Development of an optical interferometer for optical-based mechanical property microscope

光学式機械物性顕微鏡開発のための光干渉計の開発

Kazuki Tamura[1†], Ken-ya Hashimoto[2], and Seiji Yamamoto[1] ([1] Hamamatsu University School of Medicine [2], University of Electronic Science and Technology of China)

田村和輝 [1†]，橋本研也 [2]，山本清二 [1] ([1] 浜松医科大学, [2] 電子科技大学)

1. Background

The stiffness of tissue structures changes with inflammation and cancer.[1)] On the cellular scale, properties related to the volume 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. However, there is concern about contamination because the probe must be placed near or in contact with the cells. However, SAM avoids contamination while measuring the specific acoustic impedance, which is related to the bulk modulus, because the measurement is performed outside the cell culture dish.[2)]

To improve measurement speed and spatial resolution, our groups proposed a fully optical mechanical property measurement. In this report, an optical method for detecting cell culture dish vibrations was proposed as a part of the microscopy system.

2. Method

2.1 Overview of the measurement

Fig. 1 shows a schematic of the microscope for fully-optical mechanical property measurements.

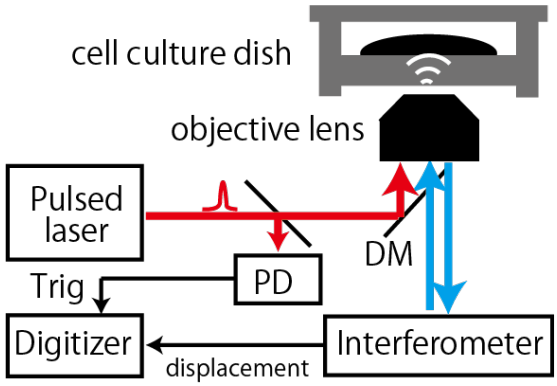

Fig. 1 Schematic of the fully-optical mechanical property measurement method. DM: dichroic mirror; PD: photodetector.

The technique is divided into two parts: vibration excitation (red optical path) and vibration detection (blue optical path). As in specific acoustic impedance measurements, the specific acoustic impedance is estimated from the amplitude of the waves reflected by a reference material and a sample on a substrate with known physical properties from the bottom of the cell culture dish. Because an inverted optical microscope is used to image the cultured cells, observation of the same sample in a observation method (phase contrast image / fluorescence image / confocal image) is possible.

2.2 Vibration detection via interferometry

Fig. 2 illustrates the interferometer for vibration detection. The vibration of cell culture dish is measured by polarized Michelson interferometer[3)] with a 633-nm continuous-wave HeNe laser (HNL210LB; Thorlabs). Optical path for sample passed an inverted optical microscope (TE2000; Nikon) and the objective lens (TU Plan Fluor EPI 20x; Nikon) via back port of the microscope. The interferometer is sensitive to sample vibrations. Differential output of the pre-amplifier is

$$A \propto P \sin\left({}^{4\pi\Delta d}/_{\lambda} - {}^{\pi}/_{2}\right),$$

where Δd is displacement, λ is laser wavelength and P is laser power. Phase unwrapping is not necessary because we assume the vibration amplitude is less than half a wavelength. For

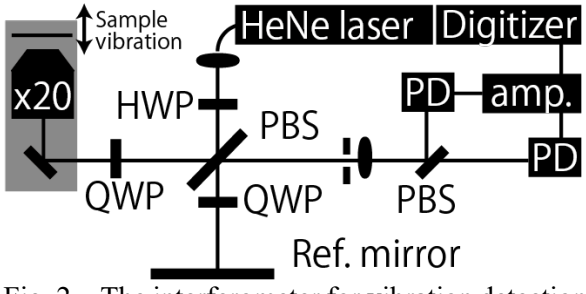

Fig. 2 The interferometer for vibration detection. The gray area is an inverted optical microscope. HWP: half waveplate; QWP: quarter waveplate; PBS: polarizing beam splitter; PD: photodetector; amp.: amplifier.

differential measurements, two photodiode modules (Craft Center SAWAKI Inc.) with matching characteristics were used, and the output was amplified by a pre-amplifier (SA-420F5; NF Corporation). The amplified output was filtered by a band-pass filter (BLP-30+; Mini-circuit) and measured by an oscilloscope (WaveSurfer3054; LeCroy).

2.3 Measurement validation

Fig. 3 shows a schematic of the validation experiment. Vibration detection was validated using pulsed waves emitted at a pulse repetition frequency of 1 kHz by a 15-MHz single-concave transducer (V328; Olympus) with focused depth 19 mm. The ultrasound pulse was excited to an acrylic tank bottom at focuses depth. The output waveform of the interferometer and the echo signal returned to the ultrasonic transducer were measured. The measurements were performed 1,024 times and averaged.

3. Result and discussion

Fig. 4 shows the echo signal and the interferometer output. The echo signal was detected 32.8 μs after pulse irradiation, which corresponds to the round-trip time between the transducer and the bottom of the tank. The interferometer output was detected at half the time of arrival of the echo signal, which corresponds to the time when the pulse has propagated through the water and reached the bottom of the tank.

This result confirms that the reflected light can be detected even though the acrylic is optically transparent (reflection coefficient:4%), and that the interferometer has sensitivity to pulse vibrations.

4. Conclusion and future work

This study verified that vibration detection using a homemade optical interferometer is possible. We validated the technique by detecting ultrasonic vibrations of the bottom of an acrylic tank. As a next step, we will test optical excitation using a nanosecond pulsed laser, as used for photo-acoustic excitation. In addition, because the Michelson interferometer is strongly affected by temperature fluctuations and the low-frequency vibrations of the experimental system, we plan to investigate other optical interferometers.

Acknowledgment

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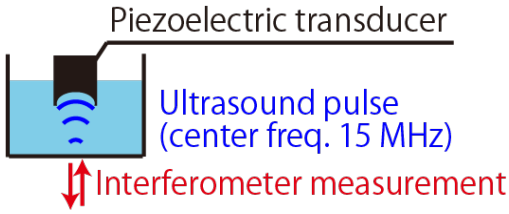

Fig. 3 Schematic of the validation experiment. The tank filled degas water.

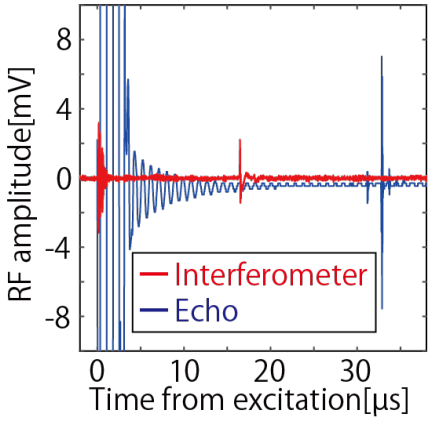

Fig. 4 Measured echo and interferometry signals.

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