A comparative study on sensitivity for biomolecular detection between gold nanoparticles coated and uncoated gold thin film using spectroscopic ellipsometry

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ABSTRACT

We investigate the plasmonic responses of gold nanoparticles (AuNP) coated and uncoated on 40nm gold thin film with surrounding medium having different refractive indices using Surface Plasmon Resonance (SPR) ellipsometry. The bulk sensitivities obtained from the measurements in both the cases are found to have same order of magnitude. However SPR dip in case of AuNP coated gold thin film has been shifted to longer wavelength (near IR) as compare to the bare gold thin film under the same environment and condition. Hence, it could provide a good platform to study biomolecular interaction where the absorption of light by the biomolucules is minimal. By monitor the induced changes in polarization state or phase of reflected light from the sample surface, which depend on surface properties of the sample, we use these changes as a sensor signal and compare that with theoretical simulation. This kind of characterization technique is non-destructive, label free and it has high sensitivity and sub-nanometer thickness resolution.

Keywords: spectroscopic ellipsometry, biosensor, surface plasmon resonance

1. INTRODUCTION

The analysis of bio-molecular interaction is a key area of research in the healthcare, pharmaceutical and biotechnology fields. Biosensing technique such as fluorescence has very high sensitivity but problems arise by using biomolecular labels such as fluorophores. These factors have motivated the research in developing label free optical detection techniques for biosensing. Most of label-free optical biosensors are based on affinity-sensor detection of small changes in refractive index near the interface. Surface plasmon resonance (SPR) sensors can be classified according to the way light interacts with the surface plasmon such as angular, intensity, wavelength, phase, or polarization modulation. Reviews on SPR biosensor can be found in reference [1, 2]. Ellipsometry refers to a class of optical experiments which is self referencing and it measures the polarization states (ellipsis of the polarization) after and before reflection from the sample. The simultaneous measurement of the ellipsometry parameters, Ψ and Δ (the magnitude and phase of the ratio of the p- and s-polarized reflectivities) spectroscopically, provides rich information about the sample under investigation, which allows quantitative analysis based on fitting theoretical predictions to experimental results. We present simple technique using a dove prism integrated with commercial ellipsometry to investigate the bulk sensitivity response on AuNP coated and uncoated gold thin film and extend our investigation to study boimolecular interaction on the AuNP coated gold thin film. Furthermore the use of dove prism in our experimental setup circumvents complicated optical setup or high precision alignment for excitation of surface plasmon resonance (see fig. 1) [3].

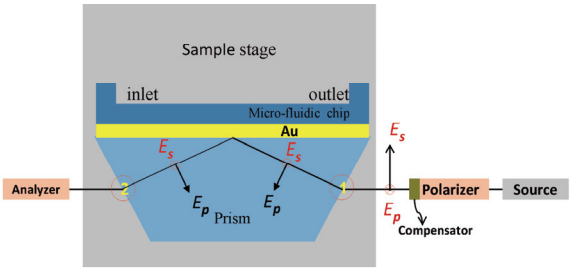

Figure1. Illustration of polarization of incident and reflected light (within the prism) for a sample attached perpendicular to the sample stage.

2. EXPERIMENTAL

2.1 Chemicals

Chemicals were obtained from commercial suppliers and used without further purification. BSA (Bovine serum albumin) and anti-BSA were purchased from Sigma Aldrich (USA). 10 mM Phosphate Buffer Saline (PBS) solutions were obtained from UniRegion Bio-Tech (Taiwan). Glycerol (99%) was purchased from Acros Organics, USA. Dove prism (BK7) was purchased from Edmund optics (Singapore). Glass slide used in all the

experiments were purchased from Gold Seal (USA). Ultra-pure water (Milli-Q Element, Millipore) was used for all experiments.

2.2 Sample Preparation

The glass substrates used in the experiment were cleaned by piranha solution (70% H_2SO_4:30% H_2O_2) followed by rinsing with ultra-pure water. Thereafter, the glass substrates were further cleaned in ultrasound bath with acetone solution for 20 minutes and with isopropyl solution for another 20 minutes, and finally rinsed with water. The clean glass substrates was stored in water and dried in oven prior to metallization. Then, 5nm thick adhesion layer (titanium) followed by 40 nm gold film were deposited on the glass substrate using e-gun evaporator (AST, Taiwan). AuNPs were prepared by reduction of chloroauric acid $H[AuCl_4]$ solution. The size of the nanoparticle has uniform distribution with mean diameter of 13nm and it is confirm with SEM. The prepared the AuNP solutions with a concentration of about 5 nM were immobilized on the APTES modified gold thin film. Fig.2 (a) shows AFM image for these 13nm diameter AuNPs sitting on the 40nm gold thin film with APTES thin film as spacer. To carry out the experiment in aqueous medium a microfluidic flow cell is prepared with a central chamber. The microfluidic flow cell consists of an acrylic plastic sandwiched between two microscopic glass slides with inlet and outlet valves. The bottom glass slide attached to the microfluidic flow cell is replaced by a freshly prepared AuNPs coated and uncoated gold thin film deposited on the glass substrate at different measurements and directly mounts in optical contact with the prism where the light is internally reflected from the gold film as shown in fig.1. It should be noted that the glass substrate used for the experiment has very close optical properties compared with the prism. To suppress the unnecessary prism–slide interference due to the air gap, an index matching liquid was used.

