Flow cytometry smaller and better

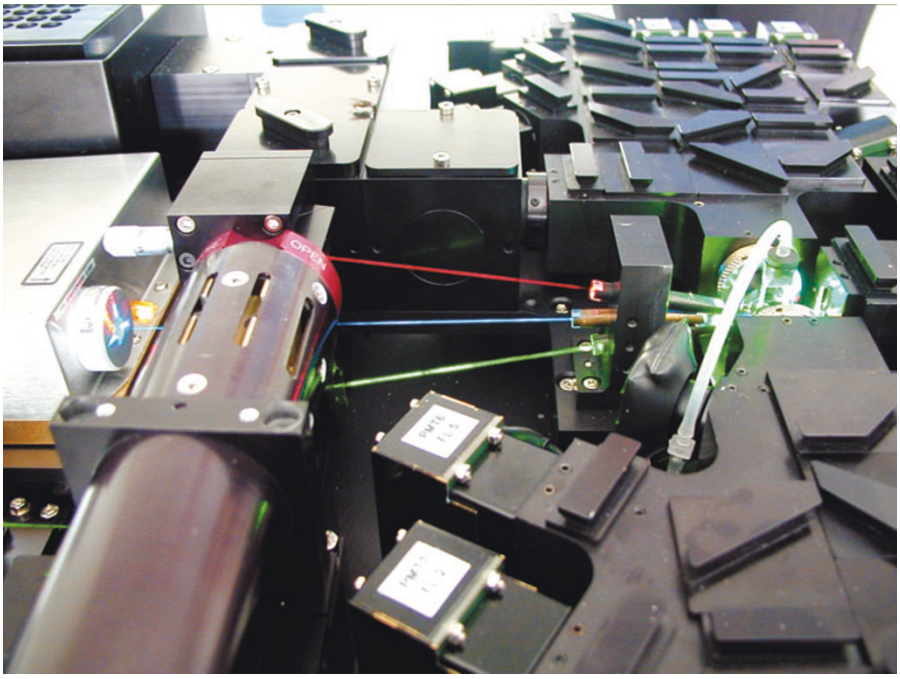
Improved lasers, optical devices and software have increased the speed, accuracy and sensitivity of flow cytometers, while decreasing their size and cost. These and other recent developments extend the reach and broaden the applications of this powerful technology. Laura Bonetta reports.

Looking like clouds of tiny insects, dot plot graphs have been adorning the pages of scientific journals for decades. They display data obtained by flow cytometry, a method for analyzing hundreds of thousands of individual cells based on their shape, consistency and other features. Once the domain of immunologists, flow cytometry applications are making their way into mainstream cell and molecular biology—from analyzing gene knockdowns with siRNA, to elucidating entire signal transduction cascades, to high-throughput screening of stable cell lines (see **Box 1**). "Flow cytometry has been around for many years, but in a way it has only just come into its own," says Tony Ward, director of the research product line at BD Biosciences, a leading supplier of flow cytometry products.

BD Biosciences, a segment of Becton, Dickinson and Company (BD), developed one of the earliest commercial flow cytometers, called a fluorescence-activated cell sorter (BD FACS), in the early 1970s. For this reason, some people still use the term FACS generically to mean flow cytometry and the instruments that perform it. Although instruments have since evolved, the basic principles of flow cytometry remain the same. The first step is to label sample cells with fluorescent dye molecules that bind specifically to components of interest. The cells are then loaded on the flow cytometer, in which, constrained to the center of a fluid stream, they pass in single file through a column. There, the instrument shines light of a given wavelength on the liquid, and detectors record what happens to the light.

In addition to measuring the fluorescence emitted from each cell, the flow cytometer collects data on how the light interacts with the cells. When the laser beam hits the edge of a cell, some of the photons of light are deflected slightly; the amount of this so-called forward scatter indicates cell size. Other photons may hit internal structures and are deflected through a wider angle. This side-scattered light is a function of the granularity of the cell—for example, granulocytes have higher side scatter than lymphocytes.

Today these measurements are taken at breathtaking speeds exceeding 70,000 cells per second with unsurpassed accuracy and sensitivity. For this reason, flow cytometry is ideally suited for studies that need to distinguish among up to several million cells based on many different parameters. The recent marriage of flow cytometers with other detection systems, such as imaging devices and multiplex bead-based assays, has greatly enhanced the amount of information that can be gathered from each cell.



