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# Selective Detection of Antigen-Antibody Reaction Using Si Ring Optical Resonators

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## 1. Introduction

An immunoassay, where the reaction between antigen and antibody is detected, is very important for the diagnosis of many infectious diseases, including HIV and many kinds of allergies. The immunoassay is usually carried out using antigen or antibody labeled with enzyme or fluorescent materials, for example in enzyme-linked immunosorbent assay (ELISA). We have been studying biosensor using Si ring optical resonators without labeling [1]. The strong reaction between biotin and streptavidin was reported [1]. The proposed biosensor chip with Si ring resonators is shown in Fig. 1, where simultaneous detection of plural kinds of antigen-antibody reaction is possible by using different kind of antibody to each ring.

In this paper we have, for the first time, demonstrated antigen-antibody reaction by using the ring resonator using the major house-dust mite allergen Der f 2 and anti-Der f 2 IgG antibody (immunoglobulin type G which is selectively bound to Der f 2). The effect of the high-temperature hydrogen annealing to improve the quality factor of the resonator is also indicated.

## 2. Experimental

An example of the ring resonator is shown in Fig. 2. The fabrication process is reported in Ref. 1. Resonance occurs when the circumference length of the ring is equal to the integral multiple of wavelength. When some substance is adsorbed on the ring, the effective refractive index of the ring is changed and the resonance wavelength is changed. The ring is coupled to the input/output waveguides separated by the small gap (0.1~0.3 μm). When the wavelength of the input light is fixed at near the resonance peak, the output intensity is sensitively changed depending on the amount of the adsorbed substance. The sensitivity is determined by the quality factor *Q* which is changed by the coupling efficiency and the waveguide loss. The sample structure with liquid fluidic channel is shown in Fig. 3 and the measurement system is shown in Fig. 4. The input/output light is guided by the lensed fibers. The light wavelength is ranging from 1480 to 1540 nm using tunable semiconductor laser. The output light is detected by InGaAs photodiode. One of the resonance characteristics with *Q* of 5400 in the pure water is shown in Fig. 5.

The major mite allergen called Der f 2 is employed as a test antigen which is fixed on the ring surface using Si binding protein (Si-tag or SBP). Figure 6 shows a preparation method of a fusion protein of Si-tag and Der f 2 which is, for the first time, produced in *E. coli* by using recombinant technology. Experimental procedure is shown in Fig. 7.

## 3. Results and Discussions

### *3.1 Detection of antigen-antibody reaction*

Figure 8 (a) shows resonance spectra after each treatment step for rabbit Der f 2 antiserum. The resonance peak shifts toward longer wavelength by ~0.25 nm after exposing to the solution containing the fusion protein of Si-tag and Der f 2, which indicates that the fusion protein is adsorbed on the ring. Next, Der f 2 antiserum diluted to 1/100 with the pure water results in further shift of 0.18 nm, which means that something is adsorbed to the receptor of the Der f 2. In order to specify what is adsorbed, the anti rabbit IgG antibody (one of the antibody which is bound to rabbit IgG) is exposed to the sample. As a result a shift of 0.11 nm is observed, indicating that the adsorbed substance is IgG. Comparing with Der f 2 antiserum, the normal rabbit serum, which does not contain Der f 2 antibody, is exposed to the sample and the result is shown in Fig. 9. The resonance peak shift is less than half (0.07 nm) and the following exposure to the anti IgG results in no detectable shift. This means that the resonance shift observed in response to the addition of the normal serum is due to the non-specific adsorption of some serum material except IgG. These phenomena are modeled in Figs. 8(b) and 9(b).

### *3.2 Improvement of Q-factor by high-temperature $H_2$ anneal*

In order to increase the sensitivity of the biosensor, the quality factor *Q* of the resonator must be improved. High temperature (~1000°C) $H_2$ anneal is reported to be effective to smooth Si surface [2]. This method was applied to improve *Q* of the Si ring resonator. Figure 10 shows *Q* in air at wavelength of 1.5 μm vs coupling length for as-fabricated ring resonators and annealed ones at 925°C for 10 min in 40 Torr $H_2$. *Q* increases about 50% by the anneal. SEM pictures of Si waveguides before and after the $H_2$ annealing are shown in Fig. 11. The initial rectangular cross section is deformed to dome shape and the Si surface seems to become smooth. The record of the propagation loss of Si waveguide is reported to be ~2 dB/cm [3]. Although our case is still much worse (12 dB/cm), the *Q* will be much improved by optimizing the $H_2$ anneal condition.

