Microfluidic MEMS Hand-Held Flow Cytometer
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ABSTRACT

Due to a number of recent technological advances, a hand-held flow cytometer can be achieved by use of semiconductor illuminators, optical sensors (all battery powered) and sensitive cell markers such as immuno-quantum dot (Qdot) labels. The specific application described is of a handheld blood analyzer that can quickly process a drop of whole, unfractionated human peripheral blood by real-time, on-chip magnetic separation of white blood cells (WBCs) and red blood cells (RBCs) and further fluorescence analysis of Qdot labeled WBC subsets.

Various microfluidic patterns were fabricated in PDMS and used to characterize flow of single cells and magnetic deflection of magnetically labeled cells. An LED excitation, avalanche photodiode detection system (SensL Technologies, Ltd., Cork, Ireland) was used for immuno-Qdot detection of WBC subsets. A static optical setup was used to determine the sensitivity of the detection system.

In this work we demonstrate: valve-less, on-chip magnetic sorting of immunomagnetically labeled white blood cells, bright Qdot labeling of lymphocytes, and counting of labeled white blood cells. Comparisons of these results with conventional flow cytometric analyses are reported. Sample preparation efficiency was determined by labeling of isolated white blood cells. Appropriate flow rates were determined for optical detection and confirmed with flowing particles.

Several enabling technologies required for a truly portable, battery powered, hand-held flow cytometer for use in future point-of-care diagnostic devices have been demonstrated. The combining of these technologies into an integrated hand-held instrument is in progress and results on whole blood cell analysis are to be reported in another paper.

Keywords: microfluidic, hand-held cytometer, Qdot labeling, magnetic deflection, valve-less sorting, avalanche photodiode; point-of-care diagnostics

1. INTRODUCTION

1.1. The need for portable, closed microfluidic flow cytometers for point-of-care diagnostics

A microfluidic flow cytometer has the potential to be a high-speed, closed-system, portable, and inexpensive device 1-3. Most current microfluidic cytometers are micro-only in the microfluidic chip. The rest of most apparatuses are decidedly macro- usually requiring microscopes or other large ancillary optical or electronic equipment 4-9. In this paper we report on progress in developing a hand-held device that can be operated by untrained, or minimally trained personnel. More importantly, it is designed to be a truly portable, battery-powered and hand-held device for point-of-care biomedical applications. In other words our goal is to bring the device to the patient rather than the patient to the device. Most existing flow cytometers are large, not portable, expensive, and usually require highly trained personnel to operate correctly. Microfluidic flow cytometers can solve many of these problems. A microfluidic cytometer can be
easily made as a closed-system device with disposable chips reducing cost, increasing sterility, and preventing contact with hazardous agents. Essentially the device becomes a “reader” with insertable and disposable reagent packs that can even be designed to talk to application-specific embedded algorithms through barcoded reagent chips. The overall portability of the device will allow for operation outside a traditional medical setting, further reducing possibilities of infection or contamination occurring from sample preparation and transport. Such a small device will require the use of novel instrument design, new photonic excitation sources, thin-film optical filters, and small sensors. In the following sections we report on the initial development stages of such a microfluidic-enabled portable flow cytometer for point-of-care applications.

2. MATERIALS AND METHODS

2.1. Proposed general concept design

The design for the hand-held blood analyzer uses a branching architecture to continuously sort white and red blood cells. The reason for on-chip sorting of white blood cells (WBCs) from red blood cells (RBCs) is to deal with the fact that there are several thousand times as many RBCs as WBCs and that different types of measurements need to be taken on the WBC subpopulations. The WBCs are actively sorted magnetically while the RBCs are passively sorted via flow distribution in the channel split. Figure 1 highlights the overall design of the cytometer. Briefly, a blood sample will be taken from a finger prick and capillary action will bring the whole blood on chip. Once on chip, the blood is mixed with fluorescent and magnetic labels. Next the blood is pushed through the PDMS channels with a micro syringe drive. The WBCs are magnetically pulled toward an analysis channel and the RBCs continue down the main channel and then passively sort into a branched channel for counting, Figure 2. The WBCs are illuminated with UV LED source and fluorescence is detected by silicon photomultipliers (SPMs). Data acquisition software analyzes the signals from the SPMs and outputs a cell count for each labeled cell type.

