Simulation Study of Amplitude-Modulated (AM) Harmonic Motion Imaging (HMI) for Stiffness Contrast Quantification with Experimental Validation

CAROLINE MALEKE,1 JIANWEN LUO,1 VIKTOR GAMARNIK,1 XIN L. LU1 AND ELISA E. KONOFAGOU1,2

Departments of 1Biomedical Engineering and 2Radiology
351 Engineering Terrace, mail code 8904
1210 Amsterdam Avenue
New York, NY 10027
ek2191@columbia.edu

The objective of this study is to show that Harmonic Motion Imaging (HMI) can be used as a reliable tumor-mapping technique based on the tumor’s distinct stiffness at the early onset of disease. HMI is a radiation-force-based imaging method that generates a localized vibration deep inside the tissue to estimate the relative tissue stiffness based on the resulting displacement amplitude. In this paper, a finite-element model (FEM) study is presented, followed by an experimental validation in tissue-mimicking polyacrylamide gels and excised human breast tumors ex vivo. This study compares the resulting tissue motion in simulations and experiments at four different gel stiffnesses and three distinct spherical inclusion diameters. The elastic moduli of the gels were separately measured using mechanical testing. Identical transducer parameters were used in both the FEM and experimental studies, i.e., a 4.5-MHz single-element focused ultrasound (FUS) and a 7.5-MHz diagnostic (pulse-echo) transducer. In the simulation, an acoustic pressure field was used as the input stimulus to generate a localized vibration inside the target. Radiofrequency (rf) signals were then simulated using a 2D convolution model. A one-dimensional cross-correlation technique was performed on the simulated and experimental rf signals to estimate the axial displacement resulting from the harmonic radiation force. In order to measure the reliability of the displacement profiles in estimating the tissue stiffness distribution, the contrast-transfer efficiency (CTE) was calculated. For tumor mapping ex vivo, a harmonic radiation force was applied using a 2D raster-scan technique. The 2D HMI images of the breast tumor ex vivo could detect a malignant tumor (20 × 10 mm2) surrounded by glandular and fat tissues. The FEM and experimental results from both gels and breast tumors ex vivo demonstrated that HMI was capable of detecting and mapping the tumor or stiff inclusion with various diameters or stiffnesses. HMI may thus constitute a promising technique in tumor detection (>3 mm in diameter) and mapping based on its distinct stiffness.

KEY WORDS: Breast; displacement; FEM; harmonic-motion imaging; modulus; radiation force; tumor.

INTRODUCTION

Palpation is a standard screening procedure for the detection of tumors based on their distinct stiffness in a clinical setting. Several elasticity-imaging techniques, such as those developed in conjunction with ultrasound and MRI, have been developed to estimate tissue stiffness and thus detect tumors using various forms of tissue perturbation for the detection of stiffer masses.1 In the field of ultrasound, Krouskop et al2 imposed an external vibration and estimated the tissue elastic modulus by measuring the resulting Doppler shift. Parker et al3 measured the tissue response to mechanical vibrations for ‘sonoelasticity imaging’ and applied it on healthy human skeletal muscle (rectus femoris and biceps brachii) in vivo.4 Ophir et al5,6 developed the method of elastography that applied a small external static compression (on the
order of 1%) and used crosscorrelation techniques on radiofrequency (rf) signals in order to estimate tissue strains resulting from the external compression. This method has been proven to produce good quality strain images (or, elastograms) in several tissues, especially in the breast and muscle in vivo.1-17

On the other hand, internal perturbation methods can produce a localized force deep inside the tissue that can be used for probing and analyzing tissue properties.1,16-20 Sugimoto et al introduced the use of a focused ultrasound transducer to produce an impulse radiation force that caused a localized static compression deep inside the tissue to evaluate localized tissue stiffness.18 The tissue displacement was estimated as a function of time by using pulse-echo methods.

Other research groups have also used the impulse radiation force to induce brief mechanical excitations locally and follow the resulting tissue response while rf data were collected during tissue relaxation (Acoustic Radiation Force Imaging; ARFI)19, 21-24 or shear-wave propagation, e.g., with Shear Wave Elasticity Imaging (SWEI),25 ARFI,26 Supersonic Shear Imaging27 and Shearwave Dispersion Ultrasound Vibrometry (SDUV).28 These techniques have been used clinically in the diagnosis of pathological tissues, such as in carotid and popliteal arteries in vivo,26, 30 human liver in vivo,31 human breast in vivo,32 human muscle in vivo33 and swine liver in vivo.28

Another approach to measure tissue mechanical properties is the use of the radiation force to generate an internal vibration. The SWEI method employs a focused ultrasound transducer and an ultrasound imaging transducer or low-frequency acoustic detector. An amplitude-modulated focused beam is used to generate a time-varying acoustic-radiation force. The modulation frequency is typically on the order of a few kHz. The shear wave resulting from the radiation force is detected by an ultrasound imaging transducer or a surface detector that is used to characterize the viscoelastic properties of the targeted medium.25

In Ultrasound-Stimulated Vibro-Acoustography (USVA),34-38 two confocal ultrasound transducers and a hydrophone are typically used. The interference of two confocal ultrasound transducers at slightly different frequencies (low kHz range) causes a vibration at the focus. The amplitude or the phase of the shear wave is recorded by a hydrophone and used to form an image (i.e., USVA), or tracked by a separate ultrasound beam and/or laser vibrometer (i.e., SDUV).28 In SDUV, the phase of the shear wave at two different locations is used to calculate the shear-wave propagation speed at different frequencies. An inverse approach is used for estimation of tissue viscosity and elasticity measurement. Among other applications, USVA has been applied on human breast in vivo34-38 and SDUV has been applied on swine liver in vivo.28

