

Au Nanoparticle Coupled- Surface Plasmon Resonance Chemical Sensor for Sensitivity Enhancement

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ABSTRACT

In order to overcome the sensitivity limitation of SPR, nanoparticle-coupled SPR biosensors have explored with tremendous interests since nanoparticles may significantly enhance the sensitivity by 1-2 orders of magnitude. M. J. Natan et al. reported that a conjugate between an antibody and Au colloidal particle produced a greater than 20-fold increase in plasmon angle shift over than observed in an assay using the unlabeled antibody. Though this colloid conjugated method has overcome the sensitivity, it does not take an advantage of SPR from the viewpoint of label-free detection. Herein we investigated the sensitivity enhancement in SPR chemical sensor coupling colloidal Au nanoparticles which have the specific size and surface density on sensor chips as label-free detection system.

Keywords: SPR, colloidal Au particle, chemical sensor

1 INTRODUCTION

Surface plasmon resonance (SPR) has been widely employed for protein-protein interaction studies in which changes in refractive index close to a thin metal surface are monitored. [1-3] The need for significant surface refractive index changes for detection, however, has limited its applications for ultra sensitive analysis. To address this limitation, several signal-amplification approaches have been developed.[4-5] Of those, the use of colloidal Au nanoparticles to enhance SPR signals associated with biochemical binding events has attracted great attention.[6-8] In this method, molecular-recognition probes are pre-conjugated to metallic nanoparticles. The binding events between the probes and their partners on the surface lead to subsequent immobilization of particles on SPR substrates. As a result, remarkably large changes in both the position of the SPR angle and the magnitude of film reflectivity are observed. The successful applications of this method have been demonstrated for sandwich immunoassays and DNA hybridization detection by various groups.[4, 6]

In addition, the effect of nanoparticle binding on SPR response has been investigated as a function of particle composition, size, surface coverage, and substrate metals.[9-10]

Though this colloid conjugated method has overcome the sensitivity, it does not take an advantage of SPR from the viewpoint of label-free detection. Herein we investigated the sensitivity enhancement in SPR chemical sensor coupling colloidal Au nanoparticles which have the specific size and surface density on sensor chips as label-free detection system.

2 EXPERIMENTAL

Reagents. In this research, we used reagents as received without further purification. All solvents used were HPLC grade. Deionized water was purified to $> 18.3 \text{ M}\Omega$ with a Millipore system. Cystamine dichloride, nitric acid, hydrochloric acid, hydrogen tetrachloroaurate, sodium citrate, ethanol, methanol were purchased from Aldrich. Glass substrates (BK7, $n = 1.517$, 18 mm x 18 mm x 0.15 mm) was purchased from Matsunami glass.

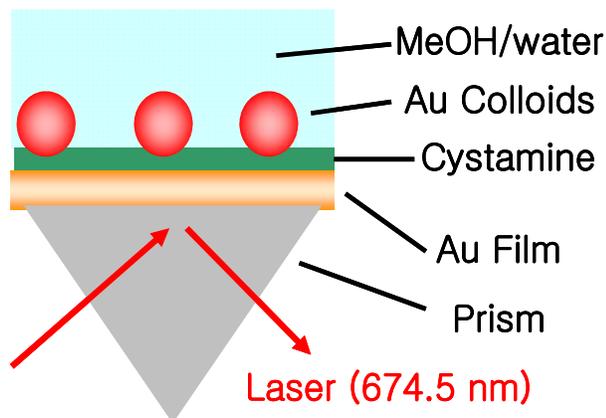
Au Colloid Synthesis. Hydrogen tetrachloroaurate 0.08 g was dissolved in 170 ml D.I. water. Hydrogen tetrachloroaurate solution was heated with stirring up to 100 °C. Added 4 mM ~ 15 mM sodium citrate aqueous solution. After 2 hr, solution was slowly cooled at room temperature. [11]

Au Film Preparation. Glass substrates were cleaned by sonicating in ethanol, D.I. water and then treated by ozone for 10 min each. Au films were deposited on the cleaned glass substrates by Au shot (99.999 %, Eiko) in Eiko IB-3 ion coater system. Au substrates (thickness 50 nm) were deposited at a pressure of 0.05 torr for 210 s and used freshly. Au films were modified by treating with 10 mM aqueous solution of cystamine dichloride for a period of 24 hr.

Fabrication of Au colloid immobilized substrate. Cystamine modified Au film on a glass substrate was exposed to Au colloids solution for 30 min at room temperature for fabrication of Au nanoparticle coupled substrate for SPR measurement (Scheme. 1).

