

# Instrumentation for flow cytometry

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## Abstract

Advances in fluidics, optics, electronics, lasers, computers and software have made flow cytometers considerably more complex, but also easier to use. Production of new fluorescent dyes has seen the development of more flexible instruments so that eight or more parameters of correlated data can be collected at once. Flow cytometers can be subdivided into two groups, analysers and sorters. A description of the commercially available analysers and sorters together with their specifications (as at October 1999) will be given. © 2000 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

The definition of a Flow Cytometer is ‘an instrument which measures the properties of cells in a flowing stream’. If this definition is applied strictly, then instruments other than those that are commonly referred to as flow cytometers would have to be included in this review, for example full blood counters. For the purposes of this review, a flow cytometer will be defined as ‘an instrument that can measure physical, as well as multi-colour fluorescence properties of cells flowing in a stream’.

Advances in fluidics, optics, electronics, lasers, computers and software have made flow cytometers considerably more complex, but also easier to use. Production of new fluorescent dyes has seen the development of more flexible instruments so that eight, 10 or more parameters of correlated data can be collected at once.

Although the amount of data that can be collected from a single cell by flow cytometry has increased enormously, the software for analysis of this data, although much improved, has not kept pace. Technology for displaying multi-dimensional data is lacking, being still essentially limited to displaying two-dimensional data. ‘Colour-coding’ of populations has helped, but analysis and displaying of minor populations can be difficult, requiring in some cases, complex gating strategies. Apart from flow cytometer manufacturers’ software, there are a number of commercial as well as shareware and freeware analysis programs available. Software will not be discussed in this review.

There are two main types of flow cytometers – analysers and sorters. Sorters have the ability not only to collect data on cells (analyse cells) but also to sort cells with particular properties (defined by the flow cytometer operator) to extremely high purities (<99%).

There are also a number of commercial flow cytometers that have been developed for particular

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analytical requirements. Partec manufacture a Ploidy Analyser and also a Cell Counter Analyser. Optoflow has developed a flow cytometer for the rapid detection, characterisation and enumeration of micro-organisms. Luminex is developing technology for multiplexed analyte quantitation using a combination of microspheres, flow cytometry and high speed digital processing. These will be described in more detail (see also article by Vignali, this issue).

Two companies, Becton Dickinson and Coulter (now Beckman Coulter) both based in the USA, have dominated the commercial flow cytometer market (with both analysers and sorters) worldwide. Cytomation, now based in the USA, has emerged recently with a sorter that is challenging the above two. Cytomation has also recently launched an analyser, which can be upgraded in several stages to the full specifications of their top-of-the-line sorter. Partec based in Germany although having both an analyser and sorter have not penetrated markets outside Europe.

## 2. Commercial flow cytometry analysers

Commercial analysers have been developed mainly for clinical analyses, being spurred on by the spread of AIDS and the need to assess the CD4 status (in particular) of patients infected with HIV. However they are also used widely for other clinical analyses such as leukemia and lymphoma phenotyping, assessment of the immune status of patients, DNA analyses of tumour material, and a range of other clinical analyses. They have also found increasing use for research where there is no need to sort cells, since they are much cheaper than a sorter (approximately half or less in price).

Most commercial analysers are built around an argon-ion laser as the light source. This limits the usefulness of analysers as they can only be used to analyse cells stained with fluorochromes that are excited at 488 nm, the emission wavelength of the small (generally about 15 mW) air-cooled argon-ion lasers used. In recent years with the development of new fluorochromes that can be excited at 488 nm (particularly the phycoerythrin (PE) tandem conjugates and peridinin chlorophyll protein (perCP)), some of the limitations have been removed.

Table 1 shows a comparison of the specifications

and performance of commercially available flow cytometry analysers.

