Three-Dimensional Flow-Focusing in Microfluidic Devices

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Introduction

Over the recent years, an interest in hand-held devices applicable in forensics and biological applications which need fast and efficient data analysis has increased through the research of lab-on-a-chip systems and bead-based detection technology [1]. In this research, three-dimensional hydrodynamic flow-focusing has been obtained in a two layer polydimethylsiloxane (PDMS) microfluidic device. Using sheath flow, this device employs a microfluidic manifold to focus fluorescent particles in three dimensions ensuring that the particles will be confined to the focal volume of the optical fibers embedded into the deoxyribonucleic acid (DNA) analysis microchip. The flow-focusing performance of this device has been characterized as a prelude to its integration into a hand-held particle-counter microchip. The geometry of the inputs and liquids of different viscosities were tested in order to achieve the most effectively focused stream. Through laser scanning confocal microscopy, the focused stream, using polyvinyl alcohol as a sheath fluid, was found to have a width of about 15 μm and a height of about 25 μm. In addition, successful calcium cross linking of sodium alginate microfibers was seen in this device with confocal microscopy through the diffusion of sheath fluid into the focused stream, within a channel of 100 μm by 100 μm cross section.

Experimental Procedure

The microfluidic device was constructed through two layer soft lithography [2]. Channels of 100 μm depth and 100 μm width were fabricated by spinning SU-8 100 photoresist onto blank silicon wafers at 2900 rpms for 35s. The distance between the inlets in the patterns did not affect the focused stream as much as the dimensions of the actual channels. After spinning the resist on the silicon wafers, the wafers were pre-baked for 10 min at 65°C and then soft-baked for 30 min at 95°C. The wafers were exposed to ultraviolet light through a patterned chrome mask at 600 mJ/cm^2 for 8s, three times. The exposed wafers were then baked for 10 min at 95°C [3]. After developing the wafer, polydimethylsiloxane (PDMS) was poured on the patterned silicon wafers and cured in an oven for 2 hr at 60°C. After curing the PDMS, the two layers of PDMS were placed into an oxygen plasma asher and then aligned under a contact aligner. Polyethylene tubing was attached to the inlets and outlets of the flow cell with epoxy. A final product of a flow cell, seen in Figure 1, shows the flow cell before being characterized.

Different geometries and different solutions of various viscosities, such as ethylene glycol, glycerol, Tris-HCL with EDTA (TE buffer), and polyvinyl alcohol were tested as sheath fluid and sample flow solutions to see the size and location of the focused stream with the fluorescent beads. The rate of the syringe pump was also considered. The velocity of the beads on the focused stream was also calculated to see how fast the beads could be detected. The focused stream was characterized under a Leica confocal microscope.

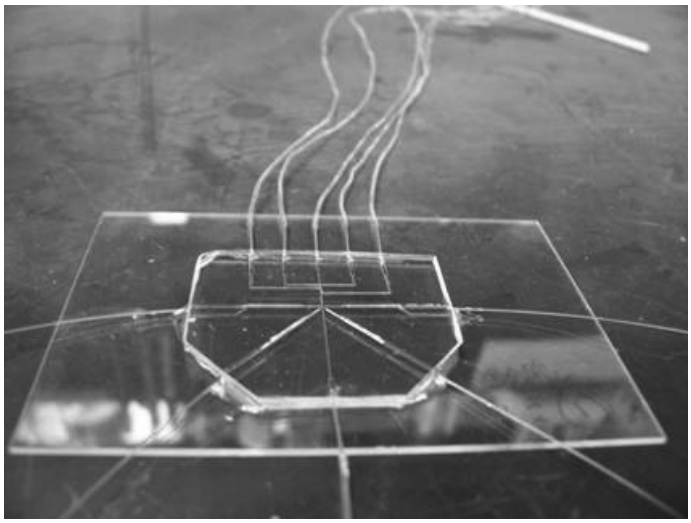

Figure 1: Assembled flow cell with channels that have a cross section of 100 µm by 100 µm.

