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Counting leukocytes from whole blood using a lab-on-a-chip Coulter counter

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Abstract— **A microfluidic lab-on-a-chip Coulter counter was demonstrated to count micro particles and leukocytes from whole blood. Instead of electroplated or deposited metal electrodes, off-the-shelf gold pins were used as electrodes to simplify fabrication process, reduce cost, enhance device durability, and above all, achieve superior uniformity in E-field distribution for improved signal quality. A custom-designed, low-cost demodulation circuit was developed to detect the AC impedance signals of the particles and cells passing the detection area defined by the microfluidic channels. A mixture of polystyrene beads with three different sizes was used to characterize the device. The results showed high throughput at 2000 particles/s and clear separation among different sizes of beads with coefficients of variation (CV) of 13.53%, 10.35% and 5.67% for 7.66µm, 10.5µm and 14.7µmbeads, respectively. To demonstrate the potential for a point-of-care or self-administered device for cancer patients going through chemotherapy, we have used the lab-on-a-chip device to count leukocytes from whole blood, generating encouraging preliminary results comparable to the results from a commercial flow cytometer.**

I. INTRODUCTION

Microfluidic cell counters hold promise for point-of-care devices because of their low cost, portability, and applications in healthcare [1-2]. Various cell detecting methods using optical, electrical, acoustic or magnetic mechanisms have been demonstrated to count and analyze cells in a flow [3-5]. Among these cell detection mechanisms in microfluidic devices, electrical impedance analysis, namely a lab-on-a-chip Coulter counter, is attractive because it is label-free, produces high throughput, and requires minimum work for sample preparation. It is particularly attractive for blood cell counts such as enumeration of white blood cells. For cancer patients going through chemotherapy and patients with compromised immune systems, frequent hospital visits to measure the lymphocyte concentration have caused high risks of hospital infection, which has taken over 90,000 lives each year in the United States. A lab-on-a-chip Coulter counter allows patients to take less than 10uL of blood, a similar amount to glucose test, for lymphocyte counts. The test can be self-administered or performed at the point-of-care clinics to reduce costs and risks of hospital infection.

Cell impedance measurements provide direct information about cell size, which can be used to distinguish lymphocytes from erythrocytes and platelets. Due to their significant volume differences, one can set the detection threshold to register only lymphocytes, thus alleviating the problem of having the system overwhelmed by the large number of erythrocytes and platelets. Although DC cell impedance measurement can be done more easily, the applied DC voltage causes hydrolysis and generates gas bubbles, which can seriously affect the signal quality and the durability of the device. Hence it becomes more desirable to detect cell impedance using AC signals. Much work has been done to measure AC impedance although most demonstrated devices have experienced some difficulties for practical point-of-care or self-administered tests. Here we summarize a few key developments in microfluidic Coulter counters as examples. Gawad [6] demonstrated differentiation of beads and also erythrocytes from ghost cells using coplanar microelectrodes. Due to the non-uniform electrical field distribution inherent to the geometry of the electrodes, the measured impedance amplitude varies widely, affecting the accuracy and reliability of cell differentiation. To improve the device performance, a flow focusing design similar to that in flow cytometers [7-8] and parallel facing electrodes [9-11] were demonstrated. However, the former approach requires sheath flow and considerably complicates the system hardware and operation, while reducing the throughput at the same time. The latter approach requires special and sophisticated fabrication process, making the device too costly to be disposable. The impedance intensity was also affected by particle positions in the micro channel.

In this work, we demonstrate a lab-on-a-chip Coulter counter with a unique electrode design that is easy and inexpensive to fabricate, and above all, produces highly uniform electric field over the detection zone. The electrodes are formed by inserting and sealing two off-the-shelf gold pins into two fluidic chambers on each side of the microfluidic channel. The distribution of the E-field is determined by the geometry of the fluidic channels crossing the main channel (Fig. 1) instead by the geometry of the electrodes themselves as in previously reported devices. In our design, the measured

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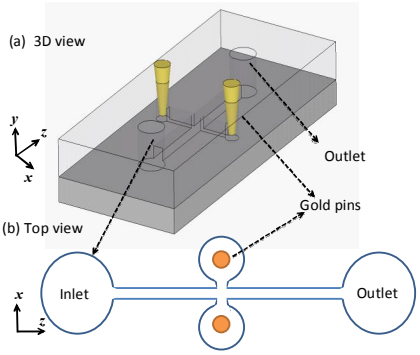

Figure 1. Schematic of the lab-on-a-chip Coulter counter. (a) 3D view and (b) Top view of the final device.

