

Improved biomolecular detection based on a plasmonic nanoporous gold film fabricated by oblique angle deposition

Nak-hyeon Kim,¹ Munsik Choi,¹ Jung Woo Leem,² Jae Su Yu,² Tae Woo Kim,³ Tae-Seong Kim,¹ and Kyung Min Byun^{1,*}

¹Department of Biomedical Engineering, Kyung Hee University, Yongin, 446-701, South Korea

²Department of Electronics and Radio Engineering, Kyung Hee University, Yongin 446-701, South Korea

³School of East-west Medical Science, Kyung Hee University, Yongin 446-701, South Korea

*kmbyun@khu.ac.kr

Abstract: We demonstrated an enhanced surface plasmon resonance (SPR) detection by incorporating a nanoporous gold film on a thin gold substrate. Nanoscale control of thickness and roughness of the nanoporous layer was successfully accomplished by oblique angle deposition. In biosensing experiments, the results obtained by biotin-streptavidin interaction showed that SPR samples with a nanoporous gold layer provided a notable sensitivity improvement compared to a conventional bare gold film, which is attributed to an excitation of local plasmon field and an increased surface reaction area. Imaging sensitivity enhancement factor was employed to estimate an overall sensor performance of the fabricated samples and an optimal SPR structure was determined. Our approach is intended to show the feasibility and extend the applicability of the nanoporous gold film-mediated SPR biosensor to diverse biomolecular binding events.

©2015 Optical Society of America

OCIS codes: (120.6660) Surface measurements, roughness; (220.4241) Nanostructure fabrication; (240.6680) Surface plasmons; (280.4788) Optical sensing and sensors.

References and links

1. S. A. Kalele, N. R. Tiwari, S. W. Gosavi, and S. K. Kulkarni, "Plasmon-assisted photonics at the nanoscale," *J. Nanophoton.* **1**(1), 012501 (2007).
2. W. Srituravanich, N. Fang, C. Sun, Q. Luo, and X. Zhang, "Plasmonic nanolithography," *Nano Lett.* **4**(6), 1085–1088 (2004).
3. K. M. Byun, "Development of nanostructured plasmonic substrates for enhanced optical biosensing," *J. Opt. Soc. Korea* **14**(2), 65–76 (2010).
4. B. Liedberg, C. Nylander, and I. Lundström, "Biosensing with surface plasmon resonance--how it all started," *Biosens. Bioelectron.* **10**(8), i–ix (1995).
5. J. Homola, S. S. Yee, and G. Gauglitz, "Surface plasmon resonance sensors: Review," *Sens. Actuators B Chem.* **54**(1-2), 3–15 (1999).
6. R. S. Moirangthem, Y.-C. Chang, and P.-K. Wei, "Ellipsometry study on gold-nanoparticle-coated gold thin film for biosensing application," *Biomed. Opt. Express* **2**(9), 2569–2576 (2011).
7. W. R. Holland and D. G. Hall, "Surface-plasmon dispersion relation: Shifts induced by the interaction with localized plasma resonances," *Phys. Rev. B* **27**(12), 7765–7768 (1983).
8. W. P. Hu, S.-J. Chen, K.-T. Huang, J. H. Hsu, W. Y. Chen, G. L. Chang, and K.-A. Lai, "A novel ultrahigh-resolution surface plasmon resonance biosensor with an Au nanocluster-embedded dielectric film," *Biosens. Bioelectron.* **19**(11), 1465–1471 (2004).
9. E. T. Arakawa, M. W. Williams, R. N. Hamm, and R. H. Ritchie, "Effect of damping on surface plasmon dispersion," *Phys. Rev. Lett.* **31**(18), 1127–1129 (1973).
10. R. W. Alexander, G. S. Kovener, and R. J. Bell, "Dispersion curves for surface electromagnetic waves with damping," *Phys. Rev. Lett.* **32**(4), 154–157 (1974).
11. A. Ghoshal, I. Divliansky, and P. G. Kik, "Experimental observation of mode-selective anticrossing in surface-plasmon-coupled metal nanoparticle arrays," *Appl. Phys. Lett.* **94**(17), 171108 (2009).
12. T. Sannomiya, C. Hafner, and J. Voros, "In situ sensing of single binding events by localized surface plasmon resonance," *Nano Lett.* **8**(10), 3450–3455 (2008).

