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A Silicon-Based Optical Thin-Film Biosensor Array for Real-time Measurement of Bio-molecular Interaction

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1. Introduction

In the recent decades, much attention has been paid to un-labeled bio-molecule detection sensors, such as surface plasmon resonance (SPR), resonant mirror detection (RMD), and reflectometric interference spectroscopy (RIfS), for real-time measurement of bio-molecular interaction [1-4]. In these sensors, the RIfS transducer is suitable for high-throughput screening applications [5].

The basic principle of bio-molecule detection using RIfS was suggested by Langmuir and Schaefer in 1937 [6]. They reported that a remarkable change of interference color was produced by adsorption of a protein monolayer onto a polished chromium slide with a multilayer barium stearate film. In 1985, Sandström et al. used a silicon-oxide film on silicon substrates for the RIfS sensor [7]. By using an opaque silicon substrate, they prevented interference by reflected light from the back surface of the substrate. In these reports, since the color change of the RIfS sensors was observed in the atmosphere after drying, real-time measurement was not achieved.

Following the above studies, Brecht et al. developed RIfS into a real-time sensing system with a sample flow system and a photodiode-array spectrometer [3]. They used interference layers on glass substrates. Birkert and Gauglitz also developed their RIfS system into 96- and 384-well RIfS microplates by gluing plastic grids onto the interference layers [4].

For real-time sensing, particularly for multi-channel sensing with an array of RIfS sensors, opaque silicon substrates are also useful, since transmitted light, which passes through the interference layer, is completely absorbed in the substrate and does not affect the observation. Further, using silicon wafers for the substrate enables the mass-production processes of the semiconductor industry to be applied to fabrication of disposable biochips, and the silicon wafers have become inexpensive.

In light of these works, the authors have developed RIfS-based real-time sensing biochips consisting of optical thin-film (i.e., interference layers) arrays on silicon substrates. We then constructed a prototype multi-channel sensing system for the biochips and used it to measure protein-protein interaction.

2. Experimental

Biochip preparation

Figure 1 shows a top view of the biochip and a cross section of one sensing spot of the biochip. Silicon wafers were used as the substrate. Titanium-oxide film (TiOx) was chosen as the optical thin film and deposited on four spots on the substrate as shown in Fig. 1. This means that four sensing spots were fabricated on the biochip. Since the refractive index of the optical thin film was 2.2, which is relatively high compared to that used by Sandström et al. [7], the interference color of the sensors was enhanced in aqueous solution. Thickness of the optical thin film was 70 nm. For immobilization of a probe protein onto the sensor surface, an amino group was introduced to the surface by means of silane coating with 3-aminopropyltrimethoxysilane (APTMS). As a probe protein, avidin was immobilized to the amino group of the two inner sensing spots by using water soluble carbodiimide (WSC) and *N*-hydroxysuccinimide (NHS). And to obtain a reference signal, bovine serum albumin (BSA) was immobilized to the outer two sensing spots in the same way. To classify the sensing spots, the two inner sensing spots are referred to as “avidin spots” and the other two, “reference spots”.

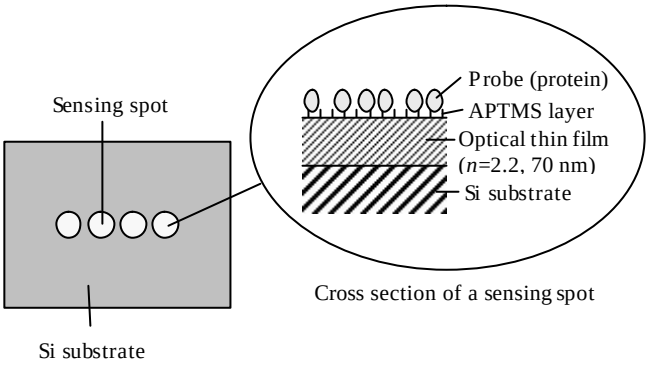

Fig. 1: Top view of the biochip. The above-right inset shows the cross section of one sensing spot of the biochip.

Real-time measurement of protein-protein interaction

Figure 2 shows a schematic diagram of the sensing system. A tungsten halogen lamp was used as a light source. Illumination light from the lamp and reflected light from each sensor spot were guided by optical fibers. The reflected light was guided to a four-channel spectrometer. Reflection spectra of all sensing spots were analyzed simultaneously by a personal computer (PC) in real time. The wavelengths of the interference peaks of each spectrum (λ_{AR}) were calculated by the PC, and the shifts of the peak wavelengths ($\Delta\lambda_{AR}$) were recorded and plotted on the PC

screen. Samples were introduced by a flow system, using a high-performance liquid-chromatography (HPLC) pump and a HPLC sample injector. The sample solutions were 1-100 µg/ml anti-avidin antibody solutions in phosphate-buffered saline (PBS). PBS was made to flow along the surface of the biochip by the pump. The sample solution was then injected into the flow cell for 10 minutes by the injector. The authors expected that $\Delta\lambda_{AR}$ would increase when the antibody of the sample solution is bound to the probe protein.

