Ellipsometry study on gold-nanoparticle-coated gold thin film for biosensing application

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Abstract: The amplified plasmonic response from various distributions of gold nanoparticles (AuNPs) coated on top of gold thin film was studied via ellipsometry under total internal reflection mode. The surface plasmon resonance dip can be tuned from the visible to near infrared by simply varying the AuNP concentration. Theoretical modeling based on effective medium theory with a multi-slice model has been employed to fit the experimental results. Additionally, this experimental tool has been further extended to study bio-molecular interactions with metal surfaces as well as in studying protein-protein interaction without any labeling. Hence, this technique could provide a non-destructive way of designing tunable label-free optical biosensors with very high sensitivity.

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References and links

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1. Introduction

The study of bio-molecular interaction is a key area of research in the healthcare, pharmaceutical and biotechnology fields. Optical bio-detection technique such as fluorescence with high sensitivity has many limitations about the stability of the fluorophors, i.e. photobleaching and need an expertise for labeling fluorescent dyes with the biomolecules or cells [1]. On other hand, surface plasmon resonance (SPR) methods have made an important contribution as a label-free technique to quantify the biomolecular interactions, in situ monitoring of nucleation and growth, surface biology, etc. [2,3]. 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. By immobilizing bio-specific capturing molecules on the surface of the biochip of the SPR device, we can monitor the real-time interactions and binding kinetics of specific biological analytes [2,4]. The interrogating intensity change and wavelength shift based on conventional SPR configurations are limited to a refractive index unit (RIU) of $5 \times 10^{-5}$RIU and $2 \times 10^{-5}$RIU, respectively, which are insufficient to monitor low concentrations of small biomolecular analytes [5]. On the other hand, SPR detection techniques measuring the phase modulation of the reflected light have been reported, showing a significant improvement with a RI resolution of the order of $10^{-7}$ RIU [6,7]. Several other approaches have been developed to overcome this drawback by utilizing external labels such as latex particles, liposomes, certain protein, etc. to enhance the sensitivity of the current technique [8]. Metallic nanoparticles have also been used to amplify the SPR response either by external labeling or immobilized on the metal surface, which considerably enhances the sensitivity of the SPR sensor [8–12]. These metal nanoparticles offer ease of preparation, high density, and high surface-to-volume ratio, large dielectric constant and biocompatibility. The enhancement or amplification of the SPR response due to immobilization of colloidal gold nanoparticles (AuNPs) on the gold thin film may arise from the combined result of greatly increased surface mass, high dielectric constant of AuNPs, and electromagnetic coupling between AuNPs and the gold film [8,9]. In addition, AuNPs can lead to strong coupling of incident light to plasmon resonances and enhance the sensitivity of the SPR biosensor [12]. The effect of the colloidal AuNPs on the SPR response leads to a shift in plasmon resonance...
wavelength, broadening of spectra, and changes in the reflectivity minima, which reflect the modulation of the surface plasmon dispersion relation. However, the strength of the modulation on the dispersion relation depends on the concentration of the AuNPs and spacing between AuNPs and gold thin film. Hence, characterizing and understanding optical properties of AuNPs for accurate prediction of the effective behavior on the optical devices are important. In this study, we employed ellipsometry as a nanometrology tool to study the optical response of the AuNPs coated on gold thin film under a total internal reflection configuration (TIR) [13]. Effective medium theory (Bruggeman approximation) with a multi-slice model was employed to treat AuNPs monolayer as a thin film [14,15]. Furthermore, we extended our investigation to explore the potential application of ellipsometry and effective medium model as a tool to quantify bio-molecular interactions by demonstrating the interaction between Bovine serum albumin (BSA) and anti-BSA.

