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Micro-Fabricated Fluorescence-Activated Cell Sorter

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Abstract—**We demonstrate a high sensitivity, high throughput microfabricated cell sorting device with the integrated piezoelectric actuator and optofluidic waveguide on a chip. For automated sorting, field-programmable-gate-array (FPGA) embedded real time control loop system is built. The sorting is performed under high flow rate (~10 cm/sec), and the sorting system can achieve a high throughput (> 1kHz) with high purity. Moreover, to enhance its sensitivity, Teflon AF coated liquid core waveguide (LCW) structure is demonstrated. Teflon AF has a lower refractive index (n=1.31) than water (n=1.33) and forms the cladding layer of the LCW. Incident light is confined and guided along the Teflon AF coated fluidic channel, and interact with samples flowing along the same channel. This enables flexible device design and gives high sensitivity to the fluorescence detection system. The preliminary results demonstrate sorting fluorescent beads at a sorting efficiency of ~70% with no false sorting.**

I. Introduction

MICRO fabricated fluorescence-activated cell sorter (μFACS) has the advantages of lower cost, reduced sample/reagent usage, portability and rapid analysis time, which make them attractive for various biotechnological applications such as single cell analysis [1]. Due to the fast growing interest in the study of single cells [2, 3] along with advances in microfabrication technology, significant progress has been made towards developing μFACS that can sort single cells with high efficiency and purity.

Various types of μFACS based on the principle of continuous laminar flow manipulation have been developed. Among those principles are electroosmotic [4, 5], dielectrophoretic [6, 7], magnetic [8, 9], and hydrodynamic [10, 11] flow manipulation sorting. Electroosmotic sorting enables precise flow manipulation, but it requires high DC voltage (e.g. hundreds of volts) for operation and suffers from low throughput. Dielectrophoretic sorting method can precisely sort suspended samples down to single cells. However, the cell differentiation capability is limited because cells with similar dielectric properties cannot be easily separated as they experience similar dielectrophoretic forces. Magnetic sorting can achieve high selectivity but the labeling of magnetic particles may cause potential cell damages. On the other hand, hydrodynamic flow manipulation can achieve high throughput sorting with simple fabrication and minimal cell damage, and thus resolving the aforementioned limitations. Bang et al. have implemented high-speed off-chip flow-switching check valve that has the response time of 2.5 msec in order to enhance sorting throughput [10]. However, those mechanisms still need bulky external actuators hindering on-chip level miniaturization. Moreover, the lack of precise real-time control system results in low efficiency and low purity of sorting.

In this paper, we describe a high throughput μFACS on a single chip based on hydrodynamic flow manipulation with the automated real-time control system and the optofluidic waveguide for enhanced sensitivity. The integrated piezoelectric PZT actuator hydrodynamically manipulates sub-nanoliter volume of flow precisely under low voltage (< $10V_{p-p}$) and low power (0.1mW) enabling manipulation down to single cell level. The integrated PZT has a much faster response time (~0.1 – 1 msec) and smaller overall size than external check valves, making the system cost-effective and portable. In order to achieve automated sorting with high efficiency, field-programmable-gate-array (FPGA) embedded closed loop control system is built. The detected signal is processed and amplified in real time, and the algorithm triggers PZT actuation for sorting. The electronic control system characterizes sorting efficiency and accuracy in real time. 70% sorting efficiency with no false sorting is achieved with our μFACS system. In addition, to suppress the noise in fluorescence detection, on chip excitation using optofluidic liquid core waveguide is implemented. The optofluidic waveguide is fabricated by coating Teflon AF on the PDMS microchannel walls. The coating forms a layer on the channel wall having a lower refractive index (n=1.31) than water (n=1.33), thus the fluidic channel also becomes an optical waveguide [12]. The fluid flowing in the channel becomes the core of the optofluidic waveguide. As the fluid is divided into several branches, the light is also split. In this architecture, fluorescence detection at multiple spots using only one excitation source can be achieved. Moreover, direct interaction between the confined light and samples in the flow results in high sensitivity. In summary, the proposed design eliminates the need for bulky actuator through the integration of PZT actuator, enhances system sensitivity through optical confinement in Teflon AF coated liquid waveguides.

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II. Device Architecture and Design

A. Sorting Mechanism

The sorter hydrodynamically deflects the targeted particle to either a center channel or selectively one of the collection channels, as seen in Fig. 1. To provide such an action, a voltage is applied to the piezoelectric PZT layer, and the PZT actuator bends upward or downward according to the polarity of the voltage. This bending induces a transverse displacement of fluid typically in an amount of sub-nanoliters per stroke. The exact amount of fluid displacement by the PZT actuator can be precisely controlled by the waveform and amplitude of the applied voltage. The main microfluidic channel is split into three branches. As the sample of interest enters the sorting junction (dotted box in Fig. 1), the particle is deflected by the instantaneous transverse flow actuated by the PZT and consequently directed down to the left or right channel. Non-targeted particles will flow straight down to the middle waste channel as the PZT actuator is in an idle state.

