A Calibration-Free Method for Measurement of Sound Speed in Biological Tissue Samples

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Abstract—A transmission method for speed-of-sound measurement in biological tissue specimens is described. The technique involves detecting the time of arrival of a transmitted pulse with a small hydrophone that penetrates the tissue sample in accurately known increments. The technique allows localized measurements to be made without the error-prone task of measuring the thickness of the specimen and without calibration of the apparatus in a reference medium with known speed of sound.

I. INTRODUCTION

SEVERAL methods for speed-of-sound measurements in tissues have been described in the literature and have been reviewed by Wells [1]. Continuous-wave interferometry techniques are capable of 0.5-percent accuracy but are usually confined to thin samples of low-attenuation liquids because of the difficulty in establishing stationary fields with adequate standing-wave ratios in highly attenuating materials [1]. Pulse transit time measurements [2] give a direct measurement of the speed of sound, but uncertainties in the length of the effective acoustic path give rise to accuracy errors of about three percent [1]. An improvement of this method [3] calibrates the path length by using a reference liquid with a known speed of sound, which results in a reduction of the errors to about 0.5 percent. A useful variation of this method is the pulse-echo substitution technique that has been used for attenuation [4] and speed of sound [5] measurements in soft tissues. However, it requires knowledge of the speed of sound in the reference medium and of the thickness of the sample.

Thus some of the common problems and limitations encountered with these techniques are as follows.

1) They may be suitable only for measurement in liquids due to the need to change the distance between the transducer and the reflector, and/or due to the presence of significant attenuation in soft tissues.
2) A reference liquid may be required to calibrate the apparatus.
3) The dimensions of the tissue sample may have to be accurately measured. Due to the softness of most tissues, this is an error prone measurement [4].

In this paper we present a transmission technique that allows accurate measurement of the speed of sound in soft tissue samples, while overcoming these limitations. A receiving hydrophone and a transmitting transducer are coaxially aligned opposite each other. The transmitting transducer is in contact with the tissue sample, while the hydrophone penetrates the tissue sample in well controlled incremental depths. The transit times of the pulse are recorded for all penetration depths of the hydrophone. These transit times are then plotted against the relative depths of the hydrophone, and a linear regression fit is made to the data. The slope of the fitted line is \( \dot{c}^{-1} \), where \( \dot{c} \) is the estimated speed of sound in the tissue sample. Thus neither calibration involving a reference medium, nor the knowledge of the thickness of the tissue sample, are required.

II. MATERIALS AND METHODS

The physical arrangement is shown in Fig. 1. An ultrasonic pulse is emitted by a 3.5-MHz 19-mm transmitting transducer attached to the bottom of a test vessel which contains the tissue sample. The pulse traverses the sample and is picked up by a 1-mm² aperture wide-band hydrophone mounted on the tip of a hypodermic needle. The hydrophone is attached to a precision slide mechanism which can step in 0.005-mm increments toward or away from the transmitting transducer. The hydrophone is shown in two positions separated by a distance \( \Delta x \). Data acquisition is controlled by a Compaq 286 personal computer as shown in Fig. 2. The hydrophone is positioned at some initial depth in the tissue and one pulse is transmitted in response to shock excitation. The received pulse is amplified by a custom RF amplifier and digitized at a 50-MHz sampling rate by a LeCroy 8-bit digitizer. The process is then repeated at several greater depths separated by increments of \( \Delta x \). The instantaneous envelope of the RF signals is derived in software from the associated analytic signal [6]. The peak of the instantaneous envelope is calculated, and its temporal position in relation to the transmitter pulse is taken as the transit time of the pulse in the tissue sample. A linear regression is performed on the values of temporal pulse delays versus hydrophone depth positions. The slope of this line is the inverse of the estimated speed of sound in the tissue. The following two experiments were performed using this technique.
An adjacent slice of 1.00 ± 0.03-cm-thickness was cut from the same liver using a special jig, ensuring uniform known thickness. This slice was used separately in a pulse-echo substitution method to measure the speed of sound in the liver at the same temperature. Details of this procedure are given elsewhere [10]. The results of the substitution measurement were compared to the hydrophone results.

