Published in final edited form as:

Chem Phys Lett. 2014 January 20; 591: 5–9. doi:10.1016/j.cplett.2013.10.081.

Sensitivity enhancement of a grating-based surface plasmon-coupled emission (SPCE) biosensor chip using gold thickness

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Abstract

We describe a novel approach to enhance the sensitivity of a grating-based surface plasmon-coupled emission (SPCE) sensor by increasing the thickness of the metal film used in this system. The calculated optical properties of grating-based SPR spectra were significantly affected by both grating depth and by gold thickness. Higher angular sensitivity could be achieved at short wavelengths and under *in situ* measurement (analysis under aqueous condition). We confirmed the predicated enhancements of SPCE response using Alexa Fluor 647-labeled anti-mouse IgG immobilized on the SPCE sensor chips. Grating-coupled SPCE sensor chips can be used as a useful tool for high contents analysis of chemical and biomolecular interactions.

1. Introduction

Surface plasmon-coupled emission (SPCE) has emerged as a novel sensor chip based technology to study chemical and biological interactions because it combines the inherent high sensitivity of fluorescence with the enhanced excitation feature of the surface plasmon [1-3]. SPCE is based on near-field interactions that occur within ~ 200 nm from the metal surface. The use of this phenomenon as a basis for biosensing has several attractive advantages because of its high directionality of emission, surface selectivity, and application in multiplexed immunoassays [4]. The optical phenomenon of SPCE is very closely related to the surface plasmon resonance (SPR) phenomenon since SPCE is the inverse process of SPR [5].

There are two frequently implemented configurations of an SPCE sensor chip platform: an attenuated total reflection (ATR) coupler-based Kretschmann configuration and a grating coupler-based configuration. The advantages of the grating-coupled sensor configuration include the simplicity of detection, the feasibility of highly parallel measurements, a strong evanescent field intensity at a gold/dielectric interface, and relative ease of chip manufacture [6]. Molded plastic grating-based sensor chips make mass-production at low cost possible using well-developed compact disk manufacturing technologies [7]. However, there can be disadvantages to molded plastic grating-based sensor chips, even if there are many advantages. As the master of a grating mold is used over time, performance of the replicated

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sensor chips degrades, as grating depth and shape of the master change. In order to increase the sensitivity of these SPR sensor chips several methods have been reported including the use of colloidal gold [8], liposomes [9], latex particles [10], and hydrogel nanospheres [11] as signal enhancers.

In this paper, we present information describing a different approach to SPCE sensitivity enhancement bioassay measurements that relies on manipulation of gold film thickness. We calculated the theoretical optical properties of an SPR spectrum with respect to a grating depth and a gold thickness using a diffraction grating solver based on the integral method [12]. Angular sensitivity of the grating-based SPR sensor chip was also investigated in both *ex situ* (analysis under air condition) and *in situ* (analysis under aqueous condition). Based on the results of these theoretical calculations, we discuss characteristics of resonance angle, reflectivity, and full-width-half-maximum (FWHM) that influence the design of the optimal sensor chip configuration. We suggest an optimal gold thickness of the grating SPCE sensor chip, and tested this projection using Alexa Fluor 647-labeled anti-mouse IgG (100 µg/ml) immobilized on an 11-mercaptopundecanoic acid (MUA)-modified gold surface. These results indicate that SPCE response enhancement can be achieved by metal film thickness optimization.

2. Surface plasmon-coupled emission (SPCE)

When transverse magnetic (TM) mode incident light couples with surface plasmons to create an evanescent field, the evanescent field excites fluorophores within the range of near-field interactions. Fluorophore emission will couple with plasmons that radiate into the analyte. The excited fluorophores located within the penetration depth of an evanescent field at a metal surface, and the emission with a high degree of p-polarization shows substantial directionality [13,14]. As shown in Fig. 1(a), the SPCE angle (θ_{SPCE}) decreases compared to the SPR angle (θ_{SPR}) measured from the normal axis. This is because the wavelength emitted by the excited fluorophore is longer than that of the light source, and there is an inverse relationship between wavelength and angle that satisfy the momentum matching condition Eq. (1) below.

