A photoacoustic technique to measure the properties of single cells

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ABSTRACT

We demonstrate a new technique to non-invasively determine the diameter and sound speed of single cells using a combined ultrasonic and photoacoustic technique. Two cell lines, B16-F1 melanoma cells and MCF7 breast cancer cells were examined using this technique. Using a 200 MHz transducer, the ultrasound backscatter from a single cell in suspension was recorded. Immediately following, the cell was irradiated with a 532 nm laser and the resulting photoacoustic wave recorded by the same transducer. The melanoma cells contain optically absorbing melanin particles, which facilitated photoacoustic wave generation. MCF7 cells have negligible optical absorption at 532 nm; the cells were permeabilized and stained with trypan blue prior to measurements. The measured ultrasound and photoacoustic power spectra were compared to theoretical equations with the cell diameter and sound speed as variables (Anderson scattering model for ultrasound, and a thermoelastic expansion model for photoacoustics). The diameter and sound speed were extracted from the models where the spectral shape matched the measured signals. However the photoacoustic spectrum for the melanoma cell did not match theory, which is likely because melanin particles are located around the cytoplasm, and not within the nucleus. Therefore a photoacoustic finite element model of a cell was developed where the central region was not used to generate a photoacoustic wave. The resulting power spectrum was in better agreement with the measured signal than the thermoelastic expansion model. The MCF7 cell diameter obtained using the spectral matching method was 17.5 μm, similar to the optical measurement of 16 μm, while the melanoma cell diameter obtained was 22 μm, similar to the optical measurement of 21 μm. The sound speed measured from the MCF7 and melanoma cell was 1573 and 1560 m/s, respectively, which is within acceptable values that have been published in literature.

Keywords: Photoacoustics, high frequency ultrasound, spectral analysis

1. INTRODUCTION

Over the past few decades, the mechanical and acoustical properties of tissue have been studied extensively. Ultrasound has been used to determine the sound speed and attenuation through tissue, where variations in these variables can be used to differentiate types of tissue, or even diagnose disease[1]. Research into ultrasonic methods to detect cancerous tumors are ongoing[2]. A promising technique uses the ultrasound backscatter spectrum to detect tumors, and evaluate the efficacy of anticancer treatments[3,4]. These methods probe bulk tissue, and it is unknown how individual cell properties affect bulk tissue properties. Several methods have been developed to measure the acoustical properties of single cells, but many require prior assumptions about the cell, require lengthy measurements or complicated normalization methods[5].

We have developed a method that can rapidly determine the size and sound speed of single cells using a combined ultrasound and photoacoustic technique without prior knowledge or assumption of any cell parameters. When irradiated with ultrasound that has a wavelength on the same order as the cell size, the resulting scattered wave has a complex scattering pattern as a function of wavelength. The power spectrum contains periodically varying minima and maxima whose shape depends directly on the size and sound speed of the cell[6]. For photoacoustics, when the cell is irradiated with a laser, the cell undergoes a rapid thermoelastic expansion resulting in the emission of a photoacoustic wave. Similar to ultrasound, the emitted photoacoustic wave has periodically varying minima and maxima that depend on the size and sound speed of the cell[7], however the frequency locations of these spectral features are different than with ultrasound. While the ultrasound and photoacoustic power spectral features both depend on the size and sound speed of the cell, the mechanism by which they are generated are different. The ultrasound wave is reflected from the cell, while the photoacoustic wave is generated within the cell.

The ultrasound and photoacoustic power spectra can be compared to theoretical models to extract the cell diameter and sound speed. For ultrasound, the Anderson scattering model describes the ultrasound reflected from a fluid-filled

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homogeneous sphere as a function of diameter, sound speed and incident angle[6]. For photoacoustics, a thermoelastic expansion model describes the photoacoustic wave generated from a homogenous sphere when irradiated with light[7]. Cells are not truly homogeneous, as they contain a complex mixture of proteins and organelles, as well as a nucleus. For ultrasound wavelengths around 200-400 MHz, the respective resolutions are approximately 8 and 4 μm, respectively[5]. At these length scales, most components of a cell cannot be resolved, and the resulting scattered wave can be described by the average sound speed within the resolution volume. The same theory holds for photoacoustic measurements, provided the optically absorbing components within the cell are homogeneously distributed. This is true for certain dyes that can permeate both the cell and nuclear membrane (such as trypan blue), however it may not hold true for other dyes or nanoparticles that are restricted to the cytoplasm only. In these cases, the analytical expressions cannot be used, and alternative methods such as finite element models (FEMs) must be used.

