ULTRASONIC VELOCITY SPATIAL DISTRIBUTION ANALYSIS OF BIOLOGICAL MATERIALS WITH THE SCANNING LASER ACOUSTIC MICROSCOPE

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The fundamental examination of biological tissue with ultrasound can lead to important diagnostic capabilities. In order to quantify tissue characteristics with ultrasound, the ultrasonic propagation properties of normal and pathological tissues must be characterized and cataloged. An important ultrasonic property is the speed of sound for characterizing tissue [1]. In this work, the Scanning Laser Acoustic Microscope (SLAM) is used to measure the spatial variation of the speed of sound in tissue, thereby providing a quantitative ultrasonic parameter for tissue characterization.

The SLAM (Sonomicroscope 100, Sonoscan, Inc.) provides three different television type images as shown in Figure 1. A laser scans the lower surface of the coverslip in order to detect mechanical disturbances induced by the 100 MHz ultrasonic energy which has passed through the specimen from below. The optical (laser scan) transmission image (Figure 1a) allows the operator to position the sample in the center of the 2 mm x 3 mm field of view. The acoustic image (Figure 1b) shows the amount of ultrasound energy passing through the sample. This signal is proportional to the envelope of the laser detector output. In this image, dark areas correspond to high areas of ultrasonic attenuation and light areas to low attenuation areas. The third image (Figure 1c), the interference image, is produced by electronically mixing the laser detector output with a 100 MHz reference signal.

The interference image consists of approximately 39 vertical light and dark bands which represent locations of constant phase contours of the ultrasonic wave after it has traversed through the

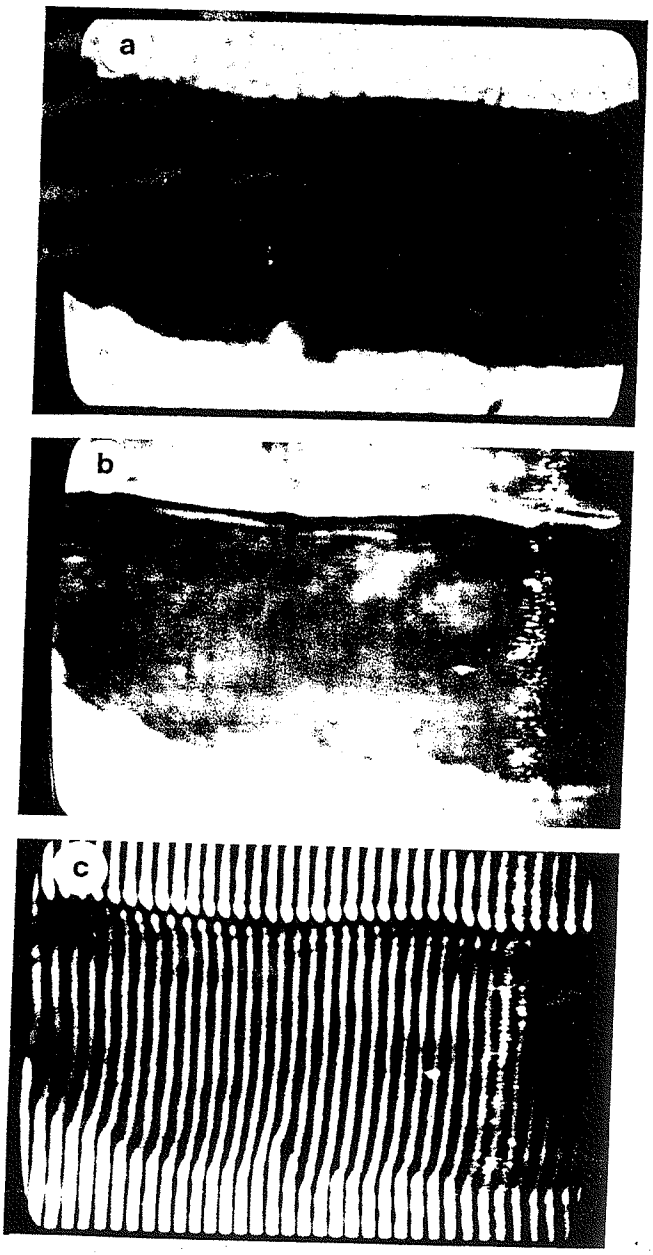


Figure 1. Photographs from the SLAM monitors of a 550 μm thick mouse liver specimen. (a) Optial Image, (b) Acoustic Image, and (c) Interference Image.