III. Results

The results of the speed-of-sound measurements in water at several temperatures are given in Table I, where they are compared to the interpolated literature values of Greenspan and Tschiegg [7]. If we take the literature results as the ground truth, we observe a systematic (accuracy) error in the measurement of about 4.5 ms⁻¹, and a precision (reproducibility) error of ±1.95 ms⁻¹. The most likely cause for most of the systematic error is a discrepancy in the actual temperature. The thermometer we used was traceable to NBS Standard to within 1°C. A 1°C discrepancy in the temperature can result in ~3 ms⁻¹ error at 23°C [7].

The results of speed-of-sound measurements in the liver sample at 23 ± 0.5°C are shown in Table II. The average speed of sound measurement over five acoustic paths in the sample separated laterally by 4 mm is 1568.89 ± 5.10 ms⁻¹ (mean ± standard deviation). Along each path, the hydrophone was advanced in increments of 4 mm, for a total of six delay time measurements over a 2-cm range of penetration into the sample. Typical pulse waveforms are shown in Fig. 3.

The speed of sound in the adjacent slice of liver was measured using the pulse-echo substitution technique described elsewhere [10]. Briefly, the arrival time $t_1$ of a steel plate echo is determined in physiological saline. Subsequently, a carefully cut liver slice is placed on the plate and allowed to reach temperature equilibrium for 1 h, after which time the new arrival time of the echo $t_3$ is recorded. The average time over five transducer positions $\bar{t}$ is computed. The speed of sound in the liver slice is then computed as

$$\frac{1}{c_l} = \frac{1}{c_s} \cdot \frac{\bar{t} - t_3}{2d},$$

where

- $c_l$ speed of sound in the liver,
- $c_s$ speed of sound in saline at 23°C = 1502.30 ms⁻¹,
- $d$ thickness of the liver slice.

The speed of sound in physiological saline was measured using a velocimeter [11] whose effective length was calibrated with distilled water, using the value of speed of sound in distilled water at 23°C of 1491.50 ms⁻¹ [7]. The results of the substitution measurement in the liver yielded a speed of sound of 1571.72 ± 3.15 ms⁻¹. The difference between these results and the hydrophone results is about 0.18 percent.
TABLE I

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>24.8</th>
<th>27.4</th>
<th>29.4</th>
<th>30.9</th>
<th>32.9</th>
<th>35.0</th>
<th>37.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of sound in water (Greenspan and Tschigg, c (ms⁻¹))</td>
<td>1496.46</td>
<td>1503.20</td>
<td>1508.06</td>
<td>1511.55</td>
<td>1515.89</td>
<td>1520.12</td>
<td>1523.93</td>
</tr>
<tr>
<td>Speed of sound in water (this technique), ( \hat{c} ) (ms⁻¹)</td>
<td>1492.54</td>
<td>1499.25</td>
<td>1504.89</td>
<td>1506.03</td>
<td>1508.29</td>
<td>1514.00</td>
<td>1522.07</td>
</tr>
<tr>
<td>Goodness of fit, ( r^2 )</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
<td>1.000</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>( c - \hat{c} ), ms⁻¹</td>
<td>3.92</td>
<td>3.95</td>
<td>3.17</td>
<td>5.52</td>
<td>7.60</td>
<td>6.12</td>
<td>1.86</td>
</tr>
</tbody>
</table>

\( r^2 \) is the coefficient of determination (\( r^2 = 1 \) indicates a perfect fit).

TABLE II

<table>
<thead>
<tr>
<th>Location</th>
<th>Speed of Sound, ms⁻¹</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1572.33</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>1564.95</td>
<td>1.000</td>
</tr>
<tr>
<td>3</td>
<td>1562.50</td>
<td>1.000</td>
</tr>
<tr>
<td>4</td>
<td>1569.86</td>
<td>1.000</td>
</tr>
<tr>
<td>5</td>
<td>1574.80</td>
<td>1.000</td>
</tr>
<tr>
<td>Average (± standard deviation)</td>
<td>1568.89 ± 5.10</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*The measurement was repeated in five locations in the sample.

