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Numerical analysis of ultrasound propagation and reflection intensity for biological acoustic impedance microscope

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ABSTRACT

This paper proposes a new method for microscopic acoustic imaging that utilizes the cross sectional acoustic impedance of biological soft tissues. In the system, a focused acoustic beam with a wide band frequency of 30–100 MHz is transmitted across a plastic substrate on the rear side of which a soft tissue object is placed. By scanning the focal point along the surface, a 2-D reflection intensity profile is obtained. In the paper, interpretation of the signal intensity into a characteristic acoustic impedance is discussed. Because the acoustic beam is strongly focused, interpretation assuming vertical incidence may lead to significant error. To determine an accurate calibration curve, a numerical sound field analysis was performed. In these calculations, the reflection intensity from a target with an assumed acoustic impedance was compared with that from water, which was used as a reference material. The calibration curve was determined by changing the assumed acoustic impedance of the target material. The calibration curve was verified experimentally using saline solution, of which the acoustic impedance was known, as the target material. Finally, the cerebellar tissue of a rat was observed to create an acoustic impedance micro profile. In the paper, details of the numerical analysis and verification of the observation results will be described.

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1. Introduction

Optical microscopy is often used to observe biological soft tissues. One of its advantages is that it provides very high spatial resolution; however, it requires a staining process to retrieve good contrast in the resulting images. This process may take several days for observation or at least several tens of minutes for a rapid observation.

Conversely, in some instances, observations using ultrasound may be a powerful measure because no staining process would be required, potentially leading to a rapid and potentially accurate observation. In ultrasonic observations, the image contrast is determined by acoustic properties such as the speed of sound and attenuation, which are reflected by visco-elastic properties.

Most acoustic microscopes are of the scanning type (SAM). SAM was developed by Quate and Lemons in 1974 with a focused ultrasonic beam formed by an acoustic lens [1]. Thanks to the development of piezoelectric copolymer films, a concave-type transducer has become available recently with a lower cost if the frequency range is as low as 100 MHz [2]. Utilization of SAM for the visualization of cells or target has been performed. The results were visualized into 2D images based on intensity, the speed of sound, attenuation and thickness [3–7].

In most SAMs for biological and medical use, a tissue slice or cells are placed on a flat substrate, and the local speed of sound can be estimated by comparing two reflections from the top and bottom surfaces. To acquire the response, focused ultrasound is transmitted across the coupling medium between the transducer and target. Because this measurement requires a thin slice of biological material, it requires a specific device for slicing the tissue. Alternatively, if the target is cultured cells, contact between the cells and transducer through a coupling media may introduce contaminants into the target.

As one of solutions to the above-mentioned problems, the authors proposed acoustic impedance microscopy in previous studies [8–10,22]. In previous studies, the acoustic wave at the

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focal point was approximated to be a plane wave propagating vertically to the target. It is assumed that the above calculation may generate error when a highly focused transducer is used. In such a case, calibration should be performed based on the results of an accurate sound field analysis.

In another previous paper [2], different angles of propagation of many beams were considered; however, frequency components were not considered.

Several numerical calculations of the sound field were performed for a certain application using a finite differential time domain (FDTD) [11–13]. Several authors also developed and improved numerical calculations based on a Fourier Transform of the so-called Angular Spectrum Method for Non-Destructive Test (NDT); this method is used to investigate the presence of a plane interface [14,15]. In a recent report, this technique is also used to solve the calculation for linear and non-linear pulsed sound fields [16,17]. A similar concept that uses the reflection coefficient method for predicting mass density was presented by Saito [18]. Fourier analysis has also been applied to calculate acoustic propagation in multilayer media by Tittmann et al. [19]; because they used an acoustic lens transducer for their measurements, the pupil function of the lens is considered.

The authors applied Fourier analysis to calculate the sound field. When an oblique incident occurs, information regarding the angle of propagation and wave number are required.

In this report, the outline of acoustic impedance microscopy and its sound propagation analysis using Fourier transform and the curve for converting reflection intensity into acoustic impedance will be described. In addition, for example, the observation and analysis results using the cerebellar tissue of a rat will be exhibited.

