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Silicon-Nitride-Coated Silicon Biochip for Real-time Optical Sensing of Biomolecular Interaction

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1. Introduction

In recent decades, much attention has been paid to unlabeled biomolecule detection sensors, such as surface plasmon resonance (SPR), resonant mirror detection (RMD), and reflectometric interference spectroscopy (RIfS), for real-time measurement of biomolecular interaction [1-4]. Of these sensors, the RIfS transducer has an advantage in arraying the sensors for high-throughput screening applications [5].

RIfS transducers are composed of an optical thin film and probe molecules that are immobilized on the surface of the film. The amount of biomolecules that are bound to the probe molecules is measured from changes in the light interference by observing reflected light from the film [3, 4, 6]. In a previous report [4], we developed a biochip that has four real-time RIfS transducers on a silicon substrate and a prototype-sensing system for real-time measurement of interference-peak shifts, and we successfully observed protein-protein interaction using them. Silicon wafers were used for the substrate, since transmitted light, which passes through the interference layer, is completely absorbed in the substrate and does not affect the observation [6]. Furthermore, for the real-time sensing, we chose titanium oxide films with a refractive index of 2.2 as the optical thin films of the biochip, since the light interference was enhanced in aqueous solutions.

It is desirable that biochips be chemically stable, especially in alkaline solutions, such as sodium hydroxide, since wet cleaning and surface-modification processes are applied to the biosensors [1]. However, silicon wafers dissolve in alkaline solutions. Furthermore, although the gradual changes in bound molecule density are measured in real-time sensing, the relationship between the density and the RIfS signal has not been supported theoretically.

In this report, we chose silicon nitride (Si_xN_y) film for the interference layer of the RIfS transducers on the silicon substrate, and we coated both surfaces of the substrate with the Si_xN_y film to prevent the silicon substrate from being etched in the alkaline solutions. We also observed protein-protein interaction using the Si_xN_y biochip and simulated sensing to ensure the relationship between the signal and the density of bound molecules.

2. Experimental

Biochip preparation

Figure 1 shows the schematics of our biochip. We fabricated four sensing spots on the biochip, which was 26 × 20 mm, as shown in Fig 1 (a). To obtain chemical resistance to alkaline solutions, we fabricated Si_xN_y films on both sides of a silicon wafer using chemical vapor deposition. The film was 70-nm thick, had a refractive index of 2.2, and was alkaline resistant. We made the sensing spots clearly visible by color by etching away the other regions on the film's obverse to roughly half of the original thickness, as depicted in Fig. 1(b).

To immobilize a probe protein on 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 affixed to the amino group of the two inner sensing spots by using water soluble carbodiimide (WSC) and *N*-hydroxysuccinimide (NHS). To obtain a reference signal, bovine serum albumin (BSA) was affixed 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 as "reference spots".

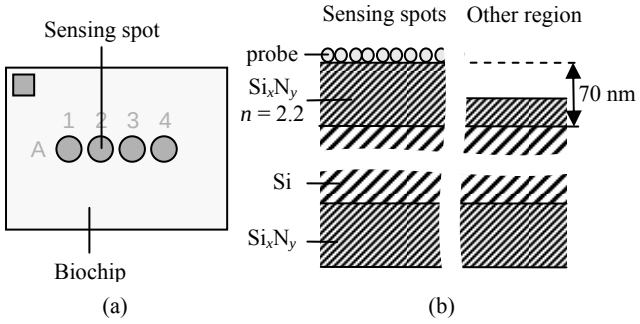

Fig. 1: Schematics of the biochip. (a) Top view. (b) Cross section.

Computer simulation

Figure 2 illustrates the biochip sensing calculation model. We assumed that the optical thin film on the silicon substrate was 70-nm thick and had a refractive index of 2.2, and that the refractive index of the background was 1.333. We assumed that the probe molecules correspond to a 10-nm-thick transparent layer, and that the sample molecules that are bound to the probe molecules correspond to another 10-nm-thick transparent layer. We fixed the refractive index of the probe layer at 1.5. We varied the refractive index of the sample layer across the range from 1.333 to 1.5. We calculated reflection spectra assuming the angle of reflection was 0º. The wavelength of the interference peak of the biochip (λ_{peak}) and their shifts ($\Delta\lambda_{peak}$) were calculated for each variant of the refractive index using the method

that we reported previously [4]. We then plotted $\Delta\lambda_{peak}$ versus density of the bound molecules. The density of the 10-nm-thick layer with a refractive index of 1.5 was approximately 16.7 ng/mm^2 [6]. Between 0 and 16.7 ng/mm^2, we applied effective mass approximation based on the Lorentz-Lorenz theory [7].

