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Two coupling media method for ultrasonic velocity measurement in biological tissues

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Measuring the ultrasonic velocity in soft materials, especially in biological tissues, is never easy. One of the main issues is that the thickness of the sample under test is badly defined. From time-of-flight measurement method uncertainties in the thickness induce important uncertainties in experimental results. This causes that the ultrasonic velocity is often a poor criteria whereas it could be a good one. This paper brings a new method to measure the ultrasonic velocity in soft materials without knowing the sample thickness The main idea of this method is to use two different coupling media for making two relative time-of-flight measurements.
The comparison of these two measurements gives the ultrasonic velocity in the sample in test. Furthermore, this comparison can give the local sample thickness of the sample. The uncertainty calculi show up that the effect on the thickness is drastically reduced by using this new ultrasound method. This method is validated on reference samples, in silicon. Before concluding, some experimental results obtained with mouse skin samples are presented.

1 Introduction

Thanks to the link between ultrasonic velocities and mechanical parameters, several authors use ultrasonic signals in order to detect mechanical pathologies [1]. Acoustic imaging leads to qualitative information [2, 3, 4, 5, 6, 7]. For quantitative studies, the longitudinal ultrasonic velocity is a key parameter [8, 9, 10].

The most common echographic techniques for measuring acoustic velocity can be ranked in two classes :

- the first need the reflection coefficient between the coupling media and the sample [11]. The coupling media is used to propagate the ultrasonic waves from the ultrasonic sensor to the sample.
- the others which are based on the time-of-flight principle. The ultrasonic waves must be transmitted through the sample.

Concerning the longitudinal velocity assessment for biological tissues, many problems occur.

First of all, the coefficient of reflection between the coupling fluid and the biological tissue is almost equal to zero. Hence, the echo reflected on the surface of the sample is often very small, mixed with noise and the mathematical treatment of this waveform can become very difficult.

Secondly, in classical time-of-flight techniques, the thickness of the sample has to be known [12, 13, 14].

Indeed, if the ultrasonic wave is plane, the velocity c is given by the well-known relation $c = e/t$ [15], where e is the sample thickness and t is the duration of the ultrasonic wave transmission. For hard materials, having a quite regular morphology, mechanical estimation of the thickness is accurate using a profilometer for instance. For biological tissues, this method generally fails and thickness evaluation is quite inaccurate [16]. Although, the less the accuracy of the thickness is, the less the accuracy of the velocity is too.

Moreover, the longitudinal ultrasonic velocity is not very different from one muscle type to another and is not very sensitive to pathologies. For instance, it is 1560 $m.s^{-1}$ for beef muscle [10], 1585 $m.s^{-1}$ for pig muscle, 1591 $m.s^{-1}$ for rat muscle [17], 1620 $m.s^{-1}$ for a healthy cardiac muscle and 1572 $m.s^{-1}$ for a sick one [18, 19, 20, 21]. Consequently, the ultrasonic velocity has to be assessed with an high accuracy in order to detect pathologies.

The main objective of this work is to propose a method of velocity measurement that is not dependant of the sample thickness.

After a presentation of our new method principle, the accuracy analysis are developed, and finally the method is applied to biological tissues.

2 Method principle description

Assuming that the ultrasonic wave is plane, that dispersive effects are negligible and that the biological tissue is a homogeneous media.

Instead of working in a transmission mode [22, 23, 24] , the double-through transmission has been chosen mainly for many reasons :

- avoiding misalignments between the ultrasonic emitter and the ultrasonic receiver [25] ,
- having a good hold of the biological tissue,
- doubling the transmission duration.

But the double-through transmission needs a reflector plate which is supposed here to be perfect (having an infinite acoustic impedance) [26, 27, 28].

The complete duration of the ultrasonic waves depends on the distance d between the ultrasonic sensor and the sample, the sample thickness e, the acoustic velocity of the coupling fluid and the sample acoustic velocity c_m . For deleting d, a reference echo must be obtained by removing the sample. For eliminating e, two similar echoes must be used with an other coupling fluid.