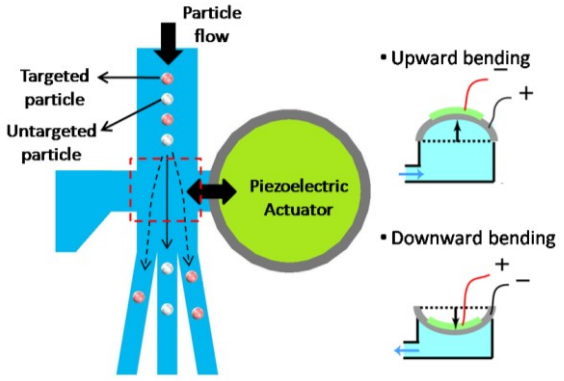

Fig. 1. As particle enters the sorting junction, bending motion of the PZT actuator will temporarily disturb fluid flow (either to the right or left), causing particles to be deflected to the left/right channels.

B. Device Fabrication

Due to the small dimensions, μFACS device is the perfect vehicle for the aforementioned PZT actuator because of the small fluid volume change actuated by the PZT actuator. The μFACS device is fabricated using the polydimethylsiloxane (PDMS) replica molding technique [13]. The device features including microfluidic channels are defined photolithographically using SU-8-50 photoresist (MicroChem) on silicon wafer. After pouring prepolymer PDMS (Sylgard 184, Dow Corning) onto the mold and thermal curing, the features of the mold are transferred to a PDMS substrate. After demolding, the patterned PDMS substrate and another plain PDMS/glass substrate are surface-activated by UV ozone. The two substrates are then bonded covalently to create microfluidic channels. The PZT actuator has a bimorph structure consisting of a PZT layer deposited on a stainless steel disk. The stainless steel disk is polished, cleaned, and UV Ozone treated before being bonded to a reservoir formed on the PDMS device. The stainless steel-PDMS bonding process is completed after the baking the sample in an 85°C oven for 4 hours. This strong, joining-layer-free bonding interface not only prevents fluid leakage but also produces fast device response and excellent power coupling between the actuator and the microfluidic channels, critical to the performance of the cell sorter.

To enhance the detection sensitivity and to allow optical detection at multiple desired positions along the fluidic channel, we have invented a novel light guiding architecture where the excitation laser light shares the same path with the analytes in the fluidic channel. This architecture greatly simplifies the light routing and enables multi-point detections. The enabling technology is a fluid-core optical waveguide. The laser excitation light is confined to the fluid in the channel as the waveguide core, and the cladding layer is formed by a low-index Teflon AF layer coated on the wall of the channel. Teflon AF is amorphous fluoropolymer that has a refractive index lower than water [12]. To achieve a uniform layer of coating, a 6% Teflon AF solution (DuPont Corp) is injected into the microfluidic channels from the inlet while the exits of the channels are vacuumed (P=-20kPa) for 20 minutes. The balance between the vacuum pressure and the adhesion of Teflon AF solution to the PDMS channel wall determines the thickness of the Teflon AF cladding layer. The device is then heated to 155°C for 20 minutes to remove the fluoroinert solvent (3M), followed by 175°C heating for additional 20 minutes. The last heating temperature is 15 °C above the glass transition temperature of Teflon AF, thus producing a smooth, optical quality Teflon AF layer with low optical loss.

C. Sorting – setup, spatial filter, closed loop control

The schematic of the automated μFACS setup is illustrated in Fig. 2. A 40mW laser (λ=488nm) is used as the excitation source. To verify the system, 10 μm fluorescent beads (Bangs Laboratory) having the excitation and emission peaks at 480 nm and 520 nm are injected to the microfluidic channel. Fluorescence emission is collected by a 20X microscope objective lens. A long pass filter (λ_{cutoff}=500nm) is used to suppress the excitation light from reaching the detector.

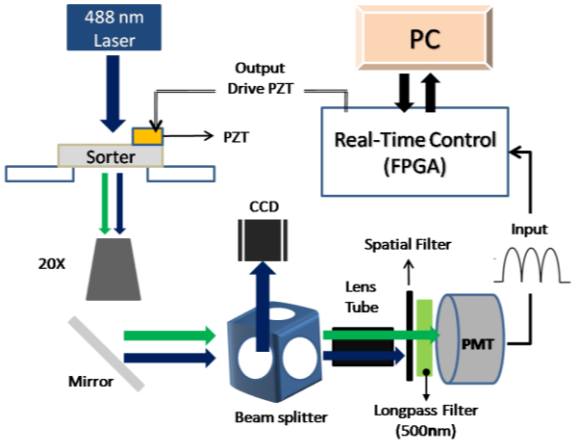

Fig. 2. A spatial filter is placed at the image plane of the sorter. This spatial filter allows transmission of fluorescence emitted only when particles pass the designed slits. A control system with embedded FPGA processes the input signals in real time and sends out an appropriate output to trigger the PZT actuator for sorting.

