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Laser flow cytometry as a tool for the advancement of clinical medicine

David Aebisher[a,*], Dorota Bartusik[b], Jacek Tabarkiewicz[c]

[a] Shorter University, Natural Sciences Department, 315 Shorter Ave., Rome, GA 30165, USA
[b] Southern Polytechnic State University, Department of Biology and Chemistry, 1100 South Marietta Parkway, Marietta, GA 30060, USA
[c] University of Rzeszów, Centre for Innovative Research in Medical and Natural Sciences, Warzywna 1A, 35-959 Rzeszów, Poland

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ABSTRACT

Flow cytometry is a classic *laser* technology. With the discovery of the cytometer, flow cytometry has become a primary tool in biodiagnostic research. This review focuses on current applications of flow cytometry to the diagnosis of disease and treatment monitoring at the single-cell level. A description of the principles of flow cytometry and a brief overview of the major applications are presented. Our criteria for selecting research papers for this review are those that show advances in biomedicine and pharmacotherapy achieved by using non-invasive flow cytometry. New concepts for diagnosis and classification based on quantitative measurements of cellular parameters and the expression of specific differentiation antigens on the surface of cells will be discussed herein.

Contents

1. Introduction

Flow Cytometry can measure both physical and chemical properties of fluorophore-labeled cells that rapidly flow in a stream through laser light. Fluorescence emission from the labeled cells and light scattering can be detected and converted to graphical data that conveys information about the cells in a sample. Cellular analysis by flow cytometry provides information about physicochemical properties such as tissue type and internal and/or external cellular composition. The information enables measuring cell size and shape and quantification of biomarkers that are attached to surface receptors or to cellular components. This information can greatly aid in the detection and diagnosis of disease as well as to quantify the effects of disease treatments. The information is conveyed between a cell and a detector within a flow cytometer by light. Light interacts with cells and particles through the process of light scattering. In a flow cytometer, a stream of cells or particles *flows rapidly* in front of a light source and parameters such as shape, size or volume are measured in seconds. Bench chemistry methods such as cell fluorescence labeling and a cytometer are employed together to obtain quantitative information based on light scattering or fluorescence emission of single cells (or single particles) in a heterogeneous population. Light scattering can differentiate cell size as large cells scatter light more effectively than smaller cells and small cells scatter higher frequency light more often than they scatter lower frequency light. Flow cytometry also provides a new route to study the phenotype of an immune cell which is an emerging aspect in cancer research.

* Corresponding author.
E-mail address: davea432@gmail.com (D. Aebisher).

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Today, flow cytometry research combines advances in software and laser technology to obtain precise measurements of single or multiple parameters of an individual cell at one time. These parameters include size, volume, granularity (refractive index) and fluorescence caused by interactions of specific fluorescent probes with cell surface antigens or cytoplasmic molecules [1]. The use of monoclonal antibodies in flow cytometry has greatly advanced the field of biodiagnostics. By using flow cytometry, the intrinsic and extrinsic properties of cells can be measured. Intrinsic cellular properties are size and granularity. Extrinsic properties include surface antigen content, total protein content, lectin binding, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) content, intracellular pH, nuclear and intracellular antigen content. Briefly, particles, which are often cells, are fluorescently labelled and excited by a laser, and emit light. Flow cytometers now range from instruments with one argon laser to clinical instruments with several output wavelength lasers which can provide rapid and quantitative clinical analysis [2].

Traditional diagnostic techniques for cancer, such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) with radiolabeled 2-fluoro-deoxyglucose, have relied on the morphological differences between normal cells and tumor cells. Flow cytometry employs probes for *in vivo* targeting of cells and *in vivo* imaging of proteins or small molecules. By use of laser and various software programs, it is possible to quantitate single or multiple parameters in one single cell in a heterogeneous population which can be resolved into smaller subpopulations. Using multi-parameter analysis, individual cells can be selected from a heterogeneous population [3,4]. The laser is one of the most important inventions of 20th century and currently lasers are employed in a wide range of medical applications including the extraction of important information from single cells using flow cytometry.

