Incredible shrinking flow cytometers

Gone are the days when flow cytometers took up entire rooms. Most of today's instruments fit on a standard laboratory bench, and some are even portable.

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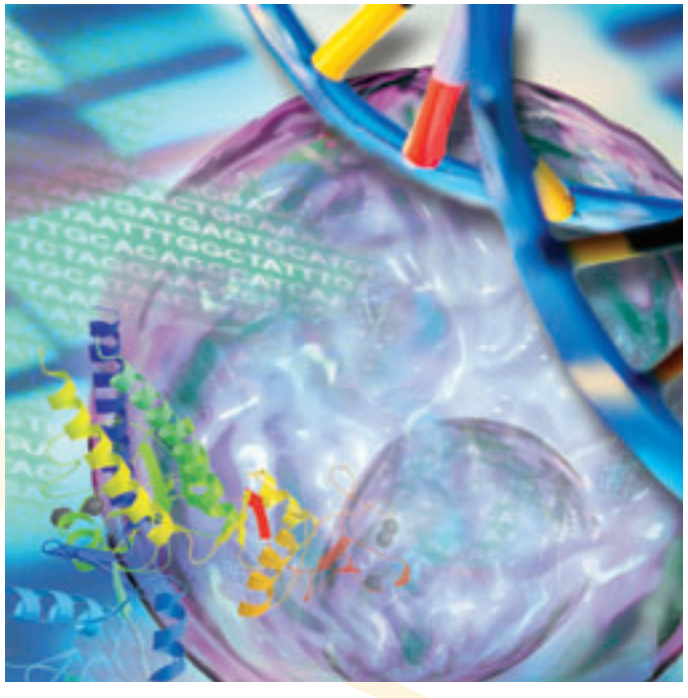

Not so long ago, flow cytometers were behemoths that sat on lots of prime laboratory real estate in a shared core facility. Walk into a core facility today, or even into your neighbor's laboratory, and you are likely to pass by a flow cytometer without realizing it. Flow cytometers are shrinking, and some are now even appearing in individual researchers' labs.

Recent innovations in laser technology have enabled instrument manufacturers to produce smaller flow cytometers. "The old paradigm was you had big water-chilled lasers that [required] a lot of bench space and special [electrical wiring]," says J. Phillip McCoy, Jr., at the National Heart, Lung, and Blood Institute. "Now, [flow cytometers] have little solid-state lasers that are basically plug-and-play."

Floor models have become increasingly rare since *Analytical Chemistry* last reviewed flow cytometers six years ago (**1999**, *71*, 755 A–759 A). Almost all flow cytometers on the market today are benchtop models. Some instruments, such as Partec's CyFlow SL, are portable. Tables 1 and 2, which are meant to be representative rather than comprehensive, list several flow cytometers that are currently available.

Flow cytometers measure optical properties of single particles, often cells, as they pass by one or more lasers. The cells flow through the instrument in a sheath fluid, in single file, at a high speed. Typically, cells are labeled with fluorescent dyes, which are illuminated by the lasers. A detector measures both emitted and scattered light. Some instruments can also sort cells into two or more populations for further analysis.

How do you choose?

Experts say that instruments fall into two general categories: expensive, top-of-the-line models that are very flexible, though difficult to use; and inexpensive, easy-to-use models that have limited capabilities. For example, high-end flow cytometers can detect nearly 20 different fluorophores simultaneously, whereas many less expensive instruments can only measure a few fluorophores at once.

Traditionally, flow cytometers are housed in core facilities of universities and research institutions. Researchers either sign up for appointments to use the instruments or hand their samples to specially trained operators who do the legwork and analysis. Experts say that many systems still require a high level of expertise and that the core facility paradigm will persist. "You can't treat [a flow cytometer] as a black box," warns Maryalice Stetler-Stevenson at the National Cancer Institute. McCoy says, "It's [the difference between] driving a Yugo or driving a Rolls Royce. You still need to know how to drive."

