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Development of the nanosecond pulsed photoacoustic wave detection system by using optical interferometry

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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. In the past, 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 specific acoustic impedance, which is related to the bulk modulus because the measurement is performed from outside the cell culture dish.[2)]

Our groups proposed a fully optical mechanical property measurement to improve measurement speed and spatial resolution.[3)] In this report, an optical method for detecting cell culture dish vibrations induced by the photoacoustic effect was proposed as a part of the microscopy system.

2. Method

2.1 Sagnac interferometer

Fig. 1 shows the basic setup of the Sagnac interferometer for detecting vertical vibration. A linearly polarized laser beam generated from a contentious laser (λ = 488 nm, Pmax = 70 mW, Spectra physics). $\lambda/2$ plate adjusted the polarization of the incident laser beam 45° off from the plane. The polarizing beam splitter (PBS) split the beam into two paths. Each beam was passed two PBSs, two mirrors, a $\lambda/4$ plate, and a sample. Clockwise (CW) and counterclockwise (CCW) propagation made a time difference at the sample surface, but the beams were detected using PD simultaneously because the beams have the same total propagation distance.

The Sagnac interferometer has frequency dependency, controlled by propagation distance lag (such as propagation time lag Δt) between CW and CCW propagation paths. Sagnac interferometer output maximum value when the largest displacement was changed inΔt. Here, Δt was 3.5 ns.

2.2 Detection of photoacoustic waves

A schematic diagram of the experiment is shown in **Fig. 2**. A 527 nm nanosecond (pulse width <10 µs) laser (TECH-527 Advanced, Laser-export) was used as the pulsed laser for photoacoustic wave excitation. The 25.6 µJ/pulse 2 kHz output was focused with a convex lens (f 100 mm) and irradiated to the sample. The sample was targeted at the bottom of a water-filled dish. The inside of the bottom was painted with black ink. The dish was made of polystyrene with a bottom thickness of 1 mm.

A commercially available inverted optical microscope (Ti2, Nikon) combined with a galvanometer mirror was used to measure the vibrations.[4)] The Sagnac interferometer[5)] was connected to the camera port via a galvanometer mirror. Measurement light of the Sagnac interferometer was focused on the outer bottom of the dish by an objective lens (TU Plan Fluor EPI 20x, Nikon). The light reflected by the attached aluminum foil on the bottom of the dish was again coupled to the same fiber following the same optical path. The output signal was measured at a photodetector (DSP-2, Graviton). The differential output of the photodetector was amplified by a low-noise

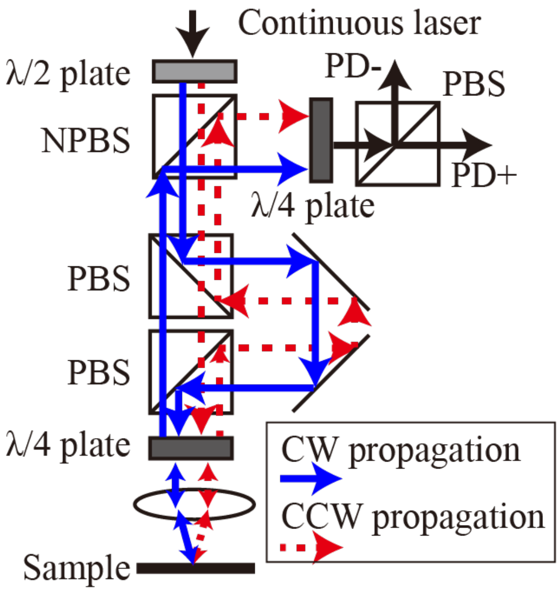

Fig. 1 The interferometer for vibration detection. The gray area is an inverted optical microscope. PBS: polarizing beam splitter; NPBS: Non polarizing beam splitter, PD: photodetector

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amplifier (SA-250F6, NF circuit block) and measured with an oscilloscope (HDO6000, LeCroy). In converting the voltage and amplitude, it was assumed that the oscillation amplitude was less than half a wavelength, and phase unwrapping was not considered. The amplitudes were averaged 1000 times.