13. K. M. Byun, S. J. Yoon, D. Kim, and S. J. Kim, "Experimental study of sensitivity enhancement in surface plasmon resonance biosensors by use of periodic metallic nanowires," *Opt. Lett.* **32**(13), 1902–1904 (2007).
14. Y. Chu and K. B. Crozier, "Experimental study of the interaction between localized and propagating surface plasmons," *Opt. Lett.* **34**(3), 244–246 (2009).
15. D. Vicka, L. J. Friedrich, S. K. Dew, M. J. Brett, K. Robbie, M. Seto, and T. Smy, "Self-shadowing and surface diffusion effects in obliquely deposited thin films," *Thin Solid Films* **339**(1-2), 88–94 (1999).
16. Y. He, J. Fu, and Y. Zhao, "Oblique angle deposition and its applications in plasmonics," *Front. Phys.* **9**(1), 47–59 (2014).
17. Y.-J. Jen, A. Lakhtakia, C.-W. Yu, C.-F. Lin, M.-J. Lin, S.-H. Wang, and J.-R. Lai, "Biologically inspired achromatic waveplates for visible light," *Nat. Commun.* **2**, 363 (2011).
18. H.-Y. Chung, C.-C. Chen, P. C. Wu, M. L. Tseng, W.-C. Lin, C.-W. Chen, and H.-P. Chiang, "Enhanced sensitivity of surface plasmon resonance phase-interrogation biosensor by using oblique deposited silver nanorods," *Nanoscale Res. Lett.* **9**(1), 476 (2014).
19. M. Tahmasebpour, M. Bahrani, and A. Asgari, "Design study of nanograting-based surface plasmon resonance biosensor in the near-infrared wavelength," *Appl. Opt.* **53**(7), 1449–1458 (2014).
20. J. DeChancie and K. N. Houk, "The origins of femtomolar protein-ligand binding: Hydrogen-bond cooperativity and desolvation energetics in the biotin-(strept)avidin binding site," *J. Am. Chem. Soc.* **129**(17), 5419–5429 (2007).
21. K. M. Byun, M. L. Shuler, S. J. Kim, S. J. Yoon, and D. Kim, "Sensitivity enhancement of surface plasmon resonance imaging using periodic metallic nanowires," *J. Lightwave Technol.* **26**(11), 1472–1478 (2008).

1. Introduction

Surface plasmon resonance (SPR) occurring at noble metal surfaces or metallic nanoparticles has been a great interest in a variety of fields such as nanoscale photonics [1], nanolithography [2], and biological sensing and imaging [3]. SPR can be defined as momentum matching between surface plasmons and polarized incidence light. As SPR condition is dependent on refractive index of dielectric medium surrounding a metallic layer, target molecules adsorbed at the metal-dielectric interface may cause a shift of resonance angle or wavelength of incidence beam. By monitoring the shift of resonance signal, we can quantify biomolecular interactions in a label-free way. SPR-based optical biosensing technique has been widely used due to rapid and real-time detection and compact and low-cost experimental setup [4,5].

In a reflection-type SPR platform based on the standard Kretschmann configuration, metal-dielectric interfaces incorporating a metallic nanostructure have been suggested to achieve better sensor sensitivity. For example, this concept includes the use of gold nanoparticles immobilized on a gold film [6], silver nanoislands on a dielectric spacer and a silver film [7], and gold nanoparticle-embedded dielectric layer on a gold film [8]. Enhanced sensing characteristics are mainly associated with shift or bending in dispersion relation of propagating surface plasmon, induced by its strong coupling with localized plasmon resonance modes in metallic nanostructures [7,9–11]. However, many of the above approaches may suffer from irregular distribution and unwanted aggregation of chemically synthesized nanostructures [12].

With an advance of nanofabrication technology, periodic metallic nanostructures such as nanodot, nanohole and nanograting have been successfully realized using top-down processes in order to acquire designable and predictable plasmonic properties [13,14]. While such engineered way allows to assure a reliability in detection signal as well as to optimize a size, shape, and distribution of metallic nanostructures, those methods require complicated and high-priced fabrication procedure, especially for finer nanostructures. Hence, it is greatly desired to develop a cost-effective SPR substrate with an enhanced sensing performance.

