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Miniaturized acoustical tweezers, A Swiss Army knife for microfluidics?

A. Riaud^{a,b,c}, L. Jami^b, O. Bou Matar^a, J.-L. Thomas^b, Michael Baudoin^a

a. Institut d'Electronique, de Microélectronique et Nanotechnologie (IEMN), LIA LICS, Université Lille 1 and EC Lille, UMR CNRS 8520, 59652 Villeneuve d'Ascq, France, michael.baudoin@univ-lille1.fr

b. CNRS UMR 7588, UPMC Université Paris 06, Institut des NanoSciences de Paris (INSP), F-75005, Paris, France, jean-louis.thomas@upmc.fr

c. Université Paris Sorbonne Cité, INSERM UMR-S1147, CNRS SNC 5014, France. Equipe labellisée ligue contre le cancer, antoine.riaud@centraliens-lille.org

Résumé :

La manipulation de particules, de cellules, de gouttelettes ou de bulles est une opération clef dans de nombreux domaines tels que la biologie cellulaire, la biologie des micro-organismes, la microfluidique, le génie chimique ou encore les microsystèmes. En particulier, la manipulation sélective d'objets micrométrique ou nanométriques ouvre des perspectives considérables telles que la réalisation de FIV sans aiguille, l'immobilisation de micro-organismes et leur imagerie haute résolution, l'étude de la mécanotransduction, des interactions cellules-cellules, ou encore l'impression cellulaire ou l'assemblage de microsystèmes en phase aqueuses. Nous avons conçu récemment des pinces acoustiques sélectives, miniaturisées, plates, facilement intégrables et bas coût permettant de manipuler sans marquage préalable des particules avec des forces considérables par rapport aux technologies actuelles (un ordre de grandeur 5 fois plus important que les pinces optiques à puissance équivalente). Cette technologie permet donc de piéger des micro objets avec des puissances réduites, réduisant ainsi l'échauffement qui peut être nuisible la viabilité des organismes biologiques, l'une des principales limitations des pinces optiques. Ces pinces acoustiques reposent sur l'utilisation d'ondes acoustiques de surface tourbillonnaires créées par des transducteurs interdigités en spirale. Ces ondes acoustiques génèrent des vortex acoustiques dans le liquide qui permettent de piéger les particules au centre du vortex via la pression de radiation.

Abstract :

Manipulation of particles, cells, droplets or bubbles is a key operation in numerous areas of science including cell biology, microbiology, microfluidics, chemical engineering and microsystems. Especially, selective manipulation of micrometric or nanometric objects opens tremendous possibilities for needleless in vitro fecundation, micro-organism capture and subsequent high resolution imaging, mechanotransduction studies, cell-cell interactions, cellular bioprinting and microsystem assembly in aqueous media. We have designed miniaturized, flat, integration-ready and low-cost acoustical tweezers allowing a label-free manipulation. The forces applied on the particles are five order of magnitude above current optical tweezers technologies. Therefore, this system allows trapping micro-objects at lower power, minimizing the deleterious heating of biological organisms that has precluded the use of optical tweezing

for decades. These tweezers are based on swirling surface acoustic waves generated by spiral-shaped interdigitated transducers. These acoustic waves radiate into acoustic vortices in the liquid, which in turn enable the capture of particles at the center of the vortex by acoustic radiation pressure.

Mots clefs : Pince acoustique, onde acoustique de surface, biomécanique, fabrication.

1 Introduction

Contactless manipulation of microscopic objects such as delicate biological cells is a considerable challenge for regenerative medicine and *in situ* micro-assembly technologies. Among various physical methods, the manipulation of a target object in a cluster of identical ones (selective manipulation) is generally achieved by the mean of optical tweezers. Optical radiation pressure \mathcal{P} is proportional to the sum of the impulsion of all the photons: $\mathcal{P} \propto I/c$ where I is the intensity of the electromagnetic wave and c is its celerity in the manipulation medium. Thus, photons yield a very small impulsion at each impact and optical tweezers require very intense light flux that jeopardize the cells viability due to overheating.