A view of the inside workings of the 16-parameter, 13-color flow cytometer CyFlow ML by Partec. (Courtesy of Partec.)

Seeing colors

A major development in flow cytometry has been the availability of an arsenal of fluorescent probes. "Today you can look at many more parameters simultaneously. We look at seven to eight colors routinely," says William Telford, who directs the Core Flow Cytometry laboratory at the US

National Cancer Institute. In addition, it is now possible to more precisely quantify the amount of signal coming from one cell relative to another. "You can detect high, medium and low expressors of a specific protein with our reagents," says BD Biosciences' Ward.

Researchers can identify receptors on the surface of cells, total DNA, the amount of mRNA for a particular gene, calcium concentrations, membrane potential and pH. Some of the newer probes allow researchers to measure the amount of protein phosphorylation in a cell to study signaling cascades. BD Biosciences alone sells approximately 25 types of fluorescent reporter dyes coupled to antibodies, probes and other reagents. Many companies provide flow cytometry reagents, including Exalpha Biologicals, Bangs Laboratories, One Lambda Inc., DakoCytomation, Molecular Probes to name a few.

The ImageStream 100-image flow cytometer distributed by Amnis takes photographs of cells in flow. (Courtesy of Amnis.)

To take advantage of the growing number of fluorescent reagents, flow cytometers have multiple lasers to stimulate different excitation wavelengths and several detectors to collect different color signals.

BOX 1 STABLE CELL LINES IN A FLASH

If you have ever had to do it, you know the trials and tribulations associated with generating a stable cell line that expresses your favorite gene. Chromocell, a startup company based in New Jersey, has found a way to deliver stable cell lines that reproducibly and reliably express high levels of functional proteins with greater ease and efficiency.

The technology uses a combination of special fluorescent probes to detect the mRNA corresponding to the gene of interest in individual transfected cells and high-speed sorting of the target cells by flow cytometry. When the probes are added to a batch of cells, only those cells that contain the target message end up with a fluorescent signal. The cytometer is then programmed to select cells with the highest amount of signal so that they can be further studied to determine whether the expressed protein is functional. For proteins made up of more than one subunit, cells are transfected with, for example, three individual genes and the cytometer simultaneously tests for the presence of all three signals in a cell. The procedure is largely automated making it possible to screen millions of cells in a matter of minutes. "When we get so many cell lines that express the gene we are interested in, we can then select the cells that meet other criteria, for example stability," says chief scientific officer Kambiz Shekdar.

In addition to stability, the researchers test the response to different treatments to see whether the expressed protein behaves as it should. "Many of our cell lines are more sensitive and more physiologically relevant than ones that have been produced by other labs," says Shekdar. He attributes the improvement to the fact that by generating so many stable cell lines at one time, it is easier to functionally identify the ones producing the different protein subunits in the right stoichiometry. Another advantage of the technology is that unlike high-throughput methods for cell selection in which transfected genes carry some kind of fluorescent tag, such as a GFP molecule, the expressed protein is as close to the native configuration as possible.

The company, which was established in 2002 by researchers from Günter Blobel's lab at The Rockefeller University, produces stable cell lines as a service and is now exploring other applications of the technology, in particular for antibody biologics production.

The top-of-the-line machine produced by BD Biosciences can detect up to 18 colors. Germany-based Partec, which started distributing flow cytometers in 1969, offers different configurations of instruments and lasers to suit customers' needs. "If someone calls and requires an instrument with 12 optical parameters and three lasers we will customize our products to this need," says Roland Göhde, HIV-AIDS project coordinator at Partec.

"There is very little limitation [for detecting more colors] on the instrument side," says Alan Saluk, director of the flow cytometry core facility at The Scripps Research Institute. But despite the boundless possibilities, only a handful of published studies look at more than eight colors simultaneously and most will use four or five. According to Saluk, complex multicolor analyses are difficult to conduct, adding that one of the main stumbling blocks is the data processing and proper interpretation (see **Box 2**).

