Volume 4, Number 2: 1-6, Octorber 2006
Review Article

International Journal of
Vascular Biomedical Engineering

Microfluidic Flow Cytometry: Principles of Cell Analysis and Applications

Sehyun Shin¶

School of Mechanical Engineering, Kyungpook National University

Abstract

Microsystems create new opportunities for conventional cell analysis by combining microfluidics and flow cytometry. This article describes recent developments in conventional flow cytometers and related microfluidic flow cytometers to detect, analyze, and sort cells or particles. Flow cytometry strongly consisted of fluidics, optics and electronics requires a large space to equip various components, which are mostly the fluidic components such as compressor, fluid handling system. Adopting microfluidics into flow cytometry enables volume- and power-efficient, inexpensive and flexible analysis of particulate samples. In this paper, we review various efforts that take advantage of novel techniques to build microfluidic cell analysis systems with high-speed analytical capability. Highly integrated microfluidic cytometry shows great promise for basic biomedical and pharmaceutical research, and robust and portable point-of-care devices could be used in clinical settings.

Keywords: Microfluidics, cytometry, blood cell.

Introduction

Flow cytometry is a technology that simultaneously measures and then analyzes multiple physical haracteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light.[1] The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence.

Even though modern flow cytometry systems provide rapid and reliable analytical performance, they are still overall bulky, expensive and mechanically complex. Also, the operation of flow cytometers often suffers from the need for highly trained personnel, multiple sample pre-treatment steps and a high volume requirement of the sample to be analyzed. Over the past decade, the drawbacks of current flow cytometers coupled with an increased need for sophisticated cellular analyses have motivated efforts to take advantage of microfabrication technologies and advanced microfluidics to achieve smaller, simpler, more innovative and inexpensive instrumentation. [2]

These micro-technologies will become increasingly implemented in applied and basic biomedical mainly because soft lithography[3] has put microfluidics within the reach of biology-focused academic laboratories. Elastomeric materials used in soft lithography, typically poly(dimethylsiloxane) (PDMS), are relatively easy to fabricate, and are compatible with most biological assays.

In fact, the use of microfluidics and microfabrication opens new opportunities to incorporate volume-efficient solid-state optics more easily with higher precision and to develop novel fluid/particle handling and sorting schemes.[4] It also confers the flexibility of integrating flow cytometry with other microfluidic cell manipulation and analysis systems that facilitate sample pre- and post-treatment as well as enable further analysis to extract additional information. The cost and complexity of fabricating fluidic components that are traditionally made of glass can be reduced by using materials such as inexpensive polymers. The increasing need for simple

¶ 1370 Sangyeok-dong Buk-gu Daegu, 702-701, KOREA
E-mail:shins@knu.ac.kr

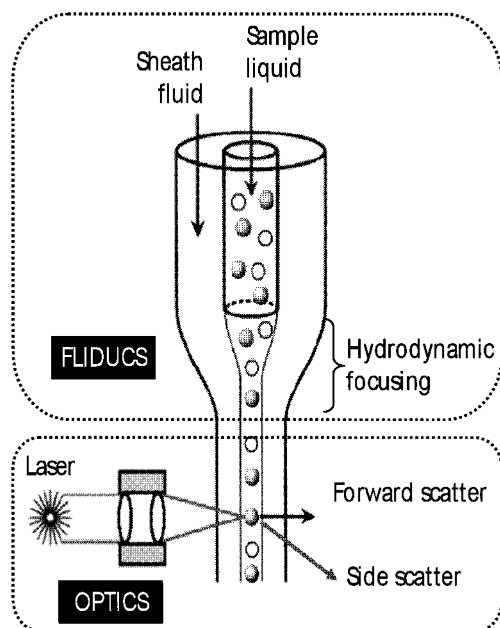


Figure 1. Schematic of conventional flow cytometry

and small instrumentation resulted in the development of flow cytometers with enhanced portability for on-site sample measurements. A reduction in volume required to make the whole system smaller is often achieved by using solid-state optics.

