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## **The Effects of Ultrasound Parameters and Microbubble Concentration on Acoustic Particle Palpation**

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1 The elasticity of tissue - an indicator of disease progression - can be imaged by ul-  
2 trasound elasticity imaging technologies. An acoustic particle palpation (APP) has  
3 recently been developed - the use of ultrasonically-driven acoustic particles (e.g., mi-  
4 crobubbles) - as an alternative method of tissue deformation. APP has the potential  
5 to improve the resolution, contrast, and depth of ultrasound elasticity imaging; but  
6 the tissue displacement dynamics and its dependence on acoustic pressure, center  
7 frequency, and microbubble concentration remains unknown. Here, we produced dis-  
8 placements of at least  $1 \mu\text{m}$  by applying ultrasound onto a microbubble solution  
9 (concentration:  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ ) placed within a tunnel surrounded by  
10 a 5% gelatin phantom. Displacements of more than  $10 \mu\text{m}$  were produced using a 1,  
11 3.5, or 5 MHz center frequency pulse with peak-rarefactional pressures of 470, 785,  
12 and 1,210 kPa, respectively. The deformation of the distal wall varied spatially and  
13 temporally according to the different parameters investigated. At low pressures, the  
14 deformation increased over several milliseconds until it was held at a nearly constant  
15 value. At high pressures, a large deformation occurred within a millisecond followed  
16 by a sharp decrease and long stabilization. Ultrasound exposure in the presence of  
17 microbubbles produced tissue deformation ( $p < 0.05$ ) while without microbubbles,  
18 no deformation was observed.

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## 19 I. INTRODUCTION

20 Changes to the elastic properties of tissue are strong indicators of disease progression.  
21 In cancer (?), liver cirrhosis (?), and other diseases, tissue stiffens over time. Quantify-  
22 ing and imaging changes in elasticity are thus major goals in imaging modalities, such as  
23 ultrasound and magnetic resonance imaging (MRI) (?). The general steps for measuring  
24 elasticity noninvasively using acoustic radiation force (ARF) is to apply the force to the  
25 tissue (i.e., palpation), monitor the resulting deformation, and derive the elasticity using  
26 a model (???). In these techniques, ARF is applied by focusing ultrasound onto a region  
27 of excitation (ROE) and is proportional to the intensity of ultrasound and the absorption  
28 coefficient of the tissue (?). The deformation can be monitored by ultrasound or other  
29 modalities, such as MRI and optical imaging. Despite the benefits of ARF-based elasticity  
30 imaging, there are diseases, such as micro-metastatic cancers and distal regions of the liver,  
31 which cannot be imaged due to poor imaging depth, contrast, or resolution (?).  
32 Lipid-shelled and gas-filled microbubbles are routinely used in the clinic as ultrasound con-  
33 trast agents to improve the quality of ultrasound images (?). This contrast enhancement  
34 takes advantage of a microbubble's unique behavior in an acoustic field: nonlinear vol-  
35 umetric oscillations that enhance scattering. Ultrasound exposure of a microbubble also  
36 generates a primary radiation force (or Bjerknes) force which is proportional to the spatial  
37 derivative of the acoustic pressure and the bubble's volume (?). Microbubbles undergoing  
38 primary radiation force move in the direction of ultrasound propagation (?). Microbubbles  
39 also experience a secondary radiation force, which is an attractive or repulsive force between

40 oscillating bubbles. This force becomes relevant as the distance between adjacent bubbles  
41 is reduced (i.e., high concentrations) and as the pressure and pulse length increase (?).  
42 Bubbles exposed to ultrasound have been previously used to measure the elasticity of vis-  
43 coelastic media (?). In one approach, large bubbles were formed by vaporization of a hydrogel  
44 (gelatin) with a laser. This laser-generated bubble had a radii between 18 and 78  $\mu\text{m}$  and  
45 was used to measure the Young's modulus of the material. In another study, the elastic  
46 properties of materials was measured by exposing a large laser-generated bubble (diameter:  
47 100-800  $\mu\text{m}$ ) to an acoustic field (?). Moreover, some experiments have been conducted to  
48 characterize the time-dependent mechanical properties of microvessels by optically imaging  
49 the tissue's response to an ultrasonically-driven microbubble collapse (i.e., inertial cavit-  
50 ation) against vessel walls (?).

51 We recently explored the use of pre-formed microbubbles undergoing primary ARF - acous-  
52 tic particle palpation (APP) - as a stress source for elasticity imaging (?). In this technique,  
53 microbubbles were administered into a vessel that modelled the bloodstream of tissue. The  
54 application of ultrasound caused the microbubbles to move in the direction of wave propa-  
55 gation and push against the distal vessel wall, resulting in tissue deformation. It was shown  
56 that a larger force was applied with APP than with ultrasound only ARF-based methods  
57 (?). Microbubbles used in this manner are acting as contrast agents for elasticity imaging.  
58 However, just as contrast agents for ultrasound imaging are not simply the enhancement of  
59 tissue contrast - it is the blood supply that is enhanced - microbubbles in APP would not  
60 enhance the tissue contrast directly. Microbubbles are contained within the vasculature and  
61 thus the vessel wall would be assessed. Thus, APP-based imaging may be able to probe

62 large vessels such as arteries or veins. The Young's modulus of arteries in human can range  
63 from 0.3 to 5.5 MPa (?) with the mass density of 1050-1075 kg m<sup>-3</sup> (?) and the speed of  
64 sound of 1560-1660 m s<sup>-1</sup> (?). These properties depend on the composition of the vascular  
65 tissue such as collagen, elastin and smooth muscle cells (?) and will change with age and the  
66 progression of vascular diseases (??). However, the vessel's properties in APP-based imaging  
67 may be far less relevant as the probed vessels approach the size of arterioles, venules and  
68 capillaries. In such small vessels, their thickness approaches a single cell and in the case of  
69 microvessels with very thin walls, the vessel takes on the elasticity of the surrounding tissue  
70 microstructures (?). For soft tissue such as liver, the Young's modulus, mass density and  
71 speed of sound are 0.6 kPa (?), 1050 kg m<sup>-3</sup> (?) and 1578 m s<sup>-1</sup> (?) respectively. APP  
72 techniques are not limited to just vessels and could potentially measure the elastic properties  
73 of the other tissue types by injecting the particles into the cerebrospinal fluid, fluid bodies or  
74 subcutaneously into the lymphatic system (?). Thus, there is a potential for measuring the  
75 stiffness of tissue microenvironments. But to make this elasticity measurement technique  
76 pragmatic, it must be safe and effective. One of the concerns with sonicated microbubbles  
77 is that high magnitudes of inertial cavitation may damage the surrounding tissue environ-  
78 ment. This occurs when the rarefactional phase of an ultrasonic pulse is high enough to  
79 cause the bubble to unstably expand to at least twice its initial radius (?), which leads to  
80 a rapid collapse due to the inertia of the surrounding fluid. This phenomenon depends on  
81 the frequency of ultrasound, peak rarefactional pressure and pulse length (?). As a result,  
82 the acoustic parameters must be below a threshold to minimize the magnitude of inertial  
83 cavitation. Although there is some debate about when in vivo bio-effects arise, studies

84 have suggested that the mechanical index (MI) should be kept below 0.4 when ultrasound  
85 is applied in the presence of certain types of microbubbles (?). The MI is proportional to  
86 the peak-rarefactional pressure and inversely proportional to the square root of the center  
87 frequency. Since APP can be achieved with very low intensities (low acoustic pressures and  
88 short duty cycles), we anticipate very low thermal index (TI) values.

