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Development of a real-time GHz resonant biosensor using asynchronous picosecond ultrasonics

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1. Introduction

To diagnose diseases and develop new drugs, it is essential to identify specific biomolecules concerning to the disease[1,2]. Biosensors are a highly effective method to detection them. Biosensors require various ability not only high sensitivity but also real-time detection and multi-channel analysis. By detecting biomolecules with high sensitivity, it becomes possible to diagnose diseases at an earlier stage. Additionally, real-time detection enables the evaluation of the binding process of target biomolecules.

There are principally two types of biosensors: labeled and label-free biosensors. The labeled biosensors show high detection sensitivity, but they are more time consuming because of the extra steps required to add and detect labels. On the other hand, the label-free biosensors, such as the quartz crystal microbalance (QCM) method[3], can directly detect target biomolecules captured on the sensor surfaces, without the labeling addition process. The QCM method detects the target molecules through the mass addition on the surface of a quartz crystal by measuring changes in the resonance frequency. However, its sensitivity is lower than those of labeled biosensors.

In this study, we develop a real-time GHz resonant biosensor using asynchronous picosecond ultrasonics. In traditional picosecond ultrasonics[4,5], delay time between pump and probe light pulses is controlled by a mechanical delay line. However, this method is unsuitable for measuring the change of the resonance frequency, amplitude, and Q-value of a resonator by biomolecules' biding because it typically takes more than 10 minutes to obtain the probe light response. To solve this issue, we utilize the asynchronous optical sampling (ASOPS)[6-8] system in picosecond ultrasonics, thereby significantly shortening the measurement time. We use free-standing nano film as the resonator, and measure its resonance-frequency change due to adsorption process of biomolecules in real time.

2. Experimental method

We use a 200-nm Si_3N_4 (SiN) free-standing film as the resonator. First, we deposited 80-nm Au on SiN using radio frequency magnetron sputtering. Next, we immobilized the ligand proteins as follows; to form self-assembled monolayers (SAM) on the SiN surface, we immersed the resonator in 10-carboxy-1-decanethiol. Then, the carboxyl terminus of the SAM was activated to immobilize protein A, which binds specifically to immunoglobulin G (IgG). To prevent nonspecific adsorption of target proteins, we blocked the residual protein A sites by bovine serum albumin (BSA). We then inject IgG solution and detect changes due to specific binding of protein A and IgG, a model of antigen-antibody reaction.

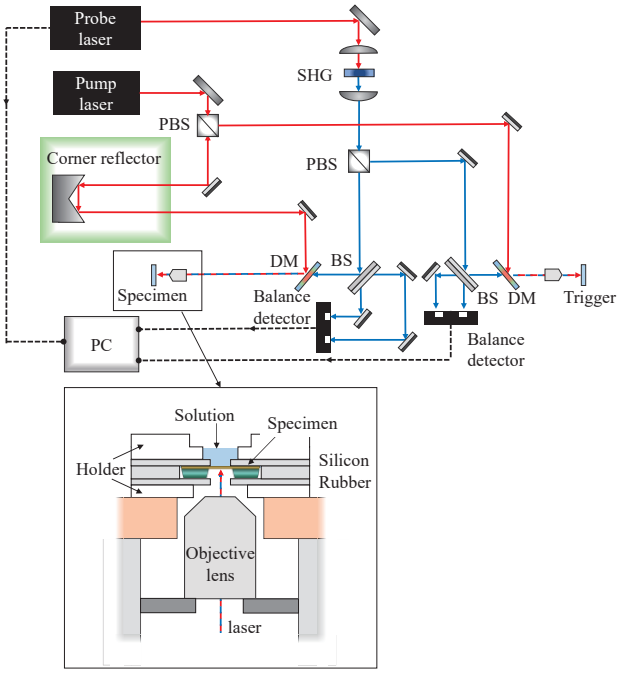

Fig. 1 Schematic of the optical systems. Red and blue arrows indicate pump and probe light, respectively. The inset is a magnified view of the area around the resonator.