## 4. Conclusions

We have developed Si ring resonator biosensors and, for the first time, succeeded in detection of selective antigen (Der f 2)-antibody (IgG) reaction. Improvement of quality factor by high-temperature $H_2$ anneal was also confirmed.

## Acknowledgments

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## References

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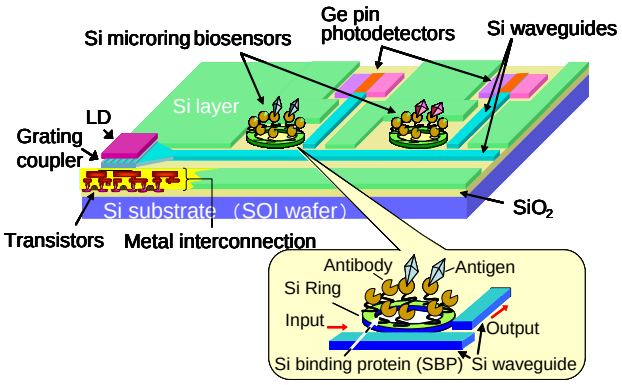


Fig. 1. Proposed biosensor chip with Si ring resonators which enable simultaneous detection of plural kinds of antigens by changing the kind of antibody attached with each Si ring.

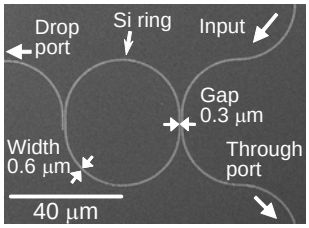


Fig. 2. Typical example of SEM photograph of fabricated Si ring resonator.

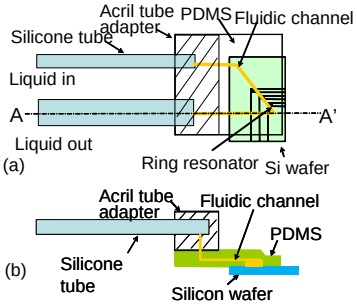


Fig. 3. Schematic (a) plan view and (b) A-A' cross section of the fluidic channel using PDMS attached to the wafer.

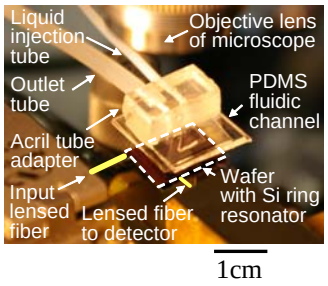


Fig. 4. Photograph of optical measurement system with fluidic channel, lensed optical fibers and optical microscope.

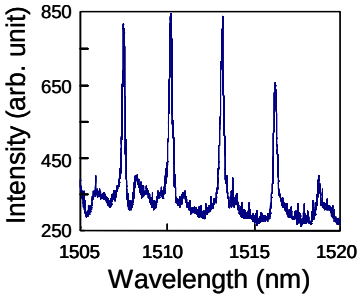


Fig. 5. Example of resonation spectrum of the ring resonator in pure water with *Q* factor of 5400.

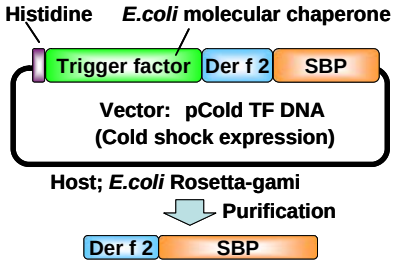


Fig. 6. Preparation method of fusion protein of Si-tag (or Si binding protein: SBP) and major mite allergen Der f 2.

