SPR Sensor Chips with Polymer Nanogratings*

Young-Ho Cho† and Young-Hyun Jin
Digital Nanolocomotion Center, Bio and Brain Engineering Department, Korea Advanced Institute of Science and Technology (KAIST),
373-1 Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea
(Received 9 November 2008; Accepted 23 April 2009; Published 4 July 2009)

This paper presents low-cost, multi-channel, nano-grating SPR (Surface Plasmon Resonance) sensor chips for applications to disposable biomolecule detection. The present SPR sensor chip consists of two fluidic channels, including a sample channel and a reference channel, each having 833 nm-pitch PDMS gratings. The SPR signals from each channel are coupled by an external mirror and the SPR dip shift in the coupled signals is detected by a single spectrometer. Compared to the previous multi-channel SPR sensors using fiber-optic switches or special prisms, the present SPR sensor chips with PDMS nanogratings result in more compact and less expensive SPR detectors. We have designed and fabricated the SPR sensor chips with two channel gratings having the refractive index sensitivity of 321.78 nm/RI and 514.26 nm/RI, respectively. From the fabricated SPR sensor chips, we measure the SPR dip shift of 1.13 ± 0.16 nm for the target biomolecules of 0.5 μM streptavidin bound on a 10% biotinylated surface. The real-time binding reaction rates of m-antibiotin has been monitored for the fixed concentration of 0.2 μM streptavidin as well as for the varying concentration range of 2-50 nM; thus obtaining the results for the fixed and varying concentration in good agreement with those obtained from a commercialized SPR sensor. It is demonstrated that the polymer nanograting SPR sensor chips offer simple and disposable biomolecule detection methods for point-of-care applications. [DOI: 10.1380/ejsnt.2009.750]

Keywords: Biomedical Engineering; Plasmon; Surface Chemical Reaction; Biological Molecules-Proteins; Nano Structure Fabrication

I. INTRODUCTION

Recently, surface plasmon resonance (SPR) biosensors [1–4] have received an increasing attention as high-speed and high-sensitive biomolecule detectors. Compared to the fluorescence-based optical biosensors, SPR sensors eliminate time-consuming labeling process and reduce molecular binding disturbance. In addition, the label-free detection capability of SPR sensors provides additional advantage to lab-on-a-chip applications.

For the reliable biomolecule detection in interfering environments, however, SPR sensors need to discriminate specific sensor responses from background refractive index variation due to temperature fluctuations, etc. Multi-channel SPR sensing configuration is required to make SPR detection insensitive to background noise, where the detection of target samples and reference samples has been made simultaneously.

The conventional multi-channel SPR sensors include scanned SPR imaging configuration [5, 6] and sequential spectral encoding configuration [7]. The scanned SPR sensors [5, 6] need a mechanical angular scanner in order to couple the incident light to the multiple channels, while capturing the reflected image using a CCD (charge-coupled device) camera. The CCD camera and mechanical angular scanner make the system bulky and expensive. In the sequential spectral encoding sensors [7], the incident light is coupled to multiple sensing channels at different incident angles using a specially-designed prism. The spectrum of the reflected light is measured by a spectrometer. However, the special prism still makes the sensing system bulky and limits its application to point-of-care devices or lab-on-a-chip systems.

In this paper, we simplify the sequential spectral encoding configuration by using a SPR sensor chip (Fig. 1) with polymer nanogratings. Compared to the sequential spectral encoding sensors, the nanograting SPR sensor replaces the special prism with integrated gratings and an external mirror, thus resulting in the smaller and the simpler multi-channel SPR sensing system. To reduce the fabrication cost of the nanograting SPR sensor chip, we develop a micromolding process for the nano-pitch PDMS gratings integrated with fluidic channels.

The paper presents the design, fabrication and characterization of the nanograting SPR sensor chips for multi-channel SPR detection insensitive to background noise, where the detection of target biomolecules is made simultaneously.

FIG. 1: The wavelength-modulated multi-channel SPR sensing system, composed of a SPR sensor chip with built-in polymer nanograting, an external mirror and a spectrometer.
channel biomolecule detection. The refractive index sensitivity and resolution of the nanograting SPR sensor are measured. We apply the SPR sensor to detect the presence and the real-time reaction rate of biotin-streptavidin binding at the fixed streptavidin concentration of 200 nM. We also report the reaction rate measurement of biotin-antibiotin binding for varying antibiotin concentration in the ranges of 2-200 nM. Finally, the results obtained from the present SPR sensor are compared with those from a commercialized SPR sensor.