specimen. In saline solution (the normal coupling medium for tissue samples) the interference lines are straight and equally spaced. When a slice of tissue (usually 400 to 800 μm thick) is placed in the saline solution, the interference lines shift to the right at the saline-tissue interface indicating the tissue has a higher speed of sound. In an area of tissue where the thickness is constant, the interference lines appearance is somewhat corrigated, that is, they do not appear straight as in the very homogeneous coupling medium. This could represent a microscopic index of refraction gradient in tissue and may represent a source of ultrasonic back scattering for clinical B-scan imaging systems. To quantify this gradient, the interference lines are subjected to an automated analysis technique which is described herein.

THE DATA ACQUISITION SYSTEM

The interference image produced by the SLAM is digitized by a data acquisition system (Figure 2) that was designed and fabricated at the Bioacoustics Research Laboratory [2]. This system consists of a video amplifier, filter, analog to digital converter and high speed buffer memory which interface the SLAM to the Perkin-Elmer 7/32 32 bit mini-computer. The video signal is monitored on an oscilliscope to assure that the signal is using the full dynamic range of the A/D converter.

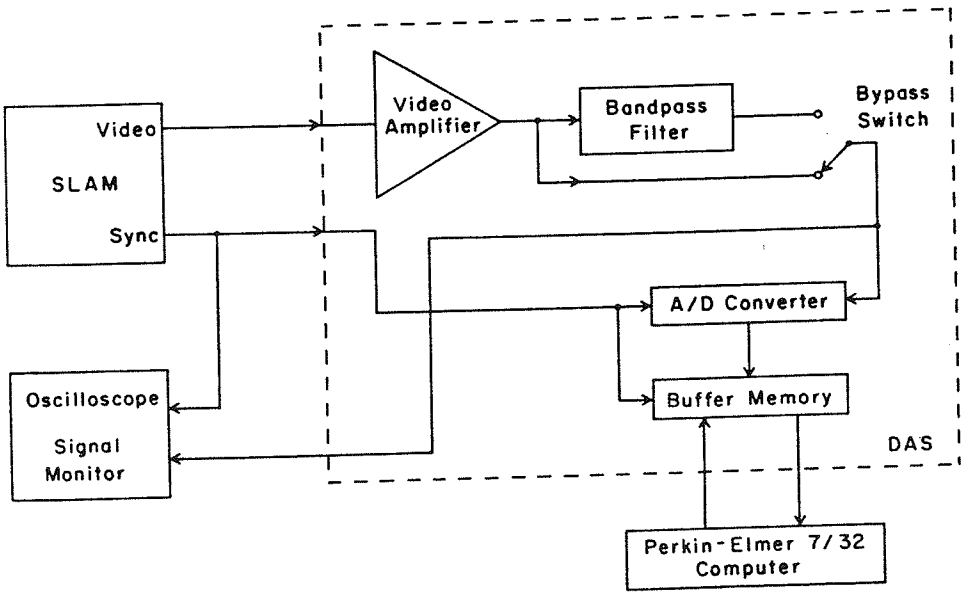


Figure 2. Block diagram of data aquisition system used to digitize the SLAM images.

