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Note: Microelectromechanical systems Coulter counter for cell monitoring and counting

Yifan Wu,[1] James D. Benson,[2] John K. Critser,[3] and Mahmoud Almasri[1]
[1]*Department of Electrical and Computer Engineering, University of Missouri, Columbia, Missouri 65211, USA*
[2]*Department of Mathematics, University of Missouri, Columbia, Missouri 65211, USA*
[3]*Comparative Medicine Center, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65211, USA*

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This note describes the design, fabrication, and testing of a novel microelectromechanical systems Coulter counter. The Coulter counter will be used to detect and monitor impedance changes of cells as a function of time in response to different experimental extracellular environments. The device consists of SU-8 (negative photoresist) microchannels, vertical electroplated electrodes, polydimethylsiloxane cover, and is divided into a passive mixing region, a focusing region using negative dielectrophoretic forces, and a measuring region defined by multiple electroplated electrode pairs. The devices were tested using both microbeads in saline water and fibroblast cells in phosphate buffered saline solution. The results show that the proposed microsystem is capable of monitoring impedance of cells at different positions along the Coulter microchannel. © *2010 American Institute of Physics.* [doi:10.1063/1.3462327]

Electronic particle counters such as the Coulter counter are standard diagnostic devices widely used in laboratory medicine and pathology.[1,2] These devices are used to perform rapid, accurate analysis of blood and cells within other bodily fluids. They are also used to characterize cells in terms of their size, and other properties that are relevant to the optimization of methods to store them at low temperatures.[3] These methods are essential for transfusion, transplantation, and reproductive medicine. Commercially available Coulter counters have a number of limitations. First, they are relatively large. Due to their rather large size, they are configured to require relatively large sample volume. This sample volume requirement limits the ability to process samples more rapidly and severely limits measurement of time sensitive cell characteristics (e.g., changes in volume in response to changes in solute concentrations). Moreover, this sample size requirement necessitates a total sample volume of more than 5 ml with a minimal density of around 10^4 cells/ml, an impractical requirement for many cell types and imposes time-to-measurement constraints when measurements are made in nonphysiologic media. These sample size and time constraints are detrimental to accurate dynamic volume measurements for some cell types. For example, mouse and rat spermatozoa are available in relatively small numbers and the cells adjust volumetrically very quickly to anisosmotic environments, equilibrating in less than 10 s in many cases, making traditional Coulter counter methods difficult or impossible to implement. Several groups have successfully demonstrated miniaturized and micromachined Coulter counters using various designs.[4–23] Miniaturized Coulter counters provide many advantages such as significantly reduced sample volume, minimal cell count, low cost, low power consumption, and portability.[4] These micromachined Coulter counters are designed to measure impedance of cells using one or two electrode pairs, and thus may only be used for cell counting purposes and static cell sizing. The main objective of this note is to develop a novel microelectromechanical systems (MEMS) Coulter counter that can ultimately detect and monitor the dynamic cell impedance changes as a function of time by using a sequence of ten electrode pairs, after mixing isolated cell populations with different extracellular media. This note reports the detection of microbeads and fibroblast cells using impedance measurement through a series of vertical electrodes. It also demonstrates and assesses the quality of fluidic mixing qualitatively, and focusing of cells using dielectrophoresis.

The Coulter counter consists of three regions: multifluidic microchannels with passive mixing of the reagents, negative dielectrophoretic focusing of the cells, and an electrical impedance based sensing mechanism. A three-dimensional schematic of the Coulter counter is shown in Fig. 1. The extracellular fluid media and cells are introduced via two inlets into a Y-shaped channel, and subsequently into the mixing region which consists of serpentine shaped channel that mixes them using chaotic advection and diffusion.[24] The Coulter counter is specifically designed to measure the cell impedance changes within 0.2 s from the start of mixing.[25–27] This time delay is determined by the time scale of the physiological processes. The mixing region is connected to the focusing region. The electrodes in this region are designed with a ramp-shape that generates a nonuniform ac electric field to focus cells to the center of the microchannel by negative dielectrophoretic (DEP) forces. This focusing region is then connected to the measuring region, and on to the outlet. The goal is to study how cell properties change as a function of time after they are exposed to a certain extracellular media. This is accomplished by placing ten electrode pairs along the Coulter channel such that each electrode pair records the impedance of cell at the time it passes through it. Thus the impedance changes can be tracked as a function of time across the channel. It is important to note that the gra-

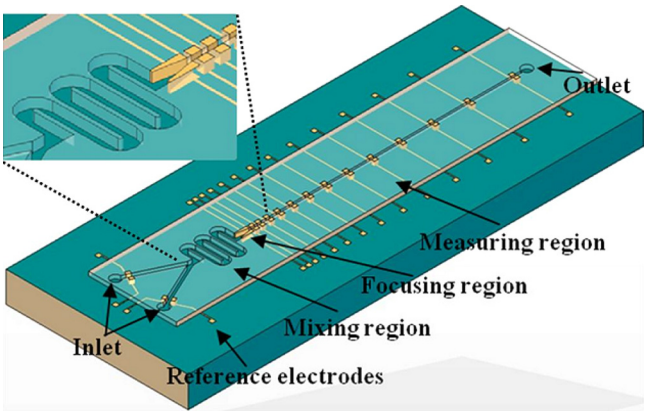

FIG. 1. (Color online) Three-dimensional schematic of the MEMS Coulter counter. The inset shows a magnified view of the serpentine mixer, ramp down electrodes for cell focusing, and couple of pairs of electrodes for impedance measurements.