Here, we present basic principle of conventional flow cytometry and recent efforts to develop microfluidics for flow cytometers and microscale flow-based cell analysis systems. The focus of this review lies in the development of flow cytometry that are not only based on conventional fluidics but also microfabricated structures and microfluidics. Although sophistication of modern microfluidic flow cytometers including sell sorting and handling are worthy to note, this topic is beyond the scope of this review. For a detailed review of microfluidic cytometry, we would recommend to see the previous reviews. [2]

Conventional Flow Cytometry

A flow cytometer as shown in Fig. 1 is made up of three main systems: fluidics, optics, and electronics. The fluidics system transports particles in a stream to the laser beam for interrogation. The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors. The electronics system converts the detected light signals into electronic signals that can be processed by the computer. For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisions to charge and deflect particles. For detailed information of cytometry, we would recommend to see the materials. [13]

Fluidics

The main function of the fluidics in flow cytometry is to transport particles in a fluid stream to the laser beam for interrogation. For optimal illumination, the stream transporting the particles should be positioned in the center of the laser beam. In addition, only one cell or particle should move through the laser beam at a given moment. To accomplish this, the sample is injected into a stream of sheath fluid within the flow chamber. The design of the flow chamber causes the sample core to be focused in the center of the sheath fluid where the laser beam will then interact with the particles. Based on principles relating to laminar flow, the sample core remains separate but coaxial within the sheath fluid. The flow of sheath fluid accelerates the particles and restricts them to the center of the sample core. This process is known as 'hydrodynamic focusing'. Figure 1 illustrates a typical hydrodynamic focusing in each type of flow cell. The sample pressure and the sheath fluid pressure are different from each other. The sample pressure is always greater than the sheath fluid pressure.

Optics

When particles or cells are aligned to pass single file through the laser intercept, they scatter laser light. The extent to which this occurs depends on the physical properties of a particle, namely its size and internal complexity. Factors that affect light scattering are the cell's membrane, nucleus, and any granular material inside the cell. Cell shape and surface topography also contribute to the total light scatter. Forward-scattered light (FSC) is proportional to cell-surface area or size. FSC is a measurement of mostly diffracted light and is detected just off the axis of the incident laser beam in the forward irection by a photodiode. FSC provides a suitable method of detecting particles greater than a given size independent of their fluorescence and is often used in immuno-phenotyping to trigger signal processing.

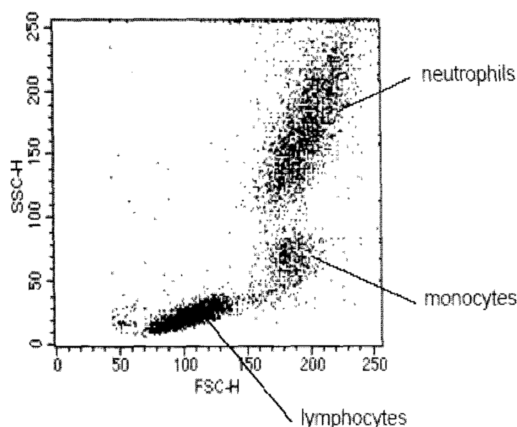


Figure 2. Cell subpopulations on based on FSC and SSC [1]

Side-scattered light (SSC) is proportional to cell\ granularity or internal complexity. SSC is a measurement of mostly refracted and reflected light that occurs at any interface within the cell where there is a change in refractive index. SSC is collected at approximately 90 degrees to the laser beam by a collection lens and then redirected by a beam splitter to the photomultiplier tubes (PMTs). Correlated measurements of FSC and SSC can allow for differentiation of cell types in a heterogeneous cell population. Major leucocyte subpopulations can be differentiated using FSC and SSC as shown in Figure 2. It is worthy to note that SSC is fluorescent light, generated by a fluorescent compound (dye) which may be conjugated to a monoclonal antibody. Thus, SSC can be used to identify a particular cell type based on the individual antigenic surface markers of the cell. The staining pattern of each subpopulation, combined with FSC and SSC data, can be used to identify which cells are present in a sample and to count their relative percentages. The cells can also be sorted if desired.