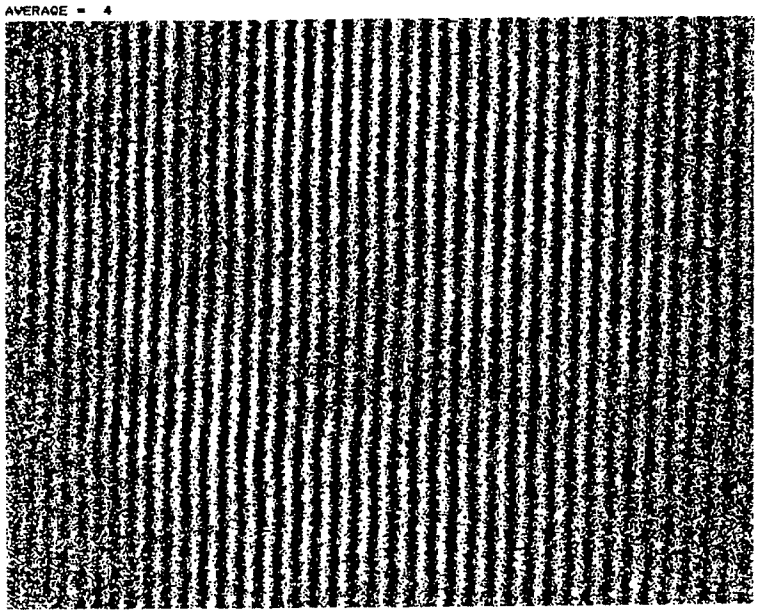
Since the interference image consists of about 39 lines, the video signal is relatively narrow-band for purposes of extracting the interference images. The video portion duration of a raster line is 52.5 μs so the average time between signal peaks is 1.35 μs or an average frequency of 740 kHz. Thus, the signal to noise ratio of the interference image can be improved significantly by the addition of a bandpass filter. A sixth order Chebyshev filter was designed for this purpose. The passband of this filter is between 700 and 900 kHz with a $\pm$ 0.5 dB ripple. The addition of this filter has allowed the speed of sound in tissue specimens with higher attentuation to be measured more accurately.

One raster line (of which there are a total of 482) at a time is digitized at 28.6 MHz (1514 data points per raster line), and stored in the buffer memory (1024 x 16 bits, 35 ns access time). The data are then transferred into the mini-computer memory. This operation is repeated for all 482 lines. Thus, the interference image consists of 729,748 picture elements.

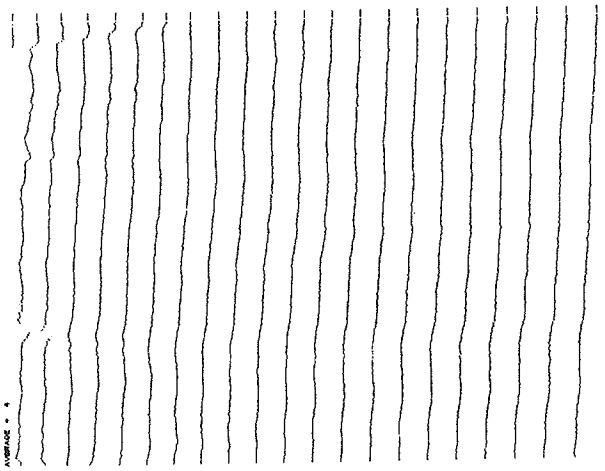
Several programs were developed to enhance the interference image. One program digitizes the raster line data, averages it n times (selectable between 1-16 times), stores the data on magnetic tape, and outputs a printer (Printonix model 300) representation of the interference image, a typical example of which is shown in Figure 3a. The averaging is accomplished by using a running average algorithm which was developed to compensate for slight and somewhat periodic horizontal jitter of the images which is produced by the SLAM.

It would be desirable to store the whole image in memory and provide for frame averaging but 1.5 megabytes of memory would be required. The present computer system has 300 kilobytes. Thus, the averaging must be performed on a block by block basis. The number of raster lines in the block is the same as the desired number of averages. The block of lines are arranged in order of position in the image. The top line of the block is averaged n-1 times, the next raster line is averaged n-2 times, and so on, until the next to the last line in the block is averaged once. The program then digitizes each line once more, adding these to the previous lines. The top line is now averaged n times and is transfered to magnetic tape. The start of the block now moves down one raster line and continues the averaging process until all 482 raster lines have been stored on magnetic tape.

By increasing n, the quality of the resulting image is improved. Unfortunately, the total digitization time is increased. Approximately 20 minutes are required to digitized the data and store a single image on magnetic tape, when four averages are taken. For eight averages the sample time is increased to 25 minutes.



(a)



(b)

Figure 3. Enhanced interference images of a blank field in which the medium was water, that is, no biological tissue specimen was present. The image data were averaged 4 times. (a) The upper image is histogram equalized to improve its contrast for the line printer. (b) The lower image has been enhanced by a correlator receiver technique.

The program then enhances the interference image with a correlated receiver algorithm which compares the waveform of each raster line to that of a reference waveform stored in memory, detects a relative maximum in the correlated result and defines the position of the interference line. The reference waveform consists of 17 data points and was obtained from a saline solution image averaged a large number of times. The enhanced interference image consists of interference lines which are each one data point in width. Each raster line now consists of about 39 data points. Figure 3b shows the results of this program. An important characteristic of this image is that it is represented by a more manageable 482 x 39 element array.

