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Measuring the Mechanical Properties of Cells using Acoustic Microscopy

Eric M. Strohm, Michael C. Kolios

Abstract—**Time resolved acoustic microscopy was used to investigate the mechanical properties of MCF7 breast cancer cells at 375 MHz. The thickness, speed of sound, acoustic impedance, density, bulk modulus and attenuation were calculated using the amplitude and time of the ultrasound backscatter from the cellular membrane and substrate. The technique was first validated by measuring the mechanical properties of a material with well characterized properties, polyvinylidene fluoride (PVDF). The measured values agreed very well (within 4.5%) with those provided from the manufacturer. The same properties of MCF7 cells were then measured using the same technique. The speed of sound, acoustic impedance, density and bulk modulus were found to have values slightly higher than the medium they were testing in. In this paper, a technique to non-invasively measure the mechanical properties of cells using scanning acoustic microscopy is presented.**

I. Introduction

Measuring the mechanical properties of cells, such as the density, elasticity, speed of sound and attenuation are important in understanding the behavior of cells. In particular, how these properties change during natural biological activities, such as mitosis, apoptosis, adhesion and locomotion can give insight into the mechanisms behind these processes [1] but also suggest methods to monitor these changes using imaging modalities sensitive to these property changes.

Numerous techniques have been used to probe the properties of cells [2], [3], including embedded particle tracking, magnetic twisting cytometry, micropipette aspiration, microneedles, optical and magnetic tweezers, and atomic force microscopy. Most of these techniques rely on a stimulus-reaction method, cell manipulation, staining and/or invasive methods which can disturb the cell and alter its natural progression [2].

Ultrasound has a major advantage over other methods of measuring the properties of cells: it is non-invasive. The intensity is low enough that it does not interfere with the cell [4], and simulations indicate negligible localized temperature increases [5].

To examine the potential of using ultrasound to monitor changes in cell structure in pre-clinical models, cells have been studied using high frequency ultrasound in the 10-60 MHz range [6], [7], however the resolution at these frequencies is unable to resolve individual cells. Using higher frequencies, such as those used in acoustic microscopy (200-1000 MHz), the resolution approaches 1 μm at 1 GHz and has the lateral and axial resolution to resolve the ultrasound backscatter from the cellular membrane and organelles [4].

Acoustic microscopy has been used since the early 1970's to study biological specimens [8], however quantitative analysis was limited due to technological limitations [9]. Due to recent advances in computer and electronic technology, the extremely small backscatter signal from cell membranes and organelles can be recorded as a function of time and position, and further enhanced using post processing techniques.

This paper describes how to use acoustic microscopy to measure the mechanical properties of cells. The technique was first validated using a material with well-known properties, PVDF, then the same technique was applied to cellular measurements.

II. Theory

A. Time Resolved Acoustic Microscopy

Time resolved acoustic microscopy requires short duration pulses to resolve echoes from the top and bottom of a cell, which are separated in the time domain [4]. As water comprises the majority of the cell volume, the reflection coefficient of a cell coupled to a fluid is very small. Consequently, the echo will have a very small amplitude and special consideration to improving the signal to noise (SNR) must be given [10].

Fig. 1 shows a typical acoustic microscope setup, with the acoustic tip positioned a distance z above the sample. Let t_1

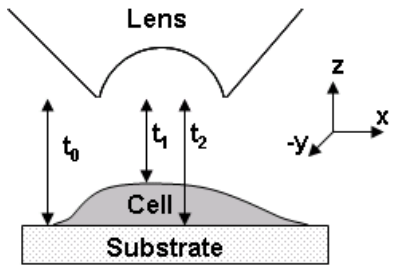

Fig. 1. The experimental setup. The time of the ultrasound echoes from the top of the sample (t_1), the sample-substrate interface (t_2) and the substrate reference (t_0) are used to calculate the thickness and speed of sound in the sample.

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E. M. Strohm and M. C. Kolios, Department of Physics, Ryerson University, Toronto, Ontario, Canada (e-mail: mkolios@ryerson.ca).

