Microfabricated Fluorescence-Activated Cell Sorter with Hydrodynamic Flow Manipulation

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ABSTRACT

Presented is a novel flow manipulation and particle detection method for the microfabricated fluorescence-activated cell sorter (μFACS). With hydrodynamic flow manipulation which includes passive flow channeling and hydrodynamic actuation with nozzle flow, we have developed a fast and robust method for utilizing the sorting function. These techniques are directly related to enabling the high throughput screening of the FACS machine. Moreover by detecting with synchronized imaging, we have also developed an on-line calibration technique for the accurate timing between detection and actuation. And this means that our system is very flexible that it can be tuned to any assay conditions with various flow speeds, particle densities and buffer or sample viscosities by real time calibration.

Keywords: micro-FACS, Lab-on-a-chip, hydrodynamic flow manipulation, plastic micromachining, PDMS

1 INTRODUCTION

Compared with conventional FACS machines, the μFACS provides higher sensitivity, lower cost, no cross-contamination and short flow path for the sorted cell viability. In this paper we present our latest results on the development of μFACS including the flow focusing and channeling concept, the design of the hydrodynamic flow manipulation, plastic microchip fabrication issues and the integration of the detection optics and signal processing system with real time system delay calibration.

For the microchip fabrication we applied plastic micromachining which is safer and easier to fabricate and suitable for mass production. And for the flow driving we chose simple negative pressure driving with vacuum suction. Actually vacuum driving setup requires only one peristaltic pump. Finally for the stream channeling and manipulation which is the main theme of this paper, we applied the passive channeling concept, hydrodynamic flow manipulation method and real time calibration with synchronized imaging technique.

The plastic microchip has three inlet ports for the sample and buffer, two outlet ports for sorted beads and waste. Between those inlet and outlet ports there is a single broad actuation channel that enables the 'timely hit' at the target cells. Simple principles of the hydrodynamic flow manipulation using off-chip valve switching technique was used to provide a much smaller size of the whole μFACS system, which resulted in a robust device design and highly reproducible focusing and sorting flow generations. Chips were evaluated by obtaining the substantial enrichments of the different fluorescent micro-beads in sorted micro chambers respect to their colors.

2 PRINCIPLE AND DESIGN

2.1 Hydrodynamic flow manipulation

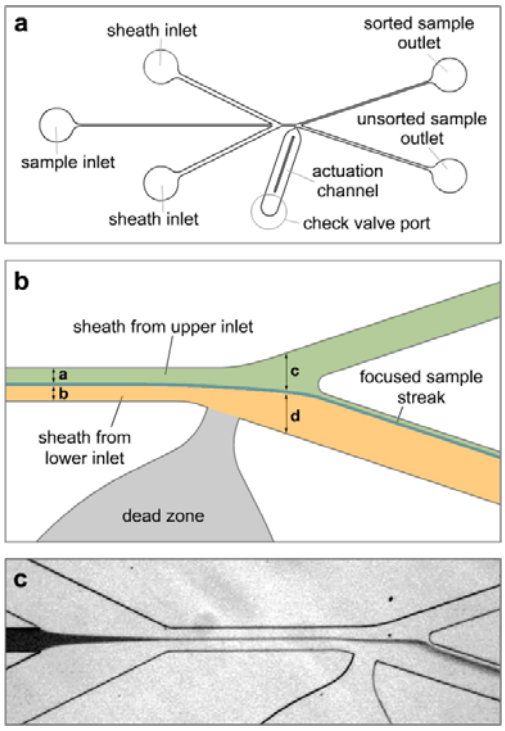

Figure 1: Channel configuration for the hydrodynamic flow switching. (a) Overall channel design with flow inlet and outlet ports (b) Passive flow channeling scheme (c) a CCD image of the focused Tryphan blue

Flow focusing is the core technology in flow cytometry. After flow focusing, the focused sample stream has to be channeled into desired outlet port for the sorting function. Then we should sort out specific particle by switching the focused stream into the sorting channel. Moreover for high throughput sorting flow switching should have high alternating frequency with good reproducibility. For this we designed a vacuum driving based fluidic network including passive flow channeling with hydrodynamic flow switching. And the device showed very fast switching ability with robust and reproducible operation.