There are two commonly implemented configurations for SPCE measurements. The first is an attenuated total reflection (ATR) coupler-based configuration. Two different types of ATR designs are possible, and differ in the orientation of incident light to the sensor chip surface. The Kretschmann design and the reverse Kretschmann design [15] are both prism-based. The second SPCE configuration is grating coupler-based. In this configuration, surface plasmons are excited by light diffracted at the metal surface. This configuration is simpler because it does not require a prism or refractive index matching fluids that are used to optically couple the prism to the sensor chip. When the wave vector of the surface plasmon matches the wave vector of the diffracted wave that is parallel to the grating surface, surface plasmons are excited on the gold grating surface [16]. The dispersion relation can be expressed as:

$$k_0 n_s \sin(\theta) + m \frac{2\pi}{L} = \pm k_0 \sqrt{\frac{\epsilon_{mr} n_s^2}{\epsilon_{mr} + n_s^2}} \quad (1)$$

where k_0 ($=2\pi/\text{wavelength}$) is the free space wave vector, n_s is the refractive index of the dielectric analyte, θ is the incidence angle, the diffraction order m is an integer, L is the pitch of grating, and ϵ_{mr} is the real part of the dielectric constant of the metal.

3. Experimental

3.1. Chemicals and reagents

11-mercaptoundecanoic acid (MUA) was purchased from Sigma (St. Louis, MO). N-ethyl-N'-(dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Pierce (Rockford, IL). Alexa Fluor 647-goat anti-mouse IgG was purchased from Molecular Probes (Eugene, OR). All other chemical reagents were of analytical grade.

3.2. SPCE Instrumentation

Fig. 1(a) shows an schematic of the grating-based SPR/SPCE sensor chip reader (Ciencia, Inc., East Hartford, CT). A 635 nm laser diode was used as the light source and a polarizer was positioned in the light path to produce p-polarized light. Images of the sensor chip are taken over a range of angles to obtain the SPR. Twelve bit grey scale bitmap images (1392×1040 pixels) from a CCD camera are used to calculate SPCE intensities for quantitative analysis at each region of interest (ROI). The summation of the intensity of each pixel is divided by the number of pixels in the region of interest to obtain an ROI's SPCE value. The field of view of the CCD camera is approximately $13 \times 13 \text{ mm}^2$ and the resolution of the image is estimated to be $19 \times 25 \text{ }\mu\text{m}^2$. Motion control, data acquisition, image processing, and display are accomplished using LabVIEW-based software.

3.3. Sensor chip

SPCE sensor chips with 500 nm pitch were purchased from Ciencia Inc. (East Hartford, CT). The gold-coated sensor chips were washed with 100% ethanol and rinsed with deionized H_2O (18 $\text{M}\Omega$) to remove any surface contamination. The SpotBot[®] 2 microarrayer (Arrayit Corporation, Sunnyvale, CA) was used to deposit proteins on the sensor chip surface, employing ArrayIt Stealth micro spotting SMP7B pins (3.1 nl delivery volume with 255 μm spot diameter and spotting density of 1,024 spot/ cm^2).

3.4. Surface modification of sensor chips with self-assembled monolayer

Fig. 1(b) represents the MUA self-assembled monolayer (SAM) on the sensor chip. Briefly, the entire sensor chip gold surface was incubated with 5 mM MUA in ethanol for 16 h. All reactions were done at room temperature. For the analysis of Alexa Fluor 647-goat anti-mouse IgG on the sensor chip, carboxyl groups of MUA were activated to form reactive N-hydroxysuccinimide esters by incubating with a solution of 50 mM N-hydroxysuccinimide and 200 mM N-ethyl-N'-(dimethylaminopropyl)-carbodiimide in water for 10 min. The anti-mouse IgG (100 $\mu\text{g}/\text{ml}$ in acetate buffer, pH 4.5) was immobilized on the NHS activated surface for 1 hour in a humid chamber. The chips were then washed with 0.1% Tween 20 in phosphate-buffered saline (PBS; 8.1 mM Na_2HPO_4 , 1.2 mM KH_2PO_4 , 138 mM NaCl, 2.7 mM KCl, pH 7.4) for 10 min, rinsed with deionized H_2O (18 $\text{M}\Omega$) and then analyzed for SPCE responses.

