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1pPAb1. Acoustic deformation of cells
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Stretching cells using optical tweezers has been previously demonstrated, enabling the mechanical properties of individual cells to be assessed with potential applications in, for example, identifying cancer cells and parasite infections such as malaria. We demonstrate a system that uses acoustic radiation forces to compress levitated cells to an extent comparable to that demonstrated with optical tweezers. While deformation of levitated droplets has been demonstrated previously, we address the challenge of producing significant forces on objects that have low acoustic contrast with their host medium and are small compared to the acoustic wavelength. Acoustic deformation can potentially be applied to many (e.g. thousands) of cells simultaneously, opening the way to higher throughput diagnostic devices. Osmotically swollen red blood cells (RBCs) are used to demonstrate the principle as they are particularly compliant. A resonance is formed in a square capillary of inner dimension, 100µm. Excited by a transducer at a half-wave resonance of 7.9 MHz, cells are both levitated and focussed laterally into a single line down the centre of the capillary prior to the compression forces being applied.

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INTRODUCTION

The mechanical stiffness of biological cells can be used to distinguish cell types and for the identification of diseases [1]. For example, a malaria infected blood cell is known to be stiffer than a healthy normal blood cell [2]. Earlier studies have reported the deformation of red blood cells (RBCs) by applying a stretching force using optical tweezers [3, 4], where a pair of laser beams were used to trap a single erythrocyte in suspension. An optical stress is exerted, causing an elongation of the cell body along the laser beam axis. However, optical tweezers are limited to single particle manipulation and there is possibility of cell damage due to exposure to high intensity optical field. Therefore acoustic manipulation, which has been demonstrated to separate, filter, sort and mix biological cells[5], has the potential to provide an alternative technique for cell deformation.

In contrast to previous experimental studies, described below, acoustically deforming a cell using radiation forces is not straightforward because the cells have low acoustic contrast with their host medium and are small compared to the acoustic wavelength. To overcome these limitations we describe here a system that work at higher frequencies than many particle manipulation devices, and uses a highly resonant cavity to achieve the required acoustic pressure amplitudes with little associated heating. The acoustic deformation can potentially be applied to many (e.g. thousands) of cells simultaneously, opening the way to higher throughput diagnostic devices.

In the past a number of studies have been carried out to demonstrate deformation of small particles such as water droplets and bubbles using acoustic radiation force. In particular, experimental and theoretical investigations were focused on understanding the phenomena and mechanisms of droplet deformation and breakup. Experimental studies have reported that the equilibrium shape of the particle can be significantly deformed for liquids with low surface tension[6, 7]. Marston presented a theoretical approach to calculate the shape of a droplet, deformed by a standing wave field in one dimension [8]: the scattered wave and resulting radiation stresses on the interface were calculated for small deformations from a spherical shape. Jackson et al. [9] extended Marston’s theory to a three-dimensional sound field, included gravity acting on the droplet as a factor affecting deformation, and wrote final expressions that were valid for particles of any size, though still only valid for small deformations.

The acoustic scattering by a non-spherical object was first included by Tian et al. to determine the static deformation of an acoustically levitated drop in air [10]. This approach was limited to calculating deformation for aspect ratios up to 2 as it did not add the spherical harmonics of the force function [11]. A number of experimental studies also supported the analytical approach, for example, Trinh and Hsu [7] reported the variations of the aspect ratios verses sound pressure of volatile liquids and aspect ratios verses drop volume at fixed sound pressure for non-volatile liquids. Anilkumar et al. [12] studied droplet deformation until breakup in order to determine the threshold of drop integration. Tian et al [10] carried out experiments to find drop position and static deformation of a droplet levitated in air by using surface tension and drop volume.

Numerical studies also investigated particle deformation, for example, Lee et al, developed a numerical method to study the drop instability and static deformation in a disk model [13]. Shi and Apfel [14] reported another numerical method to study the static shape deformation of a liquid drop in a gaseous environment. The method calculated the exterior sound field by solving the line integral form of wave equation which estimated the stress profile along the drop meridian accurately. The numerical results compared well with the experimental results by Tian et al. [10] and analytical results for small deformation range [8]. However, study was restricted to the condition when the inside liquid has high acoustic impedance compared to the outside and therefore, is not suitable to biological particle in a host fluid which have similar acoustic property.

Here we present initial experimental work demonstrating the deformation of RBCs. Numerical work to model the deformation is ongoing.

THEORY

The Radiation stress tensor at a boundary due to non-linear second order effects can be approximated in the non-viscous regime as [8]

\[
\Pi = \frac{1}{2\rho c^2} \left( p_i^2 \right) - \frac{\rho}{2} \left( v_i^2 \right) + \rho \langle u(nu) \rangle
\]  

where \( p_i \) and \( v_i \) are the first order acoustic pressure and velocity.