89 The purpose of this study is to identify a range of ultrasound and microbubble conditions  
90 that can generate APP to an extent that is relevant for elasticity imaging. Microbubbles  
91 flowing through a wall-less channel in a 5% gelatin phantom was exposed to ultrasound to  
92 cause deformation of the distal tissue wall. The Young's modulus of 5% gelatin phantom is  
93 approximately 1.5 kPa as determined in previous studies (??) and is similar to the elastic-  
94 ity of the brain in young rats (?) and liver in humans (?). The effects of different center  
95 frequencies (1, 3.5, and 5 MHz), which are below, close and above the resonance frequency  
96 of microbubbles, were investigated. A wide range of acoustic pressures were applied (peak  
97 negative of 0.3 to 2 MPa) to observe how microbubbles behaved below, at, and above the MI  
98 threshold for clinical safety. Experiments were also conducted with a range of microbubble  
99 concentrations ( $4 \times 10^6$ ,  $10 \times 10^6$  and  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$ ) to explore the possibility  
100 of palpating tissue using low microbubble concentrations.

## 101 II. MATERIALS AND METHODS

### 102 A. Tissue Mimicking Material

103 Experiments were conducted with gelatin phantoms that mimicked the elastic and acous-  
104 tic properties of tissue (?). These phantoms had a speed of sound of approximately 1540  
105  $\text{m s}^{-1}$ , a mass density of approximately  $1 \text{ g cm}^{-3}$  and a Young's modulus of approximately  
106  $10^0 - 10^2 \text{ kPa}$  (?). To prepare each phantom, 10 g of gelatin powder (Fisher Scientific UK  
107 Ltd, Loughborough, UK) was added to 200 ml of degassed and deionized water. The solution  
108 was dissolved by heating ( $42^\circ\text{C}$  for 40 minutes), stirred continuously to create a homogenous  
109 solution, and then degassed for 30 minutes under the vacuum strength of 720 mmHg in a  
110 vacuum chamber. The mixture was poured into a phantom box which had two Mylar sheets  
111 that allowed for ultrasound to enter and leave the box. An 800- $\mu\text{m}$ -in-diameter carbon rod  
112 (Hyperflight, UK) was inserted into the phantom. The box was then refrigerated overnight  
113 (approximately 12 hours) so that the solution solidified. Each phantom was left at room  
114 temperature ( $22^\circ\text{C}$ ) for an hour prior to experiments. The rod was then removed before  
115 sonication to provide a wall-less tunnel for water and microbubbles to flow through.

### 116 B. Microbubble Preparation

117 Lipid-shelled microbubbles were prepared according to a previously described method  
118 (?). Dipalmitoylphosphatidylcholine (DPPC-82%), Dipalmitoylphosphatidic acid (DPPA-  
119 8%), and dipalmitoylphosphatidylethanolamine-PEG5000 (DPPE-PEG5000-10%) (Avanti  
120 Polar Lipids Inc., AL, USA) were mixed and diluted with saline and glycerol. Each vial was

121 filled with perfluorobutane gas (FluoroMed L.P., Texas, USA) and placed in a mechanical  
122 shaker (Synergy Electronics, Scottsdale, AZ, USA) for 45 seconds to activate the bubbles.  
123 The size and population distribution of the bubbles were calculated by optical microscopy  
124 followed by image processing using a previously described technique (?). The mean diameter  
125 of the bubbles was  $2.07 \pm 1.28 \mu\text{m}$  with a range from 0.5 to  $9.87 \mu\text{m}$ . The undiluted vial  
126 concentration was  $3.83 \times 10^9$  microbubbles  $\text{ml}^{-1}$ ; approximately 2000 times the clinical  
127 dose of  $2.04 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . This clinical dose was based on a  $0.02 \text{ ml kg}^{-1}$   
128 recommendation while assuming a 5 L blood volume for a 70 kg individual (?). Diluted  
129 microbubbles solutions were prepared by diluting the vial's content with select amounts of  
130 deionized, degassed water for each experiment.

### 131 C. Experimental Setup

132 The tissue-mimicking phantom was placed in a water tank using a 3-D manual positioning  
133 system (Newport, Bloomfield, NY, USA). The tank's water was degassed and deionized. A  
134 high frame rate camera (frame rate: 1200 frames per second, field of view:  $416 \times 144$  pixels,  
135 model: Nikon 1 V3, Nikon Inc., USA) with an attached lens (Nikon VR 70-300 f/4.5-5.6)  
136 was used to record the displacement of the channel (Fig. ??). Two magnifying glasses with  
137 magnification factors of 10x were used to improve the resolution to  $10 \mu\text{m pixel}^{-1}$ . An  
138 LED light source (KL 2500 LED, SCOTT, Stafford, UK) with gooseneck guides was used  
139 for backlight illumination and to increase the contrast between the wall-less channel and the  
140 background (Fig. ??). For all experiments, microbubbles flowed across the channel using  
141 a syringe pump (70-3007, Harvard Apparatus, Cambridge, UK) and plastic tubes (R 3603,

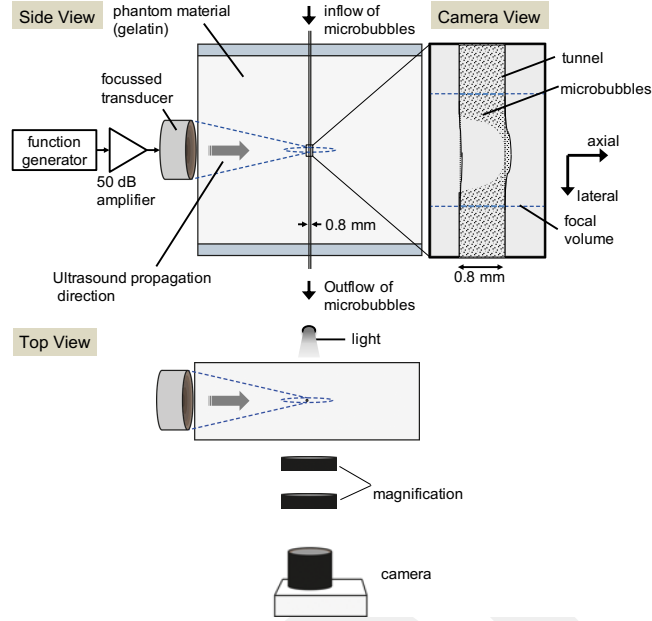


FIG. 1. Experimental setup. A solution of microbubbles flowing through a 0.8 mm wall-less channel were sonicated by a focused ultrasound transducer. The channel was created in a 5% gelatin phantom which was immersed in a water tank. The transducer was driven by a function generator and 50 dB amplifier (side view). The sonicated bubbles applied a force to the channel's wall and deformed it (Camera view). The deformation of the wall was recorded by a high frame rate camera. Two magnifying glasses were used to increase the resolution of the final image. To increase the contrast between the channel and the phantom material, the background was illuminated (top view).

