MEASURING THE ACOUSTOPHORETIC CONTRAST FACTOR OF LIVING CELLS IN MICROCHANNELS

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ABSTRACT

We report a new method, which allows for accurate measurement of the acostophoretic contrast factor Φ of different cell types, an acousto-physical parameter of fundamental importance in microchip acoustophoresis. As a test case the Φ factor is measured for undifferentiated and four-days differentiated cells from a human embryonic ventral mesencephalic cell line. The measured cell Φ factors are distributed around 0.04 and 0.07 for the two cell types, respectively. Despite a close acoustic similarity, the two cell populations are shown to be separable by acoustophoresis.

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

INTRODUCTION

The combination of ultrasonic standing waves and microfluidics is a field in rapid growth, as microsystem acoustophoresis is used in an increasing number of devices to obtain easy-applicable, gentle and label-free manipulation/separation of particles and biological cells [1–6]. An essential parameter in microchip acoustohoresis is the acoustophoretic contrast factor Φ determining the acoustic contrast between cells and their surrounding medium. As the acoustophoretic force scales linearly with cell volume and contrast factor, the latter can be used to distinguish and separate various cell populations. Measuring the acoustophoretic contrast factor is central in further advancement of acoustophoretic separation, and prior to this work there has been no systematic measurements of Φ. In this work we present a new method for accurate determination of the Φ factor assisted by the recent breakthrough in measuring the absolute acoustic energy density in microchannels presented by Barnkob, Augustsson, Laurell, and Bruus [7]. As a test case we present measurements on a living human embryonic ventral mesencephalic cell line (MESC2.10) [8]. When the cells are differentiated in special medium for 4 days (MESC-diff4d), the cells will adopt a mature neuronal phenotype with robust dopaminergic characteristics (eg. express tyrosine hydroxylase and dopamine transporter). In contrast to manufactured microbeads all having identical Φ factors, a distribution of Φ is expected for a given cell type due to biological variations between individual cells. The goal is to find out if undifferentiated and differentiated cells display differences in acoustic contrast factor that will allow for acoustophoretic separation.

THEORY

Microchip acoustophoresis uses the so-called acoustophoretic radiation force $\boldsymbol{F}_{\mathrm{ac}}$ to move particles/cells in a liquid environment toward pressure nodes of a standing ultrasonic resonance field. The acoustophoretic radiation force on a compressible and spherical particle in an inviscid liquid was admirably summarized and generalized in 1962 by Gorkov [9], the result being the gradient force $\boldsymbol{F}_{\mathrm{ac}} = -\boldsymbol{\nabla}U_{\mathrm{ac}}$, where U_{ac} is the acoustic potential. In the special case of a plane pressure wave with wave vector k_y and amplitude p_a of the form $p(x, y, z) = p_\mathrm{a} \cos{(k_y y)}$, the radiation force reduces to the one-dimensional version F_y^{ac} in the transverse direction y previously calculated by Yosioka and Kawasima in 1955 [10],

$$F_y^{\mathrm{ac}} = 4\pi a^2 (k_y a) E_{\mathrm{ac}}\, \Phi\, \sin{(2k_y y)}, \tag{1}$$

where $4\pi a^2$ is the cell surface area, $k_y a = 2\pi a/\lambda_y$ is the size-to-wavelength ratio, and E_{ac} is the acoustic energy density. The acoustophoretic contrast factor Φ is given by

$$\Phi = \frac{\rho_\mathrm{p} + \frac{2}{3}(\rho_\mathrm{p} - \rho_0)}{2\rho_\mathrm{p} + \rho_0} - \frac{1}{3}\frac{\rho_0 c_0^2}{\rho_\mathrm{p} c_\mathrm{p}^2}. \tag{2}$$

The above definition of Φ follows the original work by King [11], Yosioka/Kawasima 1955 [10], and Gorkov [9], but has in other parts of the literature been defined as 3Φ [1,7], such that the cell volume $V = \frac{4}{3}\pi a^3$ explicitly appears in Eq. (1). The one-dimensional acoustophoretic motion of a particle/cell of radius a moving with velocity u_y in a liquid of viscosity η is characterized by the (nearly perfect) balance between the acoustophoretic radiation force F_y^{ac} and the Stokes drag force $F_y^{\mathrm{drag}} = -6\pi\eta a u_y$. From this force balance the particle/cell velocity u_y can be expressed as a function of E_{ac} and k_y,

$$u_y(y) = \frac{2a^2 k_y E_{\mathrm{ac}}}{3\eta}\, \Phi\, \sin{(2k_y y)}, \tag{3}$$

and by time-integration of u_y, the position $y(t)$ can be found. In terms of E_{ac}, k_y, the time t, and the position $y(0)$ at time $t = 0$ it becomes

