Dual Characterization of Biological Cells by Optofluidic Microscope and Resistive Pulse Sensor

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Short Communication

Dual characterization of biological cells by optofluidic microscope and resistive pulse sensor

Label-free detection technique has emerged as a powerful platform for biomedical applications since it can avoid laborious multi-step sample preparation. In this paper, we demonstrate a dual analysis of biological cells using a single microfluidic system combining optofluidic microscopy and resistive pulse sensing. Both red blood cells (RBCs) and circulating tumor cells (CTCs) have been used to validate the concept of dual analysis and also to test the performance of the microfluidic device. The cell characterization by resistive pulse sensing is in good agreement with the analysis by optofluidic microscopy, further verified by the commercial Beckman-Coulter® FC500 flow cytometry. The present system has attractive merits such as simple fabrication, easy integration, high portability, and low cost. This study has great potentials for the development of innovative on-chip flow cytometry with concurrent imaging sensing and resistive sensing.

Keywords:
Label-free detection / On-chip flow cytometry / Optofluidic microscopy / Resistive pulse sensing
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Microfluidics-based resistive pulse sensor has been proved as a powerful and portable platform for the characterization of synthetic particles [1–3], bacteria [4], or biological cells such as red blood cells (RBCs) [5, 6], CD4+ T cells [7], and circulating tumor cells (CTCs) [5,6] owing to its low sample consumption and low cost. Resistive pulse sensing technique can reveal information related to the electrical properties of cells and their sizes, however, is very difficult to characterize the cell morphology like cell shape and texture. Cell analysis based on the morphologic difference is very useful in the study of chemokine-induced change in the cell shape and required for the diagnosis of certain diseases like sickle cell anemia. Conventional optical microscopic imaging technique can provide a direct vision of biological cells and is thus extensively used in various biomedical applications for many years. Up to date, cell imaging remains the most efficient method for the characterization of cell morphology. Thus, there is a great need to develop cost-effective and portable imaging systems for point-of-care diagnosis. Benefit from the advanced manufacturing technology in the semiconductor foundries, on-chip optofluidic microscopy [8–13] has been developed in recent years for high resolution imaging of biological cells [14].

In this work, we conduct a dual characterization of biological cells by optofluidic microscope and resistive pulse sensor integrated in a single device, as illustrated in Fig. 1A. Our microfluidic device is composed of a microfluidic channel and a complementary metal oxide semiconductor (CMOS) imaging sensor. The microfluidic channel with a constricted aperture was fabricated by a standard two-layer soft lithography [15]. The main channel is 200 μm in width and 70 μm in height. The sensing aperture is 30 μm in width and 30 μm in height. The microfluidic channel was subsequently bonded onto a CMOS imaging sensor after plasma treatment for surface activation. The resistive pulse sensor was constructed by placing electrodes at both ends of the main channel. When a single biological particle translocates through the aperture prefilled with conducting electrolytes, the electrical current can significantly decrease since the less-conducting particle leads to an increase in the electrical resistance. Meanwhile the CMOS sensor can continuously take images of

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Abbreviations: CMOS, complementary metal oxide semiconductor; **CTC**, circulating tumor cell; **RBC**, red blood cell

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Colour Online: See the article online to view Figs. 1–3 in colour

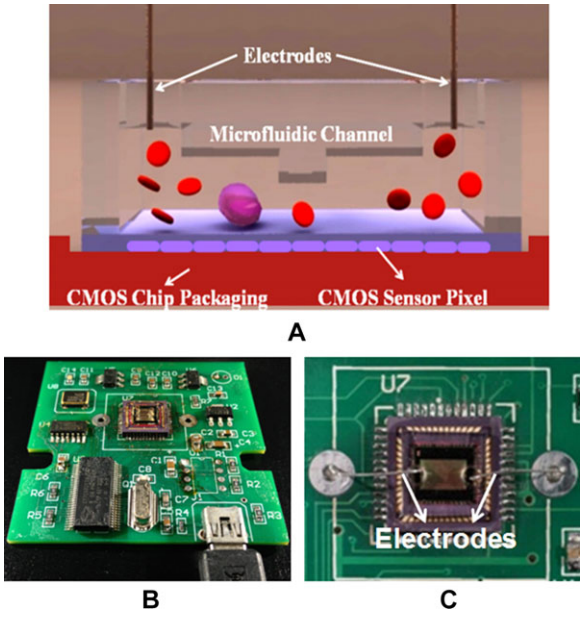

Figure 1. (A) Schematic view of the microfluidic device for dual characterization of biological cells: resistive pulse sensor (upper) and CMOS imaging sensor (bottom). (B) Photograph of the systematic board of optofluidic microscope. (C) Photograph of the microfluidic channel bonded upon the CMOS imaging sensor.

the sensing aperture to perform recognition and counting of biological cells through the microfluidic channel, referring to the imaging-based cell characterization by the optofluidic microscope. Therefore, the combination of resistive pulse sensing and cell imaging can be developed as a versatile technology for cell analysis based on both cell morphology and their electrical property.

