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A Novel Technique for Measuring Ultrasound Backscatter from Single Micron-Sized Objects

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***Abstract*—The measurement of the ultrasound backscatter from individual micron-sized objects is required to gain an understanding of the behavior of both weak (cells) and strong (contrast agents) scatterers for applications ranging from tissue characterization to molecular imaging. However, obtaining such a response remains a challenge. For instance, the presence of air bubbles in a suspension of cells during measurements of cell backscatter may lead to the incorrect interpretation of the backscattered signals. In addition, the size and shape of the single object that produces an ultrasound backscatter signal are critical input parameters to theoretical models, yet hard to be measured experimentally. In this work, a novel technique combining a Xenoworks microinjection system (Sutter, Inc., Los Angeles, CA) with co-registered Olympus IX71 inverted microscope (Olympus America, Inc., Center Valley, PA) and a VEVO770 Ultrasound imaging device (VisualSonics, Inc., Toronto, ON) was developed in which the ultrasound backscatter response from a single object was obtained under optical microscope guidance. This technique provides accurate information about the size and shape of the object. Two transducers of central frequencies of 25 and 55 MHz were used (for a total spectrum of 12-57 MHz). The foci of the optical lens and the transducer were aligned to obtain optical and ultrasonic images of the same region. The object of interest was attached to the micropipette (using negative pressure) and then released from the micropipette (using positive pressure and/or tapping on the micropipette) while imaging it both optically and ultrasonically. In order to calibrate the system, a micropipette was used to grab a 20 μm polystyrene microsphere from a suspension of microspheres in degassed water by applying a pressure of -18.9 kPa. The microsphere was released by applying a pressure of +35.0 kPa. During the release, optical and ultrasonic raw RF lines were obtained. These lines were then used to obtain the power spectral plot of individual microspheres which were compared to analytical solutions. A very good agreement was found (error of 1%) between the measured backscatter response of microspheres and that of a Faran model of an elastic sphere. Extension of this method to prostate carcinoma (PC-3) cells showed a good agreement (error of 5%) when compared to the Anderson fluid sphere model. This technique is capable of providing accurate measurements of the backscatter from individual objects and is currently being used to deduce the backscatter response from other cell lines of different sizes and from ultrasound contrast agents either in isolation or when attached to a cell. The advantages of the technique along with its future applications are discussed.**

***Keywords*—cell scattering, high frequency ultrasound scattering, tissue characterization, tumor treatment monitoring**

I. INTRODUCTION

High frequency ultrasound (20MHz - 60MHz) has the potential of detecting structural and physical changes in cell ensembles undergoing apoptosis or programmed cell death. The ultrasonic backscatter from cell ensembles treated with the chemotherapeutic drug cisplatin increased the backscatter by 9-13dB [1]. A similar increase in backscatter is detected for in-vivo models of tumor response [2]. The mechanism that causes this increase in ultrasound backscatter is not well understood. As the ultrasound wavelength in high frequency ultrasound approaches the physical dimensions of a cell, theoretical models of acoustic scattering at the cellular level are needed in order to understand this mechanism. This may help in the characterization of tissue and in determining the tumor response to treatment.

In recent studies, Baddour *et al.* [3] performed measurements of high frequency ultrasound backscatter responses from single eukaryotic cells in suspension. They found that for human acute myeloid leukemia (OCI-AML-5) cells, the ultrasound backscatter response could not accurately be modeled as a fluid sphere [4], even though they found that larger cells with a larger cytoplasm to nucleus ratio (PC-3 cells) could. During the experiments, no visual confirmation of the scattering structures could be made, as the experiments were done with cells in suspension. The presence of other scattering structures (such as air bubbles) in a suspension of cells during the ultrasonic measurements may have led to the incorrect interpretation of the acoustic signals.

In this work, a new technique is developed to measure the ultrasonic backscatter response from individual micron-sized objects. This method allows the optical detection of the scattering structures responsible for the backscatter responses measured by the ultrasound imaging devices. In order to test the methodology, the backscatter frequency responses from individual 20 μm polystyrene microspheres suspended in degassed water were measured and compared to theoretical predictions. The backscatter responses from single prostate carcinoma (PC-3) cells suspended in phosphate buffered saline were also investigated.