$$y(t) = \frac{1}{k_y} \arctan\left\{\tan\left[k_y y(0)\right] \exp\left[\frac{4\Phi}{3\eta}(k_y a)^2 E_{\mathrm{ac}}\, t\right]\right\}. \tag{4}$$
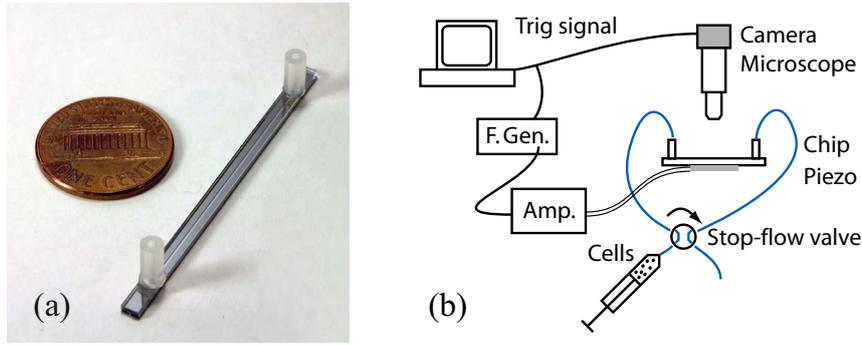
Figure 1: (a) A silicon/glass chip containing a straight microchannel of length 40 mm, width 377 μm, and height 157 μm. Vertical inlet/outlet tubings are seen at the ends of the channel. (b) Schematic of the micro-PIV and acoustophoresis setup. Before each measurement the stop-flow valve is turned to ensure a vanishing pressure drop along the channel.
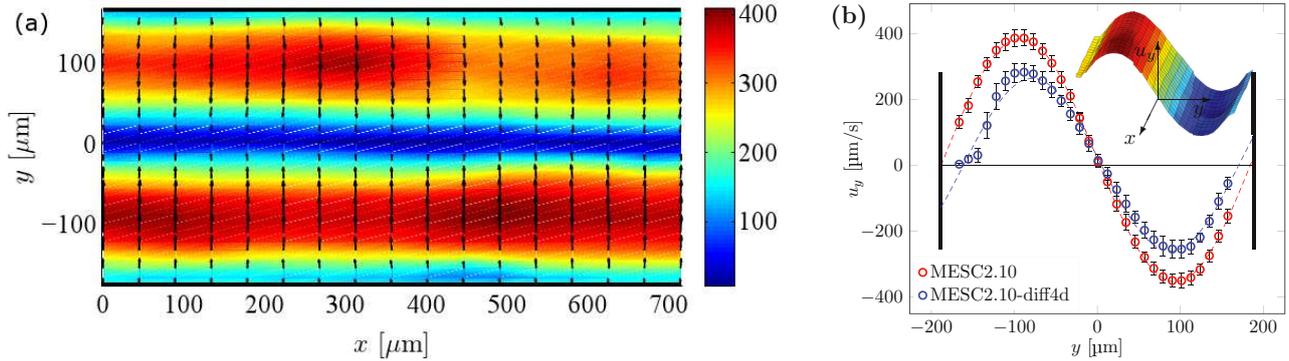

Figure 2: In situ calibration measurements of the acoustic field using polystyrene (PS) microbeads. (a) Colorplot of the average speed $|\mathbf{u}|$ and vectorplot of the average velocity field $\mathbf{u}$ at the 1.970-MHz-resonance of PS microbeads during acoustophoretic motion. (b) Average of the measured y-component u_y of the velocity (circles) and the standard deviation (error bars) for the PS microbeads calibrating the two cell-type experiments. The resonance parameters E_{ac} and λ_y are extracted from the fit (dashed line). The fit of the wavelength agrees well with the expected match to the width w between the channel walls (thick lines). The inset shows a surface plot of $u_y(x,y)$ calibrating the MESC2.10 cell type experiment.