Harmonic motion imaging (HMI) uses two focused ultrasound (FUS) transducers with an ultrasound imaging transducer39 or one (FUS) transducer with an ultrasound imaging transducer.40 The imaging and the FUS transducers are confocal and concentric. Maleke et al40 have shown that, in the two-FUS-transducer configuration, the interference of the two focused beams produced an acoustic radiation force that continuously moved across the focal region. On the other hand, in the single FUS-transducer configuration, the amplitude-modulated FUS beam (on the order of Hz) generates a time-varying radiation force at the focal region (i.e., the force did not move spatially). The resulting oscillatory motion at the focus is detected during the force application using an imaging transducer. The amplitude of the induced motion is estimated using 1D cross-correlation on the acquired rf signals.39,48 Since the induced motion is highly localized, the response of the tissue is mainly related to the underlying tissue mechanical properties.

Theoretical and experimental studies of the HMI technique using two separate FUS transducers have been previously reported.39 Finite-element and Monte-Carlo simulations were
used to simulate the oscillatory displacement within an applied force frequency range of 200 to 800 Hz. The force was applied at the specific nodes within the focal region in a finite-element model (FEM). However, the applied radiation force amplitude was not realistic because the 2D acoustic pressure field was not taken into account.

Localized Harmonic Motion (LHM) is a technique similar to HMI that uses a sequence of quasi-static excitations at a specific rate. Heikkila et al. have developed a LHM simulation framework to test the performance of LHM for lesion detection. Two configurations were simulated involving either a 1D linear phased array transducer or two confocal single-element transducers, for both sonication and imaging. A burst waveform with several repetition frequencies (e.g., 50, 100 and 150 Hz) was then used to induce dynamic excitation inside a medium. Their simulation findings indicated good agreement with the in vivo LHM experimental results in the rabbit muscle.

In this paper, we present the simulation study of HMI where the oscillatory force is generated by our previously developed technique that utilizes a single, amplitude-modulated FUS beam. A different simulation framework is considered herein to study the performance of the amplitude-modulated HMI method (i.e., using one FUS transducer) in a realistic, acoustic framework. The FUS transducer is excited by an amplitude-modulated waveform to induce a stationary vibration deep inside the tissue. The 2D pressure field of the FUS transducer is simulated in Field II. The 2D pressure field is then used as a loading condition in the FEM study. The FEM is used to evaluate the dependence of the estimated displacement on the acoustic parameters and mechanical tissue properties, such as the acoustic intensity and the tissue Young’s modulus. We examine the potential of HMI in assessing different sizes/stiffnesses of an inclusion embedded in a softer medium. One of the objectives of this study was to show that HMI could be used as a reliable tumor-mapping technique based on the tumor stiffness difference at the early onset of disease. The stiffer inclusions at various different diameters represent the tumor mass at different stages, e.g., early tumor development would entail a smaller mass.

The manuscript is organized as follows. The simulation study is first described together with the theory involving the pressure and intensity derivation for the amplitude-modulated (AM) waveform, the acoustic pressure simulation, the FEM, the image formation model, and displacement estimation method. Second, the details of the setup used for HMI experiments on polyacrylamide gels and postsurgical breast specimens, as well as mechanical testing, are provided. The results of the simulation and experiments are compared and discussed, and, finally, conclusions on the quantitative capabilities of HMI are summarized.

**MATERIALS AND METHODS**

Figure 1 shows the schematic of the simulation process to study the performance of the HMI technique. The process is as follows: (1) The 2D pressure field of the FUS transducer is simulated in Field II. (2) The 2D pressure field is then used as a loading condition in the FEM study. (3) The FEM solution yields the temporally-varying displacement (oscillatory displacement) that is used to generate a time-dependent scatterers distribution in the simulated gel. (4) The image formation model is employed to simulate the rf data. (5) One-dimensional cross-correlation is applied on the rf signals obtained to estimate the motion. (6) The peak-to-peak displacements from the FEM solution and 1D cross-correlation techniques are compared to validate the HMI motion estimates. The following sections details every step used in this simulation study.
The radiation force is caused by the change in the momentum of the acoustic wave as it propagates through a medium. In the case of a single-element focused ultrasound (FUS) transducer, the radiation force is mainly localized in the focal region. In an attenuating homogeneous medium and assuming plane wave propagation, this force can be expressed as

\[ F(t) = \frac{2\alpha I(t)}{c} \tag{1} \]

where \( t \) is time, \( F(t) \) is a volumic force [N/m^3], \( \alpha \) is the tissue absorption coefficient [1/m], \( I(t) \) is the peak-average acoustic intensity [W/m^2] and \( c \) is the speed of sound [m/s].

When an AM waveform is used to drive the FUS transducer, the radiation force is oscillating at the modulation frequency (\( \omega_m \)). The acoustic pressure \( p(t) \) of the AM wave generated at the focus can be expressed by

\[ p(t) = p_o \cdot \cos(\omega_m t) \cdot \cos(\omega_c t) \tag{2} \]

where \( p_o \) denotes the maximum instantaneous acoustic pressure, \( \omega_c \) is the carrier frequency and \( \omega_m \) is due to the modulation frequency. The spatial peak-pulse average intensity (\( I_{ppa} \)),

\[ I_{ppa} = \frac{1}{2\Delta t} \int_{t}^{t+2\Delta t} p^2(t) \, dt \]

where \( \Delta t \) is the pulse duration and \( p(t) \) is the acoustic pressure as a function of time.