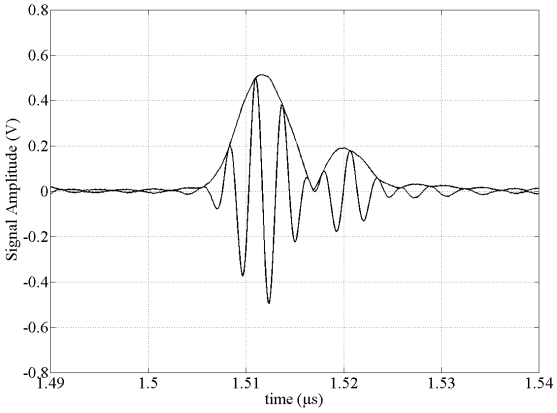

Fig. 2. An ultrasound backscatter signal (and the Hilbert transformed envelope) from PVDF of thickness 9 μm as a function of time. The first echo is from the PVDF surface, the second is from the PVDF-substrate interface.

be the time that the echo from the top of the sample is recorded, t_2 the time from the echo from the sample-substrate interface, and t_0 the reference measurement from the substrate, measured beside the sample. A typical ultrasound backscatter signal measured from PVDF is shown in fig. 2.

From these time measurements, and knowing the sound velocity in the coupling fluid c_0, the thickness d of the sample can be calculated using

$$d = \frac{c_0}{2}\left(t_0 - t_1\right), \tag{1}$$

and the sound velocity in the sample obtained from

$$c = c_0 \frac{t_0 - t_1}{t_2 - t_1}. \tag{2}$$

The signal from an interface will be maximized when the ultrasound focus is at that interface. These maxima can be obtained by measuring the amplitude of the signal as the transducer is lowered towards the sample. As the focus approaches the interface, the signal will increase until a maximum is reached at the interface. As the focus moves past the interface, the signal will then decrease. A $V(z)$ curve is then obtained by plotting the signal amplitude as a function of focus position (z) as shown in fig. 3. The signal maxima is denoted by A_1 (top of sample), A_2 (sample-substrate) and A_3 (reference). In fig. 3, the sample-substrate signal maximum appears below the substrate maximum.

The incident amplitude A_0 must be determined [11] and can be calculated using

$$A_0 = A_3 \frac{Z_s + Z_0}{Z_s - Z_0}, \tag{3}$$

where Z_s and Z_0 are the known acoustic impedances of the substrate and coupling fluid, respectively. From A_0 and A_1, the acoustic impedance of the sample can be obtained from

$$Z = Z_0 \frac{A_0 + A_1}{A_0 - A_1}. \tag{4}$$

After measuring the acoustic impedance and sound velocity in the sample, the cell density can then be calculated using

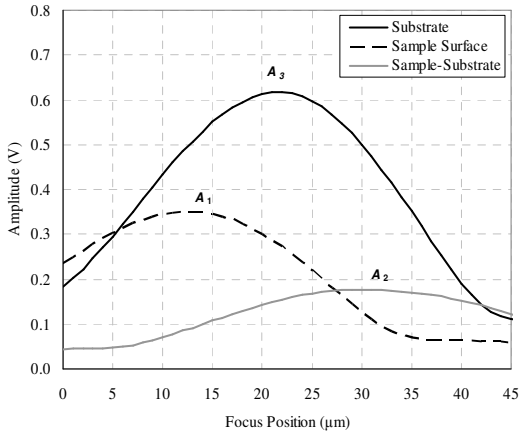

Fig. 3. A $V(z)$ curve showing the signal as a function of focus position. The focus position was normalized to the beginning of the scan, below the substrate. The maximum amplitude from each signal is used to determine the acoustic impedance in the sample.

$$\rho = \frac{Z}{c}, \tag{5}$$

and assuming shear waves are negligible [9] the bulk modulus can be calculated using

$$K = cZ = \rho c^2. \tag{6}$$

The attenuation within the sample can be calculated if the sample-substrate echo A_2 can be measured. The attenuation within the sample is

$$\alpha = \alpha_c + \frac{1}{2d}\ln\left[\frac{A_0}{A_2}\frac{Z_s - Z}{Z_s + Z}\frac{4ZZ_0}{\left(Z + Z_0\right)^2}\frac{Z_s + Z_0}{Z_s - Z_0}\right], \tag{7}$$

where α_c is the attenuation in the coupling fluid through a distance $2d$ [4]. These quantitative calculations assume homogeneity and isotropy within the sample. While cells are neither, we assume that the cell is homogeneous and isotropic at the micron length scale to enable the parameter estimation..

