Trapping and Patterning of Large Particles and Cells in 1D Ultrasonic Standing wave

Ruhollah Habibi, a Citsabehsan Devendran, a and Adrian Neild a

The use of ultrasound for trapping and patterning particles or cells in microfluidic systems offers good biocompatibility and easy integration for on chip actuation, as such the approach has been extensively studied. The vast majority of studies involve particles which are considerably smaller than that of the acoustic wavelength. In this regime, the primary forces result in particle clustering at certain locations in the sound field, whilst secondary forces, those arising due to particle-particle interaction forces, tend to assist this clustering process by attracting particles towards each other. Using a wavelength closer to the size of the particles, the features of the sound field have a size which only allows one particle to be held at each primary force minimum, thus, enabling the possibility of patterning individual particles. However, to achieve this, the influence of secondary forces needs to be carefully studied, as inter-particle attraction is highly undesirable. Here, we study the effect of particle size and material properties on both the primary and secondary acoustic forces as the particle diameter is increased towards the wavelength of the ultrasonic field. We numerically probe the ultrasonic forces acting on elastic spheres in a 1-dimensional axi-symmetric plane wave. Herein, we show that the resonance frequencies of the solid sphere has an important role in the resulting secondary forces which leads to a narrow band of frequencies that allow the patterning of large particles in a 1-D array. The knowledge regarding the naturally existent secondary forces would allow for system designs enabling single cell studies to be conducted in a biological safe manner.

1 Introduction

The ability to trap and pattern individual cells and particles within a microfluidic system allows highly detailed studies into cell responses. Such single-cell analysis, offers the advantage of preserving the information that is lost when population based averages are made in standard methods. Especially as a population inevitably includes cells with a range of sizes and chemistry result in varying response and reactions to drug or virus agents.1,2 In addition, single-cell analysis can provide detailed information about cell-cell interaction, drug screening and a platform for tunable engineered tissues or stem cell research.3–5 Given the nature and scope of the applications, it is perhaps unsurprising that a range of techniques have been developed to create single-cell arrays. These include passive methods which are typically dictated by channel geometry and flow profiles,3,4 as well as active approaches utilising optical forces,6 magnetic,7 dielectrophoresis8 and acoustophoretic.9 Whilst each of the approaches offers certain benefits, the latter, acoustic manipulation, offers excellent biocompatibility,10–13 good on-chip integration14,15 and as exploits the physical properties of the particles/cells it does not require the tagging or labeling of cells.16

Acoustophoresis is the use of ultrasound in microfluidics to generate forces required to trap cells or microparticles,17 encapsulate them in droplets,18,19 pattern them in segregated clusters,20,21 and gain control over their trajectories.22–24 The required ultrasonic fields can be generated in a range of ways, but most commonly via bulk acoustic waves, which are used to excite fluid resonances,25,26 or surface acoustic waves (SAW) in which patterned electrodes are driven at a frequency such that constructive interference between each pair of electrodes results a high amplitude wave which couples into fluid volumes in contact with the substrate.27,28 Regardless of how the waves are generated, when they impinge and are scattered by a suspended particle, forces are generated on the scattering object. This acoustic radiation force has two contributing components,
firstly the primary radiation force which arises as a result of the interaction between the incident wave and the suspended matter (i.e. particles), in contrast to the secondary radiation force which is experienced as a result of the interactions between the scattered wave from another surface (i.e. particle/wall/bubble) and the suspended matter.

The vast majority of acoustofluidic systems developed for particle handling and sorting29–31 use wavelengths which are considerably larger than the diameter of the particles being manipulated. This causes particles to cluster at defined locations within the pressure field.32 In this scenario, the primary forces drive the particles to these locations, typically the pressure nodes in a standing wave, further reinforced by the secondary forces, which are predominately attractive in this regime, assist the formation of these clusters.33 However, as Collins et al note,9 the unique requirement for acoustic single cell patterning, is that the primary force should create the patterning, whilst the secondary force must be weak enough to not promote particle clumping.

In this work, we examine the relative importance of primary and secondary forces for particle and cell manipulation over a range of particle sizes, up to half the wavelength in a one-dimensional sound field. For the primary force, whilst most previous studies assume small particle size,16,34–37 Hasegawa’s work38 is very helpful as an analytical approach capable of finding the acoustic radiation force on elastic solid sphere in both one-dimensional standing and travelling waves valid for any size range is presented. A second useful feature of previous studies is in the discussion of viscosity. Doinikov demonstrated the presence of viscosity (most studies assume an inviscid fluid) only significantly affected the forces generated on small size particles36 and Muller et al compared the magnitude of the primary force with drag from acoustic streaming in a viscous fluid, showing that the drag will not dominate for diameters larger than 0.05 of the wavelength.39 As such, we will assume an inviscid fluid and neglect the influence of acoustic streaming.