Theory

The diagram on the left with white arrows denotes the FEM method, while the diagram on the right with black arrows represents the HMI technique. Comparison between the two estimates reveals the quality of the HMI displacement.
\( I(t) \) of the AM waveform can be calculated by simply integrating \( p^2(t) \) over time, i.e., the time at which the pressure is applied:

\[
I(t) = \int_0^t \frac{p^2(\tau)}{\rho c} d\tau \tag{3}
\]

or

\[
I(t) = \frac{p_0^2}{\rho c} \int_0^t \left\{ \cos(\omega_m \tau) \cdot \cos(\omega_c \tau) \right\}^2 d\tau \tag{4}
\]

where \( \tau \) is the variable of integration and \( t \) the insonation time. The tissue parameters used were: density \( (\rho) = 1000 \text{ kg/m}^3 \) and speed of sound \( (c) = 1540 \text{ m/s} \) to simulate soft tissues. The resulting motion, which is related to the multiplication and square of the two sinusoidal functions (Eq. 4), oscillates at frequencies equal to twice the frequency of modulation \( (2\omega_m) \). This relationship was also confirmed through experimental analysis, for instance, when a 15 Hz modulation frequency was used, the harmonic displacement oscillated at a frequency equal to 30 Hz. The motion resulting from this acoustic radiation force is detected through signal processing of the rf signals acquired on an imaging i.e., a pulse-echo transducer, and then used to characterize the medium being studied.

**Acoustic pressure field simulation**

The acoustic pressure field was simulated in Field II. This framework employs a linear acoustic-propagation model to calculate the pressure field corresponding to specific transducer geometry and parameters. We modeled a single-element concave (FUS) transducer (Fig. 2a) with a circular opening in its center for the placement of a pulse-echo transducer with identical parameters used in the experiments (Fig. 2b).

The concave transducer used had a center frequency of 4.5 MHz, a focal length of 40 mm and inner and outer diameters of 30 mm and 70 mm, respectively. The aperture was divided into 1256 rectangular elements and the area of each element was equal to 1 mm\(^2\). There was a void at the center of the transducer, i.e., about 57% of the element was active. The pressure field simulation showed that the focal spot size was about 0.8 × 0.8 × 1.6 mm\(^3\) (lateral × elevational × axial), which was in agreement with the experimental beam profile. The apodization technique was applied for each element to represent active and nonactive regions. The center opening had a diameter of 30 mm and the apodization in this region was set to zero, i.e., nonactive elements, as indicated by the blue-shaded area in figure 2b. The apodization of the active area, an annular region, was set equal to one (active elements) as denoted by the pink-shaded area in figure 2b. This method was used to simulate the FUS transducer with an AM waveform, i.e., a combination of a carrier frequency \( (\omega_c) \) at 4.5 MHz and an AM frequency \( (\omega_m) \) at 15 Hz, was used to drive the transducer.

The 2D pressure field, \( p(x, z) \), was sampled at 80 MHz and calculated at the focal region. The focal region size was equal to 60 mm (axial) by 40 mm (lateral) obtained at a resolution of 0.1 mm and the attenuation coefficient \( (\alpha) \) of 0.3 dB/cm to simulate soft tissue. The acoustic intensity levels \( (I_{pp}) \) were varied between 26.39 and 986.97 W/cm\(^2\) to verify the linear relationship between the acoustic intensity and the estimated displacement. This particular acoustic intensity range was selected to match the previously-used experimental parameters for imaging and therapy purposes.
An axisymmetric FE (Comsol MultiphysicsTM, Comsol Inc., Burlington, MA, USA) cylindrical gel was constructed with a radius of 18.5 mm and a height of 20 mm. A hard spherical inclusion was defined inside the simulated phantoms with diameters equal to 3 mm, 5 mm and 10 mm. The background Young’s modulus ($E$) was equal to 10 kPa. The triangular mesh was applied to the inclusion geometry. The second-order, quadrature element with six nodes per element was used in this model. The diameter and stiffness of the inclusions were varied according to table 1. The boundary between the inclusion and background was then modeled based on the triangular discretizations. The mesh refinement was performed in order to guarantee the element density was adequate and not introducing any numerical artifacts. The model was assumed to be nearly incompressible (Poisson’s ratio of 0.499999), with zero viscosity and a density of 1000 kg/m$^3$. The ultrasound transducer was moved axially and downward with a step size of 1 mm to cover the entire phantom depth (Fig. 3). The harmonic radiation force was applied sequentially at $n$ preselected locations ($p_1, p_2, \ldots, p_n$) in and around the inclusion (Fig. 3).
The bottom boundary of the cylindrical model was constrained in the axial direction, while the remaining boundaries were free to move in both the axial and lateral directions. In total, the number of nodes and triangular elements generated was equal to approximately 7000 and 3000, respectively. This included the refined triangular elements that were specifically selected around and inside the inclusion, thus allowing sufficient resolution for the spatial variation of the corresponding tissue displacement within the transducer focus.

Image formation

The rf signals were simulated in Matlab 7.2 (Mathworks, Natick, MA, USA) using a linear convolutional scattering model. The scatterer was randomly distributed. The linear array had 64 elements, a center frequency of 7.5 MHz, a frame rate of 124 frames/s, a beamwidth of 2 mm and 60% bandwidth. In this simulation, the speckle pattern was simulated as a Rayleigh distribution and thus, the tissue motion incurred by the applied force could be estimated. The rf signals were sampled at 40 MHz. A 1D cross-correlation technique was applied on consecutively-generated rf signals at a window size equal to 1 mm and an 85% overlap. In this study, a single-element, pulse-echo transducer was used to acquire rf echoes in the experiments; thus, only the estimated displacement along the center rf line was considered in the comparison with the simulation results.