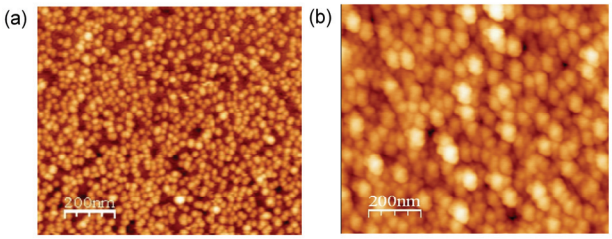

Figure2. (a) AFM image of the 13nm gold nanoparticles coated gold thin film (b) AFM image after addition of BSA anti-BSA on 13nm gold nanoparticles coated gold thin film.

2.3 Instrumentation

The SPR ellipsometry setup used in our experiment is based on the commercially available Variable Angle Spectroscopic Ellipsometry (VASE) from J.A. Woollam Company (USA) equipped with a dove prism and custom built micro-fluidic flow cell. The use of dove prism in this optical configuration and the readymade mounting of all the optical components such as source, polarizer, sample stage, compensator, and analyzer in the VASE instrument further simplifies the single-axis optical alignment and in finding the optimum angle for SPR excitation which provides a user-friendly environment. Though the present experimental setup is done with a commercial instrument, the idea can be used when developing a standalone optical instrument for sensing application.

2.4 Measurement

To determine the bulk sensitivity, various concentrations of glycerol-water mixture having different refractive indices were prepared. Subsequent measurements were done by changing the mixture solutions in the microfluidic cell, and the resulting optical signals were used to calculate the bulk sensitivity. In the biomolecular interaction study, BSA and anti-BSA were diluted in 10mM PBS buffer with concentration of 50 μM and 1 μM, respectively. Firstly, PBS buffer solution was injected into the microchip and measurements were done to obtain optical response from the buffer. Then, 50 μM BSA was injected onto the gold surface and due to the physisorption of BSA on gold surface, the BSA will form a coating on the gold thin film surface. We kept the BSA solution in the micro-fluidic cell for one hour in order to obtain sufficient BSA coverage on the gold surface, followed by washing with PBS buffer to remove unbound proteins. Finally, 1 μM anti-BSA was injected into the micro-fluidic cell and kept for 3-4 hours to undergo the protein-protein interaction, followed by washing with PBS buffer to wash away the unbound anti-BSA. The investigation was done in spectroscopic and dynamic mode with our prism-assisted SPR ellipsometry. Furthermore, all theoretical calculations used to fit experimental results were done using VASE-equipped software (WVASE32). It has to be noted that there is imperfect transmission taking place at the interfaces between air and the prism at an oblique angle, which needs to be considered, as shown in red circles in Fig. 1. The difference between measurements and model calculations has to be corrected by taking these changes due to transmission (twice) into account because the VASE-equipped software (WVASE32) can only handles the reflection from a multilayered structure.

3. RESULT AND DISCUSSION

The ellipsometry parameters are measured by applying a probe beam with a known polarization state onto a sample and then investigating the change in the polarization state of the reflected light beam. In the reflection mode, ellipsometry parameters Ψ and Δ are given as [4]:

$$\tan\Psi = \left|\frac{R_p}{R_s}\right| \quad \text{and} \quad \Delta = \delta_p - \delta_s \qquad (1)$$

