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Evaluation of Relationship between Liver Pathological Structure and Speed of Sound of Longitudinal Wave

肝臓の病理学的構造と縦波音速との関係性の評価

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1. Background

Scanning acoustic microscopy (SAM) system equipped with ultra-high-frequency ultrasound (> 100 MHz) is being used to evaluate the acoustic charactaristics of biological tissue with high resolution of several micrometer (cell level) [1]. In many cases, it was possible to evaluate only the edge of the sample because the measurement and analysis area is narrow (several hundred micrometer square). In order to solve these problems, we constructed the self-made SAM system that can measure the macro size (up to 100 mm square) of the entire sample with a micro size (several μm^2 per pixel), and proposed a highly stable analysis method that can handle a wide measurement area.

In this study, we report the examination results of speed of sound (SoS) of sliced rat liver analyzed with multi-frequency ultrasound (using three types of transducer) from the acquiring radiofrequency (RF) echo signals observed by our self-made SAM system, and acoustic characteristics were analyzed based on the tissue structure. To compare acoustic characteristics between different frequency obzavation, we used registration with pathological (PT) image.

2. Materials and Methods

2.1 Data acquisition

To acquire RF echo signal from a sliced specimen put on the glass plate, a transducer on the moving stages was scanned in two dimension (2D). In this observation, after scanning for the direction of X-axis in each scan line, the RF echo signal were transferred from the digitizer (HDO6104, Lecroy) to a computer, and then the transducer was moved for Y-axis direction.

In our SAM system, the minimum to the maximum moving pitch of X-Y stage is 0.1 μm to 100 mm. The center frequency of transmission and reception can be accepted from 1 to 500 MHz. For this study, a PVDF-TrFE transducer (Toray Engineering Co.) with a center frequency of 60, 80 MHz, and a ZnO transducer (Fraunhofer IBMT Co.) with a center frequency of 250 MHz were used. The spatial resolutions at -6 dB bandwidth of each transducer are 26, 20 and 7 μm, respectively. The amplified RF echo data (by AU-114-BNC, MITEQ) of each scan line were acquired with the sampling frequency of 2.5 GHz and digitized with 12-bit.

An excised normal and fibrosis liver of 16-week-old rat (Slc:SD, male, mixture of carbon tetrachloride was injected twice a week for ten weeks to fibrosis model rat) was fixed with formalin and sliced with 10 μm using paraffin embedding method. After removing the paraffin, that sliced tissue putted on a glass plate was put in the water tank (100 mm in lateral * 50 mm in depth * 10 mm in height) filled with degassed water.

The scanning interval for both X and Y direction was 2 μm. After scanning, the sliced tissue was stained with Hematoxylin-Eosin (HE) or Masson-Trichrome (MT) method, and a digital PT image was observed using a virtual slide scanner (NanoZoomer S60, Hamamatsu Photonics).

2.2 Speed of sound analysis

In the 3D RF echo data, the A-mode echo signal at each measurement point was up-sampled 10 times, and intensity map was acquired by normalizing with the maximum value of each X-axis in the X-Y scan. In addition, after correcting the phase on each X-axis, SoS analysis was performed by applying autoregressive (AR) model of 5 order to obtain 2D SoS map [2]. SoS values were caluculated from RF echo including not only returnning echo from glass but also from surface and glass through the sample.

2.3 Registration method

Using 2D SoS map obtained in Section 2.2 as

a reference, registration with 2D PT image of the same sample was performed, and the relationship between the accoustic characteristics and the tissue structure was confirmed. As a procedure, because of the amount of PT image was large, downsampling is performed so that the pixel size (approximately 2 μm) is the same as the scanning interval of SAM system. After that, the PT image was grayscaled, and feature point (blood vessels, etc.) were extracted from both the intensity map and PT image. Using these feature point information, PT image was affine transformed so that Jaccard index in intensity map and PT image was maximized [3].