We show the schematic of the optics in **Fig. 1**. For detection, two femtosecond pulsed lasers are used. Their pulse width, center wavelength, and repetition rate are 140 fs, 800 nm, and 80 MHz, respectively. A pump light of 800 nm wavelength is irradiates the resonator to cause the through-thickness longitudinal resonance of the resonator. A probe light with a wavelength of 400 nm, generated by a second harmonic generator (SHG), is also focused on the resonator surface to detect the

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reflectivity change caused by the strain oscillation accompanied by the resonance. The SiN is sandwiched between silicon rubbers and fixed with holders from the top and bottom. The IgG solution is dropped from the top of the holder. The resonance frequency of the specimen is measured from the reflectivity change, using a laser beam that ~~is~~ irradiates the bottom surface of the resonator.

In the ASOPS method, we shift the repetition

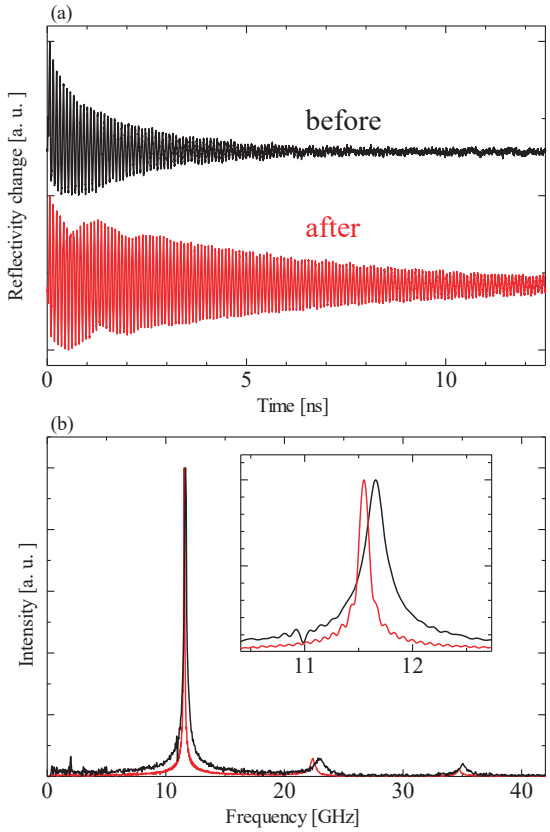

Fig. 2 (a) Reflectivity change before and after IgG solution injection. The black line shows the reflectivity change before IgG solution injection and the red line shows the reflectivity changes after IgG solution injection. (b) Corresponding FFT spectra. The inset shows a magnified view of the 1st mode.

rate of the two lasers by ~1 kHz, resulting in the delay time between the pump and probe light every pulse. The reflectivity change $R(t)$ caused by the pump pulse is detected by the probe light as $R(t_c)$, where $t_c = t \times f_r / \Delta f$ is observation time by the repetition-rate shifted probe light. We directly count f_r from the pulsing signal of the pump laser, and directly measure Δf using a trigger specimen of 100-nm Pt; we irradiate the divided pump and probe light pulses to the trigger specimen, obtaining an abrupt reflectivity change due to laser absorption, resulting in a fine trigger signal.

The magnification of the objective lens is 100, and the intensities of the pump and probe light are 10 and 3 mW, respectively.

3. Result and Discussions

We perpendicularly introduce the pump and probe light from the bottom of the holder. Measured reflectivity changes before and after IgG-solution injection are shown in **Fig. 2** with the corresponding fast Fourier transform (FFT) spectra. The concentration of the IgG is 100 ng/ml. The resonance frequencies are about 11, 23, 35 GHz, which are the through-thickness resonances of free-standing SiN/Au multilayer.

The all resonance frequencies decrease by the injection of IgG solution by -0.7 to -2.6% due to the mass increase by the adsorption of IgG on the surface. We will change the concentration of the IgG solution, to evaluate the sensitivity, reliability, repeatability, and limit of detection in further experiment.

4. Conclusion

we measure the resonance frequency change in the injection of IgG solution at a concentration of 100 ng/ml using asynchronous picosecond ultrasonics. Our next step is to create a thinner resonator and measure the resonance frequency change in low-concentration IgG solutions. Additionally, we plan to measure the IgG adsorption process in real-time by flowing protein solutions and demonstrate the usefulness of this biosensor.

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