

High frequency ultrasound imaging and spectral analysis of sea urchin oocytes

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Abstract—High frequency ultrasound measurements were made of sea urchin oocytes at 40 MHz and compared to numerical simulations. The urchin oocytes are non-nucleated cells, and have uniform size and biomechanical properties; thus, the signals measured from each cell are similar, unlike typical nucleated mammalian cells. The power spectra calculated from the measured time domain signals were periodic, with minima located approximately 10 MHz apart. This was a result of the consistent size and biomechanical properties of the cells. Numerical simulations were developed that calculated the superposition of ultrasound scattered from each cell within the transducer field of view using the Faran scattering model. Good agreement to the measured spectral features were observed. Using cepstral analysis, the size of the cells was determined to be 78.7 μm (measured) and 81.4 μm (simulated), which was similar to the size of a single cell. This work shows how ultrasound is discretely scattered from cells within a bulk homogeneous sample, with applications towards understanding the complex scattering behaviour from heterogeneous tissues.

I. INTRODUCTION

The scattering of sound waves from cells and tissues has been studied for decades, however, the scattering phenomena is not fully understood. The scattered ultrasound intensity and frequency response depends strongly on the biomechanical properties of tissues, which itself depends on the type of tissues examined [1]. The tissue types and their health can be assessed through a quantitative analysis of the ultrasound signals [2]–[4]. Changes in the ultrasound signals have been hypothesized to be due to variations in the cellular biomechanical properties, pseudorandom spatial arrangement, and/or nuclear/cell size and shape [5]–[8]. Computer simulations use estimates of distributions to calculate the ultrasound backscatter from a collection of cells. A more accurate technique is to calculate and sum the ultrasound scatter from each discrete scatterer within the ROI, but this is computationally intensive and quickly scales with the number of scatterers, limiting the volume that can be simulated.

We developed an algorithm to calculate the ultrasound scattered from individual cells within a 3D region of interest, and applies weights to the signal from each cell depending on its location relative to the transducer to account for the limited field of view (FOV). Using this technique, the superposition of

signals from each cell within the transducer FOV can be calculated, creating a matrix of ultrasound signals as a function of transducer position. The theory of scattering of sound waves from spheres is well established, with theory dating back to the 1950's [9]–[15]. The analytical solution for the far-field pressure amplitude of the scattered ultrasound wave P_s from spheres in the frequency domain is

$$P_s(\theta, f) = \frac{P_0}{akr} \sum_{n=0}^{\infty} [(-i)^n (2n+1) \sin \eta_n \exp(-i\eta_n) P_n(\cos \theta)] \times \exp(-ikr), \quad (1)$$

where P_0 is the initial ultrasound pressure, r is the distance to the observation point, n is the order of the scattered wave, P_n is the n^{th} order Legendre function, $k = 2\pi f/c$, a is the radius and η is the angle as defined in [11], [12] which itself is a function of ka of the cell and the coupling fluid, and also the Poisson ratio of the cell. This equation accounts for shear and compressional waves in the sample, and was developed by Faran in 1951 [11] and then refined by Hickling in 1962 [12]. Each cell is a viscoelastic mixture of fluids, proteins and organelles which behaves as a poroelastic material when under load [16]; they can be considered homogeneous on the length scales related to the ultrasound wavelength used in these studies.

The ultrasound signals from packed sea urchin oocyte cell pellets were examined to help understand the ultrasound scattered from cell aggregates. The oocytes are homogeneous, have a narrow size distribution and can be easily measured using commercial high frequency ultrasound systems. The sea urchin oocytes are composed mostly of fluids, protein and egg yolk, and are ideal for studying using a discrete scatterer simulation technique. Good agreement in the measured and simulated signals was observed, validating the numerical simulations. This model can be used for studying the ultrasound scattered from more complex scenarios such as heterogeneous tissues, where the scattering phenomenon is not well understood.

II. METHOD

A. Urchin cell measurements

Purple sea urchin oocytes *strongylocentrotus purpuratus* were prepared in an artificial hypertonic salt water solution containing 0.5 M KCl. The average cell diameter was 85 μm (fig. 1). Oocyte cell pellets were made by centrifuging the cells in an 8 mm diameter container for 10 minutes, creating a close-packed structure of cells mimicking normal tissue. The cell pellet was placed at the bottom of a larger container in degassed phosphate buffered saline (PBS) for ultrasound measurements at room temperature (fig. 2A). Measurements were performed using a Visualsonics VS40B Ultrasound Scanning System equipped with a 40 MHz transducer (Visualsonics Inc, Toronto, Canada). The transducer had a focal length of 9 mm, $f\#$ of 3.0, and a -6 dB bandwidth of 90%. All data was sampled at 500 MS/s and analyzed using Matlab.

