3D PULSED LASER TRIGGERED HIGH SPEED MICROFLUIDIC FLUORESCENCE ACTIVATED CELL SORTER

Yue Chen, Ting-Hsiang Wu, Yu-Chun Kung and Pei-Yu Chiou
University of California, Los Angeles, USA

ABSTRACT

We report a 3D PDMS microfluidic pulsed laser triggered fluorescence activated cell sorter for high purity and high throughput cell sorting. It is capable of sorting at a throughput of 10,000 beads/sec with 76% purity in a single channel. It is realized by exciting laser induced cavitation bubbles in a 3D PDMS microfluidic channel to create high speed liquid jets to deflect detected fluorescent particles. The ultrafast switching mechanism (20 μsec complete on-off cycle), small liquid jet perturbation volume, and the 3-dimensional sheath flow focusing for accurate timing control of fast (2 m/sec) passing particles are the three critical factors enabling high purity sorting at high throughput.

KEYWORDS

FACS, flow cytometer, pulsed laser, cavitation bubble, PDMS microfluidic channel, 3D sheath flow

INTRODUCTION

Various microfluidic FACS systems [1-3] have been demonstrated in the past decade aiming to provide a fully enclosed environment for sterile, contamination and infectious free sorting, and better downstream microfluidic integration for further analysis after sorting. The biggest challenge of μFACS systems, however, is the low sorting throughput and purity compared with commercial aerosol based FACS (90% purity at 20,000 cells/sec). Our group recently proposed a novel sorting mechanism called Pulse Laser Activated Cell Sorter (PLACS) aiming to bridge the gap of the throughput and purity between μFACS and aerosol FACS [4]. Our prior PLACS utilizes 2D sheath flow focusing and is able to achieve 90% sorting purity at 3000 cells/sec with high cell viability, but the sorting purity drops to 45% at 10,000 cells/sec due to the lack of the third dimension focusing. This creates synchronization issue between particle detection and switching at high speed flow and decreases the switching efficiency.

Here, we present a 3D PLACS (Fig. 1) that utilizes multilayer PDMS with vertical vias to achieve 3D sheath focusing. This not only solves the synchronization issue between detection and switching but also allows efficient particle switching using a smaller bubble and a shorter on-off switching cycle. 3D focusing solves the three critical parameters in high speed and high purity sorting, the synchronization timing, small perturbation volume, and short on-off switching cycle.

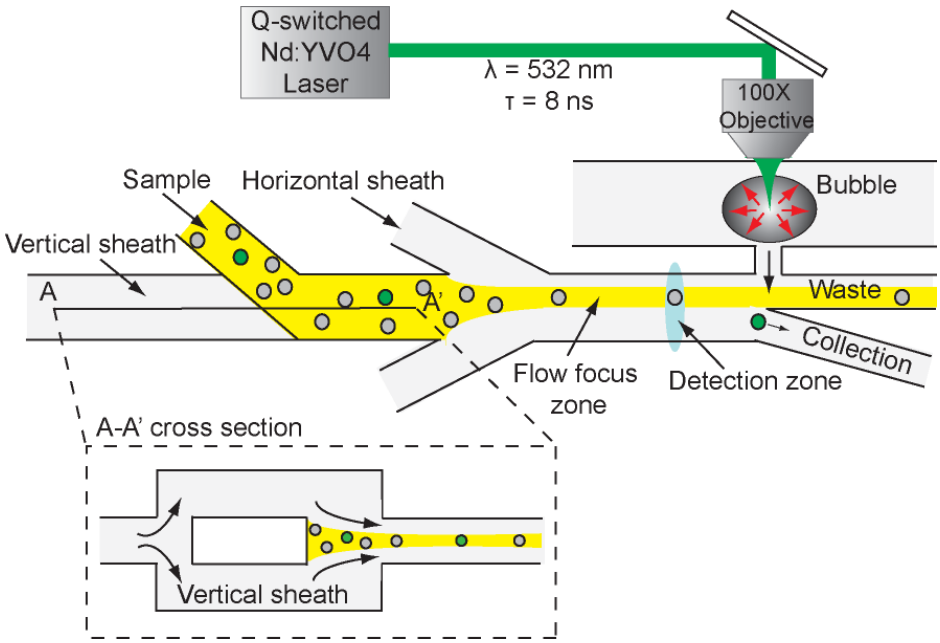

Fig. 1 Schematic of a 3D PLACS. 3D sheath flow focusing is achieved by multilayer PDMS structures with vertical vias connecting channels in different layers. Detected fluorescence particles are deflected into the collection channel by high-speed liquid jets induced by rapidly expanding laser cavitation bubbles to squeeze fluid across a micro nozzle.

