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Flow cytometry: retrospective, fundamentals and recent instrumentation

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Abstract

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Flow cytometry is a complete technology given to biologists to study cellular populations with high precision. This technology elegantly combines sample dimension, data acquisition speed, precision and measurement multiplicity. Beyond the statistical aspect, flow cytometry offers the possibility to physically separate sub-populations. These performances come from the common endeavor of physicists, biophysicists, biologists and computer engineers, who succeeded, by providing new concepts, to bring flow cytometry to current maturity. The aim of this paper is to present a complete retrospective of the technique and remind flow cytometry fundamentals before focusing on recent commercial instrumentation.

Keywords: Flow cytometry, Retrospective, Fundamentals, Instrumentation, Analyzer, Cell-sorter

Seventy seven years of technological innovation

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As flow cytometry is a technology that combines several concepts (Shapiro 2003), it is difficult to estimate a single starting point. However, the main originality residing in observation of **aligned cells one behind another into a fluid sheath was described for the first time by Moldavan (1934)** for cell counting on the 24th August 1934 in Montreal.

During World War II, the US Army was interested in developing a system that rapidly detects bacterial biowarfare agents in aerosols. Gucker and O'Konski (1947) set up and developed a photoelectronic counter which injected an air stream containing particles into a sheath stream on a dark-field microscope focal point. This first air-fluxes cytometer **detected 0.5 micron events by scattered light**. In 1942, Coons et al. used, for the first time, an anthracene-associated antibody (UV-excitabile fluorochrome) to detect *Streptococcus pneumoniae*. One decade later, Coons and Kaplan (1950) described the first fluorescein isothiocyanate (FITC) antibody association. In 1945, Coulter developed a process that permits **counting events and measuring cell size by conductance variation**. In this primary system where cells pass one by one into a very thin capillary and cross a light beam, extinction number corresponded to the cell number. However this technique led to many clogs. In 1949, Coulter precisely measured cell volume in a saline suspension based on **electrical impedance variation proportionality** due to the fact that sheath fluid and cell suspension are ionic liquids and cells surrounded by a lipid membrane are poor conductors compared to saline fluid. **Coulter's cell counters evolved into cell analyzers and were rapidly adopted by clinical laboratories for blood cell counting** (Brecher et al. 1956; Mattern et al. 1957).

In 1953, Crosland-Taylor used the laminar coaxial flux properties described by Reynolds in 1883, to develop a device counting red blood cells suspended in a sheath fluid through a capillary (Crosland-Taylor 1953). Simultaneously, photomultiplier tubes appeared, increasing optic signal precision. In the middle of the 1960s, Fulwyler described the electronic separation of cells by volume (Fulwyler 1965). Concurrently, Kametsky et al. (1965) **described a rapid cell spectrophotometer, which made rapid absorption measures**. In 1965, he also studied optical properties of cells passing across a laser beam, **such as scattered light intensity (forward scatter)** and UV-light absorption (DNA quantity) after staining. In 1967, he developed a **spectrophotometric cell sorter** (Kametsky and Melamed 1967). In the late 60s, Göhde's Partec (Münster, Germany) developed an analyzer (Dittrich and Göhde 1969) built around a Zeiss fluorescent microscope for DNA analysis with ethidium bromide (LePecq and Paoletti 1967). One year later, Phywe (Göttingen, Germany) commercialized it for the first time under the name *impulsecytophotometer ICP11*.

In 1970, Kametsky founded Bio/Physics Systems (Mahopac, NY, USA) and in 1971 sold the *Cytograph* and *Cytofluorograph* systems. For the first time, Van Dilla et al. (1969) equipped these analyzers with respectively 633 nm He–Ne laser and 488 nm argon ion lasers. Excitation by a **laser instead of a lamp allows optimal beam focusing on cells**. *Cytofluorograph* allowed the measurement of forward scatter light, green and red fluorescence emission (530 and 640 nm respectively). In 1972, Bonner et al. developed a new system named **fluorescence activated cell sorting (FACS)** which purified sub-populations (Bonner et al. 1972) and was equipped with a water-cooled argon laser to analyze FITC- and Rhodamin-coupled antibody fluorescence. To develop cell sorting, Fulwyler (1965) and Kametsky and Melamed (1967) used a **droplet deflection system developed by Sweet (1965) at Stanford University (Stanford, CA, USA)** for ink jet printers in 1965. With this droplet deflection technology, the saline sample stream was broken into droplets containing cells. Those of interest, with selected measurement values, were electrically charged at the droplet break-off point and droplets were then deflected by the means of an electric field into a collection tube. Their larger cell sorter rapidly measured scattered lights at several angles (Mullaney et al. 1969; Salzman et al. 1975a, 1975b; Steinkamp et al. 1973), fluorescence and measured cell volume by electrical impedance variation. In 1973, Hulett et al. developed a **rapid cell sorter** (Hulett et al. 1973). In 1974, the first commercial flow cytometric differential counter was the *Hemalog-D* (Technicon, Tarrytown, NY, USA), which led to the establishment of a white blood cell classification (Mansberg et al. 1974; Ornstein and Ansley 1974). The same year, Becton–Dickinson (now BD Biosciences, San Jose, CA, USA) commercialized a second cell sorter version named *FACS II*, which measured FSC and 530 nm emission fluorescence. Simultaneously, the Max Planck Institute (Göttingen, Germany) built a multiparameter cytometer cell sorter (Arndt-Jovin and Jovin 1974), and the Partec/Phywe society (Göttingen, Germany) commercialized the *Impulsecytophotometer ICP 22*. In 1975, Partec commercialized the two-parameter Particle Analyzing System PAS™ 8000. In the middle of the decade, Coulter Electronics (now Beckman Coulter, Fullerton, CA, USA), known for hematologic counters, made their first **cell sorter named two-parameter-sorter-1 (TPS-1)** with an air-cooled 35 mW argon ion laser, measuring FSC and one fluorescence. Several excitation wavelengths were introduced to flow cytometry: the first analyzer (Curbelo et al. 1976) used **five illuminating beams from a single arc lamp**; the second named Cytomat-R (Shapiro et al. 1977) with **three laser beams was built by Shapiro in order to analyze FSC, SSC and several fluorescence colors** (Shapiro et al. 1976) allowing morphological gating (Shapiro 1977). Both could detect up to **30,000 events/s**. In 1977/78, Coulter Electronics developed the *EPICS* (Electronically Programmable Individual Cell Sorter) system with a 5 watts argon laser and data multiparametric analysis. In 1978, at the Conference of the American Engineering Foundation in Pensacola, Florida, the **cytophotometry technique was renamed flow cytometry**, a term that quickly became popular. The same year, the Society for Analytical Cytology (later renamed International Society for Analytical Cytology and then International Society for Advancement of Cytometry) that edits the journal *Cytometry* was created. In 1978, Schlossman started the production of monoclonal antibodies directed against blood lymphoid antigens. New fluorochromes for multicolor flow cytometry were also developed (Reinherz et al. 1979). In 1979, Partec commercialized the PAS-II instrument. At the same time, NIH scientists added a 568 nm krypton laser to the flow cytometer and after a developmental phase, *FACS IV* was the first dual-laser cytometer created, commercialized by Becton–Dickinson (Steinkamp et al. 1979). Coulter and Ortho (that bought Bio/Physics Systems) manufactured flow cytometers that measured FSC, SSC and fluorescence, and analyzed **several thousand events/s**. After successfully performing a dual-color immunofluorescence

experiment, Loken et al. (1977) introduced fluorescence compensation in the process.

In the early 1980s, optical emission systems appeared. In the mid-1980s, Lawrence Livermore National Laboratory (LLNL) created the first high speed cell sorter prototype (Peters et al. 1985) for human chromosome separation (Gray et al. 1987). This high-speed cell sorter could sort 20,000 cells/s at 200 psi (pound per square inch) pressure, three times faster than conventional sorters (8,000 cells/s at 12 psi). In 1985, cell analyzers allowed up to three color analysis, such as EPICS C analyzer (Leif et al. 1985) (Coulter) with an arc lamp source or FACScan™ (Becton–Dickinson) with a 15 mW air-cooled argon laser source. Competition started between the different flow cytometer manufacturers concerning the simultaneous analysis capacity of fluorescence. In 1987, Partec introduced Cell Analyzer CA-II. In 1989, LLNL produced a second generation of high speed cell sorters allowing 200,000 events/s sorting at less than 100 psi pressure, with parallel organization of the data analysis process (van den Engh and Stokdijk 1989).

In 1990, flow cytometer capacity increased to measure seven fluorescences simultaneously. In 1991, Partec commercialized the two Particle Analyzing Systems PAS-III and PAS-IV. In 1994, Cytomation (Dako/Cytomation and now Beckman Coulter) commercialized the MoFlo® MLS flow cytometer developed by van den Engh, the first high-performance cell sorter for high-speed sorting applications. From the mid-90s, smaller diode and solid-state lasers were more and more often incorporated into flow cytometers. Because many objects are too large and too fragile for conventional flow cytometry, in 1998, the first Complex Object Parametric Analyzer Sorter (COPAS™) was introduced. Over the next decade, Union Biometrica expanded the COPAS platform into a family of fully automated systems for high throughput analysis, sorting and dispensing of large objects ranging from 20 to 1,500 microns.