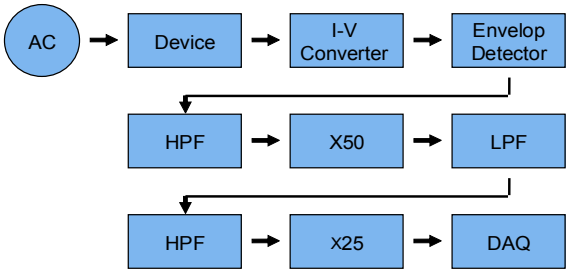

Figure 2. Block diagram of the demodulation circuit for the Coulter counter. HPF – High Pass Filter with 300 Hz cutoff frequency, LPF – Low Pass Filter with 10 kHz cutoff frequency), X50& X25 are gain of amplifier.

cell impedance value becomes independent of the cell position in the channel, so one can operate the device without any flow focusing to confine the cells.

Besides the electrode design and fabrication, we also develop a circuit to process the modulated signal of the particle. To characterize the system and to demonstrate the key functionality, we report experimental results with polystyrene beads of different sizes and leukocytes from whole blood. Comparing the results between our device and a commercial flow cytometer, we validate the device performance and demonstrate its viability as a low cost lymphocyte counter, among other applications.

II. Methods and Fabrication

A. Principle

Conventional Coulter counters measure the change in DC resistance between two fluid-filled chambers connected by a small duct [12]. When a particle passes through the duct, it displaces the conductive fluid and generates a current change. More recently, AC signals are used in microfluidic Coulter counters since at high enough frequencies, AC signals eliminate the double-layer effects at the electrode-fluid interface and provide information about the interior properties of the particle according to its dielectric properties. Similar to the DC signal, a change in the AC current due to the particle passing the sensing area gives rise to the particle impedance at a given frequency. The impedance signal is modulated at the carrier frequency and demodulation is needed to obtain the particles' impedance [13]. The change in impedance is proportional to the volume of each bead or cell, so that the device can count the absolute number of cells and discriminate sub-populations of any heterogeneous mixture. For example, leukocytes are larger than red blood cells and platelets and replaces larger volume of conductive buffer fluid such as PBS or blood plasma, thus causing larger impedance changes.

B. Device Fabrication

A microfluidic channel is fabricated by soft lithography using MicroChem SU-8-2015 negative photoresist followed by a polydimethylsiloxane (PDMS) replica molding process. The width and height of the microfluidic channels are both 30 μm so that blood cells can travel without clogging the channels. The opening of the side channel as shown in Figure 1(b) is also 30 μm wide, so that the electrical field is narrowly confined to generate a sharp increase in impedance when the conductive fluidic volume of the detection zone is replaced by each cell or bead.

A PDMS layer with microfluidic features is then permanently bonded to a microscope glass slide by UV Ozone treatment, thus creating enclosed microfluidic channels. Electrically conductive wires are then connected to the microfluidic channel via phosphate buffered saline filled side channels, thus creating three-dimensionally and uniformly confined electric field.

The fabrication technique reported in this work is simple and does not require thin film deposition of metal electrodes on the microfluidics channel walls. Since expensive semiconductor clean room facilities are not necessary, this process can lower the cost significantly. It is also well compatible with injection molding technique for mass production as well as with the well-known PDMS replica molding technique for fast prototyping. The metal wires such as gold pins or copper wires can be re-used multiple times after rinsing with DI water between each run. The low fabrication cost will make our microfluidics Coulter counter suitable for point-of-care analysis.

C. Impedance detection circuit

Due to the relatively small volume ratio between the particle and the volume of the detection zone (typically in the order of 1%), the magnitude of change in the AC signal is small and noisy, and requires a circuit to demodulate the amplitude-modulated signal. Some groups used the technique of synchronous demodulation and lock-in amplifiers to extract signals from the carrier [10, 13]. Although these approaches improve the detection sensitivity, the system becomes bulky and expensive, and less attractive to point-of-care and infrastructure independent settings. Here we developed simple, low cost demodulation circuit based on the envelope detection technique. The functional block diagram of the circuit is shown in Figure 2.