### 2.1. Beckman coulter

The EPICS<sup>®</sup> XL was the first, and is still the only, commercial flow cytometer to employ digital signal processing (DSP) electronics. The use of DSP electronics gives the EPICS<sup>®</sup> XL a unique advantage over all other flow cytometers, which employ analog signal processing electronics. To obtain logarithmic amplification of signals normally requires log amplifiers that are notoriously difficult to 'align' so they give a true logarithmic output. In the EPICS<sup>®</sup> XL all amplification is accomplished with linear amplifiers. For log signals, a log look-up table is used to convert the linear signal to logarithmic, thus avoiding the need for log amplifiers. Another advantage is that fluorescence compensation can be accomplished between the output of any pair of fluorescence photomultiplier tubes (PMT's) which is not possible with flow cytometers employing analog electronics. The EPICS<sup>®</sup> XL comes in four models, three and four colour with and without the Multi Carousel Loader (MCL), which takes a 32 tube sample carousel allowing unattended automatic sample handling.

The ability to fully compensate overlapping fluorescence signals between any pair of PMT's and the development of new 488 nm excited fluorochromes, allowed the EPICS<sup>®</sup> XL to be the first commercial analyser to analyse cells stained with four fluorochromes excited by the 488 nm line of an argon-ion laser.

Another unique parameter available on the EPICS<sup>®</sup> XL is the Prism parameter. This allows the automatic analysis and display of all possible populations with statistics in multicolour data. This can save an enormous amount of time doing complex gating to determine the number of particles in each population, particularly when collecting three or four colour data.

### 2.2. Becton Dickinson

The FACScan<sup>™</sup> was the first modern commercial benchtop flow cytometry analyser, being released in 1985. Its initial release was as a three-colour instrument. With the need to be able to analyse four

colours, Becton Dickinson upgraded the FACScan™ to a four-colour instrument. Rather than going to digital electronics to be able to compensate between the output from all four PMTs, they designed new optics based around a two-laser system to be able to analyse cells stained with four colours. The second laser is a red diode laser emitting at 635 nm, which is used to excite APC (allophycocyanin). This new instrument was called the FACSCalibur™. Becton Dickinson also designed the FACSCalibur™ to accommodate an optional sample-loading carousel that holds 40 tubes. The FACSCalibur™ can also be upgraded to sort, using the fluidic sorting module of the FACSORT™.

To fulfill a need to free up sorters for sorting and to have a multi-laser analyser at an affordable cost, Becton Dickinson has just released (mid-1999) the BD LSR. This is an analyser with a 15 mW argon-ion laser emitting at 488 nm, and an 8 mW HeCd laser emitting at 325 nm. With up to six fluorescence PMT's, this is an ideal research analyser.

### 2.3. Cytomation

Cytomation entered the flow cytometry market with the MoFlo®, a high-end sorter in 1994. Recently they have also produced an analyser, the MoFlo® BTA, with the unique capability of being upgradable to their top-of-the-line sorter in several stages. The MoFlo® range of instruments is built on a standard optical table, and modules can be added to build the analyser and/or sorter to fit the requirements of the user, which can be analysis and/or budget based. The Cytomation range is thus the most flexible on the market.

### 2.4. Partec/Dako

The Partec PAS (particle analysing system) analyser is built around dual beams, a 488 argon-ion laser and a HBO arc lamp for UV excitation. Although arc lamps are a cheap alternative to a UV laser (e.g. an air-cooled HeCd laser), they suffer from short lamp life and the instrument needing to incorporate expensive light regulation and/or intensity compensation circuitry to compensate for the fluctuating output of arc lamps. The PAS also has the

computer and monitor built into the instrument, giving it the smallest footprint of any commercial analyser.

Dako has recently released the GALAXY, which is a rebadged Partec PAS flow cytometer optimised for immunophenotyping.

## 3. Commercial flow cytometry sorters

There are two main types of sorters, droplet and fluidic. Droplet sorters operate by oscillating the flowcell at high frequencies (15–100 kHz) causing the sheath stream to break up into a regular series of droplets. When a particle is detected, its properties are compared to criteria entered into the computer by the flow cytometer operator. If the properties of the particle match the sort criteria, the last drop attached to the stream is charged just as the particle to be sorted reaches it. The droplets pass between high voltage plates and any droplet that is charged is diverted out of the main stream into a catching vessel. Within the droplet sorters there is now a further subdivision, low-speed sorters and high-speed sorters. Low-speed sorters sort at rates up to 10 000–15 000 cells/s and high-speed sorters at rates >20 000 cells/s. To achieve these high sort rates requires high pressures of 20–100 psi or greater which can in some cases have deleterious effects on cells.