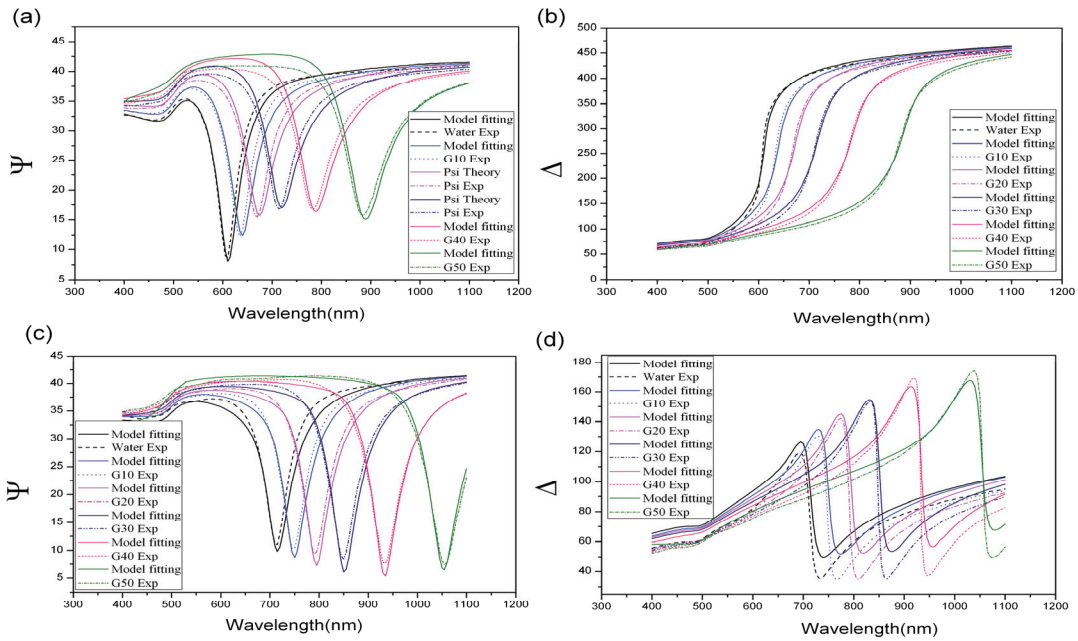

Figure3. Spectral response of the ellipsometry parameters Ψ and Δ for a 40nm gold thin (3a &3b) and for 13nm diameter gold nanoparticle coated on 40nm gold thin film (3c &3d) in surrounding aqueous medium for the glycerol-water mixture with varying refractive index.

Where R_p and R_s are the complex-valued reflection coefficients for the polarization parallel (p) and perpendicular (s) to the plane of incidence, δ_p and δ_s are the phases of R_p and R_s. Under the surface plasmon resonance (SPR) condition the surface plasmon wave generated on the gold surface is very sensitive to the change in the refractive index of the surrounding medium; hence it can monitor the changes of the surface properties. Spectroscopic measurements using SPR ellipsometry show that Ψ and Δ spectra are red shifted when the refractive index of the surrounding aqueous medium or the thickness of the biomolecular layer adsorbed on the metal surface increases and further it allow us to determine the highest-resolution wavelength. Fig.3 shows the ellipsometry spectra of Ψ(λ)and Δ (λ) for 40nm gold thin film(fig.3a,3b) and for 13nm gold nanoparticle coated on 40nm gold thin film (fig.3c,3d) in surrounding aqueous medium for the glycerol-water mixture with various refractive indices. The model fitting results are also shown together with the experimental data. We use three layer model (prism/gold/solution) with titanium as adhesive layer to fit the gold thin film experimental data and six layer model (prism/gold/EMA/Au/EMA/solutions) with titanium as adhesive layer. The gold nanoparticle layer is treated by using three layers i.e., a thin layer of gold film sandwich between two effective medium layer. As it can be seen from fig.2 (b) most of the gold nanoparticles are touching each other, so if we treat each gold nanoparticle as perfect sphere standing on the surface with the middle part touching each other and it can be treated as a thin film of gold with upper and lower part treating as effective medium layer of gold with the surrounding solution. From the fig.3a and fig 3c, it is clearly seen that the SPR dip is shifted from 610nm to 715nm for the same surrounding environment (say for water), when the gold nanoparticle are coated on the top of the thin film. This feature is very interesting and gives one possibility to tune the surface plasmon resonance dip of gold film by simply coating with gold nanoparticle on the top. The shift in this plasmon dip could be due to the increase in the surface roughness or due to the plasmonic coupling of the gold nanoparticle with gold thin film. Furthermore, the behavior of the surface plasmon dip may show differently when coated with different concentrations, sizes, shapes of gold nanoparticle on the gold thin film. Hence, a detail study is necessary and we will extend this study in our future work.