SPR Instrument. Excitation of the surface plasmon is accomplished using a 1 cm diameter triangle prism (BK 7 glass, Sigma product) which is index-matched via a microscopy immersion oil ($n = 1.516$) to a BK 7 substrate onto previously deposited Au film. This assembly is then

affixed to a home-built batch cell (volume ~1 ml) with the Au film exposed solution. The SPR excitation source is a cylindrical 5 mW HeNe laser (674.5 nm, Melles Griot) which is further polarized by 500 : 1 visible-optimized linear polarizer (Newport, 10-LP VIS). Stage rotation and data collection are controlled through the computer interface that was programmed by K-MAC Instrument. A typical SPR scan was run 0.04° resolution and a stage rotation rate of 0.2° s⁻¹.



Scheme 1. Particle-enhanced SPR sensing architectures

Atomic Force Microscopy (AFM). AFM images acquired were obtained using a PSIA Instruments XE-100 system, operated in non-contact mode with an acquisition frequency of 312 kHz and line density of 512. Standard 200 μm etched silicon probes were used.

Field Emission Scanning Electron Microscope (FE-SEM). The SEM images were obtained using a JEOL 6340F, under typical working conditions of 5 kV.

3 RESULTS AND DISCUSSTION

The dimension of the synthesized Au colloid size was measured using FE-SEM (Figure 1). It was found that the diameter of Au colloid was varied according to the change of citrate concentration in the Au solution (Table 1).

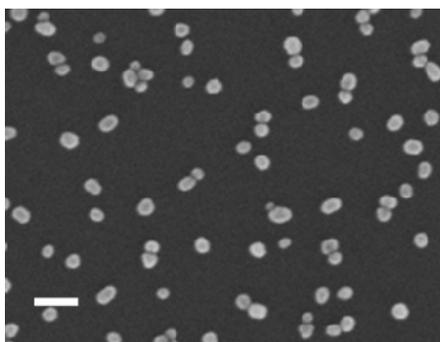


Figure 1. FE-SEM image of synthesized Au colloid of 9 mM sodium citrate concentration. Scale bar was 100 nm.

Table 1. The diameter of synthesized Au colloid of different sodium citrate concentration.

Citrate conc.	4 mM	9 mM	15 mM
Au colloid diameter (nm)	60 ± 5	30 ± 3	20 ± 3

Au colloids were deposited on the Au film with various surface densities. The surface morphology of Au colloids immobilized on Au films was evaluated using AFM (Figure 2). Surface density of Au colloids was calculated using PSIA image analysis program. The surface densities of Au colloids immobilized substrates were differ approximately 100 times by 10 times diluted Au colloids solution (Table 2).

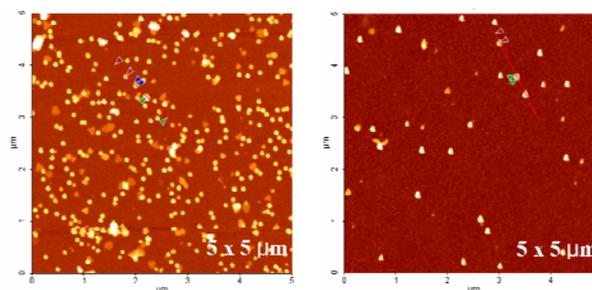


Figure 2. AFM images of immobilized Au colloid on Au film. Scan size was 5 x 5 μm.

Table 2. The surface densities of immobilized Au colloid on substrate at immobilize conditions. (ea/cm²)

Diameter	Undiluted	Diluted (x 10)
20 nm	5 x 10 ¹¹	3 x 10 ⁹
30 nm	1 x 10 ¹¹	1 x 10 ⁹
60 nm	0.2 x 10 ¹¹	0.5 x 10 ⁹

By immobilization of Au colloid, SPR curve was moved right direction and was broaden (Figure 3). Resonance angle shifts were showed linear correlation with alcohol concentration (Figure 4). SPR signal enhancement was different by changes of diameter and surface density. Because deposited Au thickness on glass substrates was maintained constant, SPR resonance shift was originated from the effect of immobilized Au colloid.

The relative signal enhancement efficiency by Au colloid diameter and surface density was summarized in Table 3. The SPR response of substrate of 20 nm Au colloid immobilized was enhanced at high surface density, but low surface density was not enhanced. The 30 nm Au colloid substrate was enhanced higher at low surface density. The SPR response was very low for 60 nm Au colloid immobilized on substrate. The maximum SPR

response was observed for Au colloid of 30 nm diameter, immobilized on substrate at low surface density.

