The acoustic radiation force of a focused ultrasound beam on a suspended eukaryotic cell

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1. Introduction

Ultrasound has been applied to manipulate [1] and lyse [2] cells since the 1920s. These were amongst the first contactless particle trapping and manipulation methods in biology and continue to find application in bio-medical research [3,4]. A particularly sensitive application of ultrasound manipulation is acoustic tweezers, which has received particular attention due to its advantages in contamination-free and label-free cell handling [5–8]. Numerous experimental, theoretical and numerical studies have shown that acoustic tweezers can be used to align, move and sort microparticles and cells [3,6–11]. Higher energy versions of these focused ultrasound technologies can be used to permeabilize membranes to ions and drugs [12–16].

Underlying all of these applications is fine control of acoustic radiation force (ARF). The study of ARF, which is the period-averaged force caused by a sound wave, is just like the optical radiation force generated by electromagnetic waves striking on electrically or magnetically responsive objects [17], therefore has a long history [18]. Investigation of ARF on microparticles dates back to King’s theoretical study of ARF on a rigid sphere in an ideal fluid subjected a planar progressive sound field [19]. Yosioka and Hasegawa extended King’s work to compressible spherical particles [20], and extensive subsequent theoretical and experimental works have shown the ARF exerted by a planar acoustic field on a microsphere [21–23] to be very sensitive to the structure and acoustic properties of the micro-particle.

Two theoretical approaches are commonly used to calculate the ARF: the partial-wave expansion method and the ray acoustics method. The ray acoustics method is limited to cases when the wavelength of the acoustic wave is far smaller than the radius of the sphere, but the partial-wave expansion method is applicable to an arbitrary frequency range [24]. The partial-wave expansion method has been used to explore a range of waves in spherical coordinates, including plane waves [20], Bessel waves [25] and Gaussian waves [26].

Gaussian waves are widely used to model optical and acoustical wavefields converging to or diverging from focal regions [26]. Particles can become trapped by a Gaussian wave in the focal region [6]. Focused Gaussian ultrasound waves have found utility in bioscience because they can trap suspended cells for quantification of the cell’s mechanical properties [27].

In existing theoretical studies of ARF in cell manipulation, cells were...
modeled as homogeneous microspheres [28]. However, eukaryotic cells are heterogeneous, and the nucleus has been reported to affect wave propagation significantly. Thus, the simple homogenous sphere model does not accurately represent eukaryotic cells.

As a first step towards understanding how cell shape and heterogeneity affect ARF, we studied an ellipsoidal cell consisting of a membrane, cytoplasm, and nucleus. This three-layered model was embedded in an ideal fluid that was subjected to a focused Gaussian ultrasound wave. The partial wave expansion method was employed to calculate the ARF on the cell. Results show that the nucleus and membrane play an important role in determining the ARF, along with the aspect ratio of the cell and the size of the cell relative to the Gaussian beam waist.

2. Theoretical model

With reference to Fig. 1, a focused Gaussian ultrasound wave is incident on a eukaryotic cell immersed in an inviscid fluid, with \( z_0 \) being the location of the center of the cell relative to the origin of the Cartesian coordination system, which is also the beam waist center. The wave with beam waist radius \( W \) propagates along the +z direction. The eukaryotic cell consists of an outer cell membrane with radius \( r_1 \), a middle layer (cytoplasm) with radius \( r_2 \), and an inner core (cell nucleus) with radius \( r_3 \). Let the mass densities and acoustic velocities of the surrounding medium, the cell membrane, the cytoplasm and the nucleus be denoted by \( \rho_1 \), \( c_1 \), \( \rho_2 \), \( c_2 \), \( \rho_3 \), \( c_3 \) and \( \rho_4 \), \( c_4 \), respectively. Corresponding acoustic impedances and wave numbers are \( Z_i = \rho_i c_i (i = 1, 2, 3, 4) \) and \( k_i = \omega / c_i (i = 1, 2, 3, 4) \), \( \omega \) being the circular frequency of the Gaussian wave.

