Development of a high-frequency phonon biosensor using graphite thin film resonator and theoretical calculation

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1. Introduction

The field of biosensing has made remarkable progress in recent years, enabling the highly sensitive detection and analysis of biomolecules for diverse applications. One such technology is the label-free detection of target molecules. We here propose a label-free ultrasensitive biosensor based on picosecond ultrasound spectroscopy (PUS)[1,2] using a graphite thin-film resonator. There are three advantages in adopting the graphite thin film as a resonator: Firstly, its thickness can be as small as ~50 nm, which significantly improves the detection sensitivity compared to conventional oscillator biosensors such as quartz-crystal-microbalance (QCM).[3-5] Since the mass sensitivity of an oscillator biosensor is inversely proportional to the square of the resonator thickness, thinning the oscillator dramatically improves the sensitivity of the biosensor.[6,7] Secondly, thermal conductivity of graphite is very low in the thickness direction and very high in the in-plane direction,[8] allowing heat generated by laser irradiation to diffuse in the in-plane direction, preventing the excessive heat transfer to proteins immobilized on the opposite sensor surface. This prevents denaturation and deactivation of proteins on the graphite resonator. Thirdly, the high absorption coefficient of graphite for 800 nm light enables the longitudinal-wave generation without additional coating materials.

In this study, we established the ultrathin-graphite-film biosensor system and detected immunoglobulin G (IgG) and C-reactive protein (CRP) for evaluating its validity. Also, because the resonator thickness (~50 nm) we use is comparable to that of the formed protein layer, we cannot use the conventional analysis for the mass-sensitive resonator, like the Sauerbrey equation.[6] We therefore propose a theoretical model for analyzing the effect of the protein capturing on the resonance frequency and the quality factor of the graphite resonator.

2. Experiment

To begin with, the graphite thin film was attached on a Si substrate with many 100 μm diameter pores with 1 mm pitch to create several graphite free-standing membranes. Subsequently, we immobilized 1-pyrenebutanoic acid succinimidyl ester, which acts as linkers, on the graphite surface, followed by the chemical modification with the ligands that specifically bind to the target molecules. The resonance frequency of the resonator is measured using the PUS system originally developed in this study. Its optics is shown in **Fig. 1**. We use titanium/sapphire pulse lasers with a wavelength of 800 nm. They independently emit the pump and probe light pulses. The intensity of pump pulse is modulated at 100 kHz by an acousto-optical (AO) modulator for the lock-in-amplifier measurement. The wavelength of the probe pulse is converted to 400 nm by a second-harmonic-generation (SHG) crystal. The probe pulse is then split by a beam splitter (BS) into a reference light and a detection light; the latter is reflected by the resonator and enters a balance detector (BD) to measure the reflectance change. Both lights are focused perpendicular to the sensor surface via an objective lens for ultrasonic excitation and detection. Their spot diameters are approximately 10 μm. The optical path of the pump light pulse is

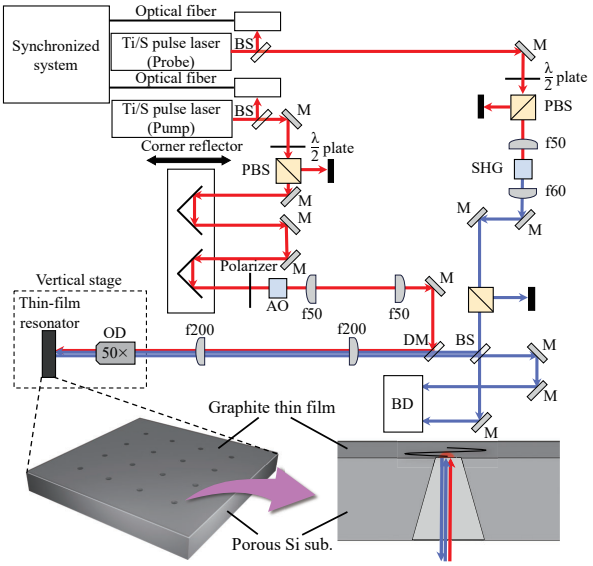

Fig. 1 Optics of picosecond ultrasound spectroscopy developed in this study. Red and blue lines denote the pump and probe light pulses, respectively.

