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Development of nano-thin film biosensors using asynchronous picosecond ultrasound method

非同期ピコ秒超音波法を用いたナノ薄膜振動子バイオセンサの開発

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1. Introduction

Highly accurate and label-free measurement is important for early diagnosis and pharmaceutical, where biomarker test plays an important role. Biomarkers are disease-specific proteins that are secreted into the blood or urine at the onset of corresponding diseases, and biosensors are widely used to determine the presence or absence of the diseases. Biosensors are capable of detecting biomarkers using the ability of biomolecules to bind specifically to specific molecules. The development of high-performance biosensors is expected to make a significant contribution to the advancement of diagnosis[1)].

Biosensors can be classified into labeled and unlabeled types. In the labeled type, biomarkers are quantified by labeling with a luminescent reagent, which is undesirable because of the structural change of the protein. Furthermore, a long-time assay is required because of additional incubation and washing procedures. However, in the case of the label-free type, these problems are solved, and a real-time measurement becomes possible. A typical label-free sensor for mass detection is the quartz crystal microbalance (QCM)[2)]. This biosensor detects a target by quantifying the change in resonance frequency caused by the adsorption of the target to the oscillator.

In this study, we replace the quartz crystal used as the oscillator with a thinner free-standing nano thin film and develop a highly sensitive biosensor that uses GHz-THz resonances. We use the picosecond ultrasound method[3)], and develop an asynchronous optical sampling (ASOPS) system to monitor the frequency changes.

2. Asynchronous picosecond ultrasound method

The picosecond ultrasound method is a pump-probe measurement using femtosecond pulsed lasers. By irradiating a sample with pulsed light (pump light), the area near the surface becomes locally hot, and longitudinal elastic wave is generated due to transient thermal expansion.

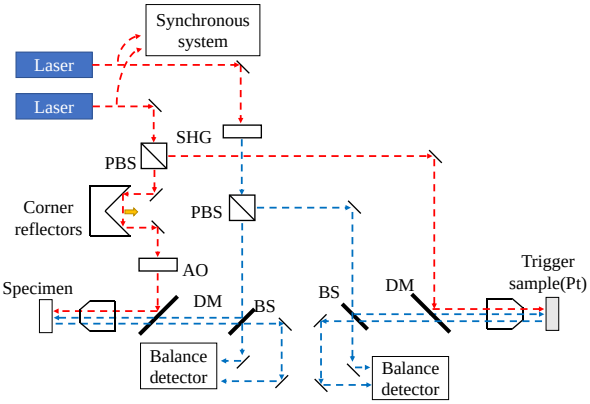

Fig. 1 Schematic of the ASOPS and MDS optics.

This changes the optical properties such as the refractive index, and the amplitude and phase of the reflected light of the probe light changes. The picosecond ultrasound method enables us to evaluate the acoustic properties of nanometer-thin films. In this method, the light path of laser pulse is controlled by a mechanical-delay line (MDL) system with a stage controller and corner reflectors. The optics of MDL and ASOPS is shown in **Fig. 1.** MDL requires mechanical operation, which takes about 40 minutes in one measurement. However, to apply this measurement method to biosensors for monitoring biological responses, it is important to shorten the measurement time.

ASOPS is a measurement system that can drastically reduce the measurement time, which enables us to observe biological reactions in real time. When a difference Δf in the pulse-repetition frequency is given ~~to~~ between the pump and probe light pulses, the difference of arrival times between them changes with each pulse and coincides again with a period of $\Delta T = 1/\Delta f$ [4)]. This schematic

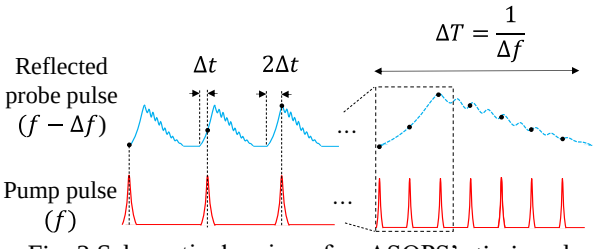

Fig. 2 Schematic drawing of an ASOPS's timing chart.

diagram is shown in **Fig. 2**. However, the reputation rates of pump and probe lasers are not constant. Usual ASOPS system uses a feed-back control to keep the reputation rates and Δf constant, resulting in a complicated system. In our ASOPS system, we use a trigger sample of ~100-nm Pt film on Si substrate to measured Δf each time to convert and average measurement data.

