TUNABLE STANDING SURFACE ACOUSTIC WAVES ACTIVATED CELL SORTING

Xiaoyun Ding[1], Chung Yu Keith Chan[1], Michael Ian Lapsley[1], Lin Wang[2], and Tony Jun Huang[1]

[1]Department of Engineering Science and Mechanics, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

[2] Ascent Bio-Nano Technologies Inc. State College, PA 16801, USA

ABSTRACT

Here we introduce a microfluidic device for cell sorting in continuous flow using tunable standing surface acoustic waves (SAWs). The device is an integrated microfluidic chip, compatible with optical detection and electronic triggering for cell sorting, and combines the potential advantages of fluorescence activated cell sorting (FACS). The sorting device utilizes the contrast in the material properties of a continuous liquid phase and the objects contained therein, so it operates in continuous flow without additional prior labeling of the objects with markers such as polarizable or magnetic beads. Our non-invasive acoustophoresis based cell sorter is capable of sorting almost all kinds of particles/cells regardless of their shape, electrical, magnetic, or optical properties.

KEYWORDS

Cell sorting, surface acoustic waves, microfluidics, lab on a chip.

INTRODUCTION

Cell sorting is essential for many fundamental cell studies, cancer research, clinical medicine, and transplantation immunology [1]. researchers have developed several microfluidic-based methods for direct cell sorting, such as magnetic-activated cell sorting, optical tweezers, electrokinetic mobilization, and hydrodynamic flow[2, 3]. These methods have pioneered many new avenues to on-chip cell sorting; however, they suffer from drawbacks such as low controllability, low cell viability and proliferation and/or requirements on bulky equipments. Here we introduce a microfluidic cell-sorting device using tunable standing surface acoustic waves (SAWs). Our approach can precisely sort cells into five separate outlets using tunable SSAW without labeling or droplet encapsulation.

EXPERIMENT

The SSAW-based cell sorting device consists of a polydimethylsiloxane (PDMS) channel and a piezoelectric substrate with a pair of chirped IDTs (Fig. 1). The finger spacing of the chirped IDTs ranges from 70 μm to 100 μm, producing a working frequency range of around 9.5 MHz to 14.5 MHz. The two parallel IDTs each generates a separate SAW and the interference between the two identical SAWs generates the standing SAW

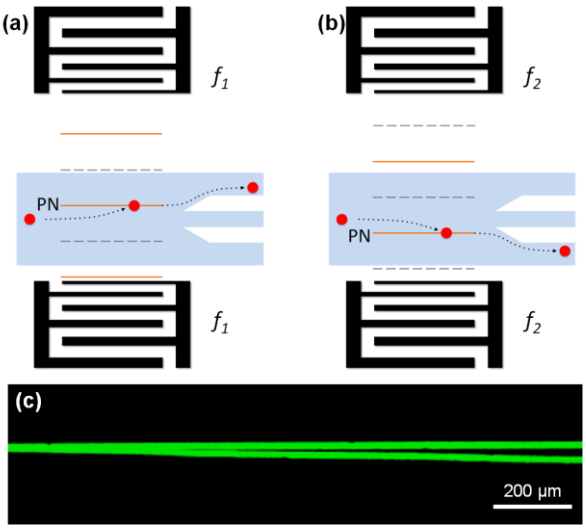

Figure 1: Device structure and working mechanism. When the SSAW is switched on, all the particles are driven to the pressure node (marked by orange solid lines) and directed in the (a) upper outlet channel, or (b) the

bottom outlet channel. (d) The trajectories of two 15 μm fluorescent polystyrene beads at two frequencies respectively. The lateral shift of the particles after switching frequency is as large as ±25 μm after passing through the sorting region.

field on the surface of the substrate. The standing SAW couples into the liquid medium and generates an acoustic radiation force on objects suspended in the liquid and pushes them to pressure nodes. Such nodes are parallel to the channel. Simply by varying the input frequency applied to the chirped IDTs, we can shift the location of the pressure nodes. As a result, particles/cells trapped in the pressure node can be translated laterally across the channel and be directed into any of the upper or lower output channels, which correspond to certain input frequency (Fig. 1a and 1b).

We first characterized our sorting device by its sorting distance. Fig. 1c shows the merged trajectories of 15 μm fluorescent polystyrene particles moving through the sorting region at input frequencies of 9.8 MHz and 10.1 MHz. A lateral shift of ~50 μm was achieved at the end of the sorting region. Precise alignment of PDMS channel and IDTs are not required due to the flexible working frequency range of the chirped IDT. With further optimization on the chirped IDT, a lateral shift greater than 50 μm can be achieved for applications that require a large sorting distance such as droplets and organisms sorting. To demonstrate the functionality of our SSAW-based sorting method, we first directed 15 μm fluorescent polystyrene beads to 3 outlet channels (Fig. 2). In our experiment, the width and height of the main channel are 200 μm and 70 μm. When an input signal was applied with a frequency of 14.5 MHz, the closest pressure node to the particles was ~25 μm above the center of the channel and directed particles to the upper outlet channel (Fig. 2a). When the frequency switched to 13.9 MHz, this pressure node shifted below the center of the channel and particles were directed to the lower outlet channel (Fig. 2c). When the SSAW was off, the particles were not shifted and followed the flow to the center outlet channel (Fig. 2b).