Using either ultrasound or photoacoustic measurements to obtain the size and sound speed alone cannot be used to uniquely determine these variables. The ultrasound scattering model and thermoelastic expansion model both use the ratio of the diameter to the sound speed; therefore the same spectral shape can be obtained for many different sets of variables provided the ratio remains constant. However since the ultrasound and photoacoustic spectra are generated via different mechanisms, calculating the diameter and sound speed that fit both spectra would give one solution, the actual diameter and sound speed of the cell.

In this study, we use the ultrasound and photoacoustic power spectrum to obtain the diameter and sound speed from a MCF7 breast cancer cell stained with trypan blue, and then an unstained B16-F1 melanoma cell. We found that the diameter and sound speed extracted for these two cells agrees with expected results. This technique requires only a single ultrasound and photoacoustic measurement; therefore it could be used to rapidly measurement a large number of cells in a short period of time.

2. METHOD

2.1 Cell Preparation

Two cell lines were used for these experiments: MCF7 (human breast cancer) and B16-F1 (murine melanoma). Cells were incubated in Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum at 37°C with 5% $C0_2$ in a cell culture flask and passed every 2-3 days to maintain confluence. Shortly before ultrasound and photoacoustic measurements, the cells were dissociated from the flask with trypsin and moved to a glass bottom dish (Mattek, USA) that were coated with approximately 200 μm of 1% agar. The agar was required to reduce back-reflections from the glass substrate and simulated a cell floating in suspension. MCF7 cells were permeabilized with 0.05% Triton-X, and then trypan blue was added as a photoacoustic absorbing agent. Trypan blue was required as the MCF7 cells do not normally absorb light at 532 nm. The B16-F1 cells did not require any treatment as the melanin within the cell act as an optical absorber.

2.2 Photoacoustic Microscope

A SASAM acoustic microscope (Kibero GmbH, Germany) was used for all ultrasound and photoacoustic measurements. An IX81 inverted optical microscope (Olympus, Japan) was modified to include a transducer positioned above the sample holder. The transducer and microscope optics were co-aligned. Ultrasound pulse-echo measurements were made by positioning the transducer over the cell to be measured. After the ultrasound measurement was completed, the photoacoustic measurements were made using a 532 nm laser (Teem Photonics, France) collimated through the side port of the microscope then focused onto the sample using a 10x optical objective. In photoacoustic mode, the transducer was used to passively record the photoacoustic signals generated from the cell. A schematic of the system is shown in figure 1A. All measurements were made with a transducer that had a center frequency of 200 MHz (60° aperture, 42% bandwidth). Acoustic pulses were generated at a pulse repetition frequency (PRF) of up to 500 kHz. The laser had a pulse width of 330 ps and PRF of 4 kHz. All signals were averaged 100 times, amplified by a 40 dB amplifier (Miteq, USA) and digitized at 8 GHz. The signals measured from directly above the single cells were normalized by removing the transducer response, windowed using a Hamming window, filtered with a bandpass filter of 100-800 MHz then the spectrum calculated using the Fast Fourier transform. Further details on the instrument can be found in reference [8], and details on the signal processing methods including normalization can be found in reference [9].

2.3 Finite Element Model

A FEM was developed to solve the photoacoustic wave emission for where the analytical thermoelastic expansion model could not be used. Melanoma cells contain melanin particles that absorb energy throughout the visible spectrum. These particles are contained within the cytoplasm only, not within the nucleus and therefore the cell is not a homogeneously absorbing particle, a requirement for the thermoelastic expansion model. A 2D-axisymmetric model was constructed using COMSOL Multiphysics, with the nucleus, cytoplasm and surrounding coupling fluid as separate elements as shown in figure 1B. The model was initiated with the cytoplasm set to a higher pressure than the nucleus or coupling fluid, and the acoustic propagation due to the pressure differential between the cytoplasm and other areas solved using the transient acoustics module. This simulated the situation immediately after the cell was irradiated with a laser, but before acoustic propagation occurred. In these simulations, the cytoplasm was set to unity pressure and the rest zero pressure. A mesh size of 0.05 µm was used.