Data Analysis

A single cell analyzed for four parameters (FSC, SSC, FITC, and PE fluorescence) generates 8 bytes of data. Once a data file has been saved, cell populations can be displayed in several different formats. A single parameter such as FSC or FITC (FL1) can be displayed as a single parameter histogram, where the horizontal axis represents the parameter's signal value in channel numbers and the vertical axis represents the number of events per channel number. A histogram allows one to view a single parameter against the number of events. A subclass control is used to determine where the markers will be placed. Two parameters can be displayed simultaneously in two dimensional plot.

Microfluidic Cytometry

The goal of incorporating microfluidics is to enable more precise and rapid manipulation of cells and to take advantage of different driving forces for controlling fluid flows. It also allows for more compact optics and volume-efficient on-chip sample detection through physical registration and integration of small solid-state optical components in close proximity to the fluidic system. In this section, we provide an overview of microfluidic sample transport and manipulation systems to perform analysis of cells or particles.

As reviewed earlier, in the conventional flow cytometers, a sheath fluid surrounds the sample flow to align cells in single file along the center of the stream and to prevent clogging of capillary tubes with cell aggregates. This hydrodynamic focusing is very important to have reliable optical analysis of cells and has been an important issue for development of microfluidic cytometry. Microfluidics is capable of stably delivering samples to a detection area, which provides with higher accuracy and better flow control compared to conventional fluidic systems.

Hydrodynamic focusing

The advantages of using microfluidics include the ability to fabricate desired microstructures rapidly and inexpensively, and to obtain optimized channel geometries that are difficult or impossible to fabricate using conventional glass machining techniques. Figure 3a shows the hydrodynamic focusing with a microfabricated sample injection system, in which a stream of sample is injected through a small inner nozzle into a converging outer nozzle with sheath flows enveloping the core stream from the sides.[5] It was found that two-dimensional hydrodynamic focusing produces a confined sample stream with a width smaller than 10 μm when the point of sample injection from the inner nozzle is located in the middle of the outer nozzle and the ratio of sheath flow rate to sample flow rate is increased. They also fabricated an inexpensive flow chamber made of poly (methylmetha -crylate) (PMMA) using a hot embossing method, and demonstrated hydrodynamic focusing of liquid streams

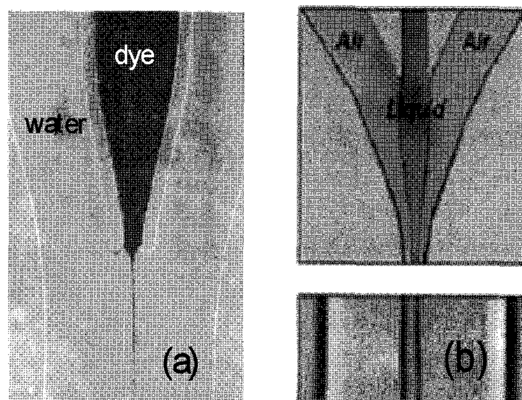
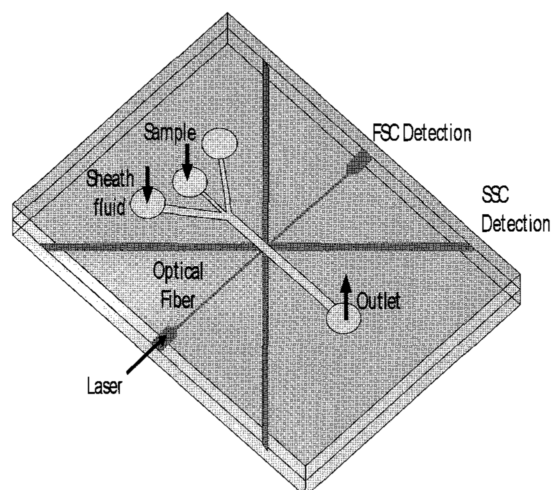


Figure 3. Hydrodynamic focusing in microchannel using sheath fluid as water (Ref. 5) and air (Ref. 6)



Figuer 4. Microfabricated PDMS flowcytometer with embedded optical-fiberwaveguides. (Ref. 12)

containing dye solution.