Size matters

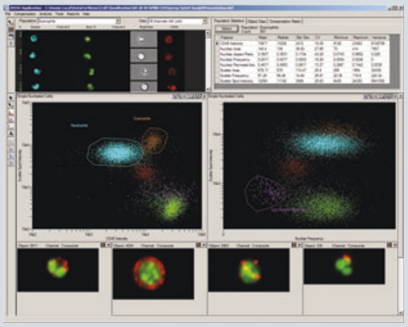
Improved lasers have not only given way to greater accuracy and sensitivity, but also smaller instruments. "Lasers are becoming smaller and cheaper and so are the instruments," says Telford—a development that manufacturers believe will take flow cytometers beyond core facilities and into individual research labs.

In the past three decades, high-end machines have gotten smaller without compromising power. DakoCytomation's CyAn cytometer contains three lasers (blue, red and violet), and can measure forward and side scatter and up to nine colors of fluorescence, all at speeds of over 30,000 cells per second. According to the company, the instrument is six to ten times smaller than earlier models with comparable capabilities. BD Biosciences sells a low-cost benchtop analyzer called BD FACSArray. With two lasers, it can measure 15,000 cells per second and visualize four colors, as well as cell size and heterogeneity.

In addition, several companies, including Guava Technologies, Beckman Coulter and Partec, sell small two-color units that are more targeted to specific applications, such as measuring cell proliferation or apoptosis. "Customers like the fact that they use their budget for the configurations that they need," says Göhde.

The most recent newcomer in this arena is Beckman Coulter's Cell Lab Quanta flow cytometry system, which began shipping in April of 2005. Built on a joint development effort with NPE Systems, the Quanta combines Coulter volume measurement (an electrical sensing method for counting and sizing cells), with two-color fluorescence capabilities. "We saw an opportunity to move from core centralized facilities to individual labs who want to do quick cell-based assays and look at

BOX 2 ANALYZE THIS



A screen shot of Amnis' IDEAS software displaying plots and images from a 5-part differential analysis of human peripheral blood. (Courtesy of Amnis.)

Every flow cytometer includes software for collecting and analyzing information gathered during an experiment. The analysis typically involves finding out how many cells from the sampled population meet a criteria of interest, and the data can be displayed in a number of different formats, most frequently dot plots or histograms. Basically, histograms quantitate intensities of scatter or fluorescence based on one parameter, whereas dot plots quantitate percentages of cells with various properties. Dot plots are typically used to detect small numbers of cells that are well separated from the main populations of the cells present. In addition, in the software packages that come with some imaging cytometers, such as Amnis' IDEAS data analysis software, the dot plots are linked to images of the cells. That means that a user can click on an individual dot to see the corresponding image or can draw a 'gate' around a population of dots to see what that cells that fall within the gate look like. "This is very useful for confirming that the dots really do represent the cells you think they do and aren't clumps of cells or other artefacts," says Amnis' Basiji.

Many companies also sell software packages separately from the instrumentation. One of the most popular products is Tree Star's FlowJo software, which can analyze data from any flow cytometer and will work in both a Windows and MacIntosh environment. FlowJo is based around 'workspace' documents—similar to pages in a notebook—that contain information about the samples, along with all the gates, graphs, statistics, tables and reports applied to the data. FlowJo provides several different analysis platforms for standard analyses such as gating and statistics, as well as more special ones, such as DNA content, cell cycle analysis, kinetics, proliferation, calibration and statistical comparisons.

Other examples include BD CellQuest, a popular commercial flow cytometry data acquisition and analysis software for the MacIntosh computer produced by BD Biosciences. FSC Express from De Novo Software is a very advanced analysis product that runs on the PC. Verity has Winlist, a comprehensive data analysis package for the PC, and Modfit, one of the main DNA and cell cycle analysis packages.