CALCULATION OF THE SPEED OF SOUND

The calculation of the specimen's speed of sound, c_x, from the interference line image can be performed by using: [3]

$$c_x = \frac{c_o}{\sin\theta_o}\left\{\tan^{-1}\left[\frac{1}{(1/\tan\theta_o) - (N\lambda_o/T\sin\theta_o)}\right]\right\} \qquad (1)$$

where c_o is the speed of sound in the reference medium, λ_o is the wavelength of sound in that same medium, θ_o is the angle of the beam from the normal in the reference medium, T is the thickness of the specimen, and N is the normalized lateral fringe shift (N=ab/ac - see Figure 4). Using Snell's Law, θ_o can be determined for the reference medium:

$$\theta_o = \sin^{-1}\left[\frac{c_o}{c_s}\sin\theta_s\right] \qquad (2)$$

where c_s is the speed of sound in the fused silica stage (5968 m/s) and θ_s is the angle at which the generated sound waves travel through the stage with respect to the normal ($\theta_s = 45^o$). In this project, the materials being examined are biological specimens; therefore, saline solution (0.9% NaCl) is the reference medium and is isotonic with the specimens. Saline has a known speed of 1507 m/s at a temperature of 22°C, hence $\theta_o = 10.2^o$.

The previous method employed [1,3] to determine the speed of sound in a specimen was performed manually by assessing the location of lines a, b, and c (see Figure 4) to calculate the normalized lateral fringe shift, N = ab/ac. For this particular method the operator had to determine the best fit of the fringe lines and choose points from which to measure the distances ab and ac. This procedure was quite time consuming and provided only a few velocity values for the specimen. The computer program that has been developed eliminates the need to do this manually,

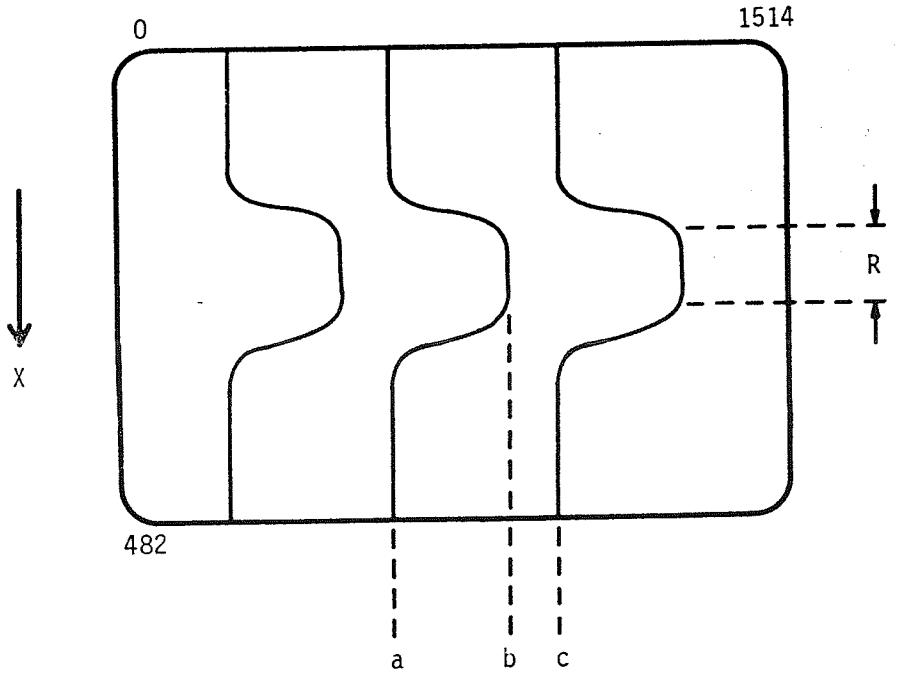


Figure 4. Schematic representation of the fringe shift, N = ab/ac.

increases the area of tissue from which the speed of sound can be calculated, and involves minimal operator interaction.

