A PORTABLE FLOW CYTOMETER WITH SIMULTANEOUS DETECTION OF IMPEDANCE AND FLUORESCENCE

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ABSTRACT

A portable flow cytometer with simultaneous detection of impedance and fluorescence was constructed incorporating polyelectrolytic gel electrodes (PGE) based impedance measurement and fluorescence measurement with integrated solid-state photonic devices. Simultaneous detection of fluorescence and impedance signal was performed with fluorescent and plain beads. The fluorescence and impedance signal were in good sync with flow of beads.

KEYWORDS: Flow cytometer, FACS, fluorescence, PGE, impedance

INTRODUCTION

Flow cytometry is widely used in biological and clinical researches because of its promising ability of counting and separation. Common types of flow cytometers are fluorescence activated cell sorter (FACS) and Coulter counter. FACS utilizes optical detection and Coulter counter employs electrical detection mechanism [1]. Optical detection requires a high-power light source for excitation of fluorescent tag, a photon detector, and other optical components, such as lens, optical filter and mirror. The combination of these parts makes an optical detection system bulky and expensive. Electrical detection of particle or cell is good for counting of them. However, counting itself does not have much information when we use mixed sample of different types.

THEORY

The key of the developed system is combining the two detection methods for flow cytometry, optical and electrical detection, onto a single system. In addition, by applying solid-state optical devices and designing electric circuits for signal amplification and processing, the size and weight of the system were minimized to provide the ability of point-of-care testing (POCT). Fig. 1 shows the configuration of the developed system. A blue light emitting diode (LED) was used as the exiting light for fluorescence and a solid-state photo multiplier (SSPM, 06184MM, Photonique SA, Switzerland) was used as the fluorescence photon detector.

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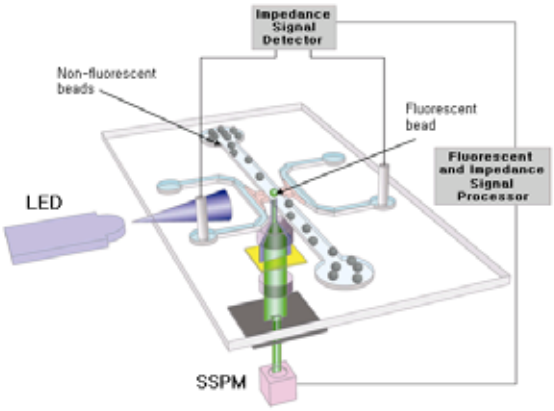

Figure 1. The overview and schematic of the developed flow cytometer device. Light from LED induces fluorescence on the poly styrene beads and the fluorescence was detected by SSPM. Impedance measurement of the PGE–microchannel–PGE series is done with the Ag/AgCl electrodes. Impedance and fluorescence data are simultaneously acquired

EXPERIMENTAL

The glass chips were prepared with conventional photolithography and wet etching techniques. Etched channel dimension is 75μm in width and 25μm in depth. After thermal bonding of the etched glass with a cover glass, a pair of PGE was formed with ultraviolet (UV) photopolymerization technique [2] and the impedance between the two electrodes was monitored. Fig. 2 shows the data flow of the developed system. Signals from the fluorescence detection circuit and the impedance detection circuit were converted by a 12 bit analog-to-digital converter (ADC, MCP3204, Microchip, USA) with sampling rate of 1 kHz. The converted signal was transferred to a microcontroller unit (MCU, ATmega8, Atmel, USA) via serial peripheral interface (SPI) and processed. The MCU communicated with a personal computer (PC) via universal serial bus (USB) interface to accept commands from a user and to send the measured data.

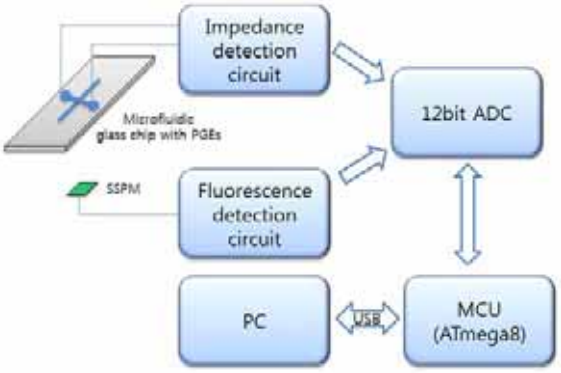

Figure 2. Schematic diagram of the developed system

RESULTS AND DISCUSSION

Pictures of the completed system are shown in Fig. 3. The size was 15 (width) × 10 (depth) × 10 (height) cm and the weight was about 800 g, which means that the device has acceptable size and weight for a POCT device. Fig. 4 shows the simultaneously acquired signals of impedance and fluorescence with fluorescent beads. Peaks from the impedance and the fluorescence signals are in perfect sync. This shows that the developed device can be used for various applications that require flow cytometer.

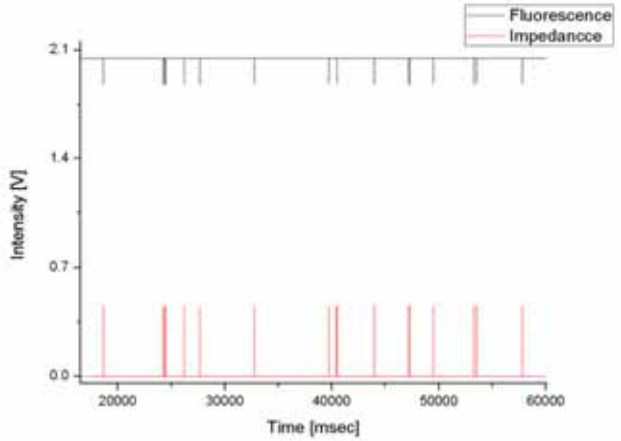

Figure 4. Fluorescence and impedance signals from 15μm-diametric fluorescent (Dragon Green) beads. The fluorescence data shows a spike at the same time level the impedance data is collected.

CONCLUSIONS

Simultaneos detection of impedance and fluorescence of particles were successfully performed with the developed portable flow cytometer. Developed cytometer could be applied to the analysis of biosamples and could be an essential part of various POCT devices. In addition, by integrating a sorting device with the developed cytometer, a true portable FACS machine will be completed.

REFERENCES

[1] G. Durack and J. P. Robinson, "Emerging tools for single-cell analysis," Wiley-Liss, New York, 2000.

[2] "Cytometry and Velocimetry on a Microfluidic Chip Using Polyelectrolytic Salt Bridges," H. Chun, T. D. Chung, and H. C. Kim, Analytical Chemistry, **77**, 2490 (2005).