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Tunable and Label-Free Bacteria Alignment Using Standing Surface Acoustic Waves

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Abstract— **This paper describes a new technique for focusing bacteria in a microfluidic channel and subsequently controlling their trajectory. Bacteria alignment is obtained using standing surface acoustic waves (SSAW) generated by two interdigitated transducer electrodes (IDTs) patterned on a piezoelectric wafer. The bacteria are focused in the standing wave pressure nodes, separated by half a wavelength, the electrode geometry and applied voltage frequency being chosen accordingly. Interestingly, the position of a pressure node can be modulated by introducing a phase shift between the electrical signals applied to both IDTs. The bacteria, trapped in this node, follow it and can therefore be deflected. This technique works with label-free bacteria in their culture medium and induces low power consumption, which is very interesting for portable devices.**

I. Introduction

Biologists are very interested in manipulating cells, and sorting them depending on their size, composition or biological function. In the field of contactless particle manipulation, four methods are widely used in microfluidic devices, namely dielectrophoresis [1], magnetophoresis [2], optical trapping [3] and acoustophoresis [4]. Dielectrophoretic techniques are not well suited to the manipulation of cells in highly conductive culture media, because of possible adverse effects due to Joule heating and because positive dielectrophoresis can not be observed in such media. Magnetic techniques require cells to be labeled with magnetic particles, which is not suitable in applications where the cell should remain unmodified. Optical tweezers enable single cell manipulation with high 3D precision but they are not adapted to the simultaneous handling of a large cell number and usually require a more complex setup. That leads us to acoustic manipulation. This technique can be used with label-free cells and theoretically in all kind of media. Moreover, the long-range effects of acoustic forces can be exploited to manipulate many particles simultaneously. Two main ways of generating acoustic waves are described in the literature. Some use bulk acoustic waves (BAW): a piezoelectric plate vibrates and initiates a wave amplified in a microchannel acting as a resonant cavity [5], [6]. The drawback of this technique is that the channel geometry imposes the boundary conditions and thus the position of the nodes and anti-nodes of the resulting wave, making the movement of those nodes impossible. Besides, in [7], Shi *et al.* have pointed out that with their poor reflection coefficient, soft polymers as polydemethylsiloxane (PDMS), widely used in microfabrication were not suited to BAWs. To avoid this, surface acoustic waves (SAW) can be used [8–10]. Electrodes are deposited on a piezoelectric substrate, on which a channel is bonded (Fig. 1). As a result, the SAW travels on a surface that is also a side of the channel, and the acoustic energy is transferred to the fluid. We have chosen this technique which also has the advantage of a lower electric consumption. To our knowledge, until recently, the possibility to move laterally a focused particle stream was not exploited. As explained above, the use of BAW intrinsically imposes the stream position. Moreover, in past studies involving SAW devices, the same electrical signal was applied to both IDTs, which prevented phase-control of particle trajectories. Yet, in a recent study, Orloff *et al.* [10] have shown that moving latex beads trapped in a pressure node was possible by moving this node playing on the phase between the electrical signals applied to the IDTs. In this paper, we demonstrate that we can focus bacteria in a microfluidic channel and control their trajectory, which can be useful in applications such as microflow cytometry or medium exchange.

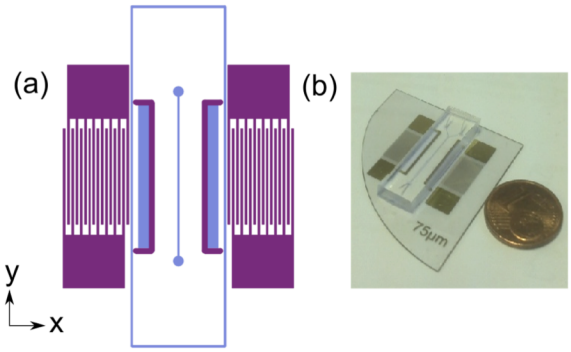

Figure 1. (a) Electrodes, microchannel and alignment marks, (b) An exemple of acoustofluidic device

