PULSED LASER ACTIVATED CELL SORTER WITH DIELECTROPHORETIC SINGLE STREAM SHEATHLESS FOCUSING

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ABSTRACT

We report a novel sheathless microfluidic fluorescence-activated cell sorter utilizing size-independent three-dimensional dielectrophoretic (DEP) single stream focusing and pulsed laser activated cell sorting (PLACS). This is realized by fabricating a 3D microfluidic device using two glass substrates with patterned electrodes sandwiching a thin and open PDMS channel. DEP forces are provided along a 4cm-long channel that focuses particles into a single stream passing through a fluorescence detection zone regardless of their sizes and types at high flow speeds. Pulsed laser-induced cavitation bubbles are used to provide rapid (~30 μs) and precise fluid perturbation to sort out particles upon the detection of fluorescence. High purity sorting has been accomplished at a throughput of 1500 particles sec^{-1}.

INTRODUCTION

Fluorescence-activated cell sorter (FACS) is widely used for single cell analysis in both fundamental biomedical research and clinic applications. In conventional FACS, cells are focused into the center of the stream by sheath flows and pass through a fluorescence detection zone. After exiting a narrow nozzle, the stream of cells is broken into individual droplets that can be selectively charged and deflected based on detected fluorescence content.. However this droplet approach can result in biohazardous aerosol formation which exposes the operator to potential contamination and infection [1]. It is also difficult to integrate additional upstream or downstream modules with conventional FACS to perform multiple functions in one single run.

Microfluidic fluorescence activated cell sorting (μFACS) systems aim to provide a fully enclosed environment for sterile cell sorting by eliminating droplet and aerosol formation. Moreover it has the advantage of integration with upstream pre-sort and downstream post-sort modules to provide flexible sample handling and versatile sample analysis. To narrow down the gap of performance between microfluidic FACS and conventional aerosol-based FACS, several active sorting mechanisms have been employed, including pneumatic valve control [2], piezoelectric actuation [3], optical force switching [4], acoustic wave actuation [5] and pulsed laser activated cell sorting (PLACS) [6]. Among them PLACS shows a great potential in achieving comparable performance to commercial aerosol-based FACS. By utilizing the rapid (~30 μs) and precise fluid perturbation from pulsed laser induced cavitation bubbles, PLACS has achieved a sorting performance of 90% sort purity at 23000 cells sec^{-1} using 3D sheath flows [7]. However due to the large sheath-to-sample flow ratio it suffers from severe sample dilution and requires a high initial sample concentration (>10^7 ml^{-1}), which is not practical for certain applications such as rare cell sorting. Hence a sheathless focusing mechanism is preferred.

Inertial focusing provides a sheathless mechanism by utilizing inertial forces on particles in high-speed flows in microfluidic channels. However, the focusing effect is size-dependent and a stable single stream focusing is difficult to obtain [8]. Tuning the focusing location is also difficult to achieve since the equilibrium position is dependent upon channel geometry and flow speed.

Here we demonstrate a new dielectrophoresis pulsed laser activated cell sorter (DEP-PLACS) featuring continuous, sheathless, size-independent, real-time tunable, dielectrophoretic single stream focusing at high speed flows for high throughput and high purity sorting.

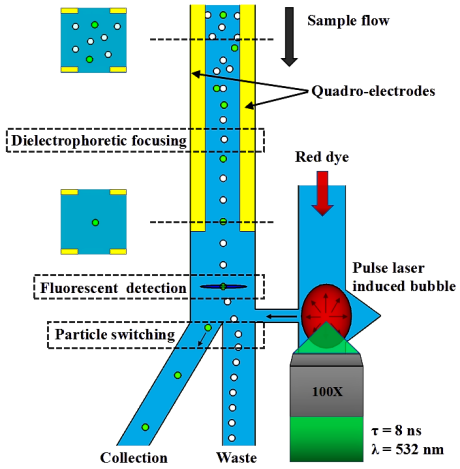

Figure 1: Schematic of the DEP-PLACS system for sheathless high throughput and high purity cell sorting. Randomly distributed particles are focused into a single stream using negative DEP and sorted into separate channels based on fluorescence properties by pulsed laser induced cavitation bubbles.