Figure 3b demonstrated the use of ambient air as an alternative sheath fluid in a stable and disposable air–liquid two-phase microfluidic system that can perform microscope -based cell/particle detection and enumeration.[6] Through an engineering of the wetting properties of microchannel surfaces made of poly(dimethylsiloxane) (PDMS) as well as the manipulation of fluid mechanical parameters, the system generates a thin (15–100 μm) and high-speed (up to 1ms−1) liquid stream transporting cells or microbeads focused by air-sheath flows in a rectangular microfluidic channel.

In addition, a highly miniaturized and power-efficient flow control system was reported for a portable flow cytometer,[7] which used electrostatically actuated silicon microvalves and highly sensitive glass-based thermal flowsensors to produce pressure-driven flows with little (~3%) pulsatility in a closed-loop flow channel system.

Instead of optical detection, the successful use of spectral impedance measurements for on-chip cell detection and counting was also reported. [8] The analyzer measures impedance signals, which are correlated with cell size and type, as hydrodynamically focused cells flow by electrodes embedded in glass channel walls and interact with an applied AC electric field. The measurements are based on the delivery of focused cells to a sensing area with multiple electrodes by laminar streams and the differential variation in impedance between two adjacent electrodes fabricated at the length scale of cells/particles to be analyzed.

Sheath-free transport system

As we reviewed, the hydrodynamic focusing is critical for reliable optical analysis of cells and the role of sheath fluid is very essential element in conventional cytometry. Recently, a sheath-free transport system was proposed. Altendorf *et al.* 9 used a sheath-free transport of blood cells in a V-groove microchannel fabricated by anisotropic wet etching of silicon to perform differential blood cell counts. The sheath-free flow cytometric system is found to be useful for monitoring biological fluids such as albumin in serum. Chau *et al.*[10] designed a silicon microfluidic chamber coupled with a fiber-optic reflection probe to take reflection–absorption measurements. The system features a simple straight flow pathway for transport of biological fluids that do not require hydrodynamic focusing or sheath fluid and a bundle of optical fibers that emit and receive light signals. A sheath-free sample transport system for portable flow cytometry was also reported by Goix *et al.* [11] The commercialized portable flow cytometer is featured by a microcapillary flow system in which the delivery of cells or microparticles to a detection location is achieved by a single liquid stream driven by a syringe pump.

This sheath-free transport system eliminates the need for hydrodynamic focusing of sample streams with sheath liquid by avoiding the use of highly concentrated samples, by using a capillary tube with a small inner diameter that

physically hinders multiple cells from entering the tube simultaneously, and by adjusting the optics to be more tolerant of slight differences in position of cells in the tube. Through the combination of a capillary with magnetic elements concentrically surrounding the tube, cells conjugated with magnetically charged dyes can also be focused along the center of the stream. By virtue of the fluidic design that does not require sheath fluid, the system allows the use of small sample volumes and produces small amounts of waste. Combined with laser-induced fluorescence and light scatter, this system has the capabilities of performing automated cell counting and viability assays, protein expression analysis and apoptosis analysis.

The use of microfabricated channel systems for the microfluidic transport of cells opens new opportunities for integrating optical waveguides and solid-state devices with the fluidics. Figure 4 shows the use of solid-state lasers and silicon-based PIN photodiode detectors combined with optical-fiber waveguides for multi-color laser excitation and fluorescence detection in a microfabricated flow cytometer made of poly(dimethyl-siloxane) (PDMS).[12] Using multiple angles of excitation and detection through the optical fibers inserted into the channel system at different angles, they studied multi-color excitation of sample particles at a single interrogation point and examined the ability of lock-in amplification techniques to increase the signal-to-noise ratio of PIN-based photodetector systems. The flow cytometric capability of this system was demonstrated by multi-color detection of hydrodynamically focused fluorescent particles and nucleic acid labeled fungus.

