BD Flow Cytometry

BD Biosciences, San Jose, CA

Technical Bulletin

Contents

Cell Analysis

Recent advances in molecular and cell biology—the study of cells including their function, the structures they contain, their interactions with their environment, and their life cycle, division, and death—have helped researchers gain a deeper understanding of human disease. Advancing understanding of the different types of cells populations, how cells work, and the differences between cell types is fundamental to the study of cellular biology and disease and is a core application of the instruments and reagents developed by BD Biosciences.

The technology underpinning of our product offerings, flow cytometry, is playing a crucial role in furthering such understanding. Flow cytometry is an advanced technology for counting, examining, and sorting individual cells. Equally important to this advance was the parallel discovery and development of monoclonal antibodies that allowed researchers to detect and label specific cell populations.

The combination of these two technologies—commercialized at roughly the same time—catalyzed the rapid growth of the flow cytometry market.

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Flow Cytometry

Flow cytometry offers three important capabilities to researchers and clinicians. First, flow cytometry analyzes a population of cells on a cell-by-cell basis, a critical capability for today's researchers and clinicians who are looking for the very few cells among the many cells in a sample (often like needles in a haystack) that will enable them to study a disease state or biological process. Second, flow cytometry is extraordinarily rapid. Routine sample analysis rates can range up to 10,000 cells per second—an incredible advance over historical methods of visually examining and counting cells. Finally, flow cytometry has the capacity to simultaneously measure multiple parameters (multiplexing) of single cells. Multiplexing allows researchers and clinicians to gather more information from a single sample faster than ever before. These capabilities have made flow cytometry a powerful tool with multiple applications for researchers and clinicians alike.

How Flow Cytometry Works

Flow cytometers contain three main systems—the fluidics, the optics, and the electronics.

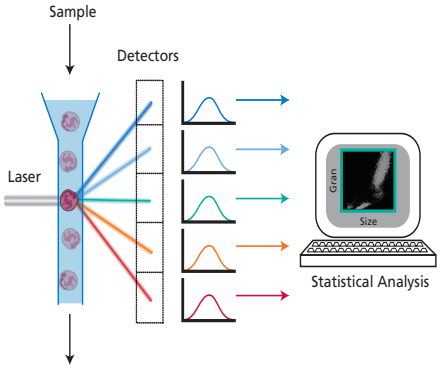

Figure 1: Flow cytometry overview

The fluidics system funnels a sample of cells (for example, a sample of human blood) into a single stream so that the cells pass one at a time through a laser beam. As each cell passes through the beam, it scatters light and may emit fluorescent light. These light signals are collected by the optics system and routed to various detectors. The signals received by the detectors then are converted into numerical values by the electronics system. Results can be displayed on the screen or saved for future analysis using specially designed software.

As each cell moves through the beam, its parameters (characteristics) are measured and recorded, along with the time that it passed through the beam. Typically data is collected for at least 10,000 cells per sample. The basic principle of how flow cytometers operate is shown in Figure 1.

Sorting

The BD FACSAria™ II system can both sort and analyze cell populations. This is valuable when a researcher needs to study a particular type of cell. First the parameters are set so that the flow cytometer can identify cells of interest. Once the cell population to be sorted has been identified, the stream of fluid containing the sample is funneled at high pressure by the fluidics system into a single stream so that cells pass one at a time through the laser beam where information about the cells is detected. If a cell matches the parameters specified, the cytometer applies an electrical charge to it. This causes the charged cell to be attracted to a collection tube. The researcher can then take the cells of interest and grow them in culture or study them in other assays. The droplets that are not charged continue in the stream to a waste container. The process is rapid and can be performed at speeds of up to 20,000 cells per second. The BD FACSAria II system can sort up to four populations at one time.

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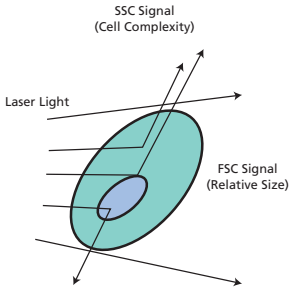

Figure 2: Light scatter

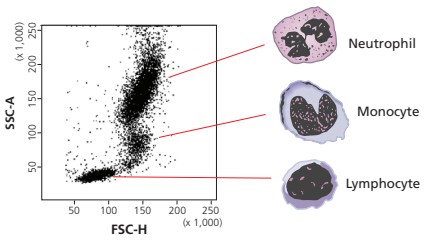

Figure 3: Dot plot showing different types of blood cells

Fluorochrome	Fluoresence Emission Color
BD Horizon™ V450	Blue
Pacific Blue™	Blue
AmCyan	Green
Alexa Fluor® 488	Green
FITC	Green
PE	Yellow
PE-Texas Red®	Orange
Texas Red®	Orange
APC	Red
Alexa Fluor® 647	Red
PE-Cy™5	Red
PerCP	Red
PerCP-Cy™5.5	Far Red
Alexa Fluor® 700	Far Red
PE-Cy™7	Infrared
APC-Cy7	Infrared
BD APC-H7	Infrared