![Figure 1: Schematic of the subcomponents and processes that constitute an integrated portable microcytometer reader, reagent packs, and disposable microfluidic chip.](image-url)
2.2. Microfluidic System

Polydimethylsiloxane (PDMS) microchips were designed and manufactured using standard photolithography and soft lithography techniques in the cleanroom of the Birck Nanotechnology Center at Purdue University. Performance of the chip was evaluated by visual observation of Hoechst stained cells flowing through the device whilst situated upon an inverted fluorescent microscope. Pictures and videos were taken of the flowing particles using this method. Quantitative data were measured by collecting the output fluids from the PDMS chip and counting cells on a macro cytometer (FC500 Beckman Coulter). PDMS chips allow for easy and quick prototyping of microfluidic devices.

2.3. Optical design

When designing the optical components it is important to remember that although the systems are independent on their own, in flow cytometry they are ‘tightly engineered’. Each system must work with the other to have a fully functional overall system, and changes of design ripple their effects through the total design. For example, a change in cell speed affects optical sensitivity and signal processing. In this design we must consider overall size, power, sensitivity, speed and the precise alignment of the components.

2.3.1. Light excitation sources

For the portable, light-weight, low-power device an LED (light emitting diode) is a promising selection. They are currently quite small and have very low power requirements. The LED that we are currently using is a high-power UV LED (NICHIA America Corporation, Wixom, MI). It produces approximately 3W of light at a peak wavelength of 365nm. The UV LED is capable of activating fluorescence in Qdot labeled white blood cells. The LED does require collimation and focusing lenses to appropriately illuminate the desired region of the PDMS chip. Potentially, a single LED could be used to illuminate several regions on the PDMS chip for different fluorescent measurements. A 405 nm laser diode is also being explored for illumination. A laser diode, similar to those found in a Blu-Ray player, is low cost and requires much less power than the UV LED. Several laser diodes could be used to illuminate several spots for fluorescent measurements and still use less power than the LED. Some other considerations are spot size, beam width, bandwidth, edge effects from slits, longevity and the ability to excite Qdots and then block the exciting light with optical filters.
2.3.2. Photodetectors

In the past the best choice for a photodetector was a photomultiplier tube (PMT) but it, although extremely sensitive, is large and power intensive. Recent developments in photonics have led to improved avalanche photodiodes (APDs) which are miniaturized analogues of the PMTs and are called silicon photomultipliers (SPMs). The SPM we have chosen is produced by SensL Inc. in Cork, Ireland. It has several desirable qualities for flow cytometry use. It is low-cost, high-gain (>10^6), and extremely sensitive. In addition, it is very robust and low power, requiring only a 30V breakdown voltage while consuming a couple of micro amperes current. The power requirement can be easily met using a battery with voltage step-up DC-DC converters. The spectral range is 400-1100 nm which makes it the ideal choice for identifying fluorescently stained cells. We are using an SPM micro™ with 1mm^2 active area that contain 100 μm microcells. Focusing and collection lenses are required to direct the fluorescent light to the detector surface. SPMs also have the potential to be placed in patterned arrays for a multi-stage design. This would allow for detection of multiple fluorescent colors or measuring the same signal at different gains and choosing the optimal result. Self-configuration of detector settings would be a key factor to simplify operation of the device. The SPMs were tested with static slides of fluorescent polystyrene beads to determine sensitivity and appropriate operating conditions. Dynamic tests were conducted using PDMS chips and flowing fluorescent beads and then Qdot labeled white blood cells at various flow rates.