These time resolved methods require that the echoes from the surface of the sample and the sample-substrate interface are sufficiently separated in time, so the two echoes do not overlap as shown in fig. 2. If there is overlap, then a deconvolution technique must be used to separate the echoes [4]. Additionally, a sufficient amplitude must be measurable from each interface, at each z-position. A transducer with a long enough field of view must be selected so the amplitude of both the echoes from the top and bottom of the sample are resolved throughout a $V(z)$ scan.

III. Materials and Methods

A. PVDF

9 μm thick PVDF from Measurement Specialties (Hampton, Virginia, USA) was used for calibration and technique verification. The material consisted of only the clear co-polymer layer, without any coatings. The properties of the PVDF were provided by the company (see table I). The PVDF was attached to glass slides (Fisher Scientific) for

measurements.

It was critical that the PVDF adhered properly to the substrate so the thickness could be accurately determined. It was found that simply pressing the PVDF onto the glass slide was the best method, which left no air between the materials. Gluing the sample onto the slide was not possible, as the glue added a small but non-negligible thickness to the material, which rendered the calculations inaccurate.

Optical observations around the region of interest ensured the PVDF was properly adhered to the glass slide, then the V(z) curves were measured. The appearance of Newton's rings in the optical images indicated the presence of a layer medium beneath the PVDF, and these regions were avoided for the analysis.

B. Cells

MCF7 breast cancer cells were grown in cell culture flasks (Sarstedt, Newton, NC) using DMEM medium (ATCC, Manassas, VA) with 10% fetal bovine serum and 0.1% insulin. Cells were incubated at 37ºC with 5% CO_2 and were passed every 3 days. Cells were dissociated using trypsin and transferred to Lab-Tek II chambers made of borosilicate glass (Nunc, Germany) 24-48 hours prior to experimentation. During experimentation, cells were kept at a constant temperature of 36ºC with 5% CO_2.

C. Acoustic Microscope

The SASAM acoustic microscope (Kibero GmbH, Saarbrücken, Germany) is an Olympus IX81 inverted optical microscope with an acoustic module attached above the sample holder. During acoustic operation, the sample is illuminated from below, allowing simultaneous optical and acoustic measurements. This is a significant advancement over other acoustic microscope versions, as it allows collection of ultrasound data from regions that can be identified from the optical imaging.

The high frequency electronics components consist of a pulse generator capable of generating monocycle pulses at a 300 MHz center frequency with 100% bandwidth and a 10 V_{pp} amplitude and a pulse repetition rate of 500 kHz, a switch, 40 dB amplifier and an A/D converter capable of digitizing the signal at 8 GHz.

The acoustic module is attached to a mechanical stage to lower the transducer to the sample. The transducer is scanned over the sample with a raster movement using an *x*-*y* piezo scanning stage with a lateral resolution of 0.1 μm. The maximum range is 100 x 100 μm. A C-scan image is created by integrating the signal at each position, and areas of strong backscatter are assigned brighter values. A B-scan is a cross sectional profile through the sample, showing the signal as a function of depth.

A transducer with a center frequency of 375 MHz, semi-aperture angle of 30º and a -6 dB bandwidth of 42% was used for these studies. The axial and lateral resolution of this transducer is about 4 μm.

The optical and acoustic components are enclosed in a climate controlled box capable of maintaining a constant temperature to within 0.02ºC.

D. Analysis

Noise was reduced by averaging up to 1000 times per measurement. A band stop filter was used to remove FM-radio interference, and post-processing filtering was used to eliminate noise outside of the transducer bandwidth.

When measuring cells, the reference signal must be measured adjacent to the cell, a certain distance from where the cell measurements were made. The substrate is typically at a slight inclination, therefore corrections must be made to rectify this tilt. By measuring the time of the echo from the four corners of the substrate, the tilt can be corrected [12].

The above correction does not need to be used when measuring the PVDF material. When the PVDF material is scanned, it is simply pulled from the substrate, and the measurement is repeated on the same position over the substrate.

IV. Experimental Results

A. PVDF Calibration

A material with known properties, PVDF, was used to verify the methodology using a 375 MHz transducer. The thickness and speed of sound was calculated by measuring the peak amplitude of the Hilbert transformed time domain signal from the top of the PVDF and the PVDF-substrate interface, as shown in fig. 2 and using (1) and (2).

The acoustic impedance was calculated using the maximum amplitude of the signals obtained from the $V(z)$ curve (A_0 and A_1 from fig. 3) and (3) and (4). Pure water was used as the coupling fluid, and fused silica glass slides as the substrate. From (5) and (6), the density and bulk modulus of the material can then be calculated.

