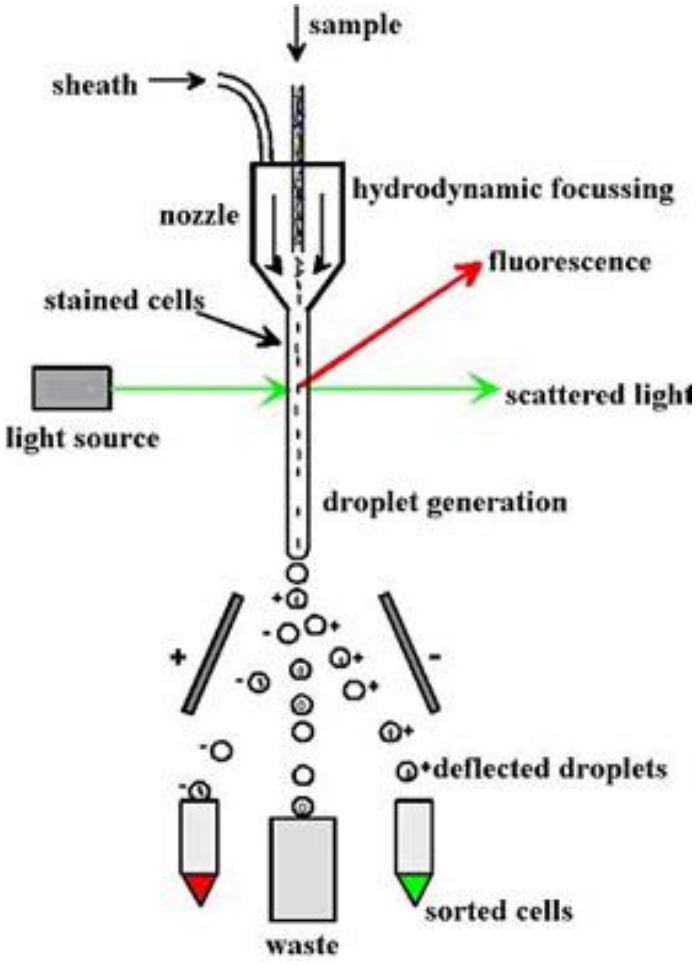
Enclosed flow chamber showing hydrodynamic focusing



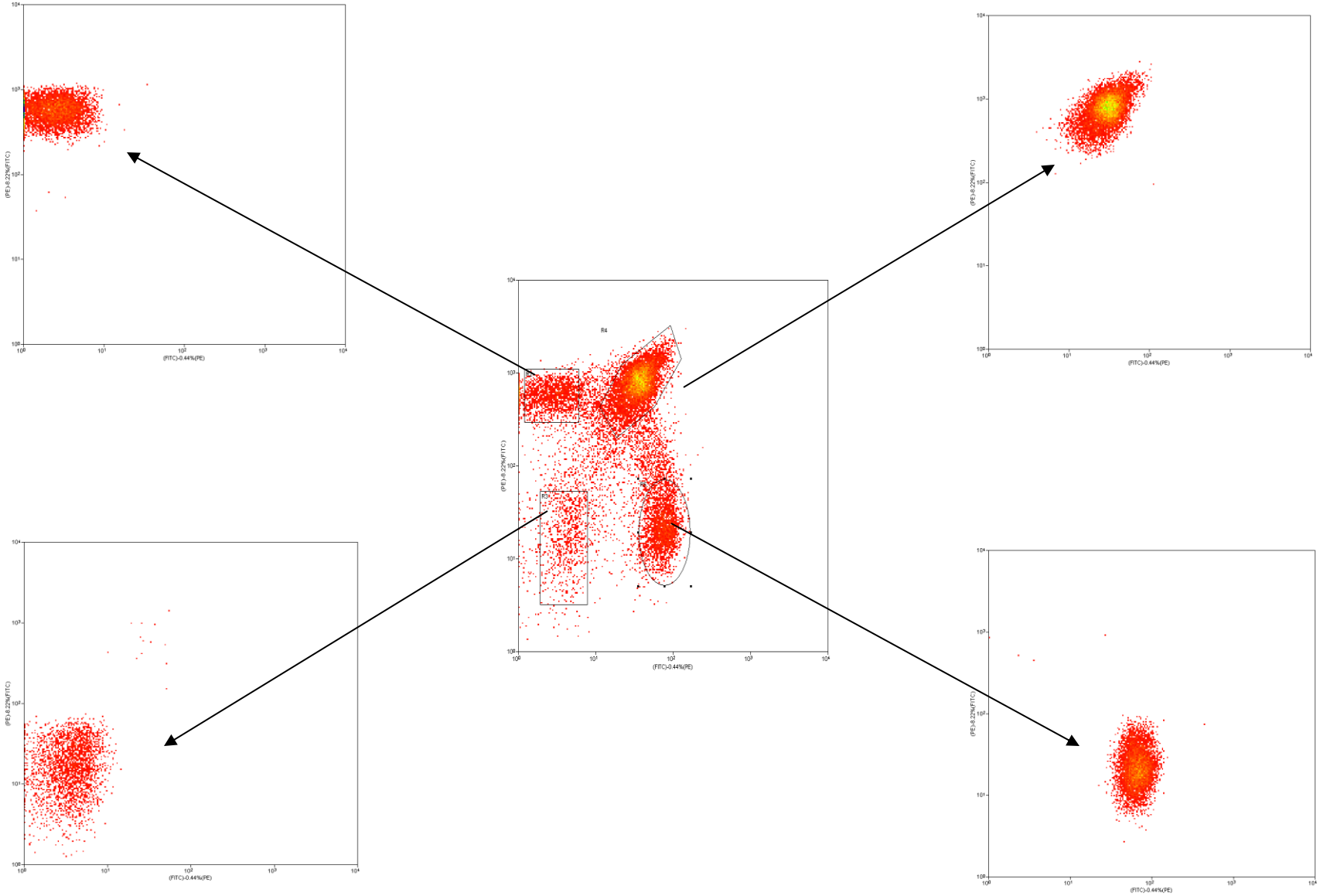
Los Alamos National Flow Cytometry Resource



