# 2Pa5-7

# Evaluation of frequency dependence of speed of sound of liver in clinical to microscopic frequency band

臨床から顕微レベルの周波数帯における肝臓音速の周波数依存性の評価

Mai Ino[1‡], Kazuma Noguchi[1], Wakana Saito[1], Kenji Yoshida[2], and Tadashi Yamaguchi[2], ([1]Grad. Sc. Sci. Eng., Chiba Univ.; [2]Center for Frontier Medical Engineering, Chiba Univ.)

伊能 舞[1‡]，野口 和馬[1]，西東 若菜[1]，吉田 憲司[2]，山口 匡[2] ([1]千葉大院 融合理工，[2]千葉大 CFME)

## 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 tissue by ultra-high frequency (50-250 MHz) using scanning acoustic microscopy (SAM) are also progressing. In this study, we evaluated speed of sound (SoS) of tissue mimicking phantoms and rat livers in wide frequency band (1-250 MHz), and clarified the relationship between SoS and tissue structure from macroscopic to morphological cell level.

## 2. Materials and Methods

### 2.1 Data acquisition

Measurement objects were 9 phantoms those included spherical shaped scatterers; mean diameters of 20 μm; concentrations of scatterers at 0 to 10.0 wt%, and excised normal and fibrosis rat livers. Each phantom was cut out as a block phantom (90 × 40 × 10 $mm^3$) for clinical and high-frequency band (1 to 50 MHz) evaluation, and as a slice phantom (thickness of about 50 μm) for ultra-high frequency band (60 to 80 MHz) evaluation.

For clinical and high-frequency band (1 to 50 MHz) evaluation, the block phantom and the excised normal and fibrosis livers of 16-week-old rat (Slc:SD, male, mixture of carbon tetrachloride was injected twice a week for ten weeks to fibrosis model rat) were fixed at a position 1 cm away from the acrylic plate that was the base, placed in a water bath for the scanning. four single element unfocused PZT transducers (OLYMPUS) with center frequencies of 3.5, 5, 10, and 15 MHz were used for measurement. The RF echo data were sampled and digitized with 100 MHz and 12-bits/sample, respectively. The scanning step was 200 μm.

For ultra-high frequency band (60 to 80 MHz) evaluation, the sliced phantom putted on a glass plate was put in the water tank filled with degassed water. The rat livers were 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 filled with degassed water. A PVDF-TrFE transducer (Toray Engineering) with a center frequency of 60, 80 MHz, and a ZnO transducer (Fraunhofer IBMT) 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 RF echo data were sampled and digitized with 2.5 GHz and 12-bits/sample, respectively. The scanning step was 2 μm. After scanning, the sliced rat liver 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 the SoS of phantoms evaluated with 3.5, 5, 10, 15, 60, 80 MHz. It was confirmed that SoS is fast according to the concentration of scatterers. Frequency dependence of SoS is small. Extremely microscopic phantom inhomogeneities are reflected especially at ultra-high frequencies.

**Figure 2** shows enlarged view of SoS maps ($1.0 \times 1.0$ 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. 2 (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 3** shows the box-plots diagram of SoS in pathological structure which picked out from **Fig. 2**. 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. 2 (a-1) - (a-3)**, **(b-1) - (b-3)**). And SoS value which acquired by lower frequency transducer was lower (**Fig. 3**). This is because how much the tissue structure is reflected 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. The large difference between 15 MHz and 60 MHz is supposed to be due to formalin fixation.

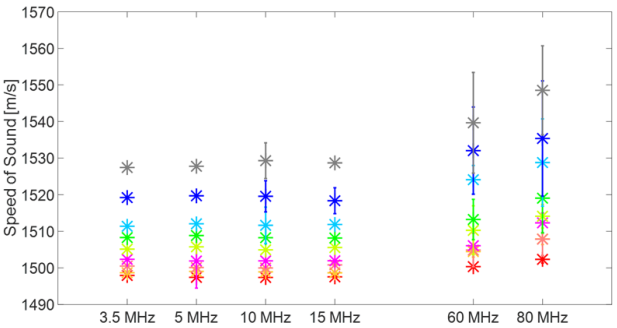


Fig 1. SoS value of phantoms (not including 250 MHz)

## 4. Conclusion

We constructed a systems and methods for evaluating the frequency dependence of the SoS of living tissue in an ultra wide band, and used it for evaluating the SoS of phantoms and rat livers. Due to the low resolution of clinical frequency band transducers compared to the structure of living tissue, SoS is evaluated as an average property of multiple tissues including soft cytoplasm. Evaluation of frequency dependence of SoS in various tissues such as kidneys and phantoms in which multiple scatterers are mixed is underway.

## Acknowledgment

This work was partly supported by JSPS Core-to-core Program, KAKENHI 19H04482, 19H04436 the Institute for Global Prominent Research at Chiba University.

## References

1. S. Irie et al.: J. Acoust. Soc. Am. **139** (2016) 512.
2. N. Tanaka: IEICE Tech. Rep. **105** (2005) 21.
3. T. Ohnishi et al.: MiCCAI COMPAY 2018 Workshop, LNCS vol. 11039, 103-111, 2018

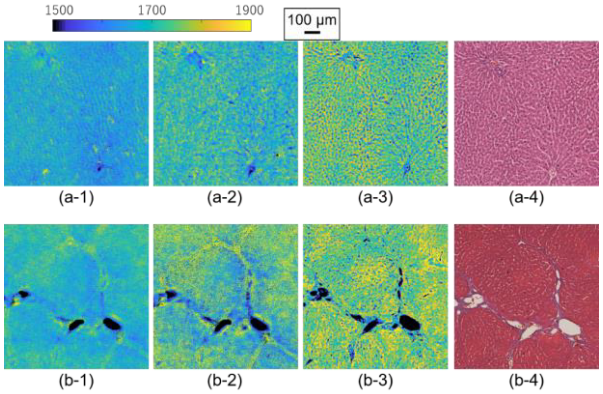


Fig 2. Observation and analysis results of rat liver. Enlarged view of SoS maps of normal model liver using 60 MHz (a-1) 80 MHz (a-2) and 250 MHz (a-3) transducers, corresponding PT image (a-4), enlarged view of SoS maps of fibrosis model liver using 60 MHz (b-1) 80 MHz (b-2) and 250 MHz (b-3) transducers,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

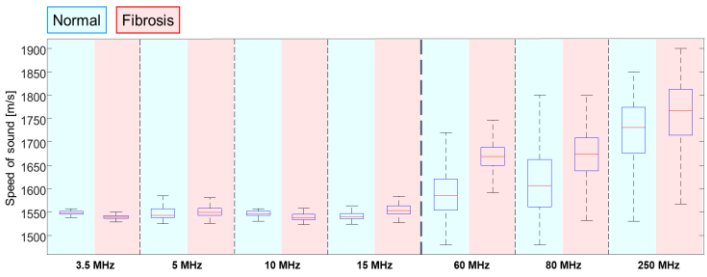


Fig 3. Box-plots of SoS of normal and fibrosis rat livers.

‡m_ino@chiba-u.jp, *yamaguchi@faculty.chiba-u.jp