4. Results and discussion

4.1. Characterization of SPR spectrum for the groove depth

To monitor the SPCE response of a gold grating-based biosensor chip, it is very important to investigate the optical characteristics of the SPR spectrum because surface plasmons excited at the gold grating surface are essential to produce a sensitive SPCE response. Theoretical analysis of SPR spectra from a diffraction grating was performed by using a diffraction grating solver PCGate (International Intellectual Group, Inc., Staten Island, NY). First, we calculated the characteristics of the SPR spectrum as a function of groove depth at a set gold thickness of 50 nm. As shown in Fig. 2(a), a series of SPR spectra were calculated as a

function of a groove depth at this thickness. The calculated optical properties of SPR spectra were similar to previous reports using a silver surface [17,18]. Differences in the SPR angle and FWHM between gold and silver-based SPR spectra reflect the different dielectric constants of these metals. The real part of the dielectric constant is related to the resonance angle shift, and the imaginary part of the dielectric constants is related to the FWHM of the SPR spectra. Even though silver has superior optical properties compared to those of gold, gold is widely used for biosensing chips because it has long-term stability in an aqueous environment [19].

Reflectivity minimum and FWHM are important factors for the sensitivity of SPR sensors. As groove depth increases, resonance angle and FWHM increase to higher values. The dip in the SPR spectra indicates the excitation of surface plasmons on the gold surface. The increment of resonance angle is due to the decrease in the phase velocity of the reflected light [18]. We observe the lowest reflectivity minimum of the SPR spectrum at a groove depth of 60 nm. At groove depths between 20 nm to 60 nm, the reflectivity minimum of SPR spectra significantly decreased with increasing groove depth. The shape of spectrum at a groove depth of 80 nm is poor compared to that of other groove depths. When the reflectivity minima and FWHM of the SPR spectra in Fig. 2(a) were recalculated as a function of the groove depth (Fig. 2(b)), the smallest FWHM with the lowest reflectivity minimum, which is the best SPR spectrum for biosensor chips, was determined. However, it is difficult to satisfy the smallest FWHM and the lowest reflectivity at the same time in Fig. 2(a). We conclude that the 60 nm groove depth of the SPR spectrum is the optimum groove depth of the gold grating-based biosensor chip as a compromise between the properties of reflectivity minima and FWHM. The 60 nm groove depth of the SPR spectrum shows the lowest reflectivity minimum among the SPR spectra indicating the most efficient excitation of surface plasmons [16].

4.2. Characterization of SPR spectrum for the gold thickness

In order to investigate the effect of gold film thickness on the SPR spectrum, we calculated SPR spectra for four different gold thicknesses (30, 50, 100, and 150 nm) with a 50 nm groove depth and analyzed the characteristics of the SPR spectra. As shown in Fig. 3(a), the reflectivity minimum and SPR angle increased as the gold thickness increased from 50 to 100 nm. Above 100 nm, the reflectivity minimum saturated. An SPR spectrum generated from a surface with a 30 nm gold thickness was far flatter compared to that of the other gold thicknesses. To clarify these observations, the properties of reflectivity minimum and FWHM were replotted as a function of gold thickness in Fig. 3(b). As shown in Fig. 3(b), the 50 nm gold thickness produced the lowest reflectivity minimum.

These results showed that gold film thickness significantly influences the properties of the SPR spectra. From the theoretical studies, the 50 nm-thick gold film was selected as the best gold thickness for the 60 nm-thick groove depth of a gold grating-based biosensor chip.