High-end instruments are as complex as they've ever been, say researchers, but some cheaper models with fewer bells and whistles do not require operators to undergo extensive training. The Guava series of instruments, for example, are marketed as personal flow cytometers. "Our whole goal is to bring flow cytometry right to the benchtop, to make it more accessible," says Kim Mulcahy at Guava. She adds that training on the Guava instruments only takes half a day, instead of the weeks required with other machines.

The application that a researcher has in mind will help determine the type of flow cytometer that he or she chooses. And it seems that the list of applications is endless. According to J. Paul Robinson at Purdue University, the flow cytometry field is multidisciplinary, and interest in the technique is growing among scientists in biochemistry, immunology, and engineering. Experts say that the method is used in the clinical laboratory to assess immune function, blood clotting efficiency,

Table 1. Selected nonsorting flow cytometers.[1]

Product	BD FACSCanto	FC500 and FC500 MPL	CyAn ADP	Guava PCA, PCA-96, and EasyCyte	CyFlow SL and ML
Company	BD Biosciences 2350 Qume Dr. San Jose, CA 95131 www.bdbiosciences.com 877-232-8995	Beckman Coulter 4300 N. Harbor Blvd. P.O. Box 3100 Fullerton, CA 92834-3100 www.beckmancoulter.com 800-742-2345	DakoCytomation 4850 Innovation Dr. Fort Collins, CO 80525 www.dakocytomation.com 800-822-9902	Guava Technologies 25801 Industrial Blvd. Hayward, CA 94545 www.guavatechnologies.com 866-448-2827	Partec GmbH Otto-Hahn-Str. 32 D-48161 Münster Germany www.partec.de +49-2534-8008
Price (U.S.D.)	INA	INA	INA	$38,000–89,000	$20,000–150,000
Research or clinical use	Research and in vitro diagnostic use	Class exempt for research, clinical research, and clinical diagnostics	Research use only	Research use only in the United States, Europe, and Japan; in vitro diagnostic use elsewhere	Research use and in vitro diagnostic use
Number of lasers	2	Up to 2	Up to 3	1	1–3, plus mercury arc lamp (UV)
Optical parameters	Up to 6 fluorescence and 2 scatter	5 fluorescence and 2 scatter	9 fluorescence and 2 scatter	PCA and PCA-96: 2 fluorescence and 1 scatter; EasyCyte: 3 fluorescence and 1 scatter	Up to 13 fluorescence and 3 scatter
Fluorescence sensitivity (MESF)	≤100 FITC and ≤50 PE	600 FITC and APC, 300 PE	<120 FITC and <60 PE with DakoCytomation 8-Peak FluoroSpheres	<600 FITC	≤100 FITC
Fluorescence resolution (%CV)	<3 for DNA analysis	Resolves 0.5-µm particles from background	<3	<7	1
Sample flow rates (µL/min)	10, 60, and 120	~10, 30, and 60	Up to 300	PCA: 6, 12, and 30; PCA-96 and EasyCyte: 35.4	10–3000
Acquisition speed (cells/s or particles/s)	10,000	~3500	Up to 50,000	250	15,000
Software	BD FACSCanto clinical software (for instrument setup and quality control)	CXP software for FC500, MXP software for FC500 MPL	Summit software v4	Guava CytoSoft software	Partec FloMax or application-specific software
Special features	The FACSCanto is designed for efficiency, optimal workflow, and performance; automated sample loading (optional) and spectral overlap correction as well as one-tube instrument setup for immunophenotyping; fixed optical alignment; an integrated fluidics cart for effortless instrument operation and maintenance; LIS-compatible	Automated application setup and analysis; FC500 MPL can hold tubes or plates and has the option of recording electronic records and signatures (for FDA 21 CFR 11); FC500 has automated quality control and customizable reporting	Complete compensation to discriminate between fluorophores; reliable, easy-to-use, walk-up operation on a small footprint; 3 models are available for a diverse range of applications, with up to 11 standard parameters and 9 colors	Microcapillary flow cell that does not require sheath fluid and provides direct absolute cell counts; small sample volumes (<20 µL); 96-well plates or tubes; turnkey assays using optimized reagents and integrated software for various applications, such as viability assessments and apoptosis	Compact, high-end benchtop instrument; single- or triple-wavelength excitation; single-platform true volumetric absolute counting; parallel 16-bit digital pulse processing; 96-multiwell-plate automatic option; flexible and modular system; ML: high sensitivity for 200 mW power at 488 nm