II. Theory

Once in the microchannel, bacteria experience several forces. Buoyancy and gravity are balanced since they have almost the same magnitude but are opposite in direction (z direction). In the y direction, bacteria are driven by the fluid flow from an inlet to the outlet but do not experience any force. Finally, in the lateral direction (x), we have two

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forces: the acoustic force we created and the viscous force, resulting from the bacteria displacement. The expression of the primary acoustic force (F_r) in a SSAW on a particle [11], [12] and the viscous force (F_v) are given by:

$$F_r = -\left(\frac{\pi {p_0}^2 V_p \beta_m}{2\lambda}\right)\phi(\beta,\rho)\, sin(2kx_0) \quad (1)$$

$$\phi(\beta,\rho) = \frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m} \quad (2)$$

$$F_v = -6\pi\eta r v \quad (3)$$

where p_0, V_p, λ, k, x_0 correspond to pressure amplitude, particle volume, ultrasonic wavelength, wave vector and distance from a pressure node. β_m, β_p, ρ_m, ρ_p are the respective compressibility and densities of the medium and particle, denoted by the subscripts "m" and "p". η, r and v represent medium viscosity, particle radius and velocity. Depending on their relative density and compressibility to the medium, particles will be pushed either to a pressure node or anti-node, which is denoted by the sign of the contrast factor Φ. In our case, bacteria have a positive contrast factor and move towards pressure nodes. Now, let examine where those pressure nodes are located. Two surface acoustic waves are generated at the same frequency and amplitude and travel in opposite direction. The resulting pressure field is given by:

$$P = \frac{p_0}{2}\cos(\omega t - kx + \Delta\varphi) + \frac{p_0}{2}\cos(\omega t + kx)$$
$$P = p_0 \cos\left(kx - \frac{\Delta\varphi}{2}\right)\cos\left(\omega t + \frac{\Delta\varphi}{2}\right) \quad (4)$$

where ω and Δφ correspond to the excitation frequency and initial phase shift between the two pressure fields. We can notice that it is similarly the initial phase-shift between the two displacement fields or electrical signals applied to the IDTs. The pressure nodes are located at x_0 verifying:

$$kx_0 - \frac{\Delta\varphi}{2} = \frac{\pi}{2} - n\pi \Leftrightarrow x_0 = \lambda\frac{\Delta\varphi}{720} + \frac{\lambda}{4} + \frac{n\lambda}{2} \quad (5)$$

where Δφ is expressed in degrees. The λ/4 factor is arbitrary because no origin on the x axis for the position has been chosen. Equation (5) tells us that pressure nodes are spaced λ/2 apart, and mostly that an initial phase shift of Δφ leads to a position shift of λ×Δφ/720 for the pressure node.

III. Device Design and Fabrication

The microfluidic device we present in this paper consists in two main components: a piezoelectric substrate on which a set of two interdigitated transducers (IDTs) are patterned and a microfluidic channel molded in PDMS (Fig. 1). The piezoelectric substrate is a 128° Y-cut lithium niobate ($LiNbO_3$) crystal, and the acoustic wave propagate along the X-axis. The operating frequency is directly linked to the finger spacing and the speed of sound in $LiNbO_3$. Fingers and fingers spacing of 75µm, which theoretically correspond to λ/4, lead to a frequency close to 13MHz. To produce the electrodes, we first patterned the $LiNbO_3$ substrate with positive photoresist (S1813, Shipley), spun at 4000 rpm for 30 s to obtain a thickness of about 2 µm. Following UV exposure (140 mJ/cm2), the resist was developed on its developer (S351, Shipley) for 60s. Then, we deposited 50nm of chromium as an adhesive layer and 150nm of gold with an e-beam evaporator (Leybold) before removing the photoresist and the metal attached with a lift-off process (Fig. 2). The PDMS microchannels were fabricated using standard soft-lithography [13]. First, a dry film photoresist (E9220, laminar) was laminated onto a glass slide, before being exposed to a UV source (75 mJ/cm2) through a plastic photomask. After development in 0.8 % sodium carbonate for 5 minutes, the resist was hard baked for a few seconds. PDMS base and its curing agent (Sylgard™ 184 kit, Dow corning) were mixed in a 10:1 weight ratio, poured on this mold, and then cured at 100°C for one hour. After drilling the inlets and outlets, surfaces on PDMS and LiNbO3 were activated with oxygen plasma (2 minutes at 20 sccm oxygen flow rate, 200 mTorr chamber pressure, 53W power). To ensure parallelism between the pressure nodes and the microchannel walls, proper alignment of the electrodes with those walls was required. A drop of ethanol was deposited between the two parts, acting as a lubricant and allowing the PDMS channel to slip on the piezoelectric substrate, until alignment was achieved. The remaining ethanol was

