The influence of inter-bubble spacing on the resonance response of ultrasound contrast agent microbubbles

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ABSTRACT

Ultrasound-driven microbubbles, typically between 1 and 8 µm in diameter, are resonant scatterers that are employed as diagnostic contrast agents and emerging as potentiators of targeted therapies. Microbubbles are administered in populations whereby their radial dynamics - key to their effectiveness - are greatly affected by intrinsic (e.g. bubble size) and extrinsic (e.g. boundaries) factors. In this work, we aim to understand how two neighbouring microbubbles influence each other. We developed a finite element model of a system of two individual phospholipid-encapsulated microbubbles vibrating in proximity to each other to study the effect of inter-bubble distance on microbubble radial resonance response. For the case of two equal-sized and identical microbubbles, each bubble exhibits a decrease between 7 and 10% in the frequency of maximum response (fMR) and an increase in amplitude of maximum response (A0) by 9–11% as compared to its isolated response in free-space, depending on the bubble size examined. For a system of two unequal-sized microbubbles, the large bubble shows no significant change, however the smaller microbubble shows an increase in fMR by 7–11% and a significant decrease in A0 by 38–52%. Furthermore, in very close proximity the small bubble shows a secondary off-resonance peak at the corresponding fMR of its larger companion microbubble. Our work suggests that frequency-dependent microbubble response is greatly affected by the presence of another bubble, which has implications in both imaging and therapy applications. Furthermore, our work suggests a mechanism by which nanobubbles show significant off-resonance vibrations in the clinical frequency range, a behaviour that has been observed experimentally but heretofore unexplained.

1. Introduction

Small gas-filled microbubbles, typically ranging in size from 1 to 8 µm and encapsulated with a thin, flexible, and biocompatible stabilizing shell, are currently employed as diagnostic ultrasound contrast agents [1–3]. Microbubbles vibrate within an ultrasound beam about their equilibrium radius with scattering cross-sections several orders of magnitude larger than a solid size-matched particle [4]. Through resonant oscillations and nonlinear harmonic and subharmonic emissions [5], microbubble signal enables the detection and separation of echoes originating from the blood - to which microbubbles are confined due to their size - from that of the much greater energy of the echoes from the surrounding tissue [6]. This vasculature-specific signal enables the quantification of blood flow and has many applications spanning from detection, diagnosis and therapy monitoring in cardiology and oncology [3,7,8]. More recently, ultrasound-stimulated microbubbles have been exploited to deliver local and targeted bioeffects under specific acoustic stimulus [9,10]. Microbubble-mediated shear stress and microstreaming are among the mechanisms behind these targeted therapies, including the transient opening of the blood–brain-barrier [11], site-specific drug/gene delivery [12–14], vascular shutdown therapy [15] and sono-reperfusion [16].

For both diagnostic and therapeutic techniques, an understanding of ultrasound-driven microbubble dynamics is critical to ensure robust and repeatable application. As has been previously well documented, microbubble behaviour is a function of both its intrinsic features [5,17–19] (e.g. bubble size, shell properties) and extrinsic environmental factors [20–24] – including fluid viscosity, fluid temperature, local boundaries and the presence of neighboring microbubbles. Indeed, there have been many mechanistic studies investigating the physics of vibrating microbubbles to elucidate the role of these factors on bubble behaviour as it relates to its imaging and therapeutic potential, the
majority of which are performed on an individual microbubble [18,22,25–28]. These investigations have explored unique physical and biophysical phenomena on an individual bubble scale, resulting in new insights towards contrast imaging [29,30] and ultrasound-mediated cellular therapies [31–33].

While it is a challenge to estimate local concentrations of contrast agent in vivo, microbubbles may not be in isolation when used diagnostically or as a therapeutic agent. Order of magnitude estimates result in clinical agent doses (~1:5000 dilution) possessing an average inter-bubble spacing of 80 µm, which can decrease due to i) acoustic radiation forces [34], ii) ultrasound-induced bubble coalescence [35] and iii) complex fluid flow patterns [36]. Furthermore, smaller ultrasound-sensitive agents are currently being investigated for both diagnostic and therapeutic application, including phase-shift nanodroplets that can acoustically vaporized into in-situ microbubbles [37], and stabilized nanobubbles – encapsulated bubbles on the order of several hundreds of nanometers in radius [38,39]. Assuming a volume-limited dose similar to clinically used micron-sized bubbles, a decrease in size by a factor of 10 translates to a 1000-fold increase in local bubble density [40].