II. ANALYSIS AND DESIGN

Surface plasmon resonance (SPR) phenomenon is related with the excitation and propagation of the electromagnetic waves along a metal-dielectric interface. On the metal-dielectric interface, the transverse-magnetic (TM)-polarized incident light, whose electric field vector is oscillating parallel to the plane of incidence, is coupled to the surface plasmon due to prisms or gratings. The coupled SPR results in the optical power loss of the reflected light. The SPR conditions depend on the properties of the prism or grating, the metal and surrounding dielectric medium, as well as the angle and wavelength of the incident light. Hence, the changes in the refractive index of the dielectric medium lead to a shift of wavelength or incident angle when SPR occurs. In this paper, we use gold metal layer for biomolecule detection because of its well-developed biomolecule immobilization techniques.

Figure 1 illustrates the working principle of the multi-channel nanograting SPR sensor chip. The incident light enters into the first grating at the incident angle of $\theta_1$, thus exciting surface plasmon and generating a narrow SPR dip (Fig. 1(a)) in the reflected light spectrum. The reflected beam from the first grating is redirected by the mirror and incident to the second grating at the incident angle of $\theta_2$, which is slightly different from $\theta_1$. Since the SPR depends on the incident angle, the reflected beam from the second gratings generates the SPR dip in a different spectral band (Fig. 1(b)). The spectrum of final reflected light shows two SPR dips as illustrated in Fig. 1(c), from which we monitor the refractive index change in each sensing channel based on each SPR dip.

The SPR coupling condition [8] for the grating, whose grooves are perpendicular to the plane of incident light, is expressed as

$$n_D \sin \theta + m \frac{\lambda}{\Lambda} = \text{Re}\left(\frac{\varepsilon_m n_D^2}{\varepsilon_m + n_D^2}\right)$$

where $n_D$ denotes the refractive index of the sample, $\theta$ and $\lambda$ indicate the incident angle and the wavelength of the light, respectively, $\Lambda$ is the pitch of the grating, $m$ means the diffraction order, and $\varepsilon_m$ is the complex dielectric constant of the metal. In the grating design process, we assume the experimental conditions of $n_D = 1.3-1.8$, $\lambda = 550-750$ nm and $\theta = 35-45^\circ$. The complex dielectric constant of gold comes from Drude's model [9]. From Eq. (1), the pitch of the grating has been decided as 833 nm, having 1,200 grooves per mm.

FIG. 2: Fabrication process of the SPR sensor chip composed of two fluidic channels with built-in PDMS nanogratings: (a) micromolding of the PDMS nanograting using a commercial master mold grating; (b) gold sputtering on the nanograting and PDMS channel sidewall formation; (c) glass cover bonding for fluidic channels; (d) fabricated SPR sensor chip.

III. FABRICATION

We fabricate PDMS (poly-dimethylsiloxane) grating using a replica molding technique [10], where the silicon wafer, etched by deep reactive-ion etching (DRIE) process is used as the grating master mold. In the pre-
vious work [10], they fabricated the grating having the minimum pitch of 10 μm, which is too large to use for SPR biomolecule detection. In this research, we fabricate nano-scale pitch grating using a commercialized grating as a master mold. Figure 2 illustrates the fabrication process for the nanograting SPR sensor chip. We treat the surface of the glass substrate with oxygen plasma for good adhesion between the glass substrate and the PDMS layer. The mixture of PDMS pre-polymer and a curing agent (Sylgard 184, Dow Corning, Midland, MI, USA) at the weight ratio of 10:1 is prepared and degassed in vacuum for 30 minutes. We use a commercialized grating having the triangular groove pitch of 833 nm and the triangular groove depth of 180 nm. In Fig. 2(a), the mixture has been poured on the glass substrate and the grating patterns of the master mold were replicated to the PDMS mixture with the mold pressure of 1.7 kPa. The PDMS curing process has been carried out at 85°C for 2 hours. The thickness of the PDMS grating has been controlled by the pre-inserted glass spacers. After the deposition of a 40 nm-thick gold layer on the nanograting, the channel sidewalls are formed using PDMS (Fig. 2(b)). The process ends with the bonding (Fig. 2(c)) of glass cover on the PDMS channel.