APC: allophycocyanine; CV: coefficient of variation; FITC: fluorescein isothiocyanate; INA: information not available; LIS: laboratory information system; MESF: molecules of equivalent soluble fluorophores; PE: phycoerythrin

[1]Some companies offer multiple instruments. Contact the vendors for their full product lines.

organ transplant suitability, and many other properties. Researchers commonly use flow cytometers to study cellular processes, such as apoptosis (cell death), signal transduction, cell division, and calcium activation. Proteomics scientists also use the technique to quantitate and localize many cellular proteins simultaneously. Routine clinical or research assays with one or two common fluorophores can be run easily and inexpensively on instruments that measure only a few parameters, but complex experiments may require a high-end instrument.

Some specialty instruments are fine-tuned for certain applications, such as the analysis of sediment in water or the detection of microbial contaminants in milk. Other instruments are

Table 2. Selected sorting flow cytometers.[1]

Product	BD FACSAria	ALTRA HyPerSort System	MoFlo High-Performance Cell Sorter	CyFlow space
Company	BD Biosciences 2350 Qume Dr. San Jose, CA 95131 www.bdbiosciences.com 877-232-8995	Beckman Coulter 4300 N. Harbor Blvd. P.O. Box 3100 Fullerton, CA 92834-3100 www.beckmancoulter.com 800-742-2345	DakoCytomation 4850 Innovation Dr. Fort Collins, CO 80525 www.dakocytomation.com 800-822-9902	Partec GmbH Otto-Hahn-Str. 32 D-48161 Münster Germany www.partec.de +49-2534-8008
Price (U.S.D.)	INA	INA	INA	$95,000
Research or clinical use	Research use only	Research use only	Research use only	Research and in vitro diagnostic use
Number of lasers	2–3	Up to 4	Up to 3	2
Optical parameters	11 fluorescence and 2 scatter	6 fluorescence and 2 scatter	9 fluorescence and 2 scatter	6 fluorescence and 2 scatter
Fluorescence sensitivity (MESF)	≤125 FITC and ≤125 PE	800 FITC and PE	<120 FITC and <100 PE with DakoCytomation 8-Peak FluoroSpheres	<100 FITC
Fluorescence resolution (%CV)	INA	Resolves 0.5-µm-diam particles from background	<3	1
Sample flow rates (µL/min)	Variable	Variable	6	10–3000
Acquisition speed (cells/s or events/s)	70,000	~25,000	Up to 100,000	15,000
Sorting speed (cells/s or events/s)	25,000	25,000	Up to 70,000	300
Sorting purity	>98%	>98%	>99%	>99%
Software	BD FACSDiva software	EXPO32 software	Summit software v4	Partec FloMax or application-specific software
Special features	First benchtop sorter with fixed-alignment cuvette flow cell; optional automated cell deposition unit for sorting to multiwell plates or microscope slides; aerosol management option	AutoClone sorting; forward fluorescence detection; large-particle sorting; application flexibility for a variety of research assays; new SortLOCK sort monitoring system provides truly walk-away, high-speed, multicolor sorting	Modular; single-cell deposition with >99% recovery; patented 4Way Sort capability; multiple sample input options; range of sorting features to improve sort results, including yield, cell viability, and collection capacity	Compact, high-end benchtop instrument; high sensitivity for 200-mW power at 488 nm; nondestructive, aerosol-free, and easy-to-operate sorting option; single-platform true volumetric absolute counting; parallel 16-bit digital pulse processing; real-time acquisition; 96-multiwell-plate automatic option; flexible and modular system configuration