3. Results and discussion

Figure 1 shows enlarged view of SoS maps (1.0×1.0 mm^2) of normal model and fibrosis liver using 60 MHz **(a-1)**, **(b-1)**, 80 MHz **(a-2)**, **(b-2)** and 250 MHz **(a-3)**, **(b-3)** transducer. **Fig. 1 (a-4)**, **(b-4)** show the PT image corresponding to SoS maps. The pixel which SoS was less than 1480 m/s is painted as black.

Figure 2 shows the box-plots diagram of SoS in pathological structure which picked out from **Fig. 1**. This box-plots excluded the value which SoS was less than 1480 m/s as glass part and more than 1900 m/s as error.

It can be confirmed that the difference of texture of SoS map between 60, 80 and 250 MHz caused from the difference of the spatial resolution (**Fig. 1 (a-1) - (a-3)**, **(b-1) - (b-3)**). And SoS value which acquired by lower frequency transducer was lower (**Fig. 2**). This is because how much the tissue structure is refrected in the evaluation results depends on the resolution of each transducer. For example, using transducer with a center frequency of 250 MHz, SoS can be evaluated at the cellular level, so the properties of hard tissues such as cell nuclei can be confirmed. Because of the resolution of other transducers are low, it is evaluated as the average characteristic of multiple tissues, and as a result, SoS value is assumed to be low.

4. Conclusion

By examining the results at lower frequencies, it is possible to confirm the frequency dependence of SoS evaluation, mainly the resolution dependence. This makes it possible to understand the relationship between echo signal characteristics at the clinical level and acoustic properties at the micro level.

Acknowledgment

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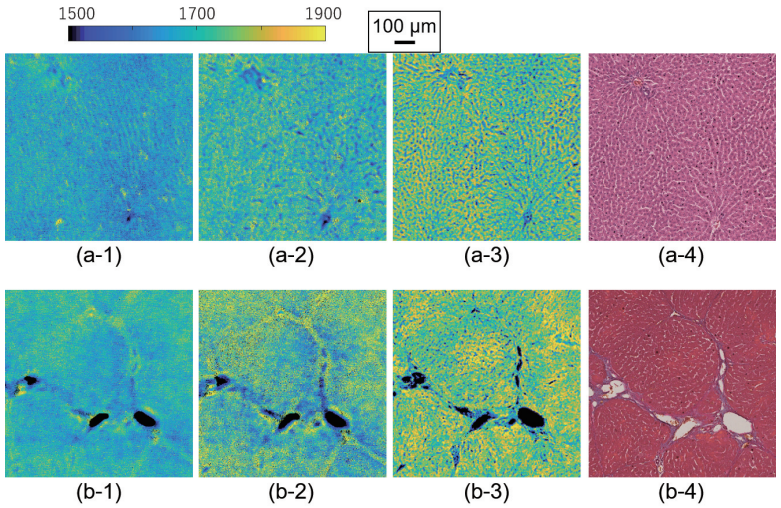

Fig. 1 Observation and analysis results of rat liver. Enlarged view of SoS maps of normal model liver using 60 MHz (a-1) 80 MHz (a-2) and 250 MHz (a-3) transducer, and corresponding PT image (a-4). And enlarged view of SoS maps of fibrosis model liver using 60 MHz (b-1) 80 MHz (b-2) and 250 MHz (b-3) transducer, and corresponding PT image (b-4).

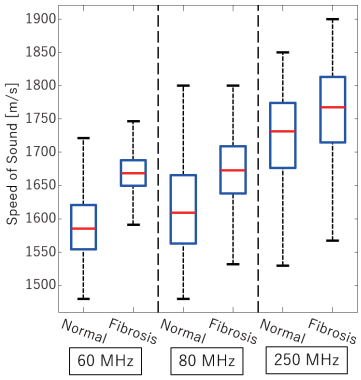

Fig.2 Box-plots of SoS map that excluded the value which SoS was less than 1480 m/s as glass part and more than 1900 m/s as error.