TEMPERATURE-CONTROLLED HIGH-THROUGHPUT (1 L/H) ACOUSTOPHORETIC PARTICLE SEPARATION IN MICROCHANNELS

C. L. Ebbesen[1], J. D. Adams[2], R. Barnkob[1], H. T. Soh[3], and H. Bruus[1]*

[1]Department of Micro- and Nanotechnology, Technical University of Denmark, DENMARK
[2]Physics Department, University of California, Santa Barbara, USA
[3]Mechanical Engineering and Materials Departments, University of California, Santa Barbara, USA

ABSTRACT

We present a microfluidic acoustophoretic separator capable of unprecedented high throughput (about 1 L/h for a whole blood sample), approximately hundred times higher than previously reported. The high throughput along the x direction in the broad and shallow channel is facilitated by a large width (17 mm) in the y direction and a standing ultrasound wave in the shallow (830 µm) z direction. The necessary high acoustic power is obtained by running the attached piezo transducer at 55 V while keeping it cooled with a Peltier element. The device is fabricated using low-cost rapid-prototyping techniques, facilitating easy and versatile application.

KEYWORDS: Acoustophoresis, Blood separation, Cell handling, High-throughput on-chip separation

INTRODUCTION

Separation and purification of target particles/cells from complex mixtures is a critical step in many diagnostic and therapeutic applications. Toward this end, there has been significant interest in microchannel acoustophoresis because it allows gentle, label-free separation based on size, density, and compressibility [1–6]. Hitherto, the inherent low throughput (below 0.03 L/h) of microfluidic systems [1], has limited its broader use in biotechnology. Here, we present a microfluidic acoustophoretic separator capable of unprecedented high throughput (about 1 L/h), about hundred times higher than previously reported [1,5]. It is fabricated using low-cost rapid-prototyping techniques, facilitating easy and versatile application.

EXPERIMENTAL

Our device consists of one broad microchannel (60 mm × 17 mm × 830 µm) equipped with two inlet/outlet pairs and