When a fluorescent particle passes through the detection region and exits the collection channel (e.g. left channel), the fluorescent signal is detected by the photo-multiplier tube (PMT) (Thorlabs Inc.) . A spatial filter is inserted at the image plane of the device, as shown in Fig. 2. By designing the patterns of the spatial filters with a transparency mask corresponding to different detection areas, one can encode the resultant detected signals (Fig. 3). In our experiment, we used 3 slots to create 3 distinct peaks. Such special signal waveforms can be processed with FIR filters to suppress the background noise and eliminate crosstalk. The spatial filter for the up-stream detection has three open slits and the filter for the down-stream optical detection has two open slits. The up-stream detection is used to decide the targets of interest for sorting, and the down-stream detection is used to verify the sorting efficiency and error rate. Because of the spatial filters, these signals have different waveforms and can share a single PMT detector.

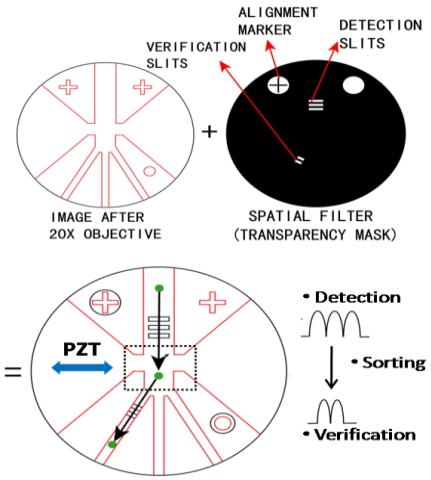

Fig.3 Overlaying the spatial filters with the image of the lab-on-chip cell sorter magnified by the 20X objective lens.

The detected signals are processed in real time as shown in Fig. 4 and an output voltage of programmed waveform and time delay is applied to the PZT actuator for sorting by an embedded FPGA system. When the particle is sorted to the designated channel, a verification signal (e.g. a signal with two peaks in Fig. 3) is expected to be detected.

The real-time electronic control system is implemented using LabVIEW Compact RIO (National Instruments) with an embedded FPGA chip, as shown in Fig. 4. The timing jitter of the system is less than 10 μsec, which is significantly shorter than the travel time of the sample from the up-stream detection position to the sorting junction (e.g. ~ 0.1 – 1 msec). With an FIR filtering algorithm, noise is suppressed and the SNR is enhanced by ~18 dB. After FIR filtering, criteria for definition of threshold and search of maximum peaks are applied. A signal above the user-defined threshold value suggests the presence of the target. A delay counter is used to specify the time delay (e.g. time elapsed for a particle to travel from the detection region to the sorting junction), and a preprogrammed output voltage signal is employed to drive the PZT actuator. After each bead or cell is sorted, a verification (e.g. double-peak) signal from the sorted bead or cell should be detected to confirm the success of the sorting event. Until the sorted particle is verified, the PZT actuator will not be fired to avoid false sorting. Finally, the system updates the record of the sorting efficiency and sorting accuracy.

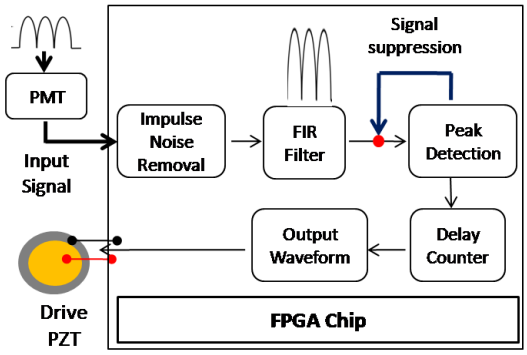

Fig. 4 The real-time control loop algorithm in the FPGA chip. It imports the detected signal and amplifies the signal using the FIR matched filter algorithm. After the peak detection and delay counters, an output waveform is generated to drive the PZT actuator for sorting.

III. Experimental Results

Laser light is fiber-coupled and confined to the microfluidic channel as shown in Fig. 5. The light confined in the main channel by the Teflon AF coated liquid waveguide can be even split at the sorting junction and still guided in sorting channels separated with a small angle (~3 degree).