At present, the Refractive Index (RI) detection sensitivity obtained from a linear fitting of the slope of SPR dip shift in experimental result for 40nm gold thin film and AuNP coated on 40nm gold thin film are 3795 RIU/nm and 4071 RIU/nm. With a wavelength resolution of 0.01nm we obtain a RI resolution of 2.6×10^{-6} and 2.4×10^{-6} respectively for the Ψ(λ) spectra. In addition the experimental results for Δ(λ) spectra indicate a phase shift at the resonance which agrees with the theory [5].To make the units on this slope clearer for the reader, we refer here to a "refractive index unit" or RIU. While this is unnecessary since the refractive index is really unitless, we believe it makes the paper easier to follow. Thus, the sensitivities in both cases turn out to have the same order of magnitude except the SPR dip in

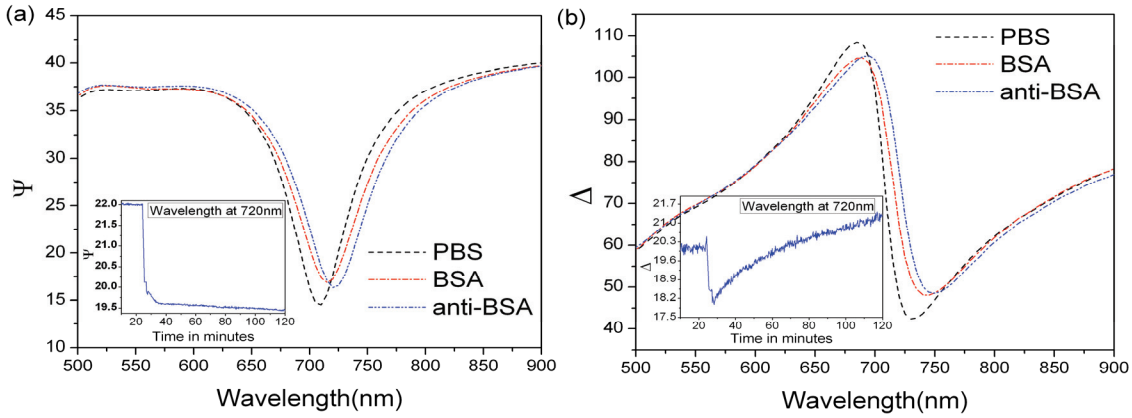

Figure4. Spectral response of the ellipsometry parameters (a) Ψ and (b) Δ for various configurations with respect to the addition of BSA and anti-BSA 13nm diameter gold nanoparticle coated on 40nm gold thin film. Inset figure is the BSA absorption on the sample surface with respect to time.

case of AuNP coated gold thin film have been shifted to longer wavelength (near IR) as compare to the bare gold thin film under the same environment and condition. Next, we perform label free bio detection of protein-protein interaction by measuring the interaction between BSA and anti-BSA on AuNP coated gold thin film. First, the PBS buffer solution was injected into the microchip and measurements were done to obtain optical response from the buffer. Next, the BSA molecules were immobilized on the metal surface followed by washing with buffer solution to remove unbound proteins. Subsequently anti-BSA molecules were injected into the chip to undergo biomolecular interaction. Spectroscopic measurements were done at various configurations with respect to the addition of BSA and anti-BSA. AFM image after addition of BSA and anti-BSA is shown in fig.2 (b). Fig.4 shows ellipsometry spectra for Ψ and Δ in surrounding aqueous medium with the addition of BSA and then anti-BSA. The absorption of BSA protein on the sample surface with time at a fixed wavelength of 720nm is also shown in the inset figure. As it can be seen from the figure that the surface plasmon dip was red shifted as the thickness/refractive index of the molecular layer on the surface of the AuNP coated gold film increases. Also the shifting of surface plasmon dip for anti-BSA in more significant because the molecular weight of anti-BSA (150kDa) is larger than that of BSA (66kDa). Similar behavior is observed for the BSA and anti-BSA interaction study on the bare gold thin film (data not shown). Hence the sensitivity to detect the large biomolucules interaction such as BSA and anti BSA does not show very significant difference between AuNP coated and uncoated gold thin films but AuNP coated gold thin film could provide a better platform for studying biomolecules as compare to the bare gold film since the SPR dip lies in the near infra red (IR) wavelength range, where the absorption of light by the biomolucules is minimal hence reduce the biomolecular damage.

4. CONCLUSION

A comparative study on the bulk sensitivity of AuNP coated and uncoated gold thin film is presented. It is found that both the samples gives the bulk sensitivity of the same order of magnitude, however the plasmon dip of AuNP coated gold thin film lies in the near IR as compare to the bare gold thin film with same surrounding medium. Through studies of BSA and anti-BSA molecular interactions, we have demonstrated both spectroscopic and dynamic measurement capabilities to sense high-affinity bimolecular interactions by using SPR ellipsometry without any labeling Additionally, AuNP coated gold thin film provide a means to tune the SPR dip which will provide an optical window to study biomolecules or other cellular interaction on the sample surface with lesser damage.

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