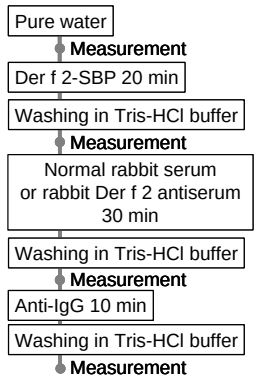


Fig. 7. Experimental procedure for detection of antigen and antibody reaction. "Measurement" means the measurement of optical resonance spectrum. Tris-HCl is $C_4H_{11}NO_3ClH$ for pH adjustment.

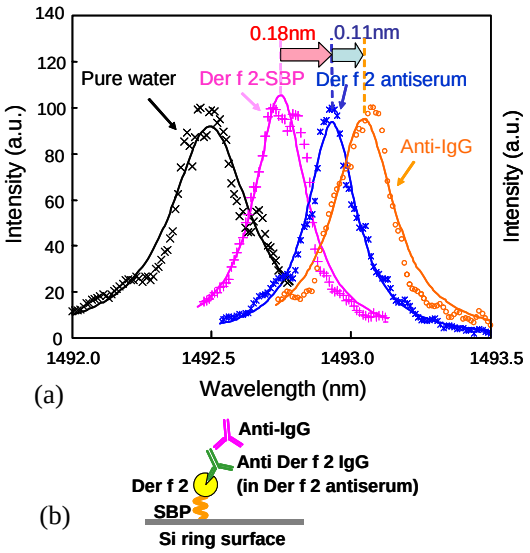


Fig. 8. (a) Resonance spectra after each treatment step for rabbit Der f 2 antiserum. (b) Schematic model for the reaction. IgG which selectively combines with Der f 2 antigen is detected in the antiserum.

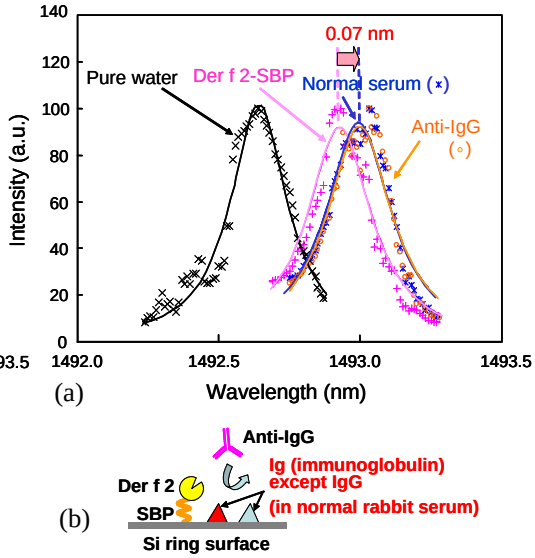


Fig. 9. (a) Resonance spectra after each treatment step for normal rabbit serum. (b) Schematic model for the reaction. IgG which selectively combines with Der f 2 antigen is not detected in the normal serum.

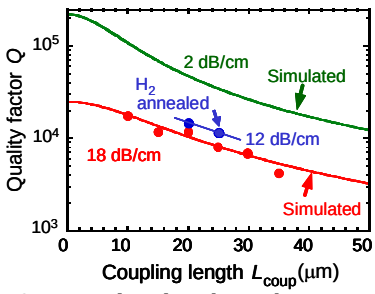


Fig. 10. *Q* vs coupling length. High-temperature (925°C) $H_2$ annealing improves *Q* by about 50%. *Q* of 15000 in air decreases to 6000 in water due to its light absorption.

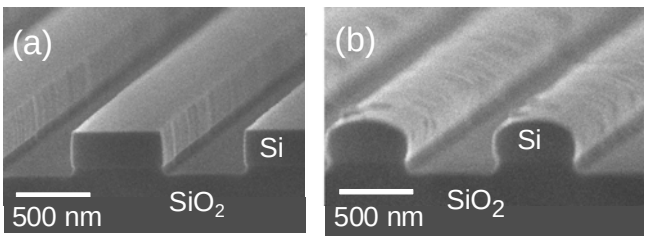


Fig. 11. Cross sectional SEM images of the Si waveguides (a) before $H_2$ anneal and (b) after $H_2$ anneal (925°C). High-temperature $H_2$ anneal leads to deformation of the waveguide and surface smoothing.