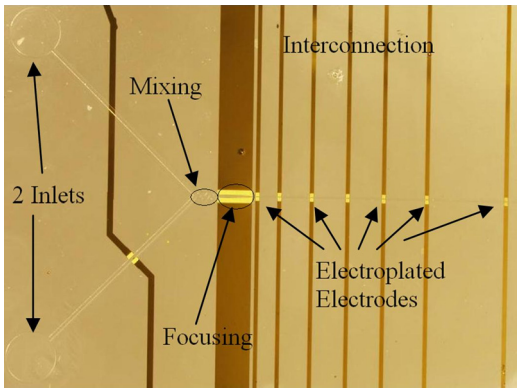

FIG. 2. (Color online) This top view optical image shows the SU-8 microchannel with the mixing region, focusing electrodes, measuring electrodes, and inlet and outlet ports (outlet port is not shown).

dient of the fluidic medium in the mixing region will not impose any measurable bias on the downstream impedance measurements because the medium will mix completely and become homogeneous by the time it reaches the measuring region. Thus, measurements are local. The microdevice is intended to measure the electrical properties of various cell types with diameters ranging from 15 to 20 μm. The electrodes are exposed in the microchannel and are in direct contact with the medium to be measured. Thus, they were partially corroded after a day of operation either by the cell medium, or electric current, or combination of them. The corrosion caused a slight dc bias shift but did not affect the magnitude of the pulses. In future work, the electrodes will be passivated by a dielectric thin film. Polydimethylsiloxane (PDMS) is chosen as a cover for the channel due to its advantages including flexibility, ease of fabrication, and transparency. Since PDMS is a rubberlike material and it is highly flexible, it conforms to the curvature of the surface it comes into contact with.

Traditionally, MEMS based Coulter counters have employed thin films of electrodes patterned on the substrate and across the microchannel.[4,5,8,15] This configuration generates nonuniform electric-field along the height of the microchannel, and most of it is close to the channel's bottom. Thus, those devices generate signal variations if identical particles pass at different heights over the electrodes.[28] In this note, the MEMS Coulter counter is fabricated with thick gold electroplated electrodes which will generate uniform E-field over the entire height of the microchannel along the direction perpendicular to the channel.[28] Multiple pairs of electrodes were distributed throughout the microchannel and the impedance of cells was monitored along the whole Coulter channel as cells pass through. The distribution of electrodes along the channel was designed such that measurements are made more frequently at the beginning, during the most transient phase of cell volume change.

The device was fabricated using the following sequence on top of a glass substrate: (1) An SU-8 layer was spin coated, exposed to UV light, and cured. The purpose of this layer is to improve the adhesion of the device to the glass substrate. (2) A bilayer of titanium (Ti) and gold (Au) were sputter deposited with thicknesses of 40 and 140 nm, respectively. Gold layer was etched to create the electrical traces and bonding pads, and seed layer for electroplating the electrodes. (3) A thick layer of photoresist was patterned to form a mold for electroplating. The electrodes were created by electroplating gold inside the mold with a thickness of around 15 μm. (4) The photoresist was washed away and the Ti layer was wet etched using gold as a mask layer. (5) The microchannel was patterned using SU-8 photoresist with a thickness of 28 μm. The microchannel width is ranged between 100 μm at the inlets and mixing region and ramped down to 24 μm at the measuring region. (6) The PDMS cover was made and cured to serve as top cover along with fluidic connectors (fluidic inlets and outlets). (7) SU-8 was spin coated onto PDMS to serve as glue, after oxygen plasma treatment. The microchannel was then aligned and bonded to PDMS cover. (8) The device was fixed and wire bonded to printed circuit board for external electrical connections. An optical image of the fabricated device without a PDMS cover is shown in Fig. 2.

The quality of mixing of the fabricated Coulter counter was tested by flowing two fluids with different colors in two streams connected using Y shape junction. The flow rate was controlled by a Harvard Apparatus PHD 2000 syringe pump. The two colors were mixed via chaotic advection and diffusion. The quality of mixing/dilution is evaluated qualitatively by observing color intensity variation of the blue dye using optical microscope at the entrance of the Coulter channel (detection zone) as shown in Fig. 3. To evaluate the focusing capability of the focusing zone in the Coulter counter, latex microbeads (Beckman Coulter, Miami, FL) with diameter of 15 μm in saline water are focused into the center of the channel by applying ac sinusoidal voltage with 2 V peak-to-peak at 100k Hz. The generated negative DEP forces on the microbeads move them to the region with low electric field in the center of the channel (see Fig. 4).