The secondary forces have also been studied widely, originally by Bjerknes who examined the forces between two oscillating bubbles.40 Furthermore, there have also been studies of bubble-particle interaction41,42 and two small rigid particles.43 None, however, provide a basis for understanding the interparticle forces as the particle size approaches the wavelength.

Utilisation of numerical simulations enables the examination of primary and secondary forces over a range of particle sizes, herein, we demonstrate the existence of distinct regimes of behaviour, with shifts in the direction of the forces as a particle resonance condition is approached. It is also described that the likelihood of successful one cell per well (OCPW) particle trapping using acoustic forces.

2 Theory

Perturbation theory is applied to obtain the second order non-zero forcing terms, the resultant acoustic radiation forces.44,45 In this approach the pressure field is assumed to consist of stationary part \( P_0 \), first order \( P_1 \), and second order \( P_2 \) expansions, in which \( P_1 \) and \( P_2 \) are assumed to be time dependent and harmonic. This expansion to the second order is sufficient when the perturbation, \( \varepsilon \) is small. Considering the stationary state density \( \rho_0 \), and velocity \( v_0 \) (which is equal to zero), pressure, density and velocity fields can be expanded as:

\[
\begin{align*}
P &= P_0 + \varepsilon P_1 + \varepsilon^2 P_2 + \ldots, \\
\rho &= \rho_0 + \varepsilon \rho_1 + \varepsilon^2 \rho_2 + \ldots, \\
v &= v_0 + \varepsilon v_1 + \varepsilon^2 v_2 + \ldots.
\end{align*}
\]

Implementing Eqn. 1 alongside the continuity and Navier-Stokes equations, and taking the time average (\( \langle \cdot \rangle \) denotes time average operator) of the terms over a harmonic cycle, yields:

\[
\begin{align*}
\rho_0 \nabla \cdot \langle v \rangle &= -\nabla \cdot \langle \rho_1 v_1 \rangle, \\
\eta \nabla^2 \langle v \rangle + \beta \eta \nabla \langle \nabla \cdot v \rangle - \nabla \langle P \rangle &= \langle \rho_1 \partial_t v_1 \rangle + \rho_0 \langle \nabla \cdot (v_1 \cdot \nabla) v_1 \rangle.
\end{align*}
\]

While the time average of static and first order components of the fields equate to zero, the time average of the first order products or second-order components of velocity and pressure fields are non-zero and thus, \( P_2 \) contributes to the acoustic radiation force.44 This force is derived from the integration of second-order pressure field over the surface of the particle (in an ideal inviscid fluid). For this reason it is also a time averaged phenomena and as such acts over numerous harmonic cycle, allowing a steady migration of particles in a rapidly oscillating sound field. The resulting force is:

\[
\langle F \rangle = \int_{S(t)} P_2 (\mathbf{n}) dS.
\]

Here, \( \mathbf{n} \) indicates the normal vector facing outward to the surface of the particle, \( S \). However, as the surface of the particle, \( S(t) \) deforms and moves under effect of the incident field, an integration over this surface is problematic. Instead, by applying Reynolds’ transport theorem and expanding second-order pressure field,45 the acoustic radiation force on the particle is found, by integration over unperturbed surface, in this form, \( S_0 \):

\[
\langle F \rangle = \int_{S_0} \left( \frac{P_0}{2} \langle v_2 \rangle \mathbf{n} - \frac{1}{2 \rho_0 c_0^2} (P_2^2) \mathbf{n} - \rho_0 (\mathbf{n} \cdot v_1) v_1 \right) dS.
\]

Here, \( c_0 \) is the wave speed in the fluid. This formula uses only the first-order velocity and pressure fields applied over the stationary surface of the particle. The integrand has three distinct parts. The first two makes the momentum flux in/out of particle volume surface while the first is kinetic and the latter is hydrostatic energy, contributing to the force. The third term is the convective
momentum flux so the particle’s motion and surface fluctuation to be taken into play. In Eqn. 4, the first-order acoustic pressure and velocity fields have two contributing parts corresponding to incident and scattered effects, as expressed here for the velocity term:  
\[ v_1 = v_i + v_s. \]  

Accordingly, the squared velocity term can be written as:
\[ v_1^2 = v_i^2 + 2v_iv_s + v_s^2. \]

The scattered wave \( v_s \) is also a first-order approximation in this case. This approach was used by Yosioka and Kawasima\(^{36}\) and later by Gor’kov\(^{34}\) to derive the formula of the force on an elastic spherical particle in an acoustic wave.

However, they assumed that the particle is much smaller than the wavelength, \( d \ll \lambda \),\(^{46}\) and as such the scattered-scattered term is very small compared to incident and scattered-incident fields, \( v_s^2 \ll v_i v_s \) (Eqn. 6). Hasegawa and Yosioka\(^{50}\) also broke the potential field into incident and scattering components and then expanded them analytically using spherical Bessel and Hankel functions. Their approach provided an analytical solution for the force on elastic particles with an arbitrary size compared to the wavelength. Similarly Doinikov\(^{36}\) derived force relations within a viscous fluid. However, for large spheres, the scattered field cannot be neglected, so a higher order of approximation is required for an analytical solution.