II. METHODS

A novel technique combining a microinjection system and synchronized optical and ultrasonic imaging devices is developed in which the ultrasound backscatter response from a

single object is obtained under optical microscope guidance. The foci of the optical lens and the transducer were aligned to obtain optical and ultrasonic images of the same region. A Xenoworks microinjection system (Sutter, Inc., Los Angeles, CA) consisting of a digital microinjector, a micromanipulator, and a micropipette puller was used. The system allows for the use of custom tailored micropipettes as well as the application of pressure gradients along the surface of the object of interest. A Retiga EXi CCD camera (QImaging, Inc., Surrey, BC) mounted on an Olympus IX71 inverted microscope (Olympus America, Inc., Center Valley, PA) and a VEVO770 Ultrasound imaging device (VisualSonics, Inc., Toronto, ON) were used to capture optical and ultrasonic images, respectively. The PC-3 cells were prepared in a degassed, dilute phosphate buffered saline (PBS) and imaged at 25 and 55 MHz. Only data from the -6-dB bandwidth of each transducer were used in the analysis which gave an overall bandwidth spanning 12-57 MHz. A single cell was initially attached to the micropipette using a pressure of ~ -0.90 kPa and then released from the micropipette (using positive pressure of +35 kPa and/or tapping gently on the pipette) while simultaneously imaging it optically and ultrasonically.

Several independent acquisitions of frame sets were performed using each transducer and only the rf lines containing the maximum value in each frame set was chosen. A Hamming window of width of ~ 0.3 μs was applied to all 8 remaining lines. The backscatter transfer function, $\mathrm{BSTF}_{\mathrm{expr}}(\omega)$, was calculated as:

$$\mathrm{BSTF}_{\mathrm{expr}}(\omega) = \frac{R_{\mathrm{expr}}(\omega)}{R_{\mathrm{ref}}(\omega)} \qquad (1)$$

where $R_{\mathrm{expr}}(\omega)$ is the Fourier transform of the average backscatter signal from a single cell. $R_{\mathrm{ref}}(\omega)$ is the Fourier transform of the reference signal (the reflection from the surface of Dow Corning® 710 Fluid placed at the transducer focus in PBS at room temperature). The values of the backscatter transfer function are shown in the form of plots expressed in decibels (dBr) relative to the backscatter intensity from the reference. Theoretical frequency responses were calculated for either an elastic sphere [3] or a fluid sphere [4] using the Faran [5] or the Anderson [6] scattering models, respectively.

III. RESULTS AND DISCUSSION

Figure 1 shows the new technique of combining a microinjection system and synchronized optical and ultrasonic imaging devices, in which the ultrasound backscatter response from a single object is obtained under optical microscope guidance. Figure 2 shows the optical images and B-scans of a single PC-3 cell initially attached to the micropipette and when released. The theoretical (Elastic sphere model parameters: d=20 μm, ρ=1.05 g/ml, c=2350 m/s, σ=0.35) and experimental backscatter frequency responses of a single 20μm polystyrene

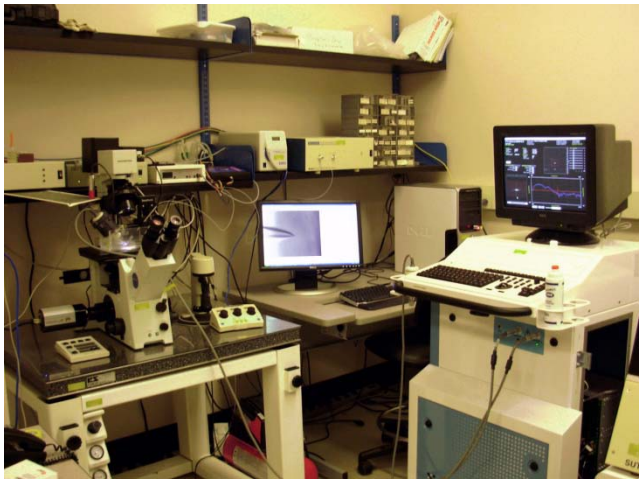

Figure 1. Experimental setup composed of an ultrasound imaging device (VEVO770) and synchronized optical imaging device (Olympus IX71).

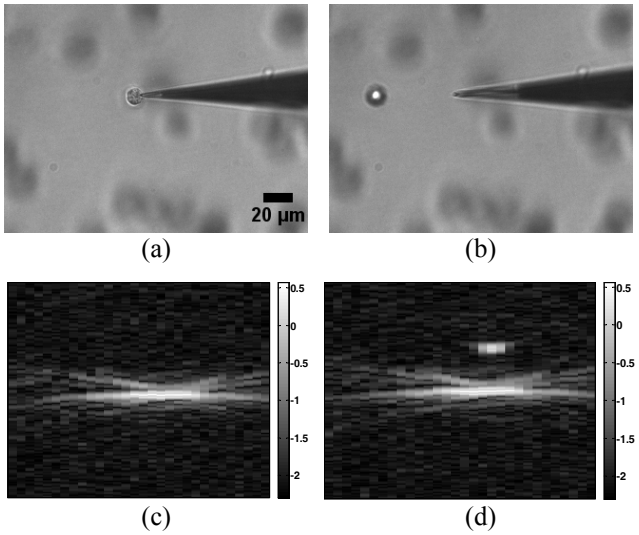

Figure 2. Optical and Ultrasonic (B-mode) images of the micropipette with a single PC-3 cell: (a) and (c) micropipette holding the cell using a negative pressure; (b) and (d) cell is released using a positive pressure. The VEVO770 ultrasound system is confocally aligned with the area in this field of view.