To begin to address this, there are limited studies exploring the physics of bubble clusters, generally performed using analytical modifications of a second-order ODE describing bubble wall motion (e.g. Rayleigh-Plesset-type equations [41]). The majority of these studies focus either on bubbles without a material encapsulation or do not take into account any of the fluid dynamic considerations of the surrounding fluid [42,43]. In this study, we propose to study the effect of bubble proximity in a system of two encapsulated microbubble contrast agents using a finite element approach to ensure the two-way coupling between bubble vibrations and the local fluid environment. Specifically, we examine the coupling between different microbubble sizes and inter-bubble spacings with a view towards the resonance response of the system, as it is one of key features that make microbubbles an ideal ultrasound agent for imaging and therapy.

2. Mathematical model

2.1. Fluid domain

In the present study, the radial oscillations of two individual microbubbles in free space are considered, situated a distance h apart – see Fig. 1. The fluid domain surrounding the microbubbles is modeled as a Newtonian fluid. Given that the acoustic wavelength is much larger than the microbubble size and that the fluid velocity is much slower than the speed of sound, the fluid was further assumed to be incompressible [44]. Under these circumstances, the fluid motion is modeled by the Navier-Stokes equations, given below:

\[ \nabla \cdot v = 0 \]  
\[ \rho \left( \frac{\partial v}{\partial t} + v \cdot \nabla v \right) = \nabla p + \mu \nabla^2 v \]  

where \( v \) is the fluid velocity, \( \rho \) is the fluid density, \( \mu \) is the dynamic viscosity of the fluid and \( p \) is the fluid pressure.

2.2. Microbubble dynamics

The gas inside each microbubble is assumed to be spatially uniform and is modeled as an ideal gas via a polytropic process [44]. The pressure difference across the bubble wall \( P_B \), is a result of the combined affects of surface tension, the surrounding fluid viscosity, and the

![Fig. 1. Finite-element model environment and data analysis description. A) A representative example of the mesh grid placement on an individual bubble, where the bubble is divided into 6 sections to allow for spatially dependent application of Eq. (3). Simulations were performed using an axisymmetric environment. Units are in micrometers. B) Schematic view of the two-microbubble system; \( h \) denotes the center-to-center distance between the two microbubbles. Units are in micrometers. C) A sample plot of a radial response of a microbubble at a given transmit frequency. Both the maximum \( R_{max} \) and the minimum \( R_{min} \) radius were used to calculate the radial excursion. D) The frequency of maximum response (\( f_{MR} \)) and amplitude of maximum response (\( A_{MR} \)) of an individual microbubble.](image-url)
pressure contributions from the viscoelastic encapsulation, and is given as follows:

\[
P_R = \left( p_0 + \frac{2\sigma_i}{R_0} \right) \left( R_0 \right)^{\frac{2}{3}} \left( 1 - \frac{3k}{R} \right) + P_t - \frac{4\mu R}{P - P_\text{visc}} - P(t) \tag{3}
\]

where \( p_0 \) is the ambient pressure, \( \sigma_i \) is the initial surface tension at the gas-liquid interface, \( k \) is the polytropic index, \( P_t \) is the vapour pressure which is considered negligible compared to the gas pressure (\( P_t = 0 \)), \( R \) and \( R_0 \) represent the bubble radius and wall velocity, respectively, \( P_\text{visc} \) and \( P_\text{elas} \) are the pressure contributions due to the viscosity and elasticity of the shell, respectively, and \( P(t) \) is the externally applied acoustic pressure at the bubble wall. Multiple models have been proposed to explain the behaviour of microbubbles characterized by a thin viscoelastic shell by incorporating elastic and viscous terms \[5,45,46\]. Perhaps the most successful nonlinear bubble models to date incorporate phospholipid monolayer dynamics – indeed, experimental lipid research highlights that the surface tension of a lipid monolayer, such as those commonly employed in contrast microbubble synthesis, decreases with increasing compression rate (i.e. decreasing intermolecular area) \[47\]. Incorporation of this physics into simplistic Rayleigh-Plesset type bubble models \[48,49\] have been shown to predict unique microbubble vibrational signatures that have been observed experimentally, including ‘compression only’ behaviour \[30,50\].

Given this, we chose to implement an encapsulation model that considers a radially-dependent surface tension, as manifested through the elastic pressure contribution \( P_\text{elas} = 2\sigma(R)/R \), with the radially dependent surface tension \( \sigma(R) \) given as:

\[
\sigma(R) = \begin{cases} 
0 & \text{if } R \leq R_b \\
\chi \left( \frac{R^2}{R_0^2} - 1 \right) & \text{if } R_b \leq R < R_c \\
\sigma_{\text{elas}} & \text{if } R \geq R_c 
\end{cases} 
\tag{4}
\]

where \( \chi \) is the shell elasticity, and \( R_b = R_0(\sigma_i/\chi + 1)^{-1/2} \) and \( R_c = R_0(\sigma_i/\chi + 1)^{-1/2} \) are defined as the ‘buckling’ and ‘rupturing’ radius, respectively, with \( R_0 \) as the equilibrium radius of the microbubble. These are the radial limits within which the shell contribution dictates a quadratic-dependence on radius \[48\]. Indeed, Eq. (4) models the repartitioning of phospholipid molecules as it is manifested through the alterations in surface tension. Further, we consider the viscous contribution as \( P_\text{visc} = \kappa_S R / R^2 \) with \( \kappa_S \) defined as the surface dilatational viscosity of the monolayer \[5\]. Note that the compressibility term proportional to \( R/c \) in Eq. (3), which was added despite our assumption of an incompressible fluid, does not play a large role in our simulation results (as \( R/c \)). It was however incorporated for validation purposes against well-known models (see below). Further, note that Eq. (4) was originally derived from surface area arguments, however was incorporated into the current study in the form presented – as has been done previously \[51\].

2.3. Model description and method of solution

The boundary conditions imposed along each bubble free surface are such that the velocity and pressure across the boundary remain continuous, namely:

\[
\nu(R) = \dot{R} \tag{5a}
\]

\[
\rho(R) = P_b \tag{5b}
\]

where \( P_b \), the pressure at the bubble wall, is given by Eq. (3). Note here that we are imposing a no-slip velocity condition and negligible shear stress in the tangential direction along this interface. In this manner, these conditions exert two-way coupling between the bubble wall motion and the surrounding fluid. In order to allow the slight perturbations deviating from spherical oscillations to influence the fluid domain, each bubble free surface was divided into 6 different sections (Fig. 1), in which each section is subjected to the local boundary conditions given above. This allows the local curvature, approximated as spherical and spatially averaged over 1/6 of the microbubble, to contribute to the neighboring fluid motion. Pilot studies using 12 segments did not yield significantly different results. The final microbubble dynamic curve calculated in our model is derived from the average of the radius changes from the different sections of a given microbubble.

The governing equations subject to the above boundary conditions, along with the boundary conditions of constant \( p_0 \) along the edges of the simulation domain, were solved computationally using the finite-element method (FEM) with COMSOL Multiphysics 5.8 (COMSOL AB, Burlington, MA). Fig. 1B illustrates the geometry of the model and Fig. 1A is a sample of the mesh grid in our FEM simulation for an individual bubble. Due to the symmetry of the two microbubbles and the computational domain, only half of the simulation space was calculated in an axisymmetric environment to minimize the computational time. The mesh size was selected to be 8–20 times smaller than the smallest bubble radius. This results in a mesh size that is much smaller than the wavelength of the acoustic wave. Further, the mesh density was much higher in the neighborhood of the microbubble wall in order to capture the salient physics of interest, and decreased further from the bubbles, where we do not expect any significant effects. The moving microbubble free surface was described using a moving mesh arbitrary Lagrangian-Eulerian (ALE) algorithm. This allows for the computational mesh to move arbitrarily to optimize the shape of the elements and for the mesh nodes to track the moving boundary. The microbubbles are considered to be inside the focal volume of a conventional ultrasound transducer. Based of the size of a typical focal volume (\( \sim \text{mm}^3 \)) and the size of contrast agent microbubbles (\( \sim \mu \text{m} \)), the microbubbles were considered to be inside a uniform domain of ultrasound energy. This energy contribution enters our model as a change in acoustic pressure on the microbubble surface \( P(t) \) in equation (3)). The transmit pressure employed was a Tukey windowed (tapered cosine) 10 cycle pulse at a sampling frequency of 500 MHz. The other parameters in this study were held constant; \( \rho = 1000 \text{kg/m}^3 \), \( k = 1.095 \), \( \mu = 0.001 \text{Pa.s} \), \( \chi = 1 \text{ N/m} \), \( \kappa_S = 1.5 \times 10^{-3} \text{kg/s} \), \( \sigma_0 = 0.072 \text{N/m} \) and \( \sigma_0 = 0.01 \text{N/m} \). We acknowledge that recent work has demonstrated transmit frequency/bubble size dependent shell properties \[52,53\]. Given that the current study explores many transmit frequency and bubble size combinations, the shell parameters adopted here were chosen to lay well within the range of previous experimental reports on phospholipid-encapsulated contrast agent microbubbles.