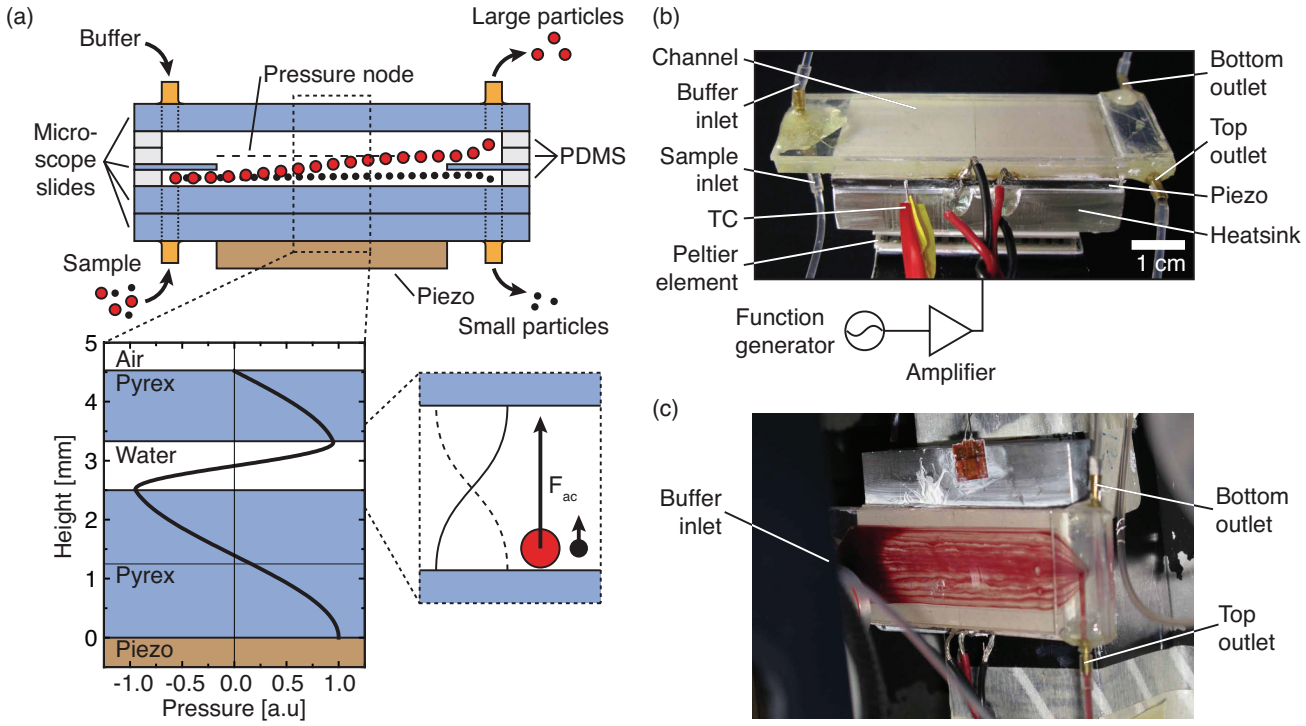

Figure 1: (a) Design schematic of the high-throughput device showing pressure resonance (black line) and acoustic radiation force (black arrows). The geometry of the device is designed to support a standing half-wave in the pressure field across the height of the microchannel (first inset). The magnitude of the acoustophoretic force driving a particle towards the nodal plane scales with the volume of the particle (second inset). (b) The device is mounted in a stable fluidic setup with 500-µm-diameter teflon tubing. The hydraulic resistance (length) of the outlet tubing is matched to ensure a correct outlet flow ratio. The temperature of the device is measured using a chromel/alumel thermocouple (TC). The device is mounted in thermal compound on an aluminum heat sink and cooled by a Peltier element. (c) The device during acoustophoretic separation of whole blood at a sample flow rate of 0.5 L/h and a total flow rate of 3.5 L/h.

fabricated from PDMS gaskets, cut from 250-µm PDMS sheets using a plotting cutter, plasma bonded between standard pyrex microscope slides (Fig. 1). The slides, bonded together using epoxy glue, act as acoustic reflectors. The central flow-divider, milled using a CNC-drill from a thin glass slide, is 70 µm thick. Two 27 mm × 27 mm piezoelectric transducers, attached beneath (using super glue) and fully covering the separation channel, induce a strong acoustic resonance in the microchannel when powered by a sinusoidal voltage at 899 kHz and an amplitude of up to 55 V (all voltages in this paper are peak-to-peak values).

THEORY

Microchip acoustophoresis uses the so-called acoustophoretic radiation force $\boldsymbol{F}_{\mathrm{ac}}$ to move particles/cells in aqueous suspensions to pressure nodes/antinodes of a standing ultrasonic resonance field [7]. In the special case of a plane pressure wave in the vertical z direction with wave vector k_z and amplitude p_{a} of the form $p(x,y,z) = p_{\mathrm{a}}\cos(k_z z)$, the acoustophoretic radiation force on a spherical particle of density ρ_{p} and compressibility K_{p} in an inviscid liquid of density ρ_0 and compressibility K_0 reduces to [8]

$$F_z^{\mathrm{ac}} = 4\pi a^2 (k_z a) E_{\mathrm{ac}} \left[\frac{\rho_{\mathrm{p}} + \frac{2}{3}(\rho_{\mathrm{p}} - \rho_0)}{2\rho_{\mathrm{p}} + \rho_0} - \frac{1}{3}\frac{K_{\mathrm{p}}}{K_0} \right] \sin(2k_z z), \tag{1}$$