142 Tygon) attached to the connectors on the phantom box. The flow rate was  $700 \mu\text{l min}^{-1}$   
 143 and the velocity was  $23 \text{ mm s}^{-1}$ .

144 Three different single element transducers (Power Series, Olympus Industrials, UK) of 1 MHz  
 145 (aperture diameter: 25.4 mm, f-number: 0.9, FWHM: 4.77 mm, focal length: 51.15 mm,  
 146 part number:18-0116-P), 3.5 MHz (aperture diameter: 19.05 mm, f-number: 3.3, FWHM:

147 1.71 mm, focal length: 52.02 mm, part number:17-0312-P) and 5 MHz (aperture diameter:  
148 25.4 mm, f-number: 4.8, FWHM: 0.83 mm, focal length: 52.30 mm, part number:18-0516-P)  
149 were used. Each transducer was calibrated in free field by a hydrophone (Precision Acoustics  
150 Ltd., Dorchester, UK) in a separate set of experiments. In order to place the transducer's  
151 focal volume over the tube, we first imaged the rod that remained embedded in the phantom.  
152 In an imaging configuration, the transducer was connected to a pulser/receiver (DPR300,  
153 JSR Ultrasonics, Pittsford, NY, USA) and oscilloscope (DPO3014, Tektronix, Inc. OR,  
154 USA) to position the transducer axially. The carbon rod was then gently removed from  
155 the phantom. The lateral targeting was conducted by imaging an air bubble that filled the  
156 channel after the rod was removed. In the APP configuration, the transducer was driven by  
157 a waveform generator (33500B Series, Agilent Technologies, Santa Clara, CA, USA) and 50  
158 dB power amplifier (Precision Acoustics Ltd, Dorchester, UK) to produce a specific range  
159 of beam characteristics (Table ??).

160 After alignment of the transducer, the control experiment (without the presence of the  
161 microbubbles) was conducted by establishing a flow of degassed, deionized water through  
162 the channel. The channel was then filled with a flow of diluted microbubbles. The channel  
163 was cleaned after each experiment by flowing an air bubble across the channel. In order  
164 to evaluate whether residual microbubbles accumulated in the channel, a second control  
165 experiment with water was conducted at the end of the experiments. No significant difference  
166 in deformation was observed between the initial and final controls. For all experiments,  
167 images were captured before, during and after ultrasound excitation (Fig. ??)

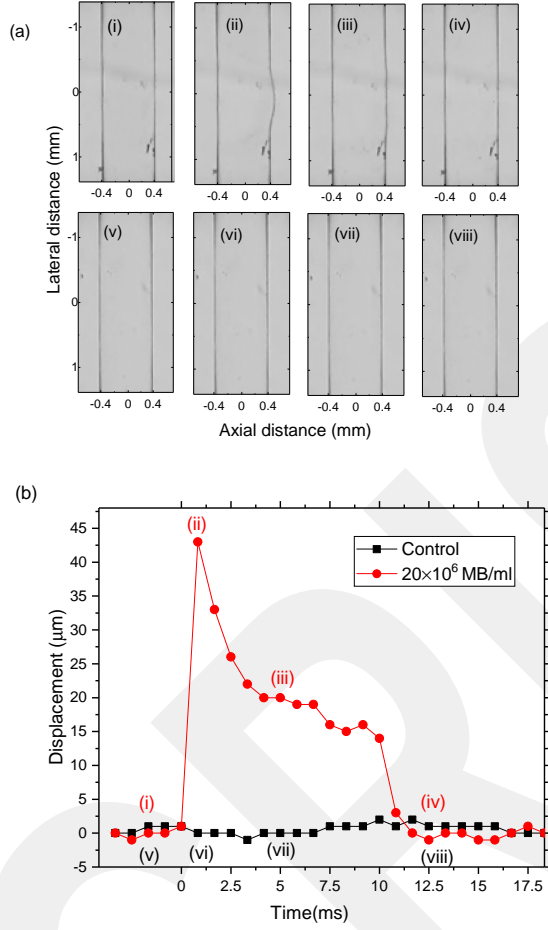


FIG. 2. (a) The images acquired by the camera of the wall less channel pre, during and post excitation (center frequency: 5 MHz, peak-rarefactional pressure: 2210 kPa, pulse length: 10 ms) with the channel filled (i-iv) with the microbubbles and (v-vii) with water alone (Control). The images are captured (i), (v) before the excitation and (ii-iv), (vi-vii) at 0.83, 4.98 and 12.45 ms after the start of the excitation. MB: microbubbles. (b) Displacement over the length of the pulse for the corresponding images.

#### 168 **D. Deformation Analysis**

169 In order to analyze the APP-induced deformation dynamics, we tracked and collected the  
170 wall deformation using the pixels from the images captured by the camera and processed  
171 with MATLAB (The Mathworks, Natick, MA, USA). Since wall deformation occurred in  
172 the direction of wave propagation, we anticipated that the deformation would occur on and  
173 orthogonal to the distal wall. Our first step was to automatically detect where the wall was  
174 located within each image. This was achieved by tracking a 1-D line of pixels along the  
175 axial axis. Once this was found, each image was interpolated by a factor of 10 along the  
176 axial dimension. The overlap of the focal volume with the distal wall was detected and the  
177 focal center of the ultrasound beam on the distal wall was determined which was assumed as  
178 the middle of the previously detected overlapped area. An average of five adjacent pixels in  
179 the focal center were considered in our deformation measurements. The displacement of the  
180 focal center pixels in each frame was measured using 1-D cross-correlation. The position of  
181 the focal center was averaged at five to ten frames before the excitation and was considered  
182 as the reference for the cross-correlation algorithm.

#### 183 **E. Statistical Analysis**

184 The mean and standard deviation values were calculated based on the deformation values  
185 for five consecutive pulses. Statistical tests, one-way analysis of variance (ANOVA) followed  
186 by post-hoc Bonferroni analysis, were performed to check the significance of the results.  
187 The data sets for different microbubble solutions at each acoustic pressure for each of the

TABLE I. Ultrasound parameters

<i>Parameters</i>	<i>Set 1</i>	<i>Set 2</i>	<i>Set 3</i>
Frequency (MHz)	1	3.5	5
Peak-negative pressure (kPa)	240 to 734	325 to 1395	325 to 1395
Pulse length (ms)	20	10	10
Pulse repetition period (ms)	200	200	200
Number of pulses	5	5	5

188 transducers were compared and a P value of 0.05 was considered to determine the significant  
189 difference. Linear regression was also performed to compare the displacement amplitudes  
190 for different solutions over all the applied acoustic pressures.