In this study, we demonstrate that the sensitivity of a reflection-type SPR platform can be further improved by using the oblique angle deposition (OAD) technique which facilitates morphological and structural control of a thin metal film [15]. Contrary to a conventional top-down fabrication combined with thermal treatment or pattern transfer processes, the OAD technique can provide a high-throughput of metallic nanopatterns by simply varying deposition time and angle. Owing to its advantages in controlling a size, shape, and composition of metallic nanostructures, it is greatly useful in plasmonic applications for

biosensing, surface-enhanced Raman scattering, and metal-enhanced fluorescence as well as in nanophotonic devices such as achromatic wave plates and antireflection coating [16–18]. Here, detection of a binding event between biotins and streptavidin molecules has been tested experimentally to verify an enhanced sensitivity of a nanoporous thin gold film fabricated by OAD method.

2. Material and methods

2-1. Sample preparation

To fabricate SPR samples via OAD technique, polished NSF10 glass of a high refractive index was used. The glass substrate with a square shape of $20 \times 20 \text{ mm}^2$ was washed by ultrasonication in piranha solution of $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 3:2$. After piranha cleaning, glass substrate was cleaned in acetone and 70% ethanol for 20 min in a sonication bath, rinsed with distilled water and dried with nitrogen gas. SPR sample was fabricated by sputtering a 45-nm thick gold film after an evaporation of a 5-nm thick titanium adhesion layer. Then, a nanoporous gold surface was produced by varying a deposition time (t_d) on the prepared template at room temperature as shown in Fig. 1. Since a highly tilted angle of incident vapor flux is beneficial to encourage nucleation formation and high self-shadowing effect of vapor flux during deposition [15], incident flux angle was fixed at 80° without rotating a substrate. The process chamber was evacuated to a base pressure of 1×10^{-6} Torr by using a cryogenic pump. Gold pellets with 99.99% purity were used as an evaporation source. The evaporation rate was kept at 3 nm/s.

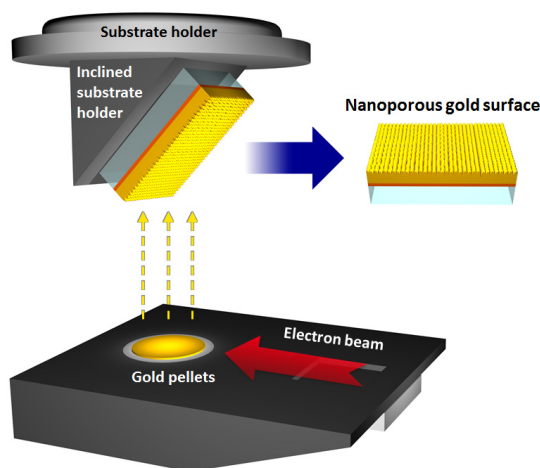


Fig. 1. Schematic illustration of OAD method via e-beam evaporation. Before OAD process, a 45-nm-thick gold film is formed on a NSF10 glass substrate after depositing a titanium adhesion film with a thickness of 5 nm. Surface morphology of a gold film can be varied depending on a deposition time of OAD when an incident flux angle is fixed at 80° .

2-2. SPR measurement system

The fabricated samples were measured by a custom-made SPR setup using an intensity-based angle-interrogation scheme to find a resonance angle. In Fig. 2, NSF10 prism was index-matched to the sensor substrate in the Kretschmann configuration. Transverse magnetic (TM) polarized He-Ne laser (05-LHP-991, Melles Griot) of $\lambda = 633 \text{ nm}$ is incident through a NSF10 prism. Dual motorized stages (SR50CC, Newport) to rotate both a prism and a photodetector (918D-SL-OD3, Newport) are employed for wide-range angle scanning. While a minimum angular resolution of the rotation stage is 0.002° , SPR curves were measured at a

resolution of 0.01° in this experiment. A syringe pump (KDS-210, KD Scientific Inc.) was used to deliver solutions into the flow cell through a fluidic channel.

2-3. Biotin-streptavidin binding interaction

The SPR sample obtained was immersed in 1 mM, 2-aminoethanethiol solution for 12 hr, followed by washing with ethanol and distilled water and drying with nitrogen gas. Surface-functionalized sensor chip was mounted on top of prism using an immersion oil. $340\ \mu\text{M}$ NHS-PEG₄-biotin (21329, Thermo Scientific) solution was injected and flowed through a fluidic channel at a flow rate of $25\ \mu\text{L}/\text{min}$ for 60 min. After immobilization of the biotins, $200\ \text{nM}$ streptavidin (21122, Thermo Scientific) molecules were injected through the fluidic channel at a flow rate of $50\ \mu\text{L}/\text{min}$ for 30 min. After individual steps in binding processes, phosphate buffered saline (PBS) was injected at a flow rate of $300\ \mu\text{L}/\text{min}$ for 5 min. During the SPR measurement, an injection pump was suspended when SPR signals were angle-scanned by rotating a prism and a photodetector simultaneously.

