HIGH THROUGHPUT FLUORESCENCE BASED FLOW CYTOMETER USING 3D MICROFLUIDICS FOR PARALLEL SHEATH FLOW FOCUSING AND EMBEDED HIGH N.A. MICROLENS

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ABSTRACT

We report a high throughput microfluidic fluorescence based flow cytometer with parallel sheath flow focusing in 32 channels using only 2 inlets. A detection throughput of 7000 beads/sec in each channel and 224,000 beads/sec for 32 channels has been accomplished. This fluorescence flow cytometer is realized by utilizing 3D soft lithography microfabrication technique to achieve multilayer PDMS structures with vertical vias connecting fluid in different layers. An array of high N.A. microball lens is also embedded in the structure to achieve parallel and high sensitivity fluorescence detection across these 32 channels.

KEYWORDS

Microlens, flow cytometry, 3D microfluidics, FACS.

INTRODUCTION

Fluorescence based flow cytometry is the gold standard for high throughput single cell analysis[1]. Commercial aerosol based flow cytometers require 3D sheath flow focusing to accurately position cells in the detection zone. It is difficult to parallelize this type of system for high throughput detection. Microfluidic based flow cytometers show the potential for parallelization since a large number of channels can be fabricated on the same chip. Using microlens array such as Fresnel lens array [2] or a high N.A. microball lens array [3], the trade-off between high sensitivity fluorescence detection and large area detection can be overcome. Another major challenge in parallel detection, however, is the difficulty of achieving parallel and uniform sheath flow focusing across many channels without facing the interconnect issues using 2D microfluidics circuits.

Here, we report a high throughput fluorescence based flow cytometer by integrating a high N.A. microball lens array with 32 parallel sample channels and 64 sheath flow channels at two different layers connected by 64 through-layer vias. Only two inlets are required, one for introducing cell samples into all 32 sample channels and the other one for delivering fluid into the 64 sheath flow channels (Fig. 1).

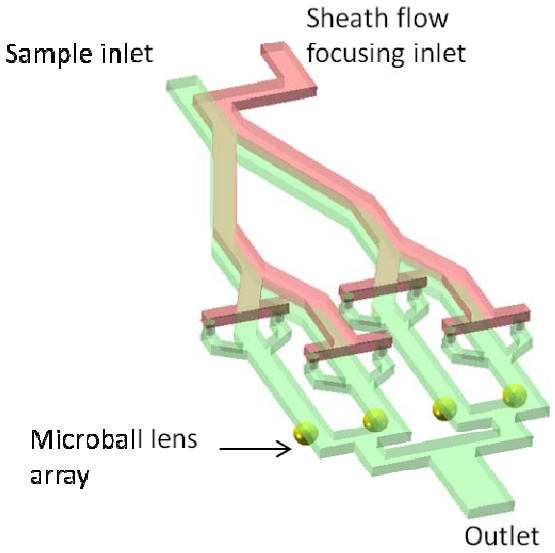

Figure 1. Schematic of 3D microfluidic channels integrating with microball lens array for high throughput fluorescence flow cytometry. Parallel 2D sheath flow focusing of 32 channels uses only two inlets, one for sample introduction and the other one for sheath flows.

EXPERIMENT

A standard photolithography process is used to fabricate an array of PDMS-based linear microwells. Glass microspheres (doped with titanium and barium) with a refractive index of 2.1 and diameter of 75 ±2 μm (XL Sci-tech Inc., USA) were used for microlens fabrication. Dry glass microspheres were spread on the surface of PDMS microwell array and swept into the pre-patterned wells. The assembled array is then baked at 100 °C for 5 minutes to dry the water trapped in wells and followed by pouring PDMS mixed with curing agent on it. This structure is then clamped by two planar solid poly(methyl methacrylate) (PMMA) surfaces and baked until all

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PDMS is fully cured. A microball lens array fully embedded in PDMS is obtained after releasing the clamp. The 3D microfluidic channel device is fabricated following the thin film PDMS process developed by Zhang et al. [4]. A piece of PDMS (about 2mm thick) treated with trichloro (1H,1H,2H,2H-perfluorooctyl)silane is used as a stamp to peel off thin film PDMS a SU-8 mold. This thin film PDMS is then transferred to either a glass or a PDMS substrate whose surface is oxygen plasma treated. Because of the trichloro (1H,1H,2H,2H-perfluorooctyl)silane surface treatment that lowers the adhesion force between the thin film PDMS and the PDMS stamp, thin film PDMS can stay on the oxygen plasma treated surface that forms strong covalent bonding. By repeating the steps mentioned above, multilayer thin film PDMS with through layer vias can be stacked to form 3D microfluidic structure as shown in Figure 2.