Displacement estimation

A cumulative axial displacement was estimated by using 1D cross-correlation on consecutive rf signals with the reference signal chosen to be the rf signal when the input force was

<table>
<thead>
<tr>
<th>Background Young’s modulus (kPa)</th>
<th>Inclusion diameter (mm)</th>
<th>Inclusion Young’s modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 3</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Type 2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Type 1</td>
<td>10</td>
<td>10</td>
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<td></td>
<td>25 and 50</td>
<td>25 and 50</td>
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![Table 1 FE model parameters.](image)

FIG. 3 (Left) 1D raster-scan diagram along the axial direction at different location, e.g., $p_1, p_2, \ldots, p_n, d_1, \ldots, d_5, \ldots, d_n$ represents the peak-to-peak displacement amplitude for each location. (Right) Graph representation of displacement amplitude vs. axial depth.
first applied. Cosine interpolation was applied around the peak of the cross-correlation function in order to improve the precision of the displacement estimation. The magnitude of cumulative displacement denoted the incurred motion. Thus, when the radiation force was applied, the tissue moved away from the transducer indicated by the increasing displacement amplitude. When the radiation force decreased, the tissue relaxed, as indicated by the decreasing displacement amplitude. The estimated cumulative displacements was estimated throughout the entire tissue depth and displayed over time.

Figure 4a shows an example of the estimated oscillatory displacement at the focus from an ex vivo canine liver to illustrate the tissue motion during the force application. The AM frequency used in this experiment was equal to 25 Hz, i.e., the optimum frequency for this particular case. The motion has a 50 Hz oscillation frequency (twice the modulation frequency; refer to Eq. (4)). A digital least-square lowpass filter (cutoff frequency of 200 Hz) was applied on the estimated displacement (shown in red) in order to show the displacement without the higher frequency noise. Figure 4 shows a transient after the application of the force (at 20 ms) before reached steady state. The motion continuously oscillated throughout the force application.

The peak-to-peak displacement amplitude denotes the HMI displacement ($D_{\text{HMI}}$) and was estimated at all depths by using a Fourier transform (FT) method. Based on the FT method, the spectral peak centered at twice the AM frequency was used to calculate the peak-to-peak displacement amplitude (HMI displacement, $D_{\text{HMI}}$) using
where $c$ is the sound speed $1540.10^6$ [µm/s], $f_s$ is the sampling frequency (80 MHz), $P_{FT}$ is the peak of the displacement spectra and $N_d$ is the displacement data points. The calculated $D_{HMI}$ (Fig. 4c) at steady state was equal to 12 µm in this case. The displacement regions were averaged within a 1D region, i.e., 1.6 mm (axially), to approximate the induced motion.

**Phantom experiments**

Four uniform and six spherical inclusion-embedded polyacrylamide gels with distinct stiffnesses were used for both mechanical testing and HMI experiments. Polyacrylamide gels were prepared using the following guidelines: premixed 40% liquid acrylamide (19:1 acrylamide:bis-acrylamide ratio) (Thermo Fisher Scientific, Waltham, MA) was diluted in deionized water to produce a range of acrylamide concentration from 25% (weight/volume) to 40% (weight/volume) in 5% increments. The percentage of acrylamide in the mixture determines the stiffness of the gel after it is polymerized. The resulting solution was dissolved (1.75 ml per total ml) in 1M trishydroxymethylaminomethane (TRIS, 1.0 ml per total ml) with deionized water (7.16 ml per total ml). 10% ammonium persulfate (APS, 8.4 µl per total µl) and N,N,N’,N’-tetramethylethylenediamine (TEMED, Sigma-Aldrich, St. Louis, MO, 0.5 µl per total ml) subsequently added. The mixture was allowed to polymerize at room temperature for approximately 15 minutes prior to use.

In order to independently measure the Young’s modulus of all four homogeneous gels, a dynamic indentation test was performed. The description of the indentation test setup is provided in the next section. Based on the test results (Table 2), 25% acrylamide concentration was used to generate the soft background ($E = 13$ kPa), that is a similar modulus value to the soft background in the simulation ($E = 10$ kPa). The acrylamide concentrations of 30% and 40% were respectively used for the hard inclusions of 25 kPa and 50 kPa. These values were chosen for quantitative comparison between simulated and polyacrylamide gels. The three inclusions used were 3 mm, 5 mm and 10 mm in diameter to simulate growing tumor dimensions.

<table>
<thead>
<tr>
<th>Acrylamide concentration (%)</th>
<th>20% pre-compression (5% dynamic loading)</th>
<th>5% pre-compression (2% dynamic loading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 Hz</td>
<td>1 Hz</td>
</tr>
<tr>
<td>25</td>
<td>13.3</td>
<td>13.4</td>
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<tr>
<td>30</td>
<td>24.2</td>
<td>29.1</td>
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<tr>
<td>35</td>
<td>36.2</td>
<td>37.1</td>
</tr>
<tr>
<td>40</td>
<td>49.9</td>
<td>48.4</td>
</tr>
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</table>

$$D_{HMI} = \frac{c \cdot P_{FT}}{f_s \cdot N_d / 2}$$

where $c$ is the sound speed $1540.10^6$ [µm/s], $f_s$ is the sampling frequency (80 MHz), $P_{FT}$ is the peak of the displacement spectra and $N_d$ is the displacement data points. The calculated $D_{HMI}$ (Fig. 4c) at steady state was equal to 12 µm in this case. The displacement regions were averaged within a 1D region, i.e., 1.6 mm (axially), to approximate the induced motion.
The HMI experiment setup is shown in figure 5. Two frequency generators (Agilent (HP) 33120A, Palo Alto, CA, USA) were used to produce a carrier frequency of 4.5 MHz and a low modulation frequency of 15 Hz, because it was determined to be the optimized oscillatory displacement throughout the stiffness range used in the study. The resulting AM waveform was amplified by 50 dB using a rf power amplifier (E&I, Rochester, NY, USA). The AM waveform was used to excite a 4.5 MHz FUS transducer (Imasonic, Voray sur l’Oignon, France) that generated the oscillatory acoustic radiation force at the focal region.

The acoustic pressure was measured using a 0.2-mm needle hydrophone (Precision Acoustics LTD, Dorchester, Dorset, UK). The positive and negative peak pressures at the focus had the same absolute amplitude of 3.15 MPa due to the applied sinusoidal force. The acoustic pressure at the focus in the gel experiments was estimated using a derating factor of 0.3 dB/cm/MHz at 4.5 MHz frequency and 1.5 cm depth to account for the attenuation through the gel. The estimated acoustic pressure at the focus was equal to 1.98 MPa and the mechanical index (MI) after taking into account the derating factor 0.3 (MIder) was equal to 0.93, i.e., below the FDA limit of 1.9. The peak average intensity (I_{ppa}) was calculated numerically using Eq. (4) with a \( p_o \) of 1.98 MPa, the gel density (\( \rho \)) was assumed to be 1000 kg/m\(^3\), c of 1540 m/s and t of 400 ms. The acoustic intensity (I_{ppa}) was equal to 32.5 W/cm\(^2\), which is also below the FDA I_{ppa} limit of 190 W/cm\(^2\). The potential temperature rise (\( \Delta T \)) during the force application can be estimated by solving the bioheat transfer equation. Here, the bioheat transfer equation is assumed to have a linear distribution of thermal sources without convection or conduction effects, i.e.,

\[
\Delta T = \frac{2\alpha I}{\gamma_v} t
\]

where \( \Delta T \) is the temperature rise in °C, I the acoustic intensity (I_{ppa}), \( \gamma_v \) the volume specific heat for tissue (4.2 J/cm\(^3\)/°C), \( \alpha \) the absorption coefficient of tissue (0.16 Np/cm, i.e., 0.3 dB/cm/MHz at 4.5 MHz) and t the duration of the oscillatory force (400 ms). For the applied
oscillatory force in a single location, the anticipated peak temperature rise was estimated to be up to $0.96^\circ C$. Note that the reported temperature rise is the maximum possible value since heat convection and blood perfusion were not taken into account.

A 7.5-MHz pulse-echo transducer (Panametrics, Waltham, MA, USA) with a diameter of 12 mm was placed through the center of the FUS transducer, with the beams of the two transducers, i.e., FUS and pulse-echo, properly aligned prior to all experiments. A pulser/receiver (Panametrics 5051PR, Waltham, MA, USA) was used to drive the pulse-echo transducer at a pulse repetition frequency (prf) of 5.4 kHz. The rf signals were acquired using a standard pulse-echo technique. An analog bandpass filter, i.e., 7th order type II Chebyshev band-pass filter, (Reactel, Inc., Gaithersburg, Maryland, USA) with cutoff frequencies of $f_{c1} = 5.84$ MHz and $f_{c2} = 8.66$ MHz (at $-60$ dB) was used to filter out the fundamental frequency of the FUS beam and its harmonics from the pulse-echo spectrum.

A silicone rubber/absorber (McMaster-Carr, Dayton, NJ, USA) was placed at 45° underneath the gel to further reduce the specular reflection from the bottom of the glass container. The FUS transducer was moved axially and downward by using a computer controlled positioner with a step size of 1 mm to cover the entire gel depth (Fig. 3). The filtered rf signals were sampled at 80 MHz and a 14-bit digitization level (CS14200, Gage Applied Technologies, Lachine, Canada). A one-dimensional cross-correlation was applied on consecutive rf signals with a kernel size equal to 1 mm and 85% overlap (as described in the previous section).

**Ex vivo experiments**

The same HMI experimental setup described above was used to image breast tumors *ex vivo*. The breast specimens were collected immediately after surgery from the Irving Pavilion of the Columbia University Medical Center. The tumor and its perilesional tissue were obtained approximately 30 minutes after excision and prior to histopathology. These tissues were immediately placed upon excision in a sterile container and submerged in degassed phosphate buffered saline (PBS) solution for the HMI experiments.

The specimens were then carefully removed from the container and placed inside a gelatin matrix for protective purposes but also to contain them and simulate the surrounding breast. All specimens were scanned using the HMI technique and the findings were compared to mammography or sonography and, finally, histopathology findings. Orientation of the specimen was provided by the surgeon. For tissue mapping, the FUS transducer was moved in a 2D raster-scanned fashion with a step size equal to 1 mm using a computer-controlled positioner (Velmex Inc., Bloomfield, NY, USA). The scan plane had a dimension of $50 \times 35$ mm², an acoustic intensity ($I_{\text{opt}}$) equal to 32.5 W/cm² with a duration of 0.2 s at each point. In order to choose the optimal AM frequency, a linear chirp test containing frequencies within the range of 10 to 30 Hz was used to investigate the optimal vibration frequency for the higher mechanical contrast between the tumor and the surrounding regions. The optimal AM frequency for the specimen presented in this paper was 15 Hz. HMI scanning lasted approximately 40 minutes. The specimens were returned to pathology for diagnosis. All procedures were approved by the Institutional Review Board (IRB) board of Columbia University prior to all studies. The lesion region was segmented based on the displacement difference between the stiff inclusion and the surrounding region.

**Mechanical testing**

A dynamic indentation test was used to assess the Young’s modulus of the polyacrylamide gels. The system consisted of an indentation probe (indenter) that vertically applied a small sinusoidal deformation on the gel surface. The indenter had a flat and rough surface to avoid
possible slippage during testing. The diameter of the indenter was equal to 6.33 mm. The loading site was selected by placing the indenter at the center of the gels. The gels were placed on a rigid cylindrical plate with a thickness of 14.3 ± 0.56 mm and a diameter of 87.1 mm, which was four times larger than that of the indenter so that the dimensionality assumptions of the theoretical model (Eq. 7) were satisfied. A small amount of deionized water was added sparingly to keep the gels moisturized. The temperature was kept within the range of 25.5 °C to 25.6 °C.