2.4. Data acquisition and sort control electronics

The detection, excitation, and powering circuitry, and data acquisition system are other subsets that need to be low-power and small in size as well. Currently, an analog detection system and powering circuitry for the SPM are assembled using discrete electronics on a PCB board, and data acquisition is currently being run by LabVIEW for prototyping. Ultimately the data acquisition system will be implemented using digital signaling processing (DSP) chips.

2.5. Sorting structures

Our goal is to sort the white blood cells from the whole blood however; the white blood cells are essentially rare cells (1:1000 ratio with respect to red blood cells). For this type of sorting and with such a large number of total cells (roughly 50 million cells), it would be nearly impossible to detect a single white blood cell and then mechanically sort it. For this reason, magnetic sorting has been used for white blood cell collection on the PDMS chip. The white blood cells are labeled with 1.5 μm CD45 superparamagnetic microparticles (Bangs Laboratories, Inc. Fishers, IN) prior to flowing through the PDMS chip. A permanent magnet (300 mT, custom wedge-shape, Quadrant Magnetics, Louisville, KY) was placed adjacent to the main channel, upstream of the WBC channel bifurcation, to divert magnetically labeled cells into the WBC channel. The effectiveness of the magnetic sorting was observed qualitatively and quantitatively. Videos were taken of Hoechst and magnetically labeled white blood cells flowing through the PDMS chip atop an inverted Nikon microscope. Cell counts of the outputs of each channel were counted using an FC500 flow cytometer (Beckman-Coulter, Inc.).

3. Experimental Results

3.1. Microfabrication

The two-stage cytometer is comprised of a main channel with two branched channels for the WBCs and RBCs. Comsol™ simulations, Figure 3(a), were performed to design the branching system in such a manner that one tenth of the total flow is diverted down the WBC channel and then one tenth of the remaining flow to the RBC channel. Figure 3(b) demonstrates the simulation result for the flow rates in the WBC channel, and after WBC branching versus the WBC channel width. Defined parameters include 10 μL/min flow in the main channel, channel heights are 30 μm, and the total width of the two channels is fixed at 300 μm. Based on the simulation results, the photomask for the microfabrication was designed using CAD-based program AutoCAD 2010 Student Version (Autodesk, Inc., San Raphael, CA), Figure 4. All channels are designed to be 30μm in height, although actual dimensions slightly differ due
to microfabrication process variability. Using the aforementioned mask, an SU-8 (Microchem, MA, USA) mold was fabricated using standard photolithography process. Then PDMS was cured against the SU-8 micro molds to establish microchannels. This microchannel embedded PDMS substrate is then bonded to a PDMS coated 500 µm thick acrylic sheet using corona charging.

![Surface Velocity Field](image1.png)

Figure 3: Comsol simulation of the flow rate distribution in the microfluidic system versus WBC channel width.

![AutoCAD Mask Design](image2.png)

Figure 4: AutoCAD mask design of 2-stage microfluidic cytometer for blood cell analysis based on Comsol simulations.

A photograph of the batch fabricated microfluidic system is depicted in figure 5 (a). After cutting an individual microchip, Tygon tubing was connected to the inlet, Figure 5 (b), and fluid was pushed through the microchannels using a syringe pump (Baby Bee Syringe Drive, BASi, West Lafayette, IN).
3.2. Cell Flow and Magnetic Sorting

To evaluate the effect of the magnetic sorting method, isolated leukocytes from a Buffy coat were stained with Hoechst 33342 and labeled with 1.5 μm magnetic particles (CD45, BioMag, Bangs Lab, Fishers, IN). Cells (4E5/mL) were flowed through the chip at a rate of 10 μL/min using a syringe pump. The magnet was positioned upstream of the WBC branch point, as illustrated in Figure 6. Videos of cell flow were recorded with the presence of the magnet and also without.