HepG2 cells (American Type Culture Collection, MD, USA) were cultured in DMEM supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, and 0.1 mM MEM nonessential amino acids. The cells were grown at 37°C under a 5% CO_2 in a T75 flask. RBCs were separated from the whole blood using optiprepRM density gradient medium. The whole blood was first mixed with optiprep at a ratio of 1:8. Tricine buffered saline (TBS: 0.85% NaCl, 200 000 mM Tricine-NaOH, pH 7.4) was layered at the top of the mixture to prevent it from mixing with the blood. The whole blood sample was subsequently centrifuged at 2000 gav for 15 min. RBCs were then collected from the bottom of the centrifuge tube. The purified RBC samples were washed three times with PBS for 10 min and were finally transferred to PBS solutions.

A mechanical pump was used to inject the cell sample through the main channel. In the cell characterization using the optofluidic microscope, a program written in Matlab controls the CMOS imaging sensor (Aptina MT9M032, San Jose, CA) to capture and record the cell images. The CMOS sensor is as small as 11.5 mm (W) × 11.5 mm (L) × 2.3 mm (H) with a pixel size of 2.2 μm × 2.2 μm. It has a full resolution array size of 1472 (H) × 1096 (V) and its frame rate is 60 frames/second (fps) at 1280 (H) × 720 (V) resolution. Figure 1B and C shows the photo of the integrated microfluidic cell characterization system. Both RBCs (~1.5×10^3 cells/mL) and CTCs (~1.5×10^3 cells/mL) samples were resuspended in 1X PBS and then were injected through the microfluidic channel, respectively. The running time is about 20 min and the flow rate is 5 μL/min. Figure 2A and B shows the captured images of RBCs and CTCs during the translocation through the sensing aperture. Based on the pixel size analysis of the captured images, the optofluidic microscope can easily distinguish the two different cell types. The reading error of the cell size is ±1 pixel, corresponding to ±2.2 μm. According to the pixel size distribution of RBCs (Fig. 2C) and CTCs (Fig. 2D), it was found that the average pixel size of RBCs and CTCs are 7.15 and 18.15 μm in diameter, respectively. In order to evaluate the performance of optofluidic microscope in distinguishing CTCs from RBCs, a mixture sample of RBCs and CTCs was introduced to the device for imaging-based characterization. Figure 2E shows the measured pixel size distribution of the mixture sample. The percentages of RBCs and CTCs are, respectively, 48 and 52% based on the analysis by the optofluidic microscope, which is in good agreement with a flow cytometric analysis (Beckman-Coulter® FC500), as shown in Fig. 2F. These results imply that this optofluidic microscope could potentially provide a relatively reliable and cost-effective platform for the detection of CTCs from blood cells.

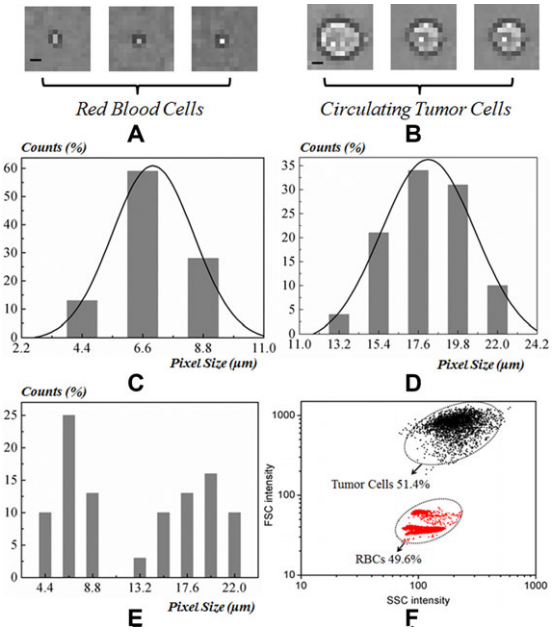

Figure 2. Captured RBC images (A) and CTC images (B) by the CMOS imaging sensor. The scale bar is 5 μm. Measured pixel size distributions of RBCs (C), CTCs (D), and a mixture of RBCs and CTCs (E) by the optofluidic microscopy. Curves in (C) and (D) are the Gaussian fittings to show the statistical size distribution of measured cells. (F) Flow cytometric scatter plots (forward scatter versus side scatter) of the mixture of RBCs and CTCs.

