Microfluidic cell counter with embedded optical fibers fabricated by femtosecond laser ablation and anodic bonding

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Abstract: A simple fabrication technique to create all silicon/glass microfluidic devices is demonstrated using femtosecond laser ablation and anodic bonding. In a first application, we constructed a cell counting device based on small angle light scattering. The counter featured embedded optical fibers for multiangle excitation and detection of scattered light and/or fluorescence. The performance of the microfluidic cell counter was benchmarked against a commercial fluorescence-activated cell sorter.

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References

1. Introduction

Microfluidic devices have enormous potential for portable, low-cost medical diagnostic tools. For instance, flow cytometry on whole blood can provide complete cell counts of red blood cells, white blood cells and platelets by scattering alone [1,2]. Particle differentiation by specific internal or external features is also possible through the introduction of a fluorophore-labeled antibody. Despite the wide applicability of this technique, commercially available flow cytometers remain costly, bulky and technical. To overcome these challenges, there has been much interest in implementing flow cytometry in a small, inexpensive and alignment-free microfluidic system. A key element to realizing this potential is the stability afforded by embedded fluid regulators and sensors within the microfluidic device. Sensitive, high throughput particle scattering measurements for particle counting and size discrimination in microfluidic devices have been demonstrated, yet many systems rely on externally mounted optics [3,4]. An important goal for applications outside the laboratory environment is to integrate the optics into the microfluidic device by removing all of the free-space optical and mechanical hardware. This integration will miniaturize the system and improve its stability by immobilizing the illumination and detection optics with respect to the fluid. One way to achieve this goal is to merge fiber optics with microfluidics. Optical delivery through embedded fibers has been demonstrated in polydimethylsiloxane (PDMS) microfluidic devices [5-7]. In fact, fabrication technology in PDMS has been extensively developed making PDMS the most common type of microfluidic material. However, certain microanalysis applications are incompatible with PDMS, and glass-based devices would be more suitable. For example, PMDS has a high solubility in many common solvents, is easily damaged by tightly focused laser light, and produces background fluorescence at some common wavelengths used for excitation. As the application base for microanalysis increases, it is becoming increasingly desirable to fabricate glass-based devices with the ease that PDMS systems are now manufactured. This would allow one to exploit the gains that come with

glass: a chemically inert medium that is optically transparent, capable of withstanding high optical powers and producing little or no background fluorescence.

The most common methods for producing glass microfluidic devices are etching and powder blasting, techniques that are unable to produce the high aspect ratios achieved in PDMS. The aspect ratio of a channel is defined as the channel’s depth divided by its width. For etching methods, the aspect ratio is \( \leq 0.7 \). The cross-sections of etched channels are half-circles or trapezoids making them non-ideal for achieving symmetric fluid flow and uniform light propagation and for incorporating standard optical fibers. Instead, we employ femtosecond laser ablation which leads to channels of higher aspect ratio. In addition, and unlike traditional construction techniques involving photolithography in both glass and PDMS, femtosecond laser ablation is not constrained to machine the fiber grooves and fluid channels at the same depth. When the focused pulse intensity is near the threshold for ablation at high numerical aperture, material removal is confined to the focal volume \([8]\). Scanning the sample beneath the laser beam creates channels. Material modification by exposure to femtosecond pulses has been used to machine cylindrical and rectangular channels, subsurface jumpers and waveguides \([9-15]\).

We demonstrate integrated optical delivery by placing commercially-available fiber optics in femtosecond laser-ablated grooves. Unlike other approaches for integrating optics in microfluidic devices \([5-7, 10,11,13, 15-23]\), ablation by femtosecond pulses is a single step process that creates both fiber grooves and microfluidic channels; it involves no chemicals; and it can be completed on a comparably short time scale \((2-4 \text{ hours})\). Optical fibers are brought directly to the fluid channel, removing the need for free space optics to focus light into waveguides or for complicated fiber/waveguide junctions. Our device is capable of multigate scattering, and the shape of the illumination region is determined by the fiber optics, available with numerical apertures up to 0.5. Embedding the optics allows us to employ anodic bonding to seal the device to silicon instead of the more complex and typically very high temperature glass-to-glass bonding. Anodic bonding poses no risk to optical fibers and fabrication is further simplified by placing the fibers into grooves in the glass prior to bonding rather than sliding the fibers into grooves after bonding. Thus, through a process of femtosecond laser machining and anodic bonding, we have developed a rapid prototyping procedure for embedding optics in all silicon/glass based devices with the ease and on the same time scale that we can fabricate PDMS systems.