In a progressive focused Gaussian ultrasound wave field, the incident wave pressure is expressed by:

\[
p_i(x, y, z, t) = \frac{p_i W}{w(z)} \exp \left[ -\left( \frac{x^2 + y^2}{w^2(z)} \right) \right] \exp \left[ -i \left( \frac{\omega}{2k} \frac{x^2 + y^2}{2R(z)} + z - \tan^{-1} \left( \frac{z}{f_c} \right) \right) \right] \exp(-i\omega t) \tag{1}\]

where \( w(z) = W \sqrt{1 + \left( \frac{z}{f_c} \right)^2} \) is the beam width, \( R(z) = f_c (z/f_c + f_c/z) \) is the radius of curvature of the isophase surface, \( \tan^{-1} (z/f_c) \) is the phase factor, and \( f_c = k W^2/2 \) is the confocal factor.

Although the phase front of the fundamental mode of the incident Gaussian wave is not planar in general, it is very nearly planar in the neighborhood of the beam waist and can be approximated as an acoustic wave with Gaussian amplitude distribution [26]:

\[
p_i(x, y, z, t) = p_i \exp(-\left( x^2 + y^2 \right)/W^2) \exp(i k_0 z) \exp(-i\omega t) \tag{2}\]

We define the wavelength in a particular medium as \( \lambda = 2\pi/k = 2\pi c_1/\omega \) and \( s = 1/(kW) \). In a spherical coordinate system, with \( x = r \sin \theta \cos \phi \), \( y = r \sin \theta \sin \phi \), \( z = r \cos \theta \), the incident acoustic wave pressure may be expanded into a generalized Rayleigh wave series, as:

\[
p_i(r, \theta, t) = p_i \sum_{n=0}^{\infty} A_n r^{n}(2n + 1) j_n (k_n r) P_n (\cos \theta) \exp(-i\omega t) \tag{3}\]

where:
\[ \Lambda_{np} = \frac{\Gamma(p+1)}{\Gamma(p+1/2)} \sum_{j=0}^{p} \frac{\Gamma(p+j+1/2)}{(p-j)!} Q_{0b}(-4Q_{0b}q^{2})^{j} \exp(-i\Omega_{\theta}z_{0}) \]  
(4)

\[ \Lambda_{np+1} = \frac{\Gamma(p+1)}{\Gamma(p+3/2)} \sum_{j=0}^{p} \frac{\Gamma(p+j+3/2)}{(p-j)!} (Q_{0b} - Q_{1b} - jQ_{2b})(-4Q_{0b}q^{2})^{j} \exp(-i\Omega_{\theta}z_{0}) \]  
(5)

Here, \( Q_{0b} = 1/(1 + 2\epsilon(1/l)), Q_{1b} = 2/\epsilon(1 - 2\epsilon(1/l)^{2}), l = \epsilon W_{j}^{2}, f_{j} (\cdot) \) is the spherical Bessel function of the first kind, \( \Gamma(\cdot) \) is the Legrendre polynomial of order \( n \), and \( \Gamma(\cdot) \) is the Gamma function.

The scattered wave field can be expressed as:
\[ p_{s}(r, \theta, t) = p_{0} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)A_{n}h_{n}^{(1)}(k_{n}r)P_{n}(\cos \theta) \exp(-i\omega t) \]  
(6)

in which \( A_{n} \) is the scattering coefficient by the boundary condition. Therefore, the total wave field outside the three-layer field (eutaryotic cell) takes the form:
\[ p_{1}(r_{0}, \theta, t) = p_{0} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)[B_{0n}h_{n}^{(1)}(k_{n}r_{0}) + C_{0n}j_{n}(k_{n}r_{0})]P_{n}(\cos \theta) \exp(-i\omega t) \]  
(7)

The acoustic wave field in the cell membrane \( p_{2} \), in the cytoplasm \( p_{3} \), and in the nucleus \( p_{4} \) can be expressed as:
\[ p_{2}(r_{2}, \theta, t) = p_{0} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)[B_{0n}h_{n}^{(1)}(k_{n}r_{2}) + C_{0n}j_{n}(k_{n}r_{2})]P_{n}(\cos \theta) \exp(-i\omega t) \]  
(8)

\[ p_{3}(r_{3}, \theta, t) = p_{0} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)[D_{0n}j_{n}(k_{n}r_{3}) + E_{0n}j_{n}(k_{n}r_{3})]P_{n}(\cos \theta) \exp(-i\omega t) \]  
(9)

\[ p_{4}(r_{4}, \theta, t) = p_{0} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)[F_{0n}j_{n}(k_{n}r_{4})]P_{n}(\cos \theta) \exp(-i\omega t) \]  
(10)

where \( j_{n} (\cdot) \) is the spherical Bessel function of the second kind.