The surface topography of the fabricated PDMS gratings was measured by AFM (Atomic Force Microscope) after gold deposition. The groove pitch of 835 ± 18 nm and the groove depth of 118 ± 16 nm were measured from the fabricated PDMS grating, showing the fabrication error less than 2.2%. Figure 2(d) shows a photograph of the fabricated SPR sensor chip with the fluidic interconnections.

IV. EXPERIMENTAL RESULTS

Polychromatic halogen light source (Navitar, Inc., USA) was used in the SPR characterization. The angle of incident light, controlled by an external mirror, was 45° and 35° for the sensing channels 1 and 2, respectively. The spectrum of the reflected light from the nanograting SPR sensor chip was measured using a fiber optic spectrometer (S2000, Ocean Optics, Inc., USA). The SPR curves were obtained from the ratio of the TM-polarized light intensity to the TE-polarized light intensity, since TE-polarized light is simply reflected on the grating surface without excitation of the surface plasmon. In the present study we measure the SPR shift due to bulk index change in order to compare the SPR characteristics of SPR chips with those obtained from commercial SPR systems; thus verifying the performance of the SPR chips. The biomolecule sample in the buffer solution was injected through fluidic interconnection ports of the SPR chips and the SPR characteristics were monitored, as follows.

A. Refractive Index Sensitivity Characterization

The refractive index sensitivity of the present SPR sensor chip is characterized using IPA solution as reference samples. The refractive index of the IPA solution [11] depends on the IPA concentration, showing the refractive index range of 1.3333-1.3600 for the IPA concentration range of 0-32%. IPA solution was injected in one of the two sensing channels and the SPR wavelength was measured. In the other sensing channel, de-ionized (DI) water was injected as a reference to monitor the environmental perturbation.

Figure 3 shows the measured SPR curves, from which we confirm the multi-channel SPR sensing performance of the fabricated SPR chips. In Fig. 3, the SPR dips for reference signal monitoring remains at the same position during the measurements, indicating that these experiments are carried out without any environmental perturbation or sensing system error. On the other hand, the SPR dips in the sample sensing channel have been shifted due to the refractive index of the IPA solutions. Figure 4 shows the plots of the SPR wavelength shift of each sensing channel with respect to the refractive indices of the IPA solutions. From the linear fitting of the data in Fig. 4(a), the refractive index sensitivity of the sensing channel 1 was measured as 321.78±8.1 nm/RI (refractive index). In sensing channel 2, the sensitivity was mea-
FIG. 4: SPR wavelength shift due to the refractive index depending on IPA concentrations measured at two sensing channels: (a) the sensing channel 1; (b) the sensing channel 2.

FIG. 5: The SPR dips measured at two sensing channels: (a) the sensing channel 1 for sample; (b) the sensing channel 2 for reference.

B. Protein Presence Detection

In order to verify the biomolecule detectability of the fabricated SPR sensor chip, we perform surface biomodification, as follows. The gold layer of the sensor chip was functionalized by the 2.1 nm-thick receptor (biotin) layer immobilized on the 1.5 nm-thick SAM layer. The 10 nm-thick target biomolecule (streptavidin) layer is ready to bind with the receptor layer. The related reagents were all purchased from commercial sources: 11-mercaptoundecanol (MUO, Sigma), 16-mercaptophexadecanoic acid (MHDA, Sigma), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Pierce), biotinamidohexanoic acid hydrazide (BACH, Sigma), and Streptavidin (SA, Sigma). Prior to the deposition of the monolayer, the SPR sensor chip was cleaned for 5 minutes by oxygen plasma. In order to adjust the binding site density for the subsequent target molecule (SA), 10%-functionalized thiol SAM (self-assembled monolayer) [12] was deposited by immersing the SPR sensor chip in an ethanolic solution of 0.9 mM MUO and 0.1 mM MHDA for 12 hours, followed by a thorough rinsing with ethanol and distilled water. For the immobilization of the receptor (biotin), the resulting sensor chip was subsequently immersed in 0.1M EDC solution in 0.1 M sodium acetate buffer (ph. 5.5) for 2 hours.

