BIOMEDICAL ACOUSTIC MICROSCOPY: RECENT RESULTS ON LIVING CELLS AND TISSUE

B. R. Tittmann
Department of Engineering Science and Mechanics, The Pennsylvania State University at University Park, USA

Abstract
Recent progress on the use of hypersonic microscopy has dramatically advanced the technique so as to allow the scanning acoustic microscope (SAM) as a viable tool for both imaging and quantitative data acquisition. Key features of the SAM for biomedical materials are in-vivo and interior visualizations. This article presents recent results with the SAM for the biomedical materials in our laboratory. First, imaging was accomplished by attenuation mapping of living cells with frequencies up to 1 GHz. Second, internal cellular structures of thickly sectioned tissues were visualized at 200 MHz. Third, with the method used an iterative procedure of matching calculations with measurements on thinly sectioned tissue mounted on a glass substrate was demonstrated. The longitudinal velocity was obtained from the acoustic signature of the material (V(z) curve). The results demonstrated the usefulness of the SAM as a quantitative and qualitative diagnostic tool.

Introduction
This article presents the use of ultrasound to image the cells in-vivo, non-destructively and in real time. An acoustic image is formed by reflected ultrasonic waves which are based on the elastic properties of the living cells and/or the tissues. Therefore, staining is not required for mechanical scanning acoustic reflection microscopy (hereinafter called simply “SAM”) [1]. Hence, living cells and tissues can be easily observed. Furthermore, SAM can observe not only the surface but also the internal structure of the specimen with sub-micrometer resolution. SAM also has capabilities for measuring the mechanical properties (e.g., attenuation, thickness, and velocity) of the cells and/or tissues [2-22]. This article presents the behavior of living cells and tissues using SAM. The results are compared with those obtained with laser scanning confocal and scanning electron microscopes.

In-vivo Observation of Living Cells with Elevated Temperature
During exposure of living healthy cells to gradual increases in heat, the cells typically undergo several stages before their death: (1) shrinkage of the cells; (2) decrease of the contact area of the cells and the substrate; (3) lift-off; and finally, (4) death as manifested as floating away. The lift-off cells were found to be badly injured, and statistically never recovered. Therefore, to determine the start for the lift-off was judged a convenient threshold between permanently injured and recovering living cells.

Biological cells and tissues have acoustic impedances close to those of the culture liquid and there is virtually no contrast caused by the difference in reflection coefficients. However, the contrast in the acoustic images can be generated from the difference in attenuation. When using a background composed of highly reflective material, the difference in attenuation of the biological specimens can be maximized in the image. This method is useful, when operating SAM with a frequency of 1.0 GHz or more. Sapphire with an acoustic impedance of 44.3 kg/m²×10⁶ was selected to be the substrate acting as background [3]. When operating SAM with frequencies ranging from 200 to 600 MHz for visualizing the biological cells and tissues, a glass slide, made of silica glass, commonly used for a conventional optical microscope, was selected as the substrate. SAM can also shed light on the adhesive condition between the cells and the substrate.

The changes in the behavior of the living cells due to the elevation of temperature in the culturing medium are shown in Figure 1. SAM images show clear effects of increasing temperature, from 37.5 to 44.5°C in terms of shrinkage, lift-off, and death.
Figure 1: SAM images show clear effects of increasing temperature, from 37.5°C to 44.5°C. The image of shrinkage, deterioration, and death of human skin cells are obtained. (a) The image of healthy cells at 37.5°C; (b) The image of the shrank and lifted off cells at 42.5°C; (c) The image of the cells, wherein some dead cells moved from the group at 44.5°C. The images were formed with frequency at 1.0 GHz. The acoustic lens was defocused at z = -1.6 μm. The bar is 50 μm.

Observation of Cellular Structure of Thick Tissue

Observation of the internal structure of the thick sections of biological tissues with the SAM is relatively difficult because we cannot apply the attenuation mapping technique with high impedance substrate to the thick specimen. Considering the previous work [20], SAM with frequency around 200 MHz may allow appropriate resolution and penetration depth to visualize a thickly sliced specimen. Therefore, in this study, the SAM with frequency at 200 MHz was used to obtain a horizontal cross-sectional image (i.e., C-scan image) of the subsurface of thick specimens sectioned from human small intestine and esophagus.

Figures 2 shows the image obtained by scanning electron microscopy (hereinafter simply “SEM”), and Figures 3(a) and 3(b) are images obtained by SAM. These images show thinly sliced human small intestine and esophagus. The images obtained by OM and SEM were compared with the acoustic images for better understandings of cellular structure. The acoustic images clearly show that the acoustic lens with frequency at 200 MHz can visualize the microstructures (e.g., goblet cells) of the specimens. Figures 4(a) and 4(b) are images obtained by SAM, and clearly show the microstructure of thickly sliced human small intestine and esophagus.