As can be seen in Figure 3b, the enhanced interference lines are not always continuous and can have a significant amount of jitter. In order to automate the speed of sound determination, it is necessary for an algorithm to identify the discrete interference lines. Thus, a computer program was written to follow the interference lines and smooth them by curve fitting. If a line is not continuous (i.e., a point is missing) then the program adds a point at a position determined by a linear interpolation based on the nearest 10 points. The program informs the user how many points have been added in which line (interference lines are numbered sequentially from left to right). The resulting continuous interference lines are then smoothed by a sliding 17 point quadratic curve fitting algorithm which can be considered a low pass filtering operation.

Two adjacent interference lines are used to determine the distance ac provided that the specimen is placed on the microscope stage within the boundaries x = 51 to x = 432 (see Figure 4). The

array for a single interference line consists of 482 data points. The top and bottom 50 points (100 points total) of each pair of interference lines are used to determine the location of lines a and c. The best fit straight lines for a and c are found by using a least squares linear curve fit. If lines a and c are perfectly vertical in orientation, the slope of these lines will be zero. The y intercept of these lines will vary from 0 to 1514, depending upon the location of the interference lines. With the equations for lines a and c defined, the distance ac is calculated for each point x = 1 to 50 and x = 433 to 482 and the average ac value determined is used for the remainder of the calculations of the speed of sound for that specimen.

The width of region R, (see Figure 4) where the distance ab is calculated, is a variable under operator control. The rationale for this is that not all of the data in the region between raster lines 51 and 432 may be useful because (1) the specimen may not occupy the entire region between raster lines 51 and 432, (2) the specimen may not be uniformly thick over this region because of edge effects and (3) it may be necessary to avoid certain inhomogeneities in the tissues, such as blood vessels. All of these can be assessed by the acoustic microscopist from the images. The horizontal distance ab is calculated R times and N is determined using each of these ab values and the average ac value previously calculated for that area of the specimen. Given the sample thickness, the speed of sound data set (R total values) is determined from equation 1.

Various statistical parameters are calculated from each speed of sound data set, namely, the standard deviation, mean, mode, median, skewness and kurtosis.

The standard deviation is defined as,

$$S = \sqrt{\frac{1}{R} \sum_{j=1}^{R} (x_j - \bar{x})^2} \qquad (3)$$

where $\bar{x}$ is the mean value, R is the number of samples, x_j is the jth speed value along the region R. The standard deviation is a measure of the concentration of values around the mean.

The mode is that value which occurs more frequently than any other value; graphically it appears as the peak of a distribution. Of a set of numbers arranged in order to magnitude, the median is the value of the exact middle position of the set. In this program, to calculate the median, the data set length is always chosen to be odd, thus requiring only one algorithm.

Skewness is an indication of the "sidedness" of a distribution with respect to its mean. By definition,

$$\text{SKEW} = \frac{3(\bar{x} - \text{median})}{S} \tag{4}$$

The distribution is said to be skewed right and SKEW will be positive if more values of the data set are to the right of the mean. Similarly if SKEW is negative the distribution is shewed left.

Kurtosis is a measure of the shape of distribution which reveals the degree of peakedness of the distribution as compared to a normal distribution. Kurtosis is defined by a dimensionless 4th moment around the mean or,

$$\text{KURTOSIS} = \frac{\frac{1}{R}\sum_{j=1}^{R}(x_j - \bar{x})^4}{S^4} \tag{5}$$

For a normal (Gaussian) distribution KURTOSIS would be zero. A distribution is said to be leptokurtic (spikey, very peaked) if KURTOSIS is greater than zero and platykurtic (flat) if less than zero.

RESULTS AND DISCUSSION

A white, ICR female mouse (Harlan Sprague Darley) approximately 6-7 months old was sacrificed by means of spinal cord dislocation so as to not introduce any drugs into the tissues. The liver was quickly excised (within 5 minutes post mortem) and immediately placed in isotonic saline solution. The sample is then trimmed with a razor blade into a rectangular piece suitable for viewing with the SLAM. The speed of sound can only be determined by this technique if there is no discontinuity of the interference line between the saline and tissue. Thus, the edges of the specimen are bevelled.

The sample is then placed in the center of the microscope slide, on the plastic sheet, and surrounded by a spacer which has a thickness slightly greater than the sample. The spacer prevents the sample from being crushed or distorted by the coverslip, insures a constant distance between the slide and the coverslip, and keeps the coverslip level with respect to the stage. The area surrounding the tissue is filled with saline, which serves as the reference media.

