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Dependence of the signal amplification potential of colloidal gold nanoparticles on resonance wavelength in surface plasmon resonance-based detection

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Abstract

We demonstrate the resonance wavelength-dependent signal of colloidal gold nanoparticles adsorbed to a planar gold surface in surface plasmon resonance (SPR)-based detection. Experimental measurements of the SPR signal as a function of particle surface coverage are presented for three different resonance wavelengths. The SPR signal due to the colloidal gold nanoparticles varies across the resonance wavelengths of 650 nm, 770 nm, and 920 nm. The experimental SPR curves show good agreement with the results of a Lorentz absorbance model at the lower particle surface coverages investigated. The results demonstrate an almost 2-fold signal difference for a subset of the experimental conditions explored.

Introduction

Signal amplification using particle tags [1–4] can be an effective method of enhancing the surface plasmon resonance (SPR)-based detection of low molecular weight or low concentration analytes at planar gold surfaces. For SPR-based detection, the particle tag will ideally be composed of a material that produces a large change in refractive index (RI) per unit volume of tag material. Because the RI of the particle tag is a wavelength-dependent quantity, the signal amplification potential of a particle tag will also depend on wavelength. The dependence of SPR signal on resonance wavelength can be especially relevant for absorptive particle tags. A recent report demonstrated a 4-fold change in the SPR signal of absorptive metal ‘nanocage’ particles adsorbed to a planar gold surface in the wavelength range of 650 nm to 950 nm. The largest signal was observed at the longest resonance wavelength due to a combination of the near-infrared (NIR) absorption peak of the particles and the inherent increase in sensitivity of SPR measurements on planar gold for NIR resonance wavelengths [5].

An alternative absorptive particle tag, in which there has been much interest, is colloidal gold. Lyon *et al.* [6–8] first demonstrated the utility of colloidal gold nanoparticles for amplification of SPR signals on planar gold surfaces. They demonstrated significant modulation of the SPR curves that included broadening and an increase in the minimum reflectivity. Since then, colloidal gold nanoparticles have been used as amplification tags in many different SPR-based

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assays including secondary antibody labels [9] in sandwich format assays and competitor labels [10] in competition format assays. However, in each of these studies, the amplification potential of colloidal gold nanoparticles was probed using a single excitation wavelength. The resonance wavelength-dependent signal of colloidal gold nanoparticles adsorbed to planar gold has not, to our knowledge, been characterized.

In this report, we describe the resonance wavelength-dependent signal of colloidal gold nanoparticles on planar gold. The SPR signal (reported as a function of particle surface coverage), varies for the resonance wavelengths of approximately 650 nm, 770 nm, and 920 nm, and is greatest at 770 nm. Modeling results using a simple absorptive Lorentz model [11,12] are shown to be in good agreement with the experimental data and are consistent with a red-shifted localized surface plasmon resonance (LSPR) absorption peak of 650 nm for the colloidal gold nanoparticles on planar gold. The results indicate that the resonance wavelength dependence of the colloidal gold nanoparticles on planar gold is a balance between this LSPR absorption peak in the visible range and the inherent sensitivity increase of SPR on planar gold in the NIR [13,14].

Experimental

Materials

The 40 nm colloidal gold particles, a gift from PATH (Program of Appropriate Technologies in Health, Seattle, WA), were synthesized according to the following protocol. First, 20 nm diameter colloidal gold particles were synthesized. Briefly, a solution of 6 ml of 1% gold chloride was added to 570 ml of water and the solution was vigorously mixed while heated to a boil. A solution of 24 ml of 1% sodium citrate was then added and the mixture continued to boil until a change in color to orange-red was observed. The 20 nm particles were then used to seed the 40 nm particle synthesis. First, 1 ml of 1% sodium citrate and 100 ml of the 20 nm particle solution were added to 500 ml of water and the solution was vigorously mixed while being heated to a boil. A solution of 10 ml of 0.2% gold chloride was then added and the mixture continued to boil until a change in color from purple to scarlet was observed. An additional solution of 10 ml of 0.2% gold chloride was added and again continued to boil until another color change from purple to scarlet was observed.