To study the effect of frequency-dependent microbubble vibration, we performed our simulations using individual tone bursts with a transmit frequency ranging from 1 to 8 MHz in increments of \( df = 25 \text{ kHz} \). The microbubble diameter \( d = 2R_0 \) range investigated in this study spanned from 0.5 \( \leq d \leq 4 \mu \text{m} \) to cover both nanobubble and traditional microbubble size ranges \[52\], with a bubble center-to-center distance varying from 2 to 32 \( \mu \text{m} \) and peak-negative pressure ranging from 1 to 120 kPa. The parameter range here was selected due to its relevance in clinical imaging and therapeutic studies.

2.4. Analysis of radial oscillations

For a given microbubble radial profile, the radial excursion was calculated based on the average of \( R_{\text{max}} \) and \( R_{\text{min}} \) over the 6 regions of the microbubble, which represent the maximum and minimum dynamic radius, respectively (Fig. 1C). To study frequency-dependent microbubble vibrations, the radial excursion was calculated for each bubble at each transmit frequency to generate a resonance curve. The metrics extracted from this curve, as shown in Fig. 1D, were the amplitude of maximum response \( (A_{\text{max}}) \) and the frequency of maximum response \( (f_{\text{max}}) \). Indeed, \( f_{\text{max}} \) represents the frequency at which the damped radial oscillations are maximal (i.e. the resonance frequency of the nonlinear
damped microbubble system) – not to be confused with other closely related ‘resonance’ frequencies, including the frequency at which maximal scattered pressure or scattering cross-sections are observed [52,54].

2.5. Validation

We employed four different metrics to validate our numerical model. Firstly, our model was validated in the limit of a single microbubble in free fluid under low acoustic pressures and compared to the well-known analytical Rayleigh-Plesset equation (RPE) under the same acoustic conditions [44,48]. Fig. 2A shows the radial oscillation profile for a microbubble \((R_0 = 1.5 \mu m)\) driven at \(f = 1\) MHz at 45 kPa. The graphs show that the result of our simulation (solid; red) and the RPE (dashed; black) are in excellent agreement with an average percent error of 0.3\%.

A second validation (Fig. 2B) was performed by assessing the resonance response of an individual microbubble as a function of acoustic pressure from 10 to 30 kPa. Our simulation generates the expected strain-softening behaviour of decreasing resonance frequency with increasing pressure and a skewing of the resonance curve – as has been observed both experimentally [29,53,55] and through numerical modeling [56].

Thirdly, under low-amplitude driving conditions (~1 kPa) where the bubble experiences small deviations about its equilibrium radius, all bubble models reduce to a similar expression for the size-dependent resonance frequency [57]. In this limit, our model results over the range of \(2.5 < d < 5 \mu m\) (red dots in Fig. 1C) show excellent agreement with an average percent error of 3.8\% as compared to the well-known equation. Finally, the fourth validation was conducted by simulating an individual microbubble adjacent to a rigid wall. Indeed, by modifying the RPE via a ‘method-of-images’ approach [44], it can be shown that the \(f_{MR}\) of an individual microbubble decreases by a factor of \(\approx \sqrt{2}/3\) and its \(A_{MR}\) increases by a factor of \(\approx \sqrt{3}/2\) as it moves in direct contact with the rigid wall (\(h = R_0\)).

We ran our simulation for a \(d = 3 \mu m\) microbubble situated at varying distances from such a rigid wall under a transmit pressure of 30 kPa. The results of this model validation are shown in Fig. 2D, resulting in a shift of \(f_{MR}\) and \(A_{MR}\) of \(\approx 13\%\) and \(\approx 10\%\) respectively in the expected direction, as the bubble sits at \(h = 4 \mu m\) from the rigid wall. While we note that the ‘method-of-images’ does not capture the complex fluid dynamics at the boundary and may not strictly serve as a validating tool, it has been employed in more simplistic microbubble modeling scenarios [58]. Indeed, as a rigid wall is not a biologically relevant boundary, we did not explore this arrangement any further.