The overall geometry and port configuration is shown in figure 1a. In figure 1a, there are two outlet channels which are connected to sort and unsorted sample outlet ports respectively. Those two channels have width of 100 μm and 150 μm respectively as shown in figure 1b. And all channels are 50 μm in depth. Because of the simple principle that streamline never crosses itself, the focused stream will roughly flow through the center line of the overall streamlines and as shown in figure 1b, the width **c** and **d** like **a** and **b** will roughly be the same. The difference in width between those two channels in the downstream makes the focused sample stream glance slightly downward the wedge structure at the diverging point.

At the diverging point it was a slight glance in flow path for the focused stream but in the end the focused stream follows through way down the lower channel and it results in drastic flow channeling. The fact that slight glance made the focused stream be channeled into sorting channel is a very important fact for flow switching because it means that a small disturbance can switch the stream up and down after the diverging point. In figure 1c you can see that focused stream of Tryphan blue is being channeled into lower channel. This concept is proved later in this paper by computer simulation. We didn't apply any active flow manipulation to channel the focused stream thus we call this concept passive flow channeling.

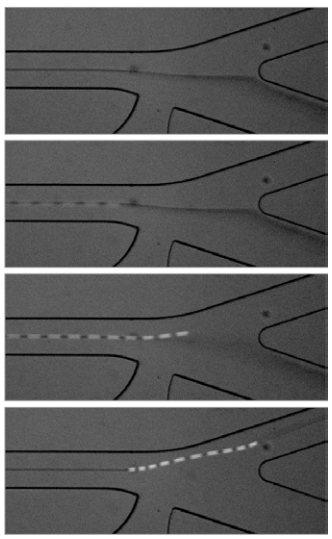

Figure 2: Focused streak of microbead being switched

As mentioned above if we just give slight disturbance toward upper channel at the diverging point, we can switch the focused stream upward. And after the disturbance has been disappeared the focused stream will rapidly switch back to its original state automatically. For this disturbance we designed an actuation channel (figure 1a) which is connected to piping from the buffer chamber which is normally blocked by check valve thus choked normally. By promptly opening and closing the check valve, the actuation channel acts as an instance disturbance source toward upper channel providing very high speed flow switching scheme.

Empirically, micro scale channel structures [1] can't afford this kind of rapid disturbance because of its laminar flow characteristics, so we designed the pressure channel to be sub-millimeter scale structure which is about 700 μm in width. The effectiveness of this millimeter scale channel structure is proved below by computer simulation. Figure 2 shows focused streak being switched by the instance disturbance method mentioned above. The application of this switching scheme and detailed explanation of the sorting function will be described with calibration method in real time sorting inspection section.

With this configuration sample stream does not run through the check valve [2], so there's no chance for the contamination or clogging inside the valve module. Other research groups and conventional FACS machines also use check valves, but they use check valves in the downstream and focused stream normally flows through it. The focused sample stream flow and blocking of it results in contamination and sample clogging inside the check valve which becomes one of main causes for system malfunction.

And our hydrodynamic flow manipulation scheme does not induce any surface modification [3] or on chip electrodes for flow manipulation [4]. And our sorting scheme occurs in a simple and continuous manner that any complicated flow driving schemes [5] are not required. This simplicity ensures the highly robust operation with easy and reproducible fabrication processes.

2.2 Simulation

Simulations were performed on a multiple-physics software package based on the computational fluid dynamics (CFD-ACE 2003, CFD Research Corporation, Huntsville Alabama, USA).

In figure 3 there are three pairs of triangular markers by the inlet and outlet of the channel. Three markers in the left inlet are used to trace streamlines in the upper, center and lower point of inlet channel respectively. On the right side of figure 3a you can see the green marker (trace 2) which traces focused sample stream of the inlet is finally in the upper side of lower channel. This proves that the channel network make the focused stream slightly glance at the diverging wedge.