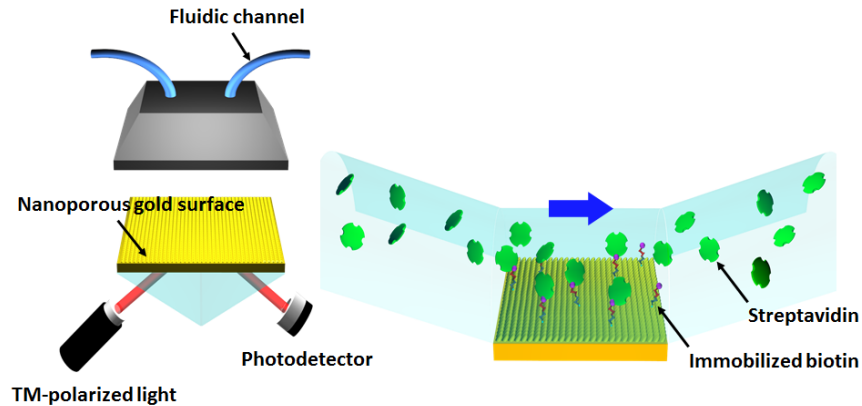


Fig. 2. Experimental schematic of the proposed SPR system to detect biotin-streptavidin reactions. TM-polarized light with $\lambda = 633\ \text{nm}$ is incident through a prism and its reflection intensity is measured by photodetector. Binding event between streptavidin and immobilized biotin occurs on the fabricated SPR sample loaded into a fluidic channel.

3. Results and discussion

Figure 3 shows side-view and top-view field emission scanning electron microscope (FE-SEM, LEO SUPRA 55, Carl Zeiss) images of nanoporous gold films obtained by OAD method. Rough gold surfaces are successfully evaporated on a planar gold film due to strong shadowing effect at a vapor incidence angle of 80° . The thicknesses of nanoporous SPR samples, denoted by OAD-A, OAD-B, and OAD-C, respectively, are measured to be 8 nm for OAD-A with $t_d = 100\ \text{sec}$, 12 nm for OAD-B with $t_d = 150\ \text{sec}$, and 16 nm for OAD-C with $t_d = 200\ \text{sec}$, indicating that an evaporation rate of OAD technique is highly linear, as estimated to be $0.08\ \text{nm}/\text{sec}$. Compared to normal flux incidence, we found that the inclined deposition with a high incident flux angle results in an extremely slow evaporation rate, which is advantageous for precise control of surface morphology. Note that, the shape of grain structure on the surface becomes more distinct with an increasing deposition time. While the results are not shown here, as a thick nanoporous gold film can lead to a considerably broad and shallow resonance signal, all the samples by OAD method are produced to have a porous gold overlayer with a thickness less than 20 nm to avoid a significant degradation of SPR characteristics.