3. Results

We examine the frequency-dependent response of a two-microbubble system in three different scenarios: i) the effect of the presence of an identical, size-matched microbubble, ii) the effect of the presence of a nearby smaller microbubble, and iii) the effect of the presence of a nearby larger microbubble. In all scenarios, the frequency-dependent radial resonance response is investigated for varying inter-bubble distances \(h\) and the response for the individual microbubble in free-space (i.e. in isolation) is shown for comparison in green to better appreciate the contributions due to the second microbubble.

3.1. Two identical, size-matched microbubbles

Fig. 3 shows the result of a simulation in a system of two identical microbubbles (\(d_1 = d_2\)) with diameters of 2, 3, and 4 \(\mu m\). Microbubbles were subjected to a series of tone bursts at a constant peak-negative pressure of 30 kPa and simulated with center-to-center distances of \(h = 8, 16\) and 24 \(\mu m\). In all examined scenarios, the results show that when a given microbubble approaches another microbubble of the same size, each bubble experiences a decrease in \(f_{MR}\) and an increase in \(A_{MR}\). Further, the extent of this effect amplifies as the microbubbles get closer to each other, with the maximal effect shown here at \(h = 8 \mu m\). Taking into account all sizes investigated here, the maximum amount of the shift from two closely-positioned microbubbles at \(h = 8 \mu m\) apart as compared to its response in free space is a decrease in \(f_{MR}\) ranging from 7 to 10\% and an increase in \(A_{MR}\) from 9 to 11\%. Note here the small
secondary peaks due to harmonic coupling (e.g. 3–4 MHz for \( d = 2 \) µm in Fig. 3A) also exhibit the same trend as the primary resonance peaks; albeit at a lower amplitude. Indeed, the presence of these harmonic peaks is a well-known and established feature of resonant bubble systems [56,59,60].

### 3.2. A microbubble in the presence of a smaller microbubble

In the following two subsections, we examine the results of two unequal sized microbubbles \( (d_1 \neq d_2) \). Fig. 4 highlights the resonance curves for the larger microbubble \( d_1 \). The following four combinations were examined: a \( d_1 = 2 \) µm bubble in close proximity to a \( d_2 = 0.5 \) µm bubble (Fig. 4A), a \( d_1 = 3 \) µm bubble in close proximity to a \( d_2 = 2 \) µm bubble (Fig. 4B), a \( d_1 = 4 \) µm bubble in close proximity to a \( d_2 = 2 \) µm bubble (Fig. 4C), and \( d_1 = 4 \) µm bubble in close proximity to a \( d_2 = 3 \) µm bubble (Fig. 4D). For the system depicted in Fig. 4A, the microbubbles were insonicated at 120 kPa, and all others were insonicated at 30 kPa. We simulated the system with center-to-center distances of 2, 4, 8, 16 and 24 µm. The results presented here indicate that, for all combinations examined, the presence of the smaller microbubble \( d_2 \) has negligible

![Image](image-url)
influence on the vibration physics of the larger microbubble $d_1$. Within the frequency resolution employed here, there is no change in $f_{\text{MR}}$ and only a slight shift towards lower $A_{\text{MR}}$ (2–3 %) as compared to its free, isolated response.