2. Principles of flow cytometry

To analyze cells in a heterogeneous population by flow cytometry, they are usually suspended in saline solution. Both the cells and saline are in laminar flow. By passage through a narrow filter, cells are hydrodynamically focused into the center of the stream. Next, cells pass in front of an extremely narrow monochromatic beam of laser light. When a cell passes through the laser beam, light is scattered in all directions. Depending on the direction of scattering, information about a designated cellular parameter is obtained [5].

Briefly, a flow cytometer has several components including one or more lasers, a sample chamber, a sample stream and a photodetector system with a photomultiplier tube (PMT). Components of a flow cytometer are shown in Fig. 1. The light scattered or the fluorescence emitted from a cell is converted to an electronic signal that is proportional to a specific parameter for that cell [6].

The amount of light reaching each detector is converted into a proportional electronic signal that can be observed by an oscilloscope. The pulse intensity (or height) is measured and converted from an analog to digital signal which is displayed as a frequency histogram. The intensity of the digitized signal is proportional to the amount of light scattered or fluorescence emitted by a cell. These digitized signals are displayed on a one parameter frequency histogram [7].

3. Probes

The optical system of a flow cytometer, comprised of one or more lasers, illuminates the particles labeled with fluorescent markers termed fluorochromes (dyes and other fluorescent probes). When laser light passes through the particle, the fluorochrome fluoresces. Fluorescence occurs when a molecule excited by light of one wavelength loses its energy by emitting light of longer wavelength. Unconjugated dyes were the first

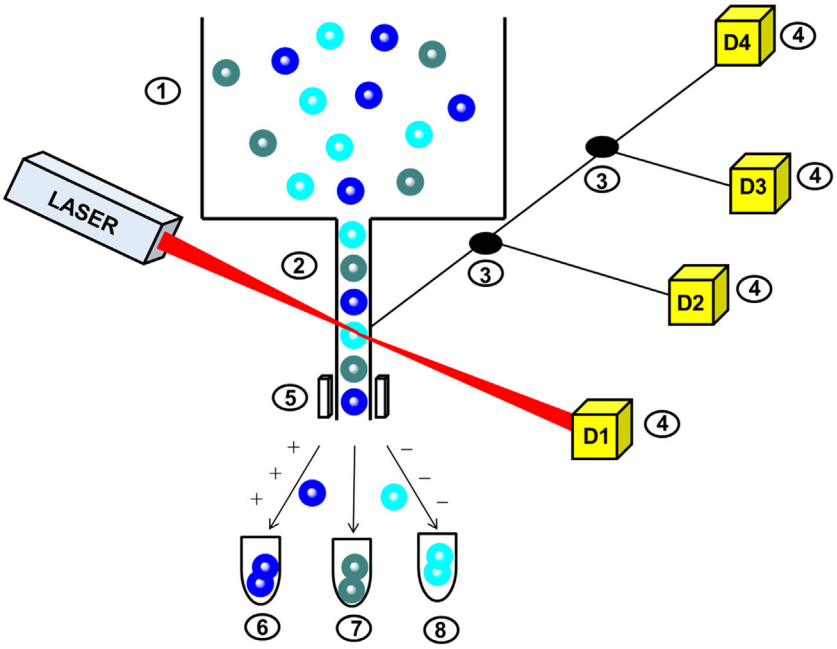

Fig. 1. Components of a flow cytometer. The components are numbered as follows: (**1**) cell suspension container; (**2**) hydrodynamic stream of cells in laminar flow; (**3**) beam splitter; (**4**) Detectors D1: light scatter detector which classify cells according to their size: D2 90° light scatter detector which classify cells accordingly to granulocity: D3 green fluorescence detector which classify cells accordingly to cell surface markers: D4 red fluorescence detector; (**5**) color charging unit; (**6**) and (**8**) samples collectors and (**7**) waste collector.