Some software packages are geared toward more sophisticated applications. Applied Cytometry Systems (ACS) has developed the StarStation software to drive the Luminex flow cytometer and help analyze the data generated from it. Bio-Rad and MiraiBio have similar software products called Bio-Plex Manager and MasterPlex, respectively. "The software takes care of calibrating the instrument, running the assays and collecting the data," says Peter Nobes, vice president for product development at ACS. In September 2005, ACS will release a new product that "reads files generated in StarStation and analyses them in custom ways according to a particular plug in application." The first plug in, called SNP-R, will be used to compare a pair of alleles to determine which one is present in a given sample. "It is similar to data obtained using a [single nucleotide polymorphism] SNP microarray but different in that the sample is in flow," says Nobes. The company plans to continue developing different types of plug-ins for customers.

things like apoptosis and cell cycle," says Ian Ley, director of the cellular analysis business center. In September 2005, the company will release a new model that can also measure light scatter and one additional color. The size and affordability of these smaller instruments, as well as the ease of use, have made it possible for them to be used in developing countries to monitor HIV infection in the population (see **Box 3**).

Sorts of sorters

Some flow cytometers can physically separate a group of cells from the rest. The instrument puts a negative or positive charge on specific cells that have defined properties, and then deflects these charged cells to separate collection tubes. Because multiple fluorochromes can be assessed simultaneously, cell sorting by flow cytometry can separate complex mixtures of cells on the basis of several markers—a capability that is increasingly exploited in stem cell research protocols. As a recent example, Sean Morrison's group at The University of Michigan, Ann Arbor, was able to isolate mouse hematopoietic stem cells of unprecedented purity from blood, based on the combined expression of the receptors of the SLAM family[1].

Beckman Coulter's Cell Lab Quanta provides flow cytometry on a smaller scale. (Courtesy of Beckman Coulter.)

The ability to do these kinds of experiments rests partly on improved sensitiv-

ity of detection. But another requirement for cell sorting, especially when dealing with a population of rare cells, is speed. DakoCytomation's MoFlo was the first true high-speed cell sorter and the first to allow separation of four different cell populations simultaneously. "It is the workhorse of the industry," says Ulrik Cordes, vice president for flow cytometry. "It has high performance in terms of yield, cell viability and purity, and runs at very high speed, reaching up to 70,000 cells per second." The sorter has an open design, which means that the user can add different kinds of lasers as new fluorechromes are developed.

Another popular cytometer with cell sorting capability is BD Biosciences' FACSVantage SE System. With a new generation of lasers and advanced optics, the company claims it provides enormous flexibility and sensitivity. It sorts up to four populations of cells simultaneously at rate of 16,000 cells per second, with sorted fractions exhibiting over 99 percent purity.

Traditionally, flow cytometers with cell sorting capability were large instruments, but that is no longer the case. In April 2003, BD Biosciences began shipping the first high-performance benchtop cytometer with electrical sorting capabilities and, according to the company, the instrument, called BD FACSAria, has taken the market "by storm." Improvements in fluidics and optics allow for higher speed sorting (25,000 events per second) with less stress on the sorted cells and enhanced overall sensitivity. The system uses three lasers to allow detection of 13 colors as well as scatter.

BOX 3 FLOW WITH A CAUSE

Flow cytometry was thrust into the spotlight by the AIDS epidemic in the 1980s as it provides a quick and accurate way to count $CD4^+$ and $CD8^+$ T cells in HIV-infected patients. Until recently flow cytometers and the necessary reagents were too expensive and too difficult to implement in developing countries—where HIV infection is ravaging the population. Thus, the development of smaller and more affordable, robust instruments has had a substantial impact in this part of the world.

Partec's CyLab unit is used for CD4 T cell counting in developing countries. (Courtesy of Partec.)

Partec has two kinds of mobile flow cytometry units: one, housed in a car, which is powered by the car battery and solar panels, and the other, part of a laboratory truck, is powered by its own generator. "Since 2002, more than 200 CyFlow instruments have been placed in Sub-Saharan Africa, offering CD4 counting at a cost of [about $2.2]," says Göhde.

In addition to CD4 counts, the Partec instruments measure the number of $CD4^+$ T cells as a percent of the total number of lymphocytes. Recent studies have revealed that in infants, CD4 counts normally decrease with age, whereas the proportion of CD4 remains stable. This means that the threshold of 200 $CD4^+$ T cells generally used to decide when to start antiviral therapy cannot be safely used for children. The decision to start therapy in pediatric patients should instead be based on the CD4 percentage value.