To determine the unknown coefficients \( B_{0n}, C_{0n}, D_{0n}, E_{0n} \) and \( F_{0n} \), we followed previous researchers in approximating the three layers as having shear resistance that is small compared to their resistance to dilatation [29,30]. Therefore, at their interfaces, the boundary condition was that the velocity and pressure must be continuous, leading to the following form for \( A_{n} \):
\[ A_{n} = \frac{\rho_{c}c_{2}j_{n}^{2}(k_{n}r_{2})[Q_{2n}(k_{n}r_{2}) - j_{n}^{2}(k_{n}r_{2})] - \rho_{c}c_{3}j_{n}^{2}(k_{n}r_{3})[Q_{2n}(k_{n}r_{3}) - j_{n}^{2}(k_{n}r_{3})]}{\rho_{c}c_{2}h_{n}^{(1)}(k_{n}r_{2})[Q_{2n}(k_{n}r_{2}) - j_{n}^{2}(k_{n}r_{2})] - \rho_{c}c_{1}h_{n}^{(1)}(k_{n}r_{2})[Q_{2n}(k_{n}r_{2}) - j_{n}^{2}(k_{n}r_{2})]} \]  
(11)

where
\[ Q_{1b} = \frac{\rho_{c}c_{3}(s_{2n}j_{n}(k_{n}r_{2}) - s_{2n}^{2}(k_{n}r_{2}))}{\rho_{c}c_{1}(s_{2n}j_{n}(k_{n}r_{2}) - s_{2n}^{2}(k_{n}r_{2}))} \]
\[ Q_{2} = \frac{\rho_{c}c_{3}(s_{2n}j_{n}(k_{n}r_{2}) - s_{2n}^{2}(k_{n}r_{2}))}{\rho_{c}c_{1}(s_{2n}j_{n}(k_{n}r_{2}) - s_{2n}^{2}(k_{n}r_{2}))} \]
\[ \]  
(12)

3. Acoustic radiation force

For a continuous focused Gaussian ultrasound wave, the ARF is obtained by integrating the excess of pressure \( (p(r, \theta, t) - p_{0}) \)
generated by the sound field over the instantaneous surface \( S(t) \) of the sphere, as:
\[ F(t) = -\int S_{1}(t) \left( p(r, \vartheta, t) - p_{0} \right) n dS \]  
(13)

where \( n \) is the outward normal to \( S(t) \). To evaluate the ARF, the excess of pressure should be taken up to second-order terms in the velocity potential. For a periodic wave, the ARF is defined as a time-averaged quantity over period of the sound field. The time-averaged force acting on a sphere immersed in an infinite ideal fluid is:
\[ \langle F \rangle = -\int S_{0} \rho \left( \langle u_{n} \rangle + v_{t} \rangle_{\nu} \right) n dS \]
\[ + \int S_{0} \left( \frac{1}{2} \frac{\partial}{\partial r} \left( \frac{\omega^{2}}{r^{2}} \right) \right) \nu dS \]  
(14)

where \( \langle \cdot \rangle \) represents the time average, \( t \) is an in-plane unit tangential vector of \( S(t) \), \( S_{0} \) is the surface of the target at its equilibrium position, \( dS = rd\theta d\phi \), and the parameters \( v_{n} \) and \( v_{t} \) are the radial and tangential components of the velocity at the surface, respectively. Here, \( \psi = \text{Re}[\phi] \), for which \( \phi \) is the velocity potential expressed as:
\[ \phi = \frac{p_{e}}{-i\omega p_{1}} \sum_{n=0}^{\infty} A_{n}i^{n}(2n + 1)[B_{0n}h_{n}^{(1)}(k_{n}r_{2})]P_{n}(\cos \theta) \exp(-i\omega t) \]  
(15)