where $4\pi a^2$ is the cell surface area, $k_z a = 2\pi a/\lambda_z$ is the size-to-wavelength ratio, and E_{ac} is the acoustic energy density. From the expression it follows that polystyrene beads, red blood cells and platelets are pushed towards nodal planes in the standing wave. An approximate expression for the standing ultrasound wave can be obtained by approximating the geometry as a parallel-plate triple layer pyrex/water/pyrex structure placed on top of a piezo transducer, and by further considering only the pressure field p (neglecting the transverse elastic waves in the glass) in the vertical z direction [9]. For ultrasound waves the viscosity of the liquid can be neglected, and p satisfies the Helmholtz wave equation $p''(z) = -[2\pi f/c(z)]^2 p(z)$, where f is the frequency and $c(z)$ is the speed of sound in the material (pyrex or water) at position z. The boundary conditions are $p' = 0$ at the bottom of the pyrex slide facing the piezo transducer, continuity of pressure and oscillation velocity at the pyrex/water interfaces, and $p = 0$ at the top pyrex slide facing the air; see inset of Fig. 1(a).

RESULTS AND DISCUSSION

To characterize the purity and throughput of the device, we prepared a sample mixture of red fluorescent 3-µm-diameter and green fluorescent 10-µm-diameter polystyrene beads (mimicking typical sizes, densities, and compressibilities of cells) suspended in a DI water buffer with 0.01 % v/v Tween-20. The sample and buffer were injected by syringe pumps into the device at flow rates of 0.25 L/h and 0.75 L/h, respectively. Outlet samples were collected in microcentrifuge tubes during separation, and the bead density was determined by flow cytometry.

Figure 2(a) shows successful separation of 3-µm and 10-µm microbeads at the high 0.25-L/h sample flow rate (1-L/h total flow rate). The mutual orthogonality of the flow direction (length), the throughput dimension (width), and the force direction (height) is the main prerequisite for our result. Furthermore, to ensure stability of the acoustic resonance, we employed two additional features: (*i*) As a novelty in microchannel acoustophoresis, Fig. 2(b) shows that the separation efficiency at a given frequency is highly temperature dependent. Our high flow rate requires a strong force field (high piezo voltage) leading to heating, which shifts the resonance frequency due to the temperature dependence of the speed of sound. Temperature stability within 1 °C is maintained using a Peltier element attached beneath the piezos. (*ii*) To reduce

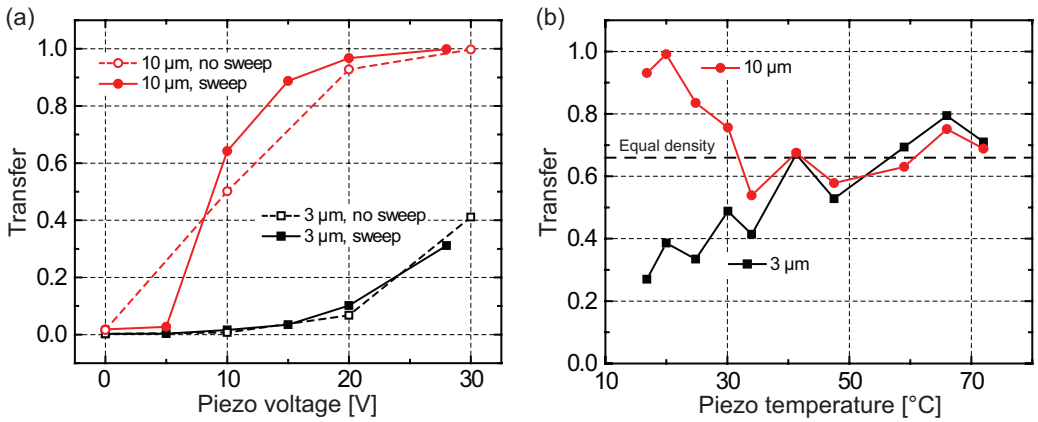

Figure 2: Measured separation of 3-µm and 10-µm polystyrene beads at 1-L/h total throughput. (a) Fraction of beads transferred from the bottom inlet to the top outlet (Transfer) showing strong separation at 15 V and f_{center} = 899 kHz. Sweeping around f_{center} increases separation efficiency. The data have been corrected for a small background, measured by running the device with buffer in both inlets. (b) Separation degradation due to heating, demonstrating the importance of temperature control. The speed of sound in water changes ≈ 10% between 0 °C and 100 °C, so heating will shift the device off resonance. Above 35 °C, we see a transfer of ≈ 0.66, corresponding to equal density in both outlets (total mixing).