Flow Cytometer – Cell Sorting



4-way sorting



Typical Commercial Cytometers

- Particle size detection (without fluorescence)--minimum 0.2 microns. Typically not larger than 50 microns, although some specialized systems can run samples that are in the 1mm size range.
- Fluorescence detection limit– typically the equivalent of 50 fluorescent molecules of fluorescein.
- Options exist for commercial instrumentation to improve detection limit

Cytometric applications

- Antigen density, immunofluorescence
- Fluorescent protein detection (GFP)
- DNA, RNA content, cell cycle
- Apoptosis
- Protein expression and total protein content
- Enzyme activity
- Molecular proximity (FRET)
- Membrane potential
- Cytokine receptors
- Microparticle/microvesicle detection and analysis
- Membrane fluidity – fluorescence polarization
- Drug uptake and efflux – multidrug resistance and “SP” cells
- Phagocytosis
- Viability
- Cell trafficking, tracking, proliferation
- Microbial, botanical and marine applications
- Changes in intracellular pH, calcium, glutathione
- Changes in oxidative burst
- Multiplexed bead assay– soluble
- Effector/target cell conjugate assay
- Cell secretion assay using gel microdrops
- Analysis of cellular organelles– mitochondria, chromosomes

Cytometry and Marine studies

- Flow cytometers on a ship
- Flow cytometers in the water

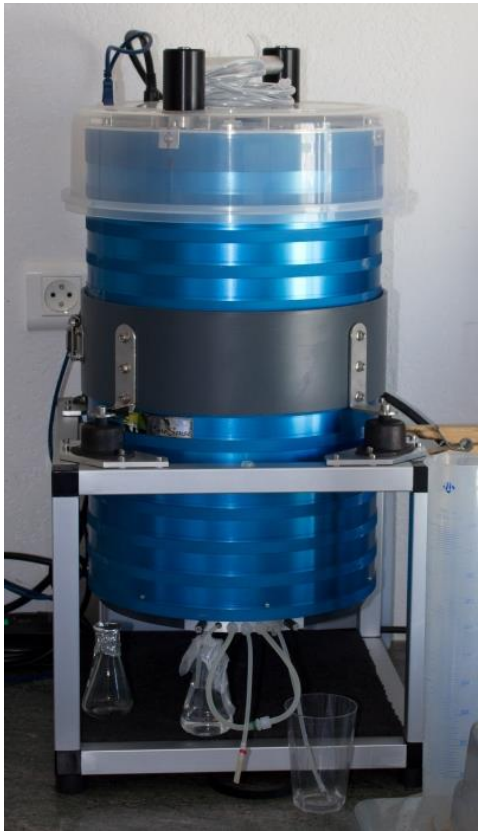
SW Chisholm, RJ Olson, ER Zettler, R Goericke, J Waterbury, and N Welschmeyer (1988).
A novel free-living prochlorophyte abundant in the oceanic euphotic zone.
Nature 334(6180): 340-343.

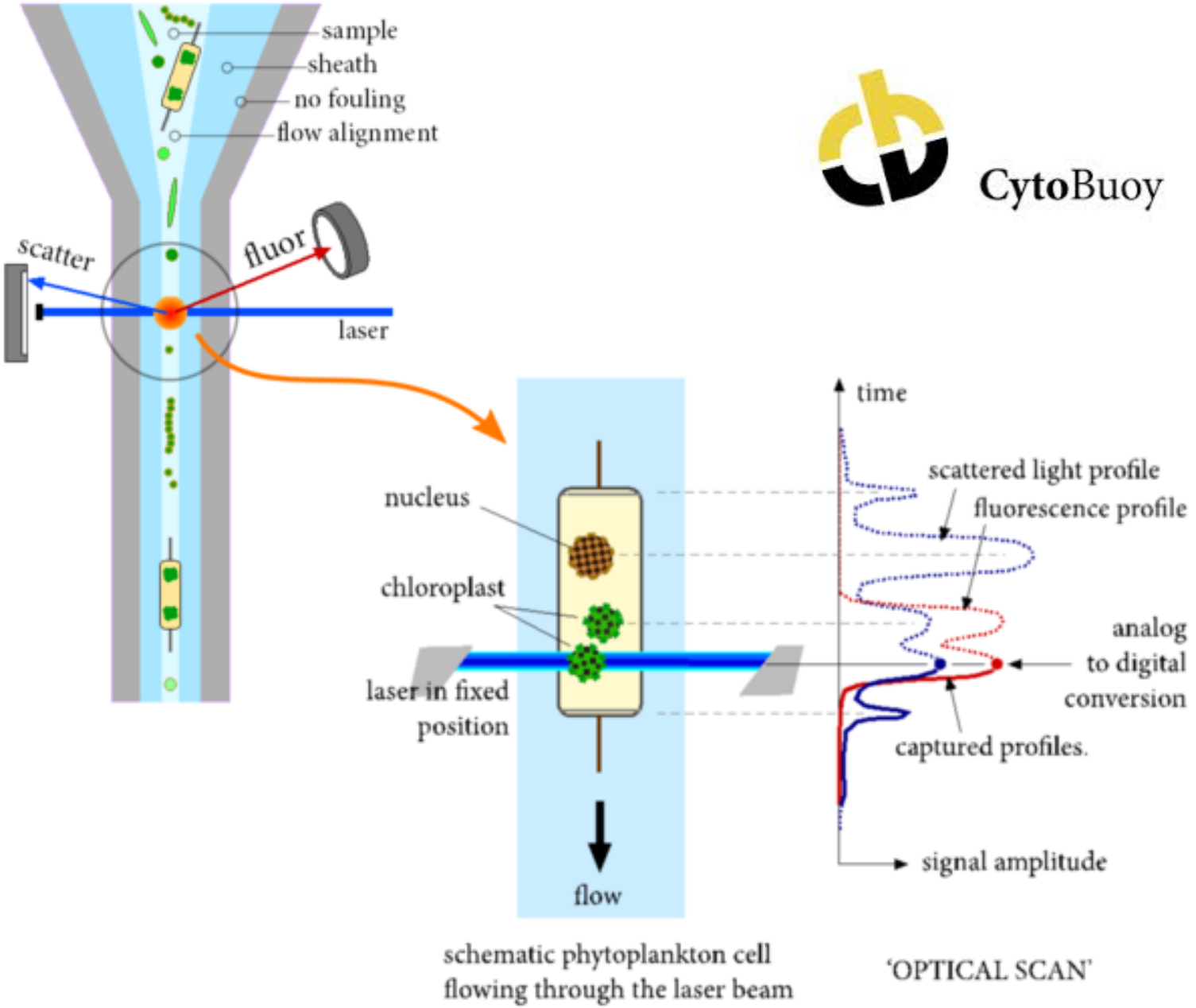
RJ Olson, D Vaulot, and SW Chisholm (1986).
Effects of environmental stresses on the cell cycle of two marine phytoplankton species.
Plant Physiology 80:918-925.