Conclusion

Flow cytometers are one of the earliest successes and the most useful instruments among microfluidic devices developed to date. For the past three decades, advances in precision technologies, dye synthesis and high-speed data-handling techniques have exerted synergistic effects on flow cytometry, bringing this powerful analytical tool into routine clinical and laboratory use in the field of cell/molecular biology, disease diagnostics, immunology, genetics and environmental monitoring. Above of all, microfluidic systems of flow cytometers have undergone rapid evolution. The emerging needs coupled with the new microfabricating technology have led to a variety of exciting developments in microfluidics for flow cytometric analysis of cells and particles. In addition to providing higher speeds, smaller sizes and lower costs, the use of microfabricated structures and microscale flow physics serves as an enabling technology to analyze and understand complex cellular processes at single-cell resolution in new ways that make use of the following advantages of microscale cellular analysis.

Although significant challenges face routine applications of 'microfluidic cytometry', tremendous advances have been realized over the past decade, and a future in which chips effectively compete with laboratory-scale technologies in the analysis of complex biological phenomena is clearly in sight. Highly integrated microfluidic cytometry will find application in basic biomedical and pharmaceutical research, whereas robust and portable point-of-care devices will be used in clinical settings.

Acknowledgement

This work was supported by Grants from the Biorheology of NRL Program and System-biodynamics NCRC program of the Ministry of Science and Technology, Korea.

References

1. Melamed MR, Lindmo T, Mendelsohn M, Flow Cytometry and Sorting, 2nd ed. Wiley-Liss, New York; 1990.
2. Huh D, Gu W and Kamotani Y, Microfluidics for flow cytometric analysis of cells and particles, Physiological Measurement 2006;26:73.
3. Xia Y and Whitesides GM, Soft lithography. Annu. Rev. Mater. Sci. 1998;28:153–184.
4. Stone HA, Stroock AD and Ajdari A, Engineering flows in small devices: Microfluidics toward a lab-on-a-chip. Annu. Rev. Fluid Mech, 2004;36:381–411.
5. Lee GB, Lin CH and Chang GL, Micro flow cytometers with buried SU-8/SOG optical waveguides, SensorsActuator A, 2003; 103:165–70.
6. Huh D, Tung YC, Wei H, Grotberg JB, Skerlos SJ, Kurabayashi K and Takayama S, Use of air-liquid two-phase flow in hydrophobic microfluidic channels for disposable flow cytometers, Biomed. Microdevices 2002; 4:141–9.
7. Cabuz E, Schwichtenberg J, DeMers B, Satren E, Padmanabhan A and Cabuz C, MEMS-based flow

controller for flow cytometry. Proc. Hilton Head 2002 Solid-State Sensor, Actuator and Microsystems wokshop, 2002; 110–111.

8. Gawad S, Schild L and Renaud P, Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing, Lab. Chip. 2001;1 :76–82.
9. Altendorf E, Zebert D, Holl M and Yager P, Differential blood cell counts obtained using a microchannel based flow cytometer, Transducers '97. 1997; 531–534.
10. Chau LK, Osborn T, Wu CC, and Yager P, Microfabricated silicon flow-cell for optical monitoring of biological fluids, Anal. Sci. 1999 ;15 :721–724.
11. Goix PJ, Method and apparatus for detecting microparticles in fluid samples, US Patent no, 2004; 6710871.
12. Tung YC, Zhang M, Lin CT, Kurabayashi K and Skerlos SJ, PDMS-based opto-fluidic micro flow cytometerwith two-color, multi-angle fluorescence detection capability using PIN photodiodes. Sensors Actuators B 2004;98:356–67.
13. BD Biosciences, Introduction to Flow Cytometry:A Learning Guide, Manual Part Number: 11-11032-01