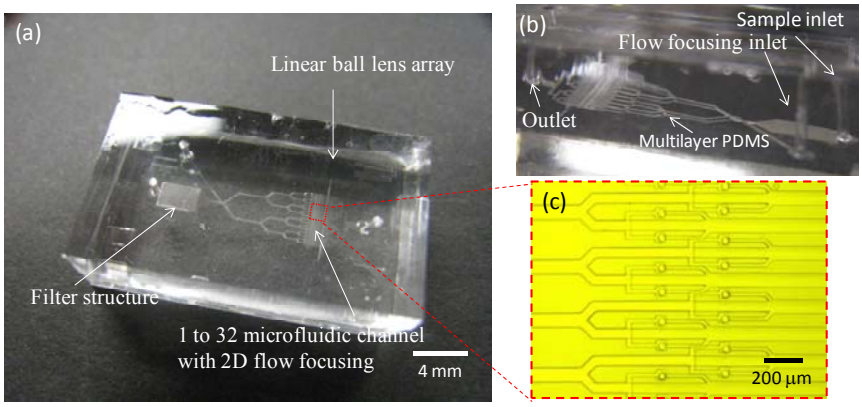

Figure 2. Images of a fabricated 3D microfluidic device for parallel high throughput flow cytometry

Figure 3 shows the optical system for large area and high sensitivity fluorescence detection using an embedded microball lens array. A light beam of 1W laser with wavelength at 445 nm is expanded and focused by a cylindrical lens on the microball lens array for fluorescence excitation. The emission light of detected samples are collected by the same ball lens array and projected onto a high speed CCD camera through a relay optics consisting of a pair of low N.A. convex lenses arranged in a telescope configuration to provide a 1:1 mapping between the ball lens and the camera.

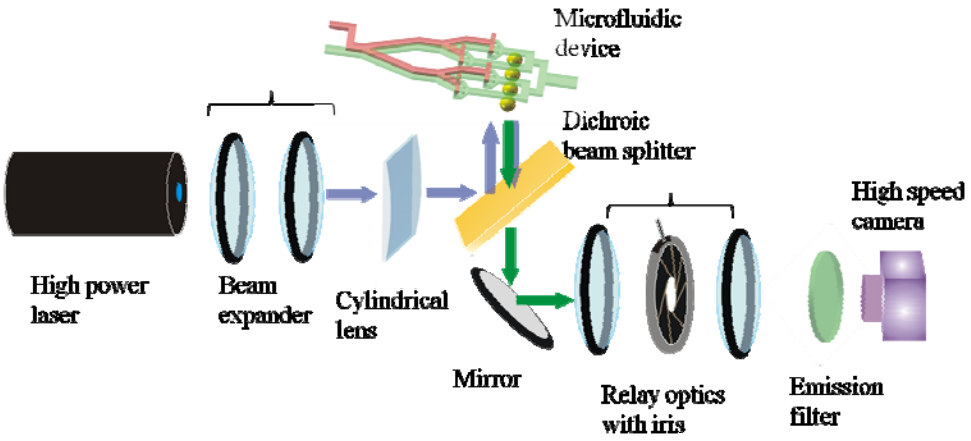

Figure 3. Schematic of the optical setup for parallel fluorescence detection. A high power 1W laser with a wavelength at 455 nm is used for fluorescence excitation. The light beam is expanded and focused by a cylindrical lens on the microball lens array for parallel excitation. The emitted signals are collected through the same lens array and projected onto a high speed camera for detection.

Figure 4 shows the focusing power of the embedded microball lens array. In this test, the microball lens is used only for focusing the excitation light. The fluorescence image is captured by a separate fluorescence microscope to check the size of the focused light spots. The full width half maximum (FWHM) spot size is 2.5 µm.

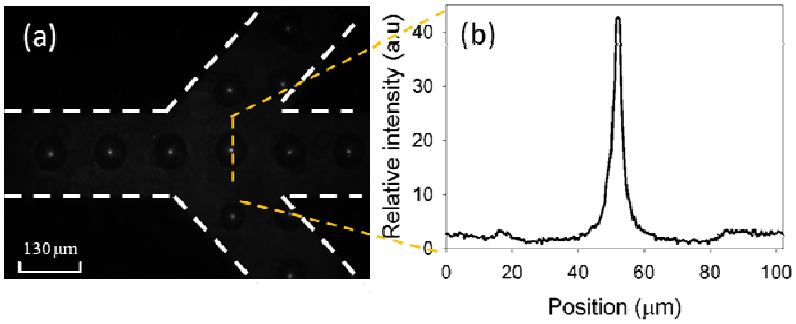

Figure 4. Test of the focusing power of embedded microball lenses. A 2.5 μm FWHM spot is obtained

Figure 5 shows the normalized fluorescence signals of 10 m fluorescent beads flowing across two of the 32 microfluidic detection channels. The highest throughput currently achieved is 7,000 beads/sec per channel and 224,000 beads/sec per device.

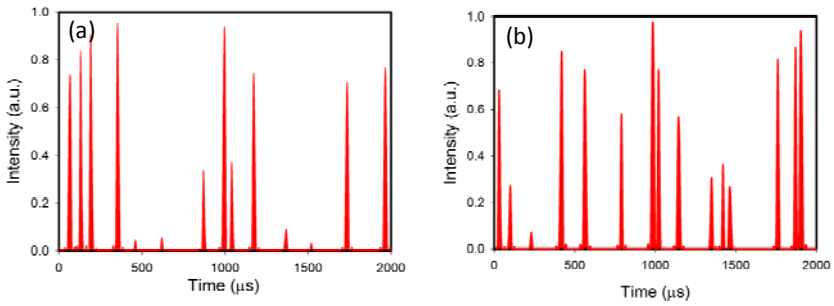

Figure 5. Normalized dynamic fluorescence signals from beads passing through two of the detection channels. The highest detection speed of 7,000 bead/sec per channel has been achieved. The overall throughput of our system is 224,000 beads/sec.

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