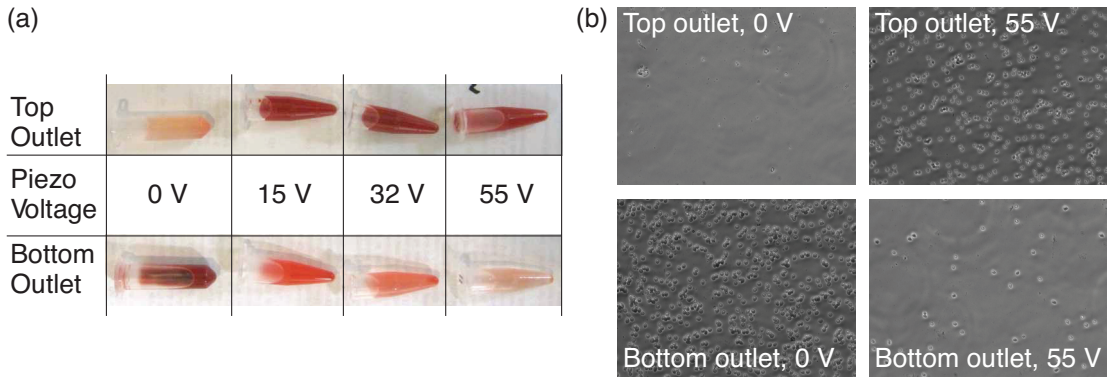

Figure 3: Preliminary separation result for a blood component mix mimicking whole blood at a sample flow rate of 0.5 L/h (total flow rate of 3.5 L/h) introduced through the bottom inlet for varying piezo voltage U_{pz}. (a) For $U_{pz} = 0$ V, 15 V, 32 V and 55 V is shown pictures of a pair of 2 mL vials filled by the top/bottom outlet, respectively. For 0 V all red blood cells (RBCs) exit through the bottom outlet. As the voltage is increased, the acoustophoretic force increases proportional to U_{pz}^2 and the amount of RBCs exiting through the top outlet also increases. At $U_{pz} = 55$ V almost all RBCs exit through the top outlet without diluting the plasma at the bottom outlet. (b) Microscope pictures of red blood cells and platelets in drops of sample drawn from the vials filled by the top/bottom outlets at $U_{pz} = 0$ V and 55 V, respectively.

separation degradation from acoustic streaming near the flow divider [9], we sweep the frequency linearly in 1-ms periods from 10 kHz below to 10 kHz above the center frequency [3].

To test the ability of our device to separate blood samples, we mixed a saline solution of human red blood cells and platelets with concentrations typical to that of whole blood. In Fig. 3 is shown the preliminary result of acoustophoretic separation of our blood mix at a flow rate of 0.5 L/h through one inlet and a pure PBS buffer at a flow rate of 3 L/h through the other inlet for increasing piezo voltage and using the above mentioned frequency sweep around the resonance frequency f_{center} = 899 kHz at a constant temperature of 23 °C. From the pictures in panel (a) of the sequence of 2-mL vials filled from the top/bottom outlet, respectively, clear indications are seen of high-purity separation of red blood cells in our high-throughput acoustophoretic separator chip are seen. This is further supported by the microscope pictures in panel (b) of the top/bottom outlet without and with acoustic actuation for a piezo voltage of 0 V and 55 V, respectively.

Using our orthogonal-direction device geometry we have demonstrated very high throughput acoustophoretic separation of blood components at whole blood concentrations at a sample flow rate of about 1 L/h. This high throughput has been achieved by combining frequency sweep with temperature control of the piezo transducer to generate a sufficiently strong acoustophoretic force.

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CONTACT

*H. Bruus, TEL: +45 4525 6399; E-MAIL: Henrik.Bruus@nanotech.dtu.dk; URL: www.nanotech.dtu.dk/microfluidics