Fluidic sorters, or fluidic switching sorters, rely on a mechanism to divert the fluidic stream into the sort collection vessel at the time a particle that fulfills the sort criteria is detected. Fluidic sorters have a distinct advantage over droplet sorters for sorting biohazardous samples. In droplet sorters the stream is broken up into a series of tiny droplets. This produces aerosols, which can potentially contaminate the operator. Fluidic sorters do not break up the stream into droplets, do not produce aerosols and thus are inherently safer for sorting biologically hazardous samples. However the speed of the stream switching mechanisms of fluidic sorters is limited (300 cells/s for the FACSCalibur and 1000 cells/s for the PAS III) limiting sort rates, and sort purity is not as good as for droplet sorters.

A disadvantage of fluidic sorters is that they can only sort single populations whereas in droplet sorters the droplets can be charged either with a

Table 1  
Specifications and performance of commercial flow cytometry analysers

	Beckman Coulter EPICS® XL	Becton Dickinson FACSCALIBUR™	Becton Dickinson LSR	Cytomation MoFlo® BTA	Partec–PAS Dako–GALAXY <sup>c</sup>
<i>Excitation</i>					
Light source	15 mW argon-ion laser, 488 nm	15 mW argon-ion laser, 488 nm; optional diode laser, 635 nm	15 mW argon-ion laser, 488 nm; 8 mW HeCd laser, 325 nm	15 mW argon-ion laser, 488 nm	20 mW argon-ion laser, 488 nm and 100 W HBO arc lamp. Red diode laser option
<i>Detectors</i>					
Scatter detectors	FS – solid state silicon SS-PMT	FS-solid state silicon SS-PMT	FS – solid state silicon SS-PMT	FS – solid state silicon or PMT SS-PMT	FS – PMT SS-PMT
Fluorescence detectors	4 PMT	3 or 4 PMT	Up to 6 PMT	Up to 9 PMT (up to 5 on beam 1, 2 on beam 2 and 2 on beam 3)	Up to 6 PMT, 4 PMT standard
<i>Fluidics</i>					
Flow cell	Quartz cuvette with integral lens	Quartz flow cell	Quartz flow cell	Nozzles 50–400 µm	Quartz flow cell
Sample flow rates	3. Low, Medium, High	3. Low, Medium, High	3. Low, Medium, High	Variable	Variable, 0–1200 µl/min
Direct absolute counting	Yes, 20 µl sample loop	No	No	No	Yes, syringe pump and sample volume detection
<i>Signal processing</i>					
Electronics	Digital Signal Processing (DSP)	Analog	Analog	Analog; optional digital signal processor (DSP) for fluorescence compensation	Analog/digital
ADC	1024 channel	1024 channel	1024 channel	4096 channel	65536 channel
Amplifiers	Linear. 4 decade Log through look up table	Linear and 4 decade Log	Linear and 4 decade Log	Linear and 4 decade Log	Linear and 3 or 4 decade Log
Fluorescence compensation	Full matrix, between any pair of fluorescence detectors	Between FL1 and FL2; FL2 and FL3; FL3 and FL4 (with FL4 option)	FL1 & FL2; FL1 & FL3; FL1 & FL6; FL2 & FL3; FL2 & FL6; FL3 & FL6; FL4 & FL5	Software compensation between any pair of fluorescence detectors (on and off line); On line 8×8 matrix with DSP	Software compensation between any pair of fluorescence detectors (on and off line)
Pulse processing	Integral and Peak available on all parameters for doublet discrimination	Width and Area available on all fluorescence parameters for doublet discrimination	Width and Area available on all fluorescence parameters for doublet discrimination	Integral and Peak available on all fluorescence parameters for doublet discrimination	Height/Width and Area available on all parameters for doublet discrimination
Time	Yes	Yes	Yes	Yes	Yes
Ratio	Yes	No	Yes	Yes	Yes
Auxiliary	Yes	No	No	No	Yes
Prism	Yes	No	No	No	No
Time-of-flight (Width)	No	Yes	Yes	Yes on FS	Yes