Total radiation stress across the boundary is the difference of the radiation stress for inner and outer surface of the boundary [8, 10]. Neglecting components parallel to the boundary, this can be written as,
\[ \Delta p = \Pi_i - \Pi_o \]  

where \( \Pi_i \) and \( \Pi_o \) are the pressure due to acoustic radiation force inside and outside respectively. This pressure difference is balance by the elastic properties of the particle to create an equilibrium shape [4].

**EXPERIMENTAL**

In our system, osmotically swollen red blood cells (RBCs) are used to demonstrate the deformation as they are particularly compliant due to the absence of a cytoskeleton. The experimental setup is shown in Figure 1. A square glass capillary of inner dimension 100 µm, and wall thickness 100 µm (Vitricom Ltd) was used as a resonant cavity. A 1 mm thick PZT plate (PZ27, Ferroperm) of dimensions 15 mm × 8 mm glued to the capillary using epoxy (Epotek 301) applied in a thin layer using an absorbent pad. The glue layer was measured as being ~ 10 µm thick. The assembly was mounted on a glass plate, with spacers to ensure the acoustic resonance is not disrupted, and PTFE tubing attached with epoxy to enable samples to be introduced.

Fresh rat blood was prepared by centrifugation to remove white blood cells and platelets. Then 50 µL of the centrifuged blood was mixed with 5 ml of a hypotonic solution of 50% PBS buffer with 50% water. The resulting uptake of water by the cells changes their morphology from the familiar bi-concave disc to spherical [4].

![FIGURE 1](a) Experimental device under the microscope. This device has two parallel capillaries (only one used at a time), which are too small to be seen, but cross the grey coloured PZT plate. The black marks are ink to reduce reflections that interfere with the imaging. (b) Schematic diagram of the device showing a cell being levitated and squashed due to the axial radiation force. Smaller lateral gradients in the velocity field align the cell in the centre of the channel.

The resonant frequency was established by exploring the frequencies in the vicinity of the predicted resonance using 10 µm fluorescent polystyrene beads (Polysciences). At 7.9 MHz a strong half-wave resonance is found such that particles are both levitated and focussed laterally into a single line down the centre of the capillary. The ultrasonic transducer was driven by an RF-amplifier (ENI 240L) driven by a sine-wave from a signal generator (TTi TG1304).

Optical phase contrast microscopy was used to view the deformation of blood cells. The blood cells were observed through the side of the capillary, which provides a more accurate view of the deformation compared to a view normal to the transducer. A 50× objective produced images captured using a CCD camera. Due to phase contrast between the blood cell and host fluid solution a dark ring was formed on the boundary of each cell (figure 2).

In order to find the best-fit ellipse corresponding to the cells’ boundaries, images were processed in MATLAB using an algorithm similar to that presented by Guck [4]. For each cell the algorithm first used a polar-to-rectangular projection about the image centre (“unwrapped”) to map the cell boundary to a line crossing the image. Next the boundary was designated as the point of minimum intensity (the centre of the black phase-contrast ring). The boundary was smoothed by taking a spatial Fast Fourier Transform (FFT) and discarding all but the lowest 8 frequency components before recombining them with an inverse FFT. The smoothed data was then mapped back to its polar form. Finally, the resulting data was fitted to an ellipse using a least squares method. The length of semi-major and semi-minor axis of the fitted ellipse represented the diameters of the deformed cell.

The acoustic pressure amplitude inside the capillary for a given drive voltage was found by balancing the weight of a 10 µm fluorescent polystyrene bead against gravity in the manner described by Spengler et al. [15]fluorescent beads. Acoustic pressure was found to be related to drive voltage by a factor of 25.7 kPa/Vp-p.
**RESULTS**

Figure 3 shows a sequence of deformed blood cell images for a range of acoustic pressure amplitude from 12.9 kPa to 978 kPa. It can be clearly observed that increasing pressure amplitude results in increasing amounts of cell deformation towards an ellipsoid. For each of n=8 blood cells a sequence of pressures were applied by varying the drive voltage. Cells were given 10s at each pressure amplitude to come to equilibrium before the corresponding image was captured for processing. Figure 4 shows the average change in aspect ratio vs acoustic pressure amplitude. It can be seen that the deformations are comparable to those found under the action of optical forces [4].
CONCLUSIONS AND FUTURE WORK

Ultrasonic standing wave fields have been shown capable of inducing deformations in red blood cells that are comparable to those demonstrated by optical methods, with little measured heating. This has promising applications in creating high throughput diagnostic devices as acoustic fields could be configured to apply forces to a large number of cells at once. Further work will continue to use numerical methods to model acoustic radiation forces in order to better understand the processes involved.

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