### 191 III. RESULTS

#### 192 A. Deformation Dynamics

193 A uniform flow of microbubbles with a concentration of  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$  was  
194 established through the channel before the excitation ((Fig. ??(a).i), (Fig. ??(b).i)). The 5  
195 MHz transducer was driven at a peak-rarefactional pressure of 2,210 kPa, which pushed the  
196 microbubbles towards the distal wall of the channel and created a large wall displacement of  
197 approximately  $43 \mu\text{m}$  ((Fig. ??(a).ii), (Fig. ??(b).ii)). The displacement decreased rapidly  
198 in the following frames ((Fig. ??(a).iii), (Fig. ??(b).iii)). Finally, the channel wall returned

199 to its initial position after the removal of ultrasound ((Fig. ??(a).iv), (Fig. ??(b).iv)). The  
200 same experiment was conducted with the channel filled with water and without the presence  
201 of the microbubbles. No deformation of the channel was observed in the control experiment  
202 ((Fig. ??(a).v-vii), (Fig. ??(b).v-vii)).

## 203 B. Acoustic Pressure

204 In order to evaluate the effect of different acoustic pressures on the APP, the 1 MHz  
205 transducer was driven at different pressures (peak-rarefactional pressure from 240 to 734  
206 kPa). As expected, higher acoustic pressure produced greater displacements. In one set of  
207 experiments with a microbubble concentration of  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ , it was ob-  
208 served that low pressure exposure (i.e. 470 kPa) produced a displacement of the wall that  
209 was nearly constant during the entire pulse duration. Increasing the pressure above 520  
210 kPa (i.e. 734 kPa) led to a displacement up to  $14 \pm 3.58 \mu\text{m}$  (0.83 ms after the start of  
211 the excitation), which rapidly decreased in the following frames (Fig ??(a)). In the control  
212 experiments at the highest pressure (i.e., 734 kPa), a very small net displacement of  $1.86 \mu$   
213 m was observed while no displacement was observed at lower pressures.

214 We evaluated similar experiments with the 3.5 MHz transducer (Fig. ??(b)). No displace-  
215 ment was observed in our control where the channel filled with water, was exposed to acoustic  
216 pulses at 1,395 kPa. In the presence of microbubbles (concentration:  $10 \times 10^6$  microbub-  
217 bles  $\text{ml}^{-1}$ ), ultrasound exposure at 785 and 1395 kPa produced a maximum displacement of  
218  $8.8 \pm 1.58 \mu\text{m}$  and  $16.2 \pm 4.39 \mu\text{m}$ , respectively. The displacement pattern where an initial  
219 peak displacement followed by a decrease in the following frames, was observed for acoustic

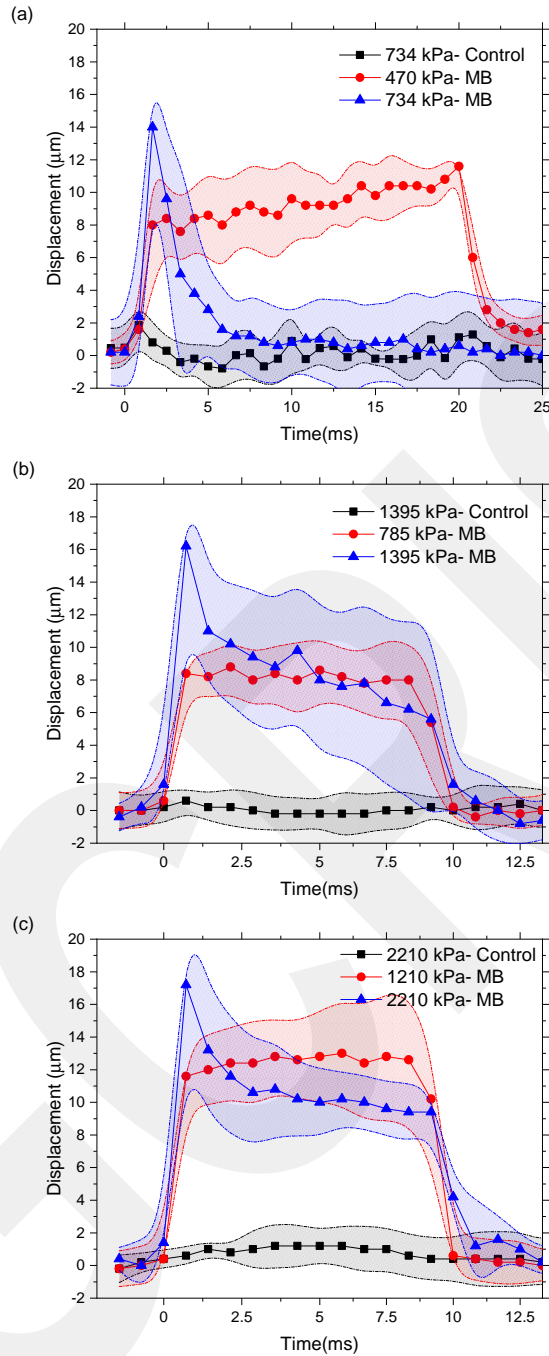


FIG. 3. Displacement over the length of the pulse. The deformation of the distal wall was tracked for different ultrasound exposure conditions (circles and triangles) with and (squares) without microbubbles. Displacements are shown as averages for five consecutive pulses using (a) 1 MHz, (b) 3.5 MHz, and (c) 5 MHz transducer. The microbubble (MB) concentration was  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ .

220 pressures above 930 kPa.

221 For the experiments using the 5 MHz transducer, no displacement was observed for the  
222 control experiments. Using a solution of  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ , higher pressures led to  
223 higher displacement of the channel. For the relatively high pressures (above 1,210 kPa), the  
224 same displacement pattern where an initial peak displacement was produced, was observed  
225 (Fig. ??(c)).

226 We evaluated the effect of three different microbubble concentrations and a range of acoustic  
227 pressures (Table ??) for each transducer. Since the results for only two different pressure  
228 values were shown in Fig. ?? for each transducer, the effect of pressure on displacements  
229 is not clearly depicted. The effect of acoustic pressure and microbubble concentration is  
230 shown in Fig. ?? in Section 2C. For each microbubble concentration, the acoustic pressure  
231 was increased and the maximum value of the displacement was measured and averaged for  
232 5 consecutive pulses. Higher acoustic pressures produced greater displacements.