B. Simulation parameters

A 3D spatial map of cells in a 5x5x1 mm volume was created using an algorithm to place cells within the volume. The algorithm sequentially placed cells randomly in the volume such that a newly placed cell was a minimum distance from its closest neighbours. For a close-packed structure of non-deformable non-overlapping spheres, the volume packing ratio is a maximum of 0.74 for a periodic face-centered cubic (FCC) structure [17]. A periodic structure is not realistic for tissues; however, for randomly packed spheres, the packing ratio drops to the 0.6 range [18]. To achieve a realistic tissue packing ratio of 0.75-0.90 [19], the spheres were allowed to overlap slightly. Using a minimum center-center distance of 55 μm , a volume packing ratio of 0.78 was achieved using 60703 cells of 85 μm diameter in a 5x5x1 mm volume.

The ultrasound scattering from the cell pellet was simulated by calculating the ultrasound scattered from each cell using the Faran scattering model (equation 1). The input parameters for the Faran model were $v_f = 1480$ m/s, $v_s = 1573$ m/s [20], $\rho_f = 1000$ kg/m³, $\rho_s = 1198$ kg/m³ [20] and a Poisson ratio of 0.499, where the subscript f denotes the coupling fluid, and the subscript s denotes the spherical cell. The algorithm stepped through every cell in the volume and if the cell was outside of the transducer FOV, then the signal from that cell was set to zero and the algorithm moved onto the next cell. For all cells within the transducer FOV, the Faran solution as a function of r and θ was found using the lookup table (fig. 2B). First the inverse Fourier Transform was calculated for each signal, then a bandpass filter of 20-55 MHz applied, and then all the signals were summed to give a radiofrequency (RF) signal from all scatterers within the FOV at that transducer position. The transducer was moved along the x-axis, and the signals from the cells within the new FOV were calculated using the same procedure. This process was repeated along the x-axis from 0 to 5 mm in steps in 10 μm . The normalized power spectrum was calculated using a reference ultrasound signal measured from a quartz plate. The power cepstrum was estimated by calculating the inverse Fourier Transform of the logarithm of the power spectrum.

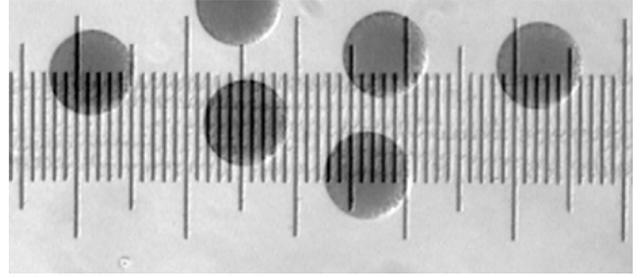


Fig. 1. Optical image of the sea urchin oocytes over the scale bar. The major division in 100 μm .

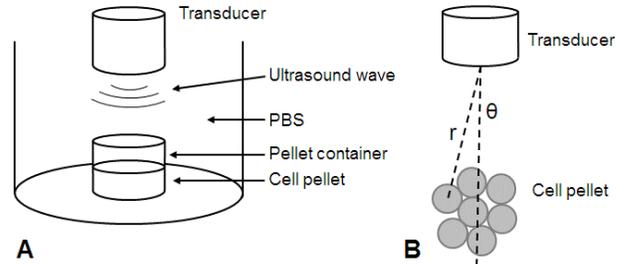


Fig. 2. (A) Schematic showing the experimental setup, with the transducer above the cell pellet. (B) Calculation of the ultrasound signal from each cell as a function of distance r and angle θ .

III. RESULTS AND DISCUSSION

A numerical simulation based on the ultrasound scattered from every cell in a 3D volume was developed to help understand the contribution that single scatterers have on the measured ultrasound signal in bulk tissues. The contribution of each cell was limited to cells within the transducer FOV only, which made the simulations more realistic, and significantly increased the computational speed. The time of the simulation for 60703 cells in a 5x5x1 mm volume using a standard Intel i7 desktop PC was about 5 minutes.

