SIMPLIFIED MONOLITHIC FLOW CYTOMETER CHIP WITH THREE-DIMENSIONAL HYDRODYANMIC FOCUSING AND INTEGRATED FIBER-FREE OPTICS

Masahiro Motosuke[1, 2*] Thomas Glasdam Jensen[2], Guisheng Zhuang[2] and Jörg P. Kutter[2]
[1]*Tokyo University of Science, JAPAN, and*
[2]*Technical University of Denmark, DENMARK*

ABSTRACT

A miniaturized flow cytometry incorporating both fluidic and optical systems has a great possibility for portable biochemical sensing or point-of-care diagnostics. This paper presents a simple microfluidic flow cytometer combining reliable 3D hydrodynamic focusing and optical detection without optical fibers in a monolithic architecture fabricated by a single photolithographic process. The vertical flow focusing is achieved by the optimized inlet geometry in a PDMS lid onto the substrate with detection channel and integrated optics. The simplified approach indicates the possibility to be applied as a portable platform of cytometer chip.

KEYWORDS: Flow cytometry, Hydrodynamic focusing, Integrated optics, Particle detection

INTRODUCTION

Flow cytometry, a measurement system of various physicochemical characteristics of biomolecules or particles, has become a standard analytical method in cell biology and medicine. A miniaturization of the instrument incorporating both fluidic and optical systems would expand the application fields toward point-of-care diagnostics and in-field environmental monitoring [1]. Efficient sample focusing, which allows a sample stream to flow surrounded by a sheath stream, is essential to ensure the quality and reliability of collected signals in flow cytometry. Most of the reported devices enabling 3D confinement, e.g., by using two-layered lamination [2, 3] or chevron-shaped patterns inside the channel [4], still need cumbersome fabrication processes using multiple photomasks and exposures. The devices also need to be coupled with optical fibers for the incident light and the signal detection [5]. The process requires sensitive handling and results in a fragile device, which is not preferred from a practical point of view, when they are to be used by non-specialists of microdevices such as medical doctors. Here, a novel cytometer chip which enables both 3D hydrodynamic focusing and fiber-free detection within a monolithic structure is presented.

THEORY

In our approach, the vertical focusing can be achieved by a "direct connection" from inlets to the main channel, as shown in Figure 1. The sample flow from a vertical inlet *b* is focused into a narrow stream by two sheath flows from inlets *a* and *c*, and then focused horizontally by the flows from *d*. This concept of 3D hydrodynamic focusing is similar to two-layered ones as in our previous devices [6], but it is a more simplified approach because it only requires standard inlets instead of an additional fluidic network in the top layer. The present device only relies on a single photolithographic process creating a main channel with horizontal focusing. The inlets *b* and *c* used for the vertical focusing are embedded in a PDMS lid sealing the channel. Figure 2 illustrates a CFD simulation of the focusing under different inlet/channel geometries. Sufficient sample focusing without distortion is obtained when the dimensions of the vertical inlets are within a certain range, such that the ratio of the inlet diameter to the width of the main channel is between 1 and 2. A secondary flow that disturbs the focusing occurs around the inlet *c* if the inlet dimensions are beyond that range.

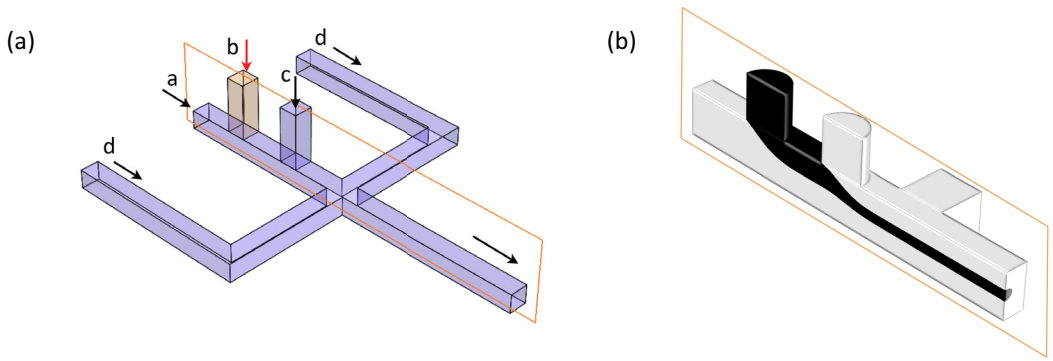

Figure 1: (a) Schematic image of a "direct connection" three-dimensional hydrodynamic focusing used for a microfluidic cytometry chip. Inlets b and c directly act as channels for vertical focusing. Monolithic channels d are for horizontal focusing. (b) Cross-sectional view of simulation of the 3D focusing. Sample solution is focused vertically and horizontally.