233 For each transducer, data sets for each pressure level were compared for all the microbub-  
234 ble concentrations with ANOVA followed by post-hoc Bonferroni analysis. For the 1 MHz  
235 transducer, the results were significantly different between all microbubble concentrations  
236 at pressure levels of 355-734 kPa except between  $10 \times 10^6$  and  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$   
237 solutions. At acoustic pressures of 240 and 300 kPa, the displacements were different except  
238 between the control and  $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$  and between  $10 \times 10^6$  and  $20 \times 10^6$   
239 microbubbles  $\text{ml}^{-1}$  solutions. For the 3.5 MHz transducer, the results were significantly  
240 different between all the microbubble concentrations at pressure levels of 475, 615 and 1395  
241 kPa except between  $10 \times 10^6$  and  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$  solutions. For the remaining

242 acoustic pressures, the results were different only between the control and the experiments  
243 with microbubbles. Finally, for the 5 MHz transducer, the presence of microbubbles in  
244 solutions yielded significantly different displacements compared to the control experiments.  
245 At the pressure of 1510 kPa, all the results were found to be significantly different. The  
246 resultant displacements from  $4 \times 10^6$  and  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$  solutions were not  
247 found to be significant at pressure levels of 1210, 1810 and 2110 kPa. For each transducer,  
248 linear regression was performed on data sets at all pressure levels for each microbubble con-  
249 centration. All the results were significantly different except for the control experiment with  
250 the 3.5 MHz transducer. In addition, slope of the linear fit was found to increase as the  
251 microbubble concentration increased for each transducer.

### 252 C. Center Frequency

253 The effect of ultrasound center frequency on the outcome of APP was investigated by  
254 keeping the microbubble concentration constant ( $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$ ) and adjusting  
255 the acoustic pressure (Fig. ??(a)). Since each transducer produced a different range of  
256 acoustic pressures, the displacement values were calculated as a function of MI. The experi-  
257 ments were repeated using microbubble concentration of  $10 \times 10^6$  (Fig. ??(b)) and  $20 \times 10^6$   
258 microbubbles  $\text{ml}^{-1}$  (Fig. ??(c)). For low MI values (below 0.6), no significant difference  
259 was observed between the displacement values generated by the transducers for a given MI.  
260 We summarized the averaged deformation values of each excitation for all the pressures,  
261 center frequencies, microbubble concentrations and control experiments in Fig. ?. Defor-  
262 mation of the wall was almost constant during excitations with moderate pressures (below

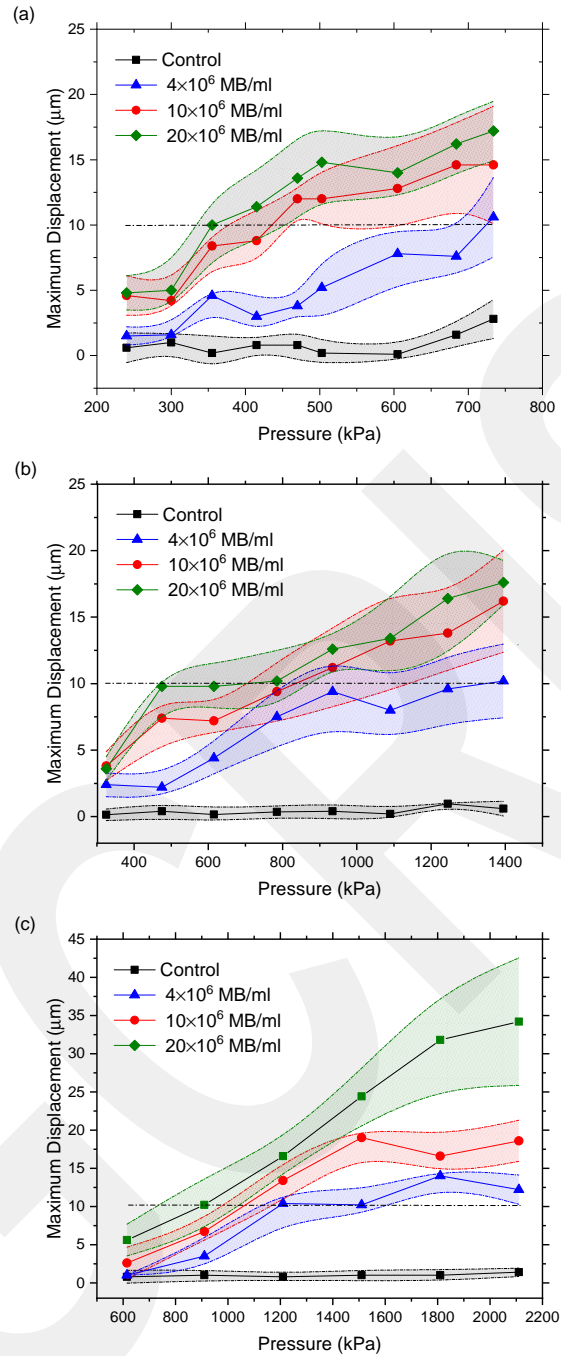


FIG. 4. Maximum displacement over acoustic pressure. The maximum displacement of the wall in each excitation is calculated and then averaged for five consecutive pulses. Values are reported for the experiments (diamonds, circles and triangles) with and (squares) without microbubbles. (a) 1 MHz transducer, (b) 3.5 MHz transducer, (c) 5 MHz transducer. MB: microbubble, Control: without microbubbles. The displacement threshold of  $10 \mu\text{m}$  is shown by the dashed lines.

263 520 kPa for 1 MHz, 930 kPa for 3.5 MHz, and 1,210 kPa for 5 MHz transducer). However,  
264 at higher pressures, the maximum displacement occurred within a few milliseconds and was  
265 followed by lower displacements thereafter. Additionally, it was observed that the deforma-  
266 tion values did not increase linearly with microbubble concentration or acoustic pressure.

267

#### 268 IV. DISCUSSION

269 We evaluated a range of ultrasound center frequencies, acoustic pressures, and microbub-  
270 ble concentrations that can produce elasticity imaging-relevant deformations using APP.  
271 A minimum axial resolution in the orders of tens of microns is required for correlation  
272 based tracking techniques according to Cramer-Rao lower band (??). The displacement of  
273 about 10  $\mu\text{m}$  was observed using  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$  by applying acoustic pressures  
274 of 350, 470 and 910 kPa for 1, 3.5 and 5 MHz transducers respectively. Thus APP re-  
275 quires lower acoustic pressures to displace tissue when compared to conventional ultrasound  
276 only ARF-based methods (?). The magnitude of displacement can be increased by using  
277 higher acoustic pressures and microbubble concentrations, which may be necessary for stiffer  
278 materials. For soft materials, small detectable displacements are enough for elasticity mea-  
279 surement purposes because the correlation between the pre- and post-compression signals  
280 tracked by ultrasound, is reduced for large strains as a result of large displacements.  
281 The displacement was not constant during a single pulse (Fig. ?? and Fig. ??). At low  
282 pressures, the displacement increased slowly. However, at high acoustic pressures, a high  
283 displacement was produced in the beginning of the pulse and was followed by a quick de-