It follows that:
\[ \psi = \text{Re}[\phi] = \frac{p_{e}}{\omega p_{1}} \sum_{n=0}^{\infty} (2n + 1)R_{n}B_{n}(\cos \theta) \]  
(16)

\[ R_{n} = \text{Re}(A_{n}i^{n}(U_{n} + iV_{n}) \exp(-i\omega t)) \]  
(17)

in which \( U_{n} \) and \( V_{n} \) are given by:
\[ U_{n} = (1 + \alpha_{n})j_{n}(\alpha_{n}r_{n}) - \beta_{n}j_{n}(\alpha_{n}r_{n}) \]
\[ V_{n} = \beta_{n}j_{n}(\alpha_{n}r_{n}) + \alpha_{n}j_{n}(\alpha_{n}r_{n}) \]  
(18)

where \( \alpha_{n} \) and \( \beta_{n} \) are the real part and imaginary part of the scattering coefficient \( A_{n} \), respectively.

In the direction of wave propagation, the total radiation force on the three-layer model is:
\[ \langle F_{1} \rangle = \langle F_{2} \rangle + \langle F_{3} \rangle + \langle F_{4} \rangle + \langle F_{5} \rangle \]  
(19)

where
\[ \langle F_{1} \rangle = \left\langle -\pi r_{1} \rho_{1} \int_{0}^{\pi} \frac{\partial \phi}{\partial r} \sin \theta \cos \theta d\theta \right\rangle \]  
(20)

\[ \langle F_{2} \rangle = \left\langle \pi r_{1} \int_{0}^{\pi} \frac{\partial \phi}{\partial \theta} \sin \theta \cos \theta d\theta \right\rangle \]  
(21)

\[ \langle F_{3} \rangle = \left\langle 2\pi r_{1} \rho_{1} \int_{0}^{\pi} \frac{\partial \phi}{\partial r} \sin \theta \cos \theta d\theta \right\rangle \]  
(22)

\[ \langle F_{4} \rangle = \left\langle -\pi r_{1} \rho_{1} \int_{0}^{\pi} \frac{\partial \phi}{\partial \theta} \sin^{2} \theta \cos \theta d\theta \right\rangle \]  
(23)

Substituting Eq. (16) into Eqs. (20)–(23) and using the following equations of time average:

\[ \]
is characteristic ARF on a cell of cross-sectional area \( \pi c \) for a wave with characteristic volumetric energy density \( E_0 = \rho_{\text{water}} c^2 / (2 \rho_{\text{water}} c_0) \), and \( Y_p \) is the dimensionless ARF amplification factor that describes the degree to which the shape and heterogeneity of the cell amplify the ARF.

The dimensionless ARF amplification factor, \( Y_p \), is thus the metric used to compare the ARF on different cells. \( Y_p \) can be calculated by:

\[
Y_p = -\frac{4}{(\zeta n)^2} \sum_{n=0}^{\infty} (n + 1) \left\{ \Re \left[ \Lambda_n \Lambda_n^{*} \right] [\alpha_n + \alpha_{n+1} + 2\alpha_n \alpha_{n+1} + 2\rho_{\text{water}} \omega \gamma_n] \right\} + \Im \left[ \Lambda_n \Lambda_n^{*} \right] [\rho_{\text{water}}(1 + 2\alpha_n) - \rho_{\text{water}}(1 + 2\alpha_{n+1})] \right\}
\]

(30)

The series of Eq. (30) can be truncated when \( \Lambda_n < 0.0001 \). ARF can be obtained by substituting Eqs. (25)–(28) and (30) into Eq. (29).

4. Parametric analyses and numerical simulations

A series of parametric analyses were performed to determine how the dimensionless ARF amplification factor, \( Y_p \), varied with the geometry and composition of cells. Finite element (FE) simulations were performed for many of these to validate the model.