RJ Olson, SW Chisholm, SL Frankel, and HM Shapiro (1983).
An inexpensive flow cytometer for the analysis of fluorescence signals in phytoplankton: Chlorophyll and DNA distributions.
J. Exp. Marine Biology Ecol. 68:129-144.

RJ Olson, D Vaulot, and SW Chisholm (1985).
Marine phytoplankton distributions measured using shipboard flow cytometry.
Deep-Sea Research 32:1273-1280.

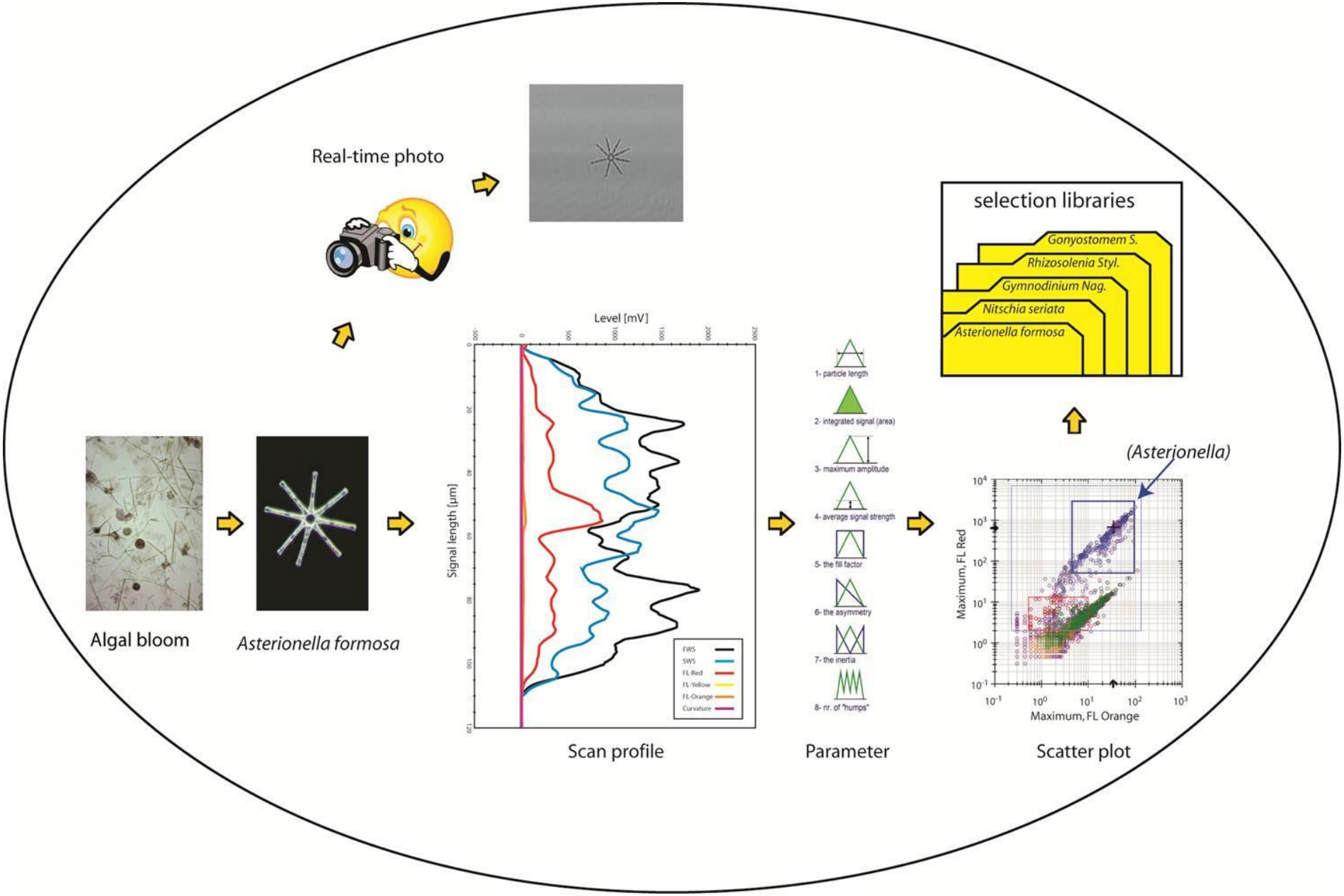
CytoBuoy





CytoBuoy

The principle of flow cytometry. One of the unique features of the CytoSense is a precise flow cytometric scan that is made of every particle.


Real-time photo
selection libraries
Gonyostomem S.
Rhizosolenia Styl.
Gymnodinium Nag.
Nitschia seriata
Asterionella formosa
Level [mV]
Signal length [µm]
FWS
SWS
FL-Red
FL-Yellow
FL-Orange
Curvature
1- particle length
2- integrated signal (area)
3- maximum amplitude
4- average signal strength
5- the fill factor
6- the asymmetry
7- the inertia
8- nr. of "humps"
(Asterionella)
Maximum, FL Red
Maximum, FL Orange
Algal bloom
Asterionella formosa
Scan profile
Parameter
Scatter plot

Flow Cytometry--State of the Art

- Smaller systems– limited capability or specialized application
- Acoustic focusing– for better alignment of particles in flow and improved CV
- CyTOF– flow cytometry and mass spectroscopy combined
- Spectral unmixing– a “new” method for multiple-color fluorescence detection
- Large particle sorting
- Imaging and real time image analysis in flow
- Microfluidic applications