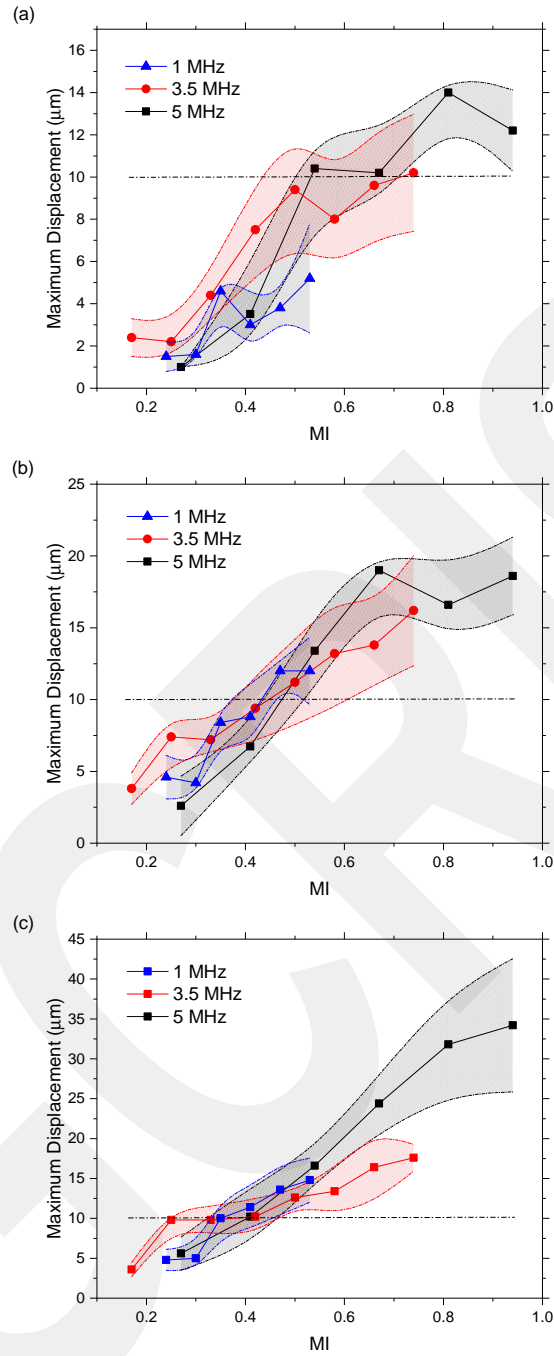


FIG. 5. Maximum displacement over mechanical index (MI). The maximum displacement value in each pulse is averaged for five consecutive pulses. The values are shown for different ultrasound exposure conditions which are center frequencies (squares: 5 MHz, circles: 3.5 MHz and triangles: 1 MHz) and acoustic pressures. The reported displacement values were obtained using microbubble concentrations of (a)  $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$ , (b)  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$  (c)  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . The displacement threshold of  $10 \mu\text{m}$  is shown by the dashed lines.

crease that stabilized over time. This behavior became more dramatic at higher acoustic pressures and microbubble concentrations. We believe that the sudden decrease of the displacement during an excitation was due to microbubbles being pushed away from the ROE or destroyed within a few microseconds or milliseconds. Previous studies have shown that high acoustic pressures can destroy microbubbles or divide them into smaller particles (?). The lower displacement values in the following frames of each pulse could be a result of a subpopulation of microbubbles that have not been destroyed. It should be noted that the microbubble solution was infused into the tunnel at a velocity of  $23 \text{ mm s}^{-1}$ , which is similar to the blood flow velocity in small arterioles (??). The pulse repetition frequency was 200 ms to ensure that unsonicated bubbles replenished the tunnel between consecutive pulses and to establish a constant microbubble concentration for all experiments. However, since the blood velocity depends on the size of the vessel, the effect of flow rate will be considered in future studies.

The amplitude of the deformations could be higher than what was measured in the experiments. The temporal resolution of the camera was limited to 0.83 milliseconds, so the maximum displacement, which could have occurred between frames, may not have been captured. A camera with a higher time resolution is suggested for future work.

For the three center frequencies tested (1, 3.5 and 5 MHz) and with a microbubble concentration of less than  $20 \times 10^6 \text{ microbubbles}^{-1}$ , a displacement of about 10 microns was obtained with mechanical indices lower than 0.4, which is defined as a potential damage threshold (?). Since the three center frequencies tested generated almost the same amount of displacements for a given MI (Fig. ??), different depths of diagnosis can be measured

306 by the careful selection of center frequencies. For example, using a low center frequency  
307 transducer could enable a high depth of elasticity imaging. Although only three center  
308 frequencies were tested in this study, other frequencies may be usable. Additionally, it was  
309 not possible to determine the optimal center frequency to use, because of the polydispersed  
310 size distribution of the microbubbles. The use of more uniformly sized microbubbles could  
311 improve the APP-induced deformation magnitude. Future work will include designing mi-  
312 crobubbles based on their size and persistence for APP imaging.

313 Different microbubble concentrations were used to palpate the phantom. In general, higher  
314 microbubble concentrations produced greater displacements. However, this rise in displace-  
315 ment with microbubble concentration was not linear. This nonlinear relationship may be  
316 due to the translational displacement of bubbles changing as the pushed microbubbles be-  
317 come increasingly dense. In other words, as the microbubbles are displaced towards the  
318 distal wall, the separation distance between bubbles reduces (?) and secondary radiation  
319 forces become greater. Therefore, the force generated by a population of the microbubbles  
320 may not equal to sum of the force applied by individual ones.

321 It was observed that maximum displacement changed sublinearly with MB concentration.

322 As an illustration, maximum displacement values of 0.2, 5.2, 12.8 and 14.8  $\mu\text{m}$  were esti-  
323 mated using no MB (Control),  $4 \times 10^6$ ,  $10 \times 10^6$  and  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$  respectively  
324 by applying an acoustic pressure of 503 kPa with the 1 MHz transducer (Fig. ??(a)). .

325 Results also showed that maximum displacement changes sublinearly with applied pressure.

326 For instance, maximum displacement values of 4.2, 8.4, 12 and 14.5  $\mu\text{m}$  were obtained by  
327 applying 300, 355, 470 and 734 kPa respectively, using a 1 MHz transducer and  $10 \times 10^6$

328 microbubbles  $\text{ml}^{-1}$ (Fig. ??(a)). A mathematical model has been recently proposed to  
329 investigate bubble displacement and tissue deformation as a result of a primary Bjerknes  
330 force on a fluid-tissue interface (?). In the same study, material stiffness dependencies were  
331 explored. A similar approach will be considered in a future study to examine the momentum  
332 transfer and particle displacement in the presence of the wall for different wall diameter and  
333 thickness, as well as for phantoms with different stiffnesses.