Acoustic waves can also apply a radiation pressure. Thanks to their low speed (relatively to light), acoustic waves momentum exceeds the one of light waves by five orders of magnitude at identical power flux. Despite ensuring cell viability [17], acoustical tweezers face a major pitfall when it comes to manipulate denser or stiffer objects than the ambient medium: a focused beam ejects any particle in its path. Indeed, the acoustic radiation force \mathcal{F} exerted by a standing field \tilde{p} on a small particle of volume V_p and density ρ_p immersed in a fluid of density ρ_f is given by [5]:

$$\mathbf{F} = -\nabla\mathcal{U}, \quad (1)$$

$$\mathcal{U} = V_p \left[f_1 \langle \mathcal{V} \rangle - \frac{3}{2} f_2 \langle \mathcal{K} \rangle \right], \quad (2)$$

$$f_1 = 1 - \frac{\rho_f c_f^2}{\rho_p (c_l^2 - \frac{4}{3} c_t^2)} \quad (3)$$

$$f_2 = 2 \frac{\rho_p - \rho_f}{2\rho_p + \rho_f}, \quad (4)$$

where \mathcal{U} is the Gor'kov potential, $\mathcal{V} = \frac{1}{2} \frac{|\tilde{p}|^2}{\rho_f c_f^2}$ and $\mathcal{K} = \frac{1}{2} \rho_f |\tilde{v}|^2$ are respectively the fluid kinetic and potential energies. c_f is the wave velocity in the fluid, c_l and c_t are the longitudinal and transverse sound speed in the particle, and $\tilde{v} = -\frac{\nabla \tilde{p}}{i\omega \rho_f}$ is the velocity field of the wave with ω the pulsation frequency and $i^2 = -1$. The monopolar contribution $f_1 \langle \mathcal{V} \rangle$ represents the buoyancy force acting on a sphere which volume doesn't change at the same pace as the fluid. The dipolar contribution $f_2 \langle \mathcal{K} \rangle$ represents the delayed dynamics of the particle center of mass relatively to the fluid motion. When the particle is harder ($f_1 > 0$) and denser ($f_2 < 0$) than the fluid, the Gor'kov potential is minimum at the pressure nodes. Thus, trapping stiffer and heavier particles than water (including biological cells [6]) requires a pressure node surrounded by a maximum amplitude region.

This problem remained unsolved until the mid 2000'th when Marston [10] suggested using helical acoustical beams called acoustic vortices to stabilize the particles along the beam axis. Indeed, these helical wavefronts twist around a zero-intensity axis where the wave self-interferes destructively. This axis is surrounded by a high intensity ring that maintains the trapped particle at the center of the beam. This

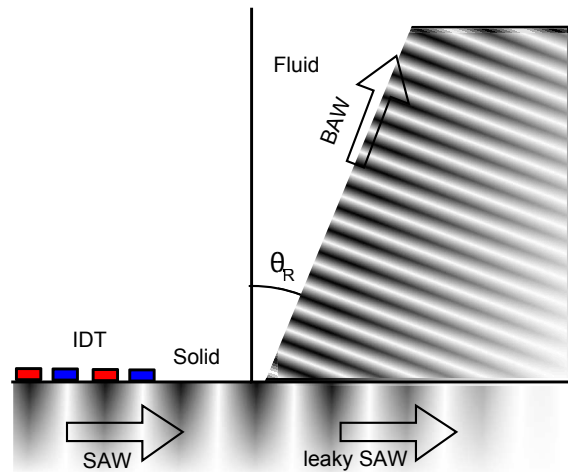


Figure 1: Generation of bulk acoustic waves from interdigitated transducers. The interdigitated electrodes generate a surface acoustic wave that travels until reaching a liquid in which it radiates along the Rayleigh angle θ_R . Beneath the liquid, the leaky SAW is attenuated with a few tens of wavelengths.

theoretical solution has been recently implemented in 2D [2] and 3D experiments with focused acoustic vortices [1]. However, helical wavefront synthesis remains challenging and requires either a high-end programmable electronics [1, 2, 15] or a tridimensional pattern [8, 11]. As the fundamental working principle of acoustical tweezers has been clearly established, our work aims to address the issues of miniaturization and possibly mass production of acoustical tweezers.

