



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

IJRSET

International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 12, Issue 10, October 2023

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA

Impact Factor: 8.423

9940 572 462

6381 907 438

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Flow Cytometry - An Overview With Focus On It's Clinical Applications & Recent Advances

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ABSTRACT : Flow cytometry is a single-cell technology that measures scatter and fluorescence to establish a set of unique cellular properties. It is used in many areas of science, like biotechnology, medicine, and in industrial applications. Flow cytometry can identify multiple phenotypic subsets from a mixture, select a single cell and even isolate that cell by a process called cell sorting. The field is currently undergoing dramatic changes. We are moving rapidly from the polychromatic flow cytometry that has been the go-to technology for 45 years to spectral flow cytometry, which is now the most significant change in nearly half a century of flow cytometry. Polychromatic flow cytometry involves measurements of signal intensity with one detector used for one dye whereas spectral flow cytometry evaluates spectral signatures. With change comes opportunity and exciting features will open up molecular diagnostics and physiology to flow cytometry.

KEYWORDS: Single Cell; Particle; Fluorescence; Light scatter; Phenotyping; Cell sorting; Lasers

I. INTRODUCTION

Flow cytometry is a technology that rapidly analyzes single cells or particles as they flow past single or multiple lasers while suspended in a buffered salt-based solution. Each particle is analyzed for visible light scatter and one or multiple fluorescence parameters.¹ A beam of light of a single wave length is directed to a continuous flow of suspended particles marked with fluorescent chemical stains. Each particle in suspension that passes by this light beam disperses light in a distinct form caught by sensors on-line with the light beam and perpendicular to it.² The main components of a flow cytometer are a flow chamber; a light source; a detector and a digital analogical converter that generates fluorescent parameters of size, complexity and signals; a system of amplification of linear or logarithmic signal; and a computer for the signal analysis.³

Evolution of Flow Cytometry²

The first automated cell counter was developed by Moldavan, and consisted of a capillary tube mounted under an optical microscope with an objective provided with a photoelectric detector which recorded the passage of cells stained according to the light change. A cell counter based on the voltage change caused by the passage of a particle through the interior of a needle was developed.

The evolution of technique was obtained when Crosland-Taylor developed a flow chamber based on the passage of cells in a liquid stream (sheath fluid) through a capillary where samples were detected by a light source. At the same time, using the technology of automated cell counter, the first differential hematology counter using cells stained in red (RBCs), in blue (leukocytes), and visible light and detectors for the blue and red light was developed.

Later, the Rapid Cell Spectrophotometer (RSC), equipment in which the cell suspension passed through a channel in a microscope slide capable of measuring the size and quantity of cellular DNA was developed. This new procedure expanded the capacity of cell count based in DNA quantity, important to study of malignant cells. Van Dilla described the first flow cytometer with orthogonal configuration using the chamber, and demonstrated the relationship between fluorescence intensity and ploidy in DNA quantification, to describe the cell cycle phases. This discovery increased the knowledge of normal and malignant cell cycle study.

Two years later, ethidium bromide was used for DNA labeling in cells analyzed by flow cytometry, contributing to study of cell cycle study. In late 60s, the Fluorescence Activated Cell Sorter (FACS), which began to be commercialized in the 70s was created. The equipment was able to add electric charge to individual cells with specific markers and separate those using electrostatic field, and presented an advantage of improved signal to noise ratio when compared to cytometers based on dyes and absorption.

In 1974, the first flow cytometer capable of identifying light scattering and the different wavelengths was commercialized. Subsequently monoclonal antibodies in the study of cell cycle phases; FACS in the identification and separation of leukocytes while light scattering using lung tumor cells was investigated.

An important application of flow cytometry technology on monoclonal antibodies to identify human T cell subsets according to their surface antigens which was earlier limited to immunohistochemistry evolved.

Later, new fluorochromes were added to the monoclonal antibody's technology, and phycobiliproteins were introduced as fluorochromes. Expanding the scope of flow cytometer technique, it was further used to study DNA from nuclei of samples preserved in paraffin, which was till then restricted to live cells, and karyotyping.

In recent decades, technological advances have led to the development of smaller instruments with basic devices for use in laboratory routine and more complex instruments, capable of measuring different properties of particles with higher sensitivity, available for the academic community, government agencies and research department of multinational industries. However, a major problem has been the high cost of equipment and reagents involved in addition to the need for training qualified personnel for its use. Due to its great potential, the use of flow cytometry has been expanded to diverse fields of biological sciences, and is routinely used in clinical diagnostics, biotechnology, and basic and applied research.

Principle Systems in Flow Cytometry Instrumentation¹

Traditional flow cytometers consist of three systems: fluidics, optics and electronics.