4.3. Sensitivity of the grating coupler-based SPR

We have also investigated the property of SPR angular sensitivity at air and aqueous interfaces. The angular sensitivity (S_θ) under water and air was calculated as described in a previous report [20] and is expressed as

$$S_\theta = \frac{d\theta}{dn_s} = \frac{1}{n_s \cos \theta} \left(\pm \left(\frac{\varepsilon_{mr}}{\varepsilon_{mr} + n_s^2} \right)^{3/2} - \sin \theta \right), \quad (2)$$

where the incidence angle θ is obtained from Eq. (1). We calculated angular sensitivity S_θ for the positive diffraction order $m=1$ in Eq. (2). As shown in Fig. 4(a), the angular

sensitivity decreases for both water and air interfaces with respect to increasing wavelength. We found that higher sensitivity could be obtained in the grating-based SPR biosensor chips operated at shorter wavelengths in the aqueous environment common to analysis of chemical and biomolecular interactions. Spectral sensitivity of ATR coupler-based SPR sensor chip increased with respect to wavelength under both air and aqueous measurement conditions, but higher sensitivity was achieved at longer wavelengths in the air measurement condition [21]. The angular sensitivity of the ATR coupler-based SPR biosensor chip was similar to that of the grating-based SPR biosensor chip [22]. Next, we calculated SPR spectra for water and air at an illumination wavelength of 635 nm. As shown in Fig. 4(b), the SPR angle increased from 8.3° to 12.4° as the refractive index of the measurement environment decreased from 1.33 (water) to 1.00 (air), and the orientation of the edge of the SPR spectrum was reversed. Interestingly, these results are opposite those of an ATR coupler-based SPR biosensor chip [21].

4.4. Sensitivity enhancement of grating-based SPCE sensor chips

We have used molded grating-based SPCE sensor chips made with a soft lithography process, by replication of a master grating. This allows inexpensive and rapid fabrication of grating replicas. However, as the master grating mold continues to be used, the performance of the replicated grating-based SPCE sensor chips degrades. When we investigated the surface properties of a recently manufactured SPCE sensor chip, we discovered that grating depth was smaller when compared to previously produced chips from the same master grating (data not shown). We assessed changing gold thickness as a way to boost performance of the degraded grating-based SPCE sensor chips that would avoid the need to replace the expensive master grating mold. As shown in Fig. 5(a), we calculated SPR spectra for 50 nm, 75 nm, and 150 nm-thick gold thickness in a four-phase (BK7 glass substrate/polycarbonate film/gold film/analyte ($RI=1.33$)) at the emission wavelength of Alexa Fluor 647 ($E_m = 665$ nm). The SPR properties in Fig. 5(a) were similar to those of the idealized grating-based SPR sensor chip in Fig. 3(a). However, the FWHM became narrower when gold films above 75 nm-thick were used, while variation of the reflectivity minimum between 50 nm and 75 nm gold thickness was negligible. As the FWHM became narrower, the evanescent field intensity of surface plasmons increases [3, 23] and this property can be applied to an enhancement of the fluorescent response of SPCE because the evanescent field was used to excite the fluorophores on the gold surface.

Finally, we confirmed whether this approach could improve SPCE responses that measure biomolecular interactions on the SPCE sensor chip. We predicted an enhancement of SPCE response because excitation of fluorophores at the gold surface is related to the evanescent field intensity of the surface plasmon [24-25]. We acquired SPCE images following chemical coupling of Alexa Fluor 647-labeled anti-mouse IgG (100 $\mu\text{g/ml}$) to the MUA-modified surface. As shown in Fig. 5(b), as gold thickness increases, the SPCE image brightness increases, as predicted by the theoretical results in Fig. 5(a). Fig. 5(c) shows that the SPCE intensity using a 150 nm gold film thickness increases as much as threefold when compared to that of the 50 nm gold thickness. The SPCE intensity became saturated above 75 nm, which was also similar to the theoretical result of Fig. 3(b). These results show that using a 150 nm-thick gold grating-based SPCE biosensor chip can optimize the sensitivity of gold grating-based SPCE biosensor chips.