The gold surface of the sensing channel 1 and the sensing channel 2 of the fabricated SPR sensor chip were mod-
ified by using the surface biomodification step described in the previous section. For the binding of the target biomolecules (streptavidin, SA), the sensing channel 1 was treated with a 0.5 μM SA solution (≈25 μg/ml) in 10 mM PBS buffer (pH 7.4) for 2 hours. For the sensing channel 2, a buffer solution without the biomolecules was injected to monitor the environmental perturbation. Figure 5 shows the SPR curves measured from both sensing channels, before and after the target biomolecule (SA) injection. Figures 5(a) and 5(b) show the enlarged views of the SPR dips obtained from sensing channel 1 and sensing channel 2, respectively. After injecting the target biomolecule, we observed the SPR wavelength of sensing channel 1 was shifted by 1.13±0.16 nm (Fig. 5(a)); thus, verifying the capability to detect the presence of the target biomolecule. It is also observed that the SPR wavelength of sensing channel 2 (Fig. 5(b)) shows no shift during the experiments, which confirmed the validity of the experiments insensitive to environmental perturbations.

C. Binding Reaction at Constant Streptavidin Concentration

Biomolecule binding reaction was monitored in real-time from the fabricated SPR sensor chips. Both sensing channels 1 and 2 were 10% biotinylated by the biomodification step described in the previous section. After injecting 0.2 μM SA solution (≈10 μg/ml) into the sensing channel 2 and PBS buffer into the sensing channel 1, we monitor the SPR wavelength shifts of both sensing channels at 10-minute intervals. The results are plotted in Fig. 6. For a period of 30 minutes, the SPR wavelength of the sensing channel 2 increased rapidly (Fig. 6(a)), indicating that SA was bound on the biotinylated surface. After 30 minutes, the SPR wavelength of the sensing channel 2 showed no further shift (Fig. 6(a)), indicating that the binding reaction between biotin and SA was saturated. During the entire experiments, the SPR wavelength of the sensing channel 1 showed no shift, as shown in Fig. 6(b), signifying that no environmental perturbation occurred.

For reference, the identical biomolecule binding process was monitored using a commercialized SPR sensing system (Biacore X) [13], as shown in Fig. 7. For the comparison of the two results, the SPR wavelength shifts in Fig. 6a are converted to the refractive index changes using the experimental refractive index sensitivity values. The resonance unit (RU) in Fig. 7(a) was also converted to its refractive index change using the well-known refractive index sensitivity [2] (0.0009 RI/1000 RU) of the commercialized SPR sensing system. Figure 7(b) compares the refractive index changes measured from the fabricated SPR sensor chip and the commercialized SPR sensing system, showing good agreement between the results. Therefore, we verify the real-time monitoring capability of the fabricated SPR sensor chip.

D. Binding Reaction for Varying Streptavidin Concentration

The concentration-dependent biomolecule binding was characterized by monitoring the SPR wavelength shifts depending on biomolecule concentrations. Monoclonal-antibiotin (m-antibiotin) was used as a target biomolecule. The sensor chip surface was biotinylated using the biomodification step described in the previous section. Different concentration m-antibiotins of 2 nM, 10 nM, 50 nM and 200 nM were injected into the sensor chip, which was washed thoroughly before each injection. The measured SPR wavelengths did not recover to the initial SPR wavelength, indicating that all of the associated m-antibiotin molecules were not dissociated from the sensor chip surface perfectly. However, after each injection of m-antibiotin solution, the SPR wavelength increased with the reaction time, indicating that m-antibiotin was binding to the immobilized biotin at the sensor chip surface. In Fig. 9, the SPR wavelength shifts for the different m-
antibiotin concentrations are compared. From Fig. 9, the reaction rates of the biotin and m-antibiotin binding were calculated as a function of the m-antibiotin concentration, as summarized in Table I. The reaction rate increases with the concentration of the m-antibiotin solution. When the concentration of m-antibiotin reaches 50 nM, however, the reaction rate no longer increases. It is noted that the binding reaction is governed not by the concentration of the injected m-antibiotin solution but by the amount of the immobilized biotin at the sensor chip surface. Due to the limited number of biotin molecules immobilized on the sensor surface, the reaction rate would not increase for higher concentration m-antibiotin.

The identical experiment was performed using a commercialized SPR sensing system (Biacore X) [13]. The SPR wavelength shifts were converted into equivalent refractive index changes using refractive index sensitivities. Figure 10 compares the refractive index change obtained from the fabricated SPR sensor chip to those measured from the commercialized SPR sensor [13]. From Fig. 10, we obtain the binding reaction rates from the fabricated nanograting SPR sensor and a commercialized SPR sensor as summarized in Table I. The binding reaction rates

| M-antibiotin concentration | Present SPR sensor | Commercialized SPR sensor |
|---------------------------|--------------------|---------------------------|
| 2 nM                      | 0.0±3.0            | 0.11                      |
| 10 nM                     | 1.554±3.0          | 4.25                      |
| 50 nM                     | 9.396±3.0          | 8.74                      |

FIG. 7: Comparison between the refractive index changes measured by the SPR sensor chip and a commercialized SPR sensing system (Biacore X), where the SPR wavelength shifts in Fig. 6 are converted into refractive index changes.

FIG. 8: SPR wavelength shift measured from the fabricated SPR sensor chip for different concentration of m-antibiotin solutions.

FIG. 9: The SPR wavelength shift as a function of the reaction times for different concentrations of m-antibiotin solution.

FIG. 10: Comparison between the refractive index change measured from the present nanograting SPR sensor chip and that from a commercialized SPR sensor.
The refractive index change measured from the fabricated SPR sensor agree well with those from the commercialized SPR sensor within the measurement uncertainty.

The refractive index changes measured from the fabricated SPR sensor chip with respect to those measured from the commercialized SPR sensing system are plotted in Fig. 11. The solid line indicates the ideal relationship between the two values. From Fig. 11, it becomes clear that the multi-grating SPR sensor chips measure the biomolecule binding within the average error of 0.00028 RI compared to the commercialized SPR sensing system. According to the literature [2], the refractive index change of 0.00028 indicates an approximate mass change of 0.28 ng/mm² of the sensor surface by biomolecule binding. The major measurement error comes from the wavelength measurement resolution (±0.16 nm) of the spectrometer.

V. CONCLUSIONS

This paper presented a disposable multi-channel nanograting SPR sensor chip for the detection of biomolecules. The SPR sensor chips with polymer nanogratings were designed in a compact and simple structure and fabricated by PDMS micromolding process for disposable use. In the experimental study, we measured the refractive index sensitivity of each sensing channel as 321.78 nm/RI and 514.26 nm/RI, respectively. Biomolecule detection capability was verified by the SPR wavelength shift of 1.13 ± 0.16 nm after the binding of 0.5 μM streptavidin on a 10% biotinylated sensor chip surface. The binding reaction of 0.2 μM streptavidin on the 10% biotinylated sensor surface was monitored in real-time. We also verified that the present sensor chip detects the concentration of antibiotic in the range of 2-50 nM. The binding reaction rates measured from the fabricated SPR sensor agreed well with those from the commercialized SPR sensor. Therefore, the nanograting SPR sensor chips show potentials as simple and low-cost biomolecule detectors for applications to lab-on-a-chip systems and point-of-care devices.

Acknowledgments

This work has been supported by the National Creative Research Initiative Program of the Ministry of Education, Science and Technology (MEST) and the Korea Science and Engineering Foundation (KOSEF) under the project title of “Realization of Bio-Inspired Digital Nanoactuators.”

Present and Future (Elsevier, Amsterdam, 2002), Chapter 7.

[1] J. Homola, S. S. Yee, and G. Gauglitz, Sensors and Actuators B 54, 3 (1999).
[2] I. Steimler, A. Brecht, and G. Gauglitz, Sensors and Actuators B 54, 98 (1999).
[3] V. Silin and A. Plant, Trends in Biotechnol. 15, 353 (1997).
[4] S. Lőşás, M. Malmqvist, I. Rönberg, E. Stenberg, B. Liedberg, and I. Lundström, Sensors and Actuators B 5, 79 (1991).
[5] G. Steiner, Anal. Bioanal. Chem. 379, 328 (2004).
[6] http://hts.mvpproof.com/
[7] J. Homola, J. Dostalek, and J. Cyto, Proc. of SPIE, 4416, 86 (2001).
[8] F. S. Ligler and C. A. R. Taitt, Optical Biosensors: Present and Future (Elsevier, Amsterdam, 2002), Chapter 7.
[9] E. D. Palik, Handbook of Optical Constants of Solids (Academic Press, San Diego, 1998), pp.294-295.
[10] K. Hosokawa, K. Hamada, and R. Maeda, J. Micromech. Microeng. 12, 1 (2002).
[11] R. C. Weast and M. J. Astle, CRC Handbook of Chemistry and Physics (CRC Press, Boca Raton, 1979), p.D-257.
[12] T. Cass and F. S. Ligler, Immobilized Biomolecules in Analysis: A Practical Approach (Oxford University Press, New York, 1998).
[13] http://www.biacore.com/lifesciences/products/systems_overview/x/system_information/index.html