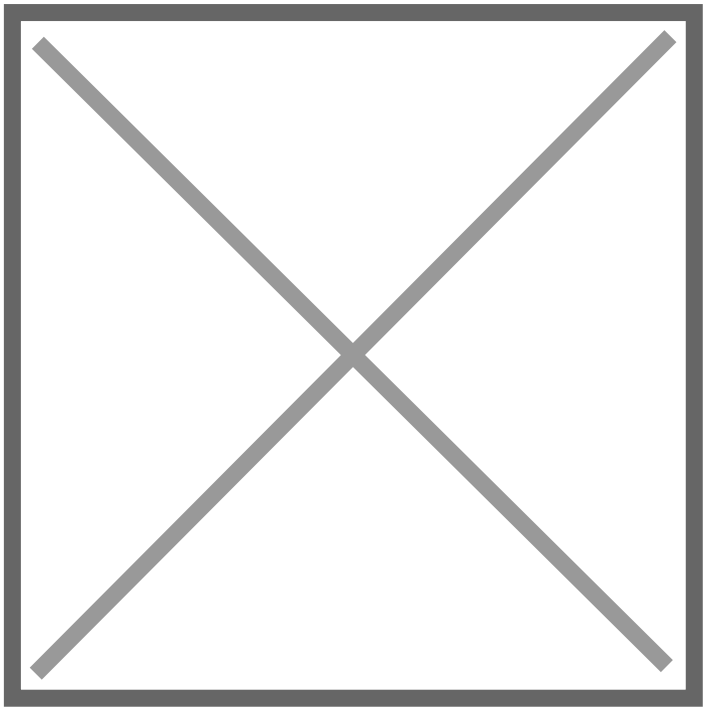
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Flow Cytometry, Simplifying Cell Selection Task

10 March 2009 | News



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Since its invention, flow cytometry has enabled scientists to analyze a variety of cell types. Today, the applications of this technology are even broader and powerful.

Flow cytometry is a technique for counting, examining and sorting microscopic particles suspended in a stream of fluid. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of single cells flowing through an optical and/or electronic detection apparatus.
Early flow cytometers were generally experimental devices, but recent technological advances have created a considerable market for the instrumentation, as well as the reagents used in analysis, such as fluorescently-labeled antibodies and analysis software.
Modern flow cytometers are capable of analyzing several thousand particles every second, in real time, and can actively separate and isolate particles having specified properties.
The new flow cytometers usually have multiple lasers and fluorescence detectors. Increasing the number of lasers and detectors allow multiple antibody labeling and can more precisely identify a target population by their phenotype. Certain instruments can even take digital images of individual cells and allow analysis of fluorescent signal location within or on the surface of cells.
Fluorescence-Activated Cell Sorting (FACS) has emerged as a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers based upon the specific light scattering and fluorescent characteristics of each cell. It has become a useful scientific instrument as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. The acronym FACS is trademarked and owned by Becton Dickinson.

The invention
The first fluorescence-based flow cytometry device (ICP 11) was developed in the year 1968 by Wolfgang Göhde from the University of Münster, Germany (patent no. DE1815352) and first commercialized in 1968-69 by German developer and manufacturer Partec through Phywe AG in Göttingen. At that time absorption methods were still widely favored by other scientists over fluorescence methods. The original name of the flow cytometry technology was pulse cytophotometry. After 10 years, in 1978, at the conference of the American engineering foundation in Pensacola, Florida, the name was changed to flow cytometry, a term which quickly became popular. Subsequently Bio/Physics Systems Inc. introduced flow cytometry instrument named Cytofluorograph in 1971. In 1973 Partec introduced PAS 8000. The first FACS instrument from Becton Dickinson came in 1974. ICP 22 from Partec/Phywe and Epics from Coulter were introduced in 1975 and 1977-78 respectively.

