Miniaturized 3D hydrodynamic focusing for flow cytometry

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This paper deals with design and fabrication of a simple, 3D hydrodynamic focusing device for microfluidic cytometry. The microfabricated device was constructed using glass, UV adhesives, and hypodermic tubing. With glass surrounding all side of the sample stream, the device should have excellent optical qualities for measurements from different angles. The coefficient of variation of the integrals of fluorescent intensities from 10 μm beads was 7.07%. The device exhibited stable 3D focusing over a range of flow rates. Our new design provides a simple method to advance microfluidic flow cytometry.

Introduction

In this work, we present a simple, inexpensive method to fabricate a true 3D microflow cytometer using glass, UV adhesive and hypodermic tubing. Flow cytometry is one of the most powerful tools in biology as it is capable of analyzing cells in a high-throughput manner. Flow cytometers are used in a wide range of both research and clinical applications: analysis of vaccine responses, hematology, quantification of phagocytosis, aquatic ecology, and the diagnosis of leukemia among others. Despite being a powerful tool in biological studies, size and cost remain barriers to broad accessibility.

Microfluidics-based cytometry can improve accessibility by reducing the fabrication cost and overall size. The first microflow cytometers used 2D focusing with two sheath flows. These 2D-focused systems have been used for studying a wide variety of specimens including algal cells, particles, E. coli, and fluorescent proteins, but have failed to achieve the type of performance achieved in full-sized flow cytometers. Particle focusing is a major factor in microfluidic cytometers lagging conventional machines in performance, because most use 2D focusing. Focusing particles in 3D has been shown to reduce CVs compared to a similar 2D focusing device. This effect is understood and predicted by Poiseuille flow estimations.

Materials and Methods

The proposed microfluidic device was fabricated using glass and UV adhesive, and then characterized using fluorescent 10 μm polystyrene beads to determine the coefficient of variation (CV).

(i) Spin coat NOA 81
(ii) Align Glass spacers
(iii) Cover with glass & cure
(iv) Flip
(v) Roll NOA 81 on spacers
(vi) Cover with glass & cure
(vii) Cut to final chip dimensions
(viii) Connect inlets
(ix) Final chip

Other microfluidics-based 3D hydrofocusing techniques have utilized a large perpendicular sheath flow, a lifting inlet, sequential microweirs, microfluidic drifting from Dean flow effects and a 3D nozzle. Many of these techniques require complicated fabrication steps or produce irregularly focused streams. A simple method for 3D focusing is needed to create affordable devices that can achieve results comparable to commercial flow cytometers. To this end, we developed a microfluidic chip that uses a sample tube with surrounding coaxial sheathing to provide 3D hydro-focusing. A similar technique has been used to create a microfluidic mixer with an inner capillary to introduce sample flow, while another design uses a “chimney” structure to create a 3D focus in a fluorescent-activated cell sorter. Our method relies on hypodermic tubing fitted inside of glass channels.

Fig. 1 (a) Top view of 2D hydrodynamic focusing using two intersecting sheath flows (b) Side view of 2D hydrodynamic focusing with velocity profile of a laminar flow and particles free to be anywhere along z-axis.

Fig. 2 Illustration of the fabrication procedure (i) Spin coat UV adhesive on each glass spacer (ii) Align glass spacers using shim (iii) Cover with 1mm thick glass and expose to 2J/cm² UV light (iv) Flip so that spacers face up (v) Roll UV adhesive selectively onto spacers (vi) Cover with No. 2 cover glass and cure with 2J/cm² UV light (vii) Cut to final dimensions with diamond tipped saw (viii) Connect luer lock needles for inlets (ix) The final chip

We adapted and developed two existing protocols to fabricate a glass chip (Fig. 1) with excellent optical properties using an UV adhesive. Four ~500 μm thick glass spacer pieces (Valley Design Corp.) were aligned and bonded with the UV adhesive NOA 81 (Norland Products) to a 1 mm thick glass substrate to...
form the basis for our channels. Adhesive was kept out of the channel area by spin coating NOA 81 on each spacer (3000 rpm for 20s). Alignment relied on .020" (508 µm) shim to space the channels and achieve ~500x500 µm channels. Alignment was checked using a 10X magnifying loupe. Vacuum chucks held the glass pieces in place while a 24x30x1mm glass substrate was placed on top, then exposed to 23/cm² of 365nm UV light (ELC 700 Electro-lite Corp.). The unit was flipped and a 24x30 mm No. 2 cover glass (Fisherbrand) was bonded using a roller method (Fig. 1v) to selectively apply UV adhesive to the four channel spacers and cured to close the chip (23/cm² at 365nm).