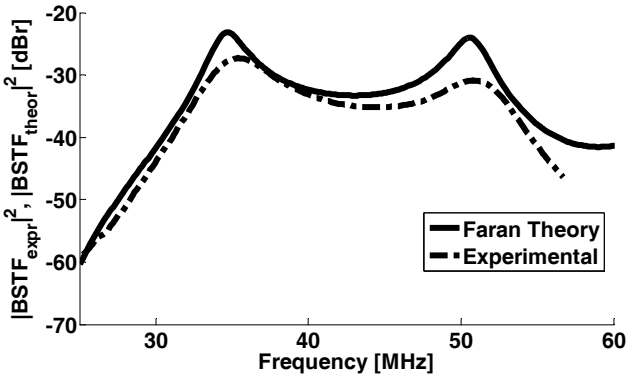

Figure 3. Theoretical (Elastic sphere model parameters: d=20 μm, ρ=1.05 g/ml, c=2350 m/s, σ=0.35) and experimental backscatter frequency responses of a single 20μm polystyrene microsphere in degassed water subject to incident pulses from a 55 MHz transducer.

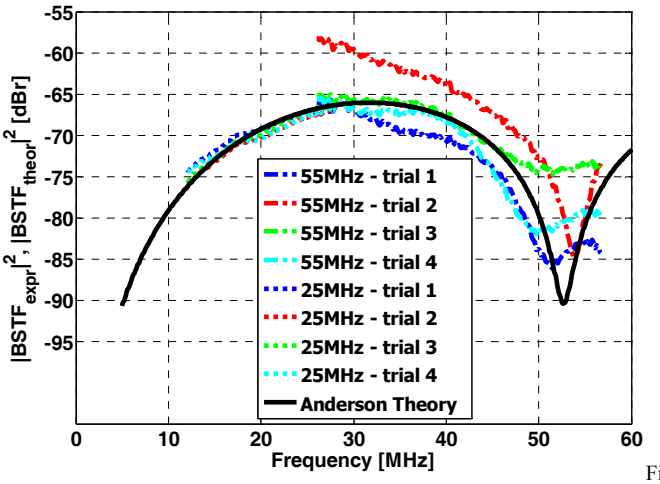

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Figure 4. Measured and theoretical (Fluid sphere model parameters: d=26 µm, ρ=1.18 g/ml, c=1523 m/s) frequency backscatter responses of a single PC-3 cell in PBS at 25 and 55 MHz.

microsphere in degassed water subject to incident pulses from a 55 MHz transducer are shown in figure 3. Figure 4 shows the measured and theoretical (Fluid sphere model parameters: d=26 µm, ρ=1.18 g/ml, c=1523 m/s) backscatter responses of a single PC-3 cell in PBS at 25 and 55 MHz.

The measurement of the ultrasound backscatter from individual biological cells is required for applications ranging from ultrasound tissue characterization to molecular imaging. However, performing such a measurement remains a challenge. For instance, the presence of air bubbles during the measurements may lead to the incorrect interpretation of the acoustic signals. Such bubbles could clearly be seen in this work when conducting the experiments with the cells. The scattering from such air bubbles distorted the scattered signals from the cells. This novel technique combining a microinjection system and synchronized optical and ultrasonic imaging devices allowed us to deduce the experimental backscatter response from a single PC-3 cell under optical guidance as shown in figures 1 and 2.

There is a very good agreement in the location of the spectral features for the polystyrene microspheres as shown in figure 3. The difference was measured to be less than 1% on average between the experimentally measured backscatter frequency response of individual microspheres and the theoretical frequency response of an elastic sphere. This result confirms the validity of the developed technique. The absence of sharp and abrupt peaks in the frequency responses of a single PC-3 cell (figure 4) reveals the absence of shear wave's propagation inside the cell. This implies that these cells behave more like fluid scatterers, in the same way that Baddour et al. [4] found for PC-3 cells and Falou et al. [7] found for sea urchin oocytes. The discrepancies found at 55 MHz between the theoretical and experimental backscatter frequency response for PC-3 cells may be due to the relatively low signal-to-noise ratio of the transducer employed. When compared to the Anderson fluid sphere model, an error of 5% on average was found between the measured and theoretical frequency backscatter responses.

IV. CONCLUSIONS AND FUTURE WORK

In conclusion, this study presented the use a novel technique in measuring the ultrasonic backscatter response from micron-size cells at high frequencies. It also showed that the backscatter response from single PC-3 cells in suspension are best modelled using the Anderson fluid sphere model. The scattering from cells will be examined for other cell types of interest to our laboratory (OCI-AML-5) as well as when the cells are responding to the effects of chemotherapeutic treatment. Future work includes the application of this methodology to investigate scattering from strong scatterers such as ultrasound contrast agents either in isolation or when attached to a cell.

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