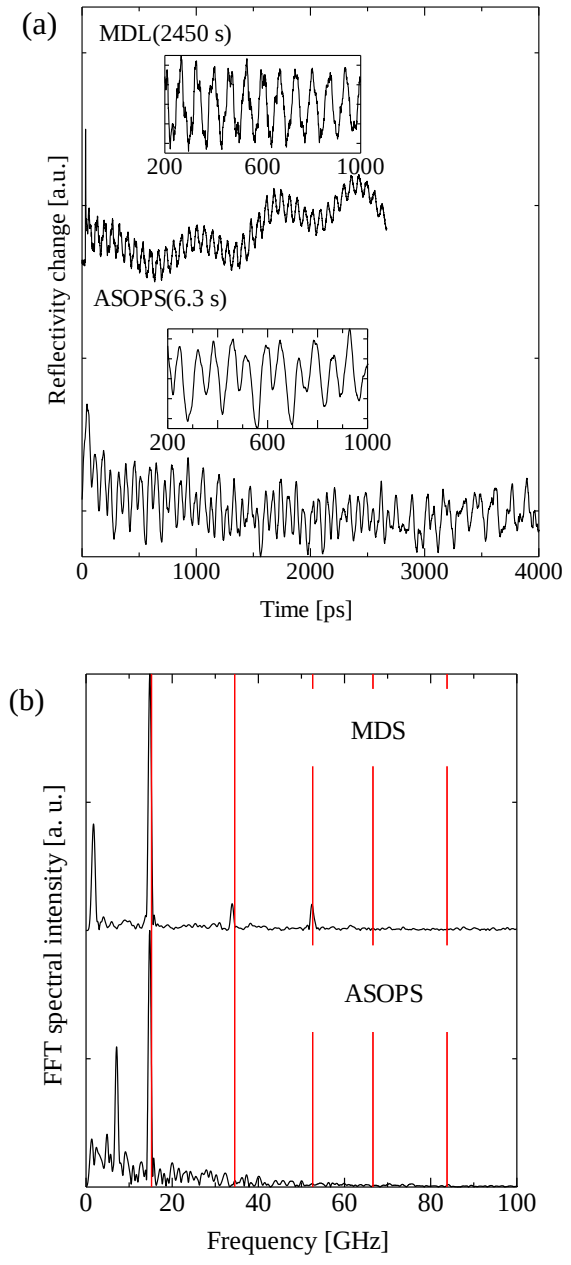

Fig. 3 (a) Reflectivity changes and (b) corresponding FFT spectra of Si_3N_4 free-standing film measured by MDL and ASOPS systems, which took 2,450 s and 6.3 s, respectively. Vertical bars represent calculated resonance frequencies.

3. Results and Discussions

We used a 100-nm Si_3N_4 free-standing film. We cleaned this film by air plasma for 1 min, and then we deposited 2-nm Cr and ~25-nm Au on one side by the RF magnetron sputtering method. At first, we measure the film-thickness resonances of the free-standing film by MDL and ASOPS systems as shown in **Fig. 3** (a). The insets show extracted resonances. The ASOPS systems requires only ~6 s to observed low-attenuation free-standing resonances when we make 1,000 times averaging. The corresponding fast Fourier transform (FFT) spectra are shown in Fig. 3 (b) with the calculated resonance frequencies. The measured and calculated fundamental-mode frequencies agree well each other, insisting on accurate measurement by the ASOPS system.

Free-standing film prevents the vibration-energy leakage to the substrate, allowing us to accurately determine the resonance frequency. In the case of a nanofilm on a substrate, the vibration usually decays within 100–200 ps, on the other hand, the free-standing films shows the resonance even after 2,500 ps. However, MDL system requires long time to observe such low-attenuation resonance, which is serious problem for a biosensor. ASOPS system enables fast and accurate measurement; we succeeded in observing the long-time resonance by the ASOPS system, which is about 390 times faster than the MDL system. By using the ASOPS system, we will monitor the frequency changes by injecting bio molecules in a flow cell.

4. Conclusion

We present a fast and accurate measurement of the free-standing resonance frequency by using the asynchronous picosecond ultrasound method. We will further show the practicality of ASOPS for real-time measurement.

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