The applied dynamic displacement was controlled by an Instron Microtester (Instron, Inc., Norwood, MA) by using a 10 N load cell (Fig. 6). The precision of the load cell was 0.5% of its total capacity (10 N). Two different tests were performed on each gel. The first test entailed a 20% precompression (i.e., 2.56 ± 0.12 mm) followed by an oscillation at a magnitude of 5% apparent strain (displacement divided by initial thickness, i.e., 0.715 ± 0.028 mm); and the second test entailed a 5% precompression (i.e., 0.715 ± 0.028 mm) followed by an oscillation magnitude of 2% apparent strain (i.e., 0.286 ± 0.0112 mm). To test reliability, each indentation test was performed and compared at two different loading frequencies of 0.1 Hz and 1 Hz (Fig. 7).

The temporally varying force was measured during the entire indentation process and these data were used to calculate the Young’s modulus of the gel samples using the equation

\[
E = \frac{2}{\sqrt{\pi}} \left( \frac{1}{2} - \frac{v^2}{4} \right) \frac{q}{a} \frac{w}{w}
\]

\[
E = \frac{2}{\sqrt{\pi}} \left( \frac{1}{2} - \frac{v^2}{4} \right) \frac{q}{a} \frac{w}{w}
\]  

(7)
where $E$ is the Young’s modulus, $\nu$ is the Poisson’s ratio (assumed to be nearly incompressible material, $\nu = 0.49999$), $q$ is the loading pressure (force per unit area), $a$ is the radius of the indenter tip and $w$ is the applied displacement amplitude.

**Contrast-transfer efficiency (CTE)**

In HMI, the applied force/stress is highly localized at the focus, typically on the order of 1 to 2 mm, and thus, the resulting displacement is related to the relative tissue modulus (stiffness) within the focal region. The difference between the displacement in the inclusion and background regions can be used to detect inclusions by their stiffnesses difference.

To quantitatively evaluate the performance of HMI, the contrast-transfer efficiency (CTE) parameter was used. Here, CTE was defined as the ratio of the average displacement contrast ($C_d$) to the elasticity contrast ($C_e$). CTE represents the percentage, at which displacement profiles depict the underlying elasticity distribution in the medium given by

$$
CTE = \frac{C_d}{C_e} = \frac{d_{\text{background}}}{d_{\text{inclusion}}} \times \frac{E_{\text{inclusion}}}{E_{\text{background}}}
$$

The average displacement contrast ($C_d$) was calculated based on the ratio between the average displacement of the background ($d_{\text{background}}$) to the average displacement of the inclusion ($d_{\text{inclusion}}$). The elasticity contrast ($C_e$) is the ratio of the inclusion Young’s modulus ($E_{\text{inclusion}}$) to the background Young’s modulus ($E_{\text{background}}$). For elasticity contrast ($C_e$) in the simulation case, the Young’s moduli were equal to the input FE values whereas, in the experiment cases, the Young’s moduli were obtained from mechanical testing.

The value of displacement contrast is nearly equivalent to the value of the elasticity contrast when the CTE is close to 1. Displacement contrast can be used to closely represent the relative stiffness of the inclusion as long as the CTE remains relatively high. Thus, the change of the elasticity at the inclusion/background regions may be used to explain the similarity between the displacement and the elasticity contrast.

A region of interest (ROI) was chosen in the middle of the inclusion and above the inclusion for the background in order to calculate the inclusion-to-background contrast; both ROIs had the same size. The ROI was always smaller than the diameter of the inclusion, and chosen around the center of the inclusion for different inclusion sizes, for instance in figure 8a, the ROI for a stiff inclusion was 1 mm from 10 to 11 mm and the ROI for the surrounding region was 1 mm between 5 and 6 mm. The ROIs for the surrounding region were chosen above the inclusion because the displacements were more uniform.
The ROI in the simulations (Fig. 8) for the 5-mm and 10-mm inclusion diameter were of size equal to 4 mm (between 9 and 12 mm) and 8 mm (between 7 and 15 mm), respectively. In the phantom experiments (Fig. 11), the ROIs for 3 mm, 5 mm and 10 mm inclusion diameters were 2 mm (between 12 and 14 mm), 4 mm (between 17 and 21 mm) and 8 mm (between 11 and 19 mm).

RESULTS

To study the performance of HMI, specific parameters are tested in order to assess its capability of estimating the entire resulting displacement range. Comparison between the FEM and HMI-estimated displacements in a homogeneous medium with a Young's modulus of 10 kPa is shown in figure 9. The highest displacement is located at the center of the focal zone (depth of 40 mm), radially spanning approximately ±3 mm from the symmetry axis.
Figure 8 depicts the relationship between the displacements and inclusion size/stiffness of the simulated phantoms. The Young’s moduli of harder inclusions were 25 kPa and 50 kPa and their diameters were equal to 3, 5, and 10 mm. The peak-positive acoustic-pressure amplitude was equal to 1.98 MPa, the same value as that used in the experimental cases. The diameter of the inclusion can be estimated based on the displacement profiles. For example, in figure 8a, the HMI displacement amplitude decreases at the axial depth of 9 mm and then increases at the axial depth of 12 mm; thus, the 3-mm-diameter inclusion is correctly depicted with a diameter of 3 mm. Figures 8b and c show the estimated inclusion sizes at 5 mm and 10 mm, respectively. As the focus of the FUS beam moves toward the center of the inclusion, the resulting displacement amplitude steadily decreases, thus correctly representing the relative stiffness of the inclusion. For instance, the displacement amplitude close to the center of the hard inclusion, i.e., between 10 and 12 mm, is the lowest (Figs. 8b, c). The displacement at the depth of 20 mm is approximately zero, because the bottom surface of the simulated gel was constrained.