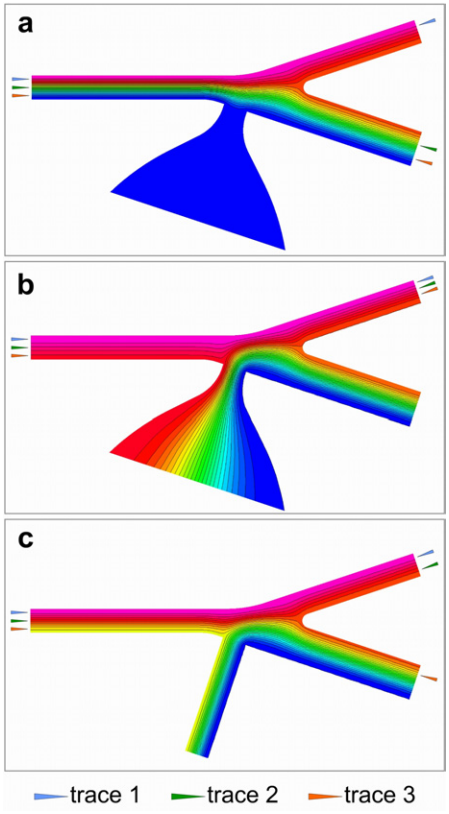

Figure 3: Computer simulation of the flow switching scheme (a) Initial state : the focused stream is passively channeled into lower channel (b) Flow actuation by the actuation channel with nozzle structure (c) Flow actuation by the actuation channel without nozzle structure

In figure 3b and 3c you can find out the effectiveness of the millimeter scale actuation channel structure. In figure 3b all markers are finally in the upper channel. But in figure 3c only green marker (trace 2) is in the upper channel which means the actuation barely switched the focused stream to the upper channel. Considering the time until the flow switching is fairly accomplished, the channel configuration in figure 3b requires much shorter time to accomplish flow switching operation than the channel configuration in figure 3c. You can see that with the same width of the opening gate, channel configuration with millimeter scale actuation channel followed by nozzle structure exerts much faster switching ability.

2.3 Real-time Sorting Inspection

Figure 4 shows overall system setup with data signal and trigger signal wiring and flow driving configuration. The cell-sorting device was mounted on an inverted microscope (IX71, Olympus, USA). A green laser (GM32-10H, Intellite, USA) was used for epifluorescence excitation. And a (USH102D, Olympus, USA)W mercury lamp provided ambient fluorescence to aid capturing particle tracks after laser detection.

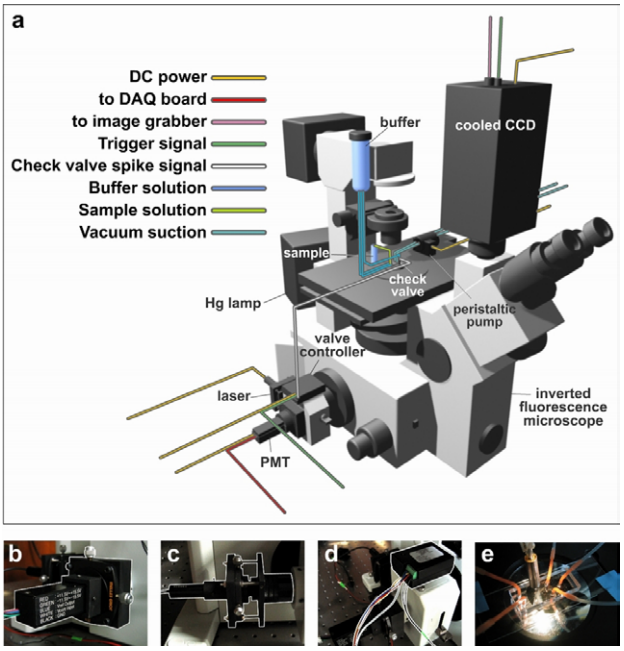

Figure 4: (a) System setup with signal and solution I/O of the μFACS system (b) XY stage and pin hole installed photo multiplier tube adaptor (c) XY stage and adjustable lens apparatus installed 532nm green laser adaptor (d) Spike signal generation kit for the check valve (e) Plastic microchip and its piping and a valve for the FACS operation