SPR substrates consisted of 2 nm of chromium and 55 nm of gold sputtered onto BK7 slides (Schott, Germany) and were coated by TTS Ltd. (Czech Republic). The coated substrates were cleaned in a 5:1:1 solution of water, 30% hydrogen peroxide, and ammonium hydroxide for approximately 40 min at room temperature and then stored in water until use. The cleaned substrates were passivated with mercaptoethylamine (MEA, Sigma Aldrich, MO) [6] by incubation in a 2 mg/ml MEA in ethanol solution for approximately 3 h, followed by sonication in ethanol for 10 min. The flowcell was composed of the MEA-treated substrate as the base, a midlayer with channels defined in 70 μ m Mylar®/adhesive, and a capping layer of acrylic.

Instrumentation

The SPR instrument was a custom built Plasmon IV (Institute of Radio Engineering and Electronics [IREE], Czech Republic) with 4 independent channels. Briefly, light from a tungsten halogen lamp (Avantes, The Netherlands) was collimated, polarized, and coupled to the sample film via a BK-7 prism. The reflected light was then collected by a multichannel spectrometer. The SPR reflectivity spectrum was calculated from the following.

$$R(\lambda) = \frac{r^{\text{TM}}(\lambda) - r^{\text{dark}}(\lambda)}{r^{\text{TE}}(\lambda) - r^{\text{dark}}(\lambda)} \quad (1)$$

Here $r^{TM}(\lambda)$ was the spectrum acquired with transverse-magnetically-polarized incident light, $r^{TE}(\lambda)$ was the spectrum acquired with transverse-electrically-polarized incident light, and $r^{dark}(\lambda)$ was the dark spectrum (acquired with no incident light). To position the resonance minimum at a given wavelength, the angle of incidence was varied by adjustment of the rotation arms on the instrument (for a given sample RI, decreasing the angle of incidence, increases the resonance wavelength).

A field-emission microscope (FEI Sirion, OR, at the Center for Nanotechnology at the University of Washington) at an acceleration voltage of 5 kV was used to image the samples. The surface coverages of colloidal gold particles were estimated by counting particles in areas greater than $70 \mu\text{m}^2$.

Procedures

The SPR experiments were conducted according to the following protocol. First water was introduced into a channel and reflectivity spectra were acquired at the three resonance wavelengths of approximately 650 nm, 770 nm, and 920 nm. Next, a colloidal gold nanoparticle solution was introduced into the channel and incubated for 30 min. The colloidal gold nanoparticle solution was then rinsed from the channel with approximately 10 channel volumes of water and reflectivity spectra were again acquired at the different resonance wavelengths. A reference channel containing water was monitored throughout the experiment and indicated that the effects of instrument drift were minimal. All solutions were hand-injected using syringes.

The experimental data were modeled using the Fresnel equations, in a two-stage process. Multiple parameters were optimized using the combined data from the three experimental resonance wavelengths, to obtain the best fit to the experimental data. Optimization was performed using a constrained branch and fit algorithm by Huyer and Neumaier [15]. The goodness-of-fit parameter used was the weighted sum of the squares of the difference between the experimental and simulated reflectivity curves. The weighting factor, the inverse square of the experimental reflectivity curve, was chosen to emphasize the region about the minimum of the SPR curve.

In the first stage, the MEA-only samples were modeled as a five-layer system composed of prism, chromium layer, gold layer, MEA layer, and water. Optimization to obtain the best fit to the experimental SPR curves for the MEA-only samples was performed simultaneously at the three resonance wavelengths. The refractive indices of the MEA layer and water, assumed to be constant with respect to wavelength, were modeled as 1.5 and 1.333, respectively, and a chromium adhesion layer thickness of 2.5 nm was assumed. The dispersion relations of the prism, chromium, and gold layers were modeled using eighth-order polynomial fits to values of the wavelength-dependent refractive indices reported in the literature. In the case of gold, the literature values corresponded to electropolished gold [110] [16]. It is expected that the RI of the sputtered gold layer used in the experiment will vary significantly from the RI of the electropolished gold. Thus, the imaginary part of the gold layer RI used in the model varied up to 30% of the above literature values. The gold thickness was allowed to vary $\pm 5\%$ of the experimental target thickness of 55 nm. Additionally, the angle of incidence for each of the resonance wavelengths was allowed to vary in order to obtain the best match with the experimental data. An experimental angle spread of 0.3° was assumed (estimate from IREE) in all cases. Table 1 lists the values of the relevant parameters.