An alternative method to synthesize ultrasonic waves is using interdigitated transducers (IDTs). These devices are patterned by photolithography and are therefore inherently suitable for miniaturization and mass production. However, IDTs have only been used to generate plane waves [3, 16] and in very few cases focused waves [9]. In this paper, we present a low-cost miniaturized spiral-shaped integrated transducer. Placing a suspension of particles above the latter, we achieve contactless manipulation of patterning of dozens of individual particles. The manipulation of biological particles and the creation of vorticity are also demonstrated.

2 Methods

2.1 Physical principle

Interdigitated transducers are interlocked arrays of electrodes patterned on a piezoelectric substrate (see Fig. 1). Charged by an alternating current of pulsation ω , the piezoelectric substrate vibrates and generates mechanical waves. In the following, we will focus on the surface acoustic waves (SAWs) guided by the solid surface, also called Rayleigh waves. Provided the spatial period of the IDT matches the wavelength of the wave that at the excitation frequency, the transducer becomes resonant and emits a beam of SAWs of amplitude $\Xi = Na\Phi$ with N of finger pairs of the IDT, a the piezoelectric coupling (in m/V) and Φ the excitation voltage amplitude. Then, these waves propagate with a velocity c_{SAW} until they meet a fluid in which they radiate into bulk acoustic waves (BAWs). Indeed, the normal vibrations of the solid are transmitted to the fluid, which damps the SAW within a few tens of wavelengths. The

BAW radiates in fluid along the Rayleigh angle:

$$\sin(\theta_R) = c_f/c_{SAW}, \quad (5)$$

where c_f is the velocity of the BAW in the liquid.

All the piezoelectric substrates are anisotropic. Thus, for a given propagation direction ψ , we have $c_{SAW}(\psi)$ and $a(\psi)$. Since acoustic vortices were only defined for isotropic media, we use a generalized expression derived in our previous work [15]:

$$\mathcal{W}_l(r, \theta) = \frac{1}{2\pi i^l} \int_{-\pi}^{\pi} e^{-il\psi - i\omega s_{SAW}(\psi)r \cos(\psi-\theta)} d\psi, \quad (6)$$

where \mathcal{W}_l is the swirling SAW vibration amplitude at the cylindrical coordinates (r, θ) , $s_{SAW} = 1/c_{SAW}$ is the SAW slowness and l is the acoustic vortex topological order. When the medium is isotropic, the integral definition of the Bessel function simplifies Eq. (6) into $W_l = J_l(\omega s_{SAW} r) e^{-il\theta}$.

A remarkable effect of the anisotropy is that the Rayleigh angle now depends on the direction of propagation. This variable Rayleigh angle distorts the helical wavefront and ultimately provokes the beam degeneration [14].

2.2 Transducer geometry

In the following, we will assume that the transducer is placed beneath a stack of n isotropic substrates of slowness $s^{(i)} = 1/c^{(i)}$. This may include fluids such as the coupling agent or water, and solids such as the glass coverslip. Swirling SAWs can be synthesized either with a programmable electronics [15] or a spiral-shaped integrated transducer. According to Riaud *et al.* [12], the electrodes of the integrated transducer run along a curve given by Eq. (7):

$$R(\theta) = \frac{\psi_0 + \omega\mathcal{T} + l\bar{\psi} + l\pi + \alpha - \frac{\pi}{4}\text{sign}(g'')}{\omega g}, \quad (7)$$

$$\mathcal{T} = \sum_{i=1}^n s_z^{(i)}(z_i - z_{i-1}), \quad (8)$$

$$\bar{\psi} = \theta + \arctan\left(\frac{s'_{SAW}}{s_{SAW}}\right). \quad (9)$$

In Eq. (7), ψ_0 is an isotropic arbitrary constant (shifted by π between the two electrical polarities), \mathcal{T} is an anisotropic pre-distortion time-delay to compensate for the vortex degeneration, α is the complex phase of $a(\bar{\psi})$, $g(\bar{\psi}, \theta) = s_{SAW}(\bar{\psi}) \cos(\bar{\psi} - \theta)$, g' denotes the derivative of g with respect to ψ and $\bar{\psi} - \theta$ is the beam stirring angle.