Becton Dickinson Accuri

Handyem HPC-100

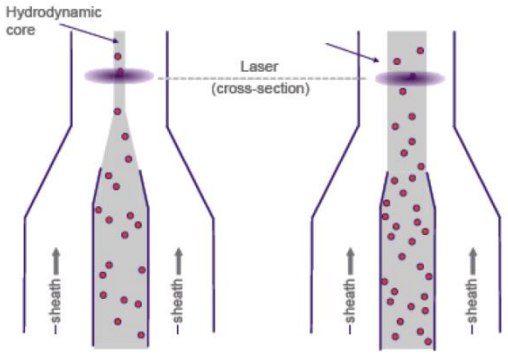
Partec Cube

Miltenyi MACSQuant

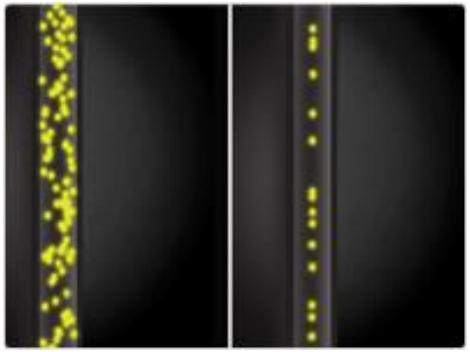
Millipore Guava

Orflo Moxi Flow

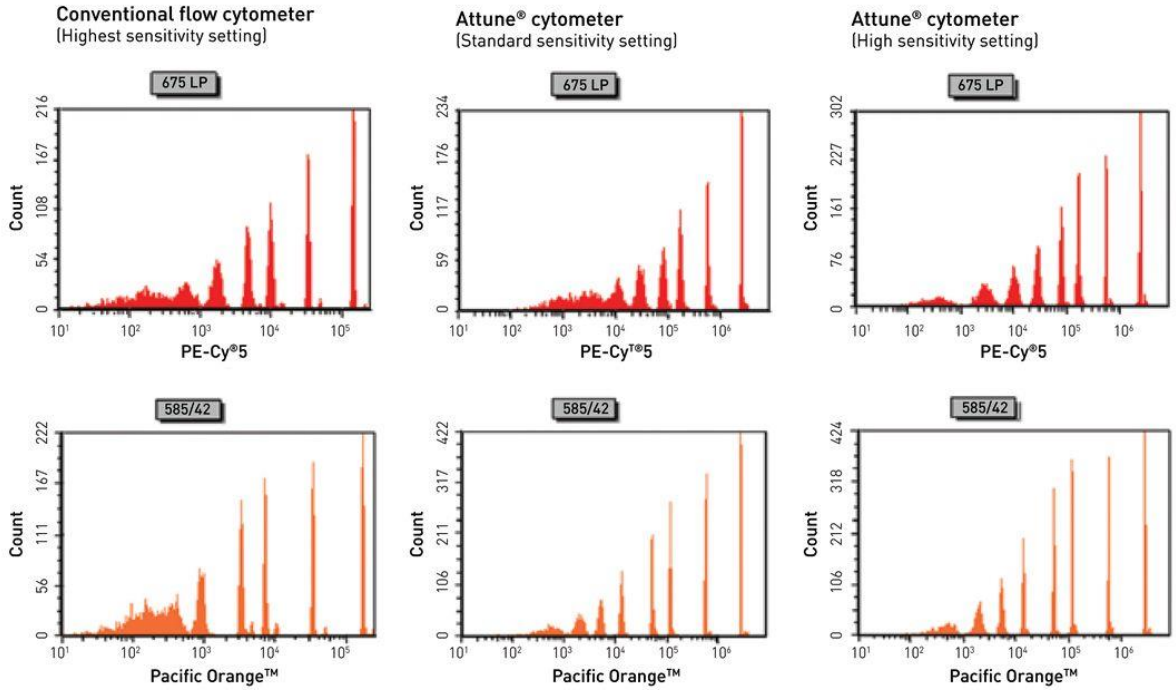
Acoustic focusing in a flow cell
Invitrogen “Attune”



Hydrodynamic focusing

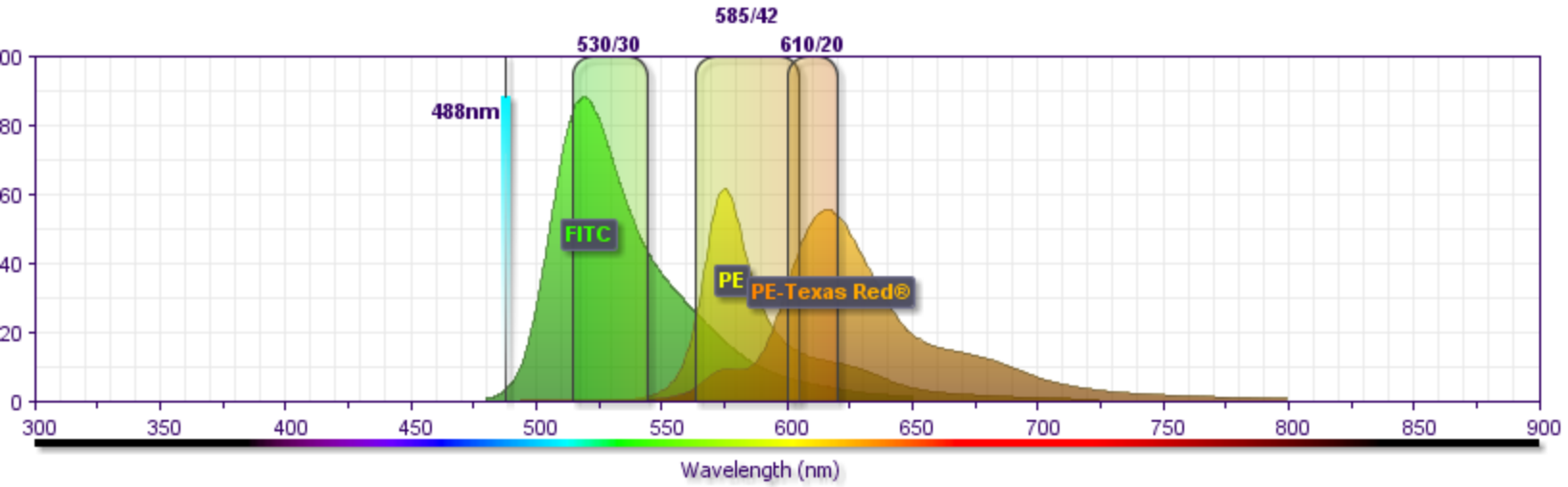


Acoustic focusing



BD Fluorescence Spectrum Viewer A Multicolor Tool

Options Curves: 4 Cytometer: Any Cytometer Excitation (nm): 488 Show Em when Ex % > 5 Cursor:



