

Development of Thin-Film Biosensors Using Picosecond Ultrasound Spectroscopy

ピコ秒超音波法を用いたナノ薄膜バイオセンサの開発

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1. Introduction

Great attention is currently focused on the quartz-crystal-microbalance (QCM) sensor as a biosensor, which can quantitatively detect biomarkers for diagnosis such as C-reactive protein for inflammation¹⁾ and amyloid- β peptides for Alzheimer²⁾. Their early detection significantly contributes to effective treatments of corresponding diseases. Additionally, the QCM biosensor can be a powerful tool for the development of drugs since it can monitor biochemical reactions, such as the antigen-antibody reaction, in real time and evaluate their affinity.

The sensitivity of a QCM biosensor is highly improved by thinning the quartz crystal because it is a mass-sensitive biosensor³⁾. Therefore, significant efforts have been carried out to make it thinner. However, a conventional QCM has gold electrodes on both surfaces, which reduce sensor sensitivity because much higher mass density of the electrodes than that of quartz causes remarkable inertia resistance, deteriorating the mass sensitivity of QCM. Recently, the wireless-electrodeless QCM⁴⁾ has been developed, which eliminates the gold electrodes and can be free from their influence, but it is difficult to make the crystal thinner than $\sim 10 \mu\text{m}$ because of the handling and strength problems.

We then focus on the pump-probe picosecond ultrasound method⁵⁾ which enables us to excite ultrahigh-frequency oscillations in metallic thin films. Using the Pt thin film as an oscillator, we intend to develop the ultrahigh-sensitive mass sensor. The thickness of the Pt thin films used in this work is $\sim 25 \text{ nm}$, being much lighter than quartz plates used for QCM sensors and promising dramatic improvement of the sensitivity. Furthermore, such sensor can realize the real-time and label-free monitoring of biochemical reactions like QCM biosensors. Here, we demonstrate the characteristic features of the Pt thin film oscillator as a biosensor by detecting human immunoglobulin G (hIgG) antibodies via staphylococcal protein A (SPA) molecules immobilized on the surface of the Pt thin film deposited on a glass substrate.

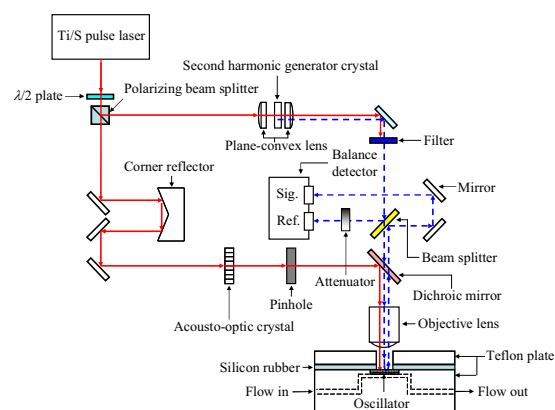


Fig. 1 Optics for the PU biosensor system. The thin-film sensor chip is set in a handmade sensor cell, which is incorporated in the flow-injection system. Dashed lines show the probe light path (400 nm), and solid lines show the pump light path (800 nm).

2. Optics

Fig. 1 shows the optics we developed for this study. We used a titanium-sapphire (Ti/S) pulse laser with 800-nm wavelength, 80-MHz repetition frequency, and 200-fs pulse width. The light pulse was split into pump and probe light pulses. The pump light was amplitude-modulated by an acousto-optic crystal with 100-kHz frequency, and the probe light was frequency-doubled by a second harmonic generator crystal. Both light pulses were focused onto the same area of the Pt thin film by using the dichroic mirror and the objective lens. The time-resolved reflectivity change represents the oscillation of the film because intensity and phase of the reflected probe light are varied by the elastic strain in the film. In this optics, the reflected probe light was received by a balance detector which can subtract the reference signal from the received signal. The 100-kHz frequency component of the output signal from the detector was extracted with a lock-in amplifier. **Fig. 2** shows measured reflectivity changes of the probe light on the 24-nm Pt thin film deposited on the amorphous SiO_2 substrate (7-mm diameter and 0.2-mm thickness), using the magnetron sputtering method. These