Fig. 3. Progression of typical received signal for six hydrophone positions in liver sample separated by 4 mm.

**IV. CONCLUSION**

We have described a transmission method that is suitable for localized speed-of-sound measurements in tissue samples. The trade-offs among the experimental parameters and their effect on the precision of the instrument are discussed in the Appendix. The technique offers several advantages over other commonly used techniques.

1) The measurements can be made in biological liquids as well as in soft tissues. This allows the use of one experimental setup for all measurements.

2) Localized speed-of-sound measurements can be made along a straight line. This would in principle allow speed-of-sound measurements in small targets such as tumor specimens which may be embedded in normal tissue.

3) The thickness of the tissue sample does not have to be known. The measurement of this quantity is necessary in some techniques. Unfortunately, soft tissues are difficult to cut and measure exactly [4]. In addition, some dimensional changes may occur due to immersion or changes in temperature.

4) Most techniques for accurate speed of sound measurement in tissues require an extra step of calibration of the apparatus in a medium of known speed of sound. Any errors arising during calibration are added to errors in the measurements itself. No calibration is necessary when using this technique.

The analysis given in the Appendix shows that the theoretical precision of this technique as implemented in the experiments reported herein is expected to be on the order of 0.1 percent. The analysis does not address errors due to uncertainties in temperature. These errors are common to all speed-of-sound measurement techniques, and as long as temperature control is maintained within ±0.5°C, a measurement error greater than 0.2 percent can be expected. The measurements in distilled water demonstrate a 0.30 ± 0.13 percent error as compared to literature values. The systematic error of 0.3 percent might be largely due to a systematic error in the measurement of the temperature. The tissue measurements using both the hydrophone and the substitution technique were performed using identical temperature control. Thus a smaller systematic error due to temperature control is expected. Indeed, the unexplained systematic error is reduced to about 0.18 percent, while the precision of the hydrophone measurement is about 0.33 percent, which is close to what is theoretically expected.

It should be noted that due to the differential nature of the measurements and the lack of need for calibration, the speed-of-sound estimation is unbiased. The precision error is further reducible (if the quality of the temperature regulation warrants it) by increasing the measurement path length, decreasing the sample spacing, and increasing the
number of paths over which the measurements are made. Expressions for the precision in terms of these parameters are derived in the Appendix.

ACKNOWLEDGMENT

The authors wish to acknowledge the contribution of Mr. T. Kontonassios in writing the data acquisition and analysis software.

APPENDIX

ERROR ANALYSIS IN SPEED-OF-SOUND ESTIMATION

We investigate the statistical parameters which affect the precision of the speed-of-sound estimation. The variance of the estimator is given as the sum of the variances due to independent errors in the measurement. Specifically, we consider errors in the spatial positioning of the hydrophone, and errors due to temporal uncertainty in the measurement of echo delays. We write

\[ \sigma_{\text{estimator}}^2 = \sigma_{\text{temporal}}^2 + \sigma_{\text{spatial}}^2. \]  

(A1)

A. Temporal Variance

In this case we consider time to be the dependent error-prone variable and space to be the independent error-free variable. Thus the variance of the slope of the linear regression fit, which represents the inverse speed-of-sound estimate, is given as [12]

\[ \text{var}_t \left( \frac{1}{\hat{c}} \right) = \frac{s_t^2}{m \sum_{i=1}^{k_1} (x_i - \bar{x})^2} \]  

(A2)

where

- \( \hat{c} \) estimated speed of sound,
- \( s_t^2 \) variance of the residuals (the vertical excursions of the data about the regression line) due to errors in temporal measurements,
- \( m \) number of fits made,
- \( x_i \) \( i \)th spatial location,
- \( \bar{x} \) center spatial location of the regression line,
- \( k_1 \) total number of temporal measurements per fit.

Assuming a uniform spacing between samples \( \Delta x = |x_i - x_{i+1}| \) it can be shown [13] that the summation

\[ \sum_{i=1}^{k_1} (x_i - \bar{x})^2 = \frac{k_1^3 - k_1}{12} (\Delta x)^2 \]  

(A3)

and thus (2) becomes

\[ \text{var}_t \left( \frac{1}{\hat{c}} \right) = \frac{12 s_t^2}{m(k_1^3 - k_1)(\Delta x)^2}. \]  

(A4)

B. Spatial Variance

We proceed in an analogous fashion to calculate the estimator variance due to uncertainties in hydrophone position. We now consider time to be the independent variable and space to be the dependent variable.