METHODS AND RESULTS

Utilizing thin film PDMS fabrication processes [9-10] we fabricated a device consisting of a microfluidic channel sandwiched by two glass substrates with a quadro-electrode layout. Fig. 2 shows the schematic of fabrication process flow of the device.

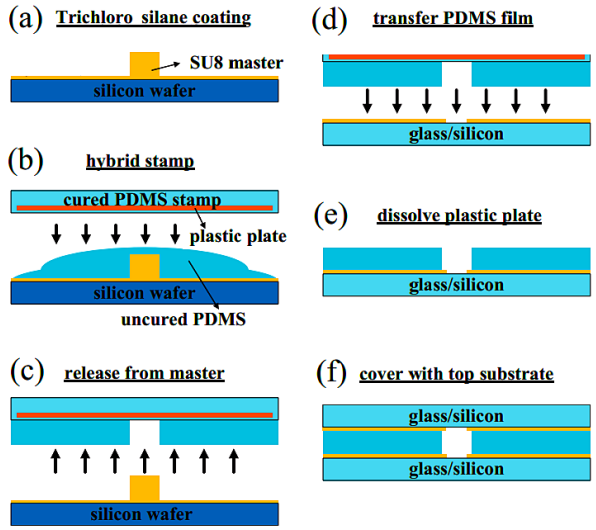

Figure 2: Schematic of the fabrication process flow using a plastic plate embedded hybrid stamp. (a) A SU-8 mold is treated with silane to facilitate later demolding. (b) Uncured PDMS mixture is poured onto the mold and pressed by a hybrid stamp. (c) PDMS thin film is demolded together with the hybrid stamp. (d) PDMS thin film is oxygen-plasma treated, aligned and bonded to a glass substrate with electrode layouts. (e) Dissolve the polystyrene plastic plate in acetone. (f) Align and cover the device with another thin glass substrate with electrode layouts to complete the fabrication process.

As shown in Fig. 1, in the DEP-PLACS system the upstream main channel carrying the sample flow is a 80μm wide and high, 4 cm long heterogeneously integrated PDMS microchannel. On top and bottom glass substrates the quadro-electrode layout spans the whole length of the main channel to provide an extremely long dielectrophoretic interaction distance for focusing cells in high-speed flows. The main channel divides into two branches in the downstream for collection and waste. Both are 80 μm in height and 40 μm in width. At the bifurcation the main channel is connected to the dye channel, which is 150 μm in width and expands to 300 μm at the bubble excitation location, by a nozzle of 60 μm in length and 20 μm in width.

When an a.c. signal is applied symmetrically to the electrodes, a tunnel-shaped electric field potential profile forms along the channel. Fig. 3(a) presents the numerically simulated electric field distribution in the microchannel with a single field minimum at the center. The location of the field minimum can also be tuned in the cross section by changing voltage combinations applied to the electrodes [11].

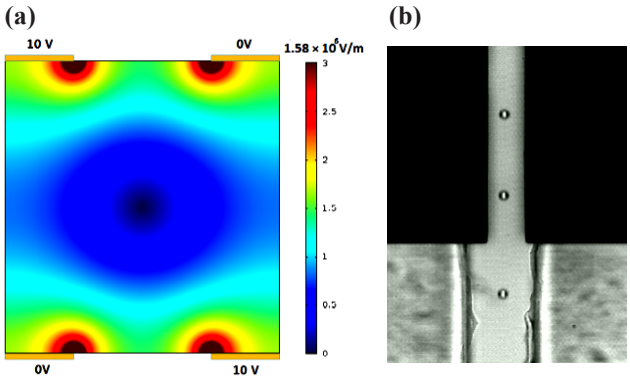

Figure 3: (a) Quadro-electrodes generate a single electric field minimum at the center of the microchannel when a.c. signal applied symmetrically. (b) A high-speed camera image of polystyrene beads focused into a single stream after 4 cm long interaction distance with a high particle speed up to 16 cm/s in a 1S/m medium with 20 $V_{p\text{-}p}$ applied. Particle speed is calculated by analyzing the bead movement and time interval between multiple image frames.

