# Evaluation of relationship between liver structure and frequency dependency of speed of sound and attenuation

肝臓の構造と音速・減衰の周波数依存性の評価

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## 1. Background

Many studies for quantitative ultrasound (QUS) assessment at frequency band of clinical studies (1-15 MHz) and high frequency band (15-50 MHz) have been reported independently. Additionally, evaluation of acoustic characteristics of biological tissues by ultra-high frequency (50-250 MHz) using scanning acoustic microscopy (SAM) are also progressing, and we aim to understand the acoustic characteristics and scattering characteristics that integrate them.

In this study, we evaluated speed of sound (SoS) and attenuation of rat livers in wide frequency band (1-300 MHz), and confirmed the relationship between the acoustic characteristics and the tissue structures of the livers.

## 2. Materials and Methods

### 2.1 Data acquisition

Measurement objects were three excised mild fatty rat livers. For under 50 MHz evaluation, RF data were acquired by two methods, the through transmission method and the reflection method.

In the measurement by the through transmission method, a pair of single element unfocused PZT transducers (OLYMPUS) with the center frequency of 3.5 MHz were employed. In a temperature-controlled water bath, two transducers for transmission and reception were placed facing each other with the liver sandwiched between them, and measurements were taken at any 10 points on the liver. The received RF signal were sampled and digitized using an oscilloscope (HDO6104, Lecroy) with a sampling frequency of 100 MHz and 12-bits quantization.

In the measurement by the reflection method, the excised rat livers were placed at a position 10 mm away from the acrylic plate settled in the temperature -controlled water bath. Four single element unfocused PZT transducers (OLYMPUS) with center frequencies of 3.5, 5, 10, and 15 MHz were used for measurement, and the recording conditions were the same as for the through transmission method. The scanning step of each transducer was 100 μm, and three-dimensional (3D) echo data sets with the depth of 60 mm (4,096 samples) and the orientation of 5 mm × 3 mm (51 × 31 lines) were acquired for each liver sample.

For 300 MHz evaluation, sliced samples with a thickness of 10 μm were measured. The sample was prepared by immersing the excised liver in a sucrose solution having a concentration of 30% for 24 hours after fixing it in formalin, embedding it in an OCT compound, instantly freezing it in liquid nitrogen via isopentane, and then slicing it. A ZnO transducer (HT-400C, Honda Electronics) with center frequency of 300 MHz was used. The scanning step was 4 μm, and 3D echo data with a depth of 768 μm (512 samples) and an orientation of 7.5 mm × 10.3 mm (1,900 × 2,600 lines) was acquired. The received signal of each scan line was sampled and digitized using an A/D board (ATS9373, Alazartech) with a center frequency of 4.0 GHz and 12-bits/sample quantization.

### 2.2 Speed of sound and attenuation analysis

For 1 to 50 MHz evaluation by the through transmission method, the SoS and the attenuation coefficient were calculated using the signal that passed through the sample and the signal that passed through only water. In the evaluation by the reflection method, the attenuation coefficient was calculated using the reflected signal from the acrylic plate that passed through the sample and the reflected signal from the acrylic plate that did not pass through the sample [1]. In addition, the SoS was calculated using the signal used to calculate the attenuation coefficient and the signals of the surface and back of the sample that were determined by detecting the maximum amplitude of the recorded data.

For 300 MHz evaluation, the A-mode echo

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signal at each scanning line was up-sampled 10 times, and intensity map was constructed by normalizing with the maximum value of each longitudinal scan line. In addition, after correcting the phase on each longitudinal scan line, the SoS was calculated from the echo signals from surface and back of the sample that detected from the original echo data by applying autoregressive (AR) model of 5 order to obtain 2D SoS map [2].

**Figure 1** shows an example of the enlarged view of SoS map of mild fatty liver analyzed using 300 MHz transducer (**Fig. 1 (a)**) and the HE stained histological image (**Fig. 1 (b)**). Since each histological image was created by staining the unstained sliced sample used for ultrasonic evaluation after measurement, the SoS map and the histological image represent exactly the same tissue.

## 3. Results and discussion

**Figure 2** shows the SoS of the mild fatty rat livers evaluated by the through transmission method with 3.5 MHz and the reflection method with 3.5, 5, 10, 15, and 300 MHz. There was no significant difference in SoS between the through transmission method and the reflection method under 15 MHz, however, SoS was fast in 300 MHz. In the previous study, SoS of the normal liver after sucrose substitution was similar to that in the frequency band of 3.5 to 50 MHz even in the ultra-high frequency band [3]. Therefore, it can be said that the characteristics of the measured fatty liver sample are shown in this result.

**Figure 3** shows the attenuation coefficient of the mild fatty rat livers evaluated by the through transmission method and the reflection method. Although multiple methods for preparing sliced specimens were tried, evaluation at 300 MHz was not performed because no sample that could be used for measurement was obtained. In the result of the reflection method, the frequency dependency is shown. However, it is desirable to evaluate by the through transmission method even in the high frequency band because there is a big difference of calculated attenuations from the through transmission method that can be evaluated with higher accuracy.

## 4. Conclusion

For the fatty rat liver, the frequency dependency of SoS and attenuation in a wide band was evaluated, however, the evaluation stability in the ultra-high frequency band for SoS and the evaluation accuracy by the reflection method for attenuation analysis remained at the stage where doubts remained. In this study, we made a new attempt to evaluate tissues containing a large amount of fat, however, sufficiently stable evaluations could not achieve because the protocol for preparing measurement samples has a large effect on fatty liver case. In current, we are reviewing the sample preparation protocols and reexamining the normal liver with few unstable factors. The evaluation of the liver containing fat and fibers will reconsider after that.

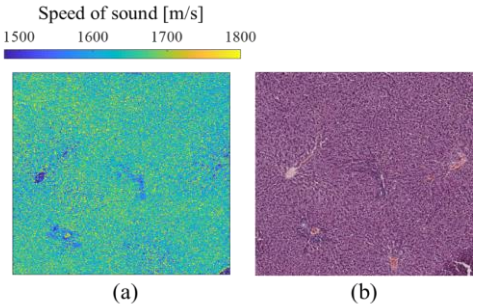


Fig 1. 2D speed of sound map of fatty rat liver (a), and histological image (b).

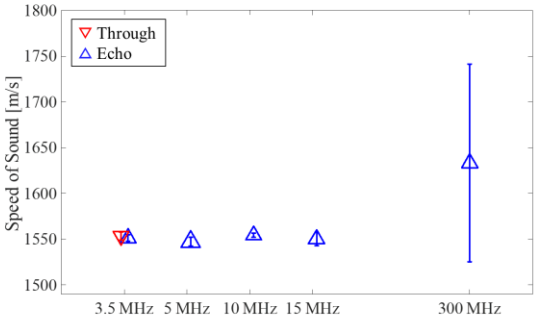


Fig 2. Speed of sound of fatty rat livers.

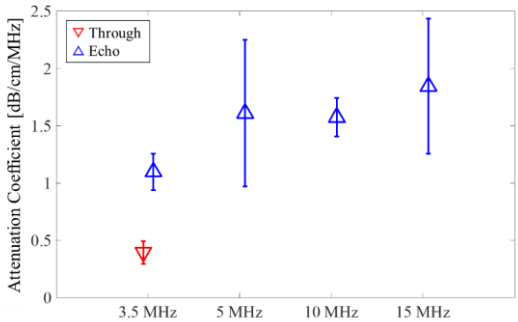


Fig 3. Attenuation coefficient of fatty rat livers (not including 300 MHz).


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