The utility and performance of our microfluidic device is illustrated with an application to optical cell counting. HeLa cells were detected through small angle scattering. Compared to previous demonstrations of particle scattering measurements in optically embedded microfluidic devices \([16,19-21]\), our system employs the lowest reported laser power \((2 \text{ mW})\) and a standard photodiode detector instead of a photomultiplier tube (PMT) or avalanche photodiode. The simplified detection significantly reduces the expense of the system. The accuracy of our system is rigorously and independently tested by subsequent counting of the same cell sample using a commercial fluorescence-activated cell sorter.

2. Experimental methods

Ablation of glass was accomplished with a home-built femtosecond Ti:Sapphire regenerative amplifier. The amplifier produced pulses with a typical duration and pulse energy of 40 fs and 30 \( \mu \text{J} \) at 800 nm with a repetition rate of 1 kHz. The laser pulses were weakly focused \((f/# = 9.2)\) to create an optimal zone of material removal for the desired channel dimensions. The lateral diameter of the ablated region per shot was 15 \( \mu \text{m} \). Channels were created in a Pyrex window \((\text{ESCO Optics})\) by scanning the sample at a rate of 1 \text{mm/s}. The fluid channel was 3 cm long, 70 \( \mu \text{m} \) wide and \( \approx 55 \mu \text{m} \) deep. Fiber grooves were 2 cm long, 90 \( \mu \text{m} \) wide and \( \approx 70 \mu \text{m} \) deep. Under these conditions the total machine time for each channel or fiber groove was \( \approx 45 \text{ min} \). Placement of a fiber is shown in Fig. 1. The fibers laid several microns below the surface of the glass in order to ensure unhindered, direct contact between the silicon and the
glass. Since the channels were machined from the top down, the ablated material was not trapped in the channel. Residual glass particles were rinsed away with water. We note that the channel depth measurements may include error arising from tip-substrate interactions when measuring channels of high aspect ratio with a standard profilometer. As such, the reported depths are lower limits.

The fluid channel is physically separated from the fiber grooves by a 50 µm thick glass barrier so as not to perturb the fluid flow. The fiber grooves terminate in short channels parallel to the fluid channel to provide a uniform surface at the optical interface. The interfaces scatter some of the light resulting in a constant background signal that is subsequently removed through electronic filtering. Figure 2 shows a white light image of the device. Multimode fibers (Polymicro Technologies, 0.22 numerical aperture (NA), 50 µm core, 55 µm cladding and 66 µm buffer) were placed by hand in the fiber grooves with the aid of a microscope. The device was then sealed by anodic bonding [24] to a 270 µm thick silicon wafer (University Wafer). Anodic bonding seals the fluid channel and holds the optical fibers in the grooves without glue.

The experimental setup is illustrated in Fig. 3. The incident laser light (2 mW at 532 nm) was coupled into the fiber with a 20X, 0.4 NA objective. (Power measurements before and after a loose fiber showed 60% coupling efficiency.) Scattered light was collected at 14 degrees as defined by the fiber groove direction and then collimated by an OFR LMU-15X-NUV objective and focused onto a photodiode (DET110, Thorlabs) by a 10 cm singlet.
In subsequent implementations, this arrangement can be simplified by employing a fiber coupled diode laser on the illumination side and by directly aligning the collection fiber onto the face of the photodiode on the collection side, completely eliminating the need for external lenses and mounts.

Figure 4 shows the overlap of the sensitive regions for the illumination fiber with the detection fiber. To characterize the overlap, the fluid channel was filled with a fluorescent dye. Light was alternately coupled into the illumination fiber and the detection fiber, and the fluorescence was imaged using a CCD camera. A lineout of the fluorescence intensity at the center of the fluid channel shows the overlap of the two regions.

The analog signal was AC filtered to remove the large DC background and then amplified prior to being digitized (PCI-6251, National Instruments) at a rate of 200 kHz. The counter triggered at a set threshold value whenever passing cells deflected light into the collection fiber generating a positive voltage signal.

Figure 4. Overlap of the illumination region with the anticipated detection region was determined by filling the fluid channel with a fluorescent dye and then imaging the fluorescence. Lineouts along the center of the fluid channel (parallel to the x axis) show the overlap of the illumination region (A) with the detection region (B).