The fluidics system - consists of sheath fluid (usually a buffered saline solution) that is pressurized to deliver and focus the sample to the laser intercept or interrogation point where the sample is analyzed. In fluidics system, cells in suspension flow in single file through an illuminated volume where they scatter light and emit fluorescence. The fluorescence can then be measured to determine the amount and type of cells present in a sample. Up to thousands of particles per second can be analyzed as they pass through the liquid stream.

The optical system - consists of excitation optics (lasers) and collection optics (photomultiplier tubes or PMTs and photodiodes) that generate the visible and fluorescent light signals used to analyze the sample. A series of dichroic filters steer the fluorescent light to specific detectors and the bandpass filters determine the wavelengths of light that are read so that each individual fluorochrome can be detected and measured. A beam of laser light is directed at a hydrodynamically-focused stream of fluid that carries the cells. Several detectors are carefully placed around the stream, at the point where the fluid passes through the light beam. One of these detectors is in line with the light beam and is used to measure Forward Scatter or FSC. Another detector is placed perpendicular to the stream and is used to measure Side Scatter (SSC). Since fluorescent labels are used to detect the different cells or components, fluorescent detectors are also in place. The suspended particles or cells, which may range in size from 0.2 to 150 μ m, pass through the beam of light and scatter the light beams. The fluorescently labelled cell components are excited by the laser and emit light at a longer wavelength than the light source. This is then detected by the detectors.

The electronic system - converts the signals from the detectors into digital signals that can be read by a computer. The detectors therefore pick up a combination of scattered and fluorescent light. This data is then analyzed by a computer that is attached to the flow cytometer using special software. The brightness of each detector (one for each fluorescent emission peak) is adjusted for this detection. Using the light measurements, different information can be gathered about

the physical and chemical structure of the cells. Generally, FSC can detect the cell volume whereas the SSC reflects the inner complexity of the particle such as its cytoplasmic granule content or nuclear structure.

General Characterization of Lasers in Flow Cytometry

Most flow cytometers use a laser to excite fluorescence molecules on cells or particles. Incorporation of an array of optical components that focus the laser and subsequent fluorescence signals, combined with light scatter signatures, means that virtually any type of cell or particle in suspension can be analyzed in great detail. It is a powerful technology that can run tens of thousands of particles per second and define subpopulations using multiparameter analysis tools.⁴ Flow cytometers may be equipped with low power air-cooled argon laser or high-power water- and air-cooled lasers. The low power lasers analyze up to four colors and physically separate selected cells in a suspension (cell sorting), whereas the high-power laser, can detect up to 18 colors simultaneously.⁵ Most equipment's use lasers as stable, monochromatic, high potency light source, which can be cooled by air or water. The mostly used light source is the low intensity (10-15 mW) air-cooled argon laser, which has an emission at 488 nm, that can excite the FITC, PE, ECD, PerCP, CyCrome, PI, 7-AAD, annexin V, ethidium bromide, acridine orange, pyronin Y, fluo-3 and rhodamines fluorochromes.²

General standards of the particles used in flow cytometry⁶

Particle standards used in flow cytometry can be classified into three major categories: Alignment standards (type I), Reference standards (type II), and Calibration standards (type III). These three categories recognize the major applications of current available standards. According to this classification system, type I alignment standards include particles utilized to align the optics of the flow cytometer; type II reference standards refer to particles with a given fluorescence intensity that are used to set up the instrument; and type III calibration standards encompasses the fluorescence particles with multiple populations used to calibrate the response of the instrument and to quantify the fluorescence signals of samples. A fourth category may also be considered, Certified blank standards (type 0), which includes particles that do not have any added fluorescent dyes and have been certified to have a fluorescence intensity lower than the autofluorescence of labeled cells (i.e., unlabeled human peripheral lymphocytes).

Applications of flow cytometry⁷

Flow cytometry has a wealth of techniques and applications that are suitable for multiple fields of study. The applications can be broadly grouped under specific disciplines, however any of these techniques can be used in all fields of study.

1. Immunology

Immunophenotyping is the most used application in flow cytometry. It utilizes the unique ability of flow cytometry to simultaneously analyze mixed populations of cells for multiple parameters. In its simplest form, an immunophenotyping experiment consists of cells stained with fluorochrome-conjugated antibodies that are targeted against antigens on the cell surface.

2. Cell Sorting

Cell sorting utilizes a flow cytometer with cell sorting capabilities to separate and purify cells or particles for further analysis. Essentially, any cell or particle that can be made fluorescent can be separated by a cell sorter. Cells can be sorted into 96 or 384 well plates, tubes and slides. A few common types of samples are transfected cells expressing a

fluorescent protein, stem cells, tumor infiltrating lymphocytes, tumor cells, and white blood cell populations. A major consideration with any cell sort is scaling up the amount of antibody needed for staining large amounts of cells.