A function generator (33522A, Agilent Technologies, Palo Alto, CA, USA) is used to generate a carrier signal at 700KHz to the gold-pin electrodes in Fig. 1. The amplitude of the signal is modulated by particles passing the detection zone. An I-V converter first transforms the current signal into a voltage signal before entering the envelope detector consisting of a rectifier and a low-pass filter. A high-pass filter is connected

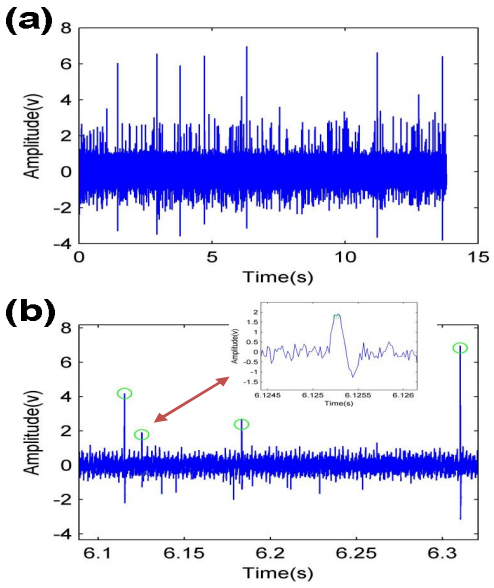

Figure 3. Beads mixture testing results with a 700 kHz, 4-volts peak-to-peak carrier. (a) Raw data of three beads mixture (7.66µm/10.5µm/14.7µm). (b) Close-up view of signals. The green circles indicate the amplitude of events. Inset: A typical waveform of an event from our device detection.

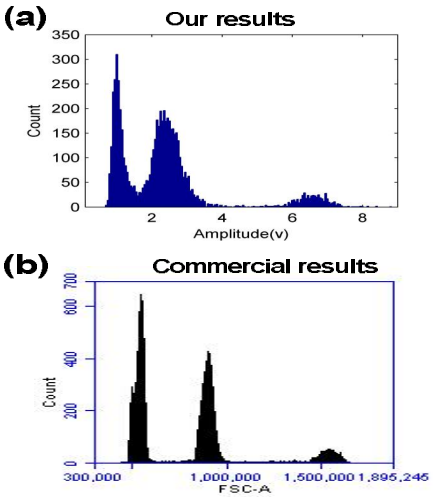

Figure 4. Histograms of the impedance signal of micro beads with three different sizes – 7.66 µm, 10.5µm, 14.7µm peak distribution by (a) our microfluidic Coulter counter and by (b) a commercial flow cytometer (Accuri C6, BD, USA). Gaussian-like distribution by the microfluidic Coulter counter is identified with the coefficient variation (CV) of 5% - 13%, which is comparable to the accuracy of commercial bench-top cell counters.

to the output of the envelope detector to remove any dc component. The signal is amplified (by 50 times) and goes through another low-pass filter to remove the AC carrier. Finally, the demodulated signal is amplified again and becomes the input of the off-the-shelf software (Signal Express 2010, National Instrument, Austin, TX, USA) with a commercial data acquisition board (DAQ USB-6251, National Instrument, Austin, TX, USA).

III. Result and Discussion

To evaluate the performance of our design, a mixture of polystyrene beads with diameters 7.66µm, 10.5µm and 14.7µm (PPS-6K, Spherotech, Lake forest, IL, USA) was injected into the microfluidic channel using a syringe pump (Pump 11 Pico Plus Elite, Harvard Apparatus, Holliston, MA,USA). The flow rate was 20µl/min. A 700 kHz sinusoidal wave with 4-volts peak-to-peak was used as a carrier. The sampling rate of the DAQ board was set at 50 kHz. An in-house peak detection algorithm based on MATLAB (Version 7.8.0.347, MathWorks, Natick, MA, USA) was developed to identify the impedance signal.

Figure 3(a) shows the raw data of the beads mixture over a period of 14 seconds. Each peak represents one event created by one particle. Figure 3(b) is a zoom-in view. The inset shows a typical waveform of the demodulated signal of a passing particle. There is a negative tail following the positive peak in each signal. In principle, a passing particle should only produce a positive peak, and the negative tail is caused by the differential effect of the high-pass filter in the circuit.

We have purposely set the parameters of the high-pass filter to create such a negative tail because it helps the algorithm detect real signals from noise, particularly when the signal-to-noise ratio (SNR) is poor. At a flow rate of 20µl/min, the average transit time of each particle across the detection zone is around500µs and the system has the capacity to support a shorter transit time. This gives an estimated throughput of 2000 particles/second, which is practical.

By means of the peak detection algorithm, a histogram of impedance amplitude for the beads mixture is shown in Figure4 (a). A total of 6210 events were recorded from a sample of145µl. The sample was collected at the outlet of the device and analyzed using a commercial flow cytometer (Accuri C6, Becton& Dickinson, Ann Arbor MI, USA). The histogram based on forward scattering intensity is plotted in Figure 4(b). Both figures show that three types of beads can be easily distinguished. By employing the gate, CVs and ratios of each bead population from both devices are summarized in Table 1.