Au colloid immobilized substrate with 30 nm diameter and 1×10^9 ea/cm² surface density was used to detect SPR signal by incremental concentration changes for around 20 % ethanol solution. In this is the typical alcoholic concentration of the consumable liquors (Figure 5). Confirmed SPR signal by alcohol concentration was enhanced using Au colloid.

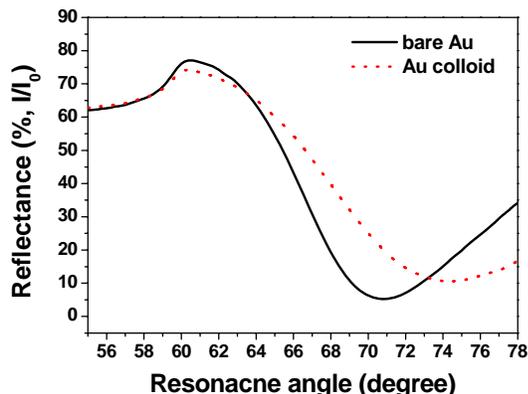


Figure 3. SPR curves obtained from a bare Au film, Au colloid immobilized surface. SPR curve were moved right direction and broaden. Au colloid diameter was 30 nm, surface density was 1×10^9 ea/cm².

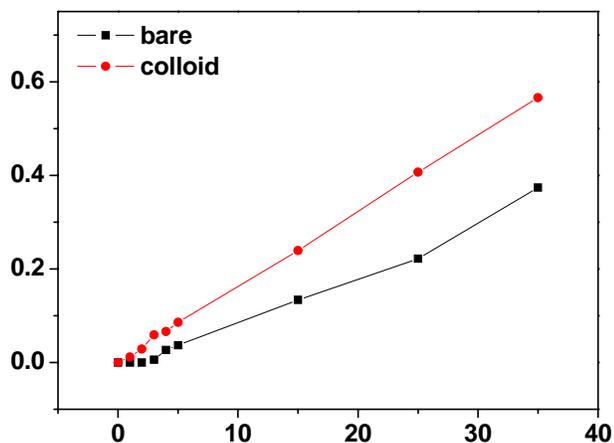


Figure 4. SPR signal was enhanced to detect concentration changes of methanol solution. Au colloid immobilized on the substrate was 30 nm diameter.

Table 3. SPR signal enhancement efficiency (%) by Au colloid immobilized condition at 25 % (v/v) MeOH/H₂O solution.

	20 nm	30 nm	60 nm
Undiluted	21.4	14.3	- 25
Diluted (x 10)	0	33	- 7.7

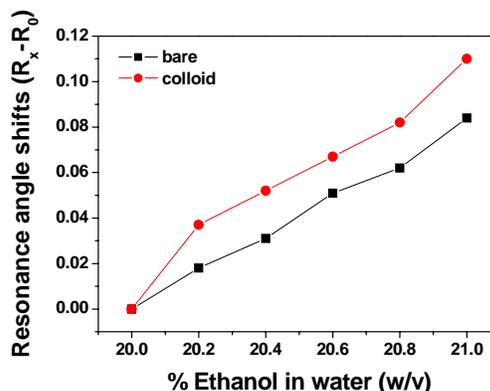


Figure 5. SPR signal changes by small changes in concentration for 20 % ethanol solution. Au colloid immobilized on substrate was with 30 nm diameter and 1×10^9 ea/cm² surface density.

In summary, we found that of the maximum SPR response was observed at 30 nm Au particle size and 1×10^9 ea/cm² surface density on the SPR substrate. The existence of electromagnetic interactions between the metallic nanoparticles and the surface, and light scattering are also thought to contribute signal enhancement. Especially for relative small separation and large particles, the electromagnetic coupling between surface and the metallic nanoparticles is expected. While quantification of the contribution from each factor was not possible in current work, our results highlight the importance of the dielectric of the particle layer on Au film.[12-14]

4 RELATION

In conclusion, to enhance the SPR signal through changed conc. of alcohol aqueous solution, we immobilized Au colloid on substrate. Enhancement of SPR signal efficiency was depended on the size and the surface density of immobilized Au colloid on substrate. A quantified equation for describing relationship between surface density and size of the Au colloids is needed further investigation.

5 ACKNOWLEDGEMENT

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