Again, performing linear regression analysis, we get

\[ \text{var}_s \left( \frac{1}{\hat{c}} \right) = \frac{s_s^2}{m \sum_{j=1}^{k_2} (t_j - \bar{t})^2} \]  

(A5)

where

- \( s_s^2 \) variance of the residuals due to errors in spatial position,
- \( t_j \) \( j \)th time,
- \( \bar{t} \) center of the total time interval,
- \( k_2 \) total number of time positions at which pulse delays were measured.

In a similar fashion to the previous analysis, we can show that

\[ \text{var}_s \left( \frac{1}{\hat{c}} \right) = \frac{12 s_s^2}{m(k_2^3 - k_2)(\Delta t)^2} \]  

(A6)

where \( \Delta t = |t_j - t_{j+1}| \).

In our particular experiments, the uncertainty in the position of the hydrophone is less than or equal to \( \pm 5 \times 10^{-6} \) m. Thus \( s_s^2 \approx (5 \times 10^{-6})^2 \). Moreover, \( m = 6 \), \( k_1 = 6 \), \( k_2 = 5 \), \( \Delta x = 4 \) mm, and \( \Delta t = 2.55 \) \( \mu \)s. The uncertainty in time measurements was estimated to be less than \( 0.03 \) \( \mu \)s, or \( s_t^2 \leq (0.03 \mu s)^2 \). The figure of \( 0.03 \mu s \) reflects the sampling interval (0.02 \( \mu \)s) plus an additional 50 percent to account for possible phase noise in the sampling frequency. Thus 0.03 \( \mu s \) represents a conservative estimate. From (A4) and (A6) we get a value for the temporal variance as follows.

\[ \text{var}_t \left( \frac{1}{\hat{c}} \right) \leq \frac{12(0.03 \mu s)^2}{6(6^3 - 6)(4 \text{ mm})^2} \]

\[ = \left( 7.32 \times 10^{-4} \frac{\mu \text{s}}{\text{mm}} \right)^2. \]

The average inverse speed of sound \( \hat{c}_{av}^{-1} = 6.377 \times 10^{-1} \) \( \mu \)s/mm. We calculate the normalized temporal precision

\[ \text{precision} \leq \left| \frac{\sqrt{\text{var}_t \left( \frac{1}{\hat{c}} \right)} - 1}{\hat{c}_{av}^{-1}} \right| \times 100 \text{ percent} \]

\[ = |0.11 \text{ percent}|. \]

Similarly, for the spatial variance,

\[ \text{var}_s \left( \frac{1}{\hat{c}} \right) \leq \frac{12(5 \times 10^{-3} \text{ mm})^2}{6(5^3 - 5)(2.55 \mu s)^2} \]

\[ = \left( 2.53 \times 10^{-4} \frac{\text{mm}}{\mu \text{s}} \right)^2. \]

and since \( \hat{c}_{av} = 1.568 \text{ mm} / \mu \text{s}^{-1} \), we get the normalized spatial precision

\[ \text{precision} \leq \left| \frac{\sqrt{\text{var}_s \left( \frac{1}{\hat{c}} \right)}}{\hat{c}_{av}} \right| = |0.02 \text{ percent}|. \]
The overall precision of the estimator (in percent) is derived from (A1) as

\[
\text{precision} \leq \sqrt{(0.11 \text{ percent})^2 + (0.02 \text{ percent})^2} = |0.11 \text{ percent}|
\]

Note that the preceding analysis ignores precision errors due to temperature fluctuations. A $\pm 0.5^\circ C$ uncertainty in the temperature of water at room temperature would translate into approximately a $\pm 0.1$-percent additional uncertainty in the estimation of the speed of sound. Clearly, this type of error exists in all measurements.

REFERENCES


J. Ophir, photograph and biography not available at time of publication.

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