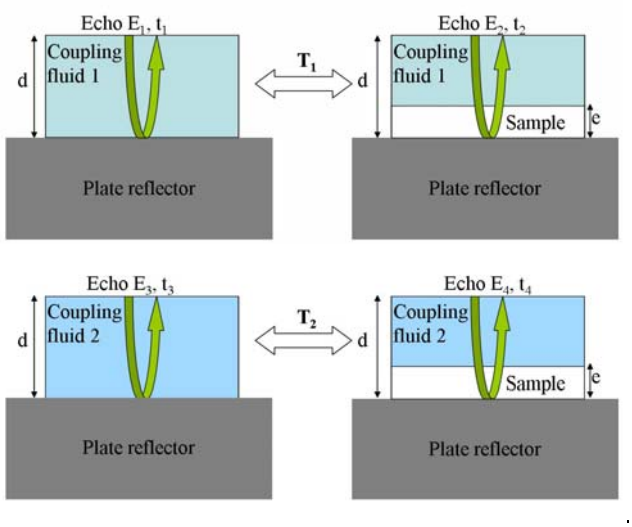

Fig.1 The method uses two coupling fluids and breaks out into four echoes.

The method is broken out of 4 steps (see Figure 1).

In the first step, the arrival time t_1 of the echo E_1 reflected on the plate reflector and obtained with the first coupling fluid of velocity c_1 is acquired :

$$t_1 = \frac{2d}{c_1} \tag{1}$$

In the second step, the sample is settled on the reflector and the arrival time t_2 of the echo E_2 is measured :

$$t_2 = \frac{2(d-e)}{c_c} + \frac{2e}{c_m} \tag{2}$$

The first difference of time of flight T_1 is deduced from t_1 and t_2 :

$$T_1 = t_1 - t_2 = 2e\left(\frac{1}{c_1} - \frac{1}{c_m}\right) \tag{3}$$

Then steps 3 and 4 are respectively identical to steps 1 and 2, but performed with an another coupling fluid of velocity c_2. From t_3 and t_4, we deduce the second difference of time-of-flight T_2, which is identical to Equation (1) with c_2 instead of c_1.

From both T_1 and T_2, the longitudinal velocity in the biological tissues c_m is assessed :

$$c_m = \frac{T_1 - T_2}{\frac{T_1}{c_2} - \frac{T_2}{c_1}} \tag{4}$$

Moreover, we note that the sample thickness *e* can be also calculated :

$$e = \frac{T_1 - T_2}{2\left(\frac{1}{c_1} - \frac{1}{c_2}\right)} \tag{5}$$

The main goal of this work is reached. The longitudinal velocity in the biological tissues c_m can be measured without knowing the thickness of the biological tissues *e* (see Equation (4)).

3 Measurement uncertainties and the coupling fluids choice

This method is useful for extracting acoustic velocity of biological tissues if the measurement uncertainties of this parameter is less than one obtained with classical methods, which need to know sample thickness.

3.1 Measurement uncertainties

From Equation (4), we deduce that the measurement uncertainties of the acoustic velocity is :

$$\left(\frac{\Delta c_m}{c_m}\right)^2 = \frac{1}{\Delta c^2}\left[\left(\frac{c_1(c_m - c_2)}{2e}\Delta T\right)^2 + \left(\frac{c_2(c_1 - c_m)}{2e}\Delta T\right)^2 + \left(\frac{c_m - c_2}{c_1}\Delta c_c\right)^2 + \left(\frac{c_1 - c_m}{c_2}\Delta c_c\right)^2\right] \tag{6}$$

where :

- Δc_m is the biological sample velocity uncertainties
- $\Delta c = c_1 - c_2$ is the difference between the two coupling fluid velocities
- ΔT is the measurement uncertainties of the arrival time
- Δc_c is the coupling fluid velocity uncertainties (supposed to be equal for the two velocities)

Equation (6) reveals that the biological sample velocity uncertainties depend on numerous parameters. The most noticeable one is that the sample thickness "reappears" in Equation 6.