The baseline geometric parameters were chosen to model an oocyte. The outer layer was taken as a homogenization of the corona radiata, zona pellucida, and vitelline membrane, with outer radius \( n = 50 \mu m \) and inner radius \( r_2 = 45 \mu m \). Because the nucleus can account for 21–50% of cell volume \([31]\), the outer radius of the nucleus was taken as \( r_3 = 30 \mu m \). Although the position of the nucleus within the cytoplasm of an oocyte can vary, it was modeled as being concentric with the other layers for simplicity.

The Gaussian ultrasound wave beam waist dimension \( W \) was set to three times the wavelength \( (W = 6\pi c / \omega) \) for an acoustic signal with angular frequency \( \omega \). The baseline acoustic material parameters used in all graphs and simulations are listed in Table 1.

FE simulations were performed using the commercial FE code COMSOL Multiphysics (COMSOL, Inc., Burlington, MA, USA). Because the Gaussian ultrasound wave field is axisymmetric, the calculation was simplified by taking advantage of axisymmetry. The “pressure acoustics” module of COMSOL was adopted to model wave propagation, and Eq. (1) was used to set the background sound field. The nucleus and surrounding medium of the FE model were meshed with linear, triangular elements, and the swept mesh method was used to create linear quadrilateral meshes for the cytoplasm and cell membrane (Fig. 2). To model an infinite medium surrounding the cell, non-reflecting boundary conditions were used. The “perfectly matched layer” routine in COMSOL was used. A set of elements around the periphery of extracellular medium introduced an acoustic field through pressure boundary conditions, but cancelled acoustic energy that was received back from the medium with minimal reflection back into the medium. Convergence studies were performed to ensure grid independence for each simulation performed. In these, each element edge length was kept smaller than one sixth of the wavelength. Acoustic pressure and velocity fields in the cell and surrounding medium were obtained directly from the FE simulations. Accordingly, based on the numerical results of

<table>
<thead>
<tr>
<th>Material</th>
<th>Density ( \rho_i ) (kg/m(^3))</th>
<th>Speed of sound ( c_i ) (m/s)</th>
<th>Impedance ( Z_i ) (MRayl)</th>
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</thead>
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<tr>
<td>Outer layer</td>
<td>970</td>
<td>1450</td>
<td>1.41</td>
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<tr>
<td>Cytoplasm</td>
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<td>1508</td>
<td>1.51</td>
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<td>Nucleus</td>
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<td>1508.5</td>
<td>2.16</td>
</tr>
<tr>
<td>Water</td>
<td>1000</td>
<td>1500</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Table 1

Acoustic parameters [29,32]

Upon substituting Eqs. (25)–(28) into Eq. (19), the radiation force on the three-layer model exerted by the incident focused Gaussian wave can be expressed as:

\[
\langle F_0 \rangle = \frac{2\pi n^2 \rho_{\text{water}}^3}{\omega^2 \rho_1} \sum_{n=0}^{\infty} \langle R_{n} R_{n+1} \rangle \hat{\gamma}_{\text{water}}
\]

(25)

\[
\langle F_0 \rangle = -\frac{2\pi n^2 \rho_{\text{water}}^3}{\omega^2 \rho_1} \sum_{n=0}^{\infty} n(n + 1) \langle R_{n} R_{n+1} \rangle \hat{\gamma}_{\text{water}}
\]

(26)

\[
\langle F_{\text{h}} \rangle = \frac{2\pi n^2 \rho_{\text{water}}^3}{\omega^2 \rho_1} \sum_{n=0}^{\infty} [n(n + 1) \langle R_{n} R_{n+1} \rangle \hat{\gamma}_{\text{water}} - (n + 1) \langle R_{n+1} R_{n+2} \rangle \hat{\gamma}_{\text{water}}]
\]

(27)

\[
\langle F_{\text{p}} \rangle = \frac{2\pi n^2 \rho_{\text{water}}^3}{\omega^2 \rho_1} \sum_{n=0}^{\infty} \langle R_{n} R_{n+1} \rangle \hat{\gamma}_{\text{water}}
\]

(28)

Upon substituting Eqs. (25)–(28) into Eq. (19), the radiation force in the three-layer model exerted by the incident focused Gaussian wave can be expressed as:

\[
\langle F_0 \rangle = Y_p f_0
\]

(29)

where \( F_0 = E_0 A_0 \) is characteristic ARF on a cell of cross-sectional area \( A_0 = \pi c^2 \) for a wave with characteristic volumetric energy density \( E_0 = \rho_{\text{water}} c^2 / (2 \rho_{\text{water}} c_0) \), and \( Y_p \) is the dimensionless ARF amplification factor that describes the degree to which the shape and heterogeneity of the cell amplify the ARF.