The optimum focus location and stability of the calculations was determined by calculating the mechanical properties through the entire $V(z)$ curve, then compared to actual values provided by the company [13]. The % error between the measured and actual values were calculated and were lowest in the region z = 15 to 25 μm, where the echoes from the three interfaces overlap (see fig. 3).

Measurements were repeated five times on the same piece of PVDF, and the average and standard deviation was

TABLE I
PVDF Calibration Results

Property	Given Value[13]	Calculated Value
Thickness (μm)	9	8.6 ± 0.4
Sound Velocity (m/s)	2200	2211 ± 59
Acoustic Impedance (MRayls)	3.92	3.95 ± 0.09
Density (kg/m^3)	1780	1786 ± 80
Bulk Modulus (GPa)	8.5	8.7 ± 0.2
Attenuation (dB/cm/MHz)	-	6.9 ± 1.9

A comparison of the mechanical properties of PVDF calculated to the values given from the manufacturer.

calculated, as shown in table I.

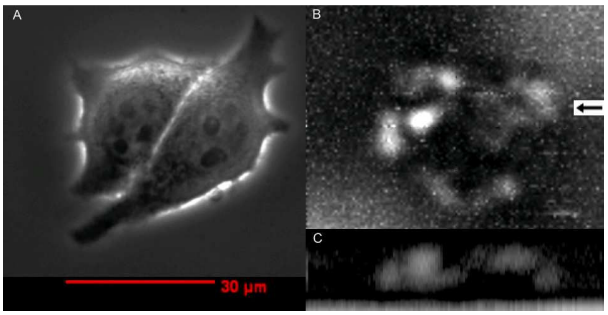

Fig. 4. A) Optical image with phase contrast. B) Ultrasound C-scan backscatter image from the cellular region. C) Ultrasound B-scan image. The substrate and cellular membrane are clearly visible.

B. Cells

MCF7 cells were scanned using the 375 MHz transducer. Optical images were taken before and after the acoustic measurements to ensure that cells did not move from the field of view. The optical and acoustic (C-scan) images are shown in fig. 4. Also shown is a B-scan, a cross section through the cell as indicated by the arrow. The cellular membrane is visible above the substrate, however discontinuous. Areas of strong backscatter signals were chosen for analysis.

The results were analyzed in a similar manner as the PVDF. The region with overlapping amplitudes of the $V(z)$ signals was used to calculate the mechanical properties of the cells. Table II shows the mechanical properties calculated for four different cells. The cells chosen for these calculations were in small clusters consisting of two or three cells.

TABLE II
MCF7 CELL CALCULATION RESULTS

Property	Cell 1	Cell 2	Cell 3	Cell 4
Thickness (µm)	16.1	11.9	12.4	11.5
Sound Velocity (m/s)	1613	1574	1557	1585
Acoustic Impedance (*MRayls*)	1.60	1.60	1.55	1.58
Density (kg/m^3)	994	1016	994	996
Bulk Modulus (GPa)	2.59	2.52	2.41	2.50
Attenuation (dB/cm/MHz)	1.56	1.43	1.79	1.56

The mechanical properties of MCF7 cells measured using time resolved acoustic microscopy.

V. DISCUSSION

As shown in table I, using PVDF as a calibration tool validated the technique and methodology outlined in this paper. The measured values were within 4.5% of the values from the manufacturer. It should be noted however that it is unknown what the tolerance on the given values are. The attenuation of PVDF was calculated but not compared to any known values measured at high frequencies.

Table II summarizes the calculations for four different MCF7 cells measured at 375 MHz. This is the first time all these properties have been measured together using one method. In all cases, the speed of sound, acoustic impedance, density and bulk modulus in the cellular region were slightly above that of the medium. These values are in agreement with published data using other cell lines, which measured a sound velocity of 1534.5 m/s in Hela cells [12] and 1571 m/s in human aortic heart muscle [14] using acoustic microscopy. A lack of published data on individual cell properties prevent a comparison of the other parameters.

Knowledge of these values as a function of the state of the cell will allow us to model ultrasound scattering from cells and cell ensembles. These values are required as input to theoretical models of scattering developed in our laboratory [15]. Finally, the acoustic microscope can be used in the study of molecular imaging and the dynamics of how ultrasound contrast agents interact with sound-fields when attached to a cell.

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