To minimize these measurement uncertainties, we must :

- decrease the ratio $\frac{\Delta T}{e}$. For that, the sample thickness *e* and the signal sampling frequency must be increased. This characteristic is common to all echographic techniques for measuring velocity,
- decrease Δc_c i.e. have a high accuracy of the coupling fluid velocities, by controlling the coupling fluid temperature for example.

But, the impact of the coupling fluid velocities on these measurement uncertainties is not clear.

3.2 The coupling fluids choice

For determining the best pair of coupling fluids, we assume that Δc is known. Then, for decreasing the measurement uncertainties of the biological velocity, we must find the minimum of $u = \left(\frac{\Delta c_m}{c_m}\right)^2$ i.e. resolving the following equation : $\frac{\partial u}{\partial c_1} = 0$.

The straightforward calculations give :

$$\frac{\partial u}{\partial c_1} = \frac{1}{\Delta c^2}\left\{\frac{1}{2}\left(\frac{\Delta T}{e}\right)^2 (c_m - 2c_1 + \Delta c)[2c_1(c_m - c_1 + \Delta c) - c_m \Delta c] + 2\Delta c_c{}^2\left[\frac{(c_1 - c_m)(c_m - \Delta c)}{(c_1 - \Delta c)^3} - \frac{(c_m - c_1 + \Delta c)(c_m + \Delta c)}{c_1{}^3}\right]\right\} \tag{7}$$

There is no analytical relation for the roots of the equation $\frac{\partial u}{\partial c_1} = 0$. Numerically, we find that the most realistic root is approximately :

$$c_1 = c_m + \frac{\Delta c}{2} \tag{8}$$

That means that the coupling fluids must have velocity on both sides of the sample velocity c_m.

But the choice of coupling fluids is limited by the biological application. Indeed, for a non-destructive biological application, no toxicological effect should be induced by the coupling fluids. For instance, the use of alcohol must be prohibited. Moreover, the ionic exchanges between coupling fluid 1 and the biological tissue have to be identical to ionic exchanges between coupling fluid 2 and the biological tissue. If it is not the case, the properties of the biological tissue could evolve during the experiment. We have not found coupling fluid with a velocity higher than the one of the sample and compatible for biological application. So we choose water H_2O for the first coupling fluid. Heavy water D_2O is a biological compatible coupling fluid and has a velocity smaller than the one of the sample one [29, 30]. So, it is chosen for the second coupling fluid.

4 Application on mouse skin

4.1 Experimental setup

The major component of the high-frequency ultrasonic system (figure 2) is the sensor (Panametrics NDT V310, 5 MHz). The sensor is stimulated by a pulse generator (Panametrics NDT 5601A/TT). A numerical oscilloscope (Lecroy LTM 374 M) acquired, with a sampling frequency of 500 MHz, the ultrasonic echoes. A three axis stepper motor (x, y, z) (Newport Motion Controller MM4005) with a motor step of 1 µm ensures the sample and sensor displacements. These different devices, which are the steppers and the oscilloscope, are linked to a computer via a GPIB contact. The sample is put on a copper plane plate. This plate can be oriented in 2 dimensions by a manual micrometer drive (M044 Polytec PI). A digital thermometer is used to measure the temperature of the coupling fluid and to evaluate its velocity.

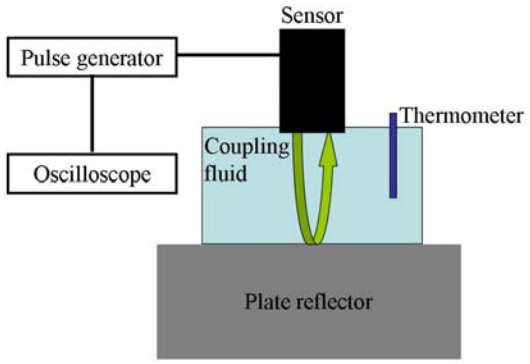

Fig.2 The experimental setup.