The whole design and fabrication process is summed up in Fig. 2. At first, we compute the slowness and coupling coefficient of the SAW. This allows evaluating numerically Eq. (7) which yields a master curve. Using a home-made object-oriented program based on the Python module Shapely, the master curve is converted into an IDT geometry and printed as a photomask. The transducer is then fabricated by a standard lift-off process: a thin layer of photosensitive resin patterned on an X-cut of lithium niobate is exposed to UV light. After developing the resin, chromium (50nm) and gold (200nm) are deposited on the surface. Finally, the lift-off step removes the excess metal.

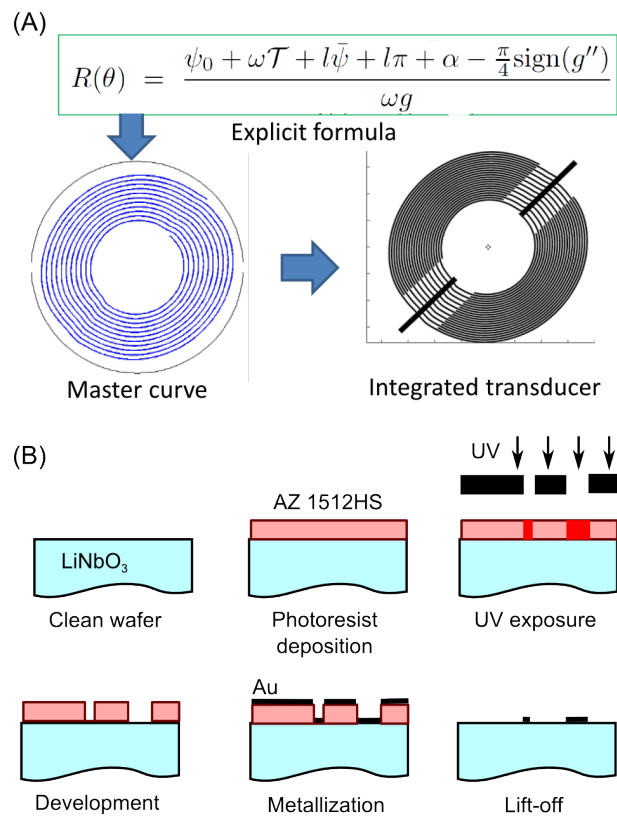


Figure 2: Design and fabrication of the acoustic vortex integrated transducers. **(A) Design:** Eq. (6) is evaluated numerically, resulting in a master curve. This master curve is converted to photomask using a home-made Python algorithm (available in the supplemental material of [12]). **(B) Fabrication:** A clean X-cut of lithium niobate is coated by the AZ 1512HS positive photoresist. Using the photomask designed above, a section of the resin is insolated and removed using a developer. 50 nm of chromium and 200 nm of gold are then deposited on the wafer by e-beam evaporation. Finally, the lift-off step removes the excess resin and metal.

2.3 Experimental setup

We installed the transducer on an inverted microscope (see Fig. 3). Similarly to an echographic probe, the sample is placed on the transducer and we add a drop of coupling fluid (silicon oil) to ensure an efficient transmission of the acoustic waves from the crystal to the sample. This sample can be a glass slide or a microchannel containing a dispersion of microscopic particles. When the transducer is turned on, the acoustic vortex captures one targeted particle in the sample. The trapping force was experimentally evaluated to a few hundreds of piconewtons [12], which is the upper range of optical tweezers but at a considerably reduced power flux. We displace the sample relatively to the transducer by the mean of a micromanipulator. Thus, the trapped particle can be displaced relatively to its neighbors.