Guava Technologies' EasyCD4 system weighs a mere 35 pounds. These systems and optimized CD4 assay kits have been sold throughout Africa, India and southeast Asia for CD4 counting at slightly more than $1 per test. Other companies offering flow cytometry products for CD4 counting in developing countries include BD Biosciences and Beckman Coulter.

Pretty pictures

An exciting advance has been the combination of flow cytometry with imaging tools and devices. Amnis Corporation pioneered multispectral image flow cytometry, a method that allows individual cells to be visualized as they zoom by the laser beam. "The Amnis instrument is a harbinger of things to come," says Telford.

BD Biosciences' FACSVantage (left) and FACSAria (right) provide powerful flow cytometry and cell sorting capabilitites. (Courtesy of BD Biosciences.)

Amnis' ImageStream 100 is similar to a flow cytometer with the important distinction that the traditional detectors have been replaced by a sensitive charge-coupled device (CCD) camera, custom designed by the company. The camera takes as many as six independent images (brightfield, darkfield and up to four fluorescent images) of each cell at a rate of about 100 cells per second. Although the speed is slow for a flow cytometer, it beats that of any high-content screening system hands down. Because it can image a large number of cells at once, the ImageStream is ideally suited to gather a statistically meaningful picture of a population of cells. "If there is any variability in a cellular response to a treatment, you will not get the necessary statistical information

using a microscope," says David Basiji CEO of Amnis Corporation.

The instrument provides the same measurements as a traditional flow cytometer but with the added benefits that "you can also calculate where the fluorescent signal is coming from in the cell," says Basiji. For example, a researcher can measure the localization of two cell-surface markers relative to one another or determine the cytoplasmic versus nuclear location of a molecule, all in a large population of cells. Albert and Vera Donnenberg at the University of Pittsburgh Cancer Institute are exploring another potential application. "We are using ImageStream to find out what [cancer stem cells] look like," says Albert Donnenberg. Many researchers believe that tumors contain a rare population of therapy-resistant resting cells, which act like stem cells to give rise to cells that continuously divide. "We are pathologists, so we don't believe anything unless we can see it," adds Donnenberg.

Variations on the theme

Laser scanning cytometers (LSC) are also capable of visualizing the location of fluorescent signals in a cell, but using a different strategy. In an LSC the sample does not move, rather the laser beam sweeps over the cells to construct an image in a manner similar to confocal microscopy. Unlike a microscope, the LSC allows the user to scan a relatively large area without the need to refocus. In addition, the LSC allows the quantification of all the fluorescent light emitted from the entire cell depth at each location in the specimen, just as a flow cytometer does. "It has the same quantitative power as flow cytometry in terms of sensitivity and precision, but with the addition of imaging and the ability to work with live or fixed adherent cells, using a variety of carrier types," says CompuCyte CEO Helena Holden.

CompuCyte's LSC has four fluorescence and two scatter detectors, to capture up to six images of cells; the sample can be either in suspension, adhering to a slide or in tissue sections. The LSC is well-suited for applications that cannot be done in flow. "It is good for studying apoptosis in adherent cells. Removing cells [from a culture dish to suspend them] can simulate cell death, so it is preferable to analyze them while they are still on their growth surface," says Telford, whose lab is also using Compucyte's LSC to detect lymphocytes that have infiltrated lung tissue.

"CompuCyte's laser scanning cytometers can analyze both fluorescent and chromatic dyes, as well as combinations of the two. This extends cytometric investigations to objective cytometric analysis of tissue sections without compromising tissue architecture and allows pathologists to view samples in the manner that they are accustomed to, coupled with additional quantitative data similar to flow cytometry reports," says Mel Henriksen, vice president of product development.

In 2006, the company will provide an additional feature with their instruments to allow the measurement of decay times of fluorescent labels. Because conditions like pH, oxygen or cation concentrations

affect the rate of decay of a fluorescent dye, decay times can provide a sensitive measure of the characteristics of a cell's microenvironment.

Another LSC system, called SurroScan, was developed at SurroMed Inc. (which was acquired in early 2005 by Pharmaceutical Product Development Biomarker Discovery Sciences, a company that provides cytometry services for clinical studies and trials.) "The system combines the best features of a hematology analyzer and a flow cytometer," says Aaron Kantor, executive director of cell and molecular biology. Samples are placed in a volumetric capillary array that holds 32 assays with three or four colors in each. As the laser scans over each capillary, the instrument gathers information to classify and quantify cells in blood or other fluids. Results include both absolute cell counts and relative levels of antigen expression. "You can process a large number of assays on a large number of samples in a reproducible way," says Kantor.