In the second stage, the MEA-plus-colloidal gold samples were modeled as a six-layer system composed of prism, chromium layer, gold layer, MEA layer, colloidal gold layer, and water. The set of best fit parameters obtained for the five-layer system in the first stage was fixed, and the MEA-plus-colloidal gold samples were modeled at the three resonance wavelengths

and for each of the particle surface coverages. The colloidal gold layer was modeled as a collection of independent Lorentz oscillators after Kurihara *et al.* [11,12] The expression used for the permittivity of the colloidal gold layer follows.

$$\epsilon_{\text{ns}} = \epsilon_{\text{ns}}^{\text{background}} + \frac{A(\omega_0) \left(\epsilon_{\text{ns}}^{\text{background}} \right)^{\frac{1}{2}} c \gamma \ln 10}{d \left(\omega_0^2 - \omega^2 - i\omega\gamma \right)} \quad (2)$$

Here $\epsilon_{\text{ns}}^{\text{background}}$ is the background permittivity of the sensing layer, ω is the angular frequency of incident light, ω_0 is the peak absorption angular frequency, $A(\omega_0)$ is the absorbance at the peak absorption frequency, d is the effective thickness of the colloidal gold layer, γ is the absorption damping frequency, and c is the speed of light in vacuum. The values of these parameters are listed in Table 1.

Results and Discussion

The experimental SPR curves for the MEA-only sample and MEA-plus-colloidal gold sample at each of the three resonance wavelengths are shown for the highest surface coverage sample in Figure 1A, 1C, and 1E. In the presence of the colloidal gold nanoparticles, the SPR curve is broadened, its minimum reflectivity is increased, and its minimum is shifted to higher wavelengths (corresponding to smaller incident angle, for angle interrogation measurements) with respect to the MEA-only sample. The corresponding difference curves of the MEA-plus-colloidal gold nanoparticles samples and the MEA-only samples of Figure 1A, 1C, and 1E are shown in Figure 1B, 1D, and 1F, respectively. It is apparent from the curves that the largest change in signal (of the three) occurs at the mid-range resonance wavelength of 770 nm. One common measure of the signal change due to absorptive particles used in previous reports [6, 17] is the maximum change in reflectivity; with this measure, the signal change due to colloidal gold nanoparticles is approximately 30% at 650 nm, 49% at 770 nm, and 47% at 920 nm. An alternative measure, which depends on the difference curve across the investigated wavelength range and more completely describes the quantifiable difference in such signals, is the sum of the absolute value of the difference curve [5]. Specifically, the sum of the absolute value of the difference curve is multiplied by the range of wavelengths interrogated and divided by the total number of data points in the range of wavelengths interrogated. The normalized sum of the absolute value of the difference curve is 39 for the resonance at 650 nm, 71 for the resonance at 770 nm, and 68 for the resonance at 920 nm. Thus, the SPR signal varies by almost 2-fold across this set of resonance wavelengths.

Additional samples were prepared with varying particle surface coverages. The surface coverage of the nanoparticles was estimated from SEM to be approximately 0.3×10^9 particles/cm², 0.6×10^9 particles/cm², 0.9×10^9 particles/cm², and 2×10^9 particles/cm², (approximately 0.4%, 0.7%, 1.5%, and 3.4% of the area density of a monolayer, respectively). Figure 2 shows the SPR signal for various particle surface concentrations for the three resonance conditions. The SPR response, i.e. SPR signal as a function of particle surface coverage, is approximately linear in this range of particle surface concentrations, and greatest for the mid-range resonance wavelength. The SPR response is also linear and of comparable magnitude for the high resonance wavelength. In contrast, the SPR response at the low resonance wavelength is significantly smaller in magnitude, by almost 2-fold, than that measured at the higher resonance wavelengths, and shows an increasing deviation from linearity at the highest surface concentration investigated.

The model and experimental SPR curves for the MEA-plus-colloidal gold samples at the three resonance wavelengths are plotted together in Figure 3 for each of the particle surface coverages. There is good agreement between the model and experimental curves for the lower surface coverages, while there is significant discrepancy at the highest particle surface

coverage. The optimized model results are consistent with a colloidal gold nanoparticle absorption peak centered at approximately 650 nm with a FWHM of approximately 280 nm when the particle is adsorbed to the MEA layer on the planar gold surface. The bulk absorption peak of the colloidal gold nanoparticle at 526 nm (data not shown) is therefore significantly red-shifted as the particle approaches the planar gold. Okamota *et al.* [18] observed a 170 nm shift in the absorption peak of approximately 40 nm diameter particles when the particles were adsorbed to an aminoethanethiol functionalized gold surface as compared to when in bulk solution. The parameters used in the model are listed in Table 1. The results suggest that in the case of the colloidal gold nanoparticles, with their LSPR absorption peak in the visible range, the resonance wavelength at which a maximum SPR signal for the particle will be obtained is determined by a balance between the particle LSPR absorption peak wavelength and the inherent greater sensitivity at longer wavelengths [5,13,14]. The LSPR absorption peak is in turn determined by many factors, including the proximity of the metal particle to the planar metal surface, and the size, shape, and composition of the particle (see [19] for a review), which will affect the choice of optimal resonance wavelength for a given system.