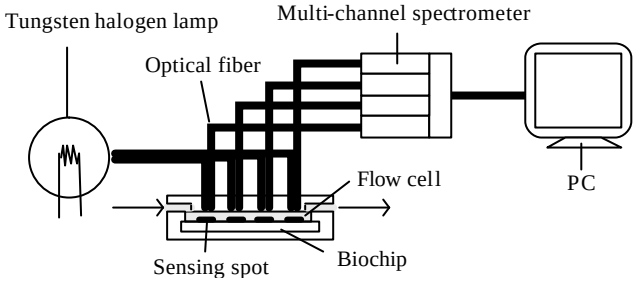

Fig. 2: Schematic diagram of the prototype sensing system.

3. Results and discussions

Figure 3 shows a reflection-absorbance spectrum of the sensing spot in the water. The interference peak appeared at a wavelength around 570 nm.

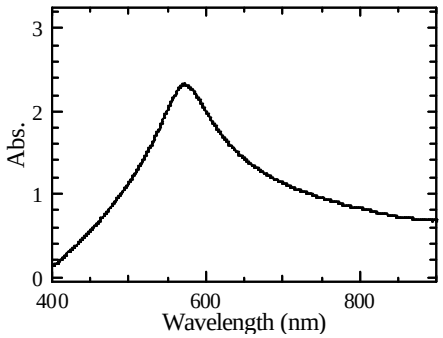

Fig. 3: Reflection-absorbance spectrum of the sensing spot.

Figure 4 shows the results of the real-time measurement of the interaction between avidin and anti-avidin antibodies. Figures 4(a), (b), and (c) show the results of the injection of the 1-µg/ml, 10-µg/ml, and 100-µg/ml antibody solutions, respectively. Solid and dotted lines show the responses of one avidin spot and one reference spot, respectively. Similar results were obtained for the other two sensing spots. Arrows indicate the start of sample injection. It is clear that $\Delta\lambda_{AR}$ of the sensing spot increased after the sample injection, while a small change was seen for the reference spot. As the concentration of the sample solution increased, $\Delta\lambda_{AR}$ of the avidin spot increased.

Numerical calculation estimated that a 0.01-nm shift of $\Delta\lambda_{AR}$ corresponds to a binding amount of protein of about 10 pg/mm^2. The minimum limit of detection for this system was therefore estimated to be in the order of 10 pg/mm^2 by comparing the numerically estimated shift and the experimentally measured shift, which is shown in Fig. 4(a).

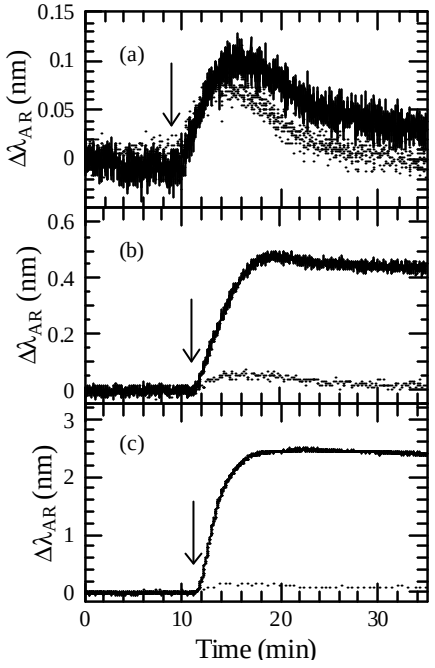

Fig. 4: Real-time measurement results of avidin-anti-avidin antibody interaction: (a), (b), and (c) show the results of the injection of 1-µg/ml, 10-µg/ml, and 100-µg/ml antibody solutions, respectively. The solid and the dotted lines show the results from the avidin spot and the reference spot immobilized with bovine serum albumin (BSA), respectively.

4. Summary

Real-time multi-channel measurement of protein-protein interaction in 1-100 µg/ml antibody solution samples was successfully demonstrated by means of an RIfS-based silicon biochip. The number of sensing channels can be increased in principle by adding more sensing spots and optical fibers to the biochip. The biochip can be fabricated by semiconductor mass-production technology and is disposable. Since the electrical property of the silicon wafer is not important, the wafer for the substrate of the biochip can be a low-grade one. Therefore, a key benefit of this biochip is its low running cost.

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