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Speed of Sound Analysis from Micro and Macro Size by Multi-Frequency Ultrasound Microscopic Measurement

複数の高周波超音波顕微鏡計測によるミクロおよびマクロサイズの音速解析

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1. Background

There are many studies for the evaluation of acoustic characteristics of biological tissue using several hundreds MHz band ultrasound which is possible to discriminate different cell organelles[1] and using several tens MHz band ultrasound which is possible to discriminate different microstructure of organs. However, there is no study to evaluate the difference of the acoustic characterintisc between several tens MHz and several hundred MHz in detail.

In this study, we report the examination results of speed of sound of sliced rat organs analyzed with multi-frequency ultrasound from the acquiring radiofrequency (RF) echo signals observed by our self-made scanning acoustic microscopy (SAM) system.

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, and the maximum position error was 429 nm. The center frequency of transmission and reception can be accepted from 1 to 500 MHz. For this study, a PVDF-TrFE transducer with a center frequency of 80 MHz, and a ZnO transducer with a center frequency of 250 MHz were used. The spatial resolutions at -6 dB bandwidth of both transducer are 20 μm 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 kidney of 17-week-old rat (Slc:SD, male) was fixed with formalin, and sliced with 7 or 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 10 μm in 80 MHz, and 4 μm in 250 MHz. After scanning, the sliced tissue was stained with Hematoxylin-Eosin (HE) method, and a digital pathological image was observed using a virtual slide scanner (NanoZoomer S60, Hamamatsu Photonics).

2.2 Speed of sound analysis

The signal from the glass surface of two positions where no tissue exists which can be observed in each scanning line (X-axis) were manually selected as the reference signals to calculate the speed of sound. Linear inclination correction with respect to the inclination in the direction orthogonal to the transducer was performed using the phase difference and the intensity difference of the RF echo signals of the glass portion as an index every one scanning. After that, autoregressive (AR) model of 5 order was applied to normalized power spectrum (measurement signals/reference signals) of each RF echo to separate the signals from the surface of the sliced tissue, bottom of the tissue (surface of the glass plate) and others. After separation of echo signals, SoS was calculated from the phase of signals from the surface and the bottom of the tissue[2].

2.3 Comparison of speed of sound

The simple moving average method and the

weighted moving average method for 3*3 pixels were used to compare the SoS values analyzed from RF echo observed by 80 and 250 MHz.

The characteristics of 250 MHz transducer was allowed as the weighted of 2D Gaussian distribution where the sum of the established densities is 1 (μ=[0 0], σ=[0.33 0; 0 0.33]). A pixel which has SoS value of less than 1480 m/s was defined as glass, and a pixel of more than 2000 m/s was defined as eliminated as error.

3. Results and discussion

Figure 1 shows SoS map of 80 MHz (a), 250 MHz (b), enlarged view (240 μm *240 μm) of SoS maps of glomerulus in 80 MHz (c) and 250 MHz (d), analysis results of simple moving average method (e) and weighted moving average method (f). The scanning area for a sample shown in Fig. 1 (a) and (b) was 21 mm *16 mm including the size of whole kidney. The pixels which SoS was less than 1480 m/s is painted as black. The internal structure of glomerulus can be seen from each SoS map.

It can be confirmed that the difference of texture of SoS map between 80 MHz and 250 MHz caused from the difference of the spatial resolution and the scanning interval on Fig. 1 (c) and Fig. 1 (d). By using the simple moving average for analysis results of 250 MHz (Fig. 1 (e)), the texture of SoS map of the glomerulus seems close to the results at 80 MHz (Fig. 1 (c)). This is because the moving average processing corresponds to equalizing the spatial resolution of measurement in both of them. On the result of the weighted moving average process (Fig. 1(d)), the boundary information of the glomerulus remains as a value close to the SoS map at 250 MHz. From these results, it can be confirmed that even if the same frequency and the same scanning interval, different SoS maps are generated if the spatial resolution is different.

Figure 2 shows box-plot diagrams of SoS in glomerulus which picked out from **Fig. 1 (c)-(f)**. The average value of simple and weighted moving average method closed to that value of 80 MHz observation after each moving averaging process. However, the result of weighted moving average is more dispersed than simple moving average as mentioned above.

4. Conclusion

The SoS maps of whole rat kidney were made from observed RF echo signal of 80 and 250 MHz, and SoS values of the glomerulus were compared. It was confirmed the difference of SoS in two frequency was caused from the difference of micro tissue structure, because the weighted moving average of 250 MHz remained detailed structure from original SoS map of 250 MHz. In the current study, other organs such as liver is using as target tissues to examine the SoS analysis for subcellular tissue.

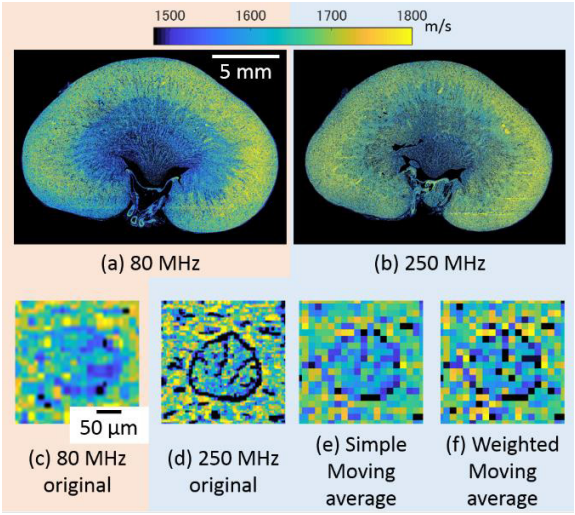

Fig. 1 Observation and analysis results of rat kidney. Whole kidney 2D SoS map of 80 MHz (a) and 250 MHz (b). Enlarged view of SoS maps of 80 MHz (c) and 250 MHz (d) in glomerulus, analysis results of simple moving average (e) and weighted moving average (f).

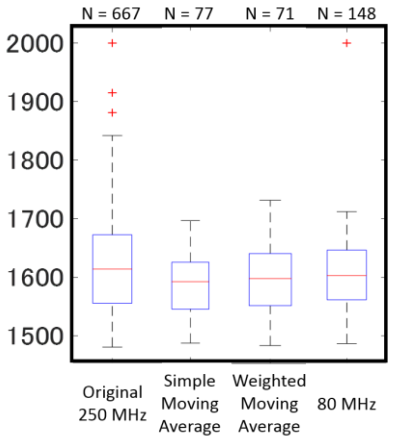

Fig. 2 Box-plot diagrams of SoS in glomerulus

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