The measured and simulated RF lines from packed oocytes are shown in fig. 3. In both the measurements and simulations, the transducer focus was approximately 1 mm below the surface of the cell pellet. The average maximum amplitude in both cases occurred around 11 μs , and then gradually decayed with time. The decay is primarily due to the limited transducer depth of field; attenuation would also contribute to the decay, but attenuation was not modeled as its contribution would be significantly less at the frequencies used. The A-scans were used to recreate the measured and simulated b-scans as shown in fig. 4. The measured ROI was 8x8 mm, while the simulated ROI was 5x5 mm, however the regions were cropped to a 2.5x1.5 mm to allow for a better comparison. Aside from the pellet surface structure, the measured and simulated images showed similar visible features. Variations in speckle intensity were observed throughout due to the random packing nature of the cells. Some voids are present in the measurements, which is likely due to uneven packing of the oocytes during the measurement.

A quantitative analysis of the cell pellet RF lines was performed to extract information about the cells. The spectra of 30 RF lines were individually calculated and then averaged (fig. 5A). There was excellent agreement in the location of the minima and maxima throughout the bandwidth of the transducer (20-60 MHz). The spectral patterns from the pellet are similar to those observed from single cells [20]. The cepstrum showed distinct peaks at 78.7 μm for the measured pellet, and 81.4 μm for the simulated pellet (fig. 5B). The cepstrum detects periodicity in the spectrum; in this case, the periodicity was mostly due to the narrow size distribution and similar biomechanical properties of the oocytes within the cell pellet. The spectral periodicity observed in this study is not normally observed in tissues due to the variations in the cell size, structure and biomechanical properties. The model developed in this study can rapidly simulate the ultrasound scattering from single cells as well as closely packed cells in minutes. The technique can be easily extended to larger volumes and smaller cells, small particle scattering as found in Rayleigh scattering, as well as photoacoustic imaging. The 3D simulation environment provides a more realistic scenario than 2D areas typically used in ultrasound scattering simulations.

IV. CONCLUSIONS

A numerical simulation of ultrasound scattering from a 3D close-packed structure of cells was developed and compared to measurements of sea urchin oocytes. Excellent agreement in the measured signals and recreated b-scan images was observed. The simulation technique is fast and easy to implement, and can be expanded to probe other tissue types, as well as other measurement systems or photoacoustic imaging.

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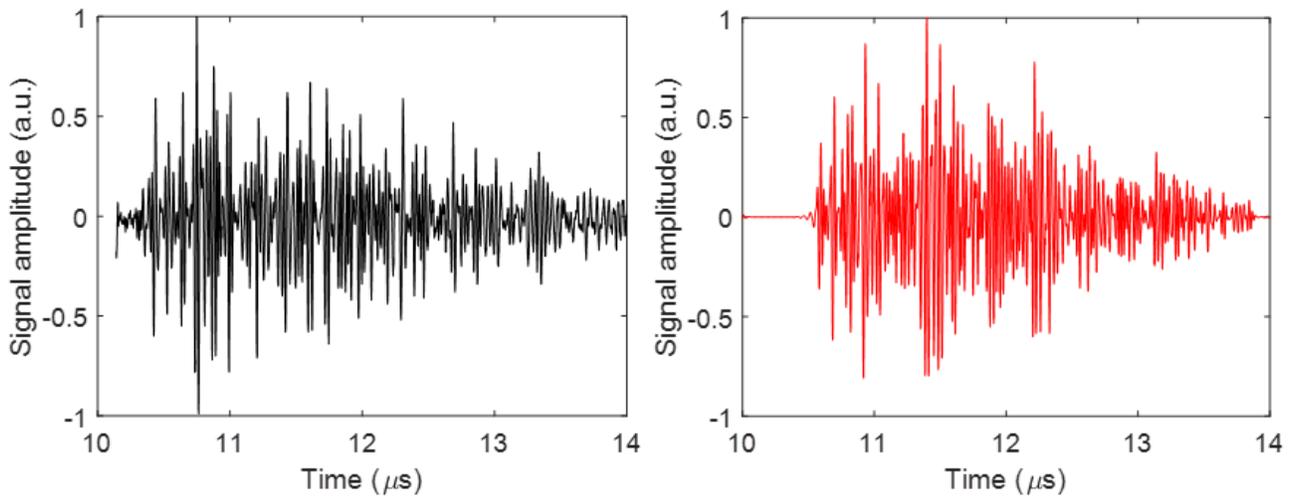


Fig. 3. Representative time domain signals from the measured (left) and simulated (right) oocyte pellets using 40 MHz ultrasound.

