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Rapid Biosensing Platform based on Monitoring Changes in the Optical Reflectance of Porous Silicon due to Penetration by Functionalized Superparamagnetic Beads

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I. INTRODUCTION

Monitoring the highly efficient optical interference spectra of light scattered from the surface of porous silicon (PSi) shows potential as means of using Psi as a material for biosensing applications [1]. Reports show the spectral positions of the Fabry-Pérot fringes to shift as a function of the refractive index of the material filling the pores of PSi. Notably, the penetration of biomolecules into pores of PSi due to either non-specific adsorption or specific binding, is observed as a shift of the Fabry-Pérot fringes to longer wavelengths [2]. This effects is due to an increase in the refractive index of the PSi layer as biomolecules displace aqueous solution occupying the pores. The optical properties of PSi, which is fabricated by electrochemical anodization, are precisely tunable by control of the geometric orientation and size of the pores [3]. Thus PSi could potentially be used for applications such as optical devices and chemical and/or biosensors. We are interested in exploiting the aforementioned optical interference properties of PSi for biosensing. To-date PSi-based biosensors have been based on a large surface area matrix covered with immobilized biomolecules such as enzymes, proteins, or DNA fragments. However, such an approach is slow, taking hours for molecules to deeply penetrate inside pore walls where complementary molecules are immobilized. Furthermore, a small concentrations of molecules and/or small molecules only produce a vanishingly small change in the scattered optical signal. Thus, a means of additional amplification is essential for higher sensitivity.

Here we report on a novel protocol utilizing functionalized superparamagnetic nanoparticles (beads) for not only amplifying the spectrum shift of the reflected optical signal, but also for enhancing the efficiency of the penetration of biomolecules into pores. Superparamagnetic beads of ~130 nm in diameter, penetrate porous silicon with pore diameters of about 500 nm. The spectrum shift of the scattered light caused by the dramatic change in index refraction of the pores due to penetration by superparamagnetic beads was readily observed by spectroscopic measurements. More importantly, due to the functionalized superparamagnetic beads, wavelength shifts of the reflection spectrum due to chemical binding reactions between the beads and the PSi was amplified and clearly observed although such reactions are extremely difficult to detect by conventional methods.

II. EXPERIMENTAL

Fig. 1 illustrates the concept of our procedure for detecting biomolecules immobilized on the walls of pores of PSi. First, NH_2 was immobilized onto the walls of pores (maximum pore diameter 400–500 nm) on a silicon substrate using an aminofunctional silane [3-propyltrimethoxysilane (APTS)] as an adhesive. Next, the functionalized substrate was placed under a light source and and photodiode, and an aqueous solution containing 130 nm superparamagnetic beads covered with COOH was dropped onto the surface of the substrate. The concentration of the beads in the solution was diluted to 10 μg/ml with a phosphate buffer solution (PBS : pH = 7.0). Subsequently, a permanent magnet was placed underneath the substrate, producing a perpendicularly aligned magnetic field with respect to the surface of the substrate to enhance the penetration of the superparamagnetic beads into the pores immobilized with NH_2, resulting in a drastic reduction of the time required for the amide reaction reaction between the magnetic beads and the NH_2 immobilized inside the pores. Next, the permanent magnet was removed and the substrate rinsed with PBS (pH 7.0) solution to remove unbound biomolecules and unattached superparamagnetic beads. Finally, we monitored the variation of the reflection spectrum with time. For the data acquisition, the samples were illuminated with a tungsten lamp, and the reflected light spectrum was measured using an Ocean Optics 4000 spectrometer fitted with a fiber optic input.

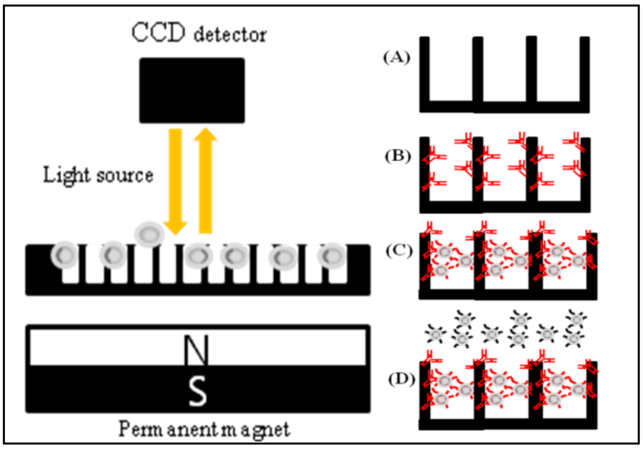

Fig. 1 Schematic diagram of the experimental set up (left) and biosensing process in a pore (right). (a) Shows the opening of pores; (b) pores immobilized with NH_2; (c) COOH functionalized beads penetrate the pores due to the action of the external magnetic field; (d) the external magnetic field is removed and substrate rinsed with PBS solution.

III. RESULTS AND DISCUSSION

The wavelength of a peak in the reflection spectrum is given by the following Bragg's equation (1),

$$m\lambda_{Bragg} = 2n_i d_i$$

where m is the spectral order of the optical fringe, λ the wavelength, n_i the refractive index of the film, and d_i its thickness. The effective refractive index (n) of a porous silicon layer is defined by the porosity and the refractive index (n) of the medium inside the pores. As the refractive index (n) of pores increases, the effective refractive index (n) of the porous silicon layer increases, causing the optical spectrum of the layer to shift to longer wavelengths (red-shift). Thus, by monitoring the reflectance spectrum, it is possible to detect the binding of molecules with functionalized superparmagnetic beads inside the pore since the capture of targets inside the pores increases the reflective index (n). Fig. 2 shows red-shift of the porous silicon reflectivity due to the penetration of superparamagnetic beads. A red-shift of about 7 nm was detected after 10 min of droping a solution of superparamagnetic beads onto the PSi. These results showed that shifts in the wavelength occurred due to the binding of superparamagnetic beads with the functionalized PSi pores.

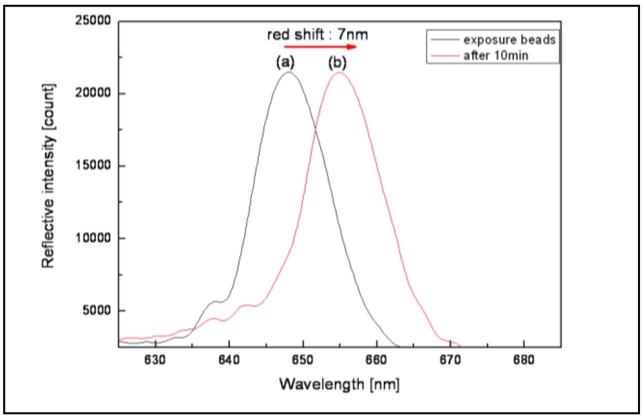

Fig. 2 Optical reflective spectra of PSi surface. (a) Initial spectrum after dropping solution of beads on PSi surface; (b) after 10 min under an external magnetic field (1000 Oe).

IV. CONCLUSION

We demonstrated the feasibility of a novel biosensing method utilizing superparamagnetic beads for medium varying index reflectivity of PSi.

REFERENCES

[1] S.-Y. Lin, K. Motesharei, K.-P. S. Dancil, M. J. Sailor, and M. R. Ghadiri, Science 278 (1997) 840.

[2] Ouyang, H. and P. M. Fauchet, Proc of SPIE. 6005 (2005) 31.

[3] Jane A, Dronov R, Hodges A, and Voelcker NH, Trends Biotechnol. 27 (2009) 230.