2. Materials and Methods

2.1. Synthesis of gold nanoparticles and immobilization on the gold thin film

Colloidal gold nanoparticles (AuNPs) were synthesized by using sodium citrate (Sigma Aldrich) reduction of chloroauric acid (H[AuCl₄], Sigma Aldrich) solution as reported earlier with slight modification in the protocol [16]. 5mM concentration of H[AuCl₄] and 38.8mM concentration of sodium citrate were prepared in deionized (DI) water (Millipore) as stock solutions. For preparing AuNPs with average sizes of 13nm, 20% (v/v %) of the stock solution of H[AuCl₄] diluted in distilled (DI) water was taken in double-neck conical flask mounted with water reflux tube. The solution were put on silicon oil bath with a temperature maintained at 130°C and stirred with a magnetic stirrer at 500 rpm for 30 minutes. 5 ml of sodium citrate were added drop-by-drop into the solution with stirring and heating and waited for 12 minutes. The color of the H[AuCl₄] solution changed from black to reddish, indicating the formation of AuNPs. The prepared colloidal AuNPs solution was cooled down to room temperature and stored in refrigerator until use. The sizes of the prepared AuNPs were confirmed with scanning electron microscope (SEM, FEI Nova). Different sizes of AuNPs can be prepared by controlling concentration of the H[AuCl₄] and sodium citrate solutions. For sample preparation, the glass substrates were cleaned by piranha solution (70% H₂SO₄:30% H₂O₂, Sigma Aldrich) followed by rinsing with DI water. The clean glass substrate was dried overnight at 60°C prior to metallization. Then, 5nm thick adhesion layer (titanium) followed by 40 nm gold (Au) film was deposited on the glass substrate using an e-gun evaporator. Amine-functionalized gold substrates were prepared by immersing the prepared gold film in a 1.0mM concentration of 2-aminoethanethiol hydrochloride (AET, Sigma Aldrich) ethanol solution for 8-12 hrs. The substrates were then removed from the solution, rinsed with ethanol, and dried with a stream of high-purity nitrogen [17]. These substrates were treated in the AuNP solution for various time spans (1, 5, 10, 20, 30, 60 and 120 minutes) and rinsed with DI water several times, followed by drying with nitrogen gas to prepare different concentrations of AuNPs on the gold film. Figure 1(a) shows the SEM image of the distribution of the 13nm AuNPs on the gold surface after 1 minute treatment of amine-functionalized gold substrate.

2.2. Instrumentation and measurements

Our experimental setup for ellipsometry measurements under total internal reflection mode was designed by integrating a commercial variable-angle spectroscopic ellipsometer (VASE) (J.A. Woollam Company) with a BK7 dove prism and custom built micro-fluidic flow cell [13]. Measurements were performed at a fixed angle of incidence over the full spectral range from 400nm to 1100nm. The angle was 72.8° in the dove prism which was larger than the total internal reflection angle at glass and air interface. The single axis alignment of optical
components minimizes the complication and avoids high precision optical alignment procedure. Though the present experimental setup is done with a commercial instrument, the idea can also be applied in a standalone optical instrument for sensing applications. To understand the optical properties from various distributions of AuNPs immobilized on the gold substrate in water, these samples were mounted on the dove prism using micro-fluidic flow cell. The layout of the experimental setup under TIR configuration is shown in Fig. 1(b). The optical response from various samples was obtained by measuring the ellipsometry signals $(\Psi, \Delta)$ with this setup.

2.3. Immobilization of BSA protein and its interaction with anti-BSA

Bovine serum albumin (BSA) and anti-BSA (Sigma Aldrich) proteins were diluted in 1X Phosphate Buffer Saline (PBS, UniRegion Bio-Tech, Taiwan) buffer solution (pH 7.4) with concentration of 50 µM and 1 µM, respectively. Firstly, PBS buffer solution was injected into the microchip and measurements were done to obtain reference signal from the buffer solution. Then, 50 µM BSA was introduced onto the AuNPs surface for 5 hrs in order to obtain sufficient BSA coverage, followed by rinsing with PBS buffer to remove unbound proteins. Due to the physisorption of BSA on gold surface, the BSA will form a coating on the AuNPs surface. Finally, 1 µM anti-BSA was injected into the micro-fluidic cell and kept for 3–4 hr to undergo the protein-protein interaction, followed by washing with PBS buffer to wash away the unbound anti-BSA. The investigation was done spectroscopically with our prism-assisted ellipsometry.