HeLa cells adapted for growth in suspension were used at a concentration of $2.5 \times 10^4$ cells/mL in Hank's Balanced Salts buffered to pH 7.4 with 20 mM HEPES (HBBSS) (Sigma Aldrich) with 1% Bovine Serum Albumin (BSA) to reduce sticking and 16% OptiPrep density gradient medium to prevent cell settling. The HeLa cell solution was placed in a pressurized reservoir and delivered to the microfluidic through tubing coupled to the device by Nanoport connectors (Upchurch Scientific). Applied pressures of 1.3 psi and 2.8 psi provided flow rates of 7.3 $\mu$L/min and 14.3 $\mu$L/min, respectively. Different aliquots of HeLa cells were run through the microfluidic device and counted independently by a commercial fluorescence-activated cell sorter (MoFlo FACS system, DakoCytomation) to test the accuracy of our microfluidic cell counter. FACS detection parameters were preset for our HeLa cells by calibration with a test sample of HeLa cells. The FACS measurement recorded the forward scatter and side scatter intensities for each cell and a total cell count.

3. Results and discussion

The average HeLa cell generated a 750 mV spike on top of +/-20 mV noise. Figure 5 shows a representative signal trace. The baseline was centered at zero by virtue of the filter. To account for intensity variation between cells, the trigger threshold was lowered to 150 mV. For an applied pressure of 1.3 psi, the average signal width was 0.7 ms, providing an estimate of the average velocity of several centimeters per second. Cells were counted in ~200 $\mu$L aliquots of ~5000 cells each and then re-counted by the FACS system for comparison. For each sample, the counts from the two methods differed by less than 5% of the total cell count.

Fig. 5. Sample data set showing the peaks from light scattered by passing HeLa cells.
The counting efficiency of the device decreased at high flow rates. At ten cells per second, the average spike amplitude and width decreased to 400 mV and 0.4 ms, which is a result of the simple integration detection scheme. Under these conditions, we counted fewer cells per sample than with FACS. The agreement with FACS decreased further when the trigger threshold was raised from 150 mV to 200 mV. The parameters and total cell count for each aliquot are provided in Table 1. The reduced efficiency at the higher flow rate and trigger threshold was possibly due to some cells passing undetected below the trigger threshold. It should be noted that cells were distributed randomly throughout the channel. One solution to increase our system's flexibility, and indeed the throughput, may be to focus the cells into a narrow streamline for more regular spacing and more uniform excitation and collection efficiency. In addition, by confining the cells to one particular region of the channel it will be possible to obtain forward and side scattering intensities that can be used to obtain comprehensive flow cytometric data on cells in an integrated, compact platform.

### Table 1

The conditions for obtaining a cell count on each aliquot are given along with the total cell count obtained with our system and with the FACS system and the percent agreement between these two counts.

<table>
<thead>
<tr>
<th>Aliquot</th>
<th>Flow rate (µL min⁻¹)</th>
<th>Time (min)</th>
<th>Trigger (mV)</th>
<th>Cell count (our system)</th>
<th>Cell count (FACS)</th>
<th>Percent agreement (%)</th>
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<tr>
<td>1</td>
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<tr>
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<td>18.8</td>
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</tr>
</tbody>
</table>

### 4. Conclusion

The development of glass microfluidics is essential for overcoming the chemical, thermal and optical limitations of PDMS. Femtosecond laser ablation is more flexible for producing features in different planes and with higher aspect ratios than competing microscale glass technologies. We have shown that embedded fiber optics in femtosecond laser ablated microfluidic devices with anodic bonding streamlines device fabrication and overall system operation, removes the need for complicated glass/glass bonding and reduces the risk of damage to fiber tips by placing the fibers in the fiber grooves prior to bonding. The efficiency of our fiber optic delivery and detection for scattering measurements from HeLa cells was rigorously tested against a commercial FACS system. Sufficient signal was achieved with even less power than for previously reported particle scattering measurements and was sensitive enough to be detected by an inexpensive photodiode rather than by an avalanche photodiode or PMT. Embedded optical illumination and detection stabilizes the interrogation region and reduces the size and complexity of microfluidic flow cytometry systems. Embedded optical delivery is a key element for realizing an inexpensive and portable analysis tool for a range of applications including point-of-care clinical diagnostics, instruments for medical research and environmental sensors.

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