In summary, for very small spherical particles, Gor’kov\(^{34}\) solution provides sufficient accuracy and his expression of force is convenient and widely used, however for larger particles it cannot provide accurate results as it ignores scattered-scattered field effects. Using perturbation theory, we are able to express the force with first-order terms. As our subject particles are in the order of the wavelength, we must consider the scattering effects as well. In this study we chose to adopt a finite element method (FEM) to solve the problem directly. Glynn-Jones et al.\(^{46}\) have used this approach and shown that calculating the acoustic radiation force (ARF) on large size particles using FEM is in good agreement with Hasegawa and Yosioka’s\(^{50}\) analytical approach.

In this work, we will apply a similar approach to Glynn-Jones’ work,\(^{46}\) using an axi-symmetric model of a particle in cylindrical coordinates, to first compute forces on a single particle in a one dimensional (1D) field. Followed by the extension of this approach to investigate secondary forces between two neighbouring identically sized spheres.

### Table 1 Basic parameters of the model. Material data are obtained from COMSOL material library otherwise stated.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluid Domain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>( \lambda )</td>
<td>100</td>
<td>( \mu m )</td>
</tr>
<tr>
<td>Density</td>
<td>( \rho )</td>
<td>997</td>
<td>( \text{kg m}^{-3} )</td>
</tr>
<tr>
<td>Speed of Sound ( c_1 )</td>
<td>( c_1 )</td>
<td>1497</td>
<td>( \text{m s}^{-1} )</td>
</tr>
<tr>
<td>Domain Height (along ( z ))</td>
<td>( L )</td>
<td>1.4( \lambda )</td>
<td>( \mu m )</td>
</tr>
<tr>
<td>Domain Width ( W )</td>
<td>( W )</td>
<td>( 1/4 \lambda )</td>
<td>( \mu m )</td>
</tr>
<tr>
<td>Frequency</td>
<td>( f )</td>
<td>14.97</td>
<td>( \text{MHz} )</td>
</tr>
<tr>
<td>Wavenumber</td>
<td>( k )</td>
<td>6.28( \times 10^4 )</td>
<td>( \text{m}^{-1} )</td>
</tr>
<tr>
<td>Acoustic Pressure Amplitude</td>
<td>( P_0 )</td>
<td>100</td>
<td>( \text{kPa} )</td>
</tr>
<tr>
<td><strong>Solid Domain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domain dimensions:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle diameter ( d )</td>
<td>( d )</td>
<td>( 2d )</td>
<td>( \mu m )</td>
</tr>
<tr>
<td>Particle radius ( R_p )</td>
<td>( R_p )</td>
<td>1190</td>
<td>( \mu m )</td>
</tr>
<tr>
<td>Density</td>
<td>( \rho_{ps} )</td>
<td>2270</td>
<td>( \text{kg m}^{-3} )</td>
</tr>
<tr>
<td>Speed of Sound (longitudinal)</td>
<td>( c_{ps} )</td>
<td>2350</td>
<td>( \text{m s}^{-1} )</td>
</tr>
<tr>
<td>Modulus of Elasticity ( E_{ps} )</td>
<td>( E_{ps} )</td>
<td>3.69</td>
<td>( \text{GPa} )</td>
</tr>
<tr>
<td>Poisson ratio ( \nu_{ps} )</td>
<td>( \nu_{ps} )</td>
<td>0.35</td>
<td>–</td>
</tr>
<tr>
<td><strong>Silica glass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>( \rho_{si} )</td>
<td>2203</td>
<td>( \text{kg m}^{-3} )</td>
</tr>
<tr>
<td>Speed of Sound (longitudinal)</td>
<td>( c_{si} )</td>
<td>5972</td>
<td>( \text{m s}^{-1} )</td>
</tr>
<tr>
<td>Modulus of Elasticity ( E_{si} )</td>
<td>( E_{si} )</td>
<td>73.1</td>
<td>( \text{GPa} )</td>
</tr>
<tr>
<td>Poisson ratio ( \nu_{si} )</td>
<td>( \nu_{si} )</td>
<td>0.17</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^{50}\) Calculated from Bulk Modulus \( K \) as \( E_{ps} = 3K_{ps}(1 - 2\nu) \) from ref. 48 and 49.