<i>Performance</i>					
Fluorescence sensitivity	<1000 MESF for FITC and PE	<750 MESF for FITC	<200 MESF for FITC	<400 MESF for FITC	<100 MESF for FITC
Scatter sensitivity	At least 0.5 $\mu$	At least 0.5 $\mu$	At least 0.5 $\mu$	At least 0.5 $\mu$	0.5 $\mu$
<i>Data management—Note Specifications current at October 1999</i>					
Computer <sup>a</sup>	FlowCenter™-IBM compatible	FACStation™ – Macintosh	FACStation™ – Macintosh	IBM compatible	IBM compatible (built in)
CPU <sup>a</sup>	Intel® PIII 550/600 MHz	G3 Power PC RISC	G3 Power PC RISC	Dual Intel® PII 333 MHz	Intel® PII 450 MHz
Memory	128 MB RAM	128 MB RAM	128 MB RAM	128 MB RAM	128 MB RAM
Data storage	13.7 GB hard disk	12 GB hard disk	12 GB hard disk	Dual 6 GB and 8 GB hard disks	18 GB hard disk
CD-ROM	Yes	Yes	Yes	Yes	Yes (read/write)
Monitor	21 in. high resolution or 15 in. or 18 in. flat panel	18 in. flat screen	18 in. flat screen	18 in. flat screen or 21 in. high resolution	15 in. built-in active LCD; optional external
Networking	Yes	Yes	Yes	Yes	Yes
Data file structure	FCS 2.0	FCS 2.0	FCS 2.0	FCS 2.0	FCS 2.0
Software	XL System II™ or EXPO32™	CELLQuest <sup>b</sup>	CELLQuest <sup>b</sup>	CyCLOPS™ SUMMIT	PAS
<i>Available options</i>					
Autosampler	Yes, 32 place MCL – Multi-carousel loader	Yes, 40 place carousel	No	No	Yes, 36 place carousel (including staining and lysing)

<sup>a</sup> Computer specifications will vary. CPU speed is per specifications at October, 1999.

<sup>b</sup> Standard acquisition and analysis software. Other software packages available for particular samples/assays.

<sup>c</sup> The Dako GALAXY is a Partec PAS flow cytometer optimised for immunophenotyping.

positive or negative charge allowing two populations to be sorted simultaneously. In the case of the MoFlo<sup>®</sup> MLS, four populations can be sorted simultaneously since droplets can be given a half or full positive or negative charge.

All of the droplet sorters have large laser benches that can house a range of lasers for different applications. The range to choose from is expanding as laser manufacturers produce new lasers suitable for flow cytometry applications. The current range of lasers includes those shown in Table 2. They can be divided into two types, air-cooled and water-cooled. In general the air-cooled lasers, can be plugged into standard power outlets, are small, relatively cheap, have a single emission line, but have limited output powers. Water-cooled lasers generally can be tuned to different emission lines, require 3-phase power, are large, expensive (to buy and to run) and of course require water-cooling.

The specifications and performance shown in Table 3 for commercial flow cytometry sorters are for the flow cytometry sorters with the high speed sorting option. The low sorting speed models generally have the same specifications except that they do

not have the modules necessary to generate and/or supply high-pressure sheath fluid (15–100 psi) to the flow cell. Maximum sort rates of the ‘slow’ speed droplet sorters are <15 000 particles/s.

### 3.1. Beckman Coulter

The Altra<sup>™</sup> can accommodate up to four lasers on its optical bench. The beams can be collinear or spatially separated. When using spatially separated laser beams, the Gated Amplifier option is required. It uses time-resolved gated amplification to measure pulses from both beams. This allows accurate measurement when the emission spectra from fluorescent dyes overlap. Since the gated amplifier introduces two auxiliary amplification circuits, it also allows the collection of fluorescence from six fluorochromes with only four fluorescence PMTs.