3 Results

We patterned a series of transducers to synthesize first-order ($l = 1$) acoustic vortices. The devices are resonant at a frequency of 10 MHz. They are powered by an arbitrary waveform generator connected to a power amplifier and a Pi-shaped 50Ω impedance matching circuit. Overall, the system consumes 1 W of electrical power to generate forces up to 219 pN [12].

In a first experiment, we dispersed 30 μm diameter polystyrene microspheres between a polymethylmethacrylate (PMMA) slide (thickness 2 mm) and a polydimethylsiloxane (PDMS) top cover that acts as a sound absorber. By grabbing, moving and dropping the particles iteratively, we arranged dozens of particles to form the word “LIFE” shown in Fig. 4(A).

Although acoustic waves in sonication bath can kill cells by cavitation, the range of frequencies and power used in contactless manipulation (megaspascals, one megahertz to one gigahertz) are much more similar to echography [17]. Thus, we used these acoustic vortices to capture and displace buccal epithelium cells suspended between a glass coverslip and a PDMS absorbing layer (see Fig. 4(B)).

When an acoustic wave attenuates in a fluid, it transfers its momentum flux to the fluid which in turn generates a steady flow [4]. It has been predicted [13] and observed experimentally [7] that isotropic acoustic vortices generated a acoustic streaming flow reminiscent to cyclones. This flow pattern can also be generated by the integrated transducers as shown in Fig. 4(C) (the velocity field was obtained by particle image velocimetry with 1 μm diameter PS particles). Therefore, this technology not only allows for contactless manipulation but also generating vorticity *in situ*.

4 Conclusion

We presented a low-cost, miniature, integrated selective acoustical tweezer. The device can be used to arrange particles and cells, and generate vorticity *in situ*. This type of technology may become the Swiss Army knife of microfluidics, and opens prospects for fundamental biology and biomechanics, regenerative medicine and *in situ* microsystem assembly. In the future, we will aim to miniaturize further the device, improve its selectivity and increase the trapping strength.

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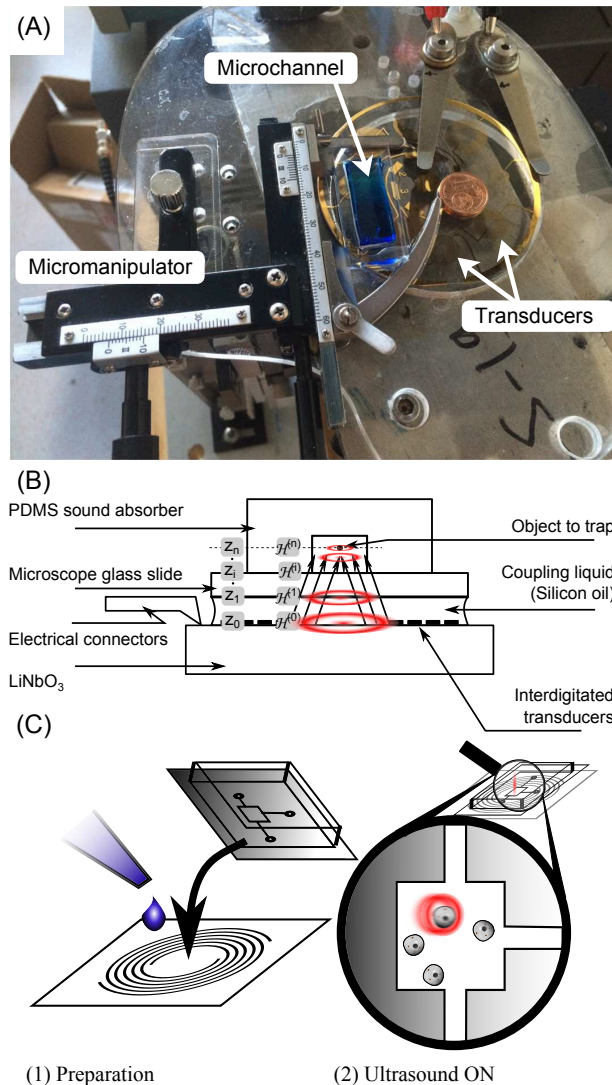