### 3 Method

#### 3.1 Establishing FEM model

To construct the finite element model, COMSOL Multiphysics\textsuperscript{®} was employed. The acoustic wavelength, \( \lambda \), in the fluid is set to 100 \( \mu m \) and all other geometrical parameters such as the particle size and particle position are made proportional to the wavelength. All spatial parameters including size, position, gap between pairs of particles and the position of gap (centre of gap) are normalised with respect to \( \lambda \). The solid material for the
particle is selected as poly(methyl methacrilate) (PMMA) while other materials such as polystyrene (PS) and silica glass (SG) will also be used for comparison. The basic parameters used in the model are listed in Table 1. Material data is taken from the COMSOL library, with the exception of polystyrene.\textsuperscript{51}

The size of the particle is used as the basis for a parametric study, with the force calculated for a range of sizes, from very small relative to the wavelength \((0.03 \lambda)\) up to one wavelength. A 2D axi-symmetric geometry model was used, with a fluid domain of dimensions as listed in Table 1. The fluid and solid domains were meshed with free triangular elements (maximum size limited to 0.4 \(\mu\)m and 0.01 \(\times R_p\), respectively). The growth rate was set to 1.05 for the fluid domain and 1.3 for the solid domain. The interface at the solid-fluid domains (the surface of the spherical particle) were meshed using an edge mesh (0.1 \(\mu\)m) to accurately capture the physics arising from the scattering effects. The standing acoustic is defined as a plane wave along the axisymetric \(z\)-axis as \(P = P_0 \cos(kz)\) (with \(P_0 = 100kPa\)) and the boundary conditions: Cylindrical Wave Radiation was used to transmit out through the modelled domain with minimal reflection (Fig. 1). The maximum ARF is experience when the sphere’s centre is located at \(z = +\lambda/8\), with \(z = 0\) located at the pressure antinode. The pressure field conditions are given in Table 1, and all the resulting forces are expressed in nN throughout this work.

3.2 Particle-Particle interaction
Following the examination of ARF on a single particle for a wide range of sizes (analogous to a range of frequencies), two equal-sized spheres were modelled to represent two proximal particles in a 1D field. As the aim is to pattern single particles individually at each node, given the fact that in 1D field inter-nodes distance is half a wavelength \((\lambda/2)\), the limiting size of the particle is \(d = \lambda/2\).

The model schematic is depicted in Fig. 1. Here, it can be seen that neighbouring particles are modelled starting with a minimum gap \((G = \lambda/100)\) around the antinode, and increasing this gap to investigate the influence of the other particle’s presence. Each particle is swept the axis toward the near node and slightly further to realize the effect of the gap on the acoustic force. Further, simulations also vary the location of the centre of this gap away from the antinode. The particle-particle interaction study was meshed in a similar manner as the previously discussed single particle study (Section 3.1), accommodating for both the solid-fluid interface present now.

4 Results and Discussions
4.1 ARF on single particle: Primary force
Fig. 2 shows the axial force on an elastic sphere of three different materials, PMMA, PS and SG positioned at \(\lambda/8\) distance from the 1D standing wave’s node/antinode, as the normalized size of the particle \((d/\lambda)\) is increased from 0 to 1. In all cases the incident pressure amplitude of the 1D wave is 100 kPa. From Fig. 2(a), it is observed that for the PMMA particle the force increases gradually with size until a local maximum at 0.25 \(\lambda\), as the size increases further there is a sharp drop in force to a minimum of about -7 nN at a size 0.4 \(\lambda\) followed by a rapid rise to second maximum with magnitude of +7.92 nN at size 0.425 \(\lambda\). It is noted that a positive force means that the particle is pushed towards the neighbouring pressure node while a negative sign indicates the particle will be moved towards the nearest antinode.

As larger sizes of PMMA is considered, we observe a repeating pattern of minima and maxima points occurring at certain (normalized) sizes which can be correlated to distinct certain frequencies. Hasegawa and Yosioka\textsuperscript{50} attribute these points to resonance frequencies of an elastic sphere. We can see a similar

![Fig. 3](image-url) In 1D standing wave field due to the geometrical constrain, one particle per node is only possible for sizes less than \(\lambda/2\). If sizes are much less than \(\lambda/4\) small and very small particles) clusters are shaped around nodes due to inter-particle attraction around node.
patterns in Fig. 2(b) and (c) for the other materials considered.

The resonance frequencies (natural frequencies or eigenfrequencies) are proportional to the Young’s modulus and inverse of a polynomial function of the Poisson ratio, \( f(\nu) = a_{i}\nu^{i} + a_{i-1}\nu^{i-1} + \cdots + a_{1}\nu + a_{0} \):\(^{52}\)

\[
\omega_{n} \propto \sqrt{\frac{E}{f(\nu)}} \tag{7}
\]

As a result the natural frequencies for the PS particle are higher than those of the PMMA particle, as PS has a lower Poisson ratio and larger \( E \). Whilst silica, with a much higher stiffness has a larger resonant frequency, indeed, Fig. 2(c) only shows two resonances within the range of \( d/\lambda < 1 \). It is clear that these eigenfrequencies strongly affect inter-particle forces and as such the patterning that can be expected. Thus, it is very important to understand the effect of the stiffness matrix components of the particle material.

