MEASURING DENSITY AND COMPRESSIBILITY OF WHITE BLOOD CELLS AND PROSTATE CANCER CELLS BY MICROCHANNEL ACOUSTOPHORESIS

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ABSTRACT

We present a novel method for the determination of density and compressibility of individual particles and cells undergoing microchannel acoustophoresis in an arbitrary 2D acoustic field. Our method is a critical advancement within acoustophoretic separation of biological cells, as the ability to determine the density and compressibility of individual cells enables the prediction and alteration of the separation outcome for a given cell mixture. We apply the method on white blood cells (WBCs) and DU145 prostate cancer cells (DUCs) aiming to improve isolation of circulating tumor cells from blood, an emerging tool in the monitoring and characterizing of metastatic cancer.

KEYWORDS: Micro-PIV, Ultrasound Resonances, Acoustophoresis, Cell Handling

INTRODUCTION

Microchannel acoustophoresis is a rapidly expanding research field allowing gentle and efficient manipulation of cells and other biological particles [1, 2]. We report a new method on how to determine the density and compressibility of individual particles and cells undergoing microchannel acoustophoresis in an arbitrary 2D acoustic field. The method is a generalization of our previously reported calibration of the acoustophoretic contrast factor of cells in a transverse 1D acoustic standing wave [3, 4]. This type of analysis paves the way for elaborate tailoring of cell medium, having acoustic properties suitable for a specific separation task. In this work the method was applied to assess the acoustic properties of WBCs and DUCs, previously reported to be amenable to separate using acoustophoresis [5].

The idea behind the method is to obtain a detailed calibration of the 2D acoustic field by measuring the acoustophoretic velocity fields for two types of calibration particles of known density and compressibility. After calibration, the size and acoustophoretic mobility of individual cells is analyzed, which in combination with the calibrated acoustic field yield the cell's density and compressibility.

THEORY

In previous work on microchannel acoustophoresis [1, 2, 3, 4, 5], the special case of a 1D transverse standing wave has been studied. Unfortunately, using this approach, both density ρ_{p} and compressibility K_{p} of a given cell enter the expression for the acoustophoretic radiation force $\boldsymbol{F}^{\mathrm{rad}}$ so that they cannot be fully determined. Instead one must work with the so-called 1D acoustophoretic contrast factor Φ. Also, an ideal 1D standing wave is difficult to realize experimentally. Here, we therefore use the expression for $\boldsymbol{F}^{\mathrm{rad}}$ on a particle of volume V in an arbitrary acoustic field with pressure p_1 and oscillation velocity $\boldsymbol{v}_1$ [6],

$$\boldsymbol{F}^{\mathrm{rad}} = -V\left[\frac{1}{2}f_1 K_0 \boldsymbol{\nabla}\langle p_1^2\rangle - \frac{3}{4}f_2 \rho_{\mathrm{o}} \boldsymbol{\nabla}\langle v_1^2\rangle\right], \tag{1}$$

where ρ_{o} and K_0 are the density and compressibility of the buffer medium, respectively, and the two prefactors $f_1 = 1 - K_{\mathrm{p}}/K_0$ and $f_2 = 2(\rho_{\mathrm{p}} - \rho_{\mathrm{o}})/(2\rho_{\mathrm{p}} + \rho_{\mathrm{o}})$ dictates the direction and magnitude of the force. The 1D contrast factor is given by $\Phi = f_1/3 + f_2/2$.

EXPERIMENTAL

The experimental setup is sketched in Fig. 1(a) showing the acoustophoresis microchip in the automated temperature-controlled micro-PIV setup capable of obtaining high-accuracy acoustophoretic velocity fields [7, 8]. The acoustophoresis microfluidic chip is shown in Fig. 1(b) and consists of a straight rectangular microchannel (35 mm $\times$ 377 µm $\times$ 157 µm) etched in a piece of $\langle 100\rangle$-silicon (35 mm $\times$ 2.52 mm $\times$ 350 µm). A piece of pyrex glass (35 mm $\times$ 2.52 mm $\times$ 1.13 mm) bonded to the silicon seals the channel. A piezoceramic transducer glued to the back of the chip actuates the ultrasound and the temperature is regulated to 25 ℃ using a Peltier element and a Pt100 thermoresistive sensor.