334

## 335 V. CONCLUSION

336 The dependence of APP-induced displacements on acoustic parameters and microbubble  
337 concentrations was investigated in this study. Ultrasound-driven microbubbles were shown  
338 to apply a force onto a region using lower acoustic pressures than is needed with only ul-  
339 trasound (control experiment). Deformations at low acoustic pressures and microbubble  
340 concentrations were on the order of microns, which is sufficient for elasticity measurements.  
341 APP produced elasticity imaging-relevant displacements for different ultrasound center fre-  
342 quencies and was nearly linear with the mechanical index. Since multiple center frequencies  
343 could generate enough displacements, elasticity imaging at different diagnosis depths may  
344 be possible. APP produced a unique deformation dynamic that varied spatially and tem-  
345 porally since microbubbles moved or were destroyed. The deformation curves varied with  
346 acoustic pressure, but was broadly classified into two dynamics: slow rise to a steady state  
347 deformation, and rapid high deformation followed by a low steady state deformation. In

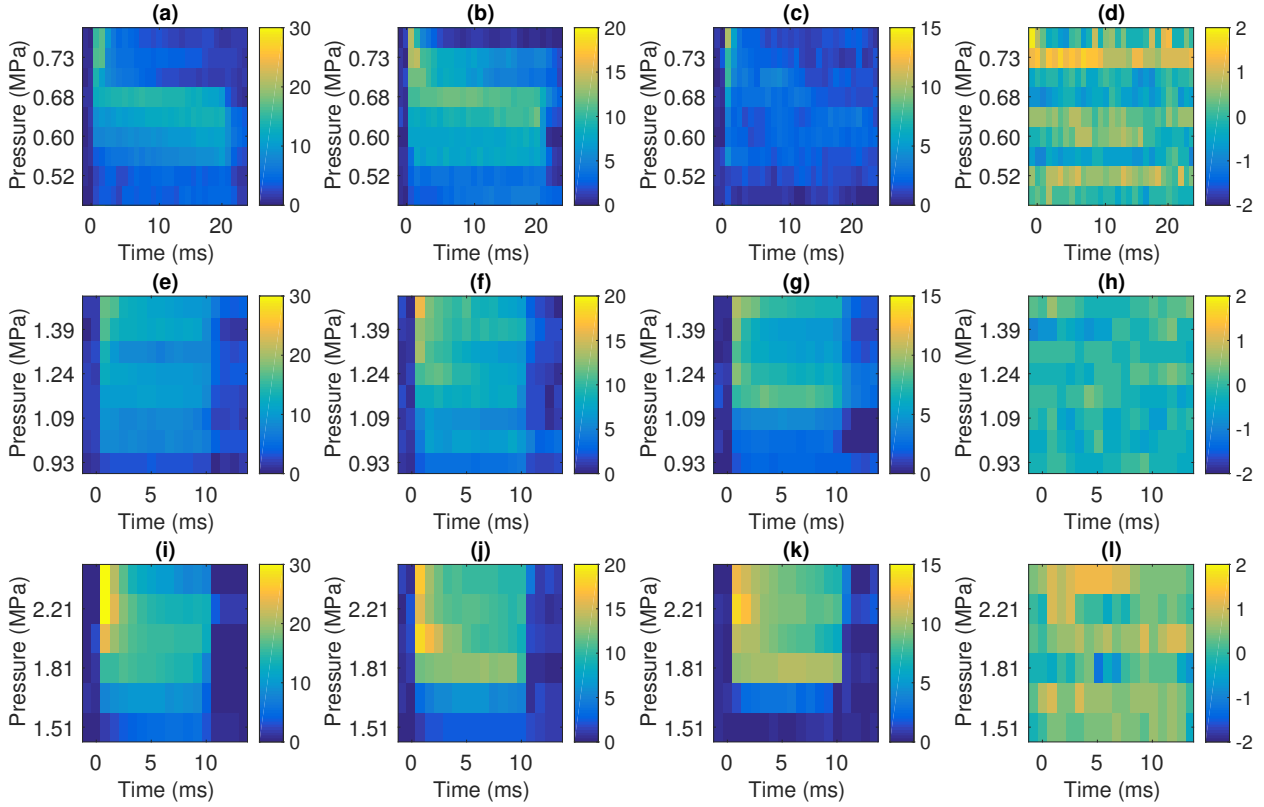


FIG. 6. Mean values of deformations for different acoustic pressures and microbubble concentrations during an excitation for each transducer. (a) 1 MHz transducer,  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (b) 1 MHz transducer,  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (c) 1 MHz transducer,  $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (d) 1 MHz transducer, Control. (e) 3.5 MHz transducer,  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (f) 3.5 MHz transducer,  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (g) 3.5 MHz transducer,  $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (h) 3.5 MHz transducer, Control. (i) 5 MHz transducer,  $20 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (j) 5 MHz transducer,  $10 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (k) 5 MHz transducer,  $4 \times 10^6$  microbubbles  $\text{ml}^{-1}$ . (l) 5 MHz transducer, Control. Control: without microbubbles.

348 conclusion, APP can produce tissue elasticity imaging relevant deformations using a wide  
349 range of acoustic parameters and microbubble concentrations.

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356  
357 Apfel, R. E. (1997). “Sonic effervescence: A tutorial on acoustic cavitation,” The Journal  
358 of the Acoustical Society of America **101**(3), 1227-1237.

359 Bouchard, R. R., Dahl, J. J., Hsu, S. J., Palmeri, M. L., and Trahey, G. E. (2009). “Im-  
360 age quality, tissue heating, and frame rate trade-offs in acoustic radiation force impulse  
361 imaging,” IEEE Trans Ultrason Ferroelectr Freq Control **56**(1), 63–76.

362 Chen, C., Robinson, D., Wilson, L., Griffiths, K., Manoharan, A., and Doust, B. (1987).  
363 “Clinical sound speed measurement in liver and spleen in vivo,” Ultrasonic Imaging **9**(4),  
364 221 – 235.

365 Chen, E., Novakofski, J., Jenkins, W., and O’Brien, W. (1996). “Young’s modulus mea-  
366 surements of soft tissues with application to elasticity imaging,” IEEE Transactions on  
367 Ultrasonics, Ferroelectrics and Frequency Control **43**(1), 191–194.

368 Chen, H., Brayman, A. A., and Matula, T. J. (2012). “Characteristic microvessel relaxation  
369 timescales associated with ultrasound-activated microbubbles.” *Applied physics letters*  
370 **101**(16), 163704.

371 Culjat, M. O., Goldenberg, D., Tewari, P., and Singh, R. S. (2010). “A review of tissue  
372 substitutes for ultrasound imaging.” *Ultrasound in medicine & biology* **36**(6), 861–873.

373 Dalecki, D. (2007). “WFUMB Safety Symposium on Echo-Contrast Agents: bioeffects of  
374 ultrasound contrast agents in vivo.” *Ultrasound in medicine & biology* **33**(2), 205–213.

375 Dayton, P., Morgan, K., Klibanov, A., Brandenburger, G., Nightingale, K., and Ferrara,  
376 K. (1997). “A preliminary evaluation of the effects of primary and secondary radiation  
377 forces on acoustic contrast agents,” *IEEE Transactions on Ultrasonics, Ferroelectrics and*  
378 *Frequency Control* **44**(6), 1264–1277.

379 Dayton, P. A., Allen, J. S., and Ferrara, K. W. (2002). “The magnitude of radiation force  
380 on ultrasound contrast agents,” *The Journal of the Acoustical Society of America* **112**(5),  
381 2183–2192.

