FREQUENCY DEPENDENCE OF TISSUE ATTENUATION MEASURED BY ACOUSTIC MICROSCOPY

C. M. W. Daft[1], G. A. D. Briggs[2], and W. D. O'Brien, Jr.[1]

[1]Bioacoustics Laboratory, University of Illinois
[2]Department of Metallurgy and Science of Materials, Oxford University, UK

ABSTRACT

A method of acoustic time domain reflectometry has been developed which allows the elastic properties of a histological section to be measured with a resolution of around 8 μm [1,2]. This is accomplished by exciting the lens of an acoustic microscope with a voltage impulse from a step recovery diode. The impulse response of the lens used provided sufficient time resolution to separate the reflections from the top and bottom surfaces of a 10-15 μm section. This A-scan can be analyzed to obtain the elastic properties of the interrogated point.

In this paper, the data from the experiment is processed so that the frequency dependence of the tissue attenuation is revealed. Comparisons are also made between SAM and SLAM data of similar tissue samples.

I. EXPERIMENTAL PROCEDURE

The lower leg muscles of an adult ICR mouse were dissected and frozen in liquid nitrogen. 60 μm frozen sections were cut transverse to the fiber direction using a Lipshaw cryostat for examination with the SLAM; adjacent 14 μm frozen sections were cut from the same block for investigation in the SAM. These sections were mounted unfixed on glass slides, and imaged in pseudo-transmission using a reflection microscope.

Leg muscle is striated, and is composed of cylindrical fibers which vary from 1 mm to 40 mm in length, and 10 μm to 100 μm in width. These fibers arise from the fusion of many myoblast cells, and are multinucleate. Each fiber is surrounded by a fine network of collagen, the endomysium. The complete muscle is enclosed by a connective tissue layer, the epimysium, which holds the muscle together. This layer contains collagen fibers, which have great tensile strength, because of the way their principal aminoacids (hydroxylysine and hydroxyproline) polymerise. Collagen has a static Young modulus 1000 times greater than most other types of tissue. In the epimysium, the fibers are interwoven into sheets. Individual fiber bundles are 1-12 μm in diameter.

II. RESULTS

Figure 1a shows a toneburst scanning acoustic microscope image obtained with the lens focused on the surface of the microscope slide, at a frequency of 425 MHz and a field of view of 425 μm. Fibres of both the soleus and gastrocnemius muscles are visible, as is the epimysium. Figure 1b is a time resolved linetrace taken as the lens moves along a line corresponding to the vertical bisector of Figure 1a (the dashed line). The scan begins at the top of the SAM image, but is 600 μm long. The horizontal axis of this image is time, with a total scan of 50 ns. The vertical axis corresponds to the position of the lens. Picture brightness is proportional to detected voltage, so the no-signal level is grey and received signals appear as a succession of black and white stripes. Two pulses are generally visible; a tissue/glass echo to the right, and a weaker tissue/water echo on the left. The black region of the SAM image in Figure 1a is connective tissue between the two muscles; it causes a large shift to the left in the second pulse on the time resolved linescan. This connective tissue is visible at lower magnification in Figure 1c, which is an optical micrograph of the adjacent frozen section cut. The tissue section is stained with toluidene blue and has a field of view of 2.8 mm. The effect of applying a Wiener filter to the data is shown in Figure 1d; the use of the filter sharpens up the time resolution of the echoes [2].

The time resolved data were analyzed by the method described in [2]. Large variations were observed in the sample thickness; a nominally 14 μm section varies from 6.2 μm to 17.5 μm. The velocity figures show typical tissue values of 1550-1600 ms^{-1} for the muscle fibers, whilst the connective tissue has a peak of slightly over 2000 ms^{-1}. The impedance is also considerably larger in the connective tissue. The mean value of the frequency averaged attenuation coefficient is 86 dB mm^{-1}, which