4.2 Experimental protocol

Eleven "wild" mice (C57BL/10) were purchased from Jackson Laboratory (Bar Harbor, USA) and supplied at 10 months of age. Just after death, their skin have been shaved, removed and directly put in the coupling biologically compatible fluid.

The results are formulated as mean ± standard deviation.

The experimental protocol is done in four steps.

Firstly, the temperature of the water is measured. Its velocity is deduced from the temperature by means of an abacus [30]. The ultrasonic sensor is immersed in this coupling fluid at the focal point on the plate. Thus, the planarity is adjusted from the maximum of reflected echo on the plate. The echo E_1 is recorded.

Secondly, the mice skin is put on the plate. The echo E_2 on the plate is acquired without moving the plate and the sensor.

Thirdly, the water is removed and the skin is dried. Then, the heavy water is put on the sample without moving the plate and the skin. The echo E_4 is recorded.

Lastly, the skin is removed. The temperature of this coupling fluid is measured to deduce its velocity [30]. The echo E_3 is acquired without moving the plate and the sensor.

Note that the oscilloscope configuration (trigger, time base, division base...) must not be changed during all manipulations.

T_1 and T_2 (see Equation 3) are obtained by intercorrelation between E_1 and E_2, and E_3 and E_4 respectively.

4.3 Experimental results and discussion

The mice skin velocity is 1533.2 ± 4.8 m.s^{-1}. The relative uncertainties are 0.31 %. This result is the same as given by [31], but with a better accuracy.

Corresponding to the experimental setup, c_1, c_2, ΔT and Δc_c are evaluate respectively as :

- $c_1 = 1494$ m.s^{-1} (water at room temperature)
- $c_2 = 1397$ m.s^{-1} (heavy water at room temperature)
- $\Delta T = 2.10^{-9}$ s (sampling frequency = 500 MHz)
- $\Delta c_c = 2.5$ m.s^{-1}

The sample thickness is estimated using Equation (5) : $e = 1.37$ mm. Note that this estimation is the mean value of the eleven results. The uncertainties are about 10 %. The biggest part of the uncertainties are due to the variations between mice.

If Equation (6) is applied with all these values, it gives $\Delta c_m = 5.0$ m.s^{-1}, which is very close to the experimental uncertainties (4.8 m.s^{-1}). Then, it is an another proof that this method does not depend on the sample thickness.

Note that if we have found the coupling fluids with velocities $c_1 = 1580$ m.s^{-1} and $c_2 = 1480$ m.s^{-1}, corresponding to the recommandation made by Equation (8), then the result uncertainties are $\Delta c_m = 2.2$ m.s^{-1}.

The velocity uncertainties are less than 1 %. This percentage is the limit to statistically discriminate a healthy tissue to a sick one. Our method permits to overcome this limit.

Only taking into account the sample thickness uncertainties, here about 10 %, the standard echographic velocity

measurements can not give results with better accuracy than $\Delta c_m = 4.0\ \mathrm{m.s^{-1}}$.

5 Conclusion

This article presents a specific acoustic method which allows an estimation of the longitudinal acoustic velocity of the biological tissues without knowing its sample thickness.

This method is based on the use of two different coupling fluids. This article has shown that the choice of the coupling fluids is fundamental for decreasing the measurement uncertainties of the acoustic velocity estimation.

The first results in mouse skin show that the relative uncertainty is less than 1%. So, this method allows the differentiation of biological tissues and furthermore to follow the evolution of a biological tissue. The main prospect of this work is to make it becoming a diagnostic tool. For that, the experimental setup must be enhanced and the plane wave approximation must be left to a more realistic one.

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