Multiplexing

New systems using multiplex bead arrays push the potential applications for flow cytometry even further. These systems simultaneously measure up to 100 compounds in suspension using very small volumes. Common applications include profiling cytokine concentrations in serum or identifying which proteins are phosphorylated after activation of a given kinase.

The flow cytometers and reagents developed by Luminex "combine the power of flow cytometry with the simplicity of an ELISA plate reader," says Grant Gibson, director of scientific alliances. "Instead of reading cell-based assays, they read a microsphere-based assays." The Luminex method uses microsphere sets carrying variable quantities of two different fluorescent dyes that produce up to 100 different shades of color. Each bead is coupled to a unique antibody or probe that recognizes a specific molecule. After the beads are mixed with a sample—for example, cell lysates—and added to the instrument, the unique color signature on each bead reveals the identity of the bound molecules.

Several companies, including Applied Cytometry Systems, Biosource International, MiraiBio, Qiagen, Upstate, LINCO Research, Marligen Biosciences and others, are licensed to sell Luminex instruments or reagents. In addition, other companies have developed similar technologies. BD Biosciences' FACSArray analyzer can process bead-based assays using the company's own brand of reagents. Beckman Coulter also produces multiplex bead assays that, instead of having two colors on one bead, use two beads per assay.

Although relatively new on the market, the power of this technology is clear. Todd Golub and Robert Horvitz used it to analyze the expression of microRNAs (miRNAs) in different human cancers[2]. As miRNAs are only about 21 nucleotides long and share similar sequences[3],

it is difficult to detect specific miRNAs using glass slide microarrays—a popular method for expression profiling. To overcome the problem, the researchers coupled oligonucleotide probes complementary to the miRNAs of interest to fluorescent beads, whereby each bead represented a single miRNA. They added the beads to miRNAs isolated from cancer cell lysates and stained with streptavidin-phycoerythrin. When the samples were run through a flow cytometer, the instrument simultaneously measured bead color (miRNA identity) and phycoerythrin intensity (miRNA abundance) to reveal a different miRNA expression profile for each sample. The profiles turned out to be an accurate depiction of the developmental lineage and differentiation states of the tumors.

Flow cytometry has benefited from many improvements on the instrument side, as well as the development of fluorescent antibodies and probes. The imaging cytometers and multiplex bead assays extend even further the power of flow cytometry by providing more information for a wider range of samples. Armed with the right software tools to analyze the data, flow cytometry is poised to pro vide an unprecedented close-up view of different cell populations and their molecular components, one cell (or one bead) at a time.

1. Kiel, M.J. *et al. Cell* **121**, 1109–1121 (2005).
2. Lu, J. *Nature* **435**, 834–838 (2005).
3. Bonetta, L. *Nat. Methods* **1**, 79–86 (2004).

Laura Bonetta is a freelance writer based in the Washington, DC area (lbonetta@nasw.org).