EXPERIMENTAL

We use the silicon/glass chip from Ref. [7] containing a single straight microchannel, see Fig. 1(a). Upon actuation at a frequency $f = 1.970\,\mathrm{MHz}$ and piezo voltage $U_{\mathrm{pp}} = 1.0\,\mathrm{V}$, the microchannel supports a strong 1D transverse half-wavelength acoustic resonance. The acoustophoretic force F_y^{ac} focuses the particles and cells in the solution to the vertical center plane of the channel. The channel is initially filled with a solution of 5 μm polystyrene (PS) microbeads of known acoustophoretic contrast factor Φ_{PS}. To determine F_y^{ac} *in situ* we first use a full-image micron-resolution particle image velocimetry (micro-PIV) setup, see Fig. 1(b), to measure the acoustophoretic 2D velocity field $\mathbf{u}(x,y)$ of the PS microbeads, see Fig. 2(a), which shows that in the field-of-view, the velocity field is close to the ideal 1D transverse resonance form in Eq. (1). We then insert the average u_y of the y-component in the force balance Eq. (3) to determine the magnitude of the acoustic energy density E_{ac} and the transverse wavelength λ_y as in Ref. [7]. This measurement constitutes the *in situ* calibration of the acoustic field. Subsequently we fill the channel with cells, measure their radius a optically, and fit each measured trajectory $y(t)$ in Eq. (4) to determine individual acoustophoretic contrast factors Φ.

RESULTS AND DISCUSSION

Fig. 2(b) shows the average and standard deviation of the transverse acoustophoretic velocities u_y for the PS beads along the microscope field-of-view. The velocities u_y align well with the 1D sine expression in Eq. (3), and the acoustic energy density is calibrated to $(44.1 \pm 0.2)\,\mathrm{J/m^3}$ and $(27.9 \pm 0.8)\,\mathrm{J/m^3}$ for the MESC2.10 and MESC-diff4d cell population experiments, respectively. The inset in Fig. 2(b) shows a surface plot of $u_y(x,y)$ for the MESC2.10 cell type experiment as that in Fig. 2(a). Fig. 3(a) shows an image of the MESC-diff4d cells undergoing the acoustophoretic motion towards the center of the channel. For each cell of measured radius a and trajectory $y(t)$, the acoustophoretic contrast factor Φ is extracted. The measured cell Φ factors are distributed around 0.04 and 0.07 for the MESC2.10 and MESC-diff4d cells, respectively. The Φ distributions for the two cell populations are shown in Fig. 3(b).

In Table 1 all measured parameter values for the two cell type experiments are summarized.

CONCLUSION

We have introduced a new method for accurate measurement of acoustophoretic contrast factor Φ. The method has been
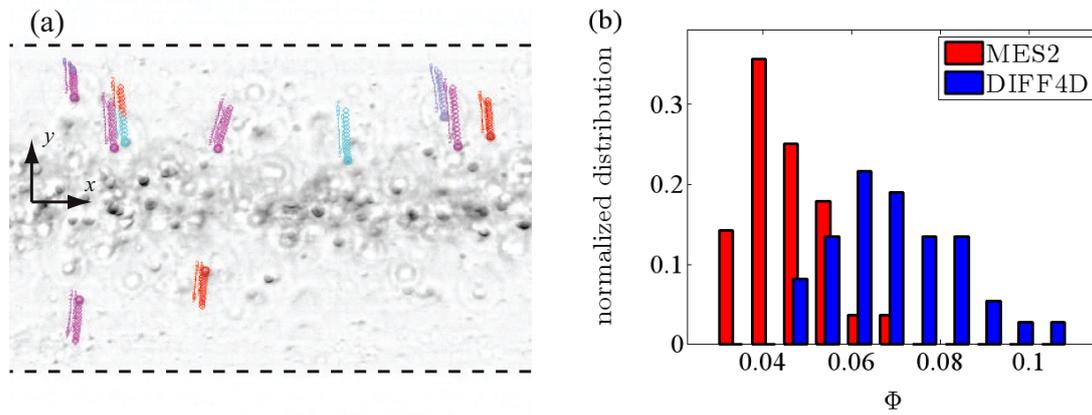
Figure 3: Cell experiments. (a) Image of the MESC-diff4d cells undergoing acoustophoresis. Each cell is individually marked (colored circles, about 20 data points for each track) and their radius a and transverse trajectory $y(t)$ are measured. (b) Distributions of the extracted acoustophoretic contrast factors Φ for the two cell populations, MESC2.10 (red, 28 cells) and MESC-diff4d (blue, 37 cells), respectively, obtained from the trajectories $y(t)$.

Table 1: Measured parameters for the two cell populations MESC2.10 and MESC-diff4d.

Parameter	Symbol	MESC2.10	MESC-diff4d
Temperature	T	26.9 °C	25.8 °C
Acoustic energy density	E_{ac}	(44.1 ± 0.2) J/m^3	(27.9 ± 0.8) J/m^3
Transverse wavelength	λ_y	(372.6 ± 1.2) μm	(331.8 ± 4.8) μm
Cell radius	a	$5.9 - 7.3$ μm	$5.2 - 7.0$ μm
Acoustophoretic contrast factor	Φ	$0.03 - 0.07$	$0.05 - 0.11$

tested on two cell populations and demonstrated that they despite a close acoustic similarity are nevertheless separable due to different distributions of Φ factors.

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