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Basic Study on Speed of Sound Analysis in Multi-scale using Hundreds MHz Band Ultrasound

数百 MHz 帯超音波によるマルチスケールでの音速解析の基礎検討

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1. Background

Many studies for evaluating acoustic characteristics of biological tissue with several μm resolution have been reported, and it is possible to discriminate different cell organelles[1]. Because the measurement and analysis areas are limited on usulal scanning acoustic microscoy (SAM), there is no study to analyze acoustic characteristics from macro size like a whole organs to micro size such as several μm^2 resolution with ultra-high frequency ultrasound.

We report the examination results of acoustic characteristics using self-made SAM system equipped with ultra-high frequency ultrasound transducer and acquiring radiofrequency (RF) echo signals from whole areas of sliced specimens of rat organs.

2. Materials and Methods

2.1 Scanning acoustic microscopy system

Figure1 shows the overview of the scanning part of our self-made inverted SAM system. To acquire RF echo signal from a sliced tissue put on the glass plate, a transducer on the side of X-Y stage was scanned in two dimension (2D). In this examination, 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. The minimum to maximum moving pitch of X-Y stage is 0.1 μm to 100 mm, and the maximum position error was 429 nm.

In this SAM system, the center frequency of transmission and reception can be accepted from 1 to 500 MHz. A ZnO single-element transducer with a center frequency of 250 MHz (spatial resolution at -6 dB bandwidth is 7 μm) was used to this study. The amplified (AU-114-BNC, MITEQ) RF echo data of each scan line was acquired with the sampling frequency of 2.5 GHz and digitized with 12-bit.

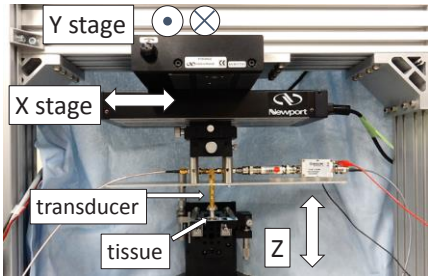

Fig. 1 Overview of self-made scanning acoustic microscopy system in the scanning part.

2.2 Materials

An excited kidney of 17-week-old rat (Slc: SD, male) was fixed with formalin, and sliced with 7 μm using paraffin embedding method. After removing the paraffin, the glass plate with the sliced tissue was put in the water tank (100 mm in lateral * 50 mm in depth * 10 mm in height) filled with degassed water. The scanning area was 21*16 mm^2 including the size of whole kidney, and the scanning interval for both X and Y direction was 4 μm. After scanning (the time of scanning was about 360 min.), the sliced tissue was stained with Masson-Trichrome (MT) method, and a digital pathological image was observed using a virtual slide scanner (NanoZoomer S60, Hamamatsu Photonics).

2.3 Speed of sound analysis

There are some problems to acquire ultra-high frequency RF echo signals in the wide area: it takes a long time to scan, and the distance from the transducer to the sliced tissue is not constant because of the mechanical displacement difference (eg. from expansion and contraction of column support due to slight temperature change). Moreover, time lag, i.e. phase shifting, is occurred in the short term such as X-axis scanning or Y-axis moving, because of the inclination of the glass plate in X-Z or Y-Z plane.

These time (distance) lags are about several nm, so this phase shifting can be possible to correct. However, the distance lag in the long term may be larger (from several μm to several 10 μm,) than in the short term scanning. If distance lag in the long term overly exceeds the resolution range in depth direction or the thickness of the sliced tissue, it assumes that large error occurs in the analysis of speed of sound (SoS).

