以銀及二氧化矽鍍膜做成之相位型表面電漿共振生醫感測器
Phase Type of SPR Biosensor with Ag and SiO_2 Coatings

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Abstract

We propose a new combination of the thin silver (Ag) and SiO_2 films to improve SPR biosensor sensitivity, using common-path heterodyne interferometry. Although the Ag film has higher sensitivity than gold (Au) film [1], it is toxic for many media and easily oxidized. To enhance the sensitivity and protect the tested medium from direct contact with Ag, we selected SiO_2 coating onto the Ag film because it is inexpensive and easily obtained. The simulations and experