AN INTEGRATED BIOPHOTONIC AND MICROFLUIDIC CHIP FOR CD4 CELL SORTING APPLICATIONS

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ABSTRACT
This paper presents an integrated and self-contained microfluidic system for monitoring the progression of the HIV infection by counting and sorting CD4 cells. The system can handle raw blood samples without the need of off-chip sample preparation. In addition, the system has integrated a laser diode onto the microchip as the light source, making it more compact and portable. Compared with the current blood analysis method performed in central laboratories, our system is low cost, portable and low requirement for maintenance, and thus could be used for point-of-care diagnosis.

Keywords: Cell Sorting, HIV Diagnosis, Microfluidics

1. INTRODUCTION
Progression from clinical latency to AIDS in persons with HIV infection is suggested by CD4 lymphocyte counts of less than 500 cells per ml. Blood analysis is currently being performed in central labs on expensive, high-maintenance equipment and can be time consuming. Therefore there is a need for portable, fast and easy-to-operate cytometer system for small clinics or even household use. Kruger \textit{et al.} demonstrated a miniaturized flow cytometer which can perform the key functions of detection, enumeration and sorting of fluorescent species [1]. Dittrich \textit{et al.} reported a microfluidic device for reaction, high-sensitivity detection and sorting of fluorescent cells and particles [2]. But for all these miniaturized flow cytometers, blood samples need to be pre-processed before they can be put into the chip. And another problem is that external laser has to be used as the light source for optical detection, which results in the difficulty of integration. We proposed a self-contained microfluidic system fabricated in poly (dimethyl siloxane) (PDMS) to monitor the progression of the HIV infection. Reagents will be preloaded in the respective reservoirs such that the system can handle the raw blood sample without any off-chip sample preparation step. Laser diode will be integrated in the chip as the light source, which eliminates the need for bulky and expensive laser system and makes the optical system more compact.
2. DESIGN, RESULTS AND DISCUSSIONS

Fig. 1 shows the schematic diagram of cell sorting system. A drop of blood is drawn into the middle input reservoir by the user. The middle input reservoir is preloaded with ethylenediamine tetra-acetic acid (EDTA) anticogulants, CD4+ antibody tagged with fluorescent dye and saline solution. Each side reservoir is filled with red blood cells (RBC) lysis buffer. At the input reservoir, CD4 cells bind to fluorescent labeled CD4+ antibody. Sample solution is transported with electoosmotic flow.

At the first intersection, continuous lysis of RBC and electrokinetic focus of the cell suspension are initiated. As shown in Fig. 2 (a), the focusing effect enables a single cell suspension along the center line of the micro-channel, which allows for more sensitive measurements to be made. The velocity field was measured at the focusing region by microscale particle image velocimetry (µ-PIV) system as presented in Fig. 2 (b). Fig. 2 (c) depicts the relationship between focus ratio and the ratio of applied voltages at focus reservoir and inlet reservoir.

![Fig. 1. Schematic diagram of cell sorting system.](image)

![Fig. 2. Electrokinetic focusing effect at the intersection. (a) Visualized sample stream. (b) Measured velocity field. (c) Focusing ratio vs. applied voltage ratio.](image)

The pre-focused sample moves down to the detection region. Fig. 3 (a) is the SEM of the micro optic system which comprises of a laser diode, a micro lens array and a photodetector. Fluorescent tagged CD4 cell is excited by the focused laser light then measured by an on-chip photodetector as shown in Fig. 3 (b). Peaks of fluorescent signal are counted, which corresponds to the number of CD4 cells. By replacing the laser by the laser diode, the cost and volume for peripheral equipment are greatly reduced.
The sorting process is realized by steering the electroosmotic flow through the switching of voltages at output reservoirs as shown in Fig. 4 (a). Sample solution is defaulted to flow to the waste reservoir. When a CD4 cell is detected, the flow is switched to the other direction for 5 ms before switched back. The switching time is very much related to the switching structure design. Fig. 4 (b) illustrates how switching time is affected by the angle between the two outlet channels. The switching time was measured by µ-PIV system.

![Fig. 4. (a) Measured velocity field when sample is switched to left. (b) Switching time vs. angle between two outlets.](image)

3. CONCLUSIONS
A self-contained microfluidic cell sorting system for counting and sorting CD4 cells has been demonstrated. On-chip sample preparation was realized and laser diode was integrated in the chip as the light source. The system can provide very high accuracy in judging the HIV progression period of patients.

REFERENCES