The area of interest in manipulating particles, and later, cell trapping in 1D fields is limited to sizes less than half wavelength due to geometrical constraints. Namely, in order to obtain one particle per acoustic force well, which is spaced at half wavelength intervals, the particles need to be less than a half of a wavelength to physically fit in the available space, this is indicated in (Fig. 3). Hence we focus on this part of force-size plots. As presented in Fig. 2 there are three distinguishable regions in the range from 0 to 0.5 \( d/\lambda \):

Region A: the ARF or the Primary force is positive, as a result particles will be pushed towards the pressure nodes. For PMMA and PS, this region covers very small to medium sized particles. However, the whole size spectrum of silica glass particles (small to large) fall in region A (highlighted in beige).

Region B: the ARF is negative, as a result it pushes the particle

\[ \begin{align*}
\text{Fig. 4} & \quad \text{Force vs Position for different sizes: black dashed line shows Primary forces on single particles along their positions sweeping from a node to other opposite node for different sizes (a) } d = 0.03\lambda \text{ (b) } d = 0.10\lambda \text{ (c) } d = 0.20\lambda \text{ (d) } d = 0.25\lambda \text{ (e) } d = 0.33\lambda \text{ (f) } d = 0.45\lambda . \text{ Red and blue solid lines show Total force on particles A (top/right side) and B (down/left side) respectively as their gap increases. All forces are in nN with 100 kPa pressure applied.}
\end{align*} \]

\[ \begin{align*}
\text{Fig. 5} & \quad \text{(a) The centre of Gap moved along the axis line to evaluate the secondary force at other positions (b) The attraction force (} F_{\text{att}} \text{) as the sum of absolute net secondary forces on each particle, as if particle A is fixed and this force pushes B toward that, in this way positive sign (arrow pointing to the right side) indicates attraction and the negative sign (arrow pointing to the left side) indicates repulsion.}
\end{align*} \]
Fig. 6 Attraction force acting on particle B toward A with different gaps (G) and with centre of gap \((C_g)\) at different positions from antinode to node, each size represents its corresponding region (a) \(d = 0.25\lambda\) for Region A (b) \(d = 0.35\lambda\) for Region B (c) \(d = 0.45\lambda\) for Region C. In the third region, attraction force is negative hence a repulsive and its repulsion even increases when \((C_g)\) is shifted toward node.

4.2 Interparticle or Bjerknes secondary Force

To investigate the role of secondary forces we add a second particle to the simulations as described in Section 3.2, whereas the total force acting on the particles is examined in order to determine whether attraction or repulsion occurs (Fig. 1).

Each sphere scatters the incident field around it, in turn results in the time-averaged phenomenon of radiation force - primary force \((F_{\text{prim}})\). This respective (scattered) field also deforms and changes the (scattered) field of other neighbouring spheres; if this deformation effect could be purely separated from primary force scenario (scattering of incident field by single particle), that is this net scattered scattering field contributes to the net inter-particle or Bjerknes secondary force \((F_{\text{sec}})\). Knowing the total and primary forces, the secondary force can be calculated indirectly:

\[
F_{\text{total}} = F_{\text{prim}} + F_{\text{sec}} \Rightarrow F_{\text{sec}} = F_{\text{total}} - F_{\text{prim}}
\]

As \(F_{\text{prim}}\) indicates the primary force measured in single particle scenario and \(F_{\text{total}}\) is the total acoustic force on each particle in the presence of the other one. \(F_{\text{sec}}\) is denoted as the Bjerknes secondary force in this case.

Fig. 7 shows the primary and total forces on single and two PMMA particles at different positions along the wave axis. Spheres with very small size (here represented by a \(d = 0.03\lambda\)) follow their primary force field even when they are very close. For sizes 0.10 and 0.20, their scattering effect on the other particle field is notable but not dominant, with the primary force still significant enough to push them to the corresponding nodes. For size 0.25 \(\lambda\) we see that when the particles are close together (Gap < 0.05 \(\lambda\)), the total acoustic radiation force, \(F_{\text{total}}\) in the opposite direction of the primary force, thus resulting in the attraction of particles.

As such, the trend in region A is that as the particle size is increased, \(F_{\text{sec}}\) increases and it acts in the opposite direction of the primary force, and as such acts to attract the particles together. At a size equal to 0.25 \(\lambda\), we can see that this effect dominates over the primary force for gaps that are small enough.

For larger sizes, moving into Region B, the trend changes significantly. The primary forces reverse in sign, acting to move the particles towards the antinode, whilst the secondary force continues to act in bringing the particles together, hence they act in the unison with each other. In addition, the magnitude of the secondary force is considerably significant at small gap sizes.