EXPERIMENT

The device consists of a main channel with two outlets, collection and waste (Fig. 1). Utilizing thin film PDMS fabrication process, hydrodynamic flow focusing can be realized in 3D. Samples are focused into the waste channel initially. When fluorescent particles flow through the detection zone, as the fluorescence are collected by a 25X N.A. 0.4 objective lens, detected by a PMT (photodetector module P30CWAD5-01 SENS-TECH) and signal processed by a NI DAQ card, a laser pulse (Q-switched Nd:YVO4, 8 nsec in pulse width, 532 nm in wavelength) is triggered to

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

activate a bubble through a 100X N.A. 0.9 objective lens with a proper delay time.

Fig. 2b demonstrates a bubble with 160 μm in diameter creating a high-speed jet to deflect a fluorescence particle into the collection channel. At 5 μsec, the bubble expands to its largest size which induces a liquid jet through the nozzle and causes ~ 100 pL perturbation. With this perturbation, detected fluorescent particles are deflected into the collection channel (Fig. 2d). The bubble collapses and fully disappears within 20 μsec.

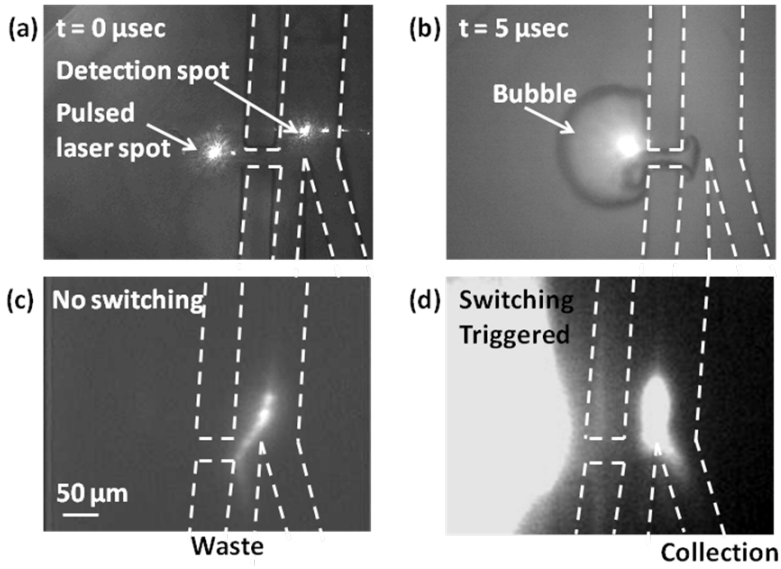

Fig. 2 Particle switching triggered by a focused laser pulse. (a) (b) Time-resolved images showing a jet created by a bubble. The maximum diameter of the bubble is 160 μm at 5 μsec delay; (c) fluorescent trace of a particle without switching and (d) of a particle when switching is triggered.

Laser pulse energy, nozzle design and the time delay to trigger the bubble are critical parameters in PLACS for high purity sorting. Fig. 3 presents the switching time window between detection and laser activation, and its relationship with pulsed laser energy and nozzle design. An optimal switching condition of using a 160 μm bubble excited by an 81 μJ laser pulse and with a nozzle 20 μm wide and 50 μm long has been used for sorting with laser trigger delay set to achieve the maximum switching efficiency. A switching efficiency of 97% and a small switching window ~25 μsec has been accomplished (Fig. 3b).