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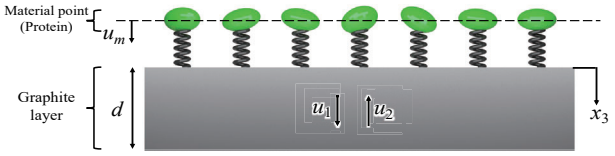

Fig. 2 One-dimensional mass-spring system model with a mass per unit area m_s adsorbed on a graphite of thickness d, where u_1 and u_2 are longitudinal waves propagating downward and upward in the film, respectively, and u_m is protein vibration.

mechanically changed with two corner reflectors to control the time difference between the arrival of both pulses at the resonator. Next, the solution containing the analyte is injected onto the sensor surface, and the resonance frequency is measured again after the appropriate immobilization time. We repeated a series of experiments with multiple concentrations of the analyte, allowing making a calibration curve for determining the concentration of an analyte from the resonance frequency change.

3. Theoretical calculation

We investigate the effect of adsorbed mass on the resonance frequency and the quality factor of the oscillator surface. In conventional oscillator biosensors, the Sauerbrey equation has been used to predict the adsorbed mass from the change in the resonance frequency, assuming that the adsorbed mass is sufficiently smaller than the mass of the resonator. However, the graphite thin film used in this experiment is extremely thin (~50 nm), and the Sauerbrey equation is unavailable. Therefore, we propose an alternative model as shown in **Fig. 2**, to simulate the change in resonance frequency and the loss of the resonator. It consists of the elastic graphite thin plate, the springs with loss on it, and the adsorbed masses on the springs. Combining the elastic-wave field inside the graphite plate and the distribution of the springs at the upper surface, we obtain the frequency equation, from which the resonance frequencies and corresponding loss of the resonator system are obtained.

4. Results and Discussion

We detected a protein by changes in resonance frequency and the quality factor caused by binding of the target materials within the laser spot located on the membrane. **Figure 3** shows examples of the measured reflectance changes before and after the injection of an analyte containing 100 ng/ml IgG, demonstrating a representative frequency change with the graphite resonator biosensor. Here, the resonance frequency decreased by 2.2% from 35.50

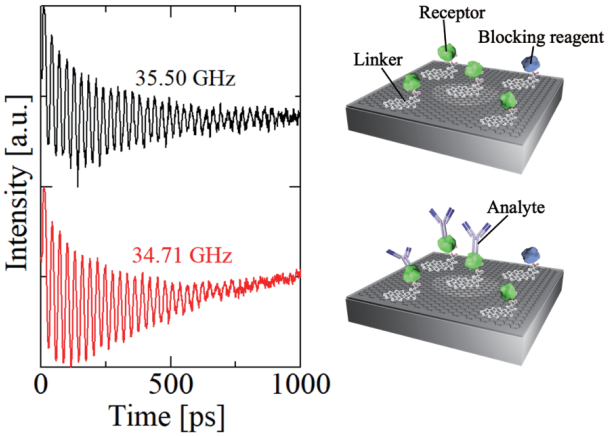

Fig. 3 Comparison of the resonance frequency of the system before (upper) and after (lower) adsorption of 100 ng/ml IgG on the graphite free-standing membrane.

GHz to 34.71 GHz, a significantly larger change compared with that observed with a very high-sensitive QCMs (<0.01%). We performed such experiments under various concentrations, ambient temperatures, and contaminant levels, and confirmed the applicability of the proposed biosensor.

Furthermore, we find that the proposed theoretical model agrees with the Sauerbrey equation when the adsorbed mass is much smaller than the resonator, but the resonance-frequency change shows a very different trend from the Sauerbrey equation with a larger amount of targets.

5. Conclusion

We have demonstrated the ultrathin-graphite-film biosensor system capable of label-free detection of target proteins. We anticipate that the biosensor will become a powerful tool for detecting extremely low concentration targets in a short time.

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