Detection of IgG by ultrasonic attenuation of free standing ^{12}C diamond thin film studied by picosecond ultrasonics

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1. Introduction

A biosensor uses the molecular recognition ability between ligands immobilized on a substrate and target proteins surrounding the biosensor chip. It plays an important role in medical diagnosis, drug discovery, and environmental monitoring. The label-free detection is preferable because of short assay time.

Quartz crystal microbalance (QCM) biosensor is a representative label-free biosensor [1], which detects the targets through the mass change of the resonator by detecting the change of its resonance frequency.

In this research, we propose a new approach for detecting target proteins through attenuation of ultrahigh-frequency ultrasound in a free standing ^{12}C diamond thin film. The thickness of the diamond thin film used in this study is much thinner than resonators used in conventional QCM biosensors, dramatic improvement in sensitivity is expected. Since diamond shows extremely high strength, the strength of the thin film can be maintained even if it is a free-standing thin film. Furthermore, diamond shows higher resistance to acid, which is needed for a biosensor chip. Thus, it is considered that the material is suitable for a biosensor. Proteins captured on the surface will adsorb phonon energies, which increases ultrahigh-frequency ultrasound attenuation. Therefore, monitoring the attenuation change, it is expected that we can detect the target proteins. Because of significantly low isotope impurities, ^{12}C diamond is expected to show very low ultrasonic attenuation even at ultrahigh-frequencies (~50 GHz), so that small attenuation change caused by the captured proteins will be detected.

In order to investigate this possibility, we measure the change in the ultrasonic attenuation by capturing immunoglobulin G (IgG) as a target protein.

2. Methodology

The experimental arrangement is shown in Fig. 1. We synthesized a ^{12}C diamond thin film on a monocrystal diamond substrate by the microwave plasma-assisted chemical-vapor-deposition method [2], and removed the diamond substrate by a gas etching method, resulting in a free-standing ^{12}C diamond thin film with thickness of ~3 μm. We deposited Pt thin films (~10 nm) on both surfaces for ultrasonic transducers. We immobilized protein A as the ligand for the target IgG on the surface of the Pt thin film and measured longitudinal-wave pulse echoes using picosecond ultrasound technique to evaluate the ultrasonic attenuation inside the diamond thin film. Picosecond ultrasound is a pump-probe technique [3]. The pump light pulse induces ultra-high frequency coherent acoustic wave via the thermal-phonon interaction. The probe light pulse reflects from the surface and detects the surface strain induced by the pump light pulse. The frequency dependence of the attenuation coefficient can be determined by the amplitude of the pulse-echo signal because it is proportional to the amplitude of the strain according to the photo-elastic effect.

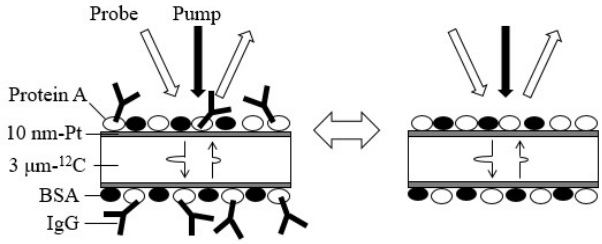

Fig. 1 Schematic diagram of the experiment.

Surface preparation for biosensor chip

In this study, rabbit IgG (IgG) was measured as a target protein. The procedure for capturing IgG on the diamond thin film as follow: The diamond thin film was first immersed in 10 mM 10-carboxy-decanthiol solution with absolute -ethanol buffer and we incubated it for 12 h at 4 °C. We then rinsed it with absolute-ethanol and ultrapure water and immersed it in a 100 mM EDC (1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide, hydrochloride) solution with 100 mM NHS (N-hydroxysulfosuccinimide sodium salt) in ultrapure water and incubated it for 1 h at 24 °C to

activate the sensor surfaces. We then rinsed it with ultrapure water and HEPES (4-(2-hydroxyethyl) -1-piperazineethanesulfonic acid) and soaked it in 200 μg/mL protein A solution with HEPES buffer for 2 h. In order to block unreacted ester sites, it was immersed in a 10 mg/mL BSA (bovine serum albumin) solution for 1 h. After we rinsed it with HEPES, it was immersed in 1 μg/mL IgG solution with HEPES buffer for 40 min. The sensor chip was then set in the picosecond ultrasonics measurement system, and we measure the pulse-echo signals. To dissociate the IgG from protein A, we immersed the sensor chip in GHB (glycine-HCl buffer) for 20 min. We then measured again the pulse-echo signals. We repeated these measurements.

3. Experiment

We used Ti-Sapphire pulse laser with 140 fs duration and 80 MHz repetition rate. The wavelength of the pump and the probe light pulses are 800 nm and 400 nm, respectively. Details of our optics are shown elsewhere [2,4]

Figure 2 shows the as-measured reflectivity change, which is proportional to the ultrasonic strain, without IgG molecules. The oscillation signal arises from the Brillouin oscillation, and we removed the non-necessity Brillouin oscillation and the background attenuation as shown in Fig. 3. Figure 4 shows FFT spectrum on each pulse-echo signal, from which we derived the frequency-dependent attenuation (Fig. 5).

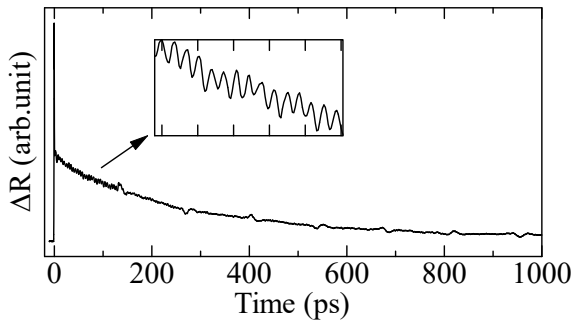

Fig. 2 A typical measured reflectivity change of the probe light in the free-standing ^{12}C diamond film.

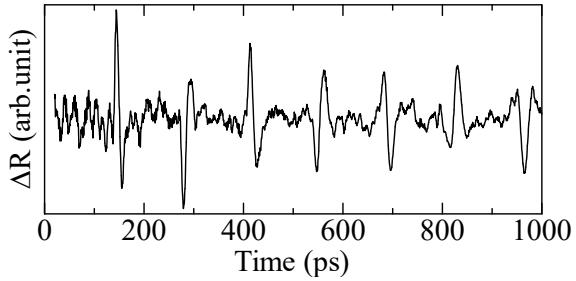

Fig. 3 The background-subtracted signal of Fig. 2.

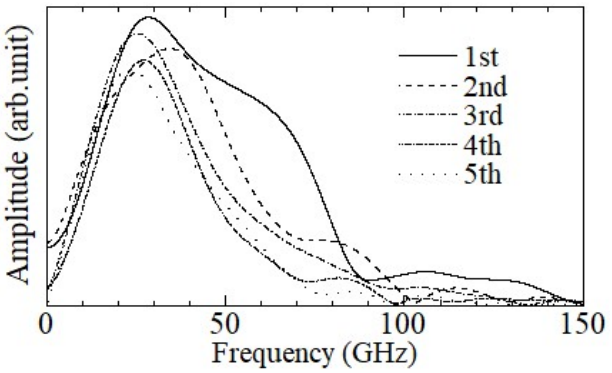

Fig. 4 The FFT spectrum on each pulse-echo signal.

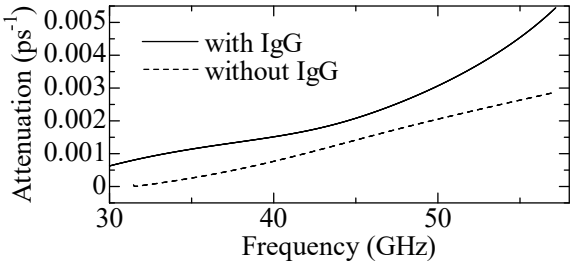

Fig. 5 The frequency-dependent attenuation with and without IgG molecules.

4. Result & Discussion

We succeeded in measuring attenuation of the free-standing diamond thin film over 30 GHz with and without the IgG target. The attenuation value with IgG molecules is significantly larger than that without IgG molecules, indicating that the IgG molecules captured on the surface adsorbed the phonon energy: The IgG molecules can behave as vibration absorbers on the resonator. Thus, we can expect that this phenomenon can be applied to a new biosensor.

Acknowledgment

This work was supported by KAKENHI Grant-in-Aid for Scientific Research A (JP19H00862).

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