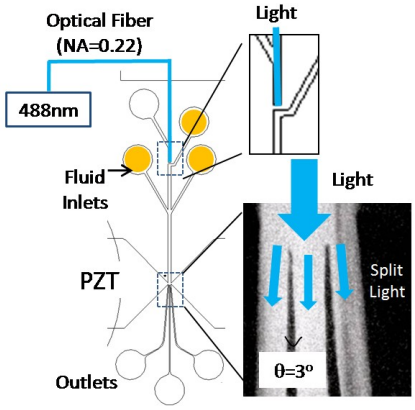

Fig. 5 Fiber-coupled incident laser input is confined to the microfluidic channel. The light can be split at the 3-way junction and remains guided along the divided channels

Under this excitation scheme, fluorescence detection at multiple spots using one excitation source can be achieved. This allows collection of fluorescent and scattering signals at different locations along the device through specially designed spatial filters or integrated waveguides and/or lenses demonstrated previously [14].

Fig. 6 shows the fluorescent signal emitted from a 10-μm bead with the incident laser power of only 0.35mW. The incident power is measured by the optical power meter at the

end of the optical fiber before being inserted to the microfluidic channel. Such a low incident power gives a raw signal with a signal-to-noise ratio (SNR) of 25dB. After FIR matched filtering, the SNR is further enhanced by 18dB. The result suggests that for many applications, we may use an LED source instead of a high power diode laser as the excitation source; or we may replace the PMT detector with semiconductor detectors.

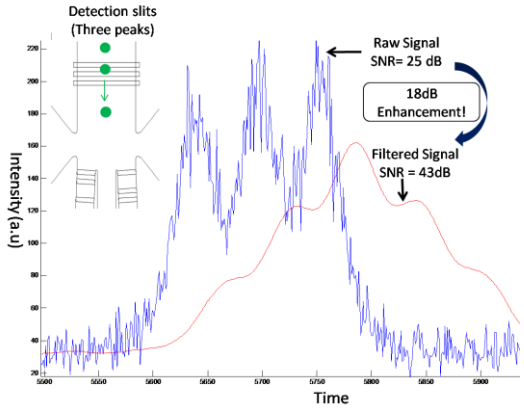

Fig. 6. Raw detection signal passing the upstream spatial filter (blue). The optical excitation power is only 0.35 mW, demonstrating the high sensitivity of the device architecture. After FIR matched filtering, the SNR is increased by 18dB (red). One division is in x-axis is 0.5 ms.

The sorting result with fluorescent beads is shown in Fig. 7. 44 beads out of 64 detected beads are successfully sorted, resulting in about 70% sorting efficiency. The sorter misses some targeted beads mainly due to imperfect PZT trigger timing as the velocity profile of the beads in the fluidic channel. For the successful sorting event, a three-peak detection signal is followed by a two-peak verification signal as shown in the graph. It should be noted that in this sorting experiment, no beads are falsely sorted.

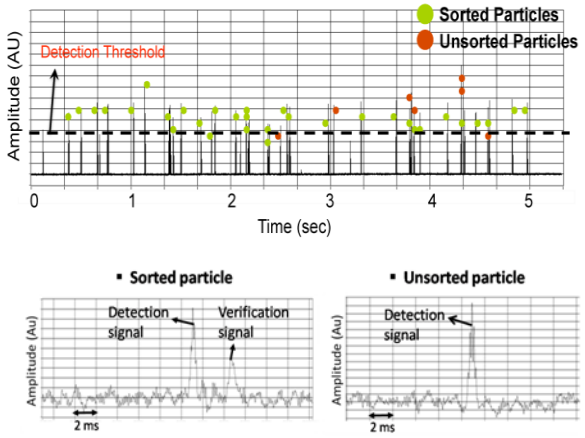

Fig. 7 Sorting efficiency is ~ 70% and error rate is 0%, which means no particles are falsely sorted. At the bottom graphs, for the sorted particle, the detected signal is always followed by a verification signal. The missing of verification signals represents lower than 100% sorting efficiency, indicated by red dots.

IV. Conclusion

We have demonstrated a lab-on-chip cell sorter with an integrated pieozoelectric actuator and fluidic optical waveguide to achieve high sensitivity. Teflon AF coated liquid waveguide confines and delivers the incident light throughout the microfluidic channels even after the channel is split into sub channels. As excitation always occurs, fluorescence can be detected at multiple point. A real-time signal processing and control system to allow close-loop sorting experiment is implemented in FPGA. The device operates under low power (<1 mW) and yields high throughput as the flow stream responds to the piezoelectric actuator at high frequency. Preliminary sorting experiment using fluorescent beads shows 70% sorting efficiency without false sorting. These attractive and unique features hold promise for high-performance, low cost, and portable lab-on-a-chip μFACS systems.

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