3.3. A microbubble in the presence of a bigger microbubble

As opposed to the results shown in Fig. 4, there is a significant effect on the smaller microbubble $d_2$ due to the presence of a neighboring larger microbubble $d_1$. Fig. 5 shows the results of the following bubble size combinations: a $d_2 = 0.5 \mu m$ bubble in close proximity to a $d_1 = 2 \mu m$ bubble (Fig. 5A), a $d_2 = 2 \mu m$ bubble in close proximity to a $d_1 = 3 \mu m$ bubble (Fig. 5B), a $d_2 = 2 \mu m$ bubble in close proximity to a $d_1 = 4 \mu m$ bubble (Fig. 5C), and $d_2 = 3 \mu m$ bubble in close proximity to a $d_1 = 4 \mu m$ bubble (Fig. 5D). As in the scenario above, the results in panel Fig. 5A were simulated at 120 kPa, while the others were insonicated at 30 kPa. We simulated the system with center-to-center distances of $h = 2, 4, 8, 16$ and $24 \mu m$. The influence of the larger bubble is most strongly felt as the two bubbles approach each other. For all combinations of microbubble sizes examined here, the results consistently indicate that the smaller microbubble of size $d_2$ exhibits a strong and significant increase in $f_{MR}$ ranging from 7 to 11 %, and a decrease in $A_{MR}$ ranging from 38 to 52 % as compared to its isolated response. In looking at Fig. 5B, for example, the isolated, free $f_{MR}$ of a $d_2 = 2 \mu m$ microbubble (green curve) at the simulated pressure is approximately 6.5 MHz. In either the presence of a neighboring $d_1 = 3 \mu m$ (Fig. 5B) or $d_1 = 4 \mu m$ (Fig. 5C) microbubble, this peak exhibits a drastic decrease in amplitude and shift to higher frequency ($\approx 7$ MHz in panel C). Note that the primary resonance of the $d_2 = 0.5 \mu m$ microbubble (i.e. a nanobubble) is well out of the range of examined frequencies ($>8$ MHz) and is thus not visible in Fig. 5A.

Another glaring and significant result stemming from the influence of a larger microbubble is the presence of a secondary, off-resonance peak that is distinct from the harmonic peak. Indeed, this secondary peak in the frequency-dependent response exhibited by the smaller bubble $d_2$, observed in all combinations of bubbles examined here, corresponds precisely to the $f_{MR}$ of the large microbubble $d_1$ and thus represents a nonlinear coupling between the two bubbles. As previously stated, while the primary resonance response from the nanobubble is not depicted, the off-resonance peak due to the neighboring $d_1 = 2 \mu m$ is clear, with its influence becoming stronger as the bubbles approach each other (Fig. 5A). The appearance of this peak, which is maximum at $h = 2 \mu m$, appears precisely at a frequency of 4.5 MHz, in excellent agreement with the $f_{MR}$ of the $d_1 = 2 \mu m$ microbubble shown in Fig. 5A. This is also readily observed in the other three panels as the inter-bubble spacing is decreased, with the $f_{MR}$ of the larger bubble ($d_1 = 3 \mu m$ in panel B; $d_1 = 4 \mu m$ in panel C&D) corresponding to 3.5 MHz and 2.1 MHz, respectively. Indeed, the final two panels highlight that this peak derived from the off-resonance nonlinear coupling of the larger bubble vibrations is distinct from the harmonic peak – a peak observed even in isolated, individual bubbles (see Fig. 2A for example). While these peaks overlap in Fig. 5D due to the specific sizes of the microbubble pair, they are clearly separated in Fig. 5C ($d_2 = 2 \mu m$), where the harmonic peak expectedly shifts up due to the decreasing size of $d_2$ in Fig. 5C versus that of Fig. 5D; whereas the off-resonance peak at 2.1 MHz remains consistent between these two scenarios since the larger microbubble size is constant between these two panels ($d_1 = 4 \mu m$). Further, this nonlinear coupling effect can result in a large magnitude effect that rivals or even exceeds the $A_{MR}$ of the primary resonance peak (e.g. Fig. 5C, black curve). Finally, Fig. 6 highlights the influence of a larger neighboring microbubble on bubble response with a particular emphasis on the transmit frequency (the panels represent the same two-bubble system as those described in Fig. 5). Indeed, clinical applications of ultrasound are conducted at a fixed transmit center frequency, varying from the lower end of the MHz range for deep targets (e.g. 1–2 MHz for abdominal imaging), to mid-range for more superficial parts (e.g. 6–10 MHz for breast imaging, carotid imaging) [6]. While clinical pulses are shorter in length (and thus more broadband) than the pulses employed here, it is still apparent that depending on the clinical application, the direction and magnitude of the influence exerted by the two-bubble system shifts.

![Fig. 5](image_url)

Fig. 5. A large microbubble exerts significant influence on the resonance characteristics of a nearby smaller microbubble. The response responses of the smaller microbubble $d_2$ in the following situations: A) $d_1 = 2 \mu m$; $d_2 = 0.5 \mu m$; B) $d_1 = 3 \mu m$; $d_2 = 2 \mu m$; C) $d_1 = 4 \mu m$; $d_2 = 2 \mu m$; D) $d_1 = 4 \mu m$; $d_2 = 3 \mu m$. Panel A was insonicated at 120 kPa, all others at 30 kPa. Individual resonance response in free-space (green curve) is shown for comparison. The primary resonance peak shifts to higher $f_{MR}$ and lower $A_{MR}$ as the inter-bubble spacing $h$ decreases. The primary resonance peak of the $d_2 = 0.5 \mu m$ nanobubble is not visible in panel A. Note the secondary, off-resonant peak occurring at the $f_{MR}$ of the larger microbubble. This strong off-resonant nonlinear coupling occurs for all bubble combinations investigated. See text for details.
as the inter-bubble spacing decreases. The fixed frequencies here are chosen to align with the main (e.g. primary) and off-resonance coupling peaks.

