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A study on measurement of blood vessel tissue by acoustic microscope using high-frequency range probe

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1. Introduction

Acoustic microscopes can obtain acoustic properties of biological tissues and cells non-invasively.[1)] However, its spatial resolution in the several hundred MHz range is lower than that of optical microscopes; thus, determining fine structures is difficult. To improve the resolution, it is necessary to increase the operating frequency of the ultrasonic probe. Therefore, we have investigated a method for measuring the high-resolution sound speed distribution using high-frequency probes. The sound speed and thickness are calculated by the pulse spectrum method,[2)] but when measuring thin tissues with high attenuation, the errors become large.[3)] In this study, the effect of attenuation on the estimated sound speed is discussed by measuring a thin biological tissue specimen and simulating the measurement signals.

2. Principle and method

The sound speed c and thickness d can be simultaneously estimated using the pulse spectrum method by measuring the reflected wave from the specimen, s_{tgt}, consisting of the reflected waves from the front surface (s_{s}) and back surface (s_{d}), and that from the substrate, s_{ref}, as the reference signal.[2)] The sound speed c and thickness d are calculated using the frequency f_{m}, phase ϕ_{m}, and the number of phase rotation, n (n: positive integer), at the local maxima or minima of the normalized amplitude spectrum, which is normalized S_{tgt} with S_{ref} in the frequency domain. When using a high-frequency range probe, the normalized amplitude spectrum in the low-frequency range cannot be obtained owing to the limited bandwidth. In such cases, the sound speed c may not be estimated accurately owing to the incorrect estimation of the number of phase rotations n. To address this issue, the periodicity of the interference components between S_{s} and S_{d} was used to estimate n. Furthermore, it is also important to appropriately estimate f_{m} and ϕ_{m} for measuring sound speed and thickness. However, when measuring biological tissues, it may be difficult to estimate f_{m} caused by the noise and low-frequency components in the normalized amplitude spectrum owing to the heterogeneity and high attenuation of biological tissues. Therefore, f_m was obtained by removing the noise component using a local parabolic approximation.[4)]

3. Experiment

The sound speed and thickness of the tissue section, which was the carotid artery of a goat embedded in paraffin sectioned to a thickness of approximately 5 µm, were measured. Ultrasound and optical images were simultaneously acquired by placing an ultrasound probe and optical lens above and under the specimen, respectively. An ultrasound probe using a ZnO transducer with a center frequency of 250 MHz was used. The two-dimensional distributions of sound speed and thickness were measured over a 2.4 mm × 2.4 mm area at intervals of 8 µm × 8 µm. The sound speed in water was assumed to be 1480 m/s.

4. Result and Discussion

Figure 1 shows the two-dimensional distribution of the sound speeds. The average thickness was 6.0 µm, which was greater than the nominal thickness of the tissue section (approximately 5 µm). The sound speed cannot be accurately determined at points where the thickness deviates significantly from 5 µm because it was calculated using the thickness value. The cause was investigated through a numerical simulation of the normalized amplitude spectra. **Figure 2** shows the amplitude spectrum and trajectory on the Gaussian plane of the normalized spectrum at point A (c = 1985 m/s and d = 6.4 µm). The normalized amplitude

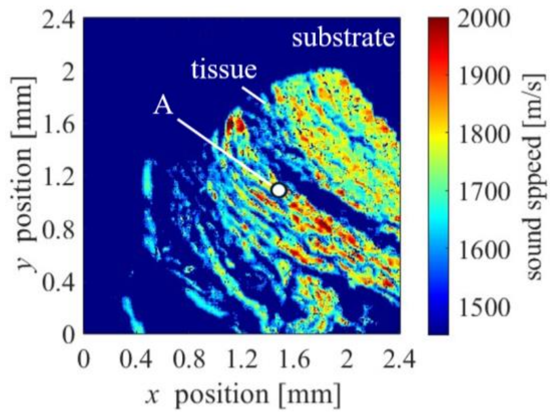

Fig. 1. Two-dimensional distribution of sound speeds of the carotid artery of a goat.