382 Doherty, J. R., Trahey, G. E., Nightingale, K. R., and Palmeri, M. L. (2013). “Acoustic radi-  
383 ation force elasticity imaging in diagnostic ultrasound.” *IEEE transactions on ultrasonics,*  
384 *ferroelectrics, and frequency control* **60**(4), 685–701.

385 Duck, F. A. (1990). *Physical Properties Of Tissue: A Comprehensive Reference Book* (Aca-  
386 demic Press), pp. 73–165.

387 Erpelding, T., Hollman, K., and O’Donnell, M. (2005). “Bubble-based acoustic radiation  
388 force elasticity imaging,” *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency*  
389 *Control* **52**(6), 971–979.

390 Fung, Y. C. (1993). *Mechanical Properties and Active Remodeling of Blood Vessels* (Springer  
391 New York), pp. 321–391.

392 Fung, Y. C., Zweifach, B. W., and Intaglietta, M. (1966). “Elastic environment of the  
393 capillary bed,” *Circulation research* **19**(2), 441–461.

394 Gefen, A., Gefen, N., Zhu, Q., Raghupathi, R., and Margulies, S. S. (2003). “Age-dependent  
395 changes in material properties of the brain and braincase of the rat,” *Journal of Neuro-*  
396 *trauma* **20**(11), 1163–1177.

397 Guyton, A. C., and Hall, John E. (John Edward), . (1996). *Textbook of medical physiology*,  
398 9th ed ed. (Philadelphia, PA: Saunders), pp. 218–220.

399 Hall, T., Bilgen, M., Insana, M., and Krouskop, T. (1997). “Phantom materials for elas-  
400 tography,” *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control* **44**(6),  
401 1355–1365.

402 Hoskins, P. R. (2007). “Physical properties of tissues relevant to arterial ultrasound imaging  
403 and blood velocity measurement,” *Ultrasound in Medicine and Biology* **33**(10), 1527 –  
404 1539.

405 Klabunde, R. E. (2005). *Cardiovascular physiology concepts* (Philadelphia, PA: Saunders),  
406 pp. 91–93.

407 Koruk, H., and Choi, J. J. (2018). “Displacement of a bubble by acoustic radiation force  
408 into a fluid-tissue interface,” *The Journal of the Acoustical Society of America* **143**(4),  
409 2535–2540.

410 Koruk, H., El Ghamrawy, A., Pouliopoulos, A. N., and Choi, J. J. (2015). “Acoustic particle  
411 palpation for measuring tissue elasticity,” *Applied Physics Letters* **107**(22), 223701.

412 Krouskop, T. A., Wheeler, T. M., Kallel, F., Garra, B. S., and Hall, T. (1998). “Elastic  
413 moduli of breast and prostate tissues under compression,” *Ultrasonic Imaging* **20**(4), 260–  
414 274.

415 Leighton, T. G., Walton, A. J., and Pickworth, M. J. W. (1990). “Primary Bjerknes forces,”  
416 *European Journal of Physics* **11**(1), 47-50.

417 Lindner, J. R. (2004). “Microbubbles in medical imaging: current applications and future  
418 directions,” *Nat Rev Drug Discov* **3**(6), 527–532.

419 McDonald, D. A. (1974). *Blood flow in arteries* (Williams & Wilkins, Baltimore, MD),  
420 1–496.

421 Neppiras, E. (1980). “Acoustic cavitation,” *Physics Reports* **61**(3), 159–251.

422 Nightingale, K. (2011). “Acoustic Radiation Force Impulse (ARFI) Imaging: a Review,”  
423 *Current medical imaging reviews* **7**(4), 328–339.

424 Nightingale, K. R., Palmeri, M. L., Nightingale, R. W., and Trahey, G. E. (2001). “On  
425 the feasibility of remote palpation using acoustic radiation force,” *The Journal of the*  
426 *Acoustical Society of America* **110**(1), 625–634.

427 Ophir, J., Céspedes, I., Ponnekanti, H., Yazdi, Y., and Li, X. (1991). “Elastography: A  
428 quantitative method for imaging the elasticity of biological tissues,” *Ultrasonic Imaging*  
429 **13**(2), 111 – 134.

430 Parker, K. J., Dooley, M. M., and Rubens, D. J. (2011). “Imaging the elastic properties of  
431 tissue: the 20 year perspective,” *Phys Med Biol* **56**(1), R1–R29.

432 Pouliopoulos, A. N., Bonaccorsi, S., and Choi, J. J. (2014). “Exploiting flow to control the  
433 in vitro spatiotemporal distribution of microbubble-seeded acoustic cavitation activity in

434 ultrasound therapy.,” *Physics in medicine and biology* **59**(22), 6941–6957.

435 Rooney, J. A., Gammell, P. M., Hestenes, J. D., Chin, H. P., and Blankenhorn, D. H. (1982).  
436 “Velocity and attenuation of sound in arterial tissues,” *The Journal of the Acoustical*  
437 *Society of America* **71**(2), 462–466.

438 Sennoga, C. A., Mahue, V., Loughran, J., Casey, J., Seddon, J. M., Tang, M., and Ecker-  
439 sley, R. J. (2010). “On sizing and counting of microbubbles using optical microscopy,”  
440 *Ultrasound in medicine & biology* **36**(12), 2093–2096.

441 Shi, W. T., Forsberg, F., Tornes, A., Østensen, J., and Goldberg, B. B. (2000). “Destruc-  
442 tion of contrast microbubbles and the association with inertial cavitation,” *Ultrasound in*  
443 *Medicine & Biology* **26**(6), 1009–1019.

444 Unger, E. C., Porter, T., Culp, W., Labell, R., Matsunaga, T., and Zutshi, R. (2004).  
445 “Therapeutic applications of lipid-coated microbubbles.,” *Advanced drug delivery reviews*  
446 **56**(9), 1291–314.

447 Varghese, T. (2009). “Quasi-Static Ultrasound Elastography.,” *Ultrasound clinics* **4**(3), 323–  
448 338.

449 Walker, W., and Trahey, G. (1995). “A fundamental limit on delay estimation using par-  
450 tially correlated speckle signals,” *IEEE Transactions on Ultrasonics, Ferroelectrics and*  
451 *Frequency Control* **42**(2), 301–308.

452 Woodard, H. Q., and White, D. R. (1986). “The composition of body tissues,” *The British*  
453 *Journal of Radiology* **59**(708), 1209–1218.

454 Yoon, S., Aglyamov, S. R., Karpiouk, A. B., Kim, S., and Emelianov, S. Y. (2011). “Estima-  
455 tion of mechanical properties of a viscoelastic medium using a laser-induced microbubble

456 interrogated by an acoustic radiation force.,” The Journal of the Acoustical Society of  
457 America **130**(4), 2241–2248.

458 Zhang, X., Qiang, B., and Greenleaf, J. (2011). “Comparison of the surface wave method  
459 and the indentation method for measuring the elasticity of gelatin phantoms of different  
460 concentrations.,” Ultrasonics **51**(2), 157–164.

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