The forerunner of the Altra<sup>™</sup>, the Elite<sup>™</sup> was the first commercial flow cytometer sorter to incorporate a video camera to view the sort stream and droplet break off point. The Altra<sup>™</sup> has a touch-screen, which can be used to set up and control the instrument settings, data output and sorting. The instru-

Table 2  
Lasers used on commercial flow cytometry sorters

Laser	Available output power	Emission wavelengths
<i>Air-cooled</i>		
Argon-ion	15–100 mW	488 nm
Red HeNe	10–35 mW	633 nm
Green HeNe	1.5 mW	544 nm
Red Diode	5 mW	635 nm
HeCd	8 to 20 mW	325 nm
Coherent DPSS (Diode pumped Nd:YAG)	10, 20, 50, 100 or 200 mW	532 nm
<i>Water-cooled–Argon-ion</i>		
Coherent Enterprise II	Up to 200 mW 488, 50 mW UV	Simultaneous 488 nm and UV (351–364 nm). Requires optics to split beams
Coherent Innova 300 series	Multiline visible 4–8 W 488 nm 1.3–2.4 W, UV 200–800 mW	Tuneable multiline UV to 514.5 nm, including UV, 457, 488, 514 nm
Coherent Innova 90 series	Multiline visible 3–6 W 488 nm 1.0–1.8 W, UV 200–500 mW	Tuneable multiline UV to 514.5 nm, including UV, 457, 488, 514 nm
Coherent Innova 70 series	Multiline visible 2–5 W 488 nm 0.7–1.5 W, UV 50 mW	Tuneable multiline UV to 514.5 nm, including UV, 457, 488, 514 nm
<i>Water-cooled–Argon/Krypton-ion mixed gas</i>		
Coherent Innova Spectrum	Multiline visible 2.5 W 488 nm 250 mW, UV 50 mW	Tuneable multiline UV to 647 nm, including UV, 457, 488, 514, 531, 568 nm

ment also contains a fan, which is exhausted through a HEPA filter, to produce laminar airflow through the sort area to minimise aerosol particle release into the environs surrounding the instrument. To maintain sort droplet stability, the Altra™ incorporates SortLOCK™, which monitors and if necessary automatically adjusts the drive to the piezoelectric crystal.

The Altra™ has available the Prism parameter to simplify multi-colour data analysis as described under the Beckman Coulter flow cytometry analyser.

### 3.2. Becton Dickinson

Up to three lasers can be mounted on the FACSVantage™ SE optical table. The three beams are spatially separated at the flow cell and the fluorescence generated from each beam is directed to its own set of PMTs requiring minimal electronic compensation.

The FACSVantage™ SE with TurboSort™, the high speed sorting option, also includes the former Becton Dickinson option, MacroSort™. MacroSort™ allows the use of flow cell nozzles up to 400 µ for large particle sorting. Becton Dickinson has, as an option, a 'machine vision' system that continuously monitors the sort droplet formation for stability called SortWatch™.

In the Becton Dickinson FACSCalibur™ equipped with the fluidic switching sort option, the sort collection tube which is normally placed eccentrically, can be rapidly moved into the centre of the stream to 'catch' the particle to be sorted. In this instrument there is a continuous flow of sheath fluid through the catching tube. A consequent disadvantage is the large volume of fluid collected with each sorted particle. Becton Dickinson has an accessory membrane filtration device to collect and concentrate the sorted particles.

### 3.3. Cytomation

Cytomation has pioneered commercial high speed flow cytometry sorting with the introduction of their MoFlo® sorter. Apart from having the fastest high speed sorter on the market (up to 100 000 particles/s), Cytomation have introduced 4-way sorting, and automated the sort set up procedure with the

SortMaster™ option. SortMaster™ automatically determines the droplet delay for each sort, as well as monitors the stream for irregularities during a sort and continuously adjusts the delay if necessary. If the droplet formation is disrupted, e.g. through a partial blockage of the nozzle, the SortMaster™ aborts the sort and issues an audible warning. Cytomation flow cytometry sorters also contain an aerosol evacuation system.

Cytomation MoFlo® flow cytometers can have up to three lasers on the optical bench. The three light paths are spatially separated and allow up to 9-fluorescence emissions (up to five from beam 1, two from beam 2 and two from beam 3) with no performance loss.

Cytomation offers the most flexible line of flow cytometers having an upgrade path from their MoFlo® BTA analyser to their top-of-the-line sorter, MoFlo® MLS.