In 2000, Apogee Flow Systems Ltd sold systems dedicated to environmental bacteria detection. The US Army chose the A40 military flow cytometer (Apogee Flow Systems) for its high sensitivity to small particles. In 2001, a violet diode laser was used for the first time on a flow cytometer (Shapiro and Perlmutter 2001). The same year, the BD LSR™ II flow cytometer proposed detection of fourteen fluorescences, a powerful tool for elucidation of the complex immune system (De Rosa and Roederer 2001; De Rosa et al. 2001). One year later, in 2002, the FACSARIA™ (BD Biosciences) became the first cell sorter with a fixed optical emission system. Partec inserted a flow cytometry laboratory into a french car: CyLab™ was the first mobile flow cytometry lab. Partec later proposed the CyLab™ Plus, an advanced mobile HIV monitoring laboratory based on a 4-wheel drive transporter vehicle using CyFlow® technology which can also run on a car battery (12 V DC power). In 2004, a Partec's CyFlow® integrated the International Space Station (ISS). The same year, new nanocrystal semi-conductor fluorochromes named Qdots® appeared. All five different nanocrystals are excited with the same long-wavelength UV lamp and their size determines their color. With Qdot® technology, Perfetto et al. (2004) realized seventeen different fluorescences per cell experiment. In 2007, van den Engh developed a new high-speed cell sorter named Influx™ (Cytospeia) and sold his society to BD Biosciences while Beckman Coulter acquired Dako/Cytomation.

In 2010, Sony, Merck Millipore and Danaher respectively acquired iCyt, Guava Technologies and Beckman Coulter. New ergonomic and compact analyzers appeared: Cube 8 (Partec), easyCyte™ 8HT (Merck Millipore) and the first acoustic focusing (Ward et al. 2009) cytometer Attune® (Applied Biosystems™ by Life Technologies™). Furthermore, new analyzers appeared LSRFortessa™ (BD Biosciences), Gallios™/Navios™ (Beckman Coulter) and a high-speed cell sorter Astrios™ MoFlo® (Beckman Coulter). This year, in 2011, BD Biosciences acquired Accuri Cytometers and commercialized a new benchtop analyzer, the FACSVerse™. Two new bench-top analyzers appeared MACSQuant® VYB (Miltenyi Biotec) and Auto40 (Apogee Flow systems). Partec produces the CyFlow® Cube Sorter, a new bench-top mechanical cell sorter.

Today, approximately seventeen societies manufacture analyzers and cell sorters. Some flow cytometers simultaneously analyze up to 32 parameters at 200,000 events/s, and sort up to 100,000 cells/s into 6-way sorting.

Flow cytometry fundamentals

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Flow cytometry is a powerful tool for the analysis of multiple individual cell parameters from heterogeneous populations. Flow cytometry is used in several multicolor applications for biology or functional studies as well as a broad range of research applications including: immuno-phenotyping, multi-parametric DNA analysis, proliferation, fluorescent protein, cell counting... Thousands of cells per second pass one by one through one or more laser beams in a flow cytometer, which measure scattered lights at several angles and fluorescence emissions. Some of them are also capable of analyzing cell volume by electrical impedance variation. Three parts constitute a flow cytometer: first, the fluidic system permits hydrodynamic focusing; second the excitation source and optical emission systems from the wavelength filters to the detectors constitute the optical part and finally the electronic system digitalizes the signal to be analyzed with specific computer software.

For flow cytometry acquisition, cells need to be in suspension in a tube or a plate. Some flow cytometers are equipped with a carousel or a rack loader (16, 30, 32 or 40 tubes capacity) containing a bar-code identification option and a vortex mixing system prior to sample aspiration. Some analyzers are equipped with a high throughput 96 or 384-well plate loader or a built-in sample tray for a 96-well microplate and/or tubes. Some systems are temperature-controlled, whereas others offer the possibility to add reagents or drugs during analysis.

Cells pass one by one across laser(s) beam(s) for individual analysis. This hydrodynamic focusing (Fig. 1 part 1) is based on coaxial laminar flow dynamic properties described in 1883 by Reynolds. To ensure a good quality of hydrodynamic focusing, the fluidic system must be very stable. The sample suspension is first pressurized and then injected into a sheath core flow before passing through a nozzle. The cellular suspension speed is dependent on the sheath fluid pressure that is fixed for an analyzer and adjustable for a cell-sorter. The higher the sample pressure the more cells have an opportunity to move laterally in the stream, causing a decrease in precision of hydrodynamic focusing, and consequently a drop in quality of the sample analysis, due to increases in the Coefficient of Variation of the peak values. The nozzle design is essential for obtaining laminar fluxes (Fulwyler 1977) in order to transport cells to the center of the stream: nozzle aperture should be narrow and perfectly calibrated (between 50 and 2,000 microns, depending on applications). This hydrodynamic focusing results from several stability developments (Steen 1990), and is used in most flow cytometers. However, other focus concepts exist, such as microcapillary technology or acoustic focusing (Ward et al. 2009) which allows alignment of cells by sound waves (Goddard et al. 2006, 2007; Goddard and Kaduchak 2005) without damage to cells (as ultrasound for fetus in utero medical exam).

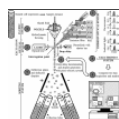


Fig. 1
Flow cytometry principle

Flow cell could be qualified as the heart of the system because it is the place where laser beam and cells interact (Fig. 1 part 2). That is the starting point of scattered lights (forward scatter and side scatter) and fluorescence signal emissions detected with an optical collection system before amplification and electronic digitalization. This intersection between excitation light source and cells is occurring in a quartz chamber or in the air at the nozzle exit. Due to the important variation of refraction indices between air and sheath

fluid, the reflection phenomenon implies lack of light signal so the amount of emission wavelength collected in the air is less important than in quartz chambers; **numerical aperture of the objective and sheath fluid pressure** are important in this case. In contrast to quartz chambers, optical **systems in the air have to be aligned daily** (Watson 1999) to ensure sensitivity and quality.

Cell illumination with an **excitation light source provides scattered (forward scatter and side scatter) and fluorescent lights**. The first flow cytometers were equipped with a mercury arc lamp, but light focalization on a small analysis area and light emissions collection were very poor, unlike those delivered by a coherent and monochromatic laser beam, direct or **fibered through prisms and optical lens** (Van Dilla et al. 1969) also used to control illumination point geometry. Indeed, contrary to circle geometry, the elliptic and now the **rectangular geometry laser** beam homogeneously illuminate cells whatever the lateral cell position in sheath fluid. The variable power intensity laser (**15–200 mW**) is function of the flow cell (**quartz or jet-in-air**). Emission fluorescence is quantitatively proportional to excitation intensity of the fluorochrome. At the present time, excitation light sources are systematically **diode lasers or lasers**, except for one analyzer which uses mercury arc-lamp for UV-excitation. Since 2001, violet laser diodes are used as light sources for cytometry (Shapiro and Perlmutter 2001). Use of several excitation sources increases the number of fluorochromes detectable simultaneously and directly impacts on the number of cellular parameters correlated (Crissman and Steinkamp 1982; Perfetto et al. 2004). Some flow cytometers were equipped with four different excitation sources (Greimers et al. 1996) or more, today cell sorters can be equipped with up to ten lasers. Laser co-linearity (several excitation sources in the same plan) implies alignment simplicity and stability but involves more fluorescence splitting than in a laser non-co-linearity system (one plan by excitation sources), which allows effective fluorescence splitting, but creates a **spatial gap, electronically converted to a temporal gap (time laser delay) in order to synchronize several light signals from one cell**.

When cell pass through the excitation source, the laser beam is refracted in all directions. Light diffusion at small angles (forward scatter light, FSC, FALS) is collected in the axis of the laser beam by a photodiode or a photomultiplier tube; the magnitude of the forward scatter light is correlated to the relative-size of the cells. A shutter bar masks the signal due to the laser beam on the detector. Some analyzers permit a choice between 1–8° and 1–19° collection of FSC signal (field stop) to detect, respectively, only large events or total events. Light diffusion at large angles (side scatter light, SSC, WALS) is collected at 90° of the laser beam like fluorescence emission lights (Fig. 1 part 3). **Orthogonal side scatter light is a combination of diffusion, reflection and refraction caused by structural complexity into the cell**. In flow cytometry, diffusion phenomena are very complex (Salzman et al. 1979). Side scatter and fluorescent lights are filtered by dichroic mirrors and adequate emission filters (band pass, short pass or long pass) (Steinkamp et al. 1974) into an emission optical system which directs lights at different wavelengths towards appropriate detectors. The role of the dichroic mirror is fundamental because it selects, reflects and transmits light signal band pass towards specific detectors collecting specific fluorescence. Light reflected by dichroic mirror is better than transmitted light, which originates in a multicolor staining, which signal passes through several filters and loses a significant part of its initial intensity. Thus, architecture of the emission optical system is essential to minimize losses and usually the user has the possibility of changing optical filters for each fluorescent measurement.