Fluorochrome	%	Ex	Em	Filters	FITC	PE	PE-Texas ...	PE-Cy7
FITC	88.0			530/30	--	--	--	--
PE	61.6			585/42	--	--	--	--
PE-Texas Red®	55.4			610/20	--	--	--	--
PE-Cy7	61.8			780/60	--	--	--	--

Applet

Reset

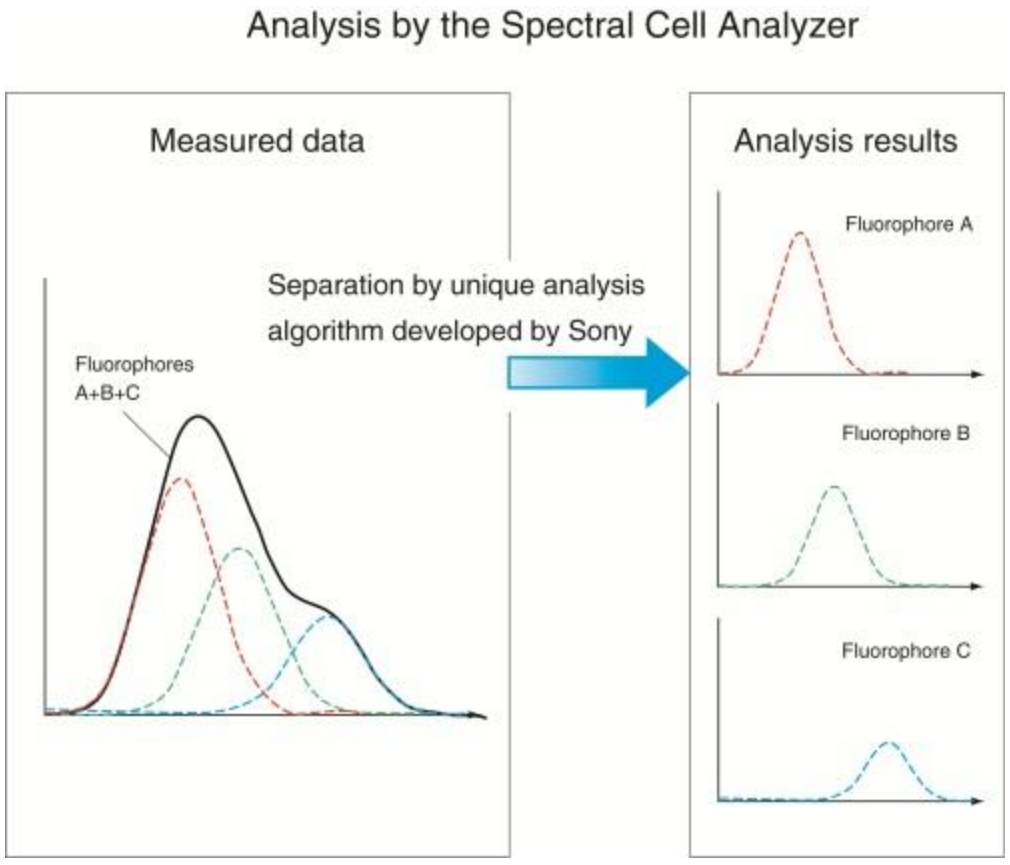
Filter List

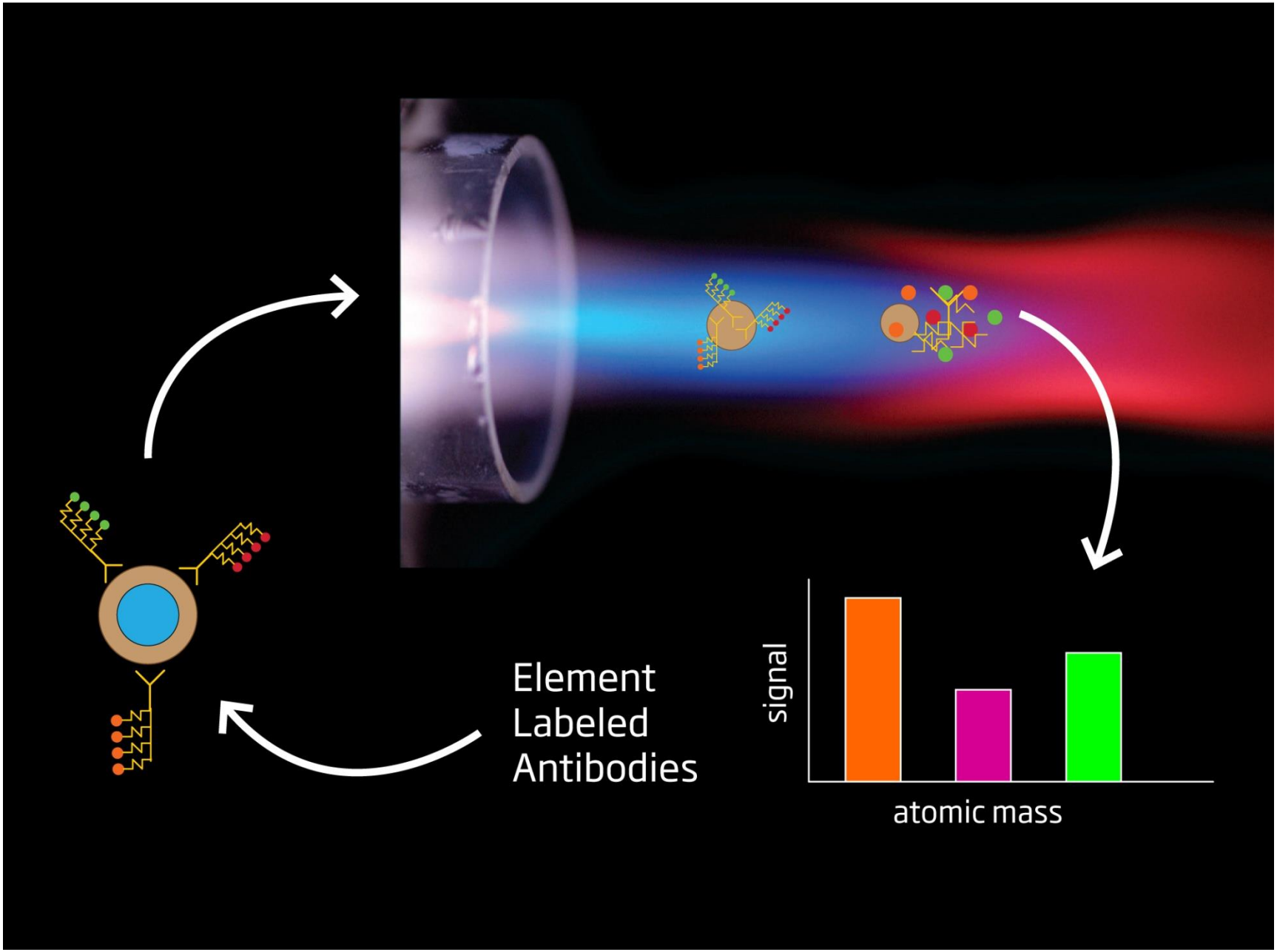
Filter: Add

Message

Click **Left Button** and drag cursor to zoom, over filter for performance. **Right Button** *more...*