5. Conclusions

We have demonstrated an approach for enhancement of sensitivity in the SPCE response for gold grating-based SPCE biosensor chips. SPR spectra were significantly affected by grating depth and gold thickness. Optimum conditions of the gold grating-based SPR sensor chip was 60 nm grating depth and 50 nm gold thickness for a grating with 500 nm pitch

illuminated with 635 nm light. Angular sensitivity of the grating-based SPR sensor chip increased at short wavelengths and in an aqueous environment. The SPCE response of the 150 nm-thick gold grating-based SPCE sensor chip increased as much as threefold compared to that of the 50 nm-thick degraded gold grating-based SPCE biosensor chip. The results suggest that SPCE response sensitivity enhancement can be achieved by optimizing metal film thickness.

Acknowledgments

This research was funded by grants for the National Institutes of Health (DK096953, ES022342 and OD016467).

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► Sensitivity enhancement of an SPCE response can be achieved by using a gold thickness. ► SPR spectrum was significantly affected by a grating depth and a gold thickness of grating-based SPR sensor chips. ► Angular sensitivity of the gratingbased SPR sensor chip increased at short wavelengths and in aqueous environment.

Fig. 1. Schematic diagram of SPCE and surface structure of the grating-based SPCE sensor chip
 (a) TM mode input light is used to excite surface plasmons at the gold/dielectric grating interface and the surface plasmons excite fluorophores on the gold surface. Directional SPCE is emitted into the analyte due to near field interactions between the fluorophores and the metal surface. (b) Alexa Fluor 647-labeled anti-mouse IgG was immobilized on a linker layer formed by MUA.

Fig. 2. SPR spectra with various thicknesses of a groove depth

(a) SPR spectra were calculated with various thicknesses of a groove depth as a function of the incidence angle at a fixed 50 nm gold thickness; SPR spectra are shown for 20 nm (solid line), 40 nm (dash line), 50 nm (dot line), 60 nm (dash-dot line), and 80 nm-thick (dash-dot-dot line) groove depths. (b) Relationship between the SPR intensity and the FWHM as a function of the groove depth.

Fig. 3. SPR spectra with various thicknesses of gold thickness

(a) SPR spectra were calculated with various gold thicknesses as a function of the incidence angle at a fixed 60 nm groove depth; SPR spectra for 30 nm (solid line), 50 nm (dash line), 100 nm (dash-dot line), and 150 nm-thick (dash-dot-dot line) gold thicknesses. (b) Relationship between the SPR intensity and the FWHM as a function of the gold thickness.

Fig. 4. Sensitivity versus the wavelength of a gold grating-based SPR sensor chip

(a) Angular sensitivity S_θ was calculated as a function of wavelength with the refractive index (RI) of 1.33 (water, solid line) and 1.00 (air, dashed line). The 500 nm pitch, and the 1st order were used for the calculation of the angular sensitivity. (b) SPR spectra were calculated with a three-phase (BK7 substrate/gold film (50 nm)/dielectric (water and air)).

Fig. 5. Confirmation of SPCE response enhancement with the gold thickness

(a) SPR spectra were calculated with various gold thicknesses for 665 nm light and with a grating pitch of 500 nm as a function of the incidence angle: SPR spectra for 50 nm (solid line), 75 nm (dash line), and 150 nm-thick (dash-dot-dot line) gold thicknesses. (b) SPCE responses are shown using Alexa Fluor 647-labeled anti-mouse IgG (100 μ g/ml) immobilized on the MUA-modified surface. The left image is the SPCE image of the 50 nm-thick gold film. The middle and right images are the SPCE images of the 75 nm and 150 nm-thick gold films. (c) Average SPCE intensities were calculated from the Fig. (b).