Fig. 2. Finite element model: (a) representative mesh for eukaryotic cell; (b) enlarged FE mesh.
The relative sizes of the nuclear and outer layers of the three-layer model affected the magnitude and the frequency dependence of the acoustic radiation force amplification factor $Y_f$ (Fig. 4). In studying these, the focus was the frequency range of 1–20 MHz relevant to standard ultrasound probes, and in particular the peak ARF observed for a spherical cell in the vicinity of 13 MHz (Fig. 3). Note that the several factors are conflated in the contour plots of Fig. 4. As above, the beam waist of the focused Gaussian ultrasound wave was fixed at $W = 3\lambda$. However, because $\lambda = 2\pi/k_b = 2\pi c/\omega$, the size of the beam and hence the relative sizes of these layers change as a function of excitation frequency.

Increasing nuclear radius $r_n$ while keeping all other dimensions at their baseline values generally increased ARF (Fig. 4a), due to the relatively high impedance of the nucleus (Table 1) and hence the relatively higher efficiency of scattering. Increasing membrane thickness, $l = r_i - r_n$, with the outer and nuclear radii fixed at their baseline values also generally increased ARF on the three-layer model (Fig. 4b). Because the contrast between the impedances of the outer layer and the medium is stronger than that between the cytoplasm and surrounding medium (Table 1), replacing cytoplasm with a thicker outer layer, increased the total acoustic scattering of the three-layer model and thus the ARF.

Note that the increases in $Y_f$ are strongly dependent upon frequency. Also, due in part to the conflation of beam waist size and frequency and in part to the vibratory nature of the ARF, certain regions can frequency and size ranges can be found in which an increase in size causes a decrease in ARF. Examples include increasing nuclear radius $r_n$ beyond 40 µm for an excitation frequency of 12 MHz, and increasing $l$ for an excitation frequency of 7.5 MHz (Fig. 4).

5.3. Influence of acoustic parameters on ARF

With all other parameters held at their baseline levels and again with $W = 3\lambda$, increasing the densities of the layers could increase or decrease the ARF, depending upon the change in contrast of the impedances and upon the vibratory nature of the problem (Fig. 5). Densities were varied ± 20% from baseline values (see Fig. 6).

Increasing the density of the outer layer over this range (776 kg/m$^3$ ≤ $\rho_2$ ≤ 1160 kg/m$^3$) while holding all other densities at baseline values decreased the ARF (Fig. 5a). This was expected because the outer layer’s acoustic impedance became closer to that of the medium and cytoplasm over most of this range (1.12 MRayl ≤ $\rho_2\epsilon_2$ ≤ 1.69 MRayl) (Table 1). A plateau in this trend was reached as the contrast diminished.

Varying the density of the cytoplasm from 800 to 1200 kg/m$^3$ led to a non-monotonic change in the ARF (Fig. 5b). As the acoustic impedance of cytoplasm increased over the range 1.20 MRayl ≤ $\rho_3\epsilon_3$ ≤ 1.80 MRayl, the ARF first decreased as acoustic impedance contrast with the outer layer and nucleus decreased, but then increased again as the acoustic impedance surpassed that of the outer layer. Although the impedance contrast with the nucleus decreased steadily over this range, the rise in ARF for higher cytoplasmic densities indicated that the contrast with the outer layer was dominant over this range.

Finally, increasing the density of nucleus from 1144 to 1716 kg/m$^3$ increased the acoustic impedance over the range 1.73 MRayl ≤ $\rho_n\epsilon_n$ ≤ 2.59 MRayl. Because this corresponded to a steady increase in contrast with the impedance of the cytoplasm, scattering and hence ARF increased monotonically with nuclear density.

