ADJUSTABLE PASSBAND PARTICLE SEPARATION DEVICE

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ABSTRACT

Acoustic separation in microchannels is a promising strategy towards high purity, label-free sorting of cells and particles. However, most device architectures only permit binary selections based on a single size-threshold (i.e. high-pass or low-pass). Here, we report a microfluidic acoustic separation device architecture that allows for separation with an independently tunable upper and lower particle size separation threshold. We demonstrate high efficiency (transfer fraction = 0.98 ± 0.02) and throughput (~10^8 particles/h) separation of an arbitrary passband of particle sizes between 3 and 10 μm in diameter.

KEYWORDS: Particle separation, Tunable separation, Ultrasonic standing waves, Acoustophoresis

INTRODUCTION

Label-free separation of cells and particles based on their physical properties is a critical preparatory step for many biotechnological applications. This need has spurred innovative device architectures for separation based on volume, polarizability, size, density, or refractive index, by means of dielectric, mechanical, hydrodynamic, acoustic and optical forces [1]. In particular, acoustophoresis in microchannels is attractive because it offers gentle separation at high throughput [2]. However, previous acoustophoretic approaches only allowed selections based on a single size threshold (i.e. high-pass or low-pass) and were incapable of purifying target particles based on a range of sizes (i.e. band-bass) [3]. In this work, we report the Acoustic Band-pass Filter (ABF), the first acoustic separation device capable of fully adjustable, size-based particle separation. Using the ABF, we demonstrate fully tunable (3 μm < diameter < 10 μm), high purity (transfer fraction = 0.98 ± 0.02) separation at high throughput (10^8 particles/hr/microchannel).

THEORY

Our device relies on a control over the acoustic radiation force $\mathbf{F}^{\mathrm{rad}}$ which is exerted on particles in the presence of a resonant acoustic field, and directs them towards the pressure nodes or antinodes. The resonant field is excited by piezotransducers coupled to the device, which generate a pressure field with a node at the channel half-width and antinodes at the channel walls. In the 1D approximation, the force on a spherical particle of radius a in the transverse y-direction across the channel is given by

$$F_y^{\mathrm{rad}} = 4\pi a^2 (ka) E_{\mathrm{ac}} \Phi \sin(2ky) = 4\pi a^2 (ka) E_{\mathrm{ac}} \left[\frac{\rho_{\mathrm{p}} + \frac{2}{3}(\rho_{\mathrm{p}} - \rho_{\mathrm{a}})}{2\rho_{\mathrm{p}} + \rho_{\mathrm{a}}} - \frac{1}{3} \frac{\rho_{\mathrm{a}} c_{\mathrm{a}}^2}{\rho_{\mathrm{p}} c_{\mathrm{p}}^2} \right] \sin(2ky), \quad (1)$$

where $4\pi a^2$ is the cell surface area, $ka = 2\pi a/\lambda$ the particle size to wavelength ratio, E_{ac} the acoustic energy density, Φ the contrast factor, ρ_{p} the particle density, ρ_{a} the medium density, c_{p} the speed of sound in the particle and c_{a} the speed of sound in the medium. For our particular particles and medium, Φ is a positive number and hence particles are directed towards the pressure node at the channel half-width, at a rate proportional to a^2. Thus, as particles travel through the device, a separation may be made between particles that successfully focus to the centre of the channel and particles that remain unfocused.

EXPERIMENTAL

The ABF is a three-input, three-output device and consists of two serially-integrated separation stages (Figure 1). In each separation stage, a piezotransducer is employed to excite a resonant pressure field such that pressure nodes exist along the half-widths of each of the channels. The different channel widths w_1 and w_2 of the two stages allow each transducer to be driven at two distinct resonant frequencies f_1 and f_2, permitting independent control over the rate of focusing in each stage [4]. Particles are introduced into the sides of the first stage along with central buffer flow. The volume dependant acoustic radiation force [2] driven at f_1 focuses the larger particles to the center of the device, transferring them to the second stage of the device. The unselected, smaller particles are eluted into the Low-pass Outlet. The second stage uses the same selection mechanism, driven at f_2, to further elute the largest particles to the High-pass Outlet while directing the target particles to the Band-pass Outlet.