Detectors (**photodiode and photomultiplier tubes**) convert and amplify light signals (photons) from cell passage into a laser beam to an electric signal. This pulse is then digitalized, recorded and treated by specific software. Photodiodes are used for high intensity signals and photomultiplier tubes (PMT) for low intensity signals that need to be amplified with dynodes succession into PMT. Some analyzers also allow increasing gain, generating significant unspecific signals (background noise) so that is preferable to first increase PMT voltage before gain for low intensity signals.

An impulsion's characteristics are **pulse height or peak value, pulse width and pulse area or integral value**. Impulsion length is also named flight time or collection integration time. Trigger is a parameter chosen by the user based on a discrimination value (threshold), often FSC, below which events are not considered by the electronic system.

The flow cytometer electronics (Fig. 1 part 4) measures **several thousand events per second**, limited however by electronic dead time (Snow 2004), which is today less than 5 µs. Most efficient cell sorters have no dead time, and thus no hard aborts. Analyzers digitalize signal with analogic to digital converter (ADC) by converting voltage value to digital value and determining the channel number: 256 (8 bits), 1,024 (10 bits), etc. on logarithmic or linear scale (Fig. 1 part 5). Some analogical and all new digital flow cytometers allow retreatment of the data and modifications of fluorescence compensation matrix post-acquisition. Flow cytometers simultaneously analyze **pulse height, width and area for each cell allowing doublet-exclusion**. Actually, the highest resolution is 32 bits (2^{32} channels), divided on a five decades logarithm scale (up to seven decades for some). Most recent cytometers offer a biexponential scale under the axis. Events visualization is characterized by exhibiting pseudo-linear like behavior for values near zero, and transitioning it to a pseudo-logarithmic behavior values distant from zero (Novo and Wood 2008; Parks et al. 2006). Thus, events with lower fluorescence intensities are grouped and cell populations appear homogeneous.

Cell sorters offer the possibility of isolating subpopulations of cells of interest with high recovery and high degree of purity from heterogeneous cell mixtures based on light scattering and fluorescent characteristics.

Two kinds of **sorting mechanisms** can be described (Ashcroft and Lopez 2000).

First, the mechanical sorter employs a **mechanical catcher tube to sort cells of interest**. The catcher tube is located in the upper portion of the flow cell and moves into the stream to collect the cells. If the cell is identified as a cell of interest, it is captured by the catcher tube and collected into a tube or a concentration module; otherwise it is dispatched to the waste tank. Ideal for pathogenic samples, this method produces no biohazardous aerosols, but is considerably slower in sorting rate (300 cells/s with BD FACSCalibur™ and CyFlow® Sorter module by Partec). The mechanical system permits sorting of only one population. These flow cytometers are designed with a closed fluid system, thereby making them ideally suited for working with potentially biohazardous samples.

Second, most high-speed cell sorters **use electrostatic deflection of droplets ("jet-in-air" method)** (Chapman 2000). The advantage of this system is the possibility to sort from one to six sub-populations of cells (depending on cell sorters) simultaneously at low- or high-speed. As sorting generates aerosols in the chamber (Schmid and Dean 1997; Schmid et al. 1997) working with potentially biohazardous samples (Perfetto et al. 2003) will require implementation of appropriate precautions.

The cell suspension is directed into a stream, which emerges from a vibrating nozzle and breaks up into individual droplets (Fig. 1 part 6). The nozzle vibration conditioned by the drop drive frequency (ddf) is the number of drops generated per second proportionally to amplitude level. The fluidic system stability allows a good evaluation of the cell localization inside the droplets (Petersen and van den Engh 2003) if all conditions are accomplished. A droplet containing a cell of interest is positively or negatively charged and goes through an electric field between two deflection plates before being deflected into collection tubes (Fig. 1 part 7).

Several parameters should be considered for cell sorting optimization. According to type of cells, it is necessary to adjust sheath fluid pressure linked to the orifice diameter of the nozzle tip; the number of drops formed per second depends upon nozzle vibration (frequency and amplitude). The design specification for a cell sorting instrument also includes set up of break-off point (point

where the stream breaks into droplets), drop delay (distance between the laser beam interception of the cell and the break-off point), and purity level (sort mode's choice). These calculations can be automatic.

Thus it is possible to sort from 1 to 6 ways simultaneously into several containers (0.6, 1.5, 2.0, 5.0, 15 or 50 mL tubes, multi-well plates until 1,536, slides).

There are many possible applications resulting from the use of high-speed cell sorters (Ibrahim and van den Engh 2003).

Recent commercial instrumentation

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The EPICS®Altra™ (Beckman Coulter), the FACScan™ and FACS Vantage™ SE (Becton–Dickinson), the PAS®III (Partec), the Galaxy Pro (Dako), and the MoFlo® BTA and the MoFlo® MLS (Cytomation) were previously described (Chapman 2000; Nunez 2001).

Small bench-top analyzers

In the last few years, new bench-top flow cytometers have appeared, combining analytical power of research dedicated cytometers into robust, ergonomic, ultra-compact and easy-to-use analyzers. These new systems are easy to handle and less demanding regarding location space, maintenance and service.

A50-Micro, A50-Universal, Auto40 (Apogee Flow systems) A50-Micro, for extremely small particles applications, with three lasers (375, 405, 488, 532 or 635 nm) detects up to 3 light scatters (3 detectors) and four fluorescence colors. The A50-Universal analyzer is equipped with up to three lasers (375, 405, 488, 532 or 635 nm) and detects FSC, SSC and four fluorescence colors simultaneously. The newest Auto40 is equipped with a laser interchangeable without realignment (488 or 532 nm) and permits analysis of FSC, SSC and three fluorescence colors simultaneously. Optical filter blocks are interchangeable without realignment. All these analyzers determine volumetric sample and give absolute counts. Analysis rate is up to 20,000 events/s. Sample acquisition (16 bits—4.8 logarithmic decades) and data analyses are performed by a software in an integrated computer. Post-acquisition compensation isn't allowed (FCS 2.0).

Accuri® C6, CSampler® (BD Biosciences) After purchase this year of Accuri Cytometers, BD Biosciences commercializes two new bench top analyzers: BD Accuri® C6 and CSampler®. Equipped with 488 and 640 nm solid-state lasers, these flow cytometers analyze up to six parameters (FSC, SSC and four fluorescences). For high throughput acquisition, Accuri® C6 can be associated with HyperCyt® (IntelliCyt™ Corporation). The CSampler® is equipped with a 48 or 96-well plate or 24 tubes capacity loader. Analysis rate is up to 10,000 events/s. The user can change bandpass and dichroic filters. Sample acquisition (24 bits—7 decades digitalization) and data analysis are performed using CFlow® software (PC), with CFlow® zoom function. Post-acquisition compensations are allowed (FCS 3.1).

Attune™ (Applied Biosystems™ by Life Technologies™) Since 2010, Life technologies™ Corporation proposes an acoustic focusing analyzer that uses sound waves to precisely control cell movement (Goddard and Kaduchak 2005; Goddard et al. 2006, 2007). Attune™ equipped with a 50 mW 405 nm and 20 mW 488 nm solid-state lasers, analyzes FSC, SSC and six colors. The user can change bandpass and dichroic filters. Sample rate is adjustable from 25 to 1,000 µL/min and electronics have the capability to run up to 20,000 events/s. Sample acquisition (six decades) and data analyses are performed by Attune™ Cytometric Software (PC). Post-acquisition compensations are allowed (FCS 3.0).

CyAn™ ADP (Beckman Coulter) After the acquisition of the Dako Cytomation society, Beckman Coulter commercializes CyAn™ ADP, a high-speed analyzer (70,000 events/s) equipped with three solid-state lasers (405, 488 and 642 nm) to analyze nine fluorescence colors simultaneously. For high throughput screening, CyAn™ ADP integrated to the HyperCyt® plate loader (IntelliCyt™ Corporation) enables 384-well plates. Fast sample acquisition (12 bits—4 decades) and high capacity data analyses (up to one hundred million event data files) are performed by Summit® software (PC). Post-acquisition compensations are allowed (FCS 3.0).

CyFlow® Cube 6/Cube 8, CyFlow® ML, CyFlow® SL (Partec) Pioneer in flow cytometry since 1968, the German society Partec has since 2000 developed flexible and modular CyFlow® cytometers. The newest CyFlow® Cube 6/Cube 8 are, respectively, equipped with two or three lasers (large choice available), analyze FSC, SSC and up to four or six fluorescence colors simultaneously.

With five light sources, CyFlow® ML detects up to sixteen optical parameters: 2 × FSC, SSC and thirteen colors simultaneously. With a 50 mW 488 nm blue solid-state laser, CyFlow® SL analyzes FSC, SSC and three fluorescent colors. CyFlow® SL can run on a car battery (12 V DC power). All cytometers determine true volumetric absolute counting. Optical filters are interchangeable. Optional RobbyWell™ permits analysis of 96-well plates. Sample acquisition (16 bits—4 decades) and data analysis with CyFlow® Cube 6 and Cube 8 are performed by CyView™ software (PC). Sample acquisition (16 bits) and data analysis with CyFlow® ML and CyFlow® SL are performed by FloMax® software (PC). Post-acquisition compensations are possible (FCS 3.0).