Changes of ± 20% to the velocity of sound had effects on the ARF identical to those in Fig. 5. This is expected because acoustic impedance is the product of the velocity of sound and the density within each constituent of the cell, and further confirms that acoustic impedance contrast is the key parameter that governs ARF. This underscores the utility of the present theoretical model in providing guidance for tuning ARF by changing the extracellular medium.

5.4. Influence of the Gaussian beam waist size

Varying the beam waist size, $W$, had little effect on the amplitude of $Y_p$, and had no effect on the locations of the frequencies for which ARF exhibited local maxima (Fig. 7). As $W$ increased, the amplitudes increased slightly, although the difference between $W = 5\lambda$, and $W = \infty$ (which is the case of a planar wave) was almost negligible (Fig. 7a). The effects of beam size can be further understood by considering the spatial distribution of the scattered wave field, which follows to form [33]:

$$I_0(f, \vartheta) = \frac{2}{\alpha_1n_1} \sum_{n=0}^{\infty} A_n (2n+1) A_n P_n (\cos \vartheta)$$

(31)
Fig. 4. Contour plots showing the effects of (a) excitation frequency and nuclear radius, \( r_3 \), and (b) excitation frequency and outer layer thickness, \( l = r_1 - r_2 \), on the acoustic radiation force amplification factor \( Y_p \) for a three-layered model. The beam waist of the Gaussian ultrasound wave was fixed at \( W = 3\lambda \). Baseline values: \( z_0 = 0 \), \( r_1 = 50\mu m \), \( r_2 = 45\mu m \) and \( r_3 = 30\mu m \).

Fig. 5. Contour plots showing the effects on the acoustic radiation force amplification factor of (a) cell membrane density, (b) cytoplasm density and (c) nucleus density. \( z_0 = 0 \), \( W = 3\lambda \), \( r_1 = 50\mu m \), \( r_2 = 45\mu m \) and \( r_3 = 30\mu m \).
For a frequency of 50 MHz, at which the maximum differential was observed in Fig. 7a for $1 \leq W \leq \infty$, the scattered wave amplitude can be seen to increase with beam waist uniformly (Fig. 7b). However, as is evident from the separation of amplitude and angular effects in Eq. (31), the changes in amplitude occur without altering the angular distribution of the scattering.

5.5. Influence of cell size on ARF

The size of the eukaryotic cell affects the acoustic radiation force amplification factor $Y_p$ (Fig. 8a). In studying this, we varied the cell radius $r_1$ while maintaining the relative dimensions so that the inner radius of the outer layer remained at $r_2 = 0.9r_1$ and the nuclear radius remained at $r_3 = 0.63r_1$. The beam waist of the Gaussian ultrasound wave was fixed at $W = 3\lambda$ (see Figs. 9 and 10).

The results in Fig. 8 indicate that, as the size of the cell increased,
the ARF peak shifted to a lower frequency, while the magnitude of this force peak remained constant. For the case of \( r_1 = 10 \, \mu m \), this peak was shifted so far that the ARF increased monotonically with frequency over the 50 MHz frequency range studied. For the other cases studied, the resonant frequencies all shifted to lower values with increasing cell size. The effects of cell size could be further understood by considering the backscattering of the scattered wave, which means that \( \theta = \pi \) in Eq. (31). Correspondingly, as the size of the cell increased, the peak of the backscattering amplitude \( f_\pi (f, \pi) \) shifted to a lower frequency without significant change in backscattering amplitude. As a result of this shift, more resonant frequencies and associated peaks appeared for larger cells over the frequency range studied. Based on these theoretical results, for smaller cells with radius ranges from 10 to 20 \( \mu m \), we need to increase the frequency of the Gaussian ultrasound wave to generate larger ARF.