Figure 2: Images obtained by Scanning Electron Microscope: Esophagus, Thickness of the specimen: 3μm
Ever since the advent of SAM, a key objective has been quantitative data acquisition besides the enhancement of the resolution in the acoustic images. For quantitative data acquisition with the SAM, the V(z) curve techniques have been developed and applied to various materials with fruitful results [18]. With the V(z) curve technique, we can obtain the velocity of the surface acoustic wave (e.g., Rayleigh wave) of the small area of the specimen. However, it is not easy to characterize biological tissues by the V(z) curve technique. First, the critical angle for the Rayleigh wave generation in bio-medial materials is generally high. Therefore, the Rayleigh wave is often not generated within a specimen, even though an acoustic lens having a high numerical aperture (e.g., 120°) is used. It means that no V(z) curve is formed. Second, the relative attenuation of biomedical materials is generally high. Therefore, the V(z) curve may not have enough oscillations for the Fast Fourier Transformation (hereinafter called simply “FFT”) analysis to measure the Rayleigh wave velocities accurately, even though the Rayleigh wave may be generated. However, the attenuation is typically frequency dependent. When an acoustic lens, having a high numerical aperture and a long working distance, with a low frequency (e.g., 10 MHz or less) is used for the biomedical materials, a V(z) curve might be formed. However, in this case, an advantage of the V(z) curve technique for characterizing a small area of the materials is lost. There may be an appropriate solution for applying the V(z) curve techniques to the tissue when the tissue is treated as a thin film coated on an isotropic substrate (e.g., sapphire, fused quartz, silica glass, or the like). Then, the reflectance function can be obtained by using the theory of ultrasonic propagation in layered media. Using the reflectance function, the V(z) curve for the thin bio-medical material mounted onto the substrate can be simulated. In the simulation, only the velocity of the longitudinal wave of the material is set by estimation. The actual velocity of the longitudinal wave of the material can be obtained by matching the V(z) curves obtained from the experiment in an iterative procedure.

A human kidney was chosen as biomedical tissue. The kidney was preserved in formalin, and then wrapped with paraffin. The wrapped specimen was sliced by a microtome. The thickness of the sliced specimen was 3 µm. The sliced specimen was located on the fused quartz glass. The positions for applying the V(z) curve technique were determined by the spherical acoustic lens (see Figure 5).

Longitudinal Wave Velocity Measurement for Thin Tissue

Ever since the advent of SAM, a key objective has been quantitative data acquisition besides the
Applying the V(z) curve technique to the points A and B, the surface acoustic wave velocities were measured as 3351 m/sec and 3277 m/sec respectively. Changing the values of the longitudinal wave velocity, the simulations were continued until the velocities of the surface acoustic waves coincided substantially with the experimental values. The longitudinal wave velocities were obtained as 1820 m/sec and 1765 m/sec, respectively. Those values are large compared to the longitudinal wave velocity obtained from the literature (1560 m/sec).

The network analyzer (Agilent Technologies; model: 8753D) was used to confirm the stability of the frequency. The specimen was located on the bottom of the glass well containing the coupling medium (i.e., distilled water). The temperature of the coupling medium was substantially kept at 20°C, and monitored by the thermocouple. The corresponding velocity of water was 1438 m/sec. The distance of movement of the acoustic lens along the Z-axis was monitored. The error caused by the non-linearity of the movement is statistically reduced by repeating the experiment. The SAW velocity was obtained from the V(z) curve through a FFT treatment. The surface acoustic velocities of fused quartz obtained by the V(z) curve in the experiments, the V(z) curve obtained by the simulation, and literature were 3443 m/sec, 3434 m/sec, and 3430 m/sec, respectively. Both experimental and simulated values are close enough for accuracy. Therefore, differences of the longitudinal velocities obtained in the experiments and the simulations were considered to stem from the conditions of the specimen.

Conclusions

In-vivo images observed with frequencies at 0.6 GHz and 1.0 GHz by SAM show clear effects (i.e., from shrinkage to lift-off) of increasing temperature. SAM images show that cells subjected to mechanical insult had little physical deformation or shrinkage and were damaged to the point of complete lift off. Scanning acoustic microscopy was found to be a useful tool for visualizing injury to skin cells.

An acoustic lens with frequency at 200 MHz permitted the imaging of surfaces and/or subsurfaces of microstructures of thickly sliced tissues. These images can be used by pathologists for diagnosing certain medical problems. The penetration depth of the acoustic beam with frequency at 200 MHz in the tissue is less than 200µm. Concerning lateral resolution, practically the penetration depth of the SAM with frequency at 200 MHz is less than 50µm. These data may be useful when developing an in vivo medical acoustic imaging device.

V(z) curves for the kidney/fused quartz specimen were simulated by developing a software program. The procedure of obtaining the longitudinal wave velocity of a thin bio-medical tissue was developed by using the V(z) curve technique. Longitudinal wave velocities of kidney tissue were obtained by the above procedure.

References

6. J. Bereiter-Hahn and H. Lüers, “Shape changes and force distribution in...