4. Discussion

The results presented here indicate that the presence of a neighboring microbubble influences the radial resonance response of an individual microbubble. We note here that a subset of studies performed on ‘clean’, unencapsulated microbubbles yield similar relationships regarding \( f_{MR} \) and \( A_{MR} \). This phenomenon plays a role not only in ascertaining the resonance response of these bubbles in clinically relevant doses, but also in the application dependent (i.e. transmit frequency-dependent) response of a system of bubbles. Specifically, the magnitude and direction of the shift in response due to bubble proximity is a strong function of the transmit frequency, a direct result of the changes in \( f_{MR} \) and \( A_{MR} \). For the simplest and idealized case of two equal sized bubbles, the frequency of maximum response for both of them shifts to lower frequencies while the amplitude of maximum response increases. This type of behaviour is similar to the effect of a rigid wall (i.e. non-biologically relevant) on the response of a single microbubble – which generates the same potential flow as two symmetrically positioned microbubbles oscillating in phase - shown theoretically using the method of images [61–63].

Of perhaps more interest is the situation of unequal microbubble sizes. In this type of two-bubble system, the smaller sized microbubble exhibits a strong shift towards higher \( f_{MR} \) and a drastic decrease in \( A_{MR} \) – in stark contrast to the equal-sized bubble scenario described above. Further, when the two bubbles are in very close proximity, the smaller microbubble exhibits a strong off-resonance response that corresponds to the resonance frequency of its larger companion microbubble, while this larger microbubble exhibits no detectable change in its radial response – neither \( f_{MR} \) nor \( A_{MR} \). These effects are shown specifically in Fig. 6 which is the result of a fixed frequency simulation for the condition of Fig. 5. Indeed, only small differences in bubble sizes are required for this drastic change in overall response. As shown in Figs. 5 and 6, only the relatively small difference in bubble diameter of 0.5 \( \mu \)m is required to switch the observed effects demonstrated for a two-bubble system of two equal sized bubbles to that of unequal sized bubbles. This is especially of interest when considering practical application of contrast microbubbles. Clinically and commercially available microbubbles (e.g. Definity, SonoVue) are characterized by polydisperse microbubble populations (e.g. [52,64]). While there is ongoing research on the design of monodisperse microbubble formulations with a view to improving contrast image sensitivity, these are still characterized by typical coefficient of variations on the order of 5% [65,66] which results in an increased likelihood of the situation presented in Fig. 5,6: unequal sized microbubbles. The phenomenon observed here also sheds insight into the recent development and characterization of sub-micron bubbles (i.e. nanobubbles). Indeed, while possessing resonance frequencies much larger than the clinical frequency range on account of their small size (linear estimates beyond \( f = 30 \) MHz [40], robust acoustic measurements have recently provided evidence of nonlinear scattering [67,68], contrast imaging, and therapeutic potentiation [69] from nanobubble populations within clinical and pre-clinical ultrasound frequency ranges. The results presented here, specifically for the nanobubble dataset (\( d = 0.5 \) \( \mu \)m), suggest a possible mechanism for this off-resonance behaviour, namely strong acoustic coupling from a neighboring micron-sized bubble (Fig. 5.A,6.A). The ‘contaminating’ microbubble need not be an artefact of bubble synthesis but can also be due to ultrasound-induced bubble coalescence within typical imaging and therapeutic pulsing schemes. In this scenario, numerous off-resonant driven nanobubbles in addition to neighboring resonant microbubbles would contribute to the observed echo at clinical frequencies. Indeed, for ultrasound therapeutics, it is the oscillation amplitude examined here that is relevant as they can be linked to sonoporation and other bioeffects e.g. [12]). In fact, there are many current investigations into nanobubble-based therapeutics [39,40,70]. However, for imaging purposes, we can estimate the far-field scattered pressure \( P_s \) at a distance \( r \) via the following relation [44]:

\[
P_s \approx \rho \left( \frac{RR^2 + 2RR^2}{r} \right) \tag{6a}
\]

where under low driving conditions, the maximum pressure reduces to.
where \( \omega \) is the angular frequency and \( \epsilon \) is the radial excursion. From the above equation, for a fixed frequency and bubble size (as is the case in Fig. 5a), the maximum scattered pressure scales proportionally to the radial excursion, and thus we expect a similar increase between a nanobubble in free-space (green curve in Fig. 5a) and a nanobubble close to a microbubble (black curve in Fig. 5b).