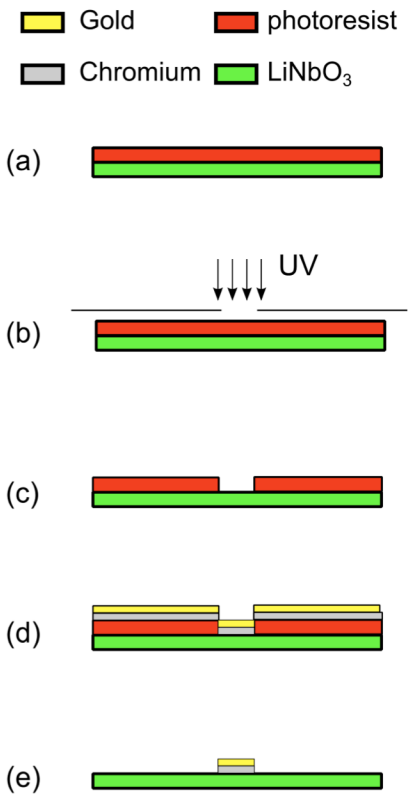

Figure 2. Electrodes deposition: (a) photoresist spincoating, (b) UV exposure, (c) development, (d) metal evaporation, (e) lift-off

evaporated by placing the chip in an oven for a few minutes (Fig. 3).

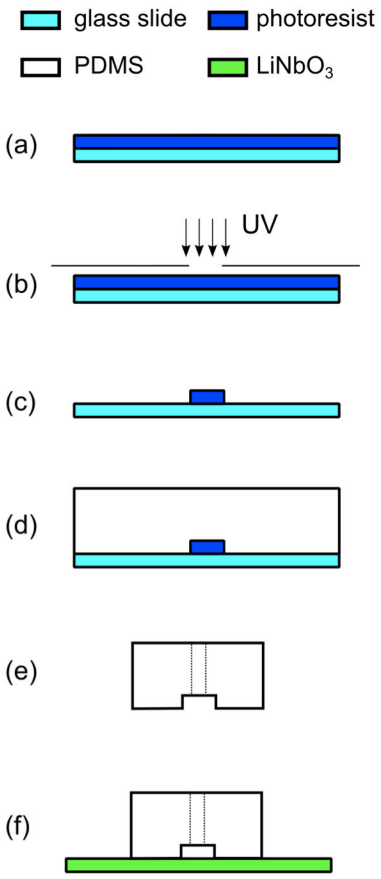

Figure 3. Channel fabrication: (a) photoresist lamination, (b) UV exposure, (c) Development, (d) PDMS molding, (e) PDMS peeling and holes drilling, (f) bonding of the channel to the LiNbO3

IV. Bacteria Sample Preparation

The bacteria used for the experiments were *Escherichia coli* (*E. coli*) DH5α cultivated in Luria Bertani (LB) medium overnight at 37°. Their final concentration was about 2×10^9 bacteria per milliliter.