CytoSense, CytoSub, CytoBuoy (CytoBuoy b.v.) CytoBuoy b.v. is a dutch company which manufactures particle analysis instruments and software for mainly aquatic and marine science. Since 2001, the bench top scanning flow cytometer CytoSense is designed for pico-, nano- and micro-plankton studies. It combines classical flow cytometry data with silico-images of the measured particles and targeted video imaging. This instrument is equipped with 2 lasers (460 or 488 or 532 or 561 nm and 445 or 635 or 640 or 660 nm) and up to 10 detectors. The CytoSense works with a hydrodynamic sheath fluid injection system with external and recirculating mode, and an auto-adaptive speed controlled from 0.2 to 20 µL/s. The CytoSense may be extended with a video imaging-in-flow at rates of up to 1,000 scans per second. The CytoSub module “Shallow” allows a submerged use in shallow waters (depth of 20 meter max.) and CytoSub module “Deep” transforms the CytoSense flow cytometer into a CytoSub instrument for submerged operation down to 200 meters. The Buoy module transforms the CytoSense into a CytoBuoy analyzer for moored operation, with 8 solar panels systems with rechargeable batteries and flashlight, telemetry and Argos transponder. CytoUSB software is used for instrument operation and storage of measured data files (8 bits—3.5 decades). CytoClus software is used for data analysis.

Eclipse™ (Sony) Since acquisition of iCyt in February 2010, Sony commercializes the Eclipse™ flow cytometer. Equipped with four fiber coupled diode solid state or DPSS lasers (30 mW 405, 25 mW 488, 30 mW 561 and 30 mW 642 nm) the Eclipse™ simultaneously analyzes FSC, SSC and five fluorescence colors up to an analysis rate of 5,000 events/s. An autoloader permits the analysis of 96- to 384-well plate and tubes (40-tube rack). Plate or rack shake at three intensity levels (5–30 s) or aspirate/dispense at three different speeds (1–12 cycles) are possible. Sample rate is adjustable from 5 to 200 µL/min. The Eclipse™ analyzer measures accurate sizing by electronic volume measurement and accurate absolute cell count with high precision syringe delivery mechanism. Sample acquisition (24 bits—6 logarithmic decades) and data analysis are performed by iFlow software (PC). Post-acquisition compensations are possible (FCS 3.0).

FACSVerse™ (BD Biosciences) FACSVerse™ is the latest machine from BD Biosciences, commercialized since June 2011. This analyzer combines a fibered three lasers excitation system, 405, 488 and 640 nm to a ten parameters collection optic (FSC, SSC and eight fluorescence). A new optical geometry design in heptagon equips this analyzer with automatic filter detection. It proposes a universal loader option that can load 96- or 384-well plates or various kinds of tubes with possibility of 30-tube rack with 1D or 2D bar-code reader, or 40-tube rack without bar-code reader. Plate or rack shakes before analysis. Sample rate is adjustable at 12, 45-55, 60 and 120 µL/min. BD FACSuite™ Software (PC) performs sample acquisition (18 bits—5 decades) and data analysis. Post-acquisition compensations are allowed (FCS 3.0). This cytometer offers bi-exponential scales.

Guava® easyCyte™ 5-5HT/6-6HT/6/2L-6HT/2L/8-8HT (Merck Millipore™) After the acquisition of Guava Technologies society, Merck Millipore™ commercializes microcapillary bench top analyzers. Guava® easyCyte™ 5-5HT and 6-6HT permits, with one blue laser, to analyze FSC, SSC and, respectively, up to three and four fluorescence colors. Guava® easyCyte™ 6/2L-6HT/2L and Guava® easyCyte™ 8-8HT flow cytometers are equipped with dual 75 mW blue and 40 mW red lasers (488 and 640 nm) and permit to analysis of simultaneous FSC, SSC and, respectively, four (3/1) and six (4/2) fluorescence colors. Sample is mixed using an automated paddle mixer. Sample acquisition and data analysis are performed using InCyte® acquisition and analysis software (PC) (equivalent 18 bits—4 decades). Post-acquisition compensations are allowed (FCS 3.0). In Guava® easyCyte™ 5HT, 6HT, 6HT/2L and 8HT flow cytometers, built-in sample tray provides walk-away automation for a 96-well microplate and ten microtubes.

MACSQuant®, MACSQuant® VYB (Miltenyi Biotec) With a tactile-screen and fluid level light warnings, MACSQuant® is an analyzer equipped with three air-cooled lasers (405, 488 and 635 nm) to detect FSC, SSC and eight colors simultaneously. MACSQuant® VYB is the newest analyzer (2011) equipped with three lasers (Violet, Yellow, Blue), respectively, 405, 561 and 488 nm to detect eight colors. Analysis rates are up to 10,000 events/s. 5, 15 and 50 mL tubes or 96-well plate can be maintained at cooling temperature. These analyzers determine volumetric or absolute cell counting. These systems incorporate a barcode reader to identify MACS reagents and their associated staining protocols. Emission filters are unchangeable. MACSQuantify™ Software (PC) performs sample acquisition (16 bits—5 decades digitalization) and data analysis. Post-acquisition compensations are allowed (FCS 3.0).

Cell analyzers

CELL LAB QUANTA™ SC/SC MPL (Beckman Coulter) The CELL LAB QUANTA™ SC or SC Multi-Platform Loader (MPL) flow cytometer is equipped with a 20 mW 488 nm air-cooled and mercury arc lamp (100 W) for UV-illumination sources (366, 405 and 435 nm). This flow cytometer simultaneously measures electronic volume by impedance variation, SSC and three fluorescence colors. The MPL version permits use of microtubes and 24-, 96- and 384-well plates. Emission filters are unchangeable. Cell Lab Quanta Collection Software (PC) performs sample acquisition (4 decades) and data analysis. Post-acquisition compensations are not allowed (FCS 2.0).

EPICS® XL™/XL-MCL™ (Beckman Coulter) Beckman Coulter manufactures since 1993 the COULTER® EPICS® XL/XL-MCL flow cytometer. The XL system features the capability to analyze FSC, SSC and four fluorescence colors with a single 15 mW 488 nm air-cooled laser. The XL-MCL system offers walk-away sample handling with the Multi Carousel Loader (MCL) (32 tubes capacity). This device incorporates positive bar-code identification and vortex mixing prior to sample aspiration. The optical emission filters are fixed. Sample acquisition (10 bits—4 decades) and data analysis are performed by XL SYSTEM II™ software or EXPO32™ ADC software (PC). Post-acquisition compensation is not allowed (FCS 2.0).

FACSCanto™, FACSCanto™ II (BD Biosciences) FACSCanto™ evolution permits obtention of an analyzer equipped with a 30 mW 405 nm solid-state laser, a 20 mW 488 nm air-cooled laser and a 17 mW 633 nm laser delivered by optic fibers to analyze eight fluorescence colors simultaneously. An automatic fluidic pump maintains fluidic stability. The optical system geometry is composed of two trigons (violet and red lasers) and one octagon (blue laser). The user can change both bandpass and dichroic filters. FACSCanto™ II offers walk-away sample handling with the carousel loader (40 tubes capacity) or high throughput sampler 96- or 384-well plate loader. Sample rate is adjustable at 12, 60 and 120 µL/min. BD FACSDiva™ Software (PC) performs sample acquisition (18 bits—5 decades) and data analysis. Post-acquisition compensations are allowed (FCS 3.0). These cytometers offer bi-exponential scales.

FC500 MCL/MPL (Beckman Coulter) FC500 MCL flow cytometer features the capability to analyze FSC (fixed stop 1–8° or 1–19°), SSC and five fluorescence colors with 20 mW 488 nm and 20 mW 635 nm air-cooled lasers. FC500 MCL system offers walk-away sample handling with the Multi Carousel Loader (MCL) (32 tubes capacity), positive bar-code identification and incorporated vortex mixing prior to sample aspiration. Sample rate is adjustable at 15, 30 and 60 µL/min. Sample acquisition (20 bits—4 decades) is performed by CXP acquisition Software. FC500 MPL version offers Multi-well Plate Loader (MPL). Sample acquisition (20 bits—4 decades) is performed by MXP acquisition software and data analysis by CXP Analysis software (PC). Post-acquisition compensations are allowed (FCS 3.0) for both.

Gallios™/Navios™ (Beckman Coulter) Recently, Beckman coulter proposed new cytometers, Gallios™ and Navios™, respectively, developed for clinical and research applications. These cytometers feature three solid-state fibered lasers (405, 488 and 638 nm) for FSC, SSC and ten fluorescence colors analysis. These systems offer walk-away sample handling with the Multi Carousel Loader (MCL) (32 tubes capacity), positive bar-code identification and incorporated vortex mixing prior to sample aspiration. The user can change bandpass as well as dichroic filters. Navios™ Software (PC) performs sample acquisition (20 bits—5 decades) at 25,000 events/s and data analysis. Post-acquisition compensations are allowed (FCS 3.0).

