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Acoustic impedance microscopy for biological tissue characterization

Kazuto Kobayashi [a,*], Sachiko Yoshida [b], Yoshifumi Saijo [c], Naohiro Hozumi [d]

[a] Honda Electronics Co., Ltd., 20 Koyamazuka, Oiwa-cho, Toyohashi 441-3193, Japan
[b] Toyohashi University of Technology, 1-1 Tempaku, Toyohashi 441-8580, Japan
[c] Tohoku University, 6-6 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan
[d] Aichi Institute of Technology, 1247 Yachigusa, Yakusa, Toyota 470-0392, Japan

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ABSTRACT

A new method for two-dimensional acoustic impedance imaging for biological tissue characterization with micro-scale resolution was proposed. A biological tissue was placed on a plastic substrate with a thickness of 0.5 mm. A focused acoustic pulse with a wide frequency band was irradiated from the "rear side" of the substrate. In order to generate the acoustic wave, an electric pulse with two nanoseconds in width was applied to a PVDF-TrFE type transducer. The component of echo intensity at an appropriate frequency was extracted from the signal received at the same transducer, by performing a time–frequency domain analysis. The spectrum intensity was interpreted into local acoustic impedance of the target tissue. The acoustic impedance of the substrate was carefully assessed prior to the measurement, since it strongly affects the echo intensity. In addition, a calibration was performed using a reference material of which acoustic impedance was known. The reference material was attached on the same substrate at different position in the field of view. An acoustic impedance microscopy with 200 × 200 pixels, its typical field of view being 2 × 2 mm, was obtained by scanning the transducer. The development of parallel fiber in cerebella cultures was clearly observed as the contrast in acoustic impedance, without staining the specimen. The technique is believed to be a powerful tool for biological tissue characterization, as no staining nor slicing is required.

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1. Introduction

In most of optical observation of biological tissue, the specimen is sliced into several micrometers in thickness, and fixed on a glass substrate. The microscopy is obtained by transmitted light through the specimen. As it is normally not easy to get a good contrast by local difference in refraction and/or transmission spectrum, the specimen is usually stained before being observed. It can be classified as a kind of chemical imaging, since only a portion that has a specific chemical property can be stained by selecting an appropriate staining material. However the staining has some disadvantages. It normally takes from several hours to several days to finish the process. Furthermore, the tissue, after being stained, often completely loses its biological functions; i.e., the observation with staining process is chemically destructive.

On the other hand, acoustic imaging can be performed without staining process; i.e., it is chemically non-destructive. The observation can be finished in a very short time, as it does not need the staining process. The idea of ultrasonic microscopy for biological tissue is based on this advantage, and it is considered to become a powerful tool for tissue characterization that can image elastic parameters. Most of ultrasonic microscopes are scanning type, in which the response to a focused acoustic signal is successively acquired as the beam is mechanically scanned [1,2].

The authors previously proposed a pulse driven ultrasonic sound speed microscopy that can obtain sound speed image in a short time [3,4]. Although a small roughness of the specimen was approved in this type of microscope, slicing the specimen into several micrometers was still required for the observation. However it is often required that the observation can be performed without slicing process, as slicing may damage some functions of the tissue.

Based on the above background, the authors newly propose the acoustic impedance microscopy that can image the local distribution of cross sectional acoustic impedance of tissue. As acoustic impedance is given as a product of sound speed and density, it would have a good correlation with sound speed, when the variance in density was not significant. In this paper, the methodology for micro-scale imaging of cross sectional acoustic impedance, and its application to the observation of cerebellar tissue of a rat will be described.

* Corresponding author. Tel.: +81 532 41 2511.
E-mail address: kazuto@honda-el.co.jp (K. Kobayashi).

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2. Preparation of specimens

The cerebellum tissue of a rat was employed as the specimen to be observed. Rats were dissected and removed their whole brains. Some of the isolated cerebellum were sliced at 200-micrometers thick using a rotor slicer (Dohan EM, Kyoto, Japan). The slices were incubated in oxygenated phosphate buffer solution (PBS) on ice for one hour. They were chemically fixed with 4% formaldehyde fixative, for 20 min. For optical observation, some slices were subjected to immunohistochemical staining against calbindin D-28k. The other specimens, the intact ones, were cut at an appropriate cross section. Both intact and fixed slices were rinsed and observed in same PBS.

All experimental procedures were approved by the committees for the use of animals in Toyohashi University of Technology, and all animal care followed the Standards Relation to the Care and Management of Experimental Animals (Notification No. 6, March 27, 1980 of the Prime Minister's Office in Japan).

The cross section of each specimen was in contact with the substrate. The substrate was a flat plastic plate made of polymethylmetacrylate (PMMA), its thickness being 0.5 mm. A reference material, of which acoustic impedance was known, was also placed on the same substrate. In many cases, the target tissue was observed together with the reference, by including it in the same field of view.