3. Blood and Bone Marrow Cells

Normal Cells and non-neoplastic Diseases: One of the first clinical applications of flow cytometry was in hematology. Cells from peripheral blood and bone marrow are easily accessible, and sequence sampling is possible without too much discomfort for the patient.

4. Lymphatic System

Lymphocytes have long been used as a diploid standard for DNA measurements by flow cytometry. Several investigations have used flow cytometric techniques for the characterization of lymphocytes and their subpopulations. This includes identification and separation of T and B cells, simultaneous measurements of surface immunoglobulins and cell cycle phase distribution, the demonstration of histamine receptors lectin binding to cell surface, fluorescence polarization and measurements of membrane potentials of individual lymphocytes. All these methods are available for human cells and for potential clinical application, but have so far mainly been used for experimental purposes.

5. Respiratory Tract

In lung carcinomas malignant cells are detached from the tumor surface and can be traced in the sputum. However, there is always a high admixture of other cell types, such as normal bronchial epithelial cells and leukocytes, and a small number of malignant cells that may be traced by flow cytometric DNA measurements.

6. Urinary Tract

Flow cytometric studies on the urinary bladder epithelium has been performed for two main reasons: first as a supplementary examination to cytologic follow-up of patients with a history of bladder tumor disease, and second to try to establish a method for screening high risk individuals, e.g., workers in the chemical industry.

7. Female Genital Tract

Uterine Cervix: Flow cytometry has been tried as screening procedure of gynecologic specimens since the introduction of the method. Conventional cytology based on morphologic criteria has an estimated false negative rate of 2.8- 26%.

8. Endometrium

Flow cytometric DNA measurements have been carried out on endometrial cells to study cell cycle changes during the menstrual cycle, showing a decrease in the fraction of cells with S and G₂ phase DNA content at onset of the secretory phase.

9. Gastrointestinal Tract

While frank carcinoma of the stomach usually is diagnosed by histology and to some extent by cytology, DNA measurements have been used as a method to predict possible premalignant changes.

10. Skin

Although the different compartments of the skin, i.e., epidermis, dermis and subcutis, are difficult to separate, several methods have been devised for the specific separation of epidermal cells for flow cytometric measurements. Flow cytometric measurements have been performed in various malignant tumors of the skin. Parallel to altered numbers of chromosomes that occur early in experimental carcinogenesis, changes in DNA content per cell may appear during development in the tumors.

11. Chromosome Analysis

Classical chromosome analysis is dependent on the presence of mitotic cells, while flow cytometric DNA measurements gives the allover ploidy of cells in all phases of the cell cycle. Small aberrations in single chromosomes may not be detected on DNA measurements, even if a high degree of resolution is obtained and, in some cases, there may even be a discrepancy between the results from chromosome analysis and DNA measurements. There exists possibility not only for diagnostic use of automated chromosome analysis in malignancy, but also for detecting cells which have been subjected to mutagenic damage.

Recent advances

Profiles of a new innovation in flow cytometry that adds fluorescence imaging and image-based decisioning to sort individual cells at exceptionally high speed, based on the visual details of each cell and not solely on the type or quantity of biomarkers that are present have been developed. The new innovation known as BD Cell View Image Technology, can capture multiple images of individual cells flowing through the system at a speed of 15,000 cells per second and also adds to a previously impossible capability of sorting cells based on detailed microscopic image analysis of individual cells at this speed.⁸

The fluorescence-based in vivo flow cytometry (IVFC) is an emerging tool to monitor circulating cells in vivo. As a noninvasive and real-time diagnostic technology, the fluorescence-based IVFC allows long-term monitoring of circulating cells without changing their native biological environment. It has been applied for various biological applications (e.g., monitoring circulating tumor cells).⁹

Advances in flow cytometry instrumentation, light sources, fluorochrome development, and basic aspects of cellular and molecular biology will continue to permit this technology to define neoplastic cells and their behavior better, resulting in both improved patient care and a better understanding of tumor biology.¹⁰

II. CONCLUSION

Flow cytometry is a powerful technique in the clinical aspect by which aberrant cells can be identified, excess or reduced populations of particular cells can be quantified and patients can be monitored for changes in tracked populations of specific cells. In the research lab, flow cytometry is used for analysis of microbial populations, human blood cells, body fluids and cultured cell lines. With recent advances, broad spectrum of manufacturers and instruments are available. However, selected instruments are preferred for certain applications. But in general, most flow cytometers are competent to perform most, if not all, of its's current applications satisfactorily.

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