SUPPLIERS GUIDE: COMPANIES OFFERING FLOW CYTOMETRY PRODUCTS AND SERVICES

Company	Address
Abcam	http://www.abcam.com/index.html
Aber Instruments	http://www.aberinstruments.com
Applied Cytometry Systems	http://www.appliedcytometry.us
Amersham Biosciences (GE Healthcare)	http://www5.amershambiosciences.com
Alternative Biomedical Services	http://www.absbiomed.com
Amnis Corporation	http://www.amnis.com
Anomeric Inc.	http://home.earthlink.net/~anomeric
Applied Cytometry Systems	http://www.appliedcytometry.com
Applied Precision, LLC	http://www.api.com
Bangs Laboratories	http://www.bangslabs.com
Beckman Coulter	http://www.beckmancoulter.com
BD Biosciences	http://www.bdbiosciences.com/index1.shtml
Bioarray Solutions	http://www.bioarrays.com
Biocytex	http://www.biocytex.fr/International.htm
Biodesign International	http://www.biodesign.com
Biodetect	http://www.biodetect.biz
BioGenex	http://www.biogenex.com
BioLegend	http://www.biolegend.com/
Biomeda	http://biomeda.com
Bio-Rad	http://www.bio-rad.com
BioSource International	http://www.biosource.com
Biostatus Limited	http://www.biostatus.co.uk
BioSure	http://www.biosure.com
Bio-Tek	http://www.biotek.com
Caltag	http://www.caltag.com/home.asp
Cell Sciences	http://cellsciences.com
Chromaprobe	http://www.chromaprobe.com
Chromocell	http://www.chromocell.com/
Clontech (BD Biosciences)	http://www.clontech.com/clontech
CompuCyte	http://www.compucyte.com
Cytek Inc.	http://www.cytek.com/cytek/index.html
CytoBuoy	http://www.cytobuoy.com
Cytometry Research, LLC	http://www.cytometryres.com
DakoCytomation	http://www.dakocytomation.com
De Novo Software	http://www.denovosoftware.com
Diatec	http://www.diatec.com
Duke Scientific	http://www.dukescientific.com
eBioscience	http://www.ebioscience.com
Exalpha Biologicals Inc.	http://www.exalpha.com
Fast systems	http://www.fastsys.com/Immunotox.php
FlowCyte Associates	http://www.flocyte.com/
Gene Logic	http://www.therimmune.com
Guava Technologies	http://www.guavatechnologies.com
Innovative Cell Technologies	http://www.innovativecelltech.com
IQ Products	http://www.iqproducts.nl/
Jackson ImmunoResearch Labs	http://www.jacksonimmuno.com/home.asp
Leinco Technologies	http://www.leinco.com
Linco Research Inc.	http://www.lincoresearch.com

Company	Address
Luminex	http://www.luminexcorp.com
Marligen Biosciences	http://www.marligen.com/products/xmap.htm
Martek Biosciences Corp.	http://www.martekbio.com/home.asp
Media Cybernetics	http://www.mediacy.com
Microbionix GmbH	http://www.microbionix.com/profile.htm
MiraiBio	http://www.miraibio.com
Molecular Probes (Invitrogen)	http://www.probes.com
Nikon	http://www.nikonusa.com
NPE Systems Inc.	http://www.npesystems.com
Olympus	http://www.olympus-europa.com/medical/206.htm
One Lambda Inc.	http://128.121.57.25/index.asp
One Cell Systems	http://www.onecell.com
Orpegen Pharma	http://www.orpegen.com/HP
Panbio Diagnostics	http://www.panbio.com.au
Partec	http://www.partec.de
Perkin-Elmer	http://www.perkinelmer.com
PPD Biomarker Discovery Sciences	http://www.ppdi.com
Pharmingen (BD Biosciences)	http://www.bdbiosciences.com/pharmingen
Phoenix Flow Systems	http://www.phnxflow.com
Polysciences	http://www.polysciences.com/shop
Q-Biogene Inc.	http://www.qbiogene.com
Qiagen	http://www1.qiagen.com/SelectCountry.aspx
R&D Systems	http://www.rndsystems.com
Research Diagnostics	http://members.aol.com/researchd/index.htm
Santa Cruz Biotechnology	http://www.scbt.com
Seradyn	http://www.seradyn.com
Serotec	http://www.serotec.co.uk/asp/index.html
Sigma-Aldrich	http://www.sigmaaldrich.com
Softflow	http://www.softflow.com
Southern Biotech	http://www.southernbiotech.com
Spectrum Computing Services	http://www.spectrum-computing-svcs.com
Spherotech	http://spherotech.com/
Stem Cell Technologies	http://www.stemcell.com
Surromed	http://www.surromed.com/technology
Synthegen	http://www.synthegen.com
Sysmex	http://www.sysmex.de
Tip labtech	http://www.ttplabtech.com/explorer
Trevigen, Inc.	http://www.trevigen.com/index.php
Tree Star, Inc.	http://www.treestar.com/
Union Biometrica	http://www.unionbio.com
Upstate	http://www.upstate.com
VayTek, Inc.	http://www.vaytek.com
Vector Laboratories	http://www.vectorlabs.com
Verity Software House	http://www.vsh.com
Zeiss	http://www.zeiss.com