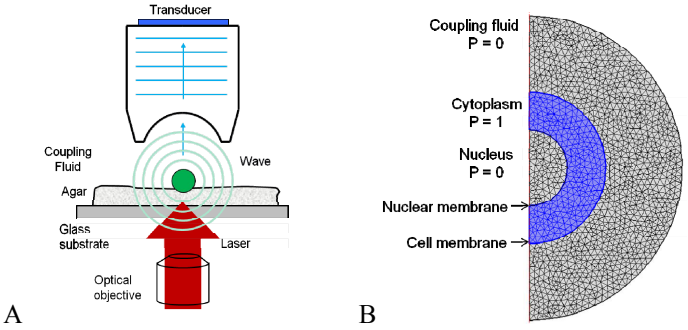

Figure 1: (A) The photoacoustic microscope. The transducer positioned above the cell records the photoacoustic waves generated from the cell after being irradiated by the laser. The microscope also operates in pulse-echo mode to record the backscattered waves from the cell. (B) The FEM construction, with the pressure values in the nucleus and cytoplasm areas identified. Optical absorption occurs in the cytoplasm only, not the nucleus.

3. RESULTS AND DISCUSSION

3.1 MCF7 Cells

Prior to any measurements, numerical simulations were completed to calculate the spectrum for various diameter and sound speed ratios using the Anderson scattering model and thermoelastic expansion model. Diameters ranging from 7 to 28 µm, and sound speeds ranging from 1500 to 1650 m/s were used. Ultrasound and photoacoustic measurements were made on a single MCF7 cell and the power spectra calculated (figure 2). The frequency locations of the spectral minima in the measured signal (approximately 160, 209 and 260 MHz) were compared to the simulation database. The top 15 diameter and sound speeds of the theoretical spectra that had the closest match to the measured spectrum were plotted in figure 3. The same method was then applied to the photoacoustic measurements, where the measured spectral minima at 121, 223, 330 and 412 MHz were compared to theoretical simulations using the thermoelastic expansion model (figure 2). The top 15 diameter and sound speeds that had the closest spectral match to the measured spectrum were plotted in figure 3A. In both models, the frequency locations of the spectral minima depend entirely on the ratio of the sound speed and diameter, so the same spectral shape could be obtained for multiple sound speed and diameter values provided the ratio remained constant. The linear representation of the sound speed and diameter for both the ultrasound and photoacoustic measurements are shown in figure 3A. It is impossible to determine the sound speed or diameter using just the ultrasound or photoacoustic measurement alone. While both the ultrasound and photoacoustic theory depends on the sound speed and diameter ratio, the resulting waves are due to different physical phenomena (ultrasound depends on scattering, while the photoacoustic wave depends on energy absorption). Therefore, the intersection of the ultrasound and photoacoustic lines of best fit should give the diameter and sound speed of the cell. In figure 3A, this gives a diameter and sound speed of 17.5 µm and 1573 m/s. The diameter is close to the optically measured value of 16 µm as shown in figure 3B. Note the cell image in figure 3B is unusually dark because of the trypan

blue stain. The sound speed falls within the range 1575±25 m/s, which was calculated from 69 cells using other ultrasonic methods[10].

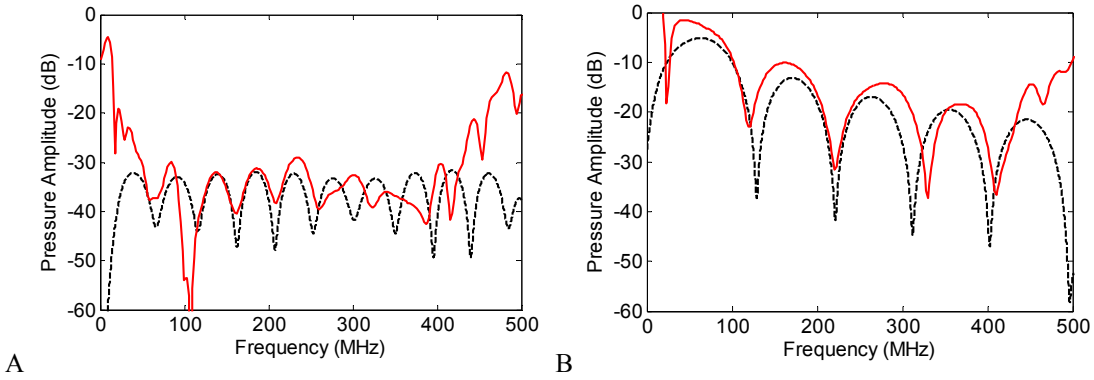

Figure 2: (A) The measured ultrasound power spectrum from a MCF7 cell (red solid line) compared to the Anderson scattering model (black dashed line). (B) The measured photoacoustic power spectrum from a MCF7 cell (red solid line) compared to the thermoelastic expansion model (black dashed line). Both models use the ratio of the sound speed and diameter; therefore multiple sound speed and diameter variables can give the same theoretical spectral pattern. The bandwidth of the transducer was approximately 100 to 350 MHz.