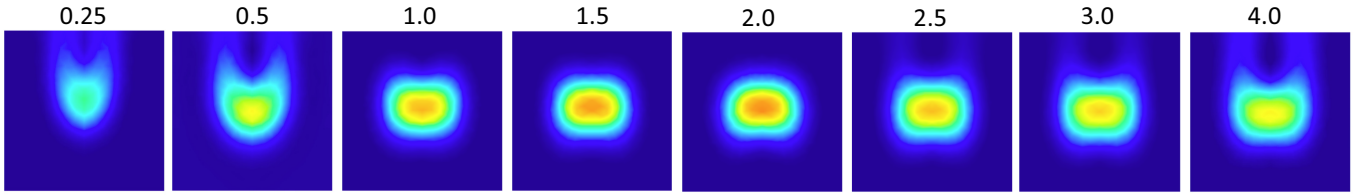

Figure 2: CFD simulation results of cross-sectional profiles of the resultant focused flow in the main channel with different inlet sizes (ratio of inlet diameter to the width of the main channel is shown). Undisturbed hydrodynamic focusing is achieved with ratios between 1 and 2.

As for the optics, on-chip lenses and mirrors for the incident laser beam and the outgoing scattered light are implemented in an SU-8 layer, thus allowing fiber-free detection in a monolithic structure. The PDMS lid placed on top of the substrate works as cladding for the planar waveguide because of a lower refractive index than SU-8. The lenses have non-spherical shape designed by an iterative optimization based on ray-tracing to minimize the aberration [7]. Since the layer works as a large planar waveguide as well as containing the main fluidic channel, the device can be fabricated in a single photolithographic step.

EXPERIMENTAL

For the validation of our simplified approach on 3D hydrodynamic focusing, flow visualization was performed using a PMMA-based prototype made by micromilling and UV-activated bonding. The main channel was 400 × 300 µm and the vertical inlets had a diameter of 500 µm. One side of the channel wall was made as thin as 200 µm to allow observation via a tiny mirror prism inserted in the optical path of the objective lens.

The cytometer chip, schematically illustrated in Figure 3, is comprised of an SU-8 layer on a glass substrate for the optics and the main channel with the horizontal focusing, and a PDMS lid for sealing of the channel, providing vertical focusing inlets and cladding of the planar waveguide. The dimensions of the main channel and vertical inlets are 100 × 75 µm and 200 µm, respectively. The incident laser beam with a wavelength of 635 nm and 5mW of power is focused by an off-chip cylindrical lens coupling to the SU-8 planar waveguide, and then focused by the on-chip lens resulting in a the beam width of approximately 15 µm (measured value). The scattered signal from the interrogation site is eventually detected by a Si photodiode after another pair of on-chip and off-chip cylindrical lenses.

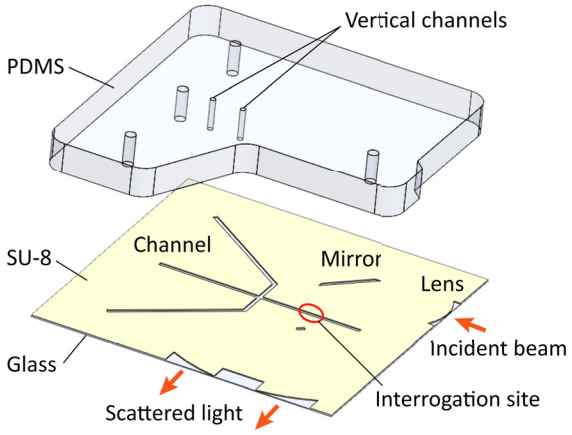

Figure 3: Schematic of the flow cytometer chip. Top PDMS layer includes vertical focusing channels. Bottom SU-8 layer has the fluidic channel and integrated optics.

RESULTS AND DISCUSSION

Figure 4 shows the flow visualization results from the PMMA chip. 1 mM of Rhodamine 6G aqueous solution was seeded from the sample inlet. A comparison of the two-cross-sections with and without focusing both in horizontal and vertical directions clearly indicates that 3D hydrodynamic focusing is attained in the device.

Figure 5 indicates the forward scattering signal of the cytometer chip obtained from non-fluorescent polystyrene particles with a diameter of 2 µm. The total flow rate in the main channel was 15 µL/min. The signal-to-noise ratio is comparable to that from our previous device with optical fibers and a PMT [6]. Consequently, the device demonstrates the applicability of the simplified monolithic structure and fiber-free optics toward cost-effective and disposable devices.