Therefore, the short term inclination in the direction of X-Z plane was linearly corrected using the phase and the intensity difference of the echo signal from glass plate areas. Moreover, to consider the nonlinear fluctuation for the direction of Y-axis, normalized power spectrum (measurement signals/reference signals) was calculated using the same scanning line in Y-axis as reference signals after correcting linear inclination. Autoregressive (AR) model of 5 order was applied to each normalized power spectrum to separate the RF echo signals from the surface with from the background of the sliced tissue. After separation of echo signals, SoS was calculated from the phase of above 2 component signals. Thus, it was possible to calculate SoS without the linear fluctuation in the short term scanning and nonlinear fluctuation occurred in the long term[2].

3. Results and discussion

Figure 2 shows the intensity image, i.e. the positive maximum of received RF echo signal (a),

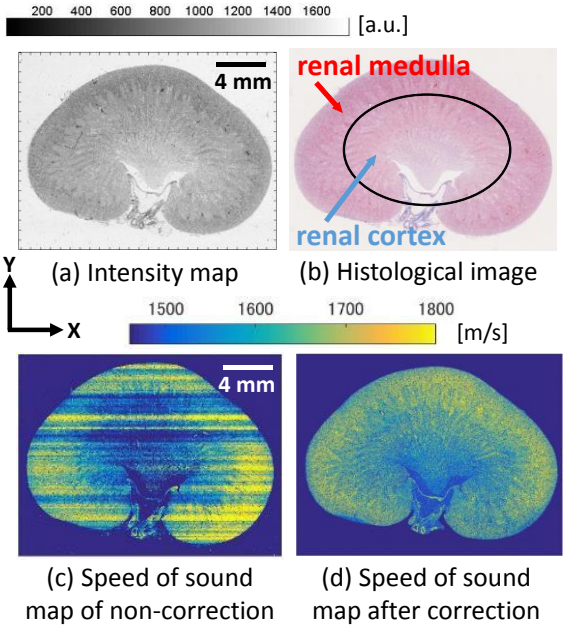

Fig. 2 Observation and analysis results of rat kidney. Intensity map (a) and corresponding histological image (b). Out of circle represents renal cortex, and inside the circle represents renal medulla. 2D SoS map of non-correction (c) and after correction (d)

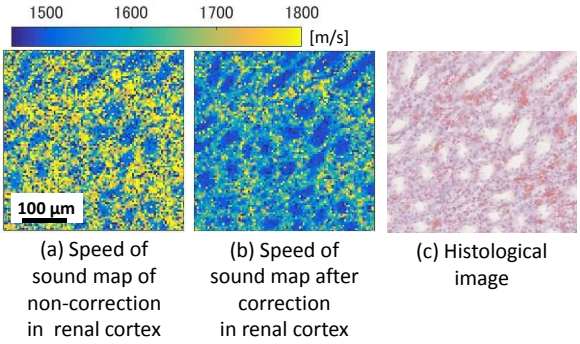

Fig. 3 Enlarged view of 2D SoS maps and corresponding histological image.

corresponding pathological image (b), SoS map of phase non-correction (c), and SoS map after phase correction (d). The texture of histopathological difference, e.g. a renal cortex of skin part, its internal, a blood vessel, and a ureter, can be seen from the intensity image. Although the discontinuous texture is especially confirmed in Y-axis of **Fig. 2(c)**, the continuous SoS map can be obtained in **Fig. 2(d)** by correcting the method of calculating SoS.

Figure 3 shows 400*400 μm^2 area in the kidney cortex in **Fig. 2**. The average ± standard deviation of SoS before and after the phase correction are 1715.6 ± 158.8 m/s and 1591.7 ± 106.8 m/s in renal cortex. The average value in the correction method is close to biological SoS in a normal kidney[3], and the standard deviation is also lower than the non-correction method.

4. Conclusion

Using a self-made SAM system with 250 MHz transducer, SoS analysis in several 10 mm^2 region of biological specimens can be accomplished. In the next step, because the depth resolution of ultra-high frequency transducer has narrow region (several 10 μm), it is necessary to suppress the mechanical displacement of the scanning system in the long term scanning, e.g. enhance stability of the metal portion not to expand and contract with temperature swings.

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