### 3.4. Partec/Dako

The Partec PAS-III and Dako Galaxy PRO use fluid switching to sort single populations from a sample. In these sorters a piezoelectric valve is opened and shut to control the fluid stream to either waste or the sort collection tube. Again sort rates are slow with a maximum rate of 1000 cells/s.

## 4. Specialised flow cytometers

### 4.1. Partec cell counter analyser (CCA)

This is a small bench top flow cytometer, which contains an HBO-100 arc lamp, built-in computer and analysis software. Cells are stained with either Partec HR-A solution or HR-B solution, and then the cells pass through a quartz flow cell where the fluorescent cells are counted. There are two models, CCA-1, which has one fluorescence parameter and CCA-2, which has two fluorescence parameters (for protein and DNA stains) together with time. This instrument is mainly used for cell cycle analysis of all kinds of cells.

**Table 3**  
Specifications and performance of commercial flow cytometry sorters

	Beckman Coulter EPICS <sup>®</sup> ALTRA <sup>™a</sup>	Becton Dickinson FACSCalibur <sup>™</sup>	Becton Dickinson FACS Vantage <sup>™</sup> SE <sup>b</sup>	Cytomation MoFlo <sup>®</sup> MLS	Partec-PAS-III Dako-GALAXY PRO <sup>c</sup>
<i>Excitation</i>					
Light source	Wide range of lasers <sup>d</sup> . Four or more lasers can be mounted on optical bench	15 mW Argon-ion laser, 488 nm; optional diode laser, 635 nm	Wide range of lasers <sup>d</sup> . For >2 lasers, third laser mount required (includes timing electronics)	Wide range of lasers <sup>d</sup> . Standard-3 spatially separated beams from 3 lasers	15 mW Argon-ion laser, 488 nm and 100 W HBO arc lamp. Red diode laser option
<i>Detectors</i>					
Scatter detectors	FS – solid state silicon or PMT (with Forward Fluorescence Detector option) SS-PMT	FS-solid state silicon SS-PMT	FS – solid state silicon SS-PMT	FS – solid state silicon or PMT SS-PMT	FS – PMT SS-PMT
Fluorescence detectors	4 or 6 PMT. Up to 8 fluorescence signals with Gated Amplifier option	3 or 4 PMT	Up to 6 PMT	Up to 9 PMT (up to 5 on beam 1, 2 on beam 2 and 2 on beam 3)	6 PMT
<i>Fluidics</i>					
Flow cell	76 µm High Velocity Quartz. 76, 100 and 140 µm SortSense <sup>™</sup> Quartz. 51–400 µm jet-in-air. 250 µm Biohazard Quartz	Quartz flow cell	Ceramic nozzles, jet-in-air, 50–100 µm. With MacroSort <sup>™</sup> option, up to 400 µm. Quartz flow cell available for biohazardous samples.	Jet-in-air nozzles, 50–400 µm	Sorting flow cells with different cross-sections are available
Sample flow rates	Variable	3. Low, Medium, High	Variable	Variable	Variable, 0–1200 µl/min from syringe pump
Sample mixing	Variable speed vortex	No	Variable speed vortex	Variable speed agitated sipper tube	Yes with Autosampler option
Direct absolute counting	No	No	No	No	Yes, syringe pump and sample volume detection
<i>Signal processing</i>					
Electronics	Analog	Analog	Analog	Analog; optional digital signal processor (DSP) for fluorescence compensation	Analog/digital
ADC	1024 channel	1024 channel	1024 channel	4096 channel	65536 channel
Amplifiers	Linear and 4 decade Log	Linear and 4 decade Log	Linear and 5 decade Log with 1 log offset	Linear and 4 decade Log	Linear and 3 and 4 decade Log
Fluorescence compensation	Between FL1 & FL2; FL1 & FL3; FL2 & FL3; FL2 & FL4; FL3 & FL4; SS PMT & AUX 1&2	Between FL1 & FL2; FL2 & FL3; FL3 & FL4 (with FL4 option)	FL1 & FL2; FL1 & FL3; FL1 & FL6; FL2 & FL3; FL2 & FL6; FL3 & FL6; FL4 & FL5	Software compensation between any pair of fluorescence detectors (on and off line); On line 8×8 matrix with DSP	Software compensation between any pair of fluorescence detectors (on and off line)
Pulse processing	Integral and Peak available on all parameters for doublet discrimination. TOF (width) on any peak	Width and Area available on all fluorescence parameters for doublet discrimination	Requires Pulse Processor option to obtain width and area for doublet discrimination.	Integral and Peak available on all fluorescence parameters for doublet discrimination	Height/Width and Area available on all parameters for doublet discrimination
Time	Yes	Yes	Yes	Yes	Yes
Ratio	Yes	No	Yes, with Pulse Processor option	Yes	Yes
Auxiliary	Yes, 2 Auxiliary channels with gated amp	No	No	No	Yes
Prism	Yes	No	No	No	No
Time-of-flight (Width)	Yes, on all signals	Yes, on all signals	Yes, on all signals	Yes on FS	Yes, on all signals



<i>Performance</i>					
Fluorescence sensitivity	<800 MESF for FITC and PE	<750 MESF for FITC	<200 MESF for FITC	<500 MESF for FITC, PE and Cy5	<100 MESF for FITC
Scatter sensitivity	At least 0.5 $\mu$	At least 0.5 $\mu$	At least 0.3 $\mu$	At least 0.5 $\mu$	0.5 $\mu$
<i>Sorting specifications and performance</i>					
Sheath pressure	4.0–100.0 p.s.i.	N/A	Up to 100 p.s.i.	Up to 100 p.s.i.	N/A
Drop drive frequency	1.0–99 kHz	N/A	1.2–99 kHz	Up to 200 kHz	N/A
Phase control	Yes	N/A	Yes	Yes	N/A
Sort rate	Up to 25 000/s	Max. 300 cells/s	Up to 25 000 cells/s	Up to 100 000 cells/s	Up to 1000 cells/s
Sort purity	>98%	>95%	>97%	>99%	99%
Sort yield <sup>c</sup>	>80%	>50%	>80%	>75%	>80%
1, 2 or 4-way sorting	2-way	1-way fluidic sorter	2-way	2-way and 4-way	1-way fluidic sorter
Large particle sorting	Large particle sorting (LPS) provides 200 $\mu$ , 300 $\mu$ & 400 $\mu$ flow cells	Yes	Yes with MacroSort™ (included in TurboSort™) Nozzle sizes to 400 $\mu$ m.	Yes, nozzles up to 400 $\mu$ m	Yes
Sort monitor	SortLOCK™. Monitors and automatically adjusts drive to maintain sort droplet stability	N/A	SortWatch™. Monitors sort drop formation for stability	SortMaster™. Automated system to determine droplet delay and continually monitor and adjust sort delay	CCD colour camera for control of sort delay and efficiency
Single-cell deposition system	AutoClone®. Deposits 1 to max number of cells to fill well in microtitre plate.	N/A	CloneCyt™ Plus. Deposits 1 to 999 999 cells per well into microtitre plates, slides	CyCLONE™. Deposits 1 to 999 999 cells per well into microtitre plates	N/A
<i>Data management—Note Specifications current at October 1999<sup>f</sup></i>					
Computer	FlowCenter™-IBM compatible	FACStation™-Macintosh	FACStation™-Macintosh	IBM compatible	Built-in IBM compatible
CPU	Intel® PIII 550/600 MHz	G3 Power PC RISC	G3 Power PC RISC	Dual Intel® PII 333 MHz	Intel® PII 450 MHz
Memory	128 MB RAM	128 MB RAM	128 MB RAM	128 MB RAM	128 MB RAM
Data storage	13.7 GB hard disk	12 GB hard disk	12 GB hard disk	Dual 6 GB and 8 GB hard disks	18 GB hard disk
CD-ROM	Yes (40×)	Yes	Yes	Yes	Yes (read/write)
Monitor	21 in. high resolution or 18 in. flat screen	18 in. flat screen	20 in. flat screen	18 in. flat screen or 21 in. high resolution	15 in. built-in active LCD; optional external
Networking	Yes	Yes	Yes	Yes	Yes
Data file structure	FCS 2.0	FCS 2.0	FCS 2.0	FCS 2.0	FCS 2.0
Software	EXPO32™	CELLQuest™ <sup>g</sup>	CELLQuest™ <sup>g</sup>	CyCLOPS™ SUMMIT	FloMax
<i>Available options</i>					
Autosampler	No	Yes, 40 place carousel	No	No	Yes, 36 place carousel (including staining and lysing)

<sup>a</sup> Specification of EPICS® ALTRA™ with HyPerSort™ option.