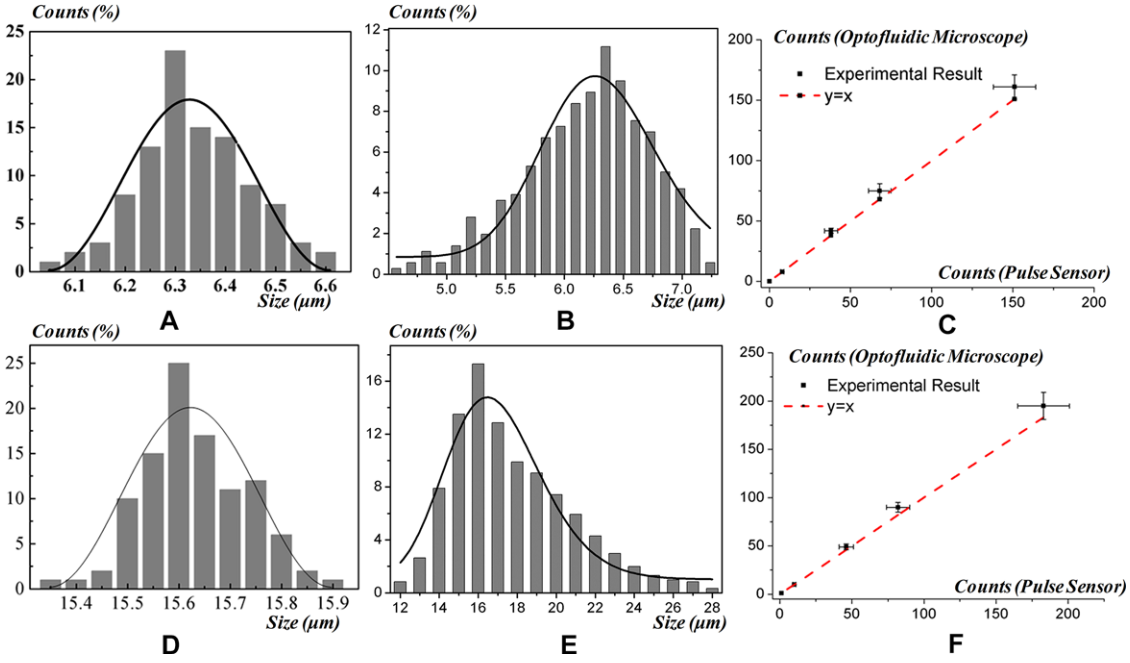

Figure 3. Measured size distribution of 6.3 μm (A) and 15.6 μm (D) polystyrene particles, RBCs (B) and CTCs (E) by the resistive pulse sensor. Comparative study of RBCs (C) and CTCs (F) by the optofluidic microscope and resistive pulse sensor.

In the cell characterization using the resistive pulse sensor, the electrical current was measured under a bias of 1 V across the main channel by a HEKA amplifier EPC 10 USB Quadro (HEKA Electronik, Lambrecht, Germany). The electrical current signals were collected with a sampling rate of 1 MHz and then were imported into a homemade MATLAB (Mathworks, MA, USA) program for postprocessing. In order to accurately measure the size of biological cells, polystyrene particles with standard sizes (6.3 and 15.6 μm) were used to calibrate the resistive pulse sensor, as shown in Fig. 3A and D. The ratio of modulated current induced by particles of different sizes is proportional to their volume ratio [4]. Therefore, the size of biological cells can be determined based on the ratio of modulated current induced by standard polystyrene particles and testing cells with a relative error of ±10%. The estimated size distributions of RBCs and CTCs are shown in Fig. 3B and E, respectively. The average size of RBCs and CTCs, determined by the resistive pulse sensing, are 6.3 and 16.5 μm, respectively. An approximate 10% variation on the measured cell size between the optofluidic microscope and resistive pulse sensor falls in a reasonable range due to the resolution of the two techniques. We also conducted a comparative study of cell characterization by the optofluidic microscope and resistive pulse sensor, in which both RBCs and CTCs with three different concentrations (~1.5×10^3 cells/mL, ~3×10^3 cells/mL, and ~6×10^3 cells/mL) were measured by the two techniques simultaneously. Figure 3C and F indicates that the two characterization techniques have a nearly identical resolution for cell counting. Furthermore, this study demonstrated that the developed system is capable of performing concurrent characterization of biological cells by the optofluidic microscopy and resistive pulse sensing, which may provide a more accurate and reliable solution for the detection and counting of cells in raw biological samples with high heterogeneity.

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