A typical output of a program which calculates all of the previously described speed of sound statistical data is shown in Figure 5 for a 460 μm thick, fresh liver specimen which was averaged 4 times. The output is shown for the 22nd and 23rd interference lines.

The slopes, y intercepts and x intercepts for lines a and c (see Figure 4) of these two interference lines are listed. Figure 5 shows graphically the velocity distribution for interference line 22 between x = 210 and x = 270 (R = 61 data points) along with the minimum speed (1561.3 m/s), the maximum speed (1571.0 m/s), the average (mean) speed (1567.4 m/s) and the standard deviation (2.3 m/s). The distribution is playtkurtic, is skewed to the right of the mean, has a median speed of 1566.5 m/s and has a mode at 1566 m/s at where there are 23 values.

Figure 6 shows the smoothed continuous fringe lines of a 550 μm liver sample. The statistics for each of the 33 line pairs are shown in Table 1. For this set of data, the standard deviation is small and the skew and kurtosis values are close to zero indicating that the liver sample is quite homogeneous. A plot of the average speed of sound, one standard deviation error bounds and the median speed versus distance across the sample is shown in Figure 7. The speed of sound is rather constant and the standard deviation is small as would be expected from a relatively homogeneous specimen.

The speed of sound for several thicknesses of mouse liver and mouse spleen has been determined [4]. In each case 61 data points were used in the center of the specimen. The average speed of sound are 1556.3 m/s for mouse liver and 1565.0 m/s mouse spleen. The average standard deviation is 2.1 m/s for liver and 3.2 m/s for spleen. These results are comparable to those cited in the literature for similar tissues using the SLAM. Frizzell and Gindorf [5] obtained values of 1565 $\pm$ 8 m/s for sheep liver and 1567 $\pm$ 13 m/s for cat liver.