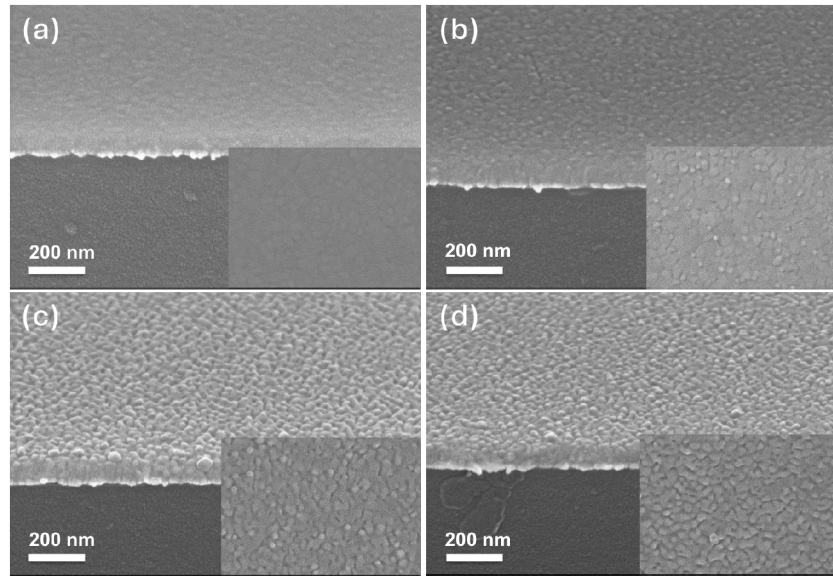


Fig. 3. Side-view and top-view FE-SEM images of bare and nanoporous SPR samples. (a) Conventional bare gold film, (b) OAD-A, (c) OAD-B, and (d) OAD-C.

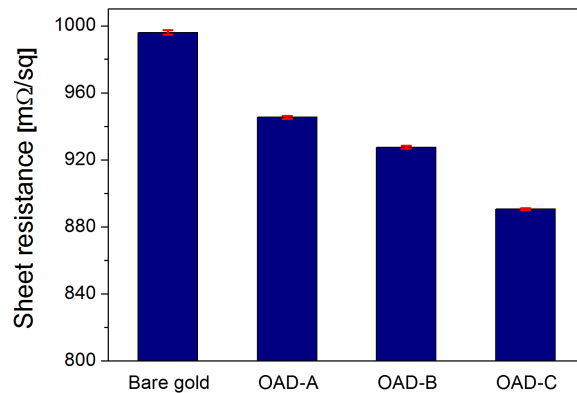


Fig. 4. Measured sheet resistance of bare and nanoporous gold films. The results obtained by four-point probe measurement show that a sheet resistance is reduced with an increasing nanoporous gold thickness.

In order to present a convincing evidence of the change in gold thickness according to deposition time, the electrical sheet resistance (R_s) is measured by four-point probe (CMT-SR2000N, AIT). Sheet resistance is an extensive property that depends upon the film thickness of the conductor being measured. To avoid a contact resistance in four-point probe measurement, a constant current is typically applied to two probes and the potential on the other two probes is measured with a high impedance voltmeter. For materials with uniform physical properties, as sheet resistance is known to be inversely proportional to a thin film thickness, Fig. 4 presents a decreasing trend of sheet resistance according to an increasing film thickness. When the nanoporous film becomes thicker, sheet resistance is determined to be 996.08 mΩ/sq for bare gold film, 945.53 mΩ/sq for OAD-A, 927.62 mΩ/sq for OAD-B,

and 890.72 mΩ/sq for OAD-C. Especially, small standard deviation indicates that the fabricated nanoporous films are spatially uniform in thickness.

In Fig. 5 obtained from $1 \times 1 \mu\text{m}^2$ atomic force microscope (AFM, XE150, PSIA) images, the OAD yields a substantially rough and porous film on the sensor surface over a large area. Mean value of surface roughness (R_a) is measured to be $R_a = 0.34 \text{ nm}$ for bare gold sample and is increased to $R_a = 1.56 \text{ nm}$ for OAD-A, $R_a = 1.89 \text{ nm}$ for OAD-B, and $R_a = 2.22 \text{ nm}$ for OAD-C. Together with an increasing surface roughness, the uniformity of grain size is improved with a deposition time. As a strong field-matter interaction at a sensor surface plays a key role in accomplishing an enhanced detection sensitivity, production of uniformly distributed metallic nanostructures is essential for effective overlap between localized surface plasmons and bound analytes. Reliable plasmonic manipulation of nanoporous gold surface is thus achievable successfully by employing OAD method, compared to using chemically synthesized nanoparticles that can be aggregated or irregularly positioned on a metal film.

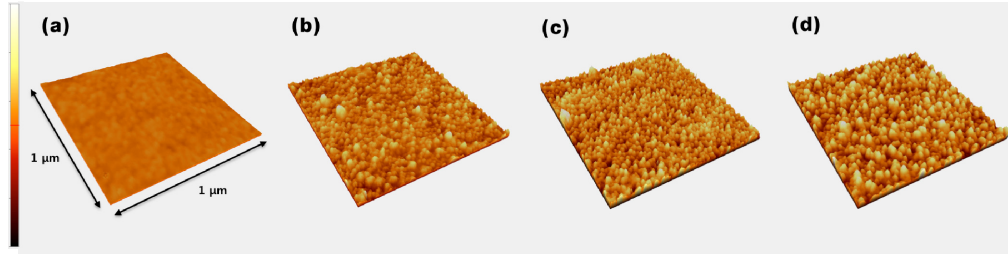


Fig. 5. AFM images of (a) conventional bare gold film, (b) OAD-A, (c) OAD-B, and (d) OAD-C.