For PMMA particles of size 0.45 \(\lambda\) located in Region C, the total force pushes particles toward the nodes, the same as the primary force does with small deviation. Secondary forces are relatively small and act repulsively. At this size, the corresponding frequency is very close to the natural frequency, resulting in an intensified acoustic radiation force (positive direction), that is, the primary force is dominant and acts to position the particles in the local pressure nodes.
Fig. 8 Patterning map of PMMA particles in 1D acoustic field showing the attraction or repulsion zones along with the primary force dominant zones for three representative sizes (a) \( d = 0.25 \), (b) \( d = 0.35 \) (c) \( d = 0.45 \). Values in the red (attraction) or black dotted (repulsion) boxes indicate the normalized size of each band.

4.3 Bjerknes secondary force at other positions

Thus far, we investigated two particles separated by a gap, the centre of which is at the antinode and varied the gap size about the gap centre, \( C_g \). In doing so, we observe that the regions of different behaviour align with the resonant frequencies of individual particles. We now examine the forces acting on two neighbouring particles which are centred around positions other than the antinode (Fig. 5). In Fig. 6 plots are given for three particle sizes, (0.25, 0.35 and 0.45) one from each of the identified regions, for each, we plot the secondary force as a function of the \( C_g \) as it is moved from the antinode to node (0 to 0.25 \( \lambda \)), in each case we do this for a minimum of three gap sizes.

Table 2 White Blood Cell (WBC) mechanical properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Properties:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>( \rho_c )</td>
<td>1070</td>
<td>kg m(^{-3})</td>
</tr>
<tr>
<td>Acoustic Impedance</td>
<td>( Z_c )</td>
<td>1.69</td>
<td>MPa m s(^{-1})</td>
</tr>
<tr>
<td>Speed of Sound(^b)</td>
<td>( c_s )</td>
<td>1579.5</td>
<td>m s(^{-1})</td>
</tr>
<tr>
<td>Solid Domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated Mechanical Properties:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poisson Ratio</td>
<td>( \nu_c )</td>
<td>0.499</td>
<td>-</td>
</tr>
<tr>
<td>Young's Modulus(^b)</td>
<td>( E_c )</td>
<td>16</td>
<td>GPa</td>
</tr>
<tr>
<td>Bulk Modulus(^c)</td>
<td>( K_c )</td>
<td>2.66</td>
<td>GPa</td>
</tr>
<tr>
<td>Rigidity Modulus(^d)</td>
<td>( G_c )</td>
<td>5.33</td>
<td>MPa</td>
</tr>
</tbody>
</table>

\(^a\) Calculated as \( c = Z_c/\rho_c \).
\(^b\) Calculated as \( E = \rho_c c_s^2 (1-\nu)/(1+\nu)(1-2\nu) \).

The attraction force is defined to determine if the total net secondary force of two particles result in attraction or repulsion (Fig. 5(b))

\[
\mathbf{F}_{\text{sec}} A = \mathbf{F}_{\text{sec}} \text{ on Particle A} = \mathbf{F}_{\text{total}} A - \mathbf{F}_{\text{prim}} A \quad (9a)
\]
\[
\mathbf{F}_{\text{sec}} B = \mathbf{F}_{\text{sec}} \text{ on Particle B} = \mathbf{F}_{\text{total}} B - \mathbf{F}_{\text{prim}} B \quad (9b)
\]
\[
\mathbf{F}_{\text{att}} = \mathbf{F}_{\text{sec}} B - \mathbf{F}_{\text{sec}} A \quad (9c)
\]

When \( \mathbf{F}_{\text{att}} \) is positive that means particle B is pushed rightward, thus secondary forces are attractive in nature, whilst a negative sign indicates a repulsive force. It is shown in Fig. 6(a and b) that \( \mathbf{F}_{\text{att}} \) decreases as the centre of the gap is moved away from the antinode, and is negative in the vicinity of the node. It appears that the main difference between these two particle sizes, representing region A and B is that particles within region B experience much larger secondary forces.

The representative of region C, size of 0.45 shows interesting behaviour. Already Fig. 4(f) revealed that two 0.45 \( \lambda \) sized spheres positioned around an antinode will be forced to their counterpart nodes and repel each other despite being in close proximity (\( C_g \approx 0.01 \)). In addition, Fig. 6(c) shows that the secondary force is repulsive even around the node (\( C_g = 0.25 \)).
This means that two neighbouring particles of this size are not brought together making them ideal candidates for single particle trapping.

In Fig. 7, we can see the impact of normalized size (or equivalently the frequency for a certain size) on secondary force for different gaps around the anti-node. Fig. 7 demonstrates that the attraction force follows a similar pattern to that of the primary force with change in size, however close to the resonating size (frequency), a large surge in its magnitude is observed resulting in a significantly larger force as compared to the primary force. Its magnitude then drops with the increase of the normalised size followed by an approach to zero after the second resonating size (frequency). Hence, at these larger sizes, its magnitude is very small compared to the corresponding primary force.

Thus far, we have analysed the resonant behaviour of individual particles demonstrating a change in the sign of the primary force at resonant sizes. For the analysed pairs of particles, the primary forces acted on particles in regions A and C such as to migrate them to the pressure nodes, whilst region B particles engender forces which act to move them to the antinodes. In addition to these primary forces, secondary forces we examined, showing that particles in region A and B follow a similar trend with the forces which are typically attractive, whilst in region C particle experience repulsive secondary forces across a full range of locations. With these trends isolated, and the link with the size regions established, we now turn to the central question: which of these particle sizes are best suited for single particle trapping? To assess this, we examine the combination of primary and secondary forces acting on a pair of particles whose starting location falls into a wavelength of the pressure field. In an ideal situation, these two particles would move to adjacent nodes or antinodes, and as such be held at positions separated by half a wavelength, this would mean that if the concentration of particles is suitable, one particle per force minima can be expected. As discussed previously, for a certain size range this is deterministically achievable. Sizes from Region C are an ideal candidate for this. However, this range is very narrow, in agreement with Collins et al. findings. In contrast, majority of particle sizes will present a more complex picture. To examine this, we place a pair of particles from each size region, in a standing wave at a range of different gap centres, and vary the gap size, at each location we examine where the particle will move to, and the outcome is plotted in Fig. 8.

In Fig. 8, each line of the figure corresponds to a different gap centre, within each of these sub-images the shading indicates where particles at each starting location will migrate to. In the case of the Region A particle, \(d = 0.25\), each sub-image has a central red box, particles starting within this box will be dominated by secondary forces and thus attracted to each other (the centre of this red box is also the centre of the gap between the two particles), the size of the box is indicated to the left of each sub-image. It should be noted that a box size of 0.26, for this particle which has a size of 0.25, means that the maximum gap between the particles at which attraction occurs is 0.01 \(\lambda\). Outside the red boxes, particles migrate to their nearest node, as such one particle per well is achieved. Note that had we examined a smaller particle size in region A, then there would also be the possibility that both particles could start in locations such that their nearest node is common, the physical size of the 0.25 particles makes this scenario impossible. If assumption of two particles per half wavelength is presumed, the probability that the particles are attracted to each other can be approximated to the size of the attraction box minuend the size of the particle divided by 0.5 (half the wavelength) minus the particle size. If we assume the centre of the gap is evenly distributed we can say the probability that they collect in different nodes is approximately 70%.

In the case of region B, there is a large attractive zone in most cases, outside of which the particles are collected in the nearest antinode. In the case of a gap centre located at the node, the particles are repelled from each other. Taking the same approach to estimate the single particle trapping probability, a value of 58% is obtained. Based on this measure the particles in this region are less well suited for single particle collection than those at the upper end of Region A, as attraction is more likely.

Finally, for region C, attraction does not occur, the secondary force is always repulsive. As such the particles are always predicted to move to the nearest node. This region, when it exists, is ideal for single particle trapping.

**4.4 Cell patterning**

We have used a study of solid particles to describe the different types of behaviour that can be expected as the particle size is increased within a 1D wave. In doing so we have identified regions of size, bounded by resonant modes, within which different types of outcomes can be expected. A significant application of trapping one object per well is in the use of ultrasound to pattern individual cells, hence one cell per well (OCPW). As such, with
the initial characterisation complete on well-defined solid materials, we now turn to examine cells. The first challenge this poses is in identifying suitable cell mechanical properties as they are not well reported in literature.

4.4.1 Cell Mechanical Properties

The term cell refers to a diverse range of biological units that vary vastly in shape, composition, construction and function. Human cells as a subset of eukaryotes are made of nuclei, cytoplasm, cytoskeleton and membrane that makes modelling of the cell as a classic mechanical system a very complicated task. Visco-elastic models of cell is generally used to predict or validate phenotyping of its physical characteristics. To understand blood cells behaviour in blood vessels arteries and hypertension, red blood cells are being studied and their biomechanical properties are of interest. The focus has been cell membrane properties which is itself very dependent to the load and speed of sample loading. Ultrasound wave can provide an average reading of the cell properties as a single unit with measuring its contrast factor or acoustic impedance.

In this study, for simplicity, the cell will be modelled as a uni-phase isotropic elastic solid spheres rather that a multilayered visco-elastic solid-liquid system. As this assumption is based on sample cells acoustic impedance, it is valid and justifiable for the physical effects being studied. Suitable values are available for both red blood cells (erythrocytes) and white blood cells (Leukocytes). Although WBC comprises a wider variety of types it was selected due to its spherical shape. Table 2 shows WBC properties. The Poisson ratio of cell has been assumed 0.499 (instead of 0.5 to avoid mathematical singularity) in several studies on mechanical modelling of cells. Measuring acoustic impedance returns in average speed of sound for the whole cell structure, $Z = \rho c_s$, taking the density as given in Table 2. With this $v$, all elastic modulii can be calculated. The resulted bulk modulus (2.66 GPa, close to the bulk moduli of water or blood) and Young's modulus are in a reasonable range, moreover in line with values cited by some literature.