LSR™, LSR™ II, LSRFortessa™, LSRFortessa™ SORP (BD Biosciences) Since 1999, the first version of LSR™ was profoundly changed to obtain LSRFortessa™ and LSRFortessa™ SORP in 2010. These new versions are equipped with five lasers (upgraded to seven with a large choice from 16 different wavelengths and a wide range of powers) delivered by fiber optics to detect FSC, SSC and up to eighteen colors simultaneously (20 PMT max.). User chooses laser wavelengths in the SORP version. The optical system has transmission pathways in trigon and octagon geometry. In addition, user can change both bandpass and dichroic filters. For screening assays, LSR™ II and LSRFortessa™ offer walk-away sample handling with high throughput sampler 96/384-well microtiter plate. Sample rate is adjustable at 12, 60 and 120 µL/min. BD FACSDiva™ Software (PC) performs sample acquisition (18 bits—5 decades) and data analysis. Post-acquisition compensations are allowed (FCS 3.0). These cytometers offer bi-exponential scales.

S1000, S1000Ex, SE500 (Stratedigm) The S1000 and S1000Ex analyzers with four lasers analyze FSC (optional PMT detector), SSC and eight or fourteen fluorescence colors respectively. S1000 and S1000Ex permit a large choice of solid-state lasers (372, 405, 488, 532, 561 and 640 nm). SE500 flow cytometer, with dual 488 and 640 nm solid-state

lasers (40 mW), analyzes FSC (optional PMT detector), SSC and four fluorescence colors. Analysis rate is 10,000 events/s for all cytometers. The CellCapTure Software (PC) performs sample acquisition and data analysis. Post-acquisition compensations are allowed (FCS 3.0) (Table 1).

Table 1

Analyzers comparison

Mechanical cell sorters

CyFlow® Cube Sorter (Partec) With two lasers, the latest CyFlow® Cube Sorter analyzes FSC, SSC and up to three fluorescence colors simultaneously and determines true volumetric absolute counting. Optical filters are interchangeable. Optional RobbyWell™ permits the analysis of 96-well plates. Sample acquisition (16 bits—4 decades) and data analysis are performed by CyView™ software (PC). Post-acquisition compensations are possible (FCS 3.0).

CyFlow® space (Partec) CyFlow® space analyzer with an open optical system and 3 lasers (375, 407, 488, 561 and 638 nm available), allows simultaneous analyzing of FSC, SSC and seven fluorescence colors. Built-in CyFlow® Sorter modules for diamond piezo-based, closed, non-destructive and non-hazardous cell and particle sorting are optionally available. Optional RobbyWell™ allows analysis of 96-well plates. Sample acquisition (16 bits—4 decades) and data analysis are performed by FloMax® software (PC). Post-acquisition compensation is possible (FCS 3.0).

CytoSense Sorter (CytoBuoy b.v.) The CytoSense Sorter is a bench top scanning mechanical sorter designed for pico-, nano-, and micro-plankton studies, combining classical flow cytometry data with silico-images of the measured particles and targeted video imaging. This instrument is equipped with 2 lasers (460 or 488 or 532 or 561 nm and 445 or 635 or 640 or 660 nm) and up to 10 detectors. The CytoSense works with a hydrodynamic sheath fluid injection system with external and recirculating mode. Auto-adaptative speed is controlled from 0.2 to 20 µL/s. The CytoSense Sorter is equipped with a piezo operated fluid switch sorter working at rates of up to 100 sorts per second. CytoUSB software is used for instrument operation and storage of measured data files (8 bits—3.5 decades). CytoClus software is used for data analysis.

FACSCalibur™ (BD Biosciences) Manufactured since 1994, FACSCalibur™ uses a 488 nm air-cooled argon gas laser (15 mW) and a smaller 635 nm diode laser for the six parameters detection (FSC, SSC, 3/1 fluorescences). An automatic fluidic pump maintains fluidic stability. Emission filters are unchangeable. FACSCalibur™ offers walk-away sample handling with the carousel loader (40 tubes capacity) or high throughput sampler 96- and 384-well plate loader. FACSCalibur™ can analyze cell suspensions at the rate of over 1,000 events per second at three different speeds (12, 35 or 60 µL/min). Unlike the traditional cell analyzer, FACSCalibur™ is equipped (in option) with a sorting module. It can sort cell suspensions at a rate of 300 cells per second and collect them in a 50 mL tube. Sample acquisition (10 bits digitalization—4 decades) and data analysis are performed by CellQuest™ Pro Software (Macintosh). Post-acquisition compensation is not allowed (FCS 2.0).

Deflection cell sorters

BioSorter™, Copas™ BIOSORT, Copas™ SELECT, Copas™ PLUS, Copas™ XL (Union Biometrica Inc) The BioSorter® is a continuous flow system capable of analyzing, sorting objects ranging in size from 10 to 1,500 µm via different interchangeable fluidic and optic core assemblies (FOCA) optimized for a particular size range (250, 500, 1,000, 2,000 µm). An axial light-loss detector measures relative axial size and optical density is determined by the total integrated signal of the light blocked. Four excitations lasers max. (405 or 445 or 460 and 488, 561, 640 or 660 nm are available); the fluorescence intensity can be simultaneously measured at three different wavelengths. Samples are introduced via a 50 mL conical tube (one and two liter sample cups are optional) with suspended stirrer. An X–Y–Z stage allows dispensing into 24-, 48- or 96-well microtiter plates, tubes and bulk receptacles. The z-direction allows adjustment for different height plates and tubes. The BioSorter™ instrument is controlled by FlowPilot-Pro™ software (PC) with real-time data acquisition (16 bits) via on-board customized electronics.

Copas™ BIOSORT, Copas™ SELECT, Copas™ PLUS, Copas™ XL are flow systems capable of analyzing, sorting and dispensing objects ranging in size from 20 to 1,500 µm via different engineered fluidic paths and optimized quartz flow cell sizes (FOCA) for a specific object size range to achieve the highest accuracy and sensitivity possible. The BIOSORT, SELECT, PLUS and XL version are respectively equipped with 250, 500, 1,000 and 2,000 µm flow cell. Multi-laser excitations (3 lasers max.) among 325, 375, 405, 488/514, 561, 635, 640 or 670 nm are available and permit to detect three fluorescence colors. All systems are equipped with an X–Y stage, which allows dispensing into 24-, 48- and 96-well microtiter plates. Only the Copas™ BIOSORT permits sorting into 384-well plate. All Copas™ systems are controlled by COPAS™ software (PC) for operation and analysis (16 bits).

FACSARIA™ III (BD Biosciences) The first BD FACSARIA™ has been available since 2003. The third version was commercialized in 2010. This high-speed cell sorter (70,000 events/s) can support six fixed alignment air cooled solid state lasers, with a 375, 405, 445, 488, 561 and 633 nm wavelength, and four spatially separated beam spots in a quartz-cuvette. Emitted light is delivered by fiber optics to twenty detectors simultaneously in a collection optics set up in patented octagon- and trigon-shaped pathways. Threshold and height, area, and width measurements are available for any parameter.

Pressure can be adjusted from 5 to 75 psi and nozzles are available in four sizes: 70, 85, 100 and 130 microns. 35- and 50-micron sample line filters can be added. Four-way sorting is possible; tube holders include sizes from micro tubes to 15 mL tubes and collection is also possible on a 384-well plate or on slide. Sample and sort collection tubes can be maintained at a cooling or heating temperature. There are several automatic menus such as BD FACS™ Accudrop (this technology assists the user in determining the best drop delay value), BD™ Aerosol Management Option (evacuates the sort collection chamber and traps aerosolized particles during sorting), Cytometer Setup and Tracking (this feature establishes baseline settings), Sweet Spot Technology (Automated clog detection and sort tube protection system) or Easy aseptic setup and cleaning.

BD FACSDiva™ Software (PC) performs sample acquisition (18 bits—5 decades) and data analysis. Post-acquisition compensations are allowed (FCS 3.0). This cell sorter offers bi-exponential scales.

Influx™ (BD Biosciences) This cell sorter was created by Cytopeia in 2007, and take over by BD Biosciences in 2008.