Widening applications
The use of flow cytometry has increased considerably during the past decade. The technology has enabled the rapid

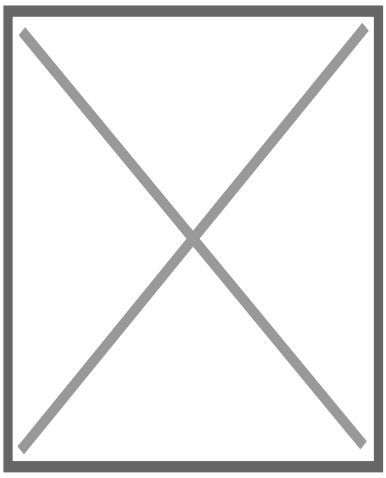
measurement and analysis of multiple characteristics of single cells. Flow cytometric DNA has been found valuable in determining the biological behavior of various tumors and predicting clinical outcomes. The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. In the field of molecular biology it is especially useful when used with fluorescence tagged antibodies. It has broad application in medicine especially in transplantation, hematology, tumor immunology and chemotherapy, genetics and sperm sorting for sex preselection. In marine biology, the auto-fluorescent properties of photosynthetic plankton can be exploited by flow cytometry in order to characterize abundance and community structure. In protein engineering, flow cytometry is used in conjunction with yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties.
Ram Sharma, managing director, BD, said, “We never imagined that flow cytometry will become core for monitoring CD4. This is now the most preferred solution for monitoring HIV/AIDS patients. Almost 90 percent of all CD4 monitoring is done using our flow cytometry. Although the technology already has a lot of clinical applications in monitoring cancer, HIV, cord blood banking, and stem cells, it has not been intensely deployed in the areas of drug discovery, life science research and basic research. Through partnerships with research institutes, we can increase the scope of our products in the life science research applications. Our primary aim lies in enhancing our scope in drug discovery.”

Market overview
Compared to other technologies, there are few players operating in the flow cytometry market space, the important ones being Beckman Coulter, Guava Technologies, Luminex and Dako which are offering products in India and globally. Talking about market competition, Rama Sharma of BD said, “Over a period of time, the technology becomes less competitive. The real difference is the quality of people who are training and educating the customers and the support that you provide to your customers. The manufacturers have to keep a constant check on the efficiency of the systems and probability of new applications for investment.”
The flow cytometry market is composed of instruments, reagents, devices, and services used across the research and clinical life sciences areas that span the academic and biomedical, biotechnology, and pharmaceutical business market sectors. According to a market research report, the global flow cytometry market in 2008 stood at $1.5 billion and is estimated to grow to $3.7 billion by 2015 with estimated growth rates of existing product. About 68 percent of the 2008 product revenue has been from instruments, and reagents make up 32 percent of the revenue. Cell-based flow cytometry is estimated at $1.3 billion with a CAGR of 10-15 percent in the coming year. Market leaders Becton Dickinson and Beckman Coulter account for about 70 percent of the research and clinical areas of the cell-based flow cytometry market. Bead-based flow cytometry is estimated at $215 million with a CAGR of 25-30 percent while Luminex and partners comprise approximately 90 percent of the bead-based flow cytometry market.

Anticipated developments
Researchers expects more fluorescent dyes to become available, with more color options, and also further technical improvements. These include dyes with much higher stoke shifts and alternatives for tandem dyes, which often suffer from inherent problems in multi-laser applications. Upgradation of some existing dyes are in the pipeline, which will offer increased brightness for conjugated antibodies. It is also predicted that new dyes will be offered together with new laser options from flow cytometer manufacturers, with an emphasis on accessibility. New solid state lasers in digital instruments are providing options in bench top machines that were previously restricted to top-end instruments.