In some cases, the surface of the substrate was treated beforehand with an atmospheric plasma for three seconds by a plasma surface treatment equipment (Keyence ST-7000 Plasma Surface Treater), in order to upgrade its hydrophilic property.

In this report, a silicone rubber, distilled water or agar was employed as a reference material, choosing one of them depending on the convenience of the measurement. In case of using silicone rubber, the observation was performed after having waited for more than 24 h since the rubber had been hardened, in order to retain the stability of the material.

3. Experimental setup

Fig. 1 illustrates the outline of the acoustic impedance microscope. Distilled water was used for the coupling medium between the substrate and transducer. A sharp electric pulse of about 40 V in peak voltage and 2 ns in width was generated by the pulse generator (AVTEC, AVP-AV-HV3-C). The maximum repetition rate of the pulse was as high as 10 kHz. The transducer was PVDF-TrFE type. It was 1.5 mm in aperture diameter, and 3.0 mm in focal length. An acoustic wave with a wide frequency component was generated by applying the voltage pulse. The acoustic wave, being focused on the interface between the substrate and tissue, was transmitted and received by the same transducer.

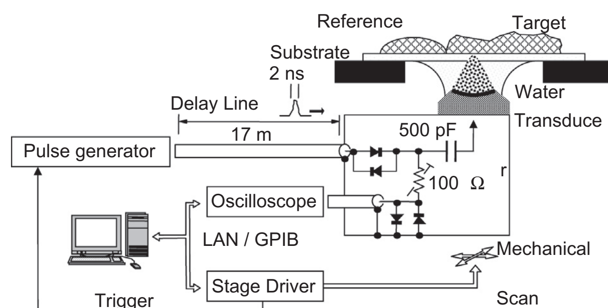

Fig. 1. Experimental setup.

The reflection was detected and digitized by the oscilloscope (Tektronix, TDS-7145B). The waveform was acquired with 2.5 G samplings/s in sampling rate, up to 1 GHz in frequency range and 8 bits in resolution. The input was terminated with 50 Ω. Considering the focal distance and the sectional area of the transducer, the diameter of the focal spot was estimated as about 26 μm at 80 MHz. The distance between the nearest two points was typically set at 10 μm, in order to retain a sufficient lateral resolution. Two-dimensional profile of acoustic impedance was obtained by mechanically scanning the transducer using the stage driver (Sigma Koki, MARK-202), keeping the focal point on the rear surface of the substrate. A typical field of view of 2 mm × 2 mm was covered with 200 × 200 pixels.

A part of the system is overviewed in Fig. 2. The transducer embedded on the coupling circuit box was mechanically scanned by the *X–Y* stage. The substrate was placed on the other stage, so that the acoustic wave can be transmitted from its bottom side. It took typically 2–3 min for one observation. In order to save the time for data transfer from the oscilloscope to the computer, the waveforms through each X-scan were once stored in the oscilloscope using its fast-frame mode before being transferred through the LAN interface. In order to reduce random noise, three times of responses at the same point were averaged.

4. Results

4.1. Waveforms

Fig. 3 shows the reflected acoustic signals. In this particular case, a water droplet was used as the reference. A part of cerebellum tissue was used as a target. The signal from the target tissue is very similar to that from the reference, suggesting the acoustic impedance of the tissue is close to that of water (1.5×10^6 Ns/m^3). Fig. 4 shows the intensity spectrum of the target signal normalized by the reference signal, and cross power spectrum of the target and reference signals. The intensity spectrum is almost flat from 15 to 100 MHz, the intensity being a little smaller than 1.0. It indicates that the impedance of the target is somehow different from the reference. The calibration method for the acoustic impedance will be described in the following section. The cross-power spectrum of the target and reference signals shows that a wideband acoustic signal had been successfully generated.

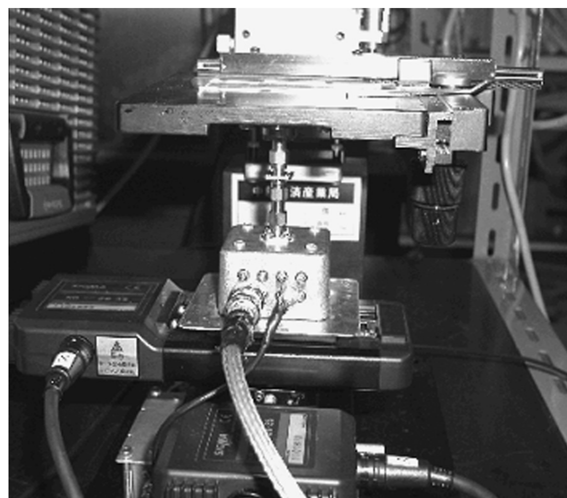

Fig. 2. Overview of a part of the experiment system.

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