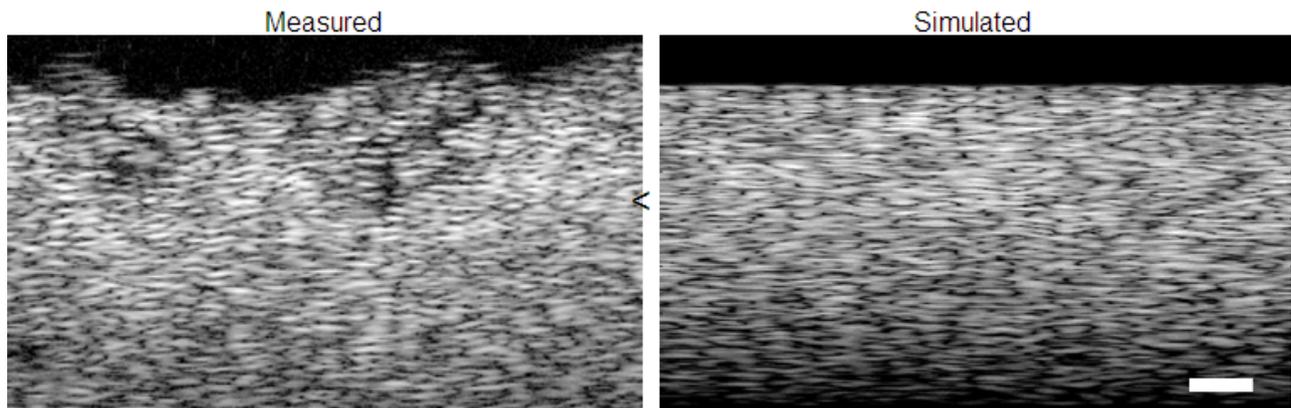


Fig. 4. B-scan images created from the measured (left) and simulated (right) oocyte pellets using 40 MHz ultrasound. The scale bar is 500 μm .

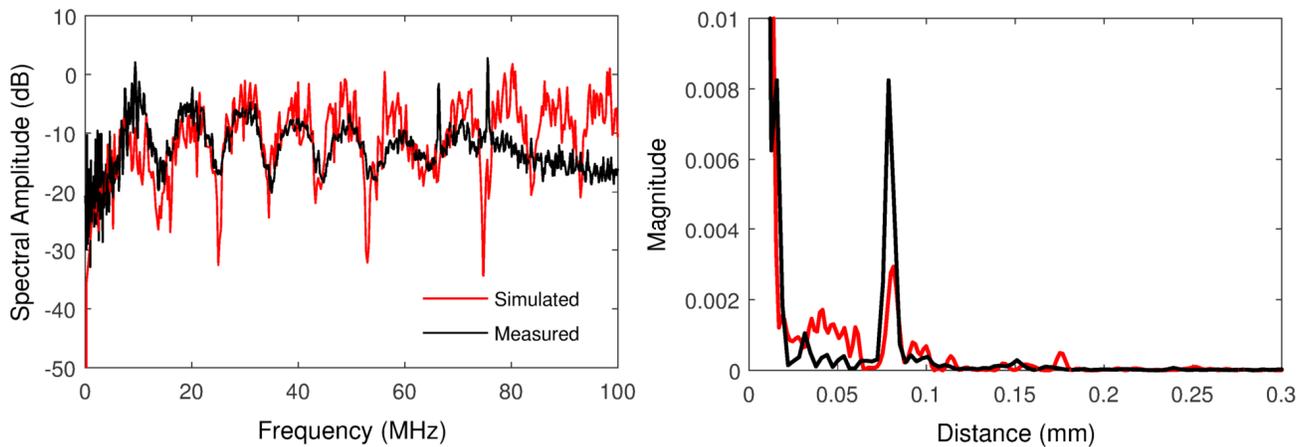


Fig. 5. (A) The power spectra from the measured (black) and simulated (red) oocyte pellets, averaged from 30 spectra. Good agreement in the location of the spectral minima and maxima was observed. (B) The cepstrum for the measured (black) and simulated (red) signals. A peak was observed at 78.7 μm (measured) and 81.4 μm (simulated).