Sheathing was achieved using a coaxial 32 gauge hypodermic tube (230 µm OD and 110 µm ID) inside of the channel with sheath flow that surrounds it. The tube was bonded inside of a 26 gauge needle that fits tightly inside the channel and is bonded into the chip so that the tube protruded a few mm beyond the point where sheath flow is introduced (Fig. 2a). 26 gauge needles were also used for connecting the sheath flow and outlet (Fig. 1vii). Laminar flow allowed for the focusing flow to completely ensheath and focus the sample stream without mixing.

10 µm beads (Flow-Check fluorospheres, Beckman Coulter) provided a check for variation due to the system. Beads were diluted in fresh water and introduced through the sample tube so that they were hydro-dynamically focused within the microfluidic chip. A forward scatter signal generated by a passing bead was detected using a using a 785 nm diode laser focused on the sample stream. An obscuration bar placed after transmission blocked this focused beam but permitted light diffracted by a passing cell to be detected with an AC-coupled Si photodiode.

Fluorescence measurements were made by exciting with 100 µs laser pulses from a 470 nm LED (Phillips Luxeon III Star 20 LXHL-LB3C; luminous flux 23 lm) and collecting fluorescence with a photomultiplier tube (PMT). Laser pulses were triggered off of forward scatter signals to detect beads. Forward scatter was detected using an obscuration bar placed after transmission through the microfluidic chip and collecting light with an AC-coupled Si photodiode. Both signals were collected using a custom Labview program (Fig. 3) to control collection parameters and output data. For alignment and focusing observation, a removable mirror directed the channel image to a CMOS camera (Marlin F131B, Allied Vision Technologies) using illumination from the FS laser diode.

Fig. 3 The front panel of the Labview data collection software used to analyze FS and FL CVs were calculated by dividing the standard deviation of fluorescence by its mean to normalize it to a dimensionless quantity. Chauvenet’s criterion was used to eliminate the calibration bead doublets from the datasets (7 of 150 data points excluded).

**Results and Discussion**

We successfully fabricated a 3D hydro-focusing prototype using our simple design (Fig. 4). The device is cheap to manufacture and durable as it is primarily made of glass and hypodermic tubing.

![Fig. 4](image-url) (a) Illustration of our microfluidic design layout (b) Image of a finished prototype

3D hydro-focusing was observed (Fig. 5) and studied using fluorescent polysterene beads. 3D hydro-focusing remained stable over a wide range of pressure ratios and sample stream sizes ranging from 35-100 µm. Since focusing depends mainly on the flow difference between the sample and sheath flow, the same focusing can be achieved at different overall flow rates. This gives the advantage of also tuning the speed of passing cells for each experiment. Experimental results indicate a CV of 7.07% for FL signals averaged over 45 µs, which puts the performance of this device near the capability of commercial cytometers and better than many reported 3D microfluidic cytometers.
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Fig. 5 Optical image of a 3D focused stream. The sample stream is filtered water while the sheath flow is saltwater to visualize focusing.

Fig. 6 shows a histogram of the beads’ fluorescence and their tight distribution. A sample stream that is focused to a defined core (Fig. 5) allows for improved data as the core becomes smaller because of more uniform particle velocity and the ability to precisely know the particle position for excitation beam alignment. This work establishes our 3D microfluidic cytometer as a viable solution to the need for smaller, cheaper flow cytometers. Future work will use the established protocol to create a chip with smaller channels and sample tube to achieve smaller, more stable sample streams.

Conclusions

In summary, we have successfully fabricated and tested a 3D microflow cytometry chip. Our simple device fabrication consists of glass pieces being bonded together using the UV adhesive NOA 81, hypodermic needles as inlet connections, and a hypodermic tube as the coaxial sample inlet. We tested the device using the fluorescence measurements of fluorescent polystyrene beads to find a CV of 7.07%. This study demonstrates proof-of-concept capability for our 3D focusing method to improve microfluidic cytometry.

Notes and references

These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

5. J. M. Peters and M. Q. Ansari, Archives of Pathology & Laboratory Medicine, 2011, 135, 44-54.