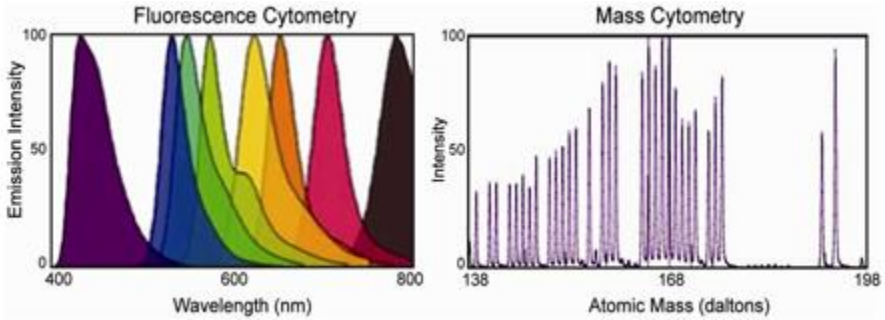
Sony Spectral Analyzer

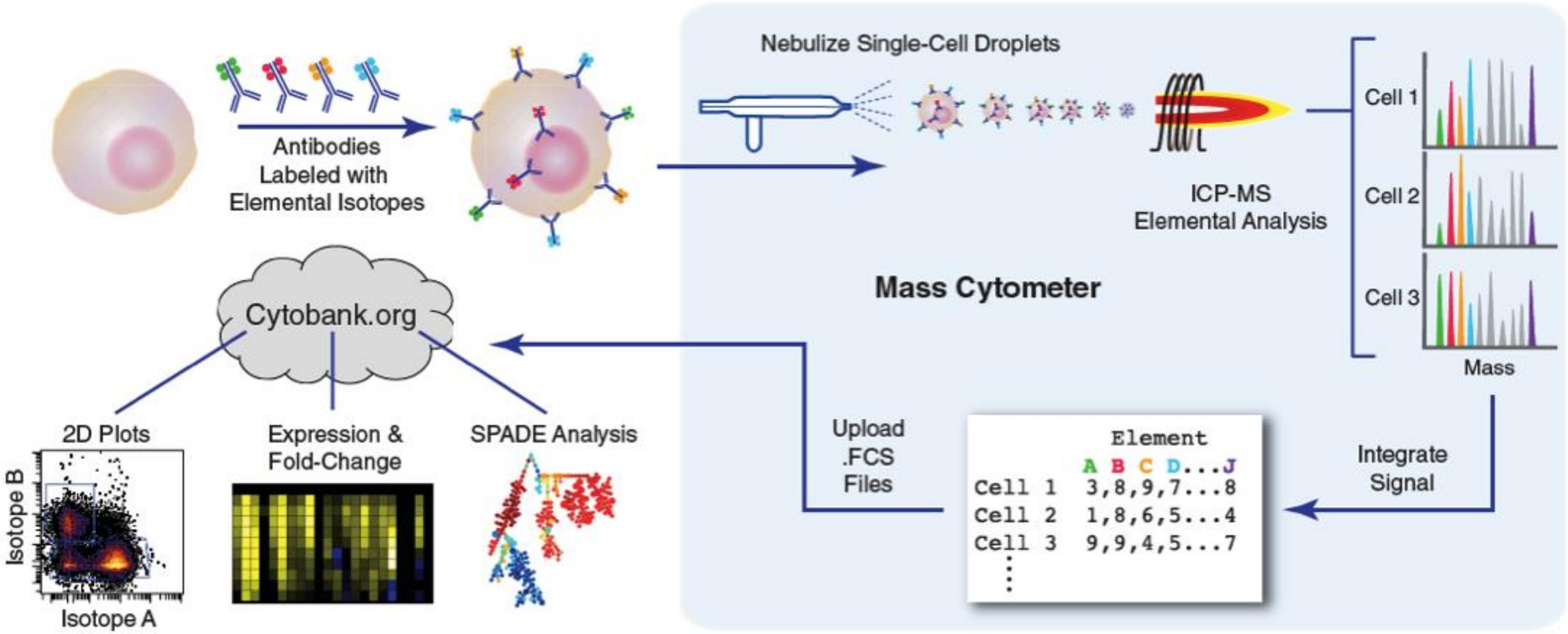



Element
Labeled
Antibodies
signal
atomic mass

DVS Sciences

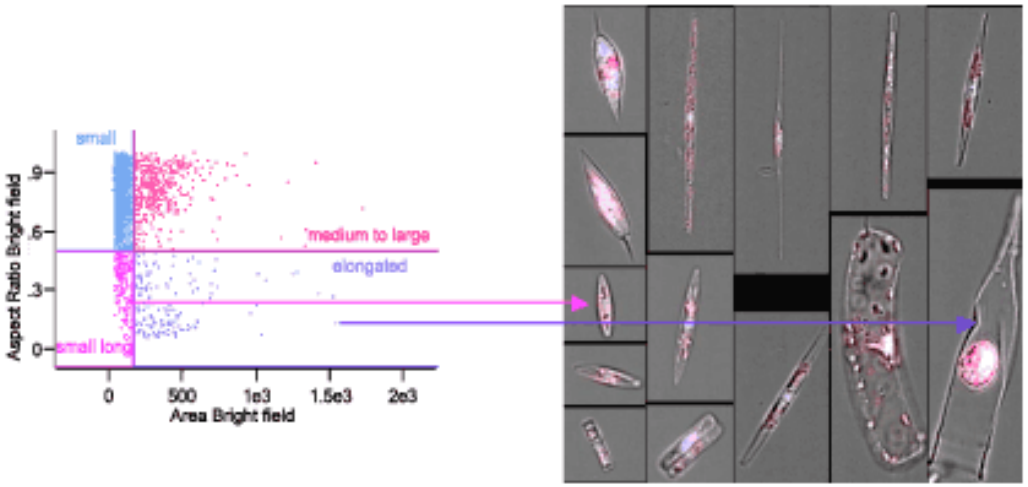
CyTOF® 2





Amnis ImageStream

Analysis of organisms in wharf tows by shape and size

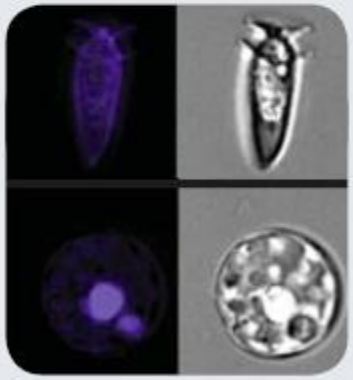


Unique species of diatoms and other plankton species from culture or from seawater can be identified and quantitated by analyzing for size, shape, fluorescence