4.4.2 Acoustic Force on Cells and the resulting patterning

As the Young's modulus of cells is less than the previously examined polymers, it might be expected that an individual white blood cell's force-size curve would be similar to those of PMMA and PS but with a leftward shift, accounting for a lower stiffness, thus, yielding lower natural frequencies. However, the natural frequencies are also affected by the Poisson ratio in a non-linear manner. Upon calculation of the force curve, shown in Fig. 9, the ARF on a single cell (with properties from Table 2) actually is more closely aligned to the case of silica than the less stiff PMMA or PS. Indeed, for sizes smaller than half wavelength ($\lambda/2$), the whole range is in region A (positive ARF), so the primary force tends to push the particles to the nearest node.

As performed previously, a study was completed using pairs of cell for size/wavelength ratios of 0.033, 0.10, 0.20, 0.25, 0.35 and 0.45 (Fig. 10). For all these sizes, the primary force tends to push the particles to the corresponding node. Similar to PMMA region A, the secondary force acts to attract two cells positioned on opposite sides of an anti-node. However, in contrast to PMMA this secondary force is not strong enough to prevail over primary forces (with the exception of size 0.03 only at gap 0.01) even for larger sizes/$\lambda$ ratios. The modelled cell material allows acoustic waves to transmit through with less scattering, consequently reducing secondary forces.

Herein, a map (Fig. 11) has been produced showing the forcing outcomes for two cells with different gaps between them for a range of locations of the centre of the gap. This too
Fig. 11 Patterning map of the cell, depicted here for size 0.25 as the representative of the whole range, shows that cells always follow the primary force field. The attraction occurs only if both cells are in same side of the antinode, mainly due to the primary force. Though, the secondary force also makes contribution toward attraction in such cases.

shows that the WBCs follow primary force field at all positions. Secondary forces always act in ‘attraction’ direction but if cells are on opposite sides of the anti-node, the secondary forces are too weak to bring them together. In this case, each cell will go to its nearest node (Cell A to Node A and Cell B to Node B). However if both cells are initially located on same side of the antinode, they will both be moved towards the same node (Node A in this case). This behaviour appears in the force map as an attraction zone, as the end result is that the cells will migrate to the same location, nonetheless, due to the primary force.

With the dominance of the primary force field, the effectiveness of the single cell patterning is dependent on the probabilities of the initial random distribution. The chance of single cells per well is equal to the chance that the cells initially are located on either side of an antinode. For two identical cells with diameter of $d$ the probability of being randomly in one half or opposite of halves of a wavelength can be derived as follows:

\[
P^\ast(H) = \frac{2(\frac{1}{2} - \delta)^2}{(1 - \delta)^2}
\]

\[
P^\ast(O) = \frac{1}{2} - \frac{\delta^2}{(1 - \delta)^2}
\]

Where $P^\ast(H)$ and $P^\ast(O)$ are the probability of two particles randomly located at same half or opposite halves of the wavelength, respectively, as a function of the normalized size, $\delta = d/\lambda$. The plot for different sizes from 0.05 to 0.5 $\lambda$ is shown in Fig. 12. This figure shows that for sizes greater than 0.35 $\lambda$ the chance of spherical and identical cell to be in the same half of a wavelength (in one side of an antinode) is less than 10% and this approaches zero with an increase in size. On the other hand, the chance of these two cells randomly positioned on the opposite halves of a wavelength is more than 90% and higher for the bigger sizes. Contrary to PMMA or PS where we had a very narrow range of sizes that patterning was possible (although guaranteed) for cells we have a much wider range of sizes that single cell patterning is possible. This can explain Collins et al. finding that cells were easier to manipulate as compared to synthetic particles; a wider range of sizes could be patterned in single cell per node.9

5 Conclusions

This analysis of the forces generated on large particles in an axisymmetric plane wave has identified regions of behaviour bounded by the resonant frequencies of the particles. Before the first resonant mode, the primary forces collect the particles at pressure nodes, and the secondary forces are attractive, however the weakness of the latter allows a good probability of single particle patterning. In the case of the white blood cells studied, this weakness is further emphasised by their very weak scattering nature making them excellent candidates for patterning. Above the first resonant mode, the primary force act to collect particles at the pressure antinodes, again this patterning is opposed by the attractive secondary forces, though in this case the strength of the latter is much more significant. Finally, the next region of behaviour, again sees the primary forces acting towards the pres-
sure nodes, but in this case the secondary forces are repulsive, so beneficial to individual patterning. Clearly the behaviour in the regimes is strongly varied, and so by establishing this framework a more robust understanding of the potential of single particle patterning using ultrasonic forces is offered.

6 Acknowledgement

The authors gratefully acknowledge the support of the Australian Research Council by way of grant DP160101263 Which has made this work possible.

References