Results and Conclusions

The most effective pattern was designed with a symmetrical setting with the sheath fluid channels joining the central channel from both sides and from the top and shortly after, sheath fluid would connect from both sides and the bottom as shown in Figure 2. The dimensions of the side sheath fluid channels were 50 μm wide, while the central channel was 100 μm wide. TE buffer

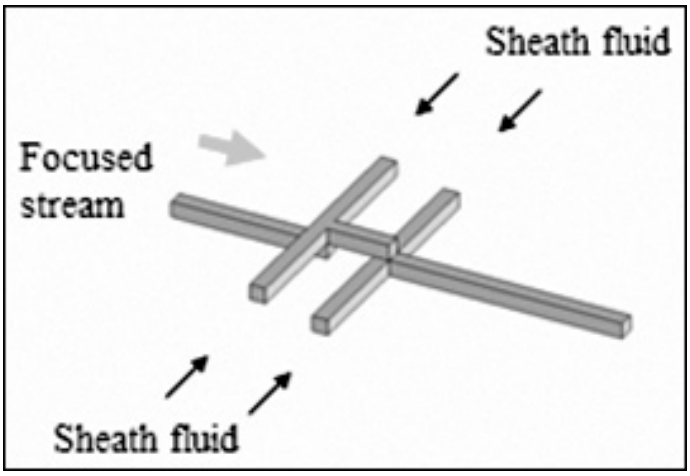

Figure 2: Schematic drawing of flow cell with 50 μm wide central channel and 100 μm wide sheath fluid channels.

(with 80% glycerol to increase its viscosity) was used to carry the sample and flowed at 3 μl/min. (TE buffer is commonly used as a buffer for DNA.) Polyvinyl alcohol was pumped as sheath fluid at 13 μl/min. This version provided a focused stream with a width of about 15 μm and a height of about 25 μm in the center of the central channel as shown in Figure 3. A more circular focused stream was characterized because the side channels did not provide high pressure in the central channel which would cause the focused stream to be more oblong.

The velocity of 5 μm-in-diameter fluorescent beads was about 10 cm/s ± 2 cm/s in the focused stream. The velocity of the beads was calculated based on 20 streak-length measurements using pixel size and the rate at which the images were scanned. Also, due to the diffusion caused in the central channel in the flow cells designed, alginate microfibers were fabricated using sodium alginate in the sample flow and calcium chloride as sheath fluid. The sample flow was pumped at 1 μl/min and the sheath fluid was pumped at 333 μl/min [4].

Future Work

Particles need to be analyzed to test the usability of the microfluidic device, by attaching the optical fibers in the microchip. Some work was done during this research to create bioluminescent microfibers using Renilla Luciferase in the sample flow and adding coelenterazine substrate to the microfibers after making them in the flow cell. However, the glow was not able to be seen.

Acknowledgements

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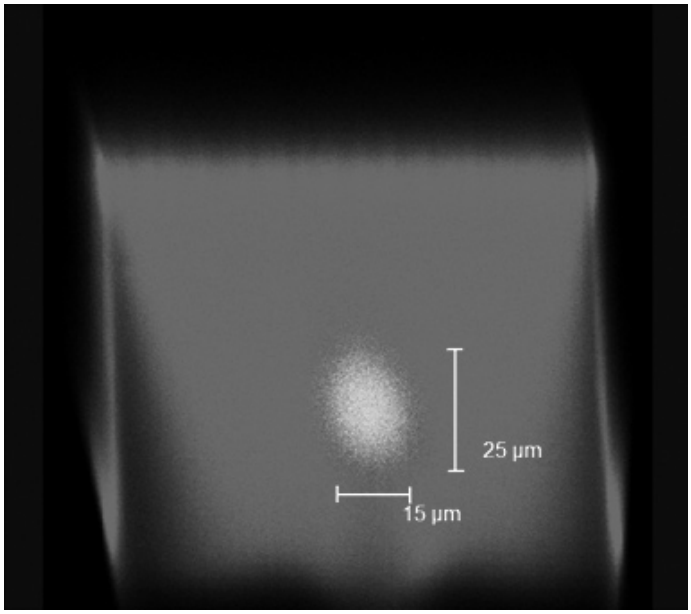

Figure 3: Cross-section view of focused stream under confocal microscope. The focused stream had a 25 μm height and a 15 μm width.