2P5-7

Improvement of reference signal generation method for larger-area measurement using scanning acoustic microscopy

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1. Background

Scanning acoustic microscopy (SAM) equipped with an ultrahigh-frequency ultrasonic transducer is utilized to evaluate acoustic properties at a cellular resolution. SAM provides two-dimensional acoustic property images by repeatedly measuring echo signals from arbitrary points within a sample with two-dimensional mechanical scanning of a single-element transducer. The spatial interval of the mechanical scan is determined according to the spatial resolution of the ultrasound transducer. In other words, finer spatial resolution necessitates finer intervals for measurement. However, this leads to a longer measurement time per unit area. Our group has successfully expanded the measurement area with the development an optimized speed of sound (SoS) estimator for a larger sample (i.e. 10 mm x 10 mm).[1)]

This paper proposes a method which incorporates a planar compensation technique using smoothing splines to compensate non-linear and non-periodic fluctuations in the distance between the transducer and the slide glass that occur during large-area measurements.

2. Methods

2.1 Data Acquisition

A paraffin-embedded healthy rat liver was used for this study. The sample was sectioned into continuous slices with a thickness of 8 μm and aligned on a slide glass. All animal protocols were approved by the Animal Experiment Committee of Chiba University (No.6-244).

A custom-built SAM system was used for this study. A ZnO transducer (HT-400C, Honda Electronics) with a center frequency of 300 MHz was excited using a pulsar (GZ1120ME-03, GEOZONDAS). Three-dimensional RF echo signals were acquired by scanning the transducer in two dimensions at a scan interval of 4 μm on the top surface of a thin slice sample, anchored in a stable-temperature water bath at 36°C. The RF data were recorded using an A/D board (ATS9373, Alazartech) with a sampling frequency of 4 GHz and 12-bit quantization.[2)] The measurement area comprised 10,000 points (40 mm) in the width direction and 1,350 slices (5.4 mm) in the lateral direction, with a total acquisition time of 4 hours. Following ultrasonic measurement, the specimen was H&E-stained, and a digital pathology image was captured using a virtual slide scanner (NanoZoomer S2.0-HT, Hamamatsu Photonics) to observe tissue structures.

2.2 Speed of Sound Analysis

SoS calculation requires the RF echo signals from both the regions where the sample is present and absent within the same scanning region. The intensity (amplitude of the RF signal) at the measurement points determines the sample's location. The intensity decreases at the locations where the sample is present due to attenuation caused by the sample’s transmission. Thresholding was applied using Otsu's binarization. This binarization finds the signal from the slide glass. The signal from this area was defined as the reference signal. Ideally, the slide glass and the ultrasonic transducer should maintain a certain distance, ensuring that the slide glass remains flat and the transducer is normal to the surface. However, maintaining consistency at the wavelength scale of 5.4 μm (@280 MHz) is challenging; thus, the obtained signal was shifted in the frequency domain to construct a pseudo-plane (see Sec.2.3). After planar compensation for each X-axis (width direction), the waveforms were separated by applying an eighth-order autoregressive (AR) model using the reference signal.[3)] The SoS was subsequently calculated from the echoes at the sample surface and back, producing a two-dimensional SoS map.

2.3 Planar Compensation Using Smoothing Splines

Since the position of the glass under the sample cannot be determined by SoS alone, it must be estimated using the surrounding reference signals. Different from the conventional measurement, the 40 mm-wide scans performed in this study cannot ignore the deformation of the slide glass and the irregularities of the mechanical stage measurement surface. Hence, compensation was required to account for fluctuations in the distance between the

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transducer and the glass surface. The conventional two-dimensional planar compensation based on three-dimensional echo data was impractical given that large-area measurements require significant time for each slice. Consequently, planar compensation was performed for each slice. The time difference between the signal of the raster in the glass region of the RF signal of an arbitrary slice and the leading raster was calculated in the frequency domain. The time difference in the region where the sample is present was estimated using a smoothing spline. Unlike cubic splines, smoothing splines allow for drawing an average curve by controlling the smoothing parameter p in point clouds with many outliers.

