Published in final edited form as:

Mater Res Soc Symp Proc. 2011 January ; 1301: . doi:10.1557/opl.2011.572.

Quantifying Micro-mechanical Properties of Soft Biological Tissues with Scanning Acoustic Microscopy

Xuegen Zhao¹, Steven Wilkinson¹, Riaz Akhtar^{1,2}, Michael J Sherratt², Rachel E B Watson³, and Brian Derby¹

¹School of Materials, the University of Manchester, Manchester, M1 7HS, United Kingdom

²School of Biomedicine, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, M13 9PT, United Kingdom

³Dermatological Sciences Research Group, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, M13 9PT, United Kingdom

Abstract

In this study we have established a new approach to more accurately map acoustic wave speed (which is a measure of stiffness) within soft biological tissues at micrometer length scales using scanning acoustic microscopy. By using thin (5 μm thick) histological sections of human skin and porcine cartilage, this method exploits the phase information preserved in the interference between acoustic waves reflected from the substrate surface as well as internal reflections from the acoustic lens. A stack of images were taken with the focus point of acoustic lens positioned at or above the substrate surface, and processed pixel by pixel using custom software developed with LABVIEW and IMAQ (National Instruments) to extract phase information. Scanning parameters, such as acoustic wave frequency and gate position were optimized to get reasonable phase and lateral resolution. The contribution from substrate inclination or uneven scanning surface was removed prior to further processing. The wave attenuation was also obtained from these images.

INTRODUCTION

Age or disease related changes in the mechanical properties of dynamic tissues such as lungs, skin, blood vessels and heart, have a profound impact on human morbidity and mortality. Global demographic shifts towards an ageing population will therefore further exacerbate the incidence of mechanical failures within the cardio-respiratory and musculoskeletal systems. However, detailed knowledge of the micro-mechanical properties of both healthy and diseased soft tissues is currently unavailable. Although acoustic methods have been used extensively to determine the bulk mechanical properties of biological tissues, little is known about their mechanical properties at microscopic length scales.

Scanning acoustic microscopy (SAM) [1] uses ultra-high frequency sound vibrations to image materials, with the contrast related to the stiffness, density, thickness and attenuation of the sample. As the acoustic beam is formed by converging propagating waves, the size of the focal spot is limited by diffraction. The use of higher frequency excitation provides higher spatial resolution but reduces signal level dramatically due to attenuation. When operated at 1GHz, the spatial resolution of the SAM is about 1 μm , which is considerably better than what is currently achievable through nanoindentation [2] and makes SAM potentially a high spatial resolution tool for the study of the micromechanical properties of biological tissue. Furthermore, SAM imaging is both non-destructive and occurs in a hydrated environment, thereby facilitating the imaging of native tissue structures.

However, to date, the vast majority of SAM investigations of biological material have been conducted on stiff, calcified tissues [1, 3-6]. In addition, most prior studies on soft tissues were conducted in the frequency range of 100-500 MHz [7-11], limiting the spatial resolution achievable. There are few reported studies carried out on unfixed soft biological tissue sections and there is currently no ideal method to relate the high resolution acoustic information to the quantitative mechanical properties of the soft tissues. The authors have previously used the $V(f)$ method (recording of voltage output vs. acoustic frequency) [12], developed by Kundu et al [13], which was thought suitable for the study of soft biological tissues because it is based on the interference between different reflections from a layered media. However, the sound waves reflected from the specimen also interfere with the stray echoes inside the lens, and this interference produces a voltage signal which is strongly dependent on acoustic frequency and the distance between the lens and the specimen. As a consequence, the $V(f)$ method may not be the most suitable approach for soft tissues. Fortunately, the interference between the stray echoes and the reflections from the specimen preserves phase (timing) information, and in this paper we make use of such interference to determine the acoustic wave speed as well as attenuation in tissues.

EXPERIMENT

Histological cryo-sections of human skin (prepared from a 6 mm diameter punch biopsy excised from a photoprotected (buttock) skin of a 35 year old female volunteer), and pig cartilage (~24 week old piglets) from the forelimb were used in this study. The samples were embedded in Optimal Cutting Temperature (OCT) medium (Sakura Finetek Europe B.V., The Netherlands), snap-frozen in liquid N₂ and immediately stored at -80°C pending cryo-sectioning. Frozen, OCT-embedded specimens were secured to cryostat chucks with OCT and sectioned to nominal thicknesses of 5 µm. The samples were mounted on glass slides.

The SAM used in this investigation was a SAM2000 (PVA Tepla, Heborn, Germany) with a frequency range from 0.1 to 2.0 GHz. Its acoustic focusing system (lens) uses a plano-concave design consisting of a sapphire rod with zinc oxide piezoelectric film deposited on one end as the transducer and a spherical cavity on the other end as an acoustic lens. For the 1 GHz high resolution lens used in this investigation, the cavity has an opening of 80 µm in diameter and a wide opening angle of 100°. The specimen slide was placed on a horizontal stage and scanned by the lens. Fluid (normally distilled water) provides acoustic coupling between the acoustic lens and the specimen.