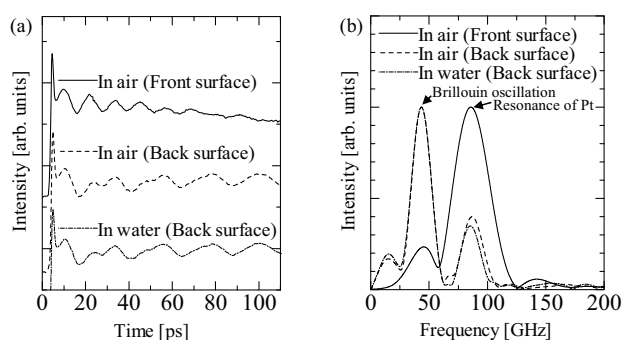


Fig. 2 (a) Time-resolved reflectivity changes observed for the 24-nm Pt thin film on the SiO₂ substrate. (b) Fourier spectra of oscillations in (a).

results indicate that we can excite ultrahigh-frequency oscillations in the Pt thin film and detect them in either case the light pulses enter from the front (Pt) or back (substrate) side of the oscillator. Even when the film contacts with water, we can observe the oscillation.

3. Experimental Section

The surface of the Pt thin film was cleaned by a piranha solution (98 % H₂SO₄ : 33 % H₂O₂ = 7:3) for 10 min and washed by ultrapure water. Then, a 2.2-mg/ml 10-carboxy-1-decanethiol (10-CDT)/ethanol solution was dropped on the surface and incubated for 24 h at 4 °C to form a self-assembled monolayer. After rinsing the surface with ultrapure water, the surface was activated with a 19-mg/ml 1-ethyl-3-[3-dimethyl-aminopropyl] carbodiimide hydrochloride solution for 1 h at RT. Next, the SPA molecules were immobilized on the Pt surface by immersing the chip into a 0.4-mg/ml SPA/phosphate buffer saline (PBS) solution (pH 7.4) for 24 h at 4 °C. Unreacted carboxyl groups of 10-CDT on the surface were blocked by a 10-mg/ml bovine serum albumin/PBS solution for 1 h at RT and rinsed by the PBS solution several times.

The surface-modified chip was set in the homebuilt reaction cell to expose its sensor surface to the solution flow. Then, we made a steady flow of the PBS solution, and injected a hIgG/PBS solution to cause the SPA-hIgG binding reaction. After the injection of the PBS solution, a glycine HCl buffer (GHB) solution (pH 2.2) was injected to dissociate the hIgG molecules from the SPA immobilized on the surface. Finally, the PBS solution was injected for rinsing the surface. In this experiment, the flow rate of all the solutions was 300 μl/min.

4. Results and Discussion

Fig. 3 shows the reflectivity change monitored at a fixed time of 31 ps during the injections of the several solutions. After the arrivals of each hIgG solution, the reflectivity decreases exponentially. Moreover, it remains in lower level

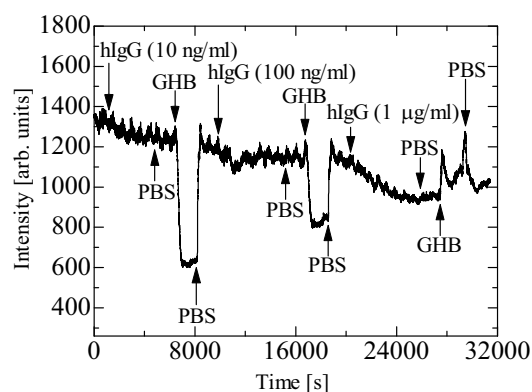


Fig. 3 Monitoring of the reflectivity at 31 ps during the sequential injections, including the SPA-hIgG reaction.

after the solutions are switched to the PBS solution. Therefore, we could monitor the binding reaction between SPA and hIgG with this Pt thin film. After the injections of the GHB solution, we observed large changes in the reflectivity. These would be caused by differences of the density and viscosity between PBS and GHB solutions, indicating that this new sensor is sensitive to the solution characteristics.

One of the highest priority issues on this measurement is the instability of the baseline. Additionally, the Q factor of the oscillator is very low due to the energy loss into the substrate. (In fact, we tried to detect the frequency change caused by the injection of the hIgG solution, but failed to observe significant change.) So, further works will be necessary to solve these problems.

5. Conclusion

Using picosecond ultrasound method, the possibility of the Pt thin film as a high-sensitive mass sensor is proposed. However, the baseline stability of the response curve is low. This problem will be resolved by utilizing a stable fiber laser as the light source. We can realize such optics by changing the light source and replacing the dichroic mirror by a polarizing beam splitter in Fig. 1. The low Q factor of the Pt oscillator will be improved by using a free-standing films, such as the Si₃N₄ self-supported film to prevent the oscillation energy loss into the substrate.

References

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