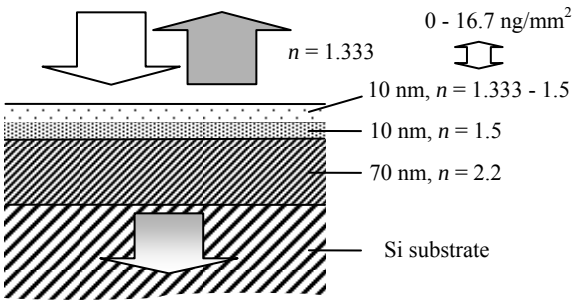

Fig. 2: Calculation model of RIfS sensing.

Real-time measurement of protein-protein interaction

We observed interaction between avidin and anti-avidin antibodies using the biochip. The values for λ_{peak} and $\Delta\lambda_{peak}$ on each sensor of the biochip were simultaneously measured using the prototype real-time-sensing system reported in our previous paper [4]. To detect changes in light interference on each sensor, illumination light from a lamp and reflected light from each sensor were guided by optical fibers. Solutions were introduced with a flow system, using a syringe pump. The sample solution was 1 μg/ml anti-avidin antibody solution in phosphate-buffered saline (PBS). The PBS was initially 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.

3. Results and discussion

Figure 3 plots the calculated results. Figure 3(a) shows the calculated reflectance-density spectrum. The λ_{peak} appeared at a wavelength of approximately 630 nm. Figure 3(b) shows the dependence of $\Delta\lambda_{peak}$ on the effective density of bound molecules. We confirmed that $\Delta\lambda_{peak}$ varies almost linearly depending on the density of bound molecules.

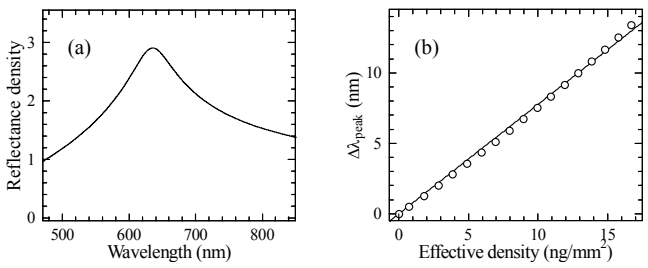

Fig. 3: Calculated results. (a) Reflection spectrum. (b) Dependence of $\Delta\lambda_{peak}$ on effective density of bound molecules.

Figure 4 plots the results of the real-time measurement of the interaction between avidin and anti-avidin antibodies. The solid line is the response of one avidin spot, and the dotted line is the response for one reference spot. Similar results were obtained for the other two sensing spots. The arrow indicates the period of the sample injection. It is clear that the $\Delta\lambda_{peak}$ of the sensing spot increased during the sample injection, while almost no change was seen for the reference spot.

We estimated the minimum limit of detection from the standard deviation σ of $\Delta\lambda_{peak}$ before the sample injection. Since the 3σ limit of detection of $\Delta\lambda_{peak}$ was approximately 0.01 nm, the minimum limit of detection was estimated to be approximately 10 pg/mm^2.

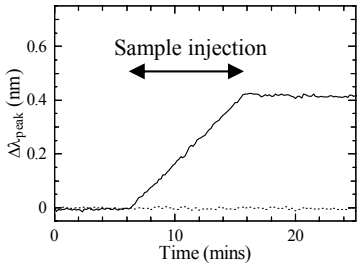

Fig. 4: Real-time measurement results of avidin-anti-avidin antibody interaction. The solid and dotted lines show the results from the avidin spot and the reference spot.

4. Summary

We developed a silicon-nitride-coated silicon biochip that has an array of real-time reflectometric-interference spectroscopic sensors. We confirmed the approximate linear relationship between the sensor response and the density of bound molecules by computer simulation. Furthermore, we successfully demonstrated the biochip's ability to detect protein-protein interaction. The minimum limit of detection was estimated to be approximately 10 pg/mm^2 of bound molecules. Its key benefit is chemical resistance to alkaline solutions, since this simplifies cleaning and surface modification. Another benefit is mass productivity, since we can apply common methods in semiconductor industries, for example chemical vapor deposition and resist processes, to fabricate the array of the sensors.

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