This high-speed sorter (200,000 events/s) can include a ten laser paths and seven pinholes optical wavelength lasers (355, 405, 445, 457, 488, 515, 532, 561, 594, 640 and 785 nm available) and requires a daily optical alignment. A pinhole camera view ensures that the fluorescence is in alignment with the detectors. It can measure 24 parameters simultaneously and calculates 24×24 compensations. In order to create droplets for sorting, the nozzle assembly is coupling to an acoustical system. A bubble detector in the sample line detects air bubbles from the sample tube. Any parameter can be used as the threshold but only from the primary laser; lasers and detectors can be switched to change laser sequence. Peak heights are measured by default; area and width measurements are available for a maximum of 8 parameters. There is a 16-bits analog-to-digital conversion. An exchangeable detector module allowing for the measurement of small particles can be equipped with polarization sensitive detector. Pressure can be adjusted from 1 to 90 psi and nozzles are available in five sizes: 70, 86, 100, 140 and 200 microns. A six-way sorting is possible; tube holders include sizes from micro tubes to 50 mL tubes and collection is also possible on 6-, 24-, 48-, 96- and 384-well plates or on slide. Sample and sort collection tubes can be cooled or heated by an optional circulating water bath. There are several automatic menus, such as BD FACS™ Accudrop (to assist the user in the best drop delay value determination) and BD™ Aerosol Management Option (to evacuate the sort collection chamber and trap aerosolized particles during sorting). Post-acquisition compensations are allowed (FCS 3.0).

JSAN™ (Bay Bioscience Co.) Optical detection fibers of the Japan-made Sorter ANalyzer (JSAN) corresponding to each excitation laser are closely fixed at the flow cell. Four lasers (375 or 405, 488, 534 or 561 and 638 nm) on the grade level with flow cell can be mounted with the spatially separated beams system. It can acquire any ten parameters simultaneously with eight fluorescences and generate 8×8 digital matrix fluorescence compensations. Pressure can be adjusted to 30 psi. The OptiDrop function permits monitoring the droplet parameters and stop sort in case of abnormality; the OptiDelay automatically calculates drop delay. The sorter provides a 2-way sorting at 20,000 events/s speed. CloneMate option device enables direct sorting of a single cell or more into each well in a multiwell plate. In option, user can choose the JSAN body color among black (default), blue, green and red color. AppSAN software (PC) performs sample acquisition (20 bits—6 decades) and data analysis. Post-acquisition compensation is not allowed (FCS 2.0).

MoFlo® Astrios™ (Beckman Coulter) This is a high-speed cell sorter (70,000 events/s) commercialized in 2010. It is controlled by a touch screen control panel for instrument set up and sort monitoring. It can combine seven lasers (355, 405, 488, 532, 561, 592 and 642 nm) for seven pinholes. Emission light is transmitted by optical fiber to thirty PMT dispatched in 49 positions. Sheath pressure range goes from 4 to 100 psi and there are eight nozzle sizes (50, 70, 80, 90, 100, 120, 150 and 200 μ m). The IntelliSort function permits to automatically calculate optimal droplet break off and to monitor it. The sort rescue function protects sample and sorted cells in case of stop sorting. A six-way sorting can be done with mixed mode sorting: each sort stream is capable of having its own sort mode (Enrich, Purify and Single) programmed. There is a variety of collection devices with temperature control for all sort outputs—1.5, 5, 15 and 50 mL tubes are accepted. The Cyclone® II module can collect into multi-well plates (from 6- to 1,536-wells) and disposes up to 1,536 spots on a slide. The sorter is equipped with an Aerosol Evacuation System (AES) filters particles from all chambers where aerosols have the potential of being generated. Additionally, the MoFlo® Astrios™ has a custom Biosafety Level II cabinet option. Summit software (PC) performs sample acquisition (32 bits—5 decades) and data analysis. It can calculate 18×18 digital compensation matrix. Post-acquisition compensations are allowed (FCS 3.0).

MoFlo® XDP™ (Beckman Coulter) This high-speed cell sorter (70,000 events/s) is compatible with an array of laser options (water cooled, free-space, fiber coupled). It can mount up to 6 lasers (3 pinholes) and 12 fluorescences. It is a 32-bits high-resolution 5-decades multi-channel digital system under the control of the Summit software, which allows an 18×18 auto-compensation matrix. Sheath pressure range goes from 4 to 100 psi and there are eight nozzle sizes (50, 70, 80, 90, 100, 120, 150 and 200 μ m). The IntelliSort II function is a beadless drop delay determination and monitoring function. The sorter provides a 4-way sorting for multiple populations, with independent sort mode capability for each tube. There are a variety of collection devices with temperature control for all sorts of outputs: 0.5, 1.0, 1.5, 5.0, 7.0, 15 and 50 mL tubes are accepted. The Cyclone® module could collect into multi-well plates up to 1,536 wells and on slides. The aerosol evacuation system (AES) removes aerosols from the sort chamber. A Biosafety cabinet option exists.

Reflection® (Sony) After the acquisition of iCyt in February 2010, Sony commercializes the Reflection cell-sorter. This high-speed parallel sorting (70,000 events/s) contains one to four Highly Automated Parallel Sort (HAPS) modules per instrument that may be operated remotely by multiple users. It can mount up to 7 lasers (355, 405, 488, 532, 561, 592 and 640 nm) and count up to 24 fluorescent detectors. Pressure can be adjusted from 5 to 100 psi and 50 to 200 μ m nozzles are available. Each module can divided sort into 4 streams and cells of interest are collected into 0.6–50 mL tubes. These modules can be mounted inside a biological safety cabinet to protect samples, products, and operators from biohazard contamination. Wide forward scatter collection angle allows FSC detection increasing. Sample acquisition (14 bits) and data analysis are performed by WinList software.

Synergy™ BTS/BSC (Sony) The Synergy™ instrument platform supports two high performance cell sorter modules, two automated cell analyzer modules, or a sorter/analyzer combination on a single bench-top frame. Configured with up to eight lasers (among 375, 405, 445, 488, 491, 532, 561, 594 and 642 nm) focused on five spatially separated interrogation points, the Synergy™ can acquire up to 24 simultaneous parameters at up to 100,000 events/s. This instrument is configured with up to 32 detectors per module: up to 26 user-configurable fluorescent detectors (PMT) and 6 scatter diodes. With the optional Sort deposition System (SDS), this high-speed cell sorter (70,000 events/s) supports sorting into popular plate formats and 4-way sorting into most popular collection tube formats. Nozzle sizes include 70, 85, 100 and 130 microns. Sample acquisition and data analysis are performed by WinList acquisition and analysis software respectively. The iCyt Synergy™ BSC can be seamlessly integrated with a Baker SterilGARD® III Advance Biological Safety Cabinet (BSC) (Table 2).

Table 2
Cell sorters comparison

Perspectives

New technologies in the cytometry field have been developed to extend possibilities.

Microfluidic cytometry (On-chip Biotechnologies) (Cho et al. 2010a) FISHMAN-R is a Japanese analyzer that enables flow cytometry on a **microfluidics chip**. With dual lasers (473 and 640 nm) it analyzes FSC, SSC and up to four colors and could detect particles size from 0.5 to 20 μm (bacteria and cells). Acquisition rate is **3,000 events/s**. The sample is not diluted with sheath fluid after analysis, so that it is possible to re-use it for other experiments afterwards. Sample acquisition and data analysis are performed using the same software (PC).

Adherent cell cytometry (Cytellect, Inc.) The Celigo™ is the first **adherent cell cytometer**, which analyzes cells in their environment with minimal sample manipulation. With three combinations of excitation LED (377, 483 and 531 nm) and block filters, the Celigo™ analyzes adherent or non-adherent cells in **brightfield and three fluorescence colors**. High-throughput acquisition is achieved by using a F-theta lens with high-speed galvanometer mirrors that scan a large field (3.5 $\times$ /NA 0.25 magnification) on 6–1,536-well microplates, T-25 and T-75 flasks. The CCD detector (2,052 $\times$ 2,052 pixels) has a resolution of **1 μm /pixel**. The Celigo™ software performs both image acquisition and analysis.

The LEAP™ (Laser-Enabled Analysis and Processing System) is a microplate-based cytometry system for non-invasive in situ process, allowing the use of various adherent and non-adherent cell types, which is capable of sorting by **killing unexpected cells (1,000 targets/s) with a UV-laser (355 nm)**. Diameter of the **laser spot is include between 10 and 50 μm** . Multiple magnifications (3 $\times$, 5 $\times$, 10 $\times$ and 20 $\times$ /0.25 NA) are possible by using a F-theta lens on specific plate formats C-Lect™ 6-, 12-, 96- and 384-well plates. The LEAP™ uses cooled emCCD detector (1,024 $\times$ 1,024 pixels). The LEAP™ software performs both image acquisition and analysis.

Imaging flow cytometry (Fluid imaging technologies; Amnis Corporation) Manufactured since 1999, the FlowCAM® (Fluid Imaging Technologies) was the first bench top digital imaging analyzer for particle or cell measurements in solution, originally developed for the oceanographic research community and now used for other applications. The FlowCAM® acquires **high resolution microscopic color or monochrome images of cells (2 $\times$, 4 $\times$, 10 $\times$ and 20 $\times$ magnification) with a size range from 2 μm to 2 mm at a rate of up to 10,000 images/min** and determines two fluorescences (488 or 532 nm laser) and up to 23 morphology parameters for each particle. Portable and Submersible FlowCAM® versions exist. The FlowCAM® V-1000 is a small analyzer with two magnifications (2 $\times$ –4 $\times$ or 4 $\times$ –10 $\times$ or 10–20 $\times$). VisualSpreadsheet® software performs acquisition and analysis for all analyzers.