The device was fabricated using standard microfabrication techniques. Briefly, the fluidic channels were etched in a Si wafer to a depth of 200 μm using a Bosch deep reactive ion etching process (770 SLR, Plasmatherm). Fluidic access holes were drilled via a CNC drill (Flashcut CNC) with a diamond bit in both the Si wafer and a Borofloat glass wafer. The glass wafer was anodically bonded (SB6, Suss Microtec AG) to the Si wafer to cap the microchannel. The wafer was then diced to yield final chip dimensions of 18 mm × 74 mm. 20 mm diameter piezotransducers (PZ26, Ferroperm Piezoceramics) were centered over each separation stage and glued to the chip using super glue. The fluidic inlets and outlets were attached with epoxy. The device was then mounted on an inverted fluorescence microscope (TE-2000S, Nikon, Melville, NY) for monitoring during operation. Sample and buffer were volumetrically pumped into the device via syringe pumps (PhD 2000, Harvard Apparatus). To excite the acoustic resonances, separate sinusoidal signals were applied to each piezotransducer via a function generator (AFG320, Tektronix) and custom amplifier based on an LT1210

operational amplifier (Linear Technology). To minimize piezotransducer heating during operation, a fan was used for cooling.

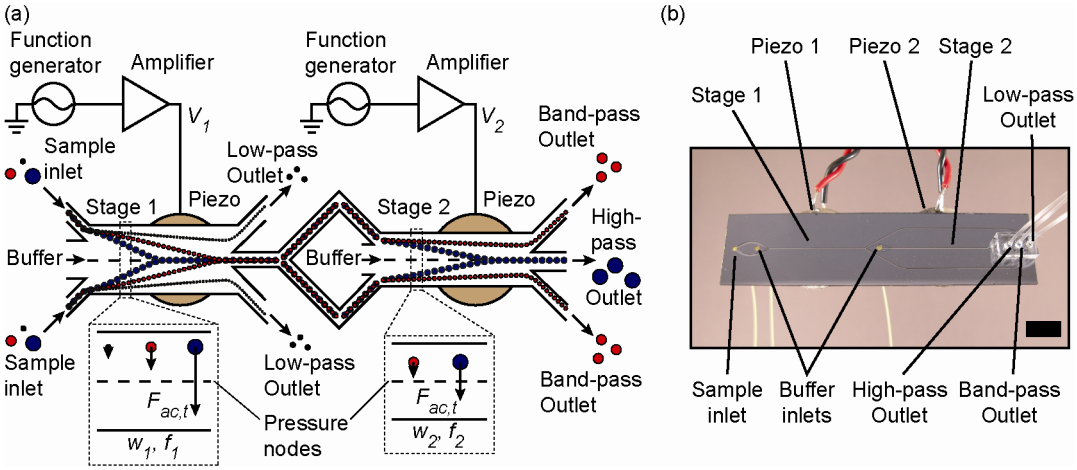

Figure 1: a) Schematic overview of the Acoustic Bandpass Filter (ABF). The device consists of two interconnected stages, each characterized by a channel width w and a driving frequency f. Two piezotranducer elements are used to independently control the amplitude of the pressure field in each separation channel; the actuation frequency is adjusted in each such that a pressure node exists along the channel half-widths. The difference in channel widths and driving frequencies ensure minimal cross-talk between channels. A particle mixture is introduced into the sides of stage 1 alongside a central buffer flow. Due to the volume dependence of the acoustic radiation force, larger particles are focused to the center stream. The selected particles from stage 1 are reintroduced into the sides of the second stage, and subject to another round of independently controlled focusing. Through adjustment of the two focusing conditions, a fully-adjustable passband of particle size are eluted through the Band-pass Outlet. b) Photograph of the ABF. The scale bar corresponds to 1 cm.

RESULTS AND DISCUSSION

By independently adjusting the actuation voltage at each stage, almost any range of particle sizes can be directed into the Band-pass Outlet. To demonstrate this novel feature, a mixture of 3, 5, and 10 μm diameter polystyrene microparticles (Microgenics Corp.) were suspended in a buffer of solution of ultrapure water with 0.01% v/v Tween-20 (Sigma-Aldrich) and pumped continuously into the device at a sample throughput of 2 ml/hr (14 ml/hr for each buffer). First, stage 1 was driven at f_1 = 2.105 MHz with variable voltage V_1 (Figure 2(a)). Next, we fixed V_1 at 43 V_{pp}, and actuated stage 2 at f_2 = 1.892 MHz with variable voltage V_2 (Figure 2(b)). By independently controlling V_1 and V_2 in this way, we show fully adjustable transfer of each particle size from the Low-pass Outlet into the Band-pass Outlet, and from the Band-pass Outlet into the High-pass Outlet respectively.