Conclusion

We have demonstrated the dependence of the SPR signal amplification potential of colloidal gold nanoparticles on resonance wavelength. The SPR response, i.e. SPR signal as a function of particle surface coverage, was presented for three different resonance wavelengths. The response due to the colloidal gold nanoparticles varied significantly for the resonance wavelengths 650 nm, 770 nm, and 920 nm, and was greatest at 770 nm. The results suggest that prudent choice of the resonance wavelength can significantly increase the signal amplification potential of colloidal gold nanoparticles, i.e., by almost 2-fold for the conditions explored in the current study.

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Figure 1.

The SPR curves of reflectivity versus wavelength for the MEA-only sample (○) and the MEA-plus-colloidal gold sample (+) at an MEA resonance wavelength of approximately 650 nm (A), 770 nm (C), and 920 nm (E). The effects of the colloidal gold nanoparticles are to broaden, increase the minimum reflectivity of, and shift the resonance wavelength of the SPR curves. The difference curve of the MEA-plus-colloidal gold sample and the MEA-only sample at the MEA resonance wavelength of approximately (B) 650 nm, (D) 770 nm, and (F) 920 nm. The normalized sum of the absolute value of the difference curve can be used to quantify the signal change due to the nanoparticles and is close to 2-fold greater at 770 nm compared to 650 nm.

Figure 2. Typical SEM images of the colloidal gold particles for the (A) lowest, 0.3×10^9 particles/cm², and (B) highest, 2×10^9 particles/cm² surface coverages.

Figure 3.

The SPR signal, the normalized sum of the absolute value of the difference curve of the MEA-plus-colloidal gold and the MEA-only samples, versus particle surface coverage for the MEA resonance wavelengths of approximately 650 nm (○), 770 nm (+), and 920 nm (×). Note that the SPR response is significantly smaller at the low resonance wavelength compared to the mid and high resonance wavelengths and also shows deviation from linearity at the highest particle surface coverage. A control channel monitored during the timescale of a typical experiment showed a signal change of 5. Surface coverage measurements on a given sample exhibited a coefficient of variation of 10% for the higher surface coverage samples and about 30% for the lower surface coverage samples.

Figure 4. Comparison of the model (solid line) and experimental (circles) curves for 4 surface coverages of colloidal gold nanoparticles, (B) 0.3×10^9 particles/cm², (D) 0.6×10^9 particles/cm², (F) 0.9×10^9 particles/cm², and (H) 2×10^9 particles/cm². Comparison of the model (solid line) and experimental (circles) curves for the MEA-only samples are shown in (A), (C), (E), and (G), respectively. Note the good agreement between the curves for the lower surface coverage samples. The significant discrepancy between the model and experimental curves for the highest particle surface coverage indicates that the simple noninteracting oscillator model is not sufficient to describe the system in this case.

Table 1

The table lists the model input parameters.

Parameter	Model value
BK7 prism RI	Schott dispersion formula
BK7 thickness	Semi-infinite
Chromium RI	8 th order polynomial fit to values from ¹⁶
Chromium layer thickness	2.5 nm (2 nm target for sputtered film by TTS)
Gold RI	8 th order polynomial fit to values from ¹⁶ for real part
Gold layer thickness	57.7 nm (55 nm target for sputtered film by TTS)
MEA RI	1.5 ¹⁶ , ¹⁷
MEA layer thickness	0.5 ¹⁸
Colloidal gold nanoparticle peak absorption frequency	650 nm
Colloidal gold nanoparticle absorption damping frequency	280 nm
Colloidal gold nanoparticle layer effective thicknesses	0.16 nm, 0.28 nm, 0.60 nm, 1.36 nm
Colloidal gold nanoparticle layer background ϵ	1.777 (RI = 1.333)
Water RI	1.333
Water layer thickness	Semi-infinite
Angle spread	0.3° (estimate from IREE)
Absorption coefficient	0.03 nm ⁻¹ , 0.06 nm ⁻¹ , 0.13 nm ⁻¹ , 0.29 nm ⁻¹