3. Results and Discussions

Ellipsometry refers to a class of optical tools, which is self-referencing, and it measures the polarization states (ellipsis of the polarization) before and after reflection from the sample. The simultaneous measurement of the spectroscopic ellipsometry parameters, $\Psi(\lambda)$ and $\Delta(\lambda)$ (the magnitude and phase of the ratio of the p- and s-polarized reflectivities), provides rich information about the sample under investigation. Quantitative analysis can be obtained from fitting theoretical predictions to experimental results. In the reflection mode, ellipsometry parameters $\Psi$ and $\Delta$ are given as [18]

$$\tan \Psi = \frac{R_p}{R_s}$$ and $$\Delta = \delta_p - \delta_s,$$

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. $\delta_p$ and $\delta_s$ are the phases of $R_p$ and $R_s$.

When the SPR effect is combined with ellipsometry in total internal reflection mode, one obtains a technique with very high sensitivity [13,19]. To study the effect of the various distributions of AuNPs on the optical properties of the gold thin film under TIR mode, AuNPs
immobilized on gold thin film samples with different treatment time were mounted on the flow cell as shown in Fig. 1(b). Since most of the biological experiments were performed in aqueous medium, we did our experiments in water and measured the effective optical response from the samples under investigation. As it can be observed from Figs. 2(a-b), the amplitude (Ψ) spectrum shows a dip at the SPR resonance wavelength, while the phase (Δ) spectrum shows a steep change at the corresponding wavelength. The SPR dip shifts towards longer wavelength and becomes broader when the gold-nanoparticles concentration is increased. The shift in the SPR spectrum and changes in the broadening of the SPR dip could be due to the electromagnetic coupling between AuNPs plasmon and SPR response from the gold thin film. However there exists a saturation point (around 60 min of treatment time) where there is no further shift in the SPR spectrum as the nanoparticle concentration is increased further. These features can also be seen from the phase signal, i.e. Δ spectra, where the slope of the phase signal shows no further changes after reaching this saturation value. Hence, the simultaneous measurement of the ellipsometry signal, Ψ and Δ helps us to understand more clearly about the optical response of the samples under investigation. Figure 2(c) shows the variation in wavelength of the SPR dip for different treatment times of the gold thin film samples in colloidal AuNP solution. In this report, we have chosen a selected size of AuNPs with average diameter of 13nm. However, the investigation can be extended to different sizes of AuNPs and study its corresponding effect on the SPR response of the gold thin film. Theoretical modeling was done using VASE equipped software (WVASE32) and the effective medium theory (EMA) was employed to treat AuNPs monolayer as a multi-slice thin film [14,15].

![Fig. 2. Spectral response of the ellipsometry parameters Ψ and Δ (a, b) for gold nanoparticle with different concentration of gold nanoparticles immobilized on top of 40nm thick gold thin film in water medium, (c) Shifting in the SPR dip obtained from Ψ spectra as a function of different concentration of AuNPs showing in terms of treatment time.](image)

We have used five or six layer model (prism/gold/EMA layers/water) with titanium as adhesive layer to fit experimental results for the samples under investigation. Fitting parameters of samples with different treatment time measured under the TIR configuration in water are given in Table 1. It can be seen from the fitting results that the calculated effective fractional volume ratio of AuNPs immobilized on the surface increases with increasing AuNP concentration. When the AuNP concentration is very dilute (e.g. for 1 minute dipping time), nanoparticles are isolated from each other, giving the lowest fractional volume ratio. As the concentration becomes denser, nanoparticles start to touch each other until a saturation point is reached. This behavior can be understood for high AuNP concentrations (dipped for 60 and 120 minutes) where the effective fractional volume ratio almost reaches saturation as indicated by the experimental results shown in Fig. 2(c). A thin layer of gold film needs to be introduced in the theoretical modeling to simulate the aggregated AuNPs for denser concentrations. When the nanoparticle concentration is low, two EMA layers were used to simulate the AuNPs monolayer for fitting the experimental spectra. Slicing of the EMA layer...
Table 1. Best-fit results of EMA parameters of samples with different treatment time exposed to water medium

