A Robust Method for Analyzing Acoustic Properties of Biological Specimens by Acoustic Microscopy

超音波顕微鏡による生体試料の音響特性のロバストな解析法の検討

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1. Introduction

Acoustic microscopy has been used to measure acoustic properties in microscopic regions of biological specimens.[1)] The pulse spectrum method, analyzing the frequency spectrum of amplitude and phase of the reflected signal measured with a pulse signal, was proposed to obtain velocity and thickness of the specimens.[2)] An analysis method using autoregression (AR) model was also proposed[3)] and installed into commercial acoustic microscopy systems.

In the measurement on the biological specimens, the amplitude of the signal S_S shown in **Fig. 1** is small. There may be scattered waves due to inhomogeneity inside the biological specimens. Then, errors in the velocity and thickness would be large. In this paper, a robust analysis method of acoustic properties of biological specimens has been discussed.

2. Analysis method

Figure 1 shows the experimental arrangement for acoustic property measurements of biological specimens. S_W, S_S, and S_B are the reflected signals from the substrate surface, the specimen surface, and the boundary between the specimen and the substrate, respectively. The power spectrum of the superposed signals of S_S and S_B was normalized by that of the signal S_W. Thickness d and velocity V_2 of the specimen were obtained by the following equations.

$$d = \frac{V_1}{4\pi f_m}\left(\phi_m + n\pi\right), \tag{1}$$

$$V_2 = \left(\frac{1}{V_1} - \frac{\phi_m}{4\pi f_m d}\right)^{-1}, \tag{2}$$

where f_m is the frequency at which the amplitude

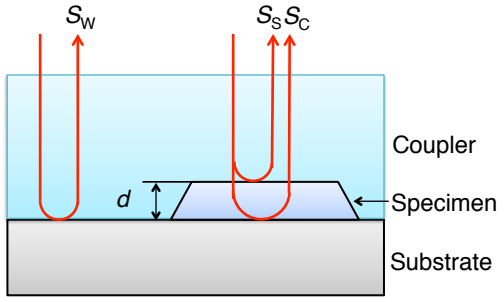

Fig. 1. Experimental arrangement for acoustic property measurements by acoustic microscopy.

spectrum becomes maxima or minima, ϕ_m is the phase of the phase spectrum at f_m, V_1 is velocity in coupler, and n is a nonnegative integer.

Eq. (3) was obtained from Eq. (2).

$$\phi_m = \frac{4\pi f_m d}{V_1} - n\pi, \tag{3}$$

Eq. (4) was obtained by substituting V_1 in Eq. (2) into Eq. (3).

$$V_2 = 4 n f_m d, \tag{4}$$

Relationships among ϕ_m, V_2, and f_m for each n can be obtained by Eqs. (3) and (4), if d is known. The relationships between V_2 and f_m are shown in **Fig. 2**. f_m exists in the frequency range between 44-106 MHz for n = 1, if d is 4-8 μm and V_2 is 1400-1700 m/s. From Eq. (4), f_m exists in the same frequency range for n = 2, if d is 8-16 μm. Therefore, frequency ranges at which maxima and minima of the amplitude spectrum occur can be predicted, if rough values of thickness and velocity of the specimen are known. Therefore, we performed the analysis according to the following procedure. First, an approximated line was

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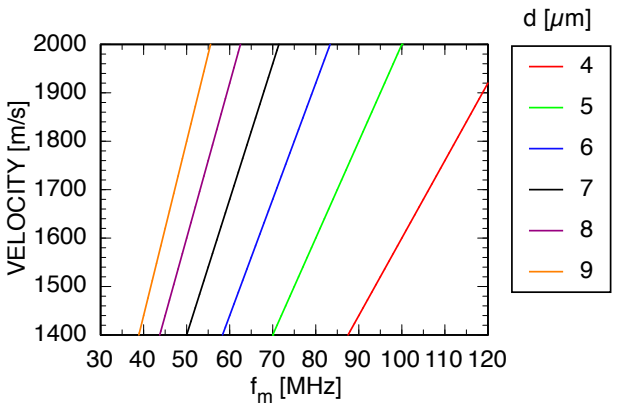

Fig. 2. Relationships between velocity of specimen and frequency at which the amplitude spectrum becomes minimum.

obtained for the amplitude spectrum. Next, the approximated line was subtracted from the amplitude spectrum. Then, the parabolic approximation was conducted for the result to find f_m. Average d and V_2 in specimen were obtained by substituting f_m and ϕ_m into Eqs. (1) and (2).

3. Experiments and Discussion

Malignant melanoma of the skin sliced with thickness of 5-7 μm was taken as a specimen. Commercial ultrasonic microscopy (AMS-50SI; Honda Electronics Co. Ltd.) with a concave transducer with a center frequency of 80 MHz was used for the measurements. Distilled water was used for the coupler. **Figure 3** shows velocity and thickness analyzed by the analysis method using AR model for the measurement results of line scanning of 2.4 mm with a step of 80 μm. There was a specimen in the range from 0 to 1.94 mm. Abnormal values, such as velocities larger than 2,000 m/s and thicknesses larger than 20 μm, were observed. It would be caused by the scattering waves due to inhomogeneity in the specimen. **Figure 4** shows the analyzed thickness for the same data by the proposed method. It is thought that accurate values were obtained, because the almost data were analyzed within 4-10 μm.

4. Conclusion

In this paper, a robust analysis method of acoustic properties of biological specimens was discussed. Average thicknesses in the specimen were obtained by the proposed method although the errors became large by the analysis method using AR model. This method is useful for analysis of acoustic properties of biological tissues and cells.

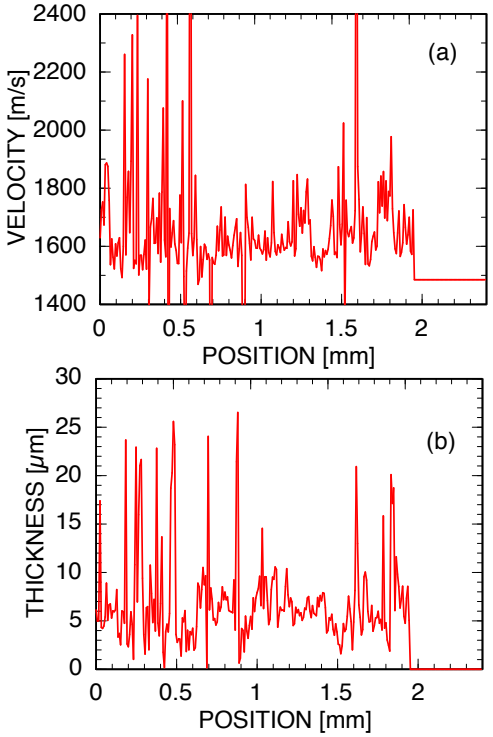

Fig. 3. Analyzed results by the analysis method using AR model. (a) velocity. (b) thickness.

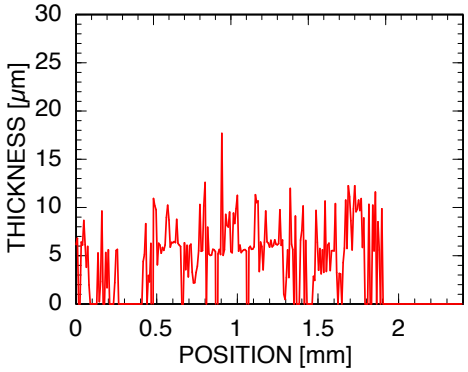

Fig. 4. Analyzed results of thickness by the proposed method.

We will discuss the analysis of velocity using the proposed method.

References

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