5.6. Influence of the cell position

The ARF could also be tuned by moving the cell with respect to the ultrasound source. To illustrate this, we calculated the ARF as \( z_0 \) was varied in a beam with waist radius \( W \) and frequency \( f \) fixed at 30 \( \mu m \) and 20 MHz, respectively. The cell studied again had fixed relative dimensions, with the inner radius of outer layer held at \( r_1 = 0.9r \) and the nuclear radius held at \( r_1 = 0.6r \).

The highly focused Gaussian ultrasound wave generated negative ARF for certain values of \( z_0 \) in an “acoustic tweezer” effect analogous to the phenomenon underlying optical tweezers. This arises from the competition between the two forces that comprise the ARF: a gradient force, which is negative and arises due to the high gradient of the extracellular sound wave field; and a scattering force, which is positive. For example, for a small cell with \( r_1 = 10 \mu m \), a positive peak and a negative valley were found for \( z_0 = 22 \mu m \) and \( z_0 = 24 \mu m \), respectively. Here, the gradient force is dominant over the scattering force and thus the ARF is negative for \( z_0 = 24 \mu m \). A similar phenomenon can be observed for the cells with \( r_1 = 20 \mu m \) and \( r_1 = 30 \mu m \). However, for the cell with \( r_1 = 40 \mu m \), the gradient force cannot counteract the scattering force and thus only positive ARF exists, which means that this kind of cell cannot be trapped by a single focused Gaussian ultrasound wave.

For a large cell with \( r_1 = 50 \mu m \), we find that negative ARF appears for \( z_0 < 0 \), which differs from the trend observed for smaller cells. This highlights the central role that the cell position \( z_0 \) plays in determining both the sign and magnitude of the ARF. Results also provide a predictive framework for tuning a highly focused Gaussian ultrasound wave for use as acoustic tweezers.

5.7. Influence of the cell shape on ARF

Although scattering by ellipsoidal objects is challenging to study analytically, the problem is of interest because most cells elongate upon spreading. We therefore used the FE model to consider two kinds of ellipsoidal three-layer models: prolate and oblate spheroids. The cell had an axis of axisymmetry aligned with the centerline of a focused acoustical Gaussian beam and was centered in the beam waist. Due to this symmetry, the ARF exists without any acoustic radiation torque. The partially enlarged view of the three-layer model is shown in Fig. 8b, with the cell membrane and cytoplasm thickness being 5 \( \mu m \) and 15 \( \mu m \). With the reference to Fig. 8c, the ARF is sensitive to the aspect ratio \( b/a \). Prolate spheroids (higher \( b/a \), with the long axis parallel to the beam axis) have dramatically larger peak ARF. For oblate spheroids, sensitivity to aspect ratio is smaller. The reason for this is that a larger value of aspect ratio \( b/a \) means a larger curvature on the illuminated side, leading to enhanced acoustic scattering and ARF.

6. Conclusions

An analytical model has been developed to predict the acoustic radiation force (ARF) generated by a focused Gaussian ultrasound beam incident on a spherical three-layered shell (three-layer model) immersed in ideal fluid. The method of finite series is employed, with the Gaussian progressive wave simulated using spherical harmonic
functions. The model is subsequently used to calculate the ARF on a eukaryotic cell suspended freely in a focused progressive Gaussian ultrasound wave. Finite element simulations are performed to validate the proposed model, with good agreement achieved. Main conclusions drawn are:

1. As the cell membrane thickness or nucleus radius is increased, the ARF increases distinctly.
2. The impedance of each constituent of the cell plays an important role in affecting the ARF: increasing the impedance of cell membrane reduces the ARF; as the impedance of cytoplasm is increased, the ARF decreases first and then increases; increasing the impedance of cell nucleus leads to enhanced ARF.
3. The influence of the beam width of the Gaussian ultrasound wave on the ARF is significant only when it is relatively small.
4. The size of the cell can significantly affect the peaks of the ARF. Larger cells show more resonant frequencies and hence more ARF peaks in the 1–50 MHz range of excitation frequencies.
5. The aspect ratio $b/a$ (= major axis/minor axis) of the spheroid three-layer model significantly affects the ARF.

The results presented in this study provide theoretical basis for the further development of acoustic control technology for cell trapping/sorting/assembling and drug delivery applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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