3. Results and Discussion

Figure 1 presents the pathology (**Fig. 1a**) and analysis results. The intensity image (**Fig. 1b**) displays morphologically consistent with that of the stained image. Additionally, the five serial sections aligned in a row exhibited similar shapes. The binarized image (**Fig. 1c**) corresponded to the shapes observed in the stained image and effectively detected the sample's position. The SoS values demonstrated a relatively consistent distribution across the five sections. (**Fig. 1d**)

Figure 2 shows the two-dimensional image of the RF signal. The cross-section is indicated by the red horizontal line in Figure 1. **Figs. 2(a)** and **(b)** display the RF signal before and after compensation, with the vertical axis representing time and the horizontal axis representing the scanning direction. The red and blue markings at the top of the image show the glass and sample regions. The uncorrected RF signal shows a concave shape (greater distance from the transducer at the center), but a generally flat plane was observed after compensation. The estimated thickness (**Fig. 2c**) showed a value of around 9 µm, while the estimated SoS (**Fig. 2d**) exhibited values distributed around 1550 m/s and 1650 m/s.

Figure 3 illustrates the power spectrum averaged from the complex spectrum of the glass echo after planar compensation for SoS estimation and the tentative reference point. Comparing the tentative reference point (black) with the average reference spectrum, the frequency bands are equivalent, and the noise components are reduced compared to the tentative reference point. These findings suggest that planar compensation using smoothing splines is effective for SoS estimation in large-area echo data.

Acknowledgment

We thank Mr. Yuki Kurita of the Advanced Research Facilities & Services (ARFS), Hamamatsu University School of Medicine, for technical assistance. This work was partly supported by JSPS Core-to-Core Program JPJSCCA20240003, KAKENHI 23H03758 and Chiba University Institute for Advanced Academic Research.

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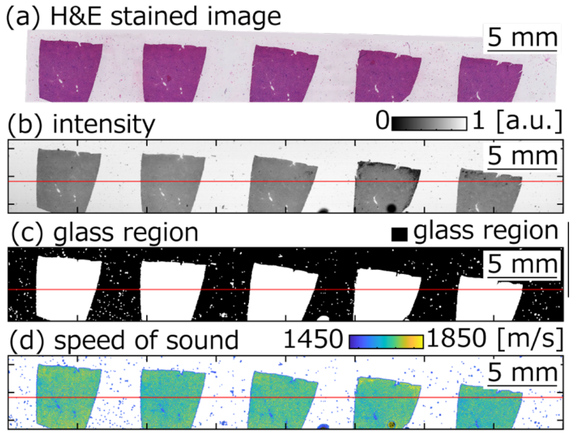

Fig. 1 Pathological photo and SAM measurement results.

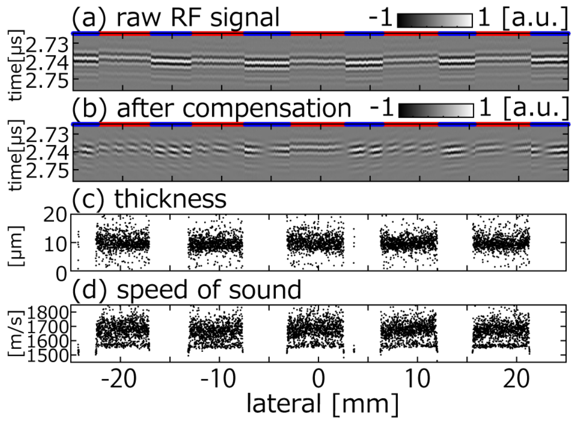

Fig. 2 Representative slice RF data and estimation results. (a) RF signal before compensation (b)(a) RF signal after compensation (c) estimated thickness (d) estimated SoS

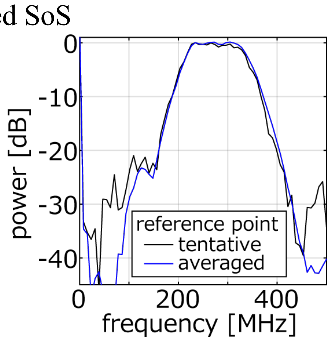

Fig.3 Frequency dependency of reference signal