The mechanical testing results show that the measured Young’s modulus increases with the concentration of acrylamide (Table 2). The calculated Young’s moduli for 25%, 30%, 35% and 40% are $13.42 \pm 0.32$, $25.4 \pm 2.46$, $37.38 \pm 1.08$ and $50.2 \pm 1.43$ kPa (mean ± SD), respectively. These results were used as a guideline to generate a hard inclusion in the gel with stiffness similar to the simulated gels. The acrylamide concentration of 25% was used to model the soft background with a Young’s modulus of $E = 13$ kPa (Table 2). We selected acrylamide concentrations of 30% and 40% to match the inclusion Young moduli ($E$) in the simulated gel, which are equal to 25 kPa and 50 kPa, respectively (Table 2). The estimated Young’s moduli obtained from mechanical testing and the inverse of the estimated HMI displacement from gel experiments exhibit a linear relationship ($r^2 = 0.97$) (Fig. 10). This further confirms that the HMI displacement can be indirectly related to the underlying tissue stiffness.

To compare the simulation results (Fig. 8) with the gel experimental findings, the force was axially moved across the spherical inclusion. RF frames were acquired for each gel in four independent iterations to test the reproducibility of the HMI technique in reliably imaging inclusion sizes. The average displacement profiles with its standard derivation among the four iterations for each gel are shown in figure 11. The small inclusions, i.e., 3 and 5 mm in diameter, are both detectable (Figs. 11a, b).

The assessment of the estimated displacement contrast that is related to the underlying elasticity distribution in the heterogeneous medium was evaluated by calculating CTE. The
CTE values from the simulated gels (triangle) and polyacrylamide gels (circle) are shown in figure 12. Here, CTE is calculated for three inclusion diameters (3, 5, and 10 mm) with two different Young’s moduli (25 kPa and 50 kPa). In this experiment, the displacement contrast is close to the elasticity contrast for larger and stiffer inclusions (CTE > 0.7). The CTE is below 0.5 for the 3-mm inclusion, even though the inclusion/background interface is clearly depicted on the HMI profile (Fig. 11a).

Figure 13a shows the HMI displacement image of *ex vivo* breast tissue sample where the displacement amplitude is in μm. The specimen was confirmed to be an invasive lobular carcinoma (ILC) by a pathologist and harvested immediately after surgery. The specimen was first imaged using mammography for pathology validation and then imaged by HMI. The ‘B’ region (i.e., \( D_{HMI} \) was above 40 μm) indicates soft breast tissue (Fig. 13a, ‘B’). The ‘T’ region (i.e., \( D_{HMI} \) was below 20 μm) indicates a stiffer region, i.e. tumor (Fig. 13a, ‘T’). Figure 13b displays a mammogram of the same specimen. The white line (Fig. 13b, ‘N’) depicts the tip of the needle attached to the lesion (tumor) after biopsy. A biopsy needle was placed prior to surgery as a guidance tool for the surgeon to identify the lesion and confirm its successful removal. Tumor size was found to be equal to approximately 20 × 10 mm². The HMI image is thus in good agreement with the mammography findings for both the size and shape of the breast and the cancer tissue. The specimen was then submitted to pathology, where a small tissue specimen was dissected for analysis and the remaining tissue was stored in the Tumor Bank Facility as required by the Institutional Review Board (IRB) of Columbia University. The histology image of the specimen was not available due to patient confidentiality agreement and the Institutional Review Board (IRB) of Columbia University regulation.
The aim of this study was to investigate the potential of the amplitude-modulated (AM) harmonic-motion imaging (HMI) technique as a noncontact method for mapping and quantifying relative tissue stiffness. Owing to the AM application, the oscillatory radiation force was sustained at the same location at a frequency twice the AM frequency (Eq. (4)). Since the FUS focal spot was small (approximately $0.8 \times 0.8 \times 1.6 \text{ mm}^3$), the force was highly localized and the probed tissue within the focal region could be indirectly associated with the underlying tissue modulus. For imaging, the radiation force could be applied in a 2D fashion using a raster-scan technique and a 3D HMI displacement image could be obtained by combining multiple 2D planes at variable depths.

**FIG. 11** $D_{\text{HMI}}$ from HMI experiments along the central axis of the FUS transducer for various inclusion sizes and two different stiffnesses (- $-30\%$ acrylamide ($\approx 25 \text{ kPa}$) and - $-40\%$ acrylamide ($\approx 50 \text{ kPa}$)). The average and standard deviation (i.e., error bars in figures) of the estimated displacement ($D_{\text{HMI}}$) were calculated based on four iterations completed for each inclusion size. (a, b and c) results for 3, 5 and 10-mm inclusion diameters, respectively. The background stiffness was 25% acrylamide ($\approx 13 \text{ kPa}$). The dotted lines indicate the diameter of the inclusions.
In the FE analysis, the axisymmetric model allowed for a 3D analysis of the pressure field applied. In this case, the geometry, tissue-mechanical properties distribution and the acoustic-radiation force were axisymmetrically distributed. This is a limitation of the axisymmetric model because biological tissues are typically asymmetrically heterogeneous. The acoustic pressure level of 1.98 MPa was applied at the center axis and moved downward with a step size of 1 mm. Since the model was linear, the behavior at higher acoustic pressure levels could be extrapolated in order to predict the resulting displacements. In this experiment, the applied pressure was approximately 1.96 MPa for 400 ms with an expected peak temperature rise of 0.9 °C. This temperature rise was considered low (ΔT<1°C) because it would not pose a risk to the patient during ultrasound exposure.\textsuperscript{65,66} This temperature rise is considered safe and acceptable for ultrasound imaging. Scanning and image generation lasted approxi-
mately one hour, which can be further improved through custom hardware implementation. Mechanical scanning and coupling to the patient will also be included as part of the new system design for clinical applications.