The ImageStream^X (Amnis Corporation) is a **multispectral imaging flow cytometer** that combines the strength of flow cytometry and fluorescence microscopy in a single platform; it can digitally image millions of cells directly in flow. This technology enables the identification of single cell based on distribution of fluorescence markers and morphology of the cell with brightfield and darkfield (SSC) at a rate of above **1,000 cells per second**. The system combines a brightfield lamp, five excitation lasers (405, 488, 560, 592 and 658 nm) and twelve channels of detection. The full Color Brightfield option provides a full spectrum brightfield light source that allows the ImageStream^X system to replicate the RGB brightfield imagery of a microscope. The MultiMag option provides 20 $\times$, 40 $\times$ and 60 $\times$ magnification. The extended depth of field technology uses a combination of specialized optics and unique image processing algorithms to project all structures within the cell into one crisp plane of focus.

The new **FlowSight (May 2011)**, based on the same technology, combines a brightfield lamp, four excitation lasers (80 mW 405, 50 mW 488, 50 mW 561 and 100 mW 642 nm) and twelve channels of detection to analyze simultaneously **brightfield, darkfield and ten fluorescence colors per cell at a rate of 2,000 cells/s**. The FlowSight operates with a pixel size of one micron (20 $\times$ /0.6NA magnification).

For high throughput analysis, the **ImageStream^X and the FlowSight** are equipped with a 96-well plate autosampler. These systems use the IDEAS software to quantify.

Mass cytometry (DVS Science) The CyTOF™ cell analyzer instrument is a **high throughput mass cytometer for individual cell analysis based on a novel elemental mass-spectrometry detection technology**. The instrument detects the stable isotopic tags attached to antibodies using labeling kits. Because there are many (up to 100) available stable isotopes, and the mass spectrometer provides highly precise resolution between detection channels, many parameters can be measured—and typically without requiring compensation. Each individual cell is analyzed for any number of parameters that the user requires, and the data is downloaded into conventional flow cytometry software for examination.

Perspectives Other optical developments appear such as **color-space-time (COST)** coding, which is a new way to detect multiple fluorescent wavelengths using a single photodetector (Cho et al. 2010b). This method of discriminating multiple fluorescent colors with a single photomultiplier holds great promise to significantly reduce the cost and size of the total system. By using COST technology, user can differentiate eleven commonly used fluorochromes in flow cytometry by using a single PMT, a major step toward the realization of compact, cost effective and multicolor flow cytometers for point-of-care applications.

For the future, users can expect that flow cytometers will continue to decrease in size and energy consumption and to increase in detection and precision measurements; but, in regard to the healthcare of users, biosafety is one of the major points which will require improvement.

Conflict of interests

Go to:

The authors state that they have no conflict of interest.

Footnotes

Go to:

Julien Picot and Coralie L. Guerin contributed equally to this work.

References

Go to:

Arndt-Jovin DJ, Jovin TM. Computer-controlled multiparameter analysis and sorting of cells and

- particles. *J Histochem Cytochem*. 1974;22:622–625. doi: 10.1177/22.7.622. [[PubMed](#)] [[Cross Ref](#)]
- Ashcroft RG, Lopez PA. Commercial high speed machines open new opportunities in high throughput flow cytometry (HTFC) *J Immunol Methods*. 2000;243:13–24. doi: 10.1016/S0022-1759(00)00219-2. [[PubMed](#)] [[Cross Ref](#)]
- Bonner WA, Hulet HR, Sweet RG, Herzenberg LA. Fluorescence activated cell sorting. *Rev Sci Instrum*. 1972;43:404–409. doi: 10.1063/1.1685647. [[PubMed](#)] [[Cross Ref](#)]
- Brecher G, Schneiderman M, Williams GZ. Evaluation of electronic red blood cell counter. *Am J Clin Pathol*. 1956;26:1439–1449. [[PubMed](#)]
- Chapman GV. Instrumentation for flow cytometry. *J Immunol Methods*. 2000;243:3–12. doi: 10.1016/S0022-1759(00)00224-6. [[PubMed](#)] [[Cross Ref](#)]
- Cho SH, Godin JM, Chen CH, Qiao W, Lee H, Lo YH (2010a) Review article: recent advancements in optofluidic flow cytometer. *Biomicrofluidics* 4:43001. doi:10.1063/1.3511706.
- Cho SH, Qiao W, Tsai FS, Yamashita K, Lo YH (2010b) Lab-on-a-chip flow cytometer employing color-space-time coding. *Appl Phys Lett* 97:093704, 1–3. doi:10.1063/1.3481695.
- Coons AH, Kaplan MH. Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody. *J Exp Med*. 1950;91(1):1–13. doi: 10.1084/jem.91.1.1. [[PMC free article](#)] [[PubMed](#)] [[Cross Ref](#)]
- Coons AH, Creech HJ, Jones RN, Brainer E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J Immunol*. 1942;45:159.
- Crissman HA, Steinkamp JA. Rapid, one step staining procedures for analysis of cellular DNA and protein by single and dual laser flow cytometry. *Cytometry*. 1982;3:84–90. doi: 10.1002/cyto.990030204. [[PubMed](#)] [[Cross Ref](#)]
- Crosland-Taylor PJ. A device for counting small particles suspended in a fluid through a tube. *Nature*. 1953;171:37–38. doi: 10.1038/171037b0. [[PubMed](#)] [[Cross Ref](#)]
- Curbelo R, Schildkraut ER, Hirschfeld T, Webb RH, Block MJ, Shapiro HM. A generalized machine for automated flow cytology system design. *J Histochem Cytochem*. 1976;24:388–395. doi: 10.1177/24.1.1254933. [[PubMed](#)] [[Cross Ref](#)]
- De Rosa SC, Roederer M (2001) Eleven-color flow cytometry. A powerful tool for elucidation of the complex immune system. *Clin Lab Med* 21:697–712, vii.
- Rosa SC, Herzenberg LA, Roederer M. 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med*. 2001;7:245–248. doi: 10.1038/84701. [[PubMed](#)] [[Cross Ref](#)]
- Dittrich W, Gohde W. Impulse fluorometry of single cells in suspension. *Z Naturforsch B*. 1969;24:360–361. [[PubMed](#)]
- Fulwyler MJ. Electronic separation of biological cells by volume. *Science*. 1965;150:910–911. doi: 10.1126/science.150.3698.910. [[PubMed](#)] [[Cross Ref](#)]
- Fulwyler MJ. Hydrodynamic orientation of cells. *J Histochem Cytochem*. 1977;25:781–783. doi: 10.1177/25.7.330728. [[PubMed](#)] [[Cross Ref](#)]
- Goddard G, Kaduchak G. Ultrasonic particle concentration in a line-driven cylindrical tube. *J Acoust Soc Am*. 2005;117:3440–3447. doi: 10.1121/1.1904405. [[PubMed](#)] [[Cross Ref](#)]
- Goddard G, Martin JC, Graves SW, Kaduchak G. Ultrasonic particle-concentration for sheathless focusing of particles for analysis in a flow cytometer. *Cytometry A*. 2006;69:66–74. [[PubMed](#)]
- Goddard GR, Sanders CK, Martin JC, Kaduchak G, Graves SW. Analytical performance of an ultrasonic particle focusing flow cytometer. *Anal Chem*. 2007;79:8740–8746. doi: 10.1021/ac071402t. [[PubMed](#)] [[Cross Ref](#)]
- Gray JW, Dean PN, Fuscoe JC, Peters DC, Trask BJ, Engh GJ, Dilla MA. High-speed chromosome sorting. *Science*. 1987;238:323–329. doi: 10.1126/science.2443974. [[PubMed](#)] [[Cross Ref](#)]
- Greimers R, Trebak M, Moutschen M, Jacobs N, Boniver J. Improved four-color flow cytometry method using fluo-3 and triple immunofluorescence for analysis of intracellular calcium ion ([Ca²⁺ + i]) fluxes among mouse lymph node B- and T-lymphocyte subsets. *Cytometry*. 1996;23:205–217. doi: 10.1002/(SICI)1097-0320(19960301)23:3<205::AID-CYTO4>3.0.CO;2-H. [[PubMed](#)] [[Cross Ref](#)]
- Gucker FT, Jr, O'Konski CT. A photoelectronic counter for colloidal particles. *J Am Chem Soc*. 1947;69:2422–2431. doi: 10.1021/ja01202a053. [[PubMed](#)] [[Cross Ref](#)]
- Hulet HR, Bonner WA, Sweet RG, Herzenberg LA. Development and application of a rapid cell sorter. *Clin Chem*. 1973;19:813–816. [[PubMed](#)]
- Ibrahim SF, Engh G. High-speed cell sorting: fundamentals and recent advances. *Curr Opin Biotechnol*. 2003;14:5–12. doi: 10.1016/S0958-1669(02)00009-5. [[PubMed](#)] [[Cross Ref](#)]
- Kamentsky LA, Melamed MR. Spectrophotometric cell sorter. *Science*. 1967;156:1364–1365. doi: 10.1126/science.156.3780.1364. [[PubMed](#)] [[Cross Ref](#)]
- Kamentsky LA, Melamed MR, Derman H. Spectrophotometer: new instrument for ultrarapid cell analysis. *Science*. 1965;150:630–631. doi: 10.1126/science.150.3696.630. [[PubMed](#)] [[Cross Ref](#)]
- Leif SB, Leif RC, Auer R. The EPICS C analyzer. An ergonomically designed flow cytometer computer system. *Anal Quant Cytol Histol*. 1985;7:187–191. [[PubMed](#)]
- LePecq JB, Paoletti C. A fluorescent complex between ethidium bromide and nucleic acids. Physical-chemical characterization. *J Mol Biol*. 1967;27:87–106. doi: 10.1016/0022-2836(67)90353-1. [[PubMed](#)] [[Cross Ref](#)]
- Loken MR, Parks DR, Herzenberg LA. Two-color immunofluorescence using a fluorescence-activated cell sorter. *J Histochem Cytochem*. 1977;25:899–907. doi: 10.1177/25.7.330738. [[PubMed](#)] [[Cross Ref](#)]
- Mansberg HP, Saunders AM, Groner W. The Hemalog D white cell differential system. *J Histochem Cytochem*. 1974;22:711–724. doi: 10.1177/22.7.711. [[PubMed](#)] [[Cross Ref](#)]