The ratios of 10.5µm and 14.7um beads from our device are very similar to the values of the commercial system except for 7.66µm beads where there is a difference of ten percent. This might be caused by the sensitivity limit of our device such that some 7.66µm beads were treated as noise by the peak detection algorithm. The impedance CVs of our device are13.53%,10.35% and 5.67% for 7.66µm, 10.5µm and 14.7

Table 1.CVs and ratios comparison between our design and commercial instrument.

	Impedance Ratios (our design)	Impedance CVs (our design)	FS Ratios (Accuri C6)	FS CVs (Accuri C6)
7.66µm	31.45%	13.53%	42.87%	4.46%
10.5µm	45.68%	10.35%	45.35%	3.37%
14.7µm	7.78%	5.67%	8.1%	2.27%

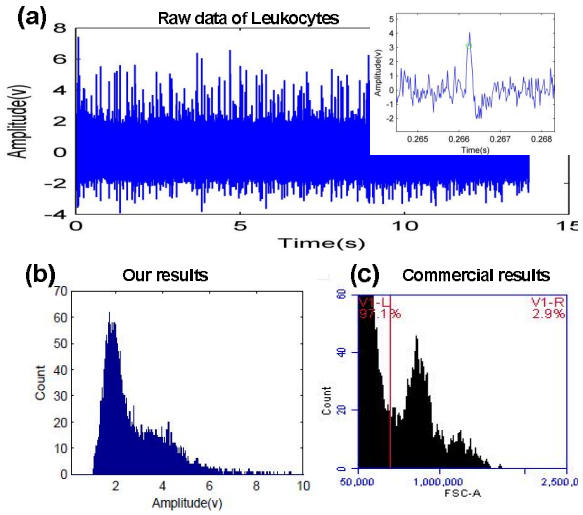

Figure 5. Leukocytes testing results with a 700 kHz, 4-volts peak-to-peak carrier. (a) The raw data of leukocytes. The inset shows the typical waveform of an event. (b) Histogram of impedance amplitude from our device. (c) Histogram of forward scattering from a commercial system.

µm beads, respectively. The CV values of 10.5µm and CVs from the commercial system. The CV value of7.66µm beads is 3 times of the value from the commercial system. Although our design can, in principle, remove the E-field non-uniformity over the cross section of the fluidic channel, the fringing field may still contribute to variations of the signals, particularly for smaller beads. That might result in the CV difference between our device and flow confined commercial system. Nonetheless, by eliminating flow focusing for a much simplified and inexpensive device, our device has produced decent CVs suitable for biomedical applications such as blood analysis discussed next.

To demonstrate point-of-care applications of the device, leukocyte counting from whole blood samples was performed in the same setup. The whole blood samples were purchased from a local blood bank (San Diego Blood Bank, CA, USA). Leukocyte samples were obtained from the whole blood after red blood cell lysing, centrifuge and dilution with 1xPBS buffer. Figure 5(a) shows a time trace for leukocytes flowing through our device. Each spike corresponds to one event of detecting leukocytes. The inset shows the waveform of the signal from a white blood cell. It is clear that the amplitude of the signal varies more significantly than the signals from beads because of a wide range of size distribution in normal leukocytes. Using the same algorithm as before, we plot the impedance histogram for leukocytes in Figure 5(b). We also collected the blood sample from the outlet of the device and plotted the histogram of forward scattering signal from the commercial flow cytometer in Figure5(c). The red line in figure 5(c) indicates the gating threshold, below which are mostly debris and impurities in the blood sample. Comparing Figure5 (b) with Figure 5(c), we observe an excellent match in the distribution of WBCs from both measurements, including the twin peak features showing the groups of lymphocytes/monocytes and granulocytes. The plots also fit well to the volume distribution of leukocytes in the literature [14]. This demonstrates the feasibility of our device for counting leukocytes.

IV. CONCLUSION

We demonstrate a lab-on-a-chip microfluidic Coulter counter for particle and leukocytes counting. Off-the-shelf gold pins are used as the electrodes in a configuration that minimizes the electrical field non-uniformity. Benefiting from the electrode design and a custom detection circuit, we can not only fabricate devices in simple and reproducible process but also achieve excellent detection sensitivity and CVs without any flow confinement. From the beads and blood experiments, we have shown that our device produces results consistent with the results from a commercial flow cytometer although our device is much cheaper, smaller, and easier to operate than flow cytometers. The device offers a promising solution for point-of-care and self-administered tests, and is particularly attractive to patients undergoing chemotherapy or having a compromised immune system.

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