The effects of inclusion size and stiffness on the displacement profile were demonstrated in simulations (Fig. 8) and experiments (Fig. 11). Three different hard inclusion diameters (3, 5, and 10 mm) were used to investigate the capability of the HMI technique in small inclusion detection. This indicates that HMI can be used for detection and mapping of small (>3 mm) tumors with a specific stiffness contrast. In the case of the 10 mm inclusion (Fig. 11c), the boundary of the inclusion is not distinct. If the area of the background region were twice larger than the inclusion like in the other cases, it is expected that the boundary of the inclusion would be more distinct. The effect of the background will be investigated in future studies.

The CTE value represented the quality of the HMI displacement, which is related to the underlying elasticity distribution in the heterogeneous medium. The results show that the CTE was higher than 0.5 but might be less than the ideal case (e.g., 1) for most inclusion cases. Thus, these CTE values indicate that the HMI displacement may be used to estimate the location and size of the lesion based on its distinct displacement but not necessarily be used to obtain its accurate stiffness contrast.

Two possible explanations are provided, i.e., that (1) different gel geometries were used and (2) the conditions of the two techniques used to measure displacement contrast by HMI and elasticity contrast by mechanical testing were different. Here, the displacement was measured by HMI where the localized force was applied deep inside the heterogeneous gels whereas the elastic modulus was measured by mechanical testing, where the force was applied on the surface of the uniform gel. The HMI and mechanical testing (i.e., dynamic mechanical testing) could not be tested within the same frequency range due to fundamental differences. In HMI, the data storage capability has been optimized for an AM frequency range above 10 Hz. The instrumentation is currently being upgraded in order to test a lower frequency range (<10 Hz). Vappou et al.\(^6\) showed that polyacrylamide gels mainly exhibit an elastic behavior and the estimated Young’s moduli remained relatively constant within the frequency range of 0.1 to 40 Hz. Therefore, we assumed that the stiffness of the polyacrylamide gels stayed constant within the range of 0.1 Hz to 15 Hz.\(^6\) In mechanical testing, when the loading frequency exceeded 1 Hz, the strain does not immediately follow the applied stress, finally causing a gap (~1 mm) between the indenter and the gel. The two techniques thus require different testing conditions and have different boundary conditions and imposed stress levels. Nevertheless, we demonstrated that the HMI displacement contrast is indirectly related to the elasticity contrast of a stiffer inclusion embedded in a soft background. The displacement contrast could be used to represent the relative inclusion stiffness, which, in turn, can be used for future tumor detection.

When the CTE value is lower, the contrast of displacement underestimates the stiffness contrast; however, as shown in figure 11a, HMI could clearly detect the 3-mm inclusion despite the relatively lower CTE. The HMI sensitivity might thus be sufficient to detect small tumors (>3 mm in a diameter). Preliminary results from postsurgical breast specimens demonstrated a good stiffness contrast between the tumor and surrounding breast tissue (Fig. 13). The tumor location was also confirmed with mammography and biopsy needle location. The histopathology results indicate that the tumor was an invasive lobular carcinoma (ILC).

In this study, the oscillatory displacement was estimated without taking into consideration the effect of reflection or any interference occurring. Shear wave and reflective waves might affect the estimated displacement. Attenuation and diffraction from these propagating waves, as well as viscosity, cannot be ignored. The effect of this interference would be mini-
mized in a viscous medium, such as tissue or gelatin gel. These effects will be further studied in simulations and experiments. The results presented herein confirm two important findings: first, the HMI technique has the potential of being successfully applied in a clinical setting for small tumor detection and diagnosis. Second, HMI could serve as an alternative noncontact indentation method that mimics the dynamic indentation testing for modulus measurement but without requiring direct contact, gel homogeneity or specific boundary conditions, with the advantage of a wider range of loading frequencies.

In this study, the experimental gels were assumed to be nearly linear elastic, to match the assumptions made in the FEM. We have also investigated the capability of HMI for measuring both the modulus and viscosity and their roles in the HMI displacement in a viscoelastic medium. Since tissue elastic properties are typically frequency dependent and the elastic moduli measurements are dependent on the excitation frequency, the modulation (or, AM) frequency can be varied to determine the degree of frequency dependence. This is one of the advantages of the HMI technique, i.e., it is capable of inducing a radiation force within a wide range of frequencies by varying the AM frequency and adapting it to the tissue type and the boundary conditions that are encountered.

**CONCLUSION**

The relationship between the HMI displacement and the tumor/inclusion Young’s modulus were validated by FEM and was also corroborated experimentally through mechanical testing on polyacrylamide gels. The study illustrated the pattern of HMI displacements and its capability of relative stiffness mapping in an inclusion-containing media. The phantom experiments showed that the oscillatory displacement was inversely proportional to the elastic modulus of the homogenous medium. Although HMI may not be able to provide clear edges of the tumor, the 2D HMI image could detect an *ex vivo* breast tumor based on its stiffness, which was consistent with mammography findings. Most importantly, good agreement was obtained between the HMI image and mammography in both the tumor shape and size. Finally, the results of the study could be used to significantly improve the design criteria, e.g., for HMI system calibration and data processing parameters, for future *in vivo* applications.

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