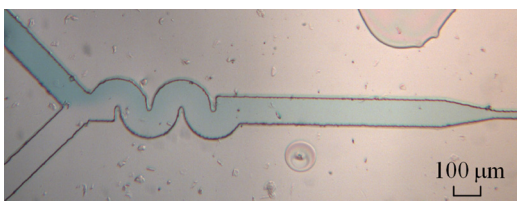

FIG. 3. (Color online) This bottom view optical image demonstrates the mixing/dilution of blue dye and de-ionized water qualitatively using passive mixing. The dilution of the two fluids at the entrance of the Coulter channel appears to be satisfactory.

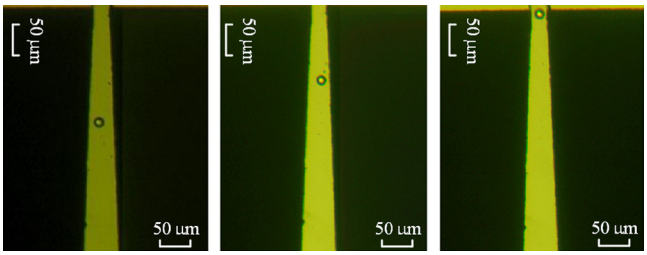

FIG. 4. (Color online) Three bottom view images display the focusing of microbeads to the center of the channel using dielectrophoresis. The upper side of each image is the exit of the focusing region (entrance of Coulter channel).

Prior to assessing the fabricated and packaged MEMS Coulter counter devices, a simple electrical circuit was designed and built in order to measure the resistance changes of microbeads and cells. The electrical testing was performed by injecting saturated (27%) saline water with 10 and 15 μm latex microbeads into the microchannel. A 9 V dc power supply and a 110 kΩ resistor for microbeads testing or 780 kΩ resistor for fibroblast cells testing were connected to the electrodes to form a voltage divider. The resistance change before and after injecting microbeads in the conductive media, measured by the electrodes can be converted to voltage change. The voltage signal is then passed into a high pass filter in order to block unwanted dc component and then amplified by an instrumentation amplifier and displayed by an oscilloscope. In case of fibroblast cells, there is no need for signal amplification. Figure 5(a) displays the measured voltage signals of the two microbeads with different sizes. The voltage amplitudes of the two voltage pulses are different and proportional to the volume of the particles. The device was also tested using fibroblast cells with diameter of 19 μm in isotonic phosphate buffered solution. Figure 5(b) shows three voltage pulses of three cells as they pass through one pair of electrodes within 0.2 s, with voltage amplitude around 2.1 V. In addition, several voltage pulses are recorded by two pairs of electrodes as shown in Fig. 6. In this testing, the ac measurement mode was selected in the oscilloscope. The width of pulse depends on the velocity of particle, and the height of pulse depends on the equivalent resistivity and volume of the particle as well as the conductivity of the medium. In this research, we will only flow the same type of dielectric particles or cells at a time.

In summary, MEMS based Coulter counter devices were designed, fabricated, and tested. The Coulter counter was fabricated using gold electroplated vertical electrodes and SU-8 microchannel. This device will be used for measuring the impedance, counting, and sizing different types and sizes of cells. This device uses passive mixing, negative dielectrophoresis to focus the cells to the center of the channel, and

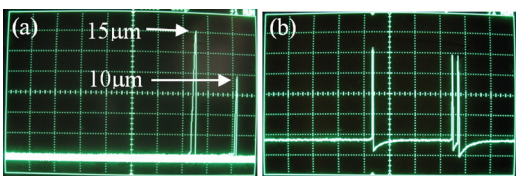

FIG. 5. (Color online) The two figures show (a) two voltage pulses with different amplitude which corresponds to microbeads with diameters of 10 and 15 μm, (b) three voltage pulses of three fibroblast cells as they pass through one pair of electrodes within 0.2 s.

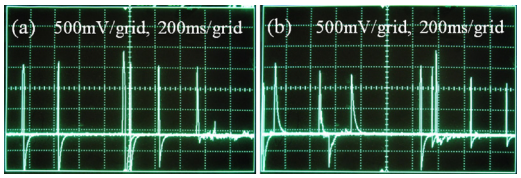

FIG. 6. (Color online) The two images display several voltage pulses of fibroblast cells measured by two pairs of electrodes. Each image represents signals captured by one pair of electrode and they were put in the same screen: (a) first pair of electrodes and (b) second pair of electrodes.

Coulter principle to detect cells based on the change in resistance when they pass through the sensing zone. Fluidic and electrical testing results using microbeads and fibroblast cells with sizes of 10 and 15 μm, and 19 μm, respectively, validate the performance of the device. Future work will include developing an automatic multichannel recording system to save the large volume of data for analysis. More types of cells would be studied and, ultimately, impedance changes of cells can be observed as a function of time after mixing with different experimental extracellular media.

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