The fluorescence was collected through the objective and detected by a photo multiplier tube (PMT) (H5784-01, Hamamatus, Japan) with pinhole installed in its adaptor. The electrical signal from the PMT was sent via a preamplifier to a DAQ board (SD-104, COMIZOA, Korea) on PC. The signal was plotted on monitor and processed by appropriate threshold level to produce check valve trigger signal. Switching the flow switching valve (INKA2424212H, The Lee Company, USA) forced the beads of interest go to the collecting channel. And the same trigger signal activated cooled CCD (PCO, Kelheim, Germany) thus grabbing particle tracks after laser detection for on line sorting verification. A peristaltic pump (P625/10k.143, Instech, USA) were used for driving the sample and the sheathing buffer.

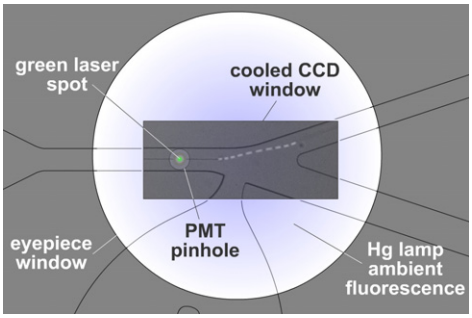

Figure 5: Light and window configuration

We focused on making a flexible detection and actuation system that can be tuned to any assay conditions with various flow speeds, particle densities and buffer or sample viscosities by real time calibration. There are lots of delay sources such as PC system delay by operating system, signal processing lag, valve actuation delay caused by mechanical characteristics of the inner valve structure and flow manipulation delay by tubing and chip (PDMS) elasticity. So if we can't perfectly predict and measure these delay sources and their degrees, we have to make flexible system that can be tune out any undesired delays.

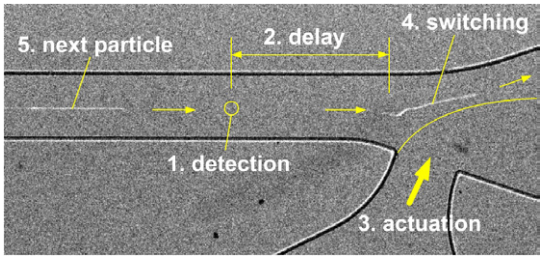

Figure 6: Sorting sequence with on line system delay calibration

Figure 6 shows how our system calibrates its delay sources. After fluorescence detection the valve trigger signal also triggers cooled CCD and the image is displayed on the monitor screen so we can inspect the verification image showing that the switching happened at appropriate moment or not. Adjusting the detection position by controlling XY stage of the microscope we can calibrate the distance between detection point and flow switching point. All the delay sources are implied in this distance, so by adjusting this distance real time sorting calibration can be accomplished. Actually it's like manipulating a jog dial watching monitor screen to calibrate the sorting operation.

3 FABRICATION

The plastic μFACS chip was fabricated in poly(dimethylsiloxane) (PDMS) using soft lithography [11] as is shown in Figure 7. The choice of the material PDMS depends primarily on its biocompatibility because this device is designed to dilute biological samples. Briefly, CAD file drawing has been patterned on a glass substrate with chrome masking. The chrome mask was used in 1:1 contact photolithography with SU-8 photoresist to generate a negative master mold, consisting of patterned photoresist on a Si wafer. Positive replicas with embossed channels were fabricated by molding PDMS against the master. Three inlet ports, two outlet ports and a actuation port (1-mm-diameter holes) for the fluids were punched out of the PDMS using a simple steel hand-puncher. The surface of the PDMS replica and a clean slide glass were activated in an oxygen plasma (2.666 x 10-2 Pa, 20 s, 25 W) and was brought together immediately after activation. An irreversible seal was formed between the PDMS and the slide glass. Finally, this assembly produced the required systems of microfluidic channels.