Figure 6: Close-up schematic of microfluidic channels and position of magnet for on-chip sorting of WBCs.

Figure 7 illustrates the effect of the magnet. Overall, in the presence of the magnet, Figure 7B, more WBCs are diverted into the WBC channel compared to when there is no magnet, Figure 7A. The next step was to quantify the WBC diversion. For quantification studies, isolated leukocytes were labeled with CD45 magnetic beads prior to flow and stained with DyeCycle Ruby (a nuclear stain visible on the FC 500 flow cytometer) after flow. The cells were flowed through the chip for 1 hour at 10 μL/min. The output from the WBC channel was collected and compared to the combined output of the other two channels.

Comparisons of cell counts were made by running the collected outputs on a macro cytometer (FC500, Beckman Coulter). The results are illustrated in Table 1. In this table WBC channel counts are represented as a percentage of the total cell output. Clearly the magnet is able to divert nearly twice as many WBCs into the branched channel than when no magnet is applied. Also the range of collected WBCs is with a 10% window, so the device provides consistent results.
Importantly, a single PDMS chip was able to be actively used for periods of an hour and rinsed with buffer and then reused without clogging. The robustness of the chip is necessary to ensure accurate cell counts.

Table 1: Effect of magnet on diverting WBCs to the small branch. Per cents calculated on the basis of ....

<table>
<thead>
<tr>
<th>Run Situation</th>
<th>Test #1</th>
<th>Test #2</th>
<th>Test #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet is positioned beside the channel</td>
<td>49.6%</td>
<td>42.1%</td>
<td>40.9%</td>
</tr>
<tr>
<td>No magnet</td>
<td>22.7%</td>
<td>18.4%</td>
<td>25.3%</td>
</tr>
</tbody>
</table>

3.3. Cell labeling

As previously seen\textsuperscript{11} isolated leukocytes were able to be labeled concurrently with both Qdots and magnetic particles. The dual labeling of leukocytes was studied quantitatively. Dual labeling of fixed, isolated leukocytes was confirmed on the FC500 flow cytometer. Percentages of labeled cells were compared to single-color controls. Dual labeling was the result of labeling fixed, isolated leukocytes with both CD45 BioMag beads (Bangs Lab, Fishers, IN) and CD4 605 nm Qdot particles (Invitrogen, CA). The dual labeling resulted in similar numbers of cells being labeled as with single color controls. There did not appear to be any steric hindrance issues with dual labeling, nor any competition for antigenic sites. Ultimately the magnetic particle labeling is the rate limiting step, as the particles are large and heavy and sedimentation becomes an issue. Mixing may help to alleviate sedimentation and reduce the required labeling time. Currently, tests have shown that 15 minutes is the minimum incubation time required to achieve sufficient magnetic labeling and collection.

3.4. Optical detection system

The optical excitation and detection system was composed of a high-power UV LED, a series of lenses and optical filters, and a 1 mm SPMmicro\textsuperscript{TM} photodiode with 100 µm microcell size. A schematic of the testing setup and the detail...
of the optical system is seen in Figure 8. The LED is pulsed to provide a modulated signal to use a lock-in amplifier (MODEL SR510, Stanford Research Systems, Sunnyvale, CA) to increase SNR. After several optical tests it was determined that with a DC signal the inherent SPM noise was too large to generate useful signal data, therefore a pulsed light source was deemed appropriate to excite the sample and also provide a basis for filtering noise. With this system we were able to distinguish individual flowing sortCAL™ beads as well as Qdot labeled leukocytes.

To test the optical detection system, a 0.5 mm wide 3 mm long slit was placed on the top of the main channel (300 µm wide) and the excitation LED (Nichia NCSUO33A UVLED) emitting at 365 nm was powered with a 100 kHz square wave with 50% duty cycle. Figure 9 shows the lock-in-amplifier’s output signals when SortCal™ beads (iCyt, IL, USA) are pushed through the microchannels at a flow rate of 10 µL/min. As can be seen in the Figure 9 (a), passing beads created signals larger than 500 mV, while the noise is around 50 mV. Hence, signal-to-noise-ratio (>10) is high enough for reliable cell counting. Also from figure 9 (b) it can be observed that the signal width is around 25 msec, as expected from calculation (dividing the excitation beam width, 0.5 mm, by the speed of the beads in the channel, 18.5 mm/sec).

Figure 8: Configuration of the testing setup (a), and detail of the optical system (b).

Figure 9: Pulse traces from the system shown in Figure 7 obtained from the Lock-in-amplifier output when SortCal™ beads are flowing into the channel with the flow rate of 10 µL/min. Each major negative-going pulse represents a stained cell traversing the excitation light source. (a) several pulses (the vertical scale is 500 mV/div and the horizontal scale is 500ms/div), (b) single pulse (the vertical scale is 500 mV/div and the horizontal scale is 25ms/div)
However, in the final cytometer design the slit width will be reduced to 100 µm to avoid multiple cell excitations. This smaller beam width will reduce the cell excitation time which can result in a weaker signal. To simulate this configuration, the flow rate was increased to 50 µL/min to generate similar excitation times. Figure 10 shows the output signal for this case. As can be seen in the figure, although the signal level was reduced, there is still enough signal to noise ratio (~5) for accurate cell counting. As can be inferred from Figure 10 (b), the signal width is approximately 7 msec which is in good agreement with the theoretical expectation.

![Figure 10: Lock-in-amplifier output signal when SortCal™ beads are flowing into the channel with the flow rate of 50 µL/min: (a) several pulses (the vertical scale is 500 mV/div and the horizontal scale is 500ms/div), (b) single pulse (the vertical scale is 500 mV/div and the horizontal scale is 25ms/div).](image)

In another experiment, CD45 Q-dot labeled WBCs were flowed through the PDMS channels. The following figure shows the lock-in-amplifier output signal when the flow rate is set to 10 µL/min. As can be seen in Figure 11, the signal level is a little lower than that of SortCal™ beads, but it is still detectable.

![Figure 11: The Lock-in-amplifier’s output signal when CD45 Q-dot labeled WBC are flowing into the channel with the flow rate of 10 µL/min. (a) several pulses (the vertical scale is 300 mV/div and the horizontal scale is 370ms/div), (b) single pulse (the vertical scale is 500 mV/div and the horizontal scale is 25ms/div)](image)

The SPMs in combination with the lock-in amplifier are able to detect Qdot labeled cells flowing at 10 µL/min. The modulated signal allowed for sufficient detection of flowing particles as well as the opportunity to sample dark noise and provide feedback to the system for noise subtraction.
4. CONCLUSIONS

Portability is the main focus of this microfluidic cytometer and we have made strides in designing and testing elements of a portable cytometer. The PDMS microchip with magnetic sorting is simplistic and performs reliably. The optical system is small enough using a combination of LEDs, SPMs, and 6 mm lenses. Currently a benchtop lock-in amplifier is being used but that will be replaced with a custom hybrid lock-in amplifier consisting of simple analog circuitry and a programmable micro chip. Pulsing the light source saves power and we calculate that the device will be able to run using a lithium battery similar to what is used by the cell phone industry. The SPMs also have the potential to be used in an array to save space and work together for multiple fluorescent signal detection. The next steps in developing the optical system include designing a custom lock-in amplifier and appropriate circuitry as well as incorporating data acquisition into the system and conducting further optimization experiments. Furthermore, a front-end design that uses capillary uptake to get a blood sample and then a simple stepper motor to mix the sample and drive fluid through the chip is also being developed. Ultimately at 10 µL/min rates, a complete run of the micro cytometer would take less than 30 minutes including on-chip sample preparation. This micro cytometer will be one of the first truly portable, point-of-care devices for whole blood analysis.

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