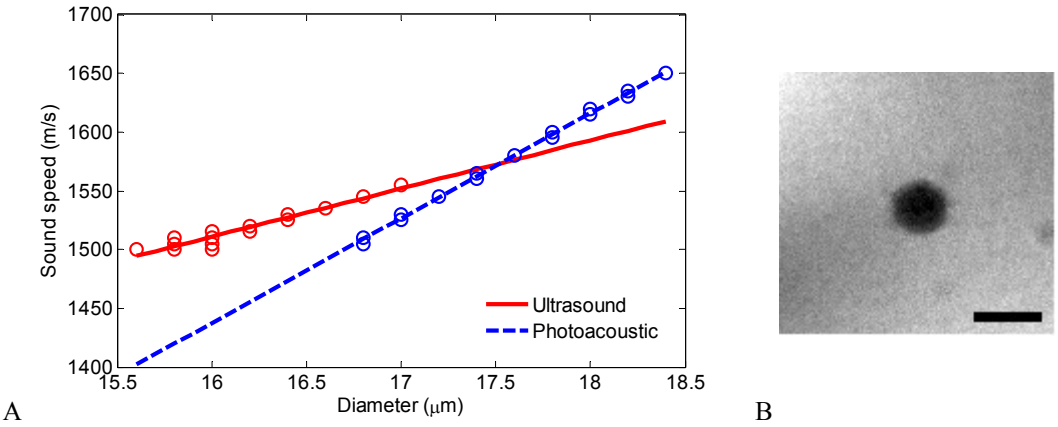

Figure 3: (A) The various combinations of cell diameter and sound speed parameters derived from the ultrasound and photoacoustic numerical simulations for where the simulated spectra closely matched the measured spectra. The sound speed and diameter are linearly related, resulting in a line of best fit for ultrasound (red solid line) and photoacoustics (blue dashed line). The intersection of these lines represents the sound speed and diameter that describe the best fit between theory and measured. The diameter and sound speed derived from this graph are 17.5 μm and 1573 m/s. (B) An optical image of the MCF7 cell dyed with trypan blue used in (A). The scale bar is 20 μm.

3.2 Melanoma Cells

The ultrasound backscatter and photoacoustic power spectrum were measured from a B16-F1 melanoma cell (figure 4). A similar method was applied to these cells as described in section 3.1 for the MCF7 cells. A database of the sound speed and diameters from theory that gave the closest spectral match to the measured spectra were found. However the photoacoustic spectrum did not match well, which is likely due to the assumption that the optical absorption occurs homogeneously throughout the cell. This is not correct, as the optically absorbing melanin particles only occur throughout the cytoplasm, not the nucleus. An analytical expression for the photoacoustic wave generation from a sphere with a central void does not exist; therefore a FEM was solved as outlined in section 2.3. Solving this model for a range of diameters and sound speeds to generate a database was not feasible, particularly as the size of the central void is

unknown. The model was initiated with best guess estimates of the diameter, sound speed and nuclear diameter (the central void), then further refined.

A comparison of the measured photoacoustic power spectrum, the solution from the analytical thermoelastic expansion model, and the solution from the FEM is shown in figure 4A. While the three spectra appear similar in shape, there are some distinct differences. The frequency width between minima was 69 MHz for the analytical thermoelastic expansion model and ranged between 72 and 79 for the FEM, resulting in a better match to the measured spectrum. Some differences were observed between the FEM and the measured spectra, however this may be due to the lack of variable optimization due to the extensive computational resources required for the simulations. A comparison of the measured and theoretical ultrasound and photoacoustic spectra are shown in figure 5. In this figure, the Anderson scattering model was used for ultrasound, while the FEM was used for photoacoustics. The diameter and sound speed that fit both the measured ultrasound and photoacoustic spectra was 22 µm and 1560 m/s, respectively. This was using a nuclear diameter of 18 µm. The diameter is similar to optical measurements of 21 µm in figure 4B, and the nuclear diameter is reasonable considering the cell size. The sound speed of melanoma cells has not been measured before, however the measured value is similar to the sound speed of the MCF7 cell measured previously.