It is insightful here to place our numerical, finite-element model within the framework of the very limited experimental data investigating the influence of a neighboring microbubble and/or a planar boundary on the radial response of an individual ultrasound contrast agent. In perhaps the only dataset to be directly comparable to our model, Garbin et al.\[23\] measured the influence of a bigger microbubble (\( d_1 = 4.8 \mu m \)) on the radial dynamics of a smaller one (\( d_2 = 4.5 \mu m \)) by employing a combination of optical trapping and ultrafast full-frame microscopy \[71\]. In this single frequency (\( f = 2.25 \text{ MHz} \)), 8-cycle acquisition, the vibrational response of the smaller bubble \( d_2 \) was significantly lower when placed \( h = 8 \mu m \) away from the larger bubble as compared to its free, isolated response (Fig. 3B in Garbin et al. \[23\]). Our simulated result within this system consistent with the measured data, with the presence of the larger bubble resulting in a 2 % decrease in maximum radius \( R_{\text{max}} \) and an 8 % decrease in minimum radius \( R_{\text{min}} \) as compared to its free response (Fig. 7). While this does not directly provide conclusive evidence of the bubble/proximity based \( f_{\text{max}} \) and \( A_{\text{MR}} \) shifts observed in the present manuscript – since no such experiment has even been conducted – it is consistent at this individual transmit frequency. Further, while the individual shell parameters for Garbin et al.’s data were not known, our simulation predicts a similar trend over a wide range of lipid shell parameter estimates.

It is also worth noting here that our model does not incorporate bubble coalescence, nor the effects of secondary Bjerknes force. While this is a noted limitation of the model, this force is likely not the dominant bubble-bubble interaction under the acoustic forcing conditions imposed here (single 10-cycle burst, \( \sim 30 \text{ kPa} \)). Indeed, in one of the only comparable experimental datasets, Garbin et al.\[72\] noted no significant translation (on the order of 100–200 nm) between two lipid-encapsulated agents situated \( h = 12.5 \mu m \) apart from each other subject to 150 kPa – higher than the transmit pressures used in the present manuscript.

5. Conclusions

For two identical microbubbles vibrating in close proximity to each other, our results show the frequency of maximum response (\( f_{\text{MR}} \)) decreases (7–10 %) and the amplitude of maximum response (\( A_{\text{MR}} \)) increases (9–11 %) as the microbubbles approach one another. For a two-bubble system of different microbubble sizes, the larger bubble shows no change in \( f_{\text{MR}} \) and a slight shift of \( A_{\text{MR}} \) (2–3 %). However, the smaller bubble exhibits an increase in \( f_{\text{MR}} \) (7–11 %) and a significant decrease of \( A_{\text{MR}} \) (38–52 %). Furthermore, in very close proximity, smaller bubbles exhibit a secondary resonance peak corresponding to the \( f_{\text{MR}} \) of the larger bubble, with amplitudes comparable to its primary resonance peak. These results have implications in both contrast imaging and microbubble-mediated therapeutic applications.

CRediT authorship contribution statement

Hossein Yusefi: Methodology, Software, Validation, Formal analysis, Investigation, Visualization. Brandon Helfield: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Supervision, Funding acquisition.

Fig. 7. Simulation results are consistent with only known experimental data of a similar system. The radius versus time of an individual \( d_2 = 4.5 \mu m \) simulated (red) in free space and (blue) in the proximity of a larger bubble (\( d_1 = 4.8 \mu m \)) situated \( h = 12.5 \mu m \) away, insonated at \( f = 2.25 \text{ MHz} \) with a single 8-cycle Hanning-windowed pulse. The trend documented here, of the bigger bubbles’ influence on the smaller one resulting in a decrease in overall radial amplitude, is consistent with the experimental work conducted by Garbin et al. – the only known such experiment.

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.

Data availability

Data will be made available on request.

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