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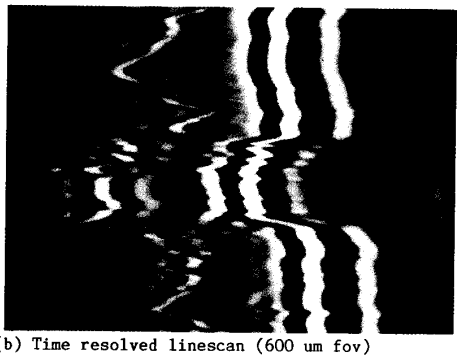
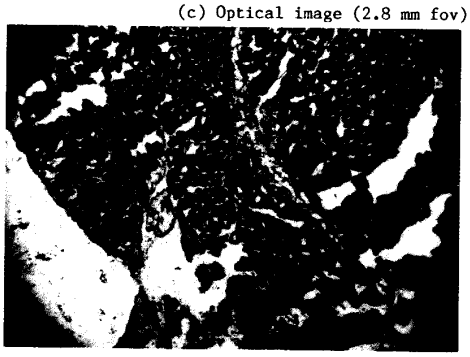
(a) SAM image (425 um fov)



(b) Time resolved linescan (600 um fov)

(c) Optical image (2.8 mm fov)



(d) Wiener filtered linescan (600 um fov)

Figure 1

Images of transverse section of mouse muscle.

(fov = field of view)

The widths of (b) and (d) are 50 ns. The vertical axes correspond to distance along the dotted line of (a).

Figure 2 Deconvolved power versus frequency

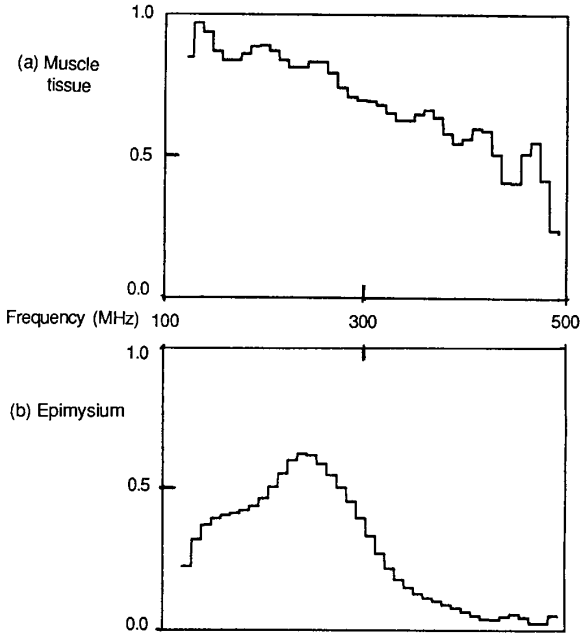
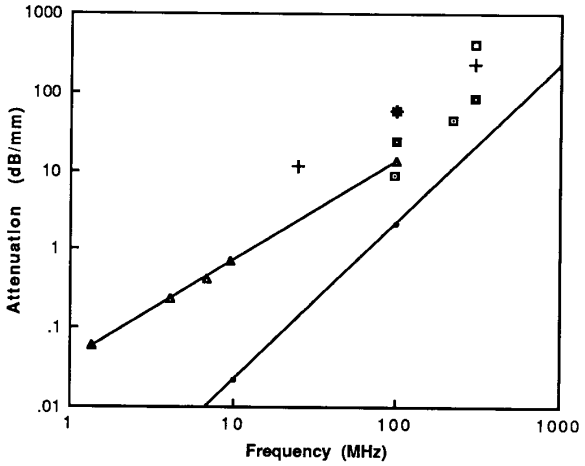


Figure 3 Comparison of SAM results with other techniques



- Kidney
- Liver
- Muscle
- Epimysium
- Skin
- Water

Liver and water data (the two lines) are included for reference. The highest epimysium value is not comparable with the other points (see text).

may be compared with the figure of 45 dB mm^{-1} obtained in kidney tissue at 222 MHz [3]. However, the peak value of attenuation coefficient in the epimysium are much higher, at 400 dB mm^{-1}, reflecting the anomalous behavior of the structural protein collagen.

Comparisons were made between these results and SLAM images. The SLAM signal processing of [4] was employed. The velocity and attenuation data (obtained from sections cut from the same tissue block) support the trends observed in the SAM. However, the range of elastic properties observed was more restricted, possibly due the lower resolution of the 100 MHz SLAM, and the thicker samples (60 μm rather than 14 μm).

The fact that the time resolution experiment can provide values for the attenuation over more than two octaves of hitherto unexplored spectrum is perhaps its major advantage. It can be seen from Figure 1b that the tissue/water and tissue/glass pulses are widely enough separated that the error involved in isolating the second signal in order to compute its Fourier transform will be small. This transform was then deconvolved with a reference signal obtained with the lens the same distance from the glass substrate, but without tissue. It can be shown [5] that this method is unaffected by changes in the lens' focal length across the frequency range. However, both the effects of velocity dispersion and the loss of energy at the tissue/water interface have been neglected. For most soft tissues this causes a very small error.

Figure 2a shows a typical muscle spectrum, averaged over 100 measurements, where the attenuation is roughly proportional to frequency. When a similar spectrum was calculated from data from a fat cell, the attenuation [6] was found to vary as $f^{1.66}$. This is the same as the accepted value for castor oil [7]. In contrast to this, very little signal is observed at low frequencies from the collagen in the middle of vertical bisector of Figure 1a. Instead, the signal rises to a maximum at around 240 MHz, and then decreases sharply (Figure 2b). The two systematic errors mentioned earlier cause this peak to be underestimated [5]. This behavior was observed throughout the middle of the connective tissue area, but not at its edges.

A peak in the received signal implies that some structure in the tissue is resonating. Since the sound velocity in this area is 1900-2000 ms^{-1}, the size of the resonator may be around 8 μm. A tentative hypothesis to explain this effect would be that there are one or more $\lambda/2$ = 4 μm collagen fibers inside the section at this point. The deconvolved spectrum of the first (tissue/water) pulse was also computed. The reflection from the collagen is again very strong, and a peak in the spectrum at 240 MHz was again observed, confirming the resonance in the second pulse. The resonance at this frequency is not a feature of collagenous tissue in general; other muscle boundaries were investigated in the same way, and different behavior was found. The resonances observed at other frequencies would also predict a characteristic dimension in the correct range for collagen fibers (1-12 μm).

III. COMPARISONS WITH PUBLISHED DATA

Figure 3 shows selected results on the frequency dependence of attenuation for five types of tissue. Data from the SAM have been averaged over the 100 to 500 MHz range, and are recorded at 300 MHz. The skin data from the SAM measurements were obtained by averaging the received signal through the dermis of a guinea pig [2]. At 100 MHz, the result is a mean value from 60 measurements on canine skin [9], whilst the 25 MHz result from the same publication was obtained from a backscattering technique [10]. The two points recorded for epimysium are not directly comparable; the 100 MHz (SLAM) measurement is a mean value for the complete muscle boundary, averaged over 10 specimens. This tissue appears to be very inhomogeneous in the higher resolution SAM results. The peak value is therefore recorded at 300 MHz on the graph. The images of the muscle fibers revealed milder variations in attenuation, and were also plotted at 100 and 300 MHz. For reference, the attenuation of water and liver [11] are included. Liver is an example of typical parenchyma, with a relatively low attenuation which is proportional to $f^{1.27}$, whereas the value for water is proportional to f^2.

It seems reasonable to suppose that tissue must always attenuate sound more than water; thus at some frequency the slope of the line drawn through the liver data must increase. Kessler [3] observed such a change in mammalian kidney (his data are shown on the graph) which contrasted with the linear dependence at lower frequencies [12]. In the present work, the behavior of both skin and muscle seem similar in the 100-300 MHz range to the 1-100 MHz liver data. The intercept of the lines with the attenuation axis varies, (skin attenuates more because of the higher structural protein content), but the slope does not approach the f^2 exhibited by water.

IV. CONCLUSIONS

Perhaps the most interesting aspect of biological acoustic microscopy is the ability of the instrument to produce data on the attenuation over more than two octaves of frequency spectrum. It appears that in tissue with high concentrations of structural protein, the attenuation behaves anomalously. Further work is needed to evalu-

ate the apparent resonances which were characteristic of some of the data. The rate at which the attenuation increases with frequency seems to vary considerably between different types of tissue, when investigated on this scale. This also requires more investigation. However, when the fine structure of the tissue is averaged out, comparisons with lower frequency techniques become possible. Finally, causality requires that an attenuation which depends on frequency is accompanied by variations in the velocity with frequency. In principle, this could be computed from the Kramers-Kronig relations [8]. The present work has not attempted to measure the velocity dispersion.

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