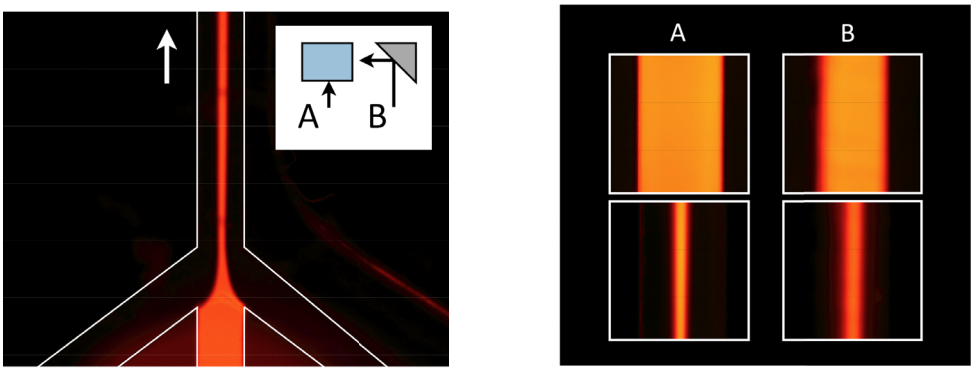

Figure 3: Fluorescent images of horizontal (A) and vertical (B) cross-sections in a PMMA prototype chip. Focused (lower panels) and unfocused images (upper panels) are shown for comparison.

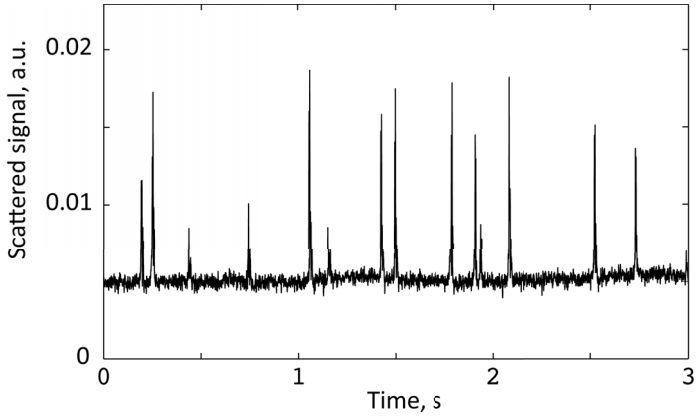

Figure 5: Forward scattering signal from 2 μm polystyrene particles detected by the simplified monolithic cytometry chip.

CONCLUSION

A simplified monolithic flow cytometer chip enabling three-dimensional hydrodynamic focusing and optical detection without fibers has been developed. Our approach only requires a single photolithographic process to fabricate the substrate with integrated optics and a PDMS lid with inlets having optimized geometry. Experimental results support the validity of the cytometer chip to be applied for portable biochemical sensing or point-of-care diagnostics.

REFERENCES

[1] D. A. Ateya, J. S. Erickson, P. B. Howell Jr, L. R. Hilliard, J. P. Golden, F. S. Ligler, "The good, the bad, and the tiny: a review of microflow cytometry," *Anal. Bioanal. Chem.*, vol. 391, pp. 1485-1898, 2008.

[2] C. C. Chang, Z. X. Huang and R. J. Yang, "Three-dimensional hydrodynamic focusing in two-layer polydimethylsiloxane (PDMS) microchannels," *J. Micromech. Microeng.*, vol. 17, pp. 1479-1486, 2007.

[3] M. J. Kennedy, S. J. Stelick, S. L. Perkins, L. Cao, C. A. Batt, "Hydrodynamic focusing with a microlithographic manifold: controlling the vertical position of a focused sample," *Microfluid. Nanofluid.*, vol. 7, pp. 569-578, 2009.

[4] J. P. Golden, J. S. Kim, J. S. Erickson, L. R. Hilliard, P. B. Howell, G. P. Anderson, M. Nasir, and F. S. Ligler, "Multi-wavelength microflow cytometer using groove-generated sheath flow," *Lab Chip*, vol. 9, pp. 1942-1950, 2009.

[5] Y. C. Tung, M. Zhang, C. T. Lin, K. Kurabayashi, S. J. Skerlos, "PDMS-based opto-fluidic micro flow cytometer with two-color, multi-angle fluorescence detection capability using PIN photodiodes," *Sensors Actuators B*, vol. 98, pp. 356-367, 2004.

[6] G. Zhuang, T. G. Jensen, J. P. Kutter, "Three-dimensional hydrodynamic focusing over a wide Reynolds number range using a two-layer microfluidic design," *Proc. μTAS*, pp. 1357-1359, 2008.

[7] T. G. Jensen, L. B. Nielsen, and J. P. Kutter, "Fiber-free coupling between bulk laser beams and on-chip polymer-based multimode waveguides," *Electrophoresis*, vol. 32, pp. 1224-1232, 2011.

CONTACT

*Masahiro Motosuke, tel: +81-3-5228-8361; mot@rs.tus.ac.jp