The SAM was used in continuous mode. An acoustic wave was generated by the transducer when an electric tone-burst signal with duration of 20 ns was applied. This acoustic wave travels through the sapphire buffer rod and is focused by the acoustic lens. The focused acoustic beam propagates through the coupling fluid and the specimen, before reaching the slide. Reflections occur at the interfaces of lens/medium, medium/tissue, and tissue/slide due to mismatch of acoustic impedances, as indicated in Figure 1. The time delay and amplitude of the reflections provide information about the elastic properties and attenuation of sound in the specimen. The reflected waves were received by the same transducer and converted into electric signals. After passing through a series of amplifiers and detectors, these electric signals produce a single voltage signal proportional to the amplitude of the reflection. The lens was scanned horizontally in X-Y dimensions by a pair of oscillator coil drives to produce C-mode two-dimensional 512×512 pixel images with a scan area 200×200 µm.

The lens was first focused at the surface of the glass slide by monitoring the maximum output of the received signal. The lens was then raised 4 µm and a stack of images were taken at different z-positions starting from this height and towards the slide surface with a

step-size of 0.1 μm over a range of 5 μm . Scanning parameters, such as acoustic wave frequency and gate position were optimized to get reasonably good signal level and lateral resolution.

The images were processed with custom software developed with LABVIEW and IMAQ (National Instruments). The gray scale value of a pixel (x, y position) was extracted from all the images to form a $V(z)$ curve. The $V(z)$ data were tapered with a Hanning window followed with Fast Fourier Transformation (FFT). The single frequency corresponding to the maximum amplitude was chosen and thus phase value determined [14]. The obtained phase image was then used for calculating sound speed in tissue. To determine the strength of transmission (inverse of attenuation) of the acoustic wave going through the specimen, the maximum of the $V(z)$ curve was used as the sum of all reflections and the average of the weakest $V(z)$ curve where the amplitude of oscillation is lower than its average, was taken as the contribution of in-lens echo.

DISCUSSION

Figure 2 shows two $V(z)$ curves extracted at different pixel positions from a stack of C-mode images of a skin sample. Periodic oscillations together with smooth backgrounds are evident for both the substrate and the epidermis. For the $V(z)$ curve of substrate, the background represents the reflection from the slide surface while the oscillation indicates the existence of in-lens reverberations.

When the acoustic beam passes through the specimen and is focused at the slide surface, the reflected wave reaches its maximum amplitude while the one from soft biological tissue surface is relatively weak and normally negligible. However there are also reverberations within the lens itself, as shown in Figure 1. Such waves can travel between the acoustic lens and zinc oxide film many times due to the low attenuation within the sapphire buffer rod and generate a sequence of electric pulse by the transducer. If the reflection from glass slide surface and any of these in-lens echoes reach the transducer film at the same time, interference occurs.

Assuming the reflections are expressed as $Ae^{i\omega t}$ for the in-lens echo and $Be^{i(\omega t + \varphi)}$ from any of the interfaces, the final wave reaches the transducer is $Ae^{i\omega t} + Be^{i(\omega t + \varphi)} = Ce^{i(\omega t + \gamma)}$ with

$\gamma = \tan^{-1} \frac{A+B \cos \varphi}{B \sin \varphi}$ as the new phase and $C = \sqrt{A^2 + B^2 + 2AB \cos(\varphi)}$ as the new amplitude which is clearly a function of the phase difference φ . $C=A+B$ when the phase difference is $n\pi$, or $A-B$ when the phase difference is $n*2\pi$. Normally when the exciting frequency and delay-time window are selected, the phase and amplitude of the in-lens echo will no longer change. However the amplitude B of the reflected wave is a function of attenuation and consequently the phase φ cannot be obtained directly from the detected amplitude C without knowing B . To overcome this problem, the lens can be moved along the z dimension, and as phase φ is a function of lens position with $\Delta\varphi = 2\pi * 2\Delta z / \lambda_{\text{water}}$, a periodic oscillation in the recorded voltage signals can be easily obtained with such a $V(z)$ scan (recording of output voltage vs. defocus distance, z).

However, when the focus of the acoustic beam is far away from the substrate surface or the attenuation of the specimen is high, reflections from the substrate surface and specimen surface could be comparable in amplitude and interfere when overlapped in time domain, which makes the $V(z)$ curve more complicated. For biological tissue characterization, only the interference between the reflection from the substrate surface and the in-lens reverberation is desired for calculating sound speed. To minimize the reflection from the specimen surface, firstly an adjustable time-delay gate with 20 ns time window can be used

to select the time specific waveform to be processed, i.e. mostly the reflection from the substrate surface, as these two reflections reach the transducer with a difference in time; and secondly the acoustic lens is positioned with the focus point under the specimen surface and near to the substrate surface, which differs from a standard $V(z)$ scan where the focus of acoustic lens is placed deeply into the substrate to make use the excited surface waves.

After processing the images pixel by pixel, a 2-D phase array is recovered and a gray scale image is generated, as shown in Figure 3. Due to the intrinsic properties of FFT, the recovered phase is limited to within $\pm\pi$, which introduces discontinuities into the image when the actual phase is beyond this limit. In order to unwrap these discontinuities, an appropriate integer multiple of 2π is added to each pixel element of the recovered phase map. The contribution from an inclined substrate, an uneven scanning plane, or both, is removed prior to further processing.