- Mattern CF, Brackett FS, Olson BJ. Determination of number and size of particles by electrical gating: blood cells. *J Appl Physiol*. 1957;10:56–70. [\[PubMed\]](#)
- Moldavan A. Photo-Electric Technique for the Counting of Microscopical Cells. *Science*. 1934;80:188–189. doi: 10.1126/science.80.2069.188. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Mullaney PF, Dilla MA, Coulter JR, Dean PN. Cell sizing: a light scattering photometer for rapid volume determination. *Rev Sci Instrum*. 1969;40:1029–1032. doi: 10.1063/1.1684143. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Novo D, Wood J. Flow cytometry histograms: transformations, resolution, and display. *Cytometry A*. 2008;73:685–692. [\[PubMed\]](#)
- Nunez R. Flow cytometry: principles and instrumentation. *Curr Issues Mol Biol*. 2001;3:39–45. [\[PubMed\]](#)
- Ornstein L, Ansley HR. Spectral matching of classical cytochemistry to automated cytology. *J Histochem Cytochem*. 1974;22:453–469. doi: 10.1177/22.7.453. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Parks DR, Roederer M, Moore WA. A new “Logicle” display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. *Cytometry A*. 2006;69:541–551. [\[PubMed\]](#)
- Perfetto SP, Ambrozak DR, Koup RA, Roederer M. Measuring containment of viable infectious cell sorting in high-velocity cell sorters. *Cytometry A*. 2003;52:122–130. doi: 10.1002/cyto.a.10033. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Perfetto SP, Chattopadhyay PK, Roederer M. Seventeen-colour flow cytometry: unravelling the immune system. *Nat Rev Immunol*. 2004;4:648–655. doi: 10.1038/nri1416. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Peters D, Branscomb E, Dean P, Merrill T, Pinkel D, Dilla M, Gray JW. The LLNL high-speed sorter: design features, operational characteristics, and biological utility. *Cytometry*. 1985;6:290–301. doi: 10.1002/cyto.990060404. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Petersen TW, Engh G. Stability of the breakoff point in a high-speed cell sorter. *Cytometry A*. 2003;56:63–70. doi: 10.1002/cyto.a.10090. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Reinherz EL, Kung PC, Goldstein G, Schlossman SF. Separation of functional subsets of human T cells by a monoclonal antibody. *Proc Natl Acad Sci USA*. 1979;76:4061–4065. doi: 10.1073/pnas.76.8.4061. [\[PMC free article\]](#) [\[PubMed\]](#) [\[Cross Ref\]](#)
- Reynolds O (1883) An experimental investigation of the circumstances which determine whether the motion of water in parallel channels shall be direct or sinuous and of the law of resistance in parallel channels. *Philosoph Trans R Soc* 174:935–982.
- Salzman GC, Crowell JM, Goad CA, Hansen KM, Hiebert RD, LaBauve PM, Martin JC, Ingram ML, Mullaney PF. A flow-system multiangle light-scattering instrument for cell characterization. *Clin Chem*. 1975;21:1297–1304. [\[PubMed\]](#)
- Salzman GC, Crowell JM, Martin JC, Trujillo TT, Romero A, Mullaney PF, LaBauve PM. Cell classification by laser light scattering: identification and separation of unstained leukocytes. *Acta Cytol*. 1975;19:374–377. [\[PubMed\]](#)
- Salzman GC, Wilder ME, Jett JH. Light scattering with stream-in-air flow systems. *J Histochem Cytochem*. 1979;27:264–267. doi: 10.1177/27.1.374583. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Schmid I, Dean PN. Introduction to the biosafety guidelines for sorting of unfixed cells. *Cytometry*. 1997;28:97–98. doi: 10.1002/(SICI)1097-0320(19970601)28:2<97::AID-CYTO1>3.0.CO;2-D. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Schmid I, Nicholson JK, Giorgi JV, Janossy G, Kunkl A, Lopez PA, Perfetto S, Seamer LC, Dean PN. Biosafety guidelines for sorting of unfixed cells. *Cytometry*. 1997;28:99–117. doi: 10.1002/(SICI)1097-0320(19970601)28:2<99::AID-CYTO2>3.0.CO;2-B. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Shapiro HM. Fluorescent dyes for differential counts by flow cytometry: does histochemistry tell us much more than cell geometry? *J Histochem Cytochem*. 1977;25:976–989. doi: 10.1177/25.8.894012. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Shapiro HM. Practical flow cytometry. Wiley-liss, New Jersey (USA): Fourth edition edn; 2003.
- Shapiro HM, Perlmutter NG. Violet laser diodes as light sources for cytometry. *Cytometry*. 2001;44:133–136. doi: 10.1002/1097-0320(20010601)44:2<133::AID-CYTO1092>3.0.CO;2-S. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Shapiro HM, Schildkraut ER, Curbelo R, Laird CW, Turner B, Hirschfeld T. Combined blood cell counting and classification with fluorochrome stains and flow instrumentation. *J Histochem Cytochem*. 1976;24:396–401. doi: 10.1177/24.1.56391. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Shapiro HM, Schildkraut ER, Curbelo R, Turner RB, Webb RH, Brown DC, Block MJ. Cytomat-R: a computer-controlled multiple laser source multiparameter flow cytophotometer system. *J Histochem Cytochem*. 1977;25:836–844. doi: 10.1177/25.7.330733. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Snow C. Flow cytometer electronics. *Cytometry A*. 2004;57:63–69. doi: 10.1002/cyto.a.10120. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Steen HB. Light scattering measurement in an arc lamp-based flow cytometer. *Cytometry*. 1990;11:223–230. doi: 10.1002/cyto.990110202. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Steinkamp JA, Fulwyler MJ, Coulter JR, Hiebert RD, Horney JL, Mullaney PF. A new multiparameter separator for microscopic particles and biological cells. *Rev Sci Instrum*. 1973;44:1301–1310. doi: 10.1063/1.1686375. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Steinkamp JA, Romero A, Horan PK, Crissman HA. Multiparameter analysis and sorting of mammalian cells. *Exp Cell Res*. 1974;84:15–23. doi: 10.1016/0014-4827(74)90374-7. [\[PubMed\]](#) [\[Cross Ref\]](#)
- Steinkamp JA, Orlicky DA, Crissman HA. Dual-laser flow cytometry of single mammalian

- cells. *J Histochem Cytochem.* 1979;27:273–276. doi: 10.1177/27.1.374585. [[PubMed](#)] [[Cross Ref](#)]
- Sweet RG. High frequency recording with electrostatically deflected ink jets. *Rev Sci Instrum.* 1965;36:131–136. doi: 10.1063/1.1719502. [[Cross Ref](#)]
- Engh G, Stokdijk W. Parallel processing data acquisition system for multilaser flow cytometry and cell sorting. *Cytometry.* 1989;10:282–293. doi: 10.1002/cyto.990100307. [[PubMed](#)] [[Cross Ref](#)]
- Dilla MA, Trujillo TT, Mullaney PF, Coulter JR. Cell microfluorometry: a method for rapid fluorescence measurement. *Science.* 1969;163:1213–1214. doi: 10.1126/science.163.3872.1213. [[PubMed](#)] [[Cross Ref](#)]
- Ward M, Turner P, DeJohn M, Kaduchak G. Fundamentals of Acoustic Cytometry. *Current Protocols in Cytometry.* 2009;1:1–12.
- Watson JV (1999) The early fluidic and optical physics of cytometry. *Cytometry* 38:2–14; discussion 1.

The latest specifications of commercial instrumentation can be found on the web pages of the manufacturers:

Amnis Corporation: www.amnis.com.
 Apogee flow systems: www.apogee-flow.com.
 Applied Biosystems by Life Technologies: www.appliedbiosystems.com.
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