Figure 4: Common fluorochromes

Measuring Cell Characteristics

As a cell passes through a laser beam, the light is scattered in different directions as shown in Figure 2. Measuring how much and in which direction light is scattered helps researchers determine both the size and internal complexity of a cell. This information is visually displayed in a type of graph called a dot plot, where each dot represents a single cell. In the dot plot shown in Figure 3, different types of blood cells—neutrophils, monocytes, and lymphocytes— can be differentiated from each other by the way they scatter light forward and sideways. Today, flow cytometry enables researchers to study more than one population of cells at a time, and obtain information beyond size and complexity.

Monoclonal Antibodies

Coincidentally, as flow cytometry emerged, the means of producing monoclonal antibodies was also pioneered. The subsequent coupling of monoclonal antibodies with flow cytometry revealed the real power of flow technology and promoted the rapid advance of flow cytometry applications.

Monoclonal antibodies allow researchers to detect and label—or "tag"—specific populations of cells. The technology involves creating an antibody reagent that will bind to a specific structure (antigen) known to be present on the kind of cell that the researchers are interested in studying. This technology is the key to developing biological reagents that could be used not only to identify cells, but also to analyze the function of that cell.

With the ability to look at multiple cells and parameters, researchers needed a way to identify and group cells. To achieve this objective, the use of fluorescence and multicolor staining was incorporated into the core technology of flow cytometry. This marriage of technologies revolutionized biological research to make it what it is today.

Fluorescence as a Marker of Cells and Cellular Activities

Monoclonal antibodies are often linked to a fluorescent dye (fluorochrome) that will emit fluorescent light when excited by a laser. The fluorochrome allows a researcher to track the cells that have bound to the antibody or marker.

Most cells do not naturally emit fluorescent light. However, if a fluorochrome-tagged monoclonal antibody is bound to an antigen present on a cell, a fluorescent light signal will be picked up when that cell passes through the laser beam of the flow cytometer.

Fluorochromes differ from each other with respect to the color of light that they will emit. For example, FITC, one of the fluorochromes BD offers, will emit green light, whereas PE, another one of the fluorochromes BD offers, will emit orange light when it is exposed to the laser used in our flow cytometers (Figure 4). The use of different fluorochromes helps researchers distinguish between cell populations or different cellular activities.

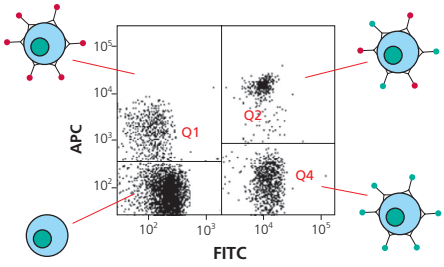

Figure 5: Fluorescence dot plot

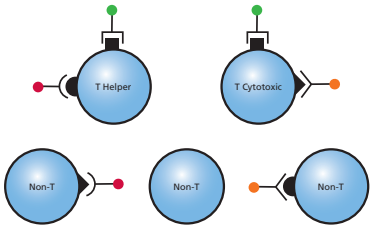

Figure 6: Three-color staining of lymphocytes

Multicolor Flow Cytometry

Cells of different types bear different combinations of antigens and will bind with different combinations of antibodies. If each monoclonal antibody used in testing a sample of cells is linked to a different fluorochrome, then the cell types can be distinguished from each other by the combinations of colors that they emit when they pass through the laser beam. Figure 5 shows a dot plot of cells stained with two different monoclonal antibody reagents, each linked to a different color fluorochrome. Four different populations were identified on the basis of how much of each color (FITC-green or APC-red) they emit.

The use of combinations of multiple colors simultaneously further enhances the ability to distinguish between different populations of cells. Figure 6 shows an example in which three different monoclonal antibodies—each linked to a different fluorochrome—were used in testing a cell sample to look for T lymphocytes. Five different types of cells were evident. The use of multiple colors not only allowed T cells to be identified, but also allowed two different types of T cells to be detected: T-helper lymphocytes and T-cytotoxic lymphocytes. Knowing the counts and ratios of these two types of T lymphocytes is very important in immunodeficiency research.

Summary

Flow cytometry combines the flexibility and sensitivity of fluorescence technology with high speed and data integration capabilities. It has become the gold standard in cell analysis and now is being used as an analytic tool across numerous sectors of life sciences.

As cell exploration continues, researchers are driving flow cytometry to support a wider range of applications. To meet this challenge, the Cell Analysis team at BD Biosciences continues to innovate—developing ever more sophisticated cell-based assays and instrumentation.

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Class I (1) laser product.

APC-Cy7: US patent 5,714,386

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23-10236-00

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