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spectrum showed low periodicity because the attenuation in the specimen caused changes of the spectrum shape then the fluctuations in the frequency at which extrema occurred. These frequency fluctuations led to estimation errors in the sound speed and thickness because they were estimated using the frequencies at extrema and the corresponding phases. **Figure 3** shows the normalized amplitude spectrum, normalized phase spectrum, and estimated sound speed corresponding to each phase rotation number *n,* obtained from numerical simulations with varying attenuation coefficients α, assuming c = 2000 m/s and d = 5.0 μm.[5)] Here, α_{w} is the attenuation coefficient of water. From Fig. 3(a), it was confirmed that larger attenuation coefficients led to greater fluctuations in the frequency at which extrema occurred, resulting in larger estimation errors in sound speed. Furthermore, as shown in Figs. 3(b) and 3(c), the errors in the phase and sound speed were larger in the high-frequency range. This was caused by the amplitude of S_{s} becoming larger than that of S_{d} in the high-frequency range around n = 5. In the case of high attenuation in the specimen, the amplitude of the reflected wave from the back surface, S_{d}, decreases as the frequency increases, whereas that from the front surface, S_{s}, remains almost constant. Consequently, in the high-frequency range, the relative amplitudes of S_{s} and S_{d} are reversed, which may disrupt the linearity of the phase and lead to significant errors in the estimated sound speed. **Figure 3**(d) shows the trajectory on the Gaussian plane of the normalized spectrum at $\alpha = 30\alpha_{\mathrm{w}}$. It was confirmed that, in the higher frequency range, the amplitude of S_{s} relative to S_{d} became larger, thus the amount of phase change increased around local minima at n = 5.

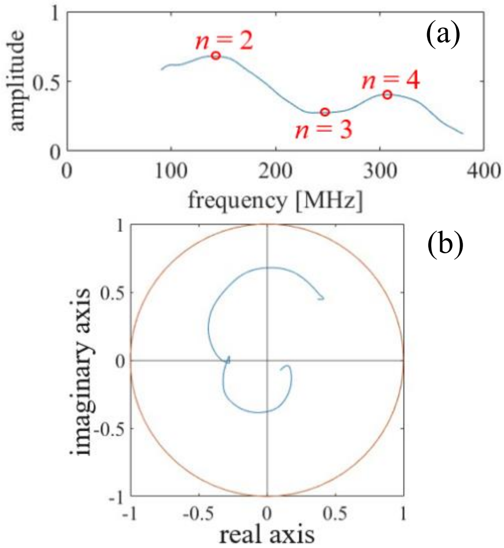

Fig. 2. (a) Amplitude spectrum and (b) trajectory in the Gaussian plane of the normalized spectrum at point A.

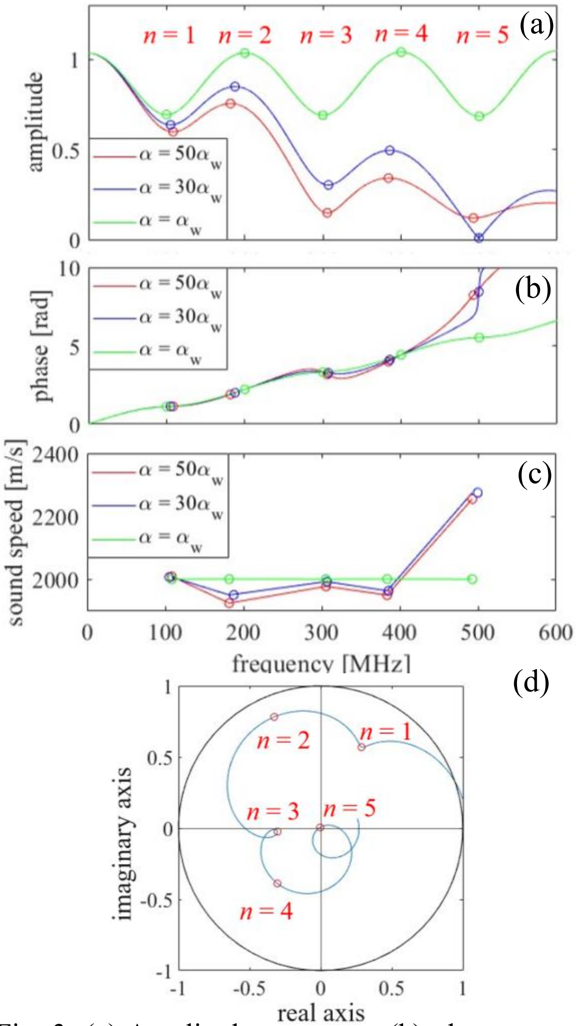

Fig. 3. (a) Amplitude spectrum, (b) phase spectrum, (c) sound speed, and (d) Gaussian plane ($\alpha = 30\alpha_{\mathrm{w}}$) of the normalized spectrum obtained by numerical simulation.

5. Conclusion

In this study, the two-dimensional distribution of sound speed in thin biological tissues was measured using an acoustic microscope. Based on this result, we investigated the cause of difficulty in accurately estimating the sound speed in thin specimens with high attenuation owing to their low periodicity and large errors in the phase. In the future, we will estimate the attenuation coefficient to achieve more accurate sound speed measurement.

References

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