V. System Setup

The acoustofluidic chip was placed on the stage of a microscope Nikon Eclipse LV150. *E.coli* cells in their culture medium or 3 µm polystyrene beads were injected in the channel through microbore tubing (S54HL, Tygon) with a microfluidic flow control system (MFCS-4C, fluigent). For all the experiments with bacteria, the pressure was set to 15mbar, which leads to a calculated flow rate of around 5 µL/min. With this flow rate, bacteria speed was slow enough to let them move toward a pressure node under acoustic force influence. For experiments with polystyrene beads, we stopped the flow. To drive the IDTs, two RF signals were created by two arbitrary waveform generators (Agilent 33250A) and amplified by two different RF amplifiers (Amplifier Research 25A250A). The two generators were synchronized and we could tune manually the initial phase

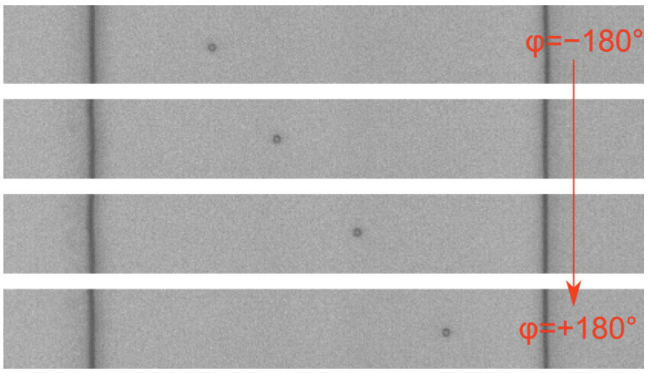

Figure 4. Phase control of a 3µm polystyrene bead, φ varying from -180° to 180°

of one of the signals. Their amplitude was kept constant at 20V peak-to-peak.

VI. Results and Discussions

First, to confirm our approach to manipulate bioparticles, we began to isolate a 3µm polystyrene bead in the middle of a 300µm microchannel. Varying the initial phase between −180° and +180° (Fig. 4), we recorded the bead trajectory. Thanks to ImageJ software, we could measure its mean amplitude. We found an amplitude of 148.9 µm. Theoretically, with a wavelength λ=300 µm and Δφ=360°

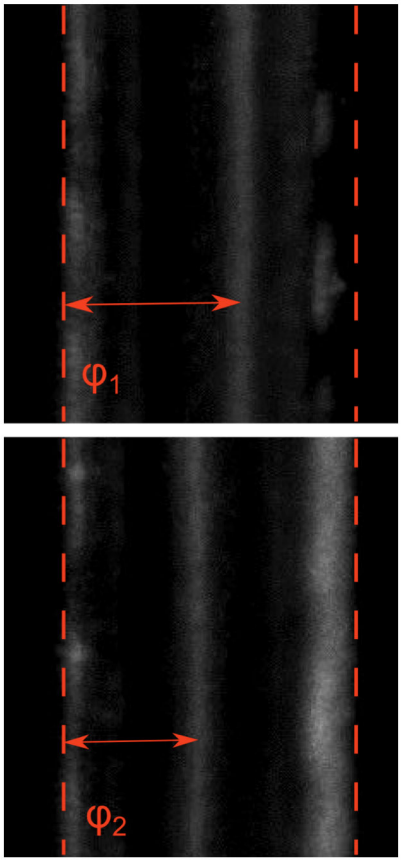

Figure 5. E. coli alignment for two different phases between the electrical signals

(5) leads to a total displacement of 150 µm. Those two values are very close, validating our theory.

Finally, we injected *E. coli* cells into a 150 µm wide microchannel this dimension being chosen to obtain only one pressure node inside. At a slow flow rate, bacteria quickly moved to the only pressure node in the channel and were trapped in it. Fig. 5 shows the alignment for two different initial phases. We can notice on the walls some bacteria that have not joined the others, which could be due to electrostatic interaction forces between them and the walls.

VII. Conclusion

We demonstrated in this paper the position control of a bacteria alignment with ultrasonic waves. This technique can be used to theoretically manipulate all kind of biological particles like eukaryote cells, bacteria, yeasts… Advantages are low power consumption and ease to use, no labeling is needed. By modulating the position of this alignment, cells can also be moved from one suspending medium to another, by exploiting laminar flows, which can be useful for filtering applications.

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