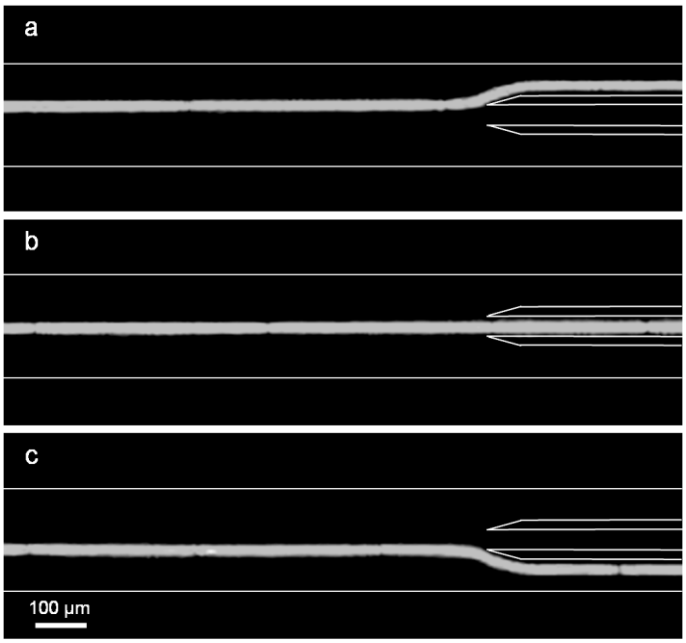

Figure 2: Sorting of 15 μm fluorescence particle in three-outlet channel upon application of two different frequencies. When the SSAW is switched on, particles passing through the sorting region move to (a) upper outlet channel at a frequency of 13.9 MHz, or (c) bottom outlet channel at frequency of 14.5 MHz. (b) When the SSAW is switched off, the particle flow in the center channel.

To further demonstrate the versatility of our method and its ability to sort bio-objects into more outlets, we tested bovine red blood cells (brbc) in our sorting device. The brbc were first hydrodynamically focused to the center of the main channel by two side flows, and then sorted to 3 specific outlets at controlled frequencies. The results of this sorting are shown in Fig. 3. In this way, we would be able to sort 3 types of cells to 3 individual outlets in a single microfluidic chip. Our device could potentially extend from current 3 outlets to a higher number of outlet channels to increase the functionality of the device for applications such as whole blood analysis and antigen-based cell diagnostics where the separation of many cell types is necessary. To demonstrate the biocompatibility of our acoustic based sorting technique, we conducted standard cell viability and proliferation assays using HeLa cells. HeLa cells were incubated for 20 h after being exposed in standing SAW field for 10 min under the input power of 23 dBm, and then metabolic activity was measured at 450 nm after 2-h BrdU labeling and following 2-h Reagent WST-1 reincubation. Subsequently, DNA synthesis was determined using Cell Proliferation ELISA to verify the cell proliferation. For control experiments, cells were

examined without standing SAW treatment and at 65°C for 1 h. The culture medium with no cell was also measured as comparison. Our test shows that no significant sign of decrease in both viability and proliferation was found after exposure to standing SAW fields for 10 min, implying no significant physiological damage was induced by our technique.

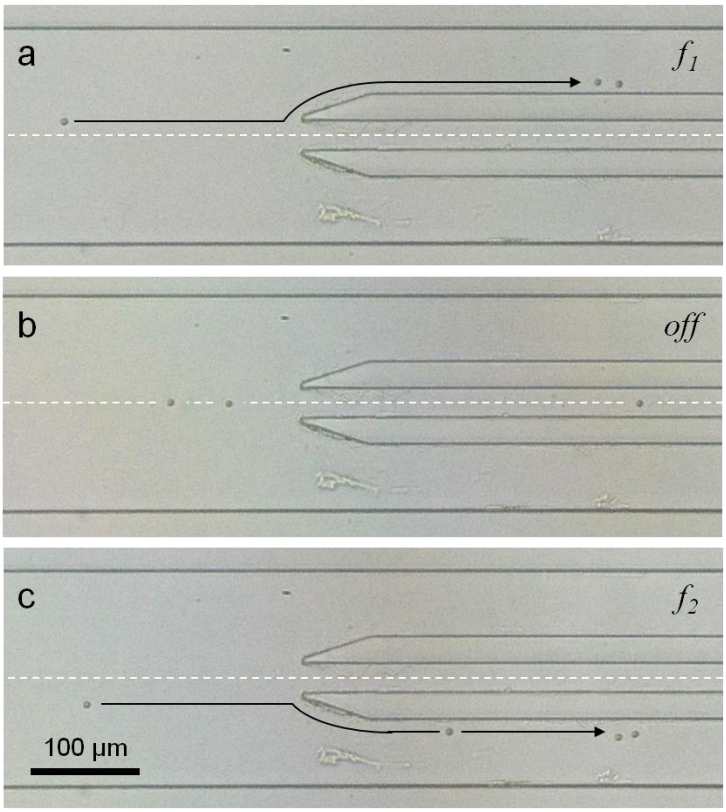

Figure 3: Sorting of bovine red blood cells into five specific outlet channels at controlled frequencies.

The standing SAW-based cell sorting technique presented here utilizes the contrast in the mechanical properties (e.g., density, compressibility) of a continuous liquid phase and the objects contained therein, so it operates in continuous flow without prior labeling of the objects with markers such as polarizable or magnetic beads. Our technique is non-invasive and is capable of sorting almost all kinds of particles/cells regardless of their shape, electrical, magnetic, or optical properties. It achieves cell sorting directly from media without prior encapsulation of cells into liquid droplet compartments required in traditional fluorescence-activated cell sorting (FACS).

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REFERENCES:

1. M. Eisenstein, "Cell sorting: Divide and conquer," *Nature*, **441**, 1179–1185 (2006).
2. A. Arora, G. Simone, G. B. Salieb-Beugelaar, J. T. Kim, A. Manz, "Latest developments in microfluidic cell biology and analysis systems," Anal. Chem. **82**, 4830–4847 (2010).
3. A. Lenshof and T. Laurell, "Continuous separation of cells and particles in microfluidic systems," Chem Soc Rev, **39**, 1203–1217 (2010).