Figure 3: Experimental setup for the contactless manipulation of microscopic particles. **(A) Photograph of the actual experiment:** 8 transducers have been patterned on an X-cut of lithium niobate. The power supply is ensured by the mean of microscope clips connected to a radiofrequency power amplifier. The sample (here a microchannel in blue) is installed on the transducer. It is hold by micromanipulator, which ensures an accurate relative motion between the sample and the and the transducer. **(B) Schematic cross section of the various layers of materials used in the experiment.** The acoustic vortex is pictured as red circles. **(C) Experimental procedure.** The experiment starts by coating the substrate with a drop of coupling fluid. The sample is then simply placed on this liquid layer. When turning the power on, the acoustic vortex captures selectively a targeted particle, which can then be displaced by the mean of the micromanipulator.

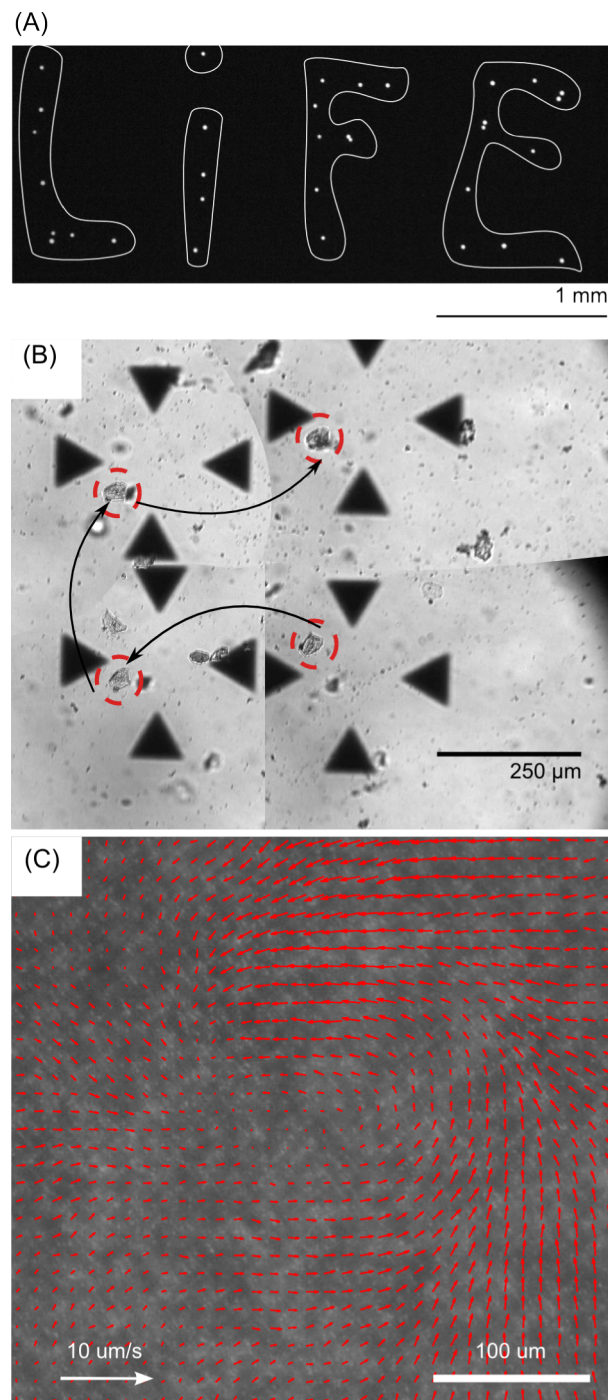


Figure 4: Manipulation of fluids and particles with an integrated acoustical tweezer. **(A) Patterning:** Final arrangement of 33 PS beads (diameter $30\ \mu\text{m}$) initially randomly dispersed. **(B) Cell manipulation:** a buccal epithelium cell is trapped by the acoustic vortex and displaced relatively to the sample. **(C) Vorticity generation:** PIV snapshot of the swirling flow motion generated by acoustic streaming around the vortex core.

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