Jahanara Parveen

“The Future Belongs to Cell-based Therapies and Flow Cytometry is the Central Tool”



-Sunit Trivedi, director, BD Biosciences, BD India

How is flow cytometry making a difference to biotechnology research ?
The life science research in the last few years has moved from genomics and proteomics to single cell analysis. Flow cytometry’s power of single cell analysis makes this new research possible. Flow cytometry posses the ability to sort cells with absolute precision in terms of purity, recovery and viability. It has the power of measuring everything in the cell including cell surface antigens, membrane proteins, intracellular proteins (cytokines), signal transduction pathways, cell signaling, nuclear proteins, apoptosis, DNA cell cycle, and ploidy analysis and co-relates all these assays to answer critical questions in healthcare such as stem cell enumeration, leukemia and lymphoma phenotyping, and HIV monitoring.
The future belongs to cell-based therapies with flow cytometry as its integral part. “Cytomics” is the combination of cytometry technology with other cell biology and imaging techniques.

How has flow cytometry contributed to the development of research ?

Flow cytometry has become the central tool. Almost every question can be answered with flow cytometry. This technology helps to answer questions at single cell level, probe what it is and then understand what it is doing. One can also see the working of a cell and observe how the cell is being affected by a drug. It enables us to identify samples with many flurochromes at the same time.

Many colors lead to more answers and more answers lead to more questions. At the end of the day, researchers are getting to a point where they can do an analysis on rare samples and understand much more completely what has happened. If you compare cytometry 20 years ago, with what researchers can do today, you can't really say enough about what the technology can do and where you can go with it.

Researchers can do things that they were never able to do before and that is really because of the speed of computer processing, the availability of new solid state lasers with more power, more control over the lasers and the size of lasers. Now researchers can do 17 colors simultaneously because the processing speed of computers lets researchers collect the data at that speed. The power, lasers and flexibility let researchers identify large number of cells, using different flurochromes and different areas of spectrum that were previously inaccessible.

Flow cytometers can allow measurements of particles as small as 200nm and it finds applications in marine biology, microbiology, environmental biology, and nanotechnology. Its capability is used in applications such as chlorophyll detection and analysis of sperm cells.

Flow cytometry with spectral analyzer capabilities are used in marine biology applications and could be used to measure fluoroscence energy transfer in fluorescent protein pairs. Few of the versions also features special brackets that easily mount the instrument shipboard.

Quick- exchange fluidics with single use sort assemblies allow researchers to achieve true aseptic conditions and prevent possible cross-contamination between samples which may lead to therapeutic cell sorting and effective cell therapy using stem cells, a much needed breakthrough required in many incurable diseases.

Development in technology has made it possible to build flow cytometers on fixed alignment with flow cell which reduce startup time, improve reproducibility, improve sensitivity and efficiency. But the most important advantage is ease of use that has upgraded flow cytometry as a user-friendly sorting device which is so often required for cellular researchers.

A quick and intuitive alignment procedure has been designed in the system with pin hole camera to bring near optical alignment within seconds without using beads. Two way and four way sorting can be optimized for applications in genomics and proteomics. For instance, cells can be deflected according to their DNA content to arrange them by the phase in the cell cycle. While mRNA hybridization to the stripe of cells of increasing DNA content might serve to study differential gene expression during the cell cycle.

Software improvements play a major role in flow cytometry and make researcher's job easier. Softwares like Cytometer Setup and Tracking (CS&T) automates flow cytometer setup, adjusts instrument variability and establishes baseline settings. Chances of operator error are reduced, and results are more consistent. The setup features let users define a configuration baseline and run a performance check. The software will then automatically set up the cytometer to this established baseline. To match the exact baseline, the CS&T software automatically adjusts reproducible performance of the instrument. This ensures the greatest possible consistency from one day to the next, saves time, and improves reliability of experiment results. The software also allows creation of application-specific settings for rapid performance of routine experiments in a more consistent manner.

Instruments can also be customized to meet customer requirements via the Special Order Research Products (SORP) programs taking care of special research needs and configurations.

What are some of the advances that you foresee?

More dyes will take advantage of larger spectra, moving from visible color spectrum to far infrared to get more information out of a cell.

How can flow cytometry technology be further promoted?

Education and training are the key. Organizations should open training centers and collaborate with leading institutions to facilitate training of larger group to maximize versatile utility of flow technology.