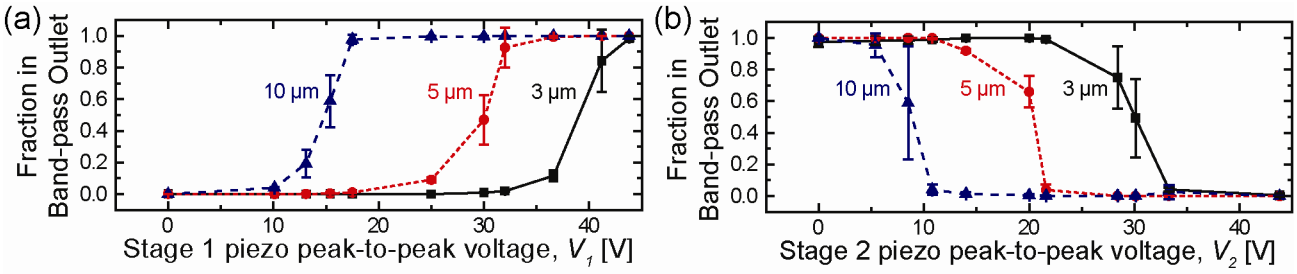

Figure 2: a) Selective transfer of particles in stage 1: the stage 1 piezotransducer was driven with a sinusoidal signal at 2.105 MHz and amplitude between 0 and 43 V_{pp}; the stage 2 piezotransducer was not actuated. Increasing V_1 transfers successively smaller particles into stage 2 (and then eluted through the Band-pass Outlet). At V_1 = 43 V_{pp}, > 99% of all particles elute through the Band-pass outlet. b) Selective transfer of particles in stage 2. The stage 1 piezotransducer was driven with a sinusoidal signal at 2.105 MHz and 43 V_{pp}, thus initially directing all particles into the Band-pass Outlet. The stage 2 piezotransducer was driven with a sinusoidal signal at 1.892 MHz and peak-to-peak amplitude between 0 and 43 V_{pp}. Increasing V_2 transfers successively smaller particles into the High-pass Outlet. At 43 V_{pp}, < 0.2% of all particles elute through the Band-pass Outlet.

Through a combined adjustment of V_1 and V_2, the ABF is capable of continuously separating particles with an adjustable size range. In order to demonstrate this feature, we suspended a mixture of 1, 3, 5, and 10 μm diameter polystyrene microparticles (Microgenics Corp.) at a concentration of ~10^8 particles/mL in the same buffer as above. Though the 1 μm diameter particles are too small to be focused by the acoustic radiation force, they were included to demonstrate capability of the device to reject smaller-than-desired particles. Sample and buffer were again pumped into the device at flowrates of 2 mL/h and 14 mL/h (for each buffer), and V_1 and V_2 adjusted to select for each of the possible particle size

ranges (Figure 3). The average transferred fraction of desired particles into the passband was 0.98 ± 0.04, and only 0.02 ± 0.04 for non-target particles.

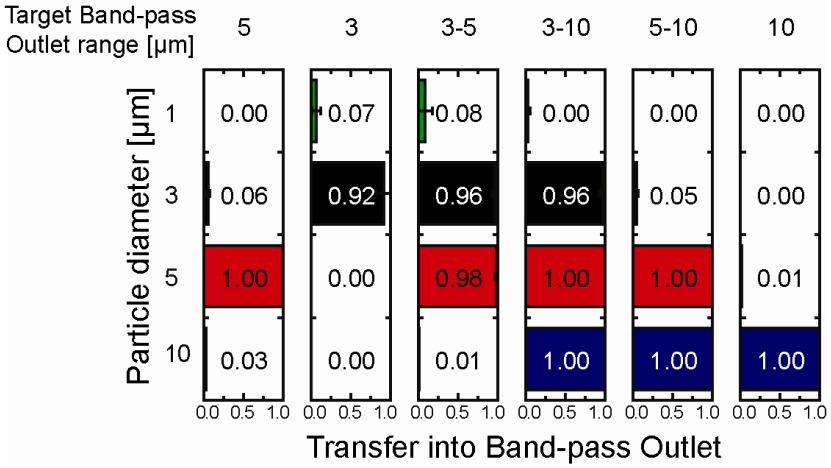

Figure 3: Fully tunable,size-based acoustic separation is achieved by independently controlling V_1 and V_2, . Here, a mixture of 10, 5, 3, and 1 µm particles was separated at 10^8 particles/hr throughput. The applied voltage to both transducers was adjusted for each possible combination of 10, 5 and 3 µm particles in the passband. The average transfer fractions for target and non-target particles in the passband are 0.98 ± 0.04, and 0.02 ± 0.04 respectively.

CONCLUSION

In conclusion, we have developed an acoustic separation device capable of fully adjustable, label-free, size-selectable particle separation and demonstrate a tunable passband of particle sizes between 3 and 10 µm in diameter. We believe our architecture may enable novel approaches for sample preparation at the point of care.

ACKNOWLEDGEMENTS

We are grateful for the financial support of the ARO Institute for Collaborative Biotechnologies, Office of Naval Research, and National Institutes of Health. We thank Hans C. Meyer and Henrik Bruus for helpful discussions.

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