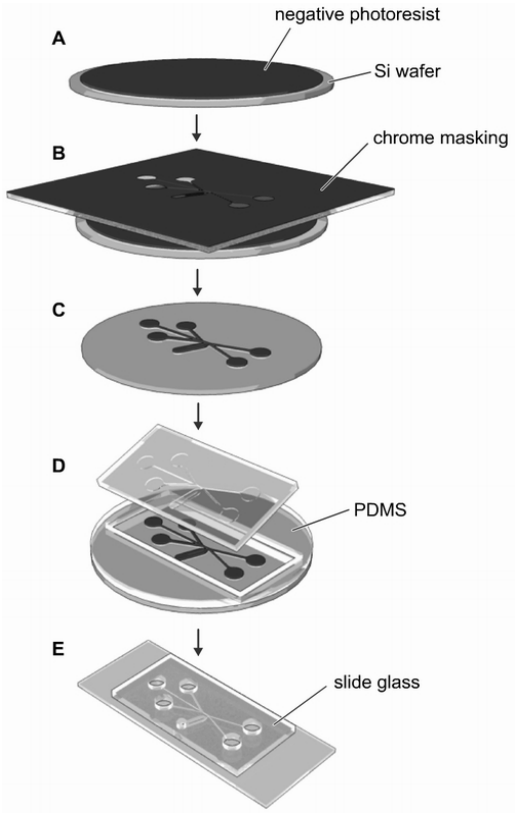

Figure 7: Scheme describing the steps involved in the fabrication process of PDMS-glass hybrid microchip

The fabricated microchip is smaller than a slide glass (14 mm x 29 mm x 4 mm, in width, length and thickness). The microchip has six holes five of which will latterly be connected to polyethylene tubing making inlet and outlet piping and one for the check valve connection. The check valve tip is very small thus directly inserted into the actuation hole. You can see fabricated plastic μFACS chip in figure 8.

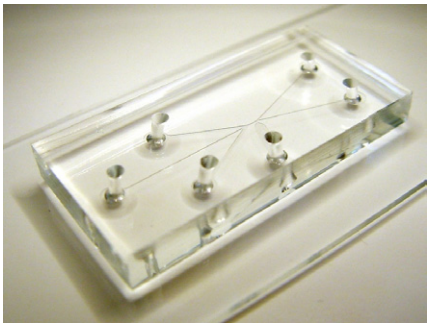

Figure 8: Fabricated plastic μFACS chip

4 RESULTS AND DISCUSSION

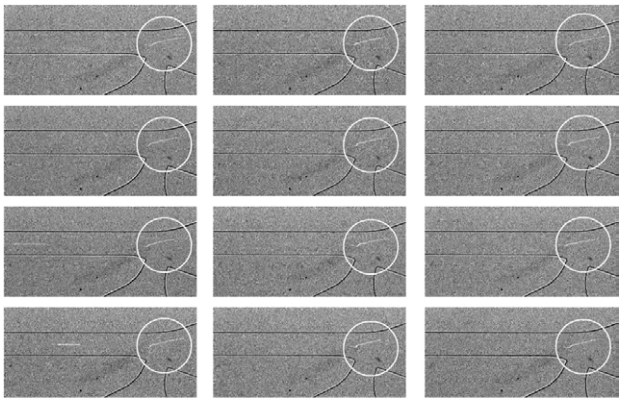

Figure 9: Micro beads being sorted out

Every time sorting happens the system leaves an image for the verification of its operation. Once the total system delay has been calibrated by the feedback inspection of the images, all detected particles are sorted out to the sorting channel. Figure 9 shows sequence of images after total system delay calibration, and you can see that all particles are sorted out to upper (sorting) channel.

Still for the on line calibration there's a limit in lateral flow speed. To take simultaneous images while sorting, there should be enough exposure time. So if the lateral flow speed is too fast particle streak images have to be taken in a very short time interval. And low exposure results in dark images which are so vague to be distinguished. But if we use conventional calibration method which is verifying the system delay by inspecting collected samples after each run or use fixed lateral flow speed, we can also calibrate the whole assay condition like other systems.

Our μFACS system is a very flexible one that it can be tuned to any assay conditions with various flow speeds, particle densities and buffer or sample viscosities by real time calibration. And its passive channeling and hydrodynamic flow manipulation concept ensures robust, fast and highly reproducible operation. We believe this kind of μFACS system will find a place next to conventional FACS machine as an essential facility of cell biology laboratory while cellomics is needed.

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