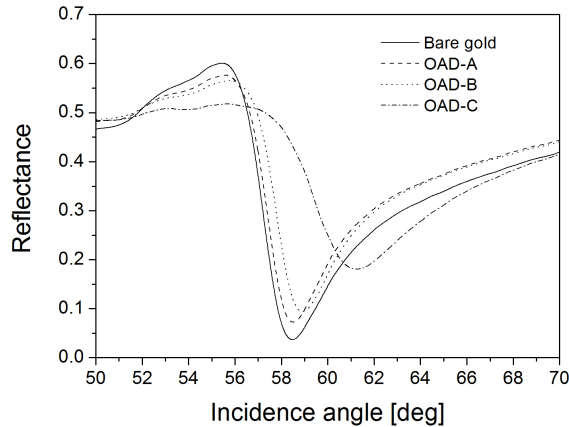


Fig. 6. SPR curves of the fabricated samples obtained before biotin-streptavidin binding experiment. Resonance angles in PBS ambience are found to be 58.48° for bare gold film, 58.52° for OAD-A, 58.93° for OAD-B, and 61.23° for OAD-C, respectively.

Figure 6 shows the experimental reflectance spectra for conventional and nanoporous SPR substrates in PBS ambience. As the thickness of nanoporous gold film increases, deformation of SPR curve characteristic seems more noticeable, compared to that of a bare gold film. More specifically, the resonance condition shifts to a higher momentum and the resonance dip becomes shallower. This is attributed to the bending effect of dispersion relation, which implies that the dispersion curve moves toward the horizontal axis of incidence angle [10,19]. Such displacement of dispersion curve often occurs when an absorptive structure is applied on a thin gold film [7]. In our case, as a nanoporous gold film may produce a local field

enhancement by localized surface plasmons (LSPs) and lead to an interference between LSPs and propagating surface plasmons, the SPR characteristic at a smooth gold film will be influenced significantly by damping and coupling with LSP modes. As a result, change in dispersion relation can be measured as a form of distorted reflectance curve.

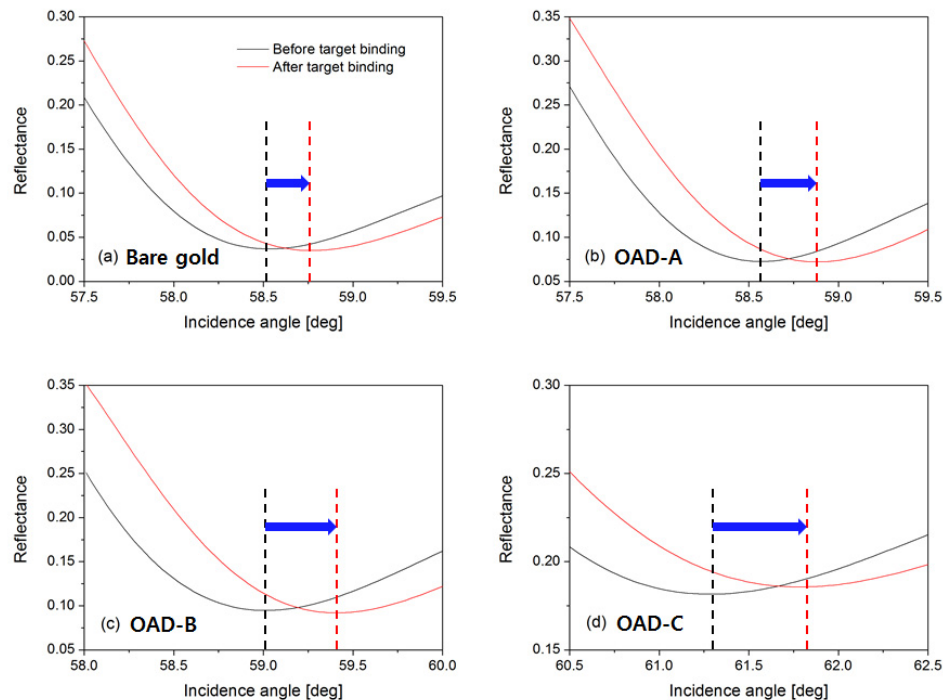


Fig. 7. Measured SPR curves for determining the resonance angle change by biotin-streptavidin binding event. The black and red lines indicate the curves before and after streptavidin molecules are bound to immobilized biotins.