In conclusion, an automated technique for determining the speed of sound at many points across a sample has been developed. The speed of sound data set which is generated is much larger than could be generated manually, thus enabling one to use quantitative statistical parameters to study the degree of tissue specimen heterogeneity.

```
NUMBER OF AVERAGES = 4
THICKNESS(M) = 0.00046
LINE#    SLOPE      YBAR      XBAR
 22      -0.02     798.88    241.50
LINE#    SLOPE      YBAR      XBAR
 23      -0.02     838.32    241.50
NS = 210    NE = 270    N = 61
AVERAGE FN = 1.19533
NMAX = 1.26496    NMIN = 1.08027
VMIN(M/S) = 1561.348    VMAX(M/S) = 1571.029
AVERAGE SPEED(M/S) = 1567.367
STANDARD DEVIATION(M/S) = 2.313
PLATYKURTIC  -0.55
MEDIAN SPEED(M/S) = 1566.490
SKEWED RIGHT  1.137
MODE OCCURS AT 1566M/S    VALUE OF 23.00
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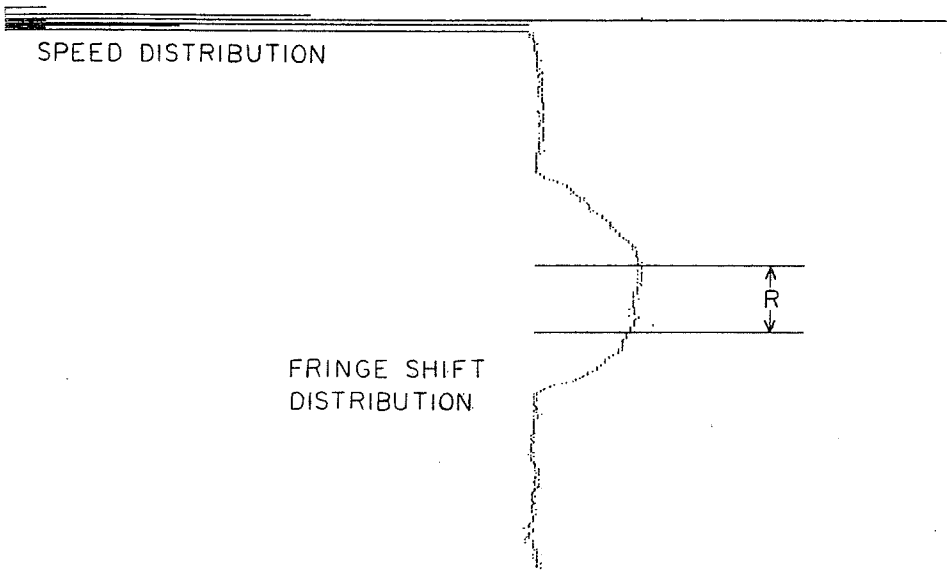

Figure 5. Typical output of the program which calculates the speed of sound statistical data for one pair of interference lines. (Data for lines 22 and 23 are shown.)

Figure 6. Smoothed interference lines after running line fitting program for a 550 μm thick liver sample.

Table 1. Statistical Speed of Sound Data for 550 μm Thick Liver Sample Averaged Over 121 Points Horizontally. Thirty-three Points Across the Sample are Shown.

NS=180 NE=300 N=121
THICKNESS(M) = 0.00055

LINE #	SLOPE1	YBAR1	SLOPE2	YBAR2	AVE N	NMIN	NMAX	VMIN	VMAX	VAVE	SIGMA	KURTOSIS	VMED	SKEW
2	0.01	127.0	0.01	162.5	0.9453	0.8006	1.0280	1540.30	1550.02	1546.48	2.60	-0.02	1546.82	-0.397
3	0.01	162.5	0.00	198.5	0.9431	0.8368	1.0221	1541.84	1549.77	1546.39	1.91	-0.09	1546.35	0.051
4	0.00	198.5	0.00	235.1	0.9315	0.8554	1.0015	1542.64	1548.88	1545.89	1.56	-0.62	1545.24	1.250
5	0.00	235.1	0.00	271.8	0.9262	0.8391	0.9820	1541.94	1548.05	1545.66	1.61	-0.78	1545.60	0.115
6	0.00	271.8	0.00	308.6	0.9139	0.8438	0.9837	1542.14	1548.12	1545.13	1.52	-0.80	1544.59	1.083
7	0.00	308.6	0.00	345.6	0.9023	0.8444	0.9580	1542.16	1547.02	1544.64	1.39	-0.83	1544.61	0.075
8	0.00	345.6	-0.00	383.0	0.8907	0.8375	0.9465	1541.87	1546.53	1544.14	1.04	-0.82	1544.21	-0.189
9	-0.00	383.0	-0.00	421.1	0.8818	0.8140	0.9175	1540.96	1545.29	1543.76	0.96	-0.03	1544.12	-1.117
10	-0.00	421.1	-0.00	459.4	0.8702	0.8102	0.9361	1540.71	1546.08	1543.26	1.11	0.25	1543.80	-1.448
11	-0.00	459.4	0.00	497.7	0.8635	0.8013	0.9284	1540.33	1545.75	1542.98	1.21	0.63	1543.48	-1.248