intensity, texture, and location/co-location of fluorescent labels and/or chloroplasts.

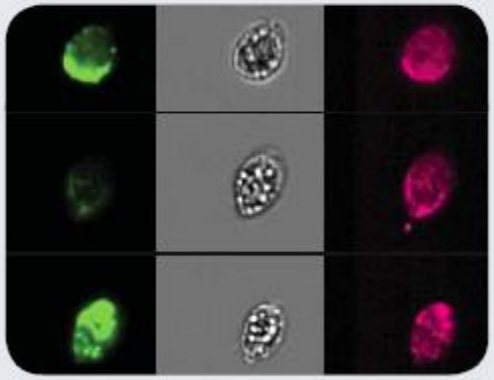
Study Highlights

Analyzed morphology and levels of silicification in diatoms labeled with Rhodamine 123.

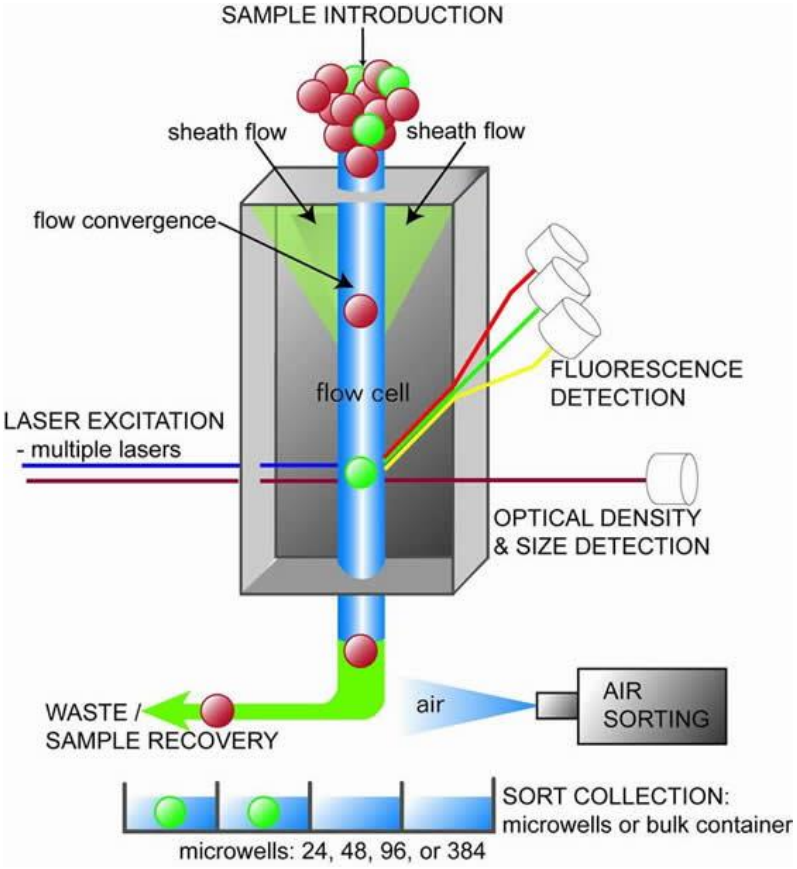
Classify organisms based on overall morphology and fluorescence intensity of chloroplasts and labeled DNA.



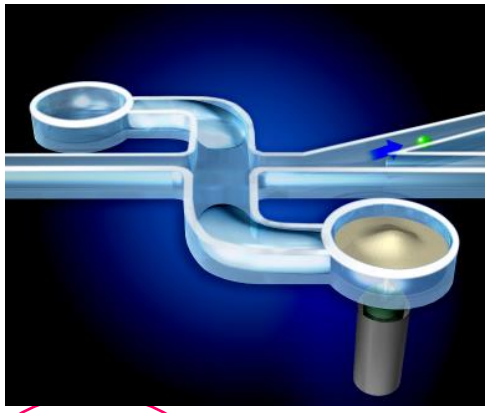
Measure nutrient stress based on chloroplast morphology and fluorescence intensity.