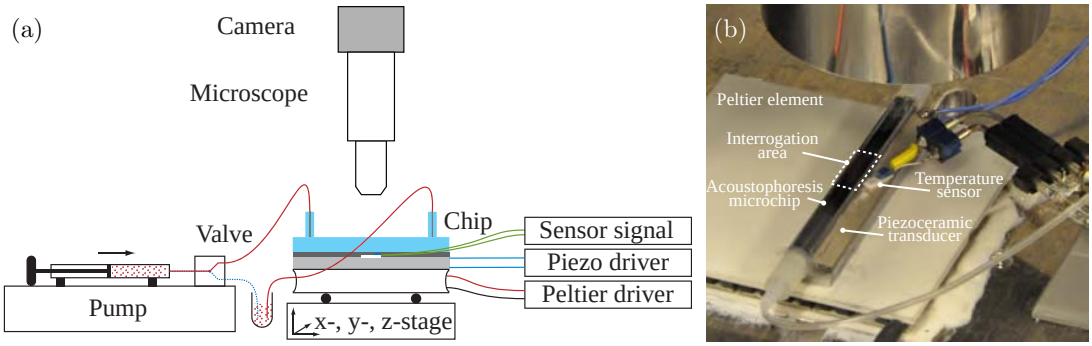

Figure 1: (a) Schematic of the automated temperature-controlled micro-PIV setup. (a) Photograph of the acoustophoresis silicon/glass straight-channel microchip in the micro-PIV setup.

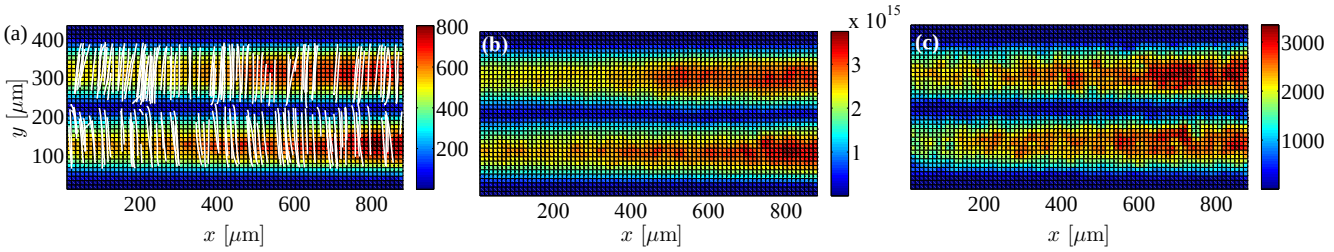

Figure 2: Calibration of the 2D acoustic field by PS and MR microbeads: (a) Colorplot of the acoustophoretic velocity magnitude u [μm/s] superimposed with plots of the individual WBC tracks (white lines). Colorplot of the magnitude of (b) the derived gradient field $\boldsymbol{\nabla}\langle p_1^2 \rangle$ [Pa^2 m^{-1}] and (c) the derived gradient field $\boldsymbol{\nabla}\langle v_1^2 \rangle$ [m s^{-2}].

All samples were prepared in phosphate buffered saline (PBS). Suspensions of calibration microparticles of either polystyrene ($d = 5.16$ μm) or melamine resin ($d = 4.83$ μm) were prepared at a concentration of ~ 3 g/L. Cells were fixated in paraformaldehyde (PFA) and resuspended in PBS prior to the experiment. WBCs from healthy donors were prepared from red-blood-cell-lysed whole blood and DUCs were cultured at 37 ℃ in a humidified atmosphere with 5% CO_2, according to ATTC recommendations[1].

The velocity data was acquired by injecting bead- or cell-samples into the microchannel, stopping the flow temporarily, and activating the ultrasound, while a camera recorded the acoustophoretic motion of the beads or cells. For the calibration beads, only a few frames were recorded from each focus experiment, but in order to get sufficeint statistics for the velocity field analyses, the focusing experiments were repeated 100 times. For the cells, the full focusing to the channel vertical center plane were recorded to get the full cell trajectories. The velocity fields of the bead suspension was derived by performing average correlation PIV with central difference window shifting in an iterative scheme. The motion of cells were analyzed using a multi-frame correlation based particle tracking method that also evaluates the size of each individual cell.

RESULTS AND DISCUSSION

In Fig. 2(a) is shown the acoustophoretic velocity field magnitude u (colorplot) for the PS calibration particles superimposed with plots of the individual WBC tracks (white lines). From the PS- and MR velocity fields $\boldsymbol{u}$ we obtained detailed measurements of the radiation force field $\boldsymbol{F}^{\mathrm{rad}} \propto \boldsymbol{u}$ and we determined the gradient fields $\boldsymbol{\nabla}\langle p_1^2 \rangle$ and $\boldsymbol{\nabla}\langle v_1^2 \rangle$ shown in Fig. 2(b) and Fig. 2(c), respectively. From the cell tracks and the calibrated gradient field, we determined the prefactors f_1 and f_2 for each cell, and from that we could derive the individual density and compressibility. In Fig. 3 is shown the measured distributions of volume, density, and compressibility of the two cell lines based on a set of cell tracks of 182 WBCs and 202 DUCs.