CV: coefficient of variation; FITC: fluorescein isothiocyanate; INA: information not available; MESF: molecules of equivalent soluble fluorophores; PE: phycoerythrin
[1]Some companies offer multiple instruments. Contact the vendors for their full product lines.

tailored for bead-based multiplexed assays. Instead of analyzing one cell at a time, the COPAS flow cytometer from Union Biometrica analyzes and sorts entire organisms, such as embryos and larvae of the fruit fly *Drosophila melanogaster* and the roundworm *Caenorhabditis elegans*, and other types of cell clusters. Rock Pulak at Union Biometrica says that COPAS allows researchers to analyze samples of these larger objects more rapidly than by microscopy, although the instrument is not as fast as traditional flow cytometers.

Another important consideration is whether the researcher needs to sort cells and particles. "If you have a cell sorter, you can physically separate a population of cells, which is unbelievably powerful," says Robinson. "So now you can take that population that you've identified, and you can [perform] a series of biochemical analyses or whatever you want to do with those purified populations." He adds that cell sorting is a complex procedure that cannot be done without extensive training.

Most sorters separate cells by an electrostatic or a deflected-drop mechanism. After analysis, the flow stream passes through a nozzle, which breaks the stream into uniform droplets. On the basis of the fluorescence and scatter analyses, the droplets are assigned to a population and are given a charge. Charged

deflection plates attract oppositely charged droplets, which are shunted to collection tubes or microwell plates. A few sorters use a much slower piezoelectrical mechanism whereby a pressure pulse temporarily diverts the flow stream into a collection chamber or a catcher device moves into the stream to collect a bit of the liquid. Although sorting is widely touted as not harmful to cells, McCoy cautions that one cannot make that assumption. He says that some cells are sensitive to these manipulations and that researchers should test a small amount of sample in a sorter before running a big experiment.

Cell concentration is difficult to ascertain using most flow cytometers, says Mulcahy, because the cells become diluted with an unknown amount of sheath fluid. Researchers who need this value typically run a known number of reference beads through the instrument prior to the sample run. However, this step increases running costs and labor. In the past few years, Guava and Partec have taken different approaches to avoid the use of reference beads and obtain accurate concentration values. Guava instruments do not use sheath fluid at all. Instead, a pump draws up a precisely measured volume of sample, which is directly analyzed. Partec's instruments measure volume by true volumetric absolute counting. In this process, two electrodes in the sample tube directly measure the sample volume over a precise distance.

On the horizon

Experts predict that the flow cytometers of the future will be even smaller and easier to operate. Connectivity to laboratory information systems and databases will become more efficient. And some company representatives say that more application-specific instruments may appear on the market.

Additional fluorophores may be developed for use on flow cytometers. McCoy says, "It sounds like we're on the cusp of getting nanocrystal technology, which might allow us to do 50 or 100 colors instead of 13 or so. That would just be mind-boggling."

Robinson looks forward to advances in mathematical analyses. "We have cells from patients, and those cells tell a story," he says. "We need to be able to put that story together quickly, effectively, [and] accurately. Computational analysis is absolutely going to play a role."

Many experts are excited by the prospect of marrying microscopy with flow cytometry in some fashion—often referred to as the Holy Grail of the field. The first imaging flow cytometer to hit the market is produced by Amnis. The ImageStream, which was launched in November 2004, captures six images from each cell and obtains flow cytometry data. Robinson says, "I think this is an instrument that has great promise and opens up yet another frontier that says to us, 'Look, flow cytometry is alive and well on planet Earth.'"

Experts say that flow cytometry is here to stay because it's so useful for so many applications and fields of science. Flow cytometry, Robinson says, is "no longer an exclusive club. It's out there for all to use."

Katie Cottingham is an associate editor of Analytical Chemistry.