Figure 3 shows example images obtained from sections of human skin and porcine cartilage. The three main histological layers (dermis, epidermis and the outermost cornified layer) can be distinguished clearly in the skin images. The images of cartilage sample also demonstrate the capability of high resolution quantitative mapping.

Denoting the interference wave length as $\lambda_{interference}$ and the interference phase of the reflection wave from the slide surface as φ_{sub} , the interference phase from interface between the tissue and the substrate as φ_{tissue} , the following relations hold:

$$\lambda_{interference} = c_{water} / (2f) \quad 1$$

$$\varphi_{tissue} - \varphi_{sub} = 2\pi f (2d/c_{water} - 2d/c_{tissue}) \quad 2$$

where d is the tissue thickness, c_{water} and c_{tissue} are the sound speeds in water and tissue respectively, and f is the acoustic wave frequency. Finally, the sound speed in the tissue can be calculated from equations 1 and 2, as demonstrated in Figure 4 for skin sample, where sound speeds along a line drawn traversing the different histological layers are calculated and an x-z scan image has been reconstructed for comparison from the image stack. The cornified layer has the highest sound speed ($>1900 \text{ ms}^{-1}$) whereas in the epidermis sound speed is around 1650 ms^{-1} .

CONCLUSIONS

We report a new SAM method that can be used to determine micron-scale variations in soft biological tissues and demonstrate its application on cartilage and skin. The method uses thin ($5 \mu\text{m}$ thick) histological sections and exploits the phase information preserved in the interference between acoustic waves reflected from the substrate surface as well as internal reflections from the acoustic lens.

Acknowledgments

The authors are grateful to the Wellcome Trust, Research into Ageing / Age UK and the British Heart Foundation for funding. Mr Thomas Howard and Dr Abigail Langton must be thanked for providing the cartilage and skin samples respectively.

REFERENCES

1. Briggs, A. Acoustic Microscopy. Clarendon Press; Oxford: 1992.
2. Akhtar R, Schwarzer N, Sherratt MJ, Watson REB, Graham HK, Trafford AW, Mummery PM, Derby B. J. Mater. Res. 2009; 24:638. [PubMed: 20396607]

3. Hasegawa K, Turner CH, Recker RR, Wu E, Burr BB. Bone. 1995; 16:85. [PubMed: 7742089]
4. Litniewski J. Ultrasound Med. Biol. 2005; 31:1361. [PubMed: 16223639]
5. Raum K, Kempf K, Hein HJ, Schubert J, Maurer P. Dent. Mater. 2007; 23:1221. [PubMed: 17178152]
6. Saied AA, Raum K, Leguerney I, Laugier P. Bone. 2008; 43:187. [PubMed: 18407822]
7. Daft CM, Briggs GA. J. Acoust. Soc. Am. 1989; 85:416. [PubMed: 2921421]
8. Jørgensen CS, Assentoft JE, Knauss D, Gregersen H, Briggs GAD. Ann. Biomed. Eng. 2001; 29:1059. [PubMed: 11853256]
9. Hattori K, Sano H, Saijo Y, Kita A, Hatori M, Kokubun S, Eiji E. J. Pediatr. Orthop. 2007; B16:357.
10. Saijo Y, Hozumi N, Lee C, Nagao M, Kobayashi K, Oakada N, Tanaka N, dos Santos Filho E, Sasaki H, Tanaka M, Yambe T. Ultrasonics. 2006; 44:e51. [PubMed: 16844175]
11. Hitomi H, Kiyomoto H, Hashimoto M, Aki Y, Uchida K, Takahashi N, Fukunaga M, Mizushige K, Senda S, Sakamoto H, Matsuo H, Yuasa S. Ultrasound Med. Biol. 2000; 26:571. [PubMed: 10856619]
12. Akhtar R, Sherratt MJ, Watson REB, Kundu T, Derby B. Mater. Res. Soc. Symp. Proc. 2009; 1132E:Z03.
13. Kundu T, Bereiter-Hahn J, Karl I. Biophys. J. 2000; 78:2270. [PubMed: 10777725]
14. Grandke T. IEEE Trans Instrum. Meas. 1983; IM-32:350.

Figure 1.

Schematic diagram showing reflections from acoustic lens/medium, specimen/medium, specimen/substrate, and medium/substrate interfaces when a sound wave is generated and propagates through acoustic lens, medium and specimen.

Figure 2. Illustration of $V(z)$ curves extracted from an image stack and taken at different z-positions for a skin sample.

Figure 3.

Images of a skin sample (3.1) and a cartilage sample (3.2), image size 200×200 μm, taken at 1GHz. a) typical image from $V(z)$ stack; b) strength of reflection from substrates (transmission); c) recovered phase image; d) unwrapped phase image.

Figure 4. Line profile in (a) across dermis (D), epidermis (E), cornified layer (C) and substrate (S) and the corresponding sound speed variations (b). Inset: reconstructed $V(x, z)$ image.