Development of 30-GHz phonon biosensor using graphite thin-film resonator

多層グラフェンを用いた 30 GHz を超えるフォノンバイオセンサーの開発

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1. Introduction

Recent advances in biosensor technologies have established the label-free detection of target molecules. The quartz-crystal-microbalance (QCM) biosensor is widely recognized as a typical label-free biosensor.[1,2] Since the mass sensitivity of QCM is inversely proportional to the square of the resonator thickness, the sensitivity of QCM biosensor can be greatly improved using a thinner quartz resonator. However, further decrease in the crystal thickness is never straightforward, because the robustness and strength of the crystal significantly deteriorate with thinning.

To resolve this problem, we propose an ultrasensitive biosensor with a graphite thin-film resonator, whose vibration is excited and detected using the picosecond ultrasound spectroscopy (PUS).[3][4] There are two advantages for adopting the graphite thin film: (i) The graphite thin-film (~50 nm) can be much thinner than the quartz plate used in conventional QCM biosensors (~100 μm), making it possible to achieve the ultrasensitive resonator biosensor.[5] (ii) Graphite shows very low thermal conductivity in the out-of-plane direction, and extremely high thermal conductivity in the in-plane direction.[6] Therefore, the heat generated by the laser irradiation in PUS diffuses in the in-plane direction, suppressing heating in the out-of-plane direction and preventing heat-induced deactivation of proteins immobilized on the surface. Previously, metallic thin films such as Pt were used as resonators[7], but if the thickness is thin, the heat induced by the light pulses deteriorated biomolecules on them.

In this study, we establish the ultrathin-graphite-film biosensor system and detect immunoglobulin G (IgG) for evaluating its validity.

2. Experimental procedure

The graphite thin film substrate (~50 nm thick) used in the experiments was prepared by graphitization of polyimide film, and the graphite basal plane was oriented parallel to the film surface.[8,9] **Figure 1** illustrates the laboratory-built sensor cell and shows **(a)** the 3D image of the cell and **(b)** a schematic drawing of the biosensor using the cell. We first deposit graphite thin-film on a Si substrate with many holes to make many free-standing graphite-film membranes. We then immobilize receptor proteins on the graphite film through linker material as follows. Graphite thin-film was immersed for 1 h at room temperature in a methanolic solution of 1 mM 1-pyrenebunanoic acid succinimidyl ester, which serves as a linker. The pyrenyl group of the linker interacts strongly with the graphene surface via π-stacking. Thus, we can immobilize the receptor molecules on the graphene surface.[10] After rinsing the linker with ultrapure water and phosphate-buffered solution (PBS), the graphite thin-film was immersed in a 200 μg/mL solution of the protein A in PBS overnight at 4 ℃. Finally, the remaining activated terminals was blocked by 5 mg/mL bovine serum albumin (BSA) in PBS solution for 1 h at room temperature, and we rinsed the sensor surface with the ultrapure water several times. Afterwards, IgG solutions with different concentrations in PBS solution were injected. Because IgG and protein A bind specifically, we can detect IgG with this biosensor. The PUS measurement was performed in air after drying the graphite thin-film with N_2 gas each time.

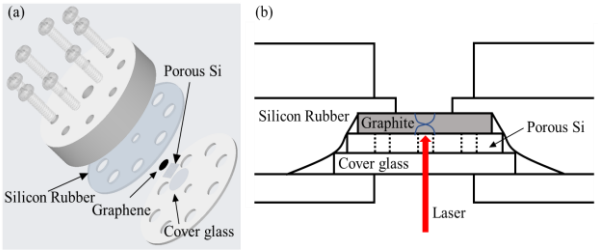

Fig. 1 (a)The 3D image of the sensor cell, and (b) its cross-section illustration.

3. Results and Discussion

Both pump and probe light pulses were perpendicularly focused on the back surface of the

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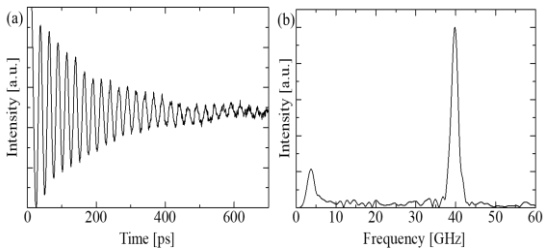

Fig. 2 (a) Measured resonance vibration and (b)its FFT spectrum.

graphite membrane with an objective lens. Their spot diameters were about 10 μm. **Figure 2(a)** shows an example of measured vibration signal of the graphite thin-film membrane, and **Figure 2(b)** shows the corresponding Fast Fourier transformation (FFT) spectrum. We observed about 40-GHz resonance frequency. The binding reactions between protein A and IgG were evaluated by change in the quality factor (Q value). **Figure 3** shows the relationship between the change in the Q value of the resonator and the concentration of IgG solutions. As the concentration of IgG increases, the Q value decreases. We obtain great correlation between 0.01 and 10 μg/mL, demonstrating the validity of the proposed graphite-film biosensor.

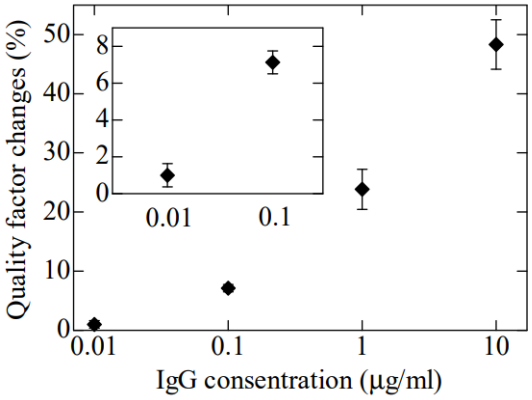

Fig. 3 The comparison of quality factor changes at different IgG solutions.

4. Conclusion

We developed 30-GHz phonon biosensor using graphite thin-film resonator. Compared to other biosensors, we expect high detection sensitivity.

References

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