<sup>b</sup> Specification for FACS Vantage™ SE with TurboSort™ Plus option.

<sup>c</sup> The Dako GALAXY PRO is a Partec PAS flow cytometer optimised for immunophenotyping.

<sup>d</sup> See Table 2, list of lasers used on flow cytometry sorters.

<sup>e</sup> Sort yield will depend on a number of factors including data rate, abort rate, mode of operation, particles being sorted and their concentration.

<sup>f</sup> Computer specifications will vary. CPU speed is at October, 1999.

<sup>g</sup> Standard acquisition and analysis software. Other software packages available.

#### 4.2. Partec ploidy analyzer

Like the CCA this is a small benchtop, single parameter, arc lamp based flow cytometer. It is used for the automatic measurement of ploidy levels in plants using UV excited DNA dyes including DAPI, Hoechst, PI or EtBr. It gives a rapid result (less than 2 min) on any plant material with an accuracy of  $\pm 1$  chromosome. The plant material is chopped in Partec HR-A solution, filtered, stood at room temperature for 15 min, Partec HP-B solution added and the sample analysed.

#### 4.3. Optoflow Microcyte<sup>®</sup>

The Microcyte<sup>®</sup> is a specially developed flow cytometer for microbiology. This instrument uses a 5 mW red diode laser emitting at 635 nm and has a light scatter sensitivity of 0.4 to 15  $\mu$ . It also has a single avalanche photodiode fluorescence detector. It can analyse 1, 10 or 100  $\mu$ l volumes thus giving counts in absolute numbers. The instrument is powered by 12 VDC making it ideal for fieldwork. It is ideal for rapid enumeration of microorganisms and animal cells, algae in water samples and detection of contaminating organisms in food and beverages.

#### 4.4. Luminex

Luminex is developing a system for the rapid analysis of multiple analytes (up to 64 currently) in a single sample (see also review by Dr Vignali, this issue). The system consists of a flow cytometer, microspheres and computer hardware and software. The polystyrene microspheres are made with different levels of red and/or orange fluorescence. They are modified by binding to them reactive components such as antigens, antibodies, or oligonucleotides. The modified beads are then mixed to form a multiplexed

assay set. The individual components of the set can be determined by passing through a flow cytometer and measuring their levels of red and orange fluorescence. The assay set are mixed with the sample and the amount of analyte bound to each bead can be determined using a green label to bind to unbound reactive components on each group of beads. Luminex state that it will be possible to analyse up to 100 analytes in a single tube using their technology.

The technology is exciting but still in development. Luminex has found that existing flow cytometers are not adequate for separating and rapidly counting the bead populations. They are developing their own hardware and software to overcome these difficulties.

### 5. Further reading

Further reading about flow cytometry instrumentation can be found in other articles in this issue, and in the following reference works:

Melamed, M.R., Lindmo, T., Mendelsohn, M.L. (Eds.), 1990. *Flow Cytometry and Sorting*, 2nd Edition. Wiley-Liss, New York.

Robinson, J.P. (Managing Ed), 1997. *Current Protocols in Cytometry*. Wiley, New York.

Shapiro, H.M. 1995. *Practical Flow Cytometry*, 3rd edition. Wiley-Liss, New York.

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

Beckman Coulter: [www.coulter.com](http://www.coulter.com)

Becton Dickinson: [www.bdfacs.com](http://www.bdfacs.com)

Cytomation: [www.cytomation.com](http://www.cytomation.com)

Luminex: [www.luminexcorp.com](http://www.luminexcorp.com)

Optoflow: [www.optoflow.com](http://www.optoflow.com)

Partec: [www.partec.de](http://www.partec.de)