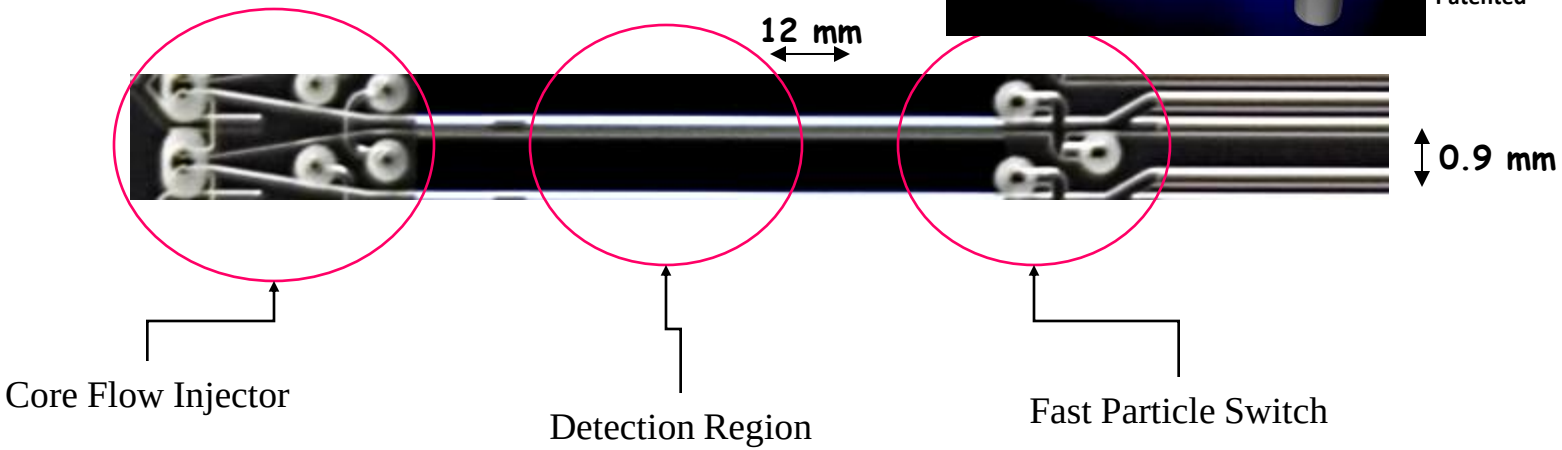
Large Particle Sorting—Union Biometrica “BioSorter”



FAST MICROFLUIDIC SWITCH SORTING
~2,000 Ev/Sec



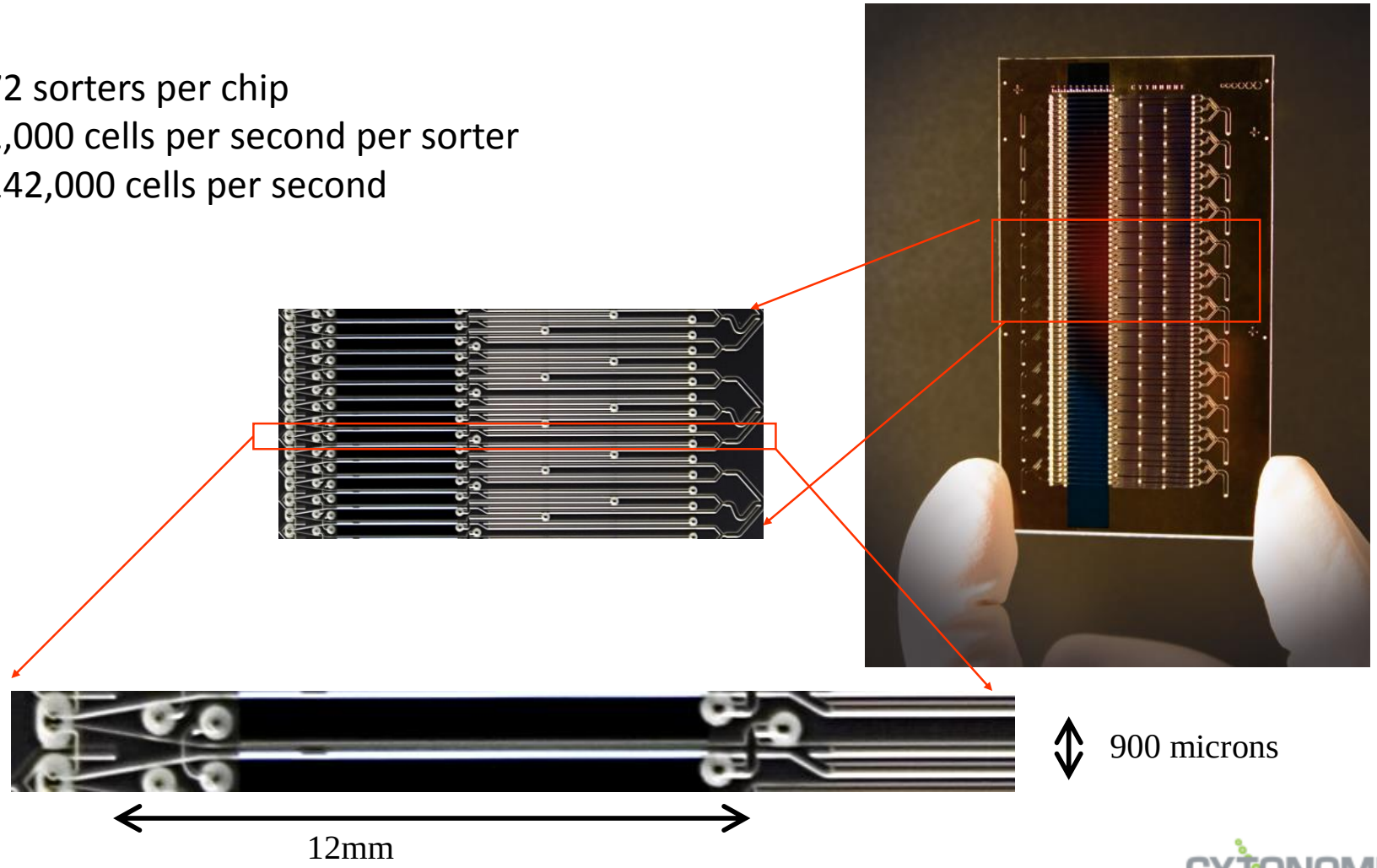
Patented



CYTONOME ST

HIGH-THROUGHPUT THROUGH PARALLEL IMPLEMENTATION OF MICROSORTERS

72 sorters per chip
2,000 cells per second per sorter
142,000 cells per second



CYTONOME ST