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Development of High-Sensitive Biosensor using Diffusion of Ultrahigh-Frequency Phonons in Ultrathin Films

ナノ薄膜内の超高周波フォノンの拡散現象を用いた高感度バイオセンサの開発

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1. Introduction

Irradiation of ultrathin films with ultrafast light pulses generates ultrahigh-frequency phonons in films, which are diffused in-plane and out-of-plane directions. This diffusion process can be detected by a time-delayed ultrafast probe light pulse through change in its reflectivity because thermal phonons modifies material's reflectivity. We predict that diffuison behavior of phnons is sensitive to the physicochemical change of the film surface. When proteins are bound to a thin film through the corresponding receptor proteins immobilized on the film, energies of phonons in the film will leak to outside via the captured proteins. We then propose a new biosensor utilizing this phenomenon. We used a femtosecond fiber laser for light source of the excitation and detection system of high-frequency phonons, because it shows much higher output stability than conventional Ti-sapphire pulse lasers[1]. This biosensor has a high potential as a real-time and label-free biosensor.

2. Optics

Figure 1 shows the optics we originally developed in this study. The linearly polarized light pulse at the output has a wavelength of 1064 nm, pulse duration of 150 fs, pulse repetition rate of 50 MHz. Considering the absorption coefficient of light, this wavelength is too long to excite ultrahigh-frequency phonons in metallic films, and we focus it on a second-harmonic-generator crystal to obtain a visible 532-nm light pulse. It propagates through a λ/2 wavelength plate which rotates the polarization direction, and it is separated into the pump and probe light pulses by a polarizing beam splitter (PBS). The pump light pulses with the perpendicular polarization pass through a mobile stage controller, which controls the optical length of the pump light pulse to produce difference in optical lengths between pump and probe lights. The intensity of pump light pulses is modulated at 1

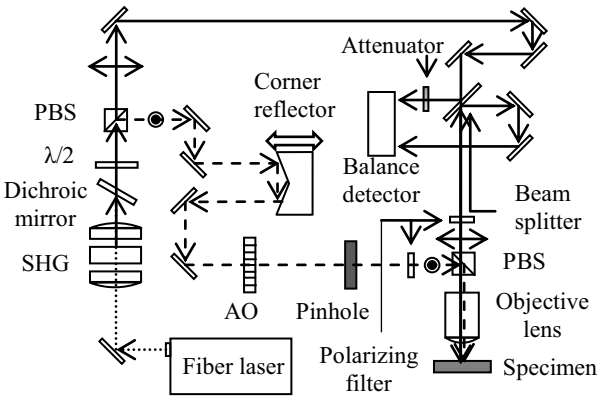

Fig. 1 Optics for the pump-probe technique with the fiber laser.

MHz by an acousto-optics modulator. They are reflected by the other PBS and irradiate the specimen perpendicularly, which generate ultrahigh-frequency phonons. The time-delayed probe light pulses with the in-plane polarization transmit the PBS crystals and irradiate the specimen perpendicularity to the surface to detect the diffusion phenomenon of phonons. The reflected probe light pulses transmit the PBS again and enter the balance detector. The baseline-subtracted output from the detector enters a lock-in amplifier, which extracts the modulation-frequency components, providing information on the phonons inside the specimen.

3. Specimens

As the specimen, we choose Au/Si_3N_4 free-standing film on the Si substrate. A 90 nm Si_3N_4 free-standing film was deposited by the chemical-vapor-deposition method, and center rectangular part of Si substrate (2×2 mm^2) was removed by anisotropic etching with a potassium-hydroxide solution to fabricate partially free-standing Si_3N_4 film. The 12 nm Au film is then deposited on the Si_3N_4 film by the magnetron

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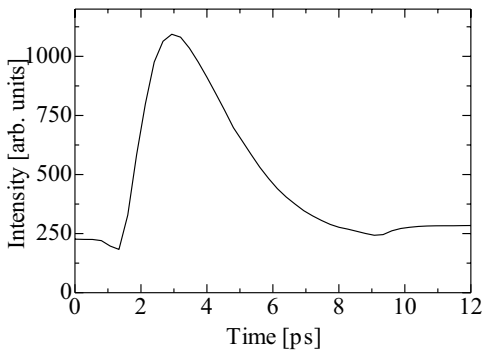

Fig. 2 Reflectivity change from the Au/Si_3N_4 free-standing film.

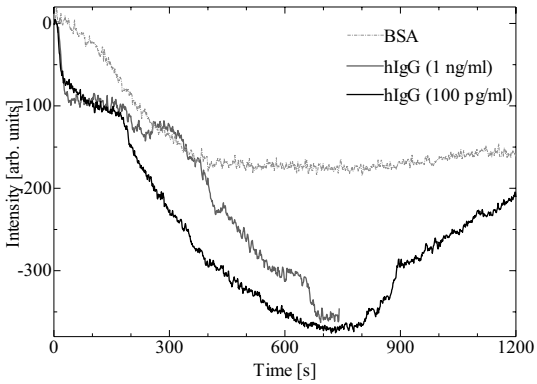

Fig. 3 Binding curves for the BSA solution and different hIgG-concentration solutions.

sputtering method. We contacted the solution with Au surface and irradiated the specimen from the silicon-nitride side with light pulses.

4. Biosensor experiments

We detected the human immunoglobulin G (hIgG) as an antigen with an anti-hIgG antibody as a receptor immobilized on the Au surface of the specimen. The modification of the metal surface was performed in the chemical method with the self-assemble-monolayer technique.

The phosphate buffered saline (PBS) solution was used for dilution of hIgG and as a buffer in whole flow injection analysis. We also used a 10 mg/ml of bovine serum albumin (BSA) in PBS as a control, and flowed in order of BSA, PBS, hIgG (100 pg/ml), PBS and hIgG (1 ng/ml) continuously.

5. Results and Discussion

Figure 2 shows the time-resolved reflectivity changes of the probe light observed from the Au/Si_3N_4 free-standing film. The peak intensity of a signal at about 3 ps is four times higher than the baseline level, indicating that high-frequency phonons were successfully generated and observed by the optics with a fiber laser. After the peak, the intensity is lowered quickly, indicating fast diffusion of phonons in Au film.

Figure 3 shows the changes in the phonon intensity of the Au/Si_3N_4 free-standing film at a fixed time of 3 ps during the antigen-antibody reaction. The intensity decreases after both concentrations of hIgG solutions arrive at the sensor surface. We expect that energy of phonons leaks to the solution via the binding of hIgG on the surface, and the result in Fig. 3 indicates that the binding reaction between hIgG and anti-hIgG is successfully monitored with ultrathin films. Both binding curves are similar, which confirmed specific binding is successfully detected.

The intensity decreased slowly as BSA solution arrived. This is attributed to the non-specific binding to the surface area.

The detection of a 100 pg/ml hIgG solution is difficult with a conventional label-free biosensor owing to the low concentration of hIgG. For example, the detection limit of conventional quartzcrystal-micro-balance biosensor and surface-plasmon-resonance biosensor for an antibody is of the order of 10 ng/ml[2-3]. Thus, the high-sensitivity potential involved in the present biosensor is remarkable.

6. Conclusion

We have successfully developed a newly biosensor using diffusion of ultrahigh-frequency phonons in ultrathin films. The diffusion process of ultrahigh-frequency phonons in the Au film is successfully detected by the optics using a fiber laser as a light source. Using diffusion of phonons, we could detect antigen-antibody binding reaction at very low concentration of antigen. This suggests a high potential as a high-sensitive label-free biosensor.

References

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