Dielectrophoresis (DEP) is the phenomenon in which a force is exerted on a dielectric particle when it is subjected to a non-uniform electric field. It is widely used to manipulate, transport, separate and sort different types of particles. For a homogeneous sphere of radius r and complex permittivity ε^*_p in a medium with complex permittivity ε^*_m the time-averaged DEP force can be modeled as

$$\left\langle F_{DEP} \right\rangle = 2\pi r^3 \varepsilon_m \,\mathrm{Re}\left\{\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right\} \nabla \left| \vec{E}_{rms} \right|^2$$

For a particle more polarizable than the medium, it experiences positive DEP forces and tends to migrate to electric field maximum; otherwise for a particle less polarizable than the medium, it experiences negative DEP and migrates to field minimum. In regular physiological buffers with high conductivity (>1S/m) dielectric particles and mammalian cells only show negative DEP responses at the frequency ranging from kHz to MHz and the DEP forces are weak. Generally it's challenging to manipulate or focus particles in high conductivity media using DEP. However for our device due to the extremely long (4 cm) interaction distance, microparticles/cells experience negative DEP forces continuously and can be focused into a single stream in high-speed flows [12]. 10 μm polystyrene spheres suspended in phosphate buffer solution (PBS) with a conductivity of 1S/m can be focused into a single stream at the particle flow speed up to 16cm/s when a 20 $V_{p\text{-}p}$ a.c. signal is applied symmetrically to the quadro-electrodes as shown in Fig. 3(b).

To enhance the DEP response, microparticles/cells can be suspended in low ionic buffers with the conductivity of 0.01S/m to 0.1S/m, in which polystyrene spheres still only show negative DEP but mammalian cells can exhibit either

negative or positive DEP responses at different frequencies. Higher voltage can also be applied in such media without introducing electrolysis or severe heating issues to increase DEP forces and the maximum flow rate in which microparticles/cells can still be focused into a single stream.

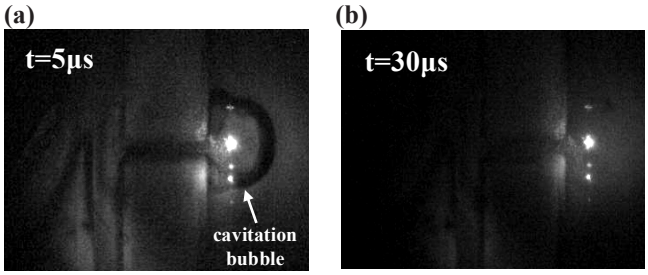

Figure 4: Time resolved images showing a micro cavitation bubble induced by a ns laser pulse (a) expands to its maximum at 5 µs and (b) collapses at 30 µs. A liquid jet through the connection nozzle is formed during the rapid expansion process.

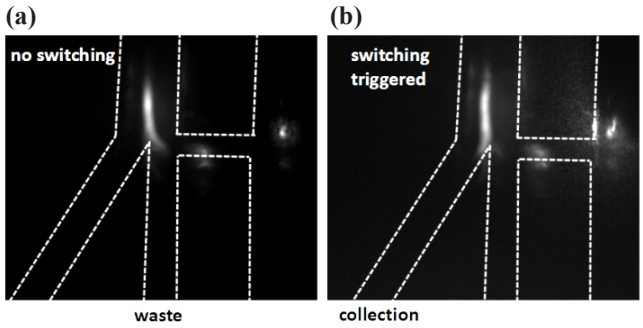

Figure 5: Fluorescent particle traces showing (a) focused particles are initially biased into the waste channel without laser triggering and (b) successful particle switching into the collection channel. This switching is achieved by triggering a cavitation bubble in the dye channel with a ns laser pulse upon fluorescence detection.