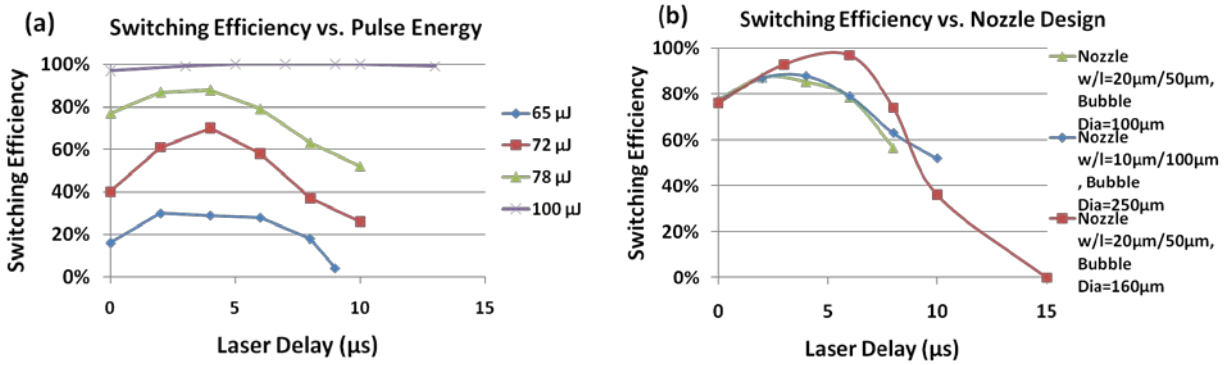

Fig. 3 (a) Switching efficiency at different pulse energy and delay time. Larger pulse energy generates a larger bubble for higher switching efficiency but also a larger switching window that could deflect nontarget particles into collection. (b) Optimization of the nozzle design helps increasing the switching efficiency yet narrowing the switching window using a smaller bubble for high purity sorting.

The sorting is performed using mixed polystyrene beads with initial green fluorescent beads purity of 2.3% and a final concentration of 4×10^6 beads/ml for 1000 beads/sec throughput and 4×10^7 beads/ml for 10,000 beads/sec throughput. The samples after sorting are collected and confirmed by a conventional flow cytometer (FACSCantoII, BD). A final purity achieved is 90% at 1000 beads/sec throughput and 76% at 10,000 beads/sec throughput. Compared to our prior 2D PLACS, the sorting purity has increased from 45% to 76% at a throughput of 10,000 beads/sec (Table. 1).

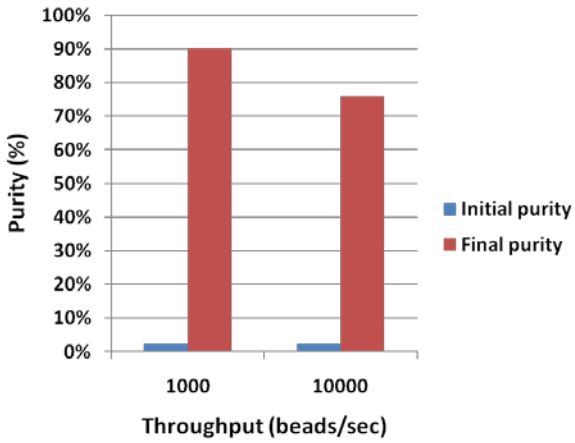

Fig. 4 Sorting results. With initial mixture of 2.3% purity, a final purity of 90% is achieved at a throughput of 1000 beads/sec and 76% at a throughput of 10,000 beads/sec.

Table 1. Comparison of 3D PLACS with 2D PLACS.

Device	Throughput	Final collection purity
3D PLACS	1000/sec	90%
3D PLACS	10,000/sec	76%
2D PLACS	3000/sec	90%
2D PLACS	10,000/sec	45%

In Sum, by utilizing 3-dimensional sheath flow focusing, the particle position is more precisely controlled, which contributes to the improvement in the sorting purity from 45% to 76% at 10,000 beads/sec throughput and at 2 m/sec particle speed.

ACKNOWLEDGEMENT

This work was supported in part by NSF ECCS-0901154 and NSF DBI-0852701, a UC Discovery/Abraxis BioScience Biotechnology Award (#178517), and by the Broad Center of Regenerative Medicine and Stem Cell Research at UCLA.

REFERENCES

[1] M. W. Wang et al., "*Microfluidic sorting of mammalian cells by optical force switching*", Nat. Biotechnol., **23**, pp. 83-87 (2005).

[2] A. Wolff, et al., "*Integrating advanced functionality in a microfabricated high-throughput fluorescent-activated cell sorter*", Lab Chip, **3**, pp. 22-27 (2003).

[3] S. H. Cho et al., "*Human mammalian cell sorting using a highly integrated μFACS*", Lab Chip, **10**, pp. 1567-1573 (2010).

[4] T. -H. Wu et al., "*Pulsed laser triggered high speed microfluidic fluorescence activated cell sorter*", Lab Chip, **12**, pp. 1378-1383 (2012).

CONTACT

Yue Chen 1-310-871-2436 or katechen@ucla.edu, Pei-Yu Chiou 1-310-825-8620 or pychiou@seas.ucla.edu