2. System setup

Fig. 1 shows the block diagram system for an acoustic impedance microscope [2,20]. A PVDF-TrFE concave type transducer with an aperture with a 2.4-mm diameter and a focal length of 3.2 mm was used. Its half angle of focusing was 22 degrees. As a coupling medium, pure water was chosen and had a pressure wave speed of 1480 m/s at 20 °C. A polystyrene substrate (Nunc #150318 Petri-dish, speed of sound = 2340 m/s) with a thickness of 0.8 mm was used to mount a target, and the substrate was coupled with the transducer through the coupling medium.

A stage driver sent a control command to the x–y scanning machine and sent a trigger to the pulse transceiver to generate a short electric pulse that was applied to the transducer and converted into an ultrasound wave.

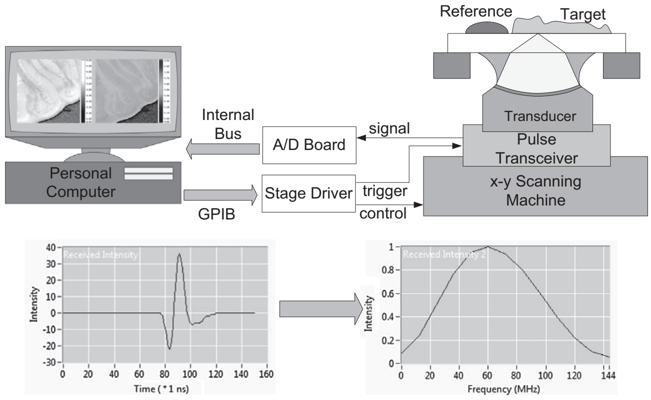

Fig. 1. Schematic diagram of the measurement system and typical acoustic waveform reflected from the target [2,20].

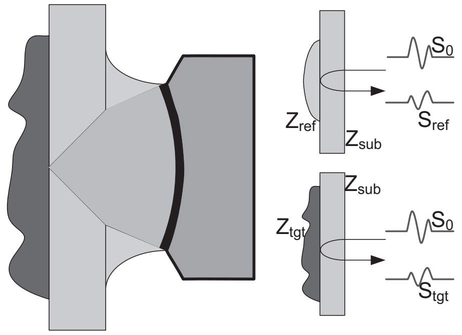

Fig. 2. Illustration for calibration of the acoustic impedance assuming perpendicular incidence.

A focused ultrasound beam with a frequency between 30 and 100 MHz was transmitted from the front side of the substrate, the rear side of which was in contact with the target (Fig. 1, the front is in the bottom of the figure, and the rear is at the top of the figure). The ultrasound propagated through the coupling medium and substrate, reflected at the interface between the substrate and target and then finally returned to the same transducer. The reflection intensity from the interface between the substrate and target, on which the beam is focused, was acquired and interpreted into the absolute value of the characteristic acoustic impedance of the target. During measurement, the room temperature was maintained by an air conditioner at 20–21 °C. Measurements were performed under ambient pressure, but the pressure was not controlled.

3. Method and analysis

3.1. Normal incident

To perform quantitative observations, both the target tissue and reference material should be observed. These two materials may be observed in the same field of view; however, if this is impractical, they may be observed separately under the same conditions, as shown in Fig. 2. If the incident angle of the sound can be approximated as perpendicular to the target, the intensity of the reflected wave can be described as:

$$S_{ref} = \frac{Z_{ref} - Z_{sub}}{Z_{ref} - Z_{sub}} S_0, \tag{1}$$

and:

$$S_{tgt} = \frac{Z_{tgt} - Z_{sub}}{Z_{tgt} - Z_{sub}} S_0, \tag{2}$$

where S_0 is the transmitted sound, S_{tgt} is the reflection from the target, S_{ref} is the reflection from the reference, and Z_{tgt}, Z_{ref} and Z_{sub} are the acoustic impedances of the target, reference and substrate, respectively. Substituting Eq. (1) into Eq. (2), the acoustic impedance of the target can be calculated as shown in Eq. (3):

$$Z_{tgt} = \frac{1 - \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 + \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} \cdot Z_{sub} \tag{3}$$

3.2. Focused beam with oblique incidences

In a practical measurement, a focused transducer is preferable to improve image quality; a focused transducer provides higher intensities as well as image resolution. Because the beam is focused, Eq. (3) does not yield accurate acoustic impedance,

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