In order to analyze the sensing characteristics of the fabricated samples, biotin-streptavidin interaction is employed, which epitomizes a biomolecular reaction due to its strong binding affinity. Streptavidin, a tetrameric protein, with four binding sites produces the biotin-streptavidin complexes with superior stabilization by cooperative hydrogen-bonding effect [20]. Since a refractive index in the vicinity of sensor surface changes by streptavidin immobilization onto biotin-functionalized surface, the sensitivity of individual samples can be measured by finding a resonance angle shift before and after injecting a 200-nM streptavidin in PBS environment. Figure 7 displays that, for a traditional bare gold sample, the resonance angle shifts from 58.54° to 58.78°, thus, the total change is 0.24°. On the other hand, for proposed nanoporous gold substrates, the resonance angle change is 0.30° for OAD-A, 0.40° for OAD-B, and 0.50° for OAD-C sample. A larger shift in resonance angle shows that introduction of a rough and porous structure on the smooth sensor surface can be an effective way for increasing the sensitivity because any corrugation, even at the nanometer scale, can provide more binding sites than an ideally flat surface. It seems that a bended dispersion curve by LSP modes excited at the nanoporous gold surface is also responsible for the enhancement.

Here, as a performance measure, we employ the angular sensitivity enhancement factor (ASEF), which is defined as a ratio of SPR angle shift for conventional bare gold system during refractive index change at the binding region of biotin-streptavidin interaction, to that for SPR samples with a nanoporous gold film. However, since ASEF cannot reflect signal

quality factors such as SPR curve width and minimum reflectance at resonance, we intend to introduce imaging sensitivity enhancement factor (ISEF) which is a quantitative measure of sensitivity improvement in SPR imaging scheme, defined as a ratio of maximum reflectance change between conventional and proposed SPR systems [21]. As the change in reflectance at a fixed incidence angle becomes greater for a larger resonance shift and a deeper and narrower SPR curve, ISEF can be an effective parameter to evaluate an overall sensor performance. In SPR imaging, two incidence angles with positive and negative peak gradients, at which the change in the reflectance signal reaches a maximum, are found. Because the gradient in reflectivity is generally steeper for the incidence angle preceding the resonance angle, the magnitude of the positive peak is larger than that of the negative one. Hence, the predetermined incidence angle in ISEF is equivalent to the condition in which a positive peak is obtained. From the SPR curves in Fig. 8, change in reflectance is determined to be a maximum of 0.060 at 57.23° for bare gold film, 0.076 at 57.48° for OAD-A, 0.096 at 57.83° for OAD-B, and 0.052 at 59.96° for OAD-C, respectively.

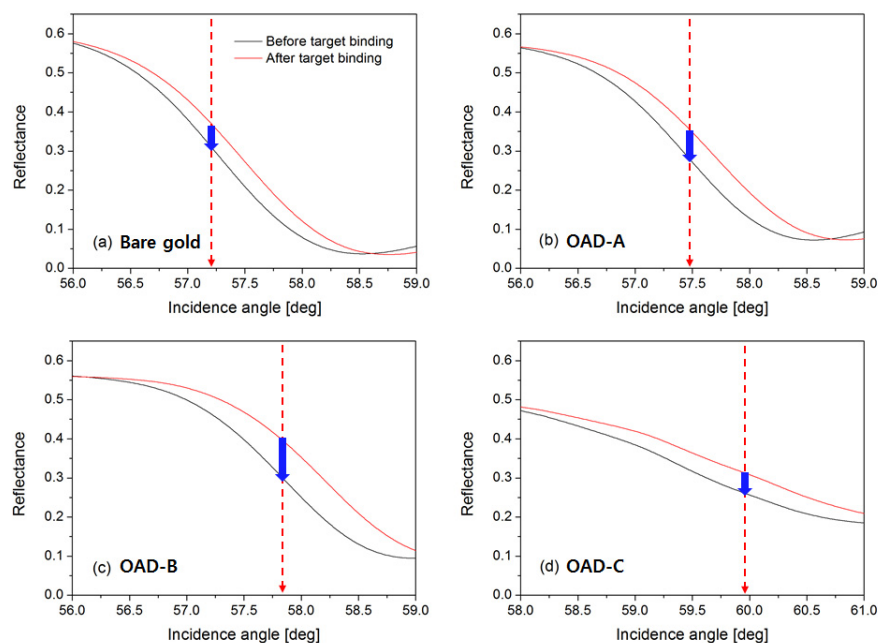


Fig. 8. SPR curves for determining the peak reflectance change by biotin-streptavidin binding event. The dashed red line indicates the incidence angle with a maximum change in reflectance.