| Dipping Time (minutes) | Calculated EMA Film Thickness (nm) | % $f_{water}$ | % $f_{gold}$ | Effective Fractional Volume Ratio of AuNPs |
|------------------------|-----------------------------------|--------------|-------------|-----------------------------------------|
| 1 EMA1                 | 35.36                             | 97.70        | 2.29        | 0.81                                    |
| 5 EMA1                 | 10.65                             | 91.45        | 8.54        | 0.91                                    |
| 10 EMA1                | 12.08                             | 90.30        | 9.69        | 1.17                                    |
| 30 EMA1                | 4.84                              | 87.15        | 12.84       | 2.01                                    |
| EMA2                   | 41.63                             | 96.66        | 3.33        | 4.57                                    |
| 60 EMA1                | 6.38                              | 44.47        | 55.52       | 4.57                                    |
| Au film                | 0.15                              |              |             |                                         |
| 120 EMA1               | 11.60                             | 92.40        | 7.59        | 4.81                                    |
| Au film                | 0.80                              |              |             |                                         |
| EMA2                   | 17.51                             | 92.66        | 7.33        |                                         |

allows us to model the small clusters or aggregations adequately where the single EMA layer fails to do so. For the case of very high concentration, corresponding to longer treatment time, the AuNPs layer can be simulated by three layers with a thin layer of gold film sandwiched between two EMA layers of gold and water medium. This model for high concentration treats AuNPs as perfect spheres with their middle portion touching each other. Thus the effective medium theory with a two- or three-slice model allows us to fit the experimental results well without using sophisticated theoretical modeling. It is worth noting that the localized plasmon mode of AuNPs expected at wavelength around 550nm (on substrate) does not appear in the experimental result, nor does it in the theoretical result as seen in Fig. 2(a).

The immobilized AuNPs help to increase surface-to-volume ratio or surface roughness as compared to the gold thin film. This greatly enhances the sensitivity of the SPR sensing device. The possible mechanism of this amplification is discussed elsewhere [9]. In order to calibrate our SPR device based on ellipsometric signals $\Psi$ and $\Delta$, bulk sensitivity measurements were performed by injecting glycerol-water mixtures of different concentrations, having different refractive indices, onto the sensing surface through the micro fluidic flow cell as shown in Figs. 3(a-b). The refractive indices for different glycerol-water mixtures were measured by using a refractometer (Misco, USA). Based on the wavelength scanning ellipsometric measurement near SPR, the calculated slope of the SPR dip vs. refractive index gives a value of 4701 RIU/nm for the refractive index (RI) detection sensitivity [see Fig. 3(c)]. With a wavelength resolution of 0.01nm as adopted from literature [6], we obtain a RI resolution of $2.1 \times 10^{-6}$ RIU for $\Psi(\lambda)$ spectra, which shows one order of magnitude improvement from the previously reported result using a similar detection scheme.
and comparable to the detection limit achieved by the commercial SPR machine with high precision angular resolution [for example, SPR-Navi (BioNavis Ltd., Finland)] or a complicated interferometric SPR sensor [21]. Further improvement can be made on our current detection technique by employing ellipsometric phase signal, because phase change at the SPR minima is far more rapid as a function of wavelength than the change in reflected intensity, thereby enabling a higher sensitivity to the change of refractive index [7]. In the study of biomolecular interactions, we demonstrate here the interaction between BSA and anti-BSA proteins on the AuNPs coated substrate with 30-min treatment time. Spectroscopic responses of the ellipsometry signals with fitted result at various configurations with respect to the PBS buffer solution, addition of BSA and anti-BSA are shown in Fig. 4 (b-c). It is seen that the surface plasmon dip is red shifted as the thickness/refractive index of the biomolecular layer on the metal nanostructure surface increases. The shift in surface plasmon dip for anti-BSA is more significant due to the larger molecular weight of anti-BSA (150kDa) as compared to that of BSA (66kDa). Furthermore, the phase signal in Fig. 4(c) changes even more significantly at the SPR minima, which will be useful for investigating the biomolecular interaction. Figures 4(e-f) show the AFM images of the bare AuNPs sitting on gold film and after attachment of the BSA + anti-BSA. In theoretical modeling, the optical constants of PBS buffer, BSA, and anti-BSA as calibrated on the gold thin film [Fig. 4 (a)] were used to fit the experimental data in Figs. 4(b-c). A Cauchy parameterization model was used to describe the refractive index (n) of the BSA layer by treating it as a transparent layer (i.e. no extinction) over the spectral range of concern. However, the extinction coefficient can also be considered as a fitting parameter in the Cauchy model and the best-fit result also indicates that the extinction co-efficient for BSA is nearly zero [22]. It is noted that our calibrated refractive index (n) of BSA obtained from fitting the data gives a close value to that reported in [22,23] but slightly lower than that reported in [24,25]. The inconsistency in the results could be due to many factors such as the pH value, concentration of the biomolecules, measuring environment (dry or liquid), surface charge effect of the substrate etc., which would affect the measured refractive index [22–26]. Hence a more detailed investigation concerning all these factors over the whole spectroscopic range will be useful for improving the theoretical model, which we intend to do in the future. Based on the theoretical model described above, two EMA layers were used and their optical constants were determined for the AuNPs layer in the PBS buffer. The obtained effective optical constant of the AuNPs in PBS buffer is used as a host medium for the EMA layer in order to fit the spectra obtained after immobilization of BSA. Hence this EMA layer consists of fractional volume of host medium (% fPBS + Au) and fractional volume of the BSA molecule (% fBSA). We intend not to use separate fractional volumes of the PBS buffer, AuNPs, and BSA molecules simultaneously as a fitting parameter, because it gives a strong correlation in the fitting parameters. For the anti-BSA, we have treated it as a single layer by assuming that the entire AuNPs surface was covered with the BSA molecules and used the pre-calibrated optical constant to determine the thickness of the biomolecular layer. The fitting parameters such as fractional volumes and thickness in each configuration due to the addition of BSA, and its subsequent interaction with anti-BSA on the AuNPs surface in the buffer medium are given in Table 2.