CONCLUSION

The obtained results on the density and compressibility of cells in suspension pave the way to further enhance the separation quality by controlled alteration of the suspending medium. Such tuning of the medium may enhance the differences in $\boldsymbol{F}^{\mathrm{rad}}$ between the different cells, and it can even lead to a subset of the DUCs undergoing acoustophoresis with negative sign of $\boldsymbol{F}^{\mathrm{rad}}$ enabling binary separation of them from WBCs.

[1]ATTC: The Global Bioresource Center (`http://www.lgcstandards-atcc.org/`

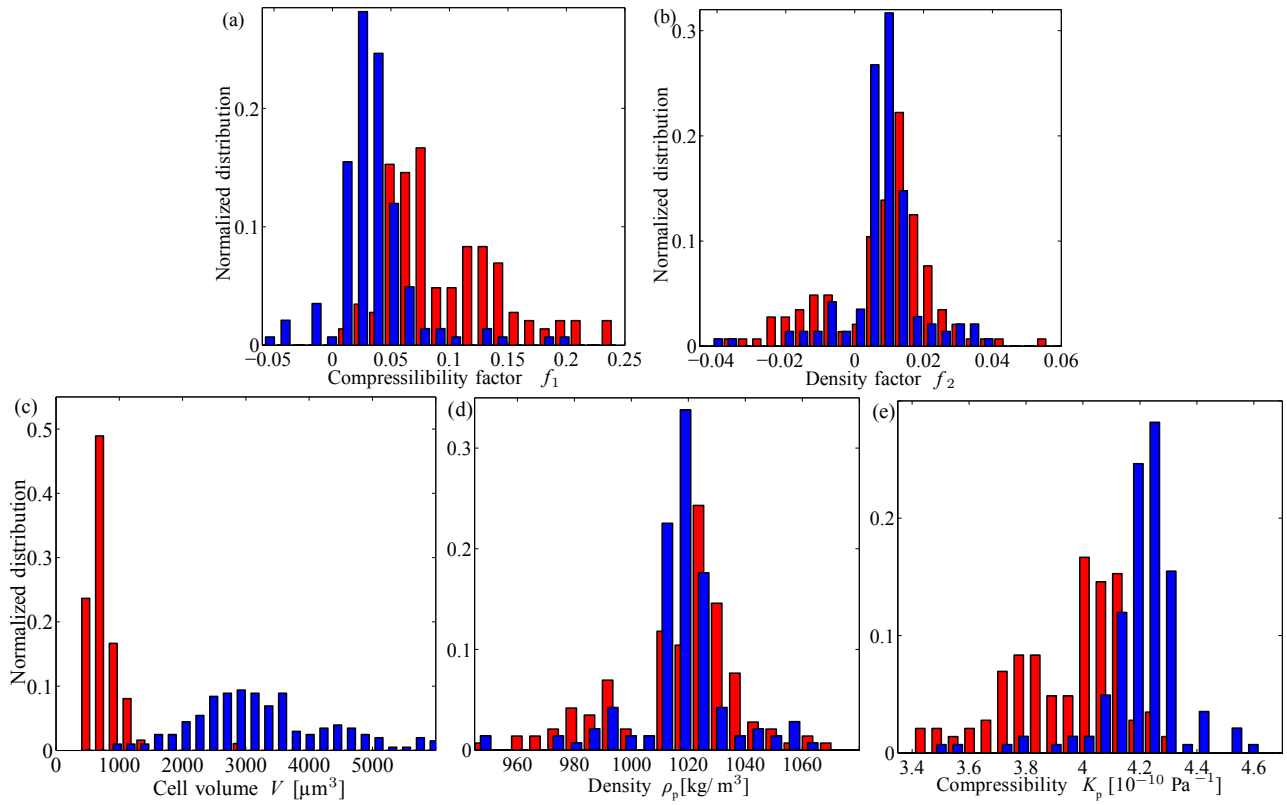

Figure 3: Cell measurements: Normalized distributions of WBCs (red bars) and DUCs (blue bars) as function of (a) compressibility factor f_1, (b) density factor f_2, (c) cell volume V, (d) density ρ_p, and (e) compressibility K_p.

Table 1: Summary of the acoustical parameters for the calibration particles and the measured cells.

Type	ρ_p [kg m^{-3}]	K_p [Pa^{-1}]	f_1	f_2	Φ
PS	1050	1.72×10^{-10}	0.61	0.03	0.22
MR	2730	8.89×10^{-11}	0.80	0.25	0.39
WBCs	1019 ± 1	$(3.995 \pm 0.012) \times 10^{-10}$	0.0816 ± 0.0028	0.0091 ± 0.0007	0.0318 ± 0.0010
DUCs	1018 ± 1	$(4.239 \pm 0.006) \times 10^{-10}$	0.0254 ± 0.0013	0.0086 ± 0.0004	0.0128 ± 0.0005

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