3 Result and discussion

Fig. 3 shows the results of vibration measurements on the bottom of the dish. Vibrations were measured at (A) 0.40 µs and (B) 1.19 µs after the Q-switch trigger, which indicates the timing of the pulse laser emission. The oscillation peak frequency in (A) is about 8.0 MHz (4.0 MHz- 19.2 MHz for -6 dB bandwidth).

Since the polystyrene dish is 1 mm thick and the longitudinal wave propagation velocity is 2330 m/s, the propagation time in the thickness direction is 0.43 µs. Therefore, the vibration (A) measured at 0.40 µs was the propagation of photoacoustic waves generated inside the dish and propagating to the dish's outer surface. The vibration (B) measured at 1.12 µs is the photoacoustic wave reflected at the dish's inner side and at the outer side of the dish again. Therefore, propagation distance was three times of dish bottom thickness.

The center frequency of the photoacoustic wave was 8.0 MHz. Thus, the wavelength in polystyrene is 291µm. In this experiment, the light is focused by a convex lens, and the photoacoustic wave is generated from a spot with a Gaussian beam and full width half maximum was less than the wavelength of the photoacoustic wave. In other words, it is considered spherically diffused in propagation of the thickness direction.

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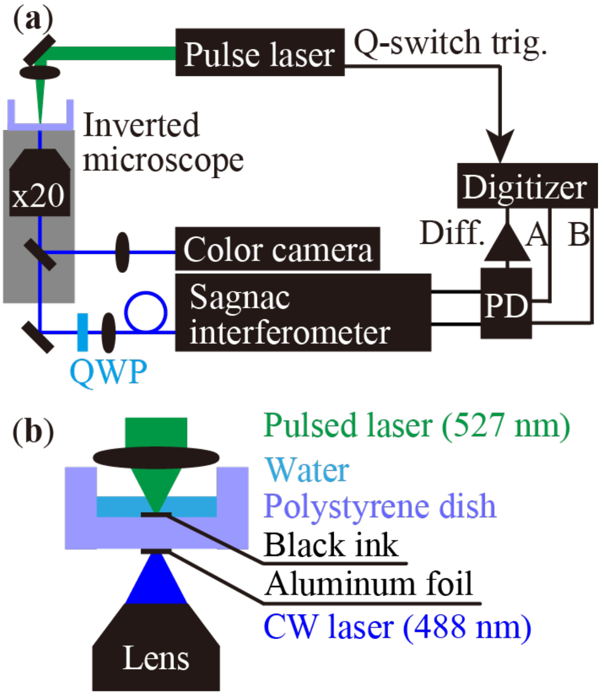

Fig. 2 Experimental setup (a) The Sagnac interferometer with an inverted optical microscope. (b) optical configuration around the dish.

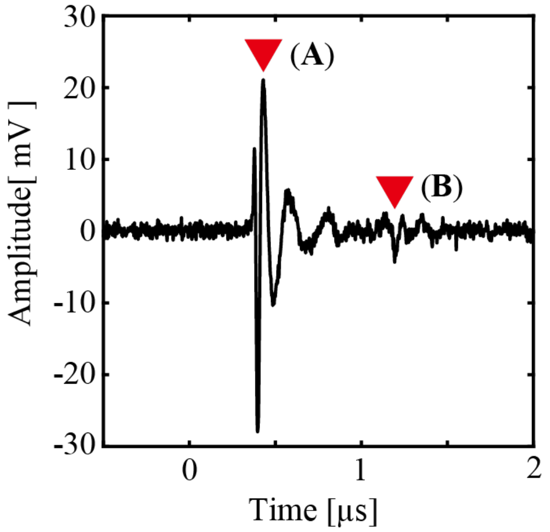

Fig. 3 Measured Sagnac interferometry signals.

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