Hence, spectroscopic ellipsometry measurements with theoretical modeling allow us to understand the optical responses from the metal nanostructure surface and provide quantitative analysis of the biomolecules adsorbed on the metal nanostructure surface. Thus, we have achieved a label free technique with very high sensitivity.
Fig. 4. (a) Optical constants of the PBS buffer solution, BSA and anti-BSA bio-molecules as calibrated on the gold thin film under TIR configuration. Spectral response of the ellipsometry parameters (b) $\Psi$ and (c) $\Delta$ for various configurations with respect to the addition of BSA and anti-BSA on 13nm diameter gold nanoparticles coated onto 40nm gold thin film. (d) Shows the model describing the EMA layers representing the AuNPs in buffer medium with BSA and an additional layer of anti-BSA. An AFM image of the bare AuNPs sitting on gold film is shown in (e) and after attachment of the BSA + anti-BSA in (f).

Table 2. Best-fit results of EMA parameters with different configurations with respect to the addition of BSA and its subsequent interaction with anti-BSA

| Slice         | Thickness (nm) | % $f_{\text{BSA}} \cdot \Delta$ | % $f_{\text{BSA}}$ | Thickness (nm) |
|---------------|----------------|----------------------------------|-------------------|----------------|
| EMA layer 1   | 7.34           | 68.48                            | 31.52             | 63.32          |
| EMA layer 2   | 23.21          | 71.40                            | 28.61             |                |

4. Conclusions

The optical responses of various distributions of AuNPs on the gold thin film under total internal reflection mode were studied by using the spectroscopic ellipsometry setup integrated with a dove prism and microfluidic flow cell. The SPR spectra show red shift when the gold nanoparticle concentration on the gold surface is increased and the effect saturates when the concentration of AuNPs on the gold thin film is high enough (with a treatment time > 60 min). By changing the concentration of AuNPs on gold surface, it provides a means to tune the SPR dip from visible to near infra-red (IR). Theoretical modeling based on the effective medium theory with a few slices fits the experimental results very well, which indicates the adequacy of the model used as compared to more sophisticated models. In addition to the basic functionalities of the ellipsometry as a characterization tool, we have successfully demonstrated spectroscopically to sense high-affinity bio-molecular interactions on metal nanostructure surface as well as its specific interaction with antibody without any labeling. Additionally, gold-nanoparticle coated gold thin film could provide an optical window for studying biomolecules or other cellular interactions on the sample surface with lesser damage due to its SPR dip occurring in near IR wavelength.

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