12	0.00	497.7	0.00	536.4	0.8656	0.8075	0.9120	1540.59	1545.05	1543.07	1.27	-0.46	1542.82	0.592
13	0.00	536.4	0.00	575.8	0.8744	0.7999	0.9310	1540.27	1545.86	1543.44	1.56	-0.70	1543.60	-0.301
14	0.00	575.8	0.01	614.9	0.8899	0.8028	0.9534	1540.39	1546.82	1544.11	1.79	-0.99	1544.54	-0.717
15	0.01	614.9	0.01	654.3	0.8860	0.8054	0.9465	1540.51	1546.53	1543.94	1.95	-1.06	1544.43	-0.751
16	0.01	654.3	0.00	693.8	0.8771	0.7940	0.9315	1540.02	1545.88	1543.56	1.96	-1.37	1543.75	-0.291
17	0.00	693.8	0.00	732.6	0.8901	0.8254	0.9578	1541.36	1547.01	1544.12	2.02	-1.59	1544.70	-0.870
18	0.00	732.6	0.01	771.9	0.8998	0.8452	0.9534	1542.20	1546.82	1544.53	1.75	-1.66	1544.71	-0.305
19	0.01	771.9	0.00	811.7	0.8949	0.8230	0.9542	1541.26	1546.85	1544.32	1.57	-1.07	1544.58	-0.487
20	0.00	811.7	0.00	851.5	0.8913	0.8295	0.9591	1541.53	1547.07	1544.17	1.44	-0.82	1544.02	0.211
21	0.00	851.5	0.00	891.1	0.8912	0.8476	0.9460	1542.30	1546.51	1544.17	1.34	-0.89	1543.45	1.594
22	0.00	891.1	0.00	931.4	0.8768	0.8212	0.9135	1541.18	1545.11	1543.55	1.02	-0.91	1543.22	0.974
23	0.00	931.4	0.00	972.4	0.8540	0.7988	0.8900	1540.23	1544.11	1542.57	1.09	-1.02	1542.98	-1.110
24	0.00	972.4	0.00	1012.3	0.8511	0.7926	0.0907	1539.96	1544.14	1542.45	1.16	-1.20	1543.03	-1.485
25	0.00	1012.3	0.00	1052.0	0.8702	0.8254	0.9227	1541.35	1545.51	1543.27	1.00	0.21	1543.38	-0.342
26	0.00	1052.0	0.00	1092.1	0.8835	0.8413	0.9235	1542.03	1545.54	1543.83	1.02	-0.82	1544.26	-1.247
27	0.00	1092.1	0.00	1132.5	0.8751	0.8207	0.9153	1541.16	1545.19	1543.47	0.86	-0.14	1543.84	-1.278
28	0.00	1132.5	0.00	1172.4	0.8865	0.8207	0.9165	1541.16	1545.24	1543.96	0.82	0.81	1544.07	-0.384
29	0.00	1172.4	-0.00	1212.7	0.8990	0.8357	0.9343	1541.80	1546.00	1544.50	1.25	-0.71	1544.87	-0.893
30	-0.00	1212.7	0.00	1253.2	0.8844	0.8201	0.9691	1541.13	1547.50	1543.88	1.67	-0.43	1543.33	0.979
31	0.00	1253.2	0.00	1293.6	0.8680	0.8102	0.9609	1540.71	1547.14	1543.18	1.78	-0.39	1542.93	0.412
32	0.00	1293.6	0.00	1333.9	0.8767	0.8006	0.9549	1540.30	1546.89	1543.54	1.82	-0.63	1543.56	-0.033
33	0.00	1333.9	0.00	1374.6	0.8815	0.8079	0.9614	1540.61	1547.16	1543.75	1.83	-1.14	1544.08	-0.542
34	0.00	1374.6	0.01	1415.0	0.8717	0.7956	0.9520	1540.09	1546.76	1543.33	1.95	-1.13	1543.65	-0.482

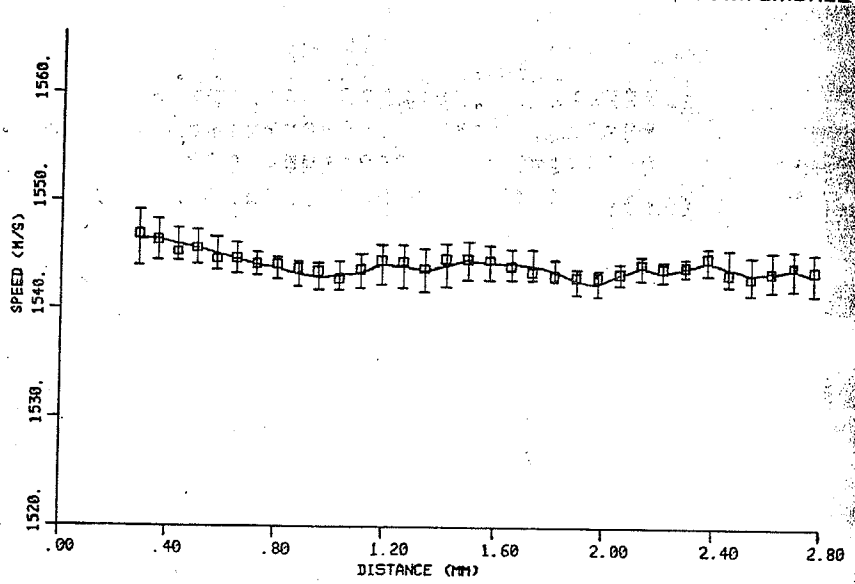

Figure 7. Speed of sound versus distance acrosse sample.
—— average speed.
□ median speed.
I ± 1 sigma error bounds.

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