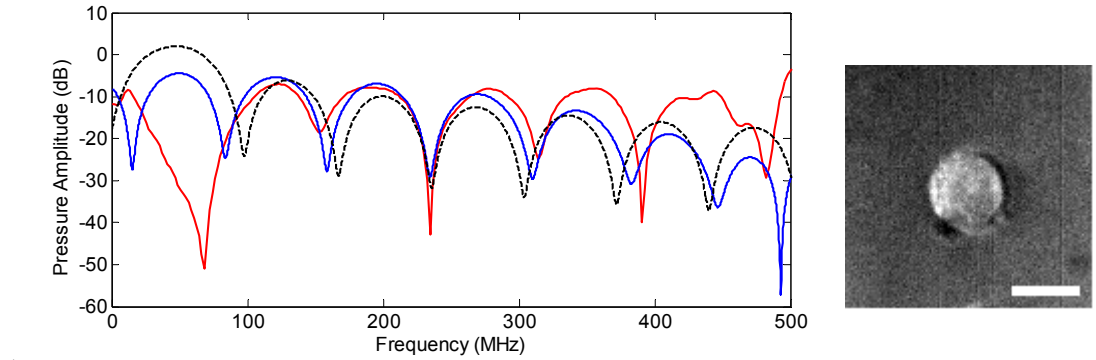

Figure 4: (A) The power spectrum measured directly from a melanoma cell (red dashed line) compared to the analytical thermoelastic expansion model solution (black dashed line) and the FEM representation of the thermoelastic expansion model (blue solid line). The FEM accounts for the lack of photoacoustic wave generation from the nucleus of the cell. Using the ultrasound and photoacoustic spectra, the best fit diameter and sound speed were 22 µm and 1560 m/s, respectively. The nucleus diameter used in the model was 18 µm. (B) An optical image of the melanoma cell used in (A). The scale bar is 20 µm.

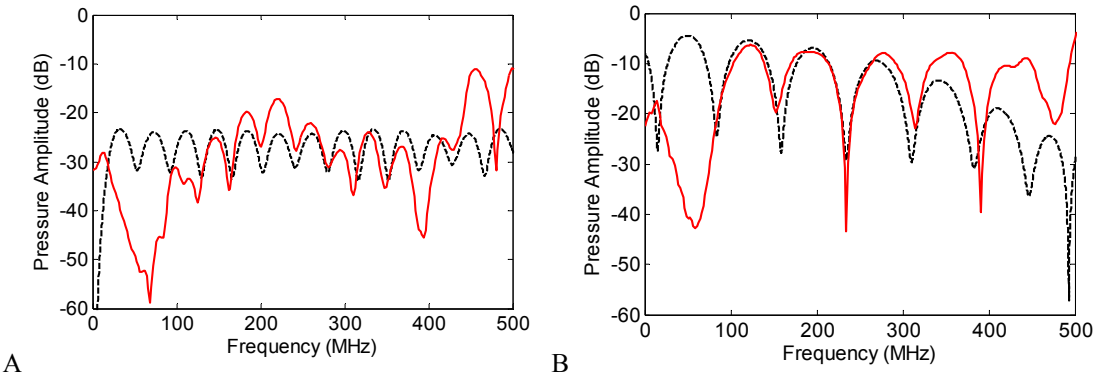

Figure 5: (A) The measured ultrasound power spectrum from a melanoma cell (red solid line) compared to the Anderson scattering model (black dashed line). (B) The measured photoacoustic power spectrum from a melanoma cell (red solid line) compared to a FEM implementation of the thermoelastic expansion model (black dashed line). The bandwidth of the transducer was approximately 100 to 350 MHz.

4. CONCLUSIONS

In this paper, we have shown that the diameter and sound speed can be extracted from a single cell using a single ultrasound and photoacoustic measurement provided the cell absorbs light at the interrogating wavelength. No prior knowledge of the cell parameters or any separate normalization methods were required. The measured ultrasound and photoacoustic spectra were compared to the Anderson scattering model (for ultrasound) and the thermoelastic expansion model (for photoacoustics). The diameters obtained were similar to those measured optically (17.5 vs. 16 μm for the MCF7 cell, and 22 vs. 21 μm for the melanoma cell). The sound speeds obtained were similar to those observed from other studies (1573 m/s for the MCF7 and 1560 m/s for the melanoma). These methods could be automated to rapidly characterize single cells to determine the size and sound speed distributions of a large sample size.

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