During cell sorting experiments focused particles are initially biased into the waste channel at the bifurcation by either adjusting the flow ratio between the main channel and dye channel, or changing the voltages applied to electrodes. A 50mW, 488nm CW laser (CrytaLaser, DL-499-50) is focused through a 25X/N.A. 0.4 objective lens into the microchannel before the bifurcation to excite fluorescence. The emission fluorescence is collected through the same objective lens and detected by a photomultiplier tube (PMT, Sens-Tech P30CWAD501) connected to a DAQ card (National Instrument, PCI 7831R) for signal acquisition and processing. Upon the detection of fluorescence a cavitation bubble is generated in the parallel dye flow channel induced by a 532nm ns laser pulse (Q-switched Nd;YVO4, EKSPLA, Jazz 20) focused by a 100X objective lens (N.A. 0.9). The bubble expands rapidly through the connection nozzle between the main and the dye channels (Fig. 4), forming a high-speed liquid jet which pushes the fluorescent particle over the Y-junction into the collection channel to achieve successful switching (Fig. 5). The cavitation bubble collapses within 30µs after triggering, which ensures fast and precise particle sorting.

Sorting of microparticles was performed to characterize the performance of DEP-PLACS system. 10µm green fluorescent polystyrene beads (Fluoro-Max G1000) and 9µm non-fluorescent polystyrene beads (Duke Standards 4209A) were mixed at a ratio of 1:100 and the total concentration of 5.6×10^6 beads/ml. The beads were suspended in an isotonic buffer with a conductivity of 0.1S/m. The sample solution was sent into the main channel by a syringe pump (Harvard Apparatus, PHD 2000) at the flow rate of 1ml/h, which corresponds to a throughput of 1,500 particles sec^{-1}. Allura Red dye (67 mg/ml, Sigma-Aldrich) was pumped into the dye channel to reduce the laser energy threshold for bubble generation. A $20V_{p-p}$, 1MHz a.c. signal was applied symmetrically to the quadro-electrodes to focus particles into a single stream at the center of the channel. With synchronized fluorescence detection and pulsed laser triggering, fluorescent beads can be successfully switched into the collection. Initial mixture sample and sorted sample were analyzed by a commercial flow cytometer (BD, FACSCantoII). With the precise single stream focusing and fast particle switching, DEP-PLACS has achieved a sorting purity of 91% for polystyrene beads at a throughput of 1,500 particle sec^{-1} (Fig. 6). The fluorescent beads were enriched from an initial mix ratio of 0.01 to a final ratio of 10.36, corresponding to a 1,036-fold enrichment.

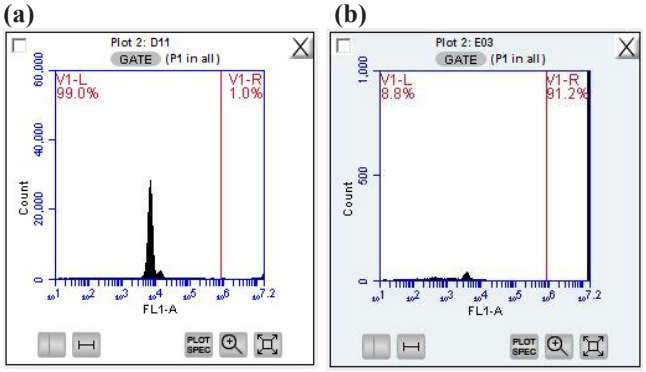

Figure 6: Particle sorting purity is verified by a commercial flow cytometer. (a) 10µm green fluorescent and 9µm non-fluorescence polystyrene beads are mixed before sorting at a ratio of 0.01 with a particle concentration of 5.6×10^6 beads/mL, sample flow rate 1ml/h, particle moving speed 10cm/s. (b) After sorting sample collected has a 91% purity.

CONCLUSION

We demonstrated a new dielectrophoresis pulsed laser activated cell sorter (DEP-PLACS) featuring continuous, sheathless, size-independent, real-time tunable, dielectrophoretic single stream focusing at high speed flows for high throughput and high purity sorting. By utilizing the extremely long (4 cm) DEP interaction channel and the rapid (~30 µs) fluid perturbation, 91% sorting purity has been achieved at a throughput of 1,500 particles sec^{-1} with polystyrene beads.

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