Figure 9 exhibits the ASEF and ISEF characteristics of three nanoporous gold substrates and several interesting findings need to be addressed. First, for nanoporous film thickness less than 10 nm, ASEF is well consistent with ISEF. At this thickness range, nanoscale structural perturbation for an excitation of LSP mode is not significant and the propagating surface plasmon mode at a thin gold film is still dominant. As a result, the SPR curves for OAD-A and OAD-B samples can produce a narrow and deep resonance band, compared to a smooth bare gold film. Second, when a thickness of nanoporous gold film is increased, ASEF is improved continuously with a deposition time while ISEF value has a peak value at the sample of OAD-B. Since the amplitude in reflectance and the SPR curve width are greatly influenced by a resonant excitation of LSP mode, enhancement in SPR shift and ISEF cannot be positively correlated for nanoporous gold film thicker than 10 nm. Especially, for OAD-C sample with a shallow and broad SPR curve as shown in Fig. 6, a degraded ISEF is found

despite the highest ASEF of 208%. Third, by taking both ASEF and ISEF into account, an optimal structure is determined to be OAD-B sample. While its enhancement of about 170% appears to be less outstanding than expected, further optimization, e.g., use of a longer wavelength in infrared band, could provide a potential to realize a greater sensitivity.

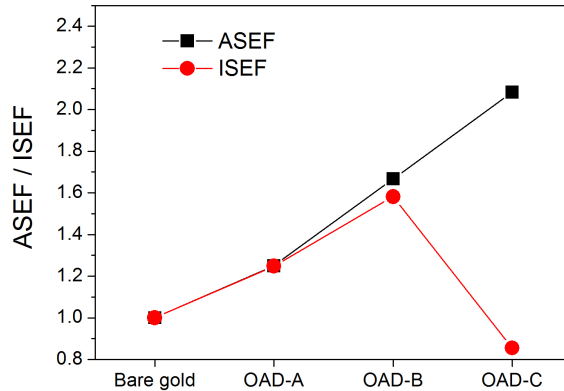


Fig. 9. ASEF and ISEF characteristics of the four SPR samples. The ASEFs (squares in black) and ISEFs (circles in red) are obtained to be 1.25 and 1.25 for OAD-A, 1.67 and 1.58 for OAD-B, and 2.08 and 0.86 for OAD-C, respectively.

4. Conclusion

In this study, we investigated a novel SPR biosensor with a nanoporous gold surface by using a simple and low-cost OAD method and demonstrated its enhanced sensitivity by detecting a binding reaction between biotins and streptavidin molecules. The fabricated sample showed a large-area nanoporous pattern with a highly uniform distribution. The thickness and surface roughness was found to be controllable in a nanoscale dimension by varying a deposition time. Compared to a conventional bare gold film, the proposed SPR substrates with a nanoporous gold overlayer lead to a larger SPR shift by more than 2 times due to locally enhanced plasmon field and increased surface reaction area. However, as ISEF is strongly influenced by broad and shallow SPR curve, which is accompanied by a resonant excitation of localized plasmons occurring at the nanoporous film, the peak ISEF of 158% was achieved for an optimal structure with 12-nm thick nanoporous gold film on a 45-nm thick gold film. To obtain a greater sensitivity, further optimization in fabrication procedure and wavelength selection is currently underway. The proposed SPR structure is expected to provide a potential for application to diverse biomolecular reactions.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2013R1A1A1A05011990). Tae-Seong Kim acknowledges the support by the National Research Foundation of Korea (NRF) grant funded by the South Korean government (MEST) (2014R1A2A2A09052449). Tae Woo Kim acknowledges the support by the National Research Foundation of Korea (NRF) grant funded by the South Korean government (MEST) (2013R1A2A2A04016066). Jae Su Yu acknowledges the support by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2014-069441).