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Fully optic characterization of acoustic impedance implementable in optical microscope

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1. Background

The stiffness of tissue structures changes with inflammation and cancer.[1)] On the cellular scale, properties related to the elastic modulus also change depending on the cell type and stage in the cell cycle. Atomic force microscopy (AFM) and scanning acoustic microscopy (SAM) have been used to measure parameters related to the bulk modulus at the cellular scale. AFM has achieved a spatial resolution of about 1 nm, which enables us to obtain high-resolution values for the mechanical properties. SAM avoids contamination while measuring the intrinsic tic acoustic impedance (Z), which is related to the bulk modulus because the measurement is performed from outside the cell culture dish.[2)] However, the current scanning geometry does not allow optical-acoustical simultaneous measurement, which may prevent laboratory practice installation. This study proposed a Z-measure method using a common optical microscope setup.

2. Method

2.1 Thermoelastic wave-enhanced substrate

Thermoelastic (TE) waves are generated by the volume expansion that occurs by heat converted from light. Propagation in the direction normal to the flat plate surface is small. Therefore, a black ink layer for light absorption and a resin material layer with a large thermal expansion coefficient were laminated on the surface of a flat plate. **Figure 1(a)** illustrates the TE wave enhancement substrate. TE waves are generated at Boundary A, where the black ink and resin layers are laminated, and propagate in the thickness direction through a polystyrene plate with 1 mm thickness. The generated TE waves propagated to the inner boundary of the petri dish (Boundary B, Fig.1(a)) and reflected at boundary B, with a reflection coefficient R. To optically pick up the propagating waves at boundary A, aluminum foil was attached as a mirror. Measurements were made for air-filled and water-filled petri dishes.

2.2 Echo signals amplitude estimation

The echo returned from the sample and substrate boundary was determined by the reflection coefficient R_{sample}, which was calculated from the difference in impedance (Z_{sampe}) between the substrate (Z_{sub}) and the sample using Equation 1.

$$R_{sample} = \frac{Z_{sampe} - Z_{sub}}{Z_{sampe} + Z_{sub}} \quad \text{Eq.1}$$

Two samples with different Z were measured under the same conditions, and if Z of one was known, Z of the other was calculated. The sample with known Z was called the reference sample (Z_{ref}), and the echo amplitude was S_{ref}. The echo signal amplitude of the sample (S_{terget}) was acquired with unknown Z (Z_{target}), Z_{target} was obtained by the following formula. [2)] Here, Z_{ref} and Z_{sub} were 410 kg/m²/s and 2.37×10^{6}kg/m²/s, respectively.

$$Z_{target} = \frac{1 - \frac{S_{target}}{S_{ref}} \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 + \frac{S_{target}}{S_{ref}} \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} Z_{sub} \quad \text{Eq.2}$$

2.3 Echo signal measurement

The light source for TE wave excitation was the 527 nm pulsed laser (TECH-527 Advanced, Laser-export). The pulse width was shorter than 10 ns and the pulse energy was 70 uJ/pulse. The pulse repetition frequency was 2 kHz. The out-of-plane displacement generated by the echo was measured with a self-built Sagnac interferometer [3)] using a 488 nm contentious light laser (Spectra physics) adjusted

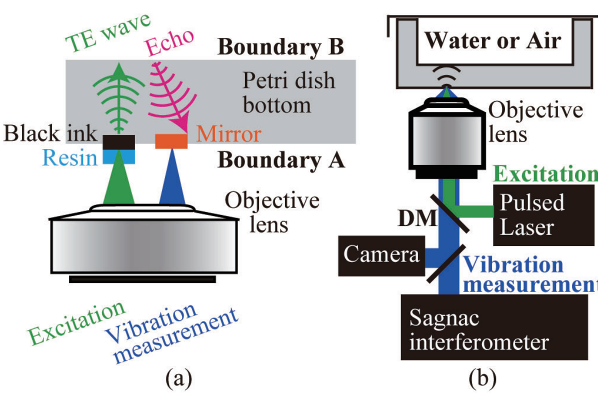

Figure 1: Measurement configuration

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80 mW at the laser output. The Sagnac interferometer has frequency dependency, controlled by propagation distance lag (such as propagation time lag Δt) Here, Δt was 7.67 ns. The Sagnac interferometer output was measured at a photodetector (DSP-2, Graviton). The differential output of the photodetector was amplified by a low-noise amplifier (SA-250F6, NF circuit block) and measured with an oscilloscope (HDO4034, LeCroy). The amplitudes were averaged 100 times synchronous to the pulse laser excitation.

Figure 1(b) shows how the excitation light and the displacement measurement light were placed in the field of view. The two lights were connected to a biological optical microscope (Ti2, Nikon), and the dichroic mirror (DM) arranged the excitation and vibration measurement light to the objective lens. (Plan Fluor 10x, Nikon)

3. Result and discussion

Figure 2 shows the acquired time waveforms. The upper signal shows the echo signal with a sample as the air, and the lower signal shows the echo signal with a sample as the water.

The times where pulse-type echo signals appear were named t_1 to t_5. The longitudinal and transverse propagation velocities of the polystyrene substrate are 2330 m/s and 1150 m/s, respectively, so the one-way propagation times were 429 ns and 869 ns. The pulse wave at t_1 showed surface propagation to the measurement point. The pulse wave at t_2 corresponded to the TE wave reflected at boundary B and returned to boundary A. It is consistent with round-trip longitudinal wave propagation time. The pulse wave at t_3 corresponded to the propagation time, which included mode conversion has occurred in the reflection. The pulse wave at t_4 was the signal of a round-trip transverse wave or two round-trips of a longitudinal wave. The pulse signal at t_5 appears only for the water sample, the time corresponded to the water/air surface.

The reflected wave amplitude was larger in air-filled than in a water-filled petri dish at t_2. The amplitude of the air pulse was 29.6 mV and that of the water pulse was 4.42 mV. The ratio of these values was 0.15. These values are close to the ratio 0.24 (= $R_{water}/\ R_{air}$) of the reflection coefficients R_{water} = 0.24 and R_{air}= 1.00 for water and air as calculated in Eq. 1. The Z_{terget} calculated by Eq. 2 was 1.75 $\times 10^6$kg/m^2/s.

4. Conclusion

This study estimated sample Z using the propagation of thermoacoustic waves generated by a pulsed laser. The calculated Z of water was 1.75 $\times 10^6$kg/m^2/s, showing an error of 17 % with respect to the true value.

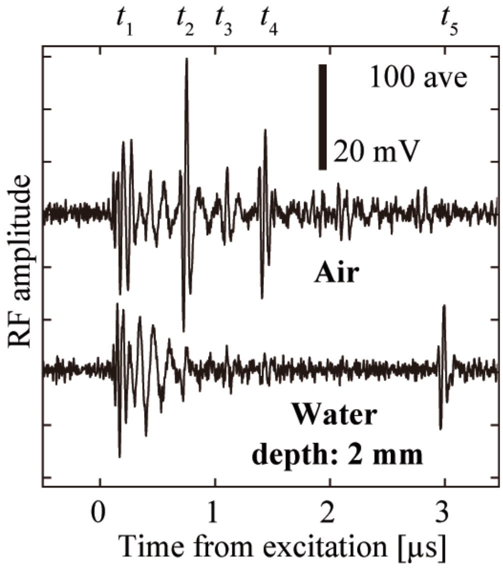

Figure 2: Echo signal of air and water sample

This study showed the concept of fully optical acoustic impedance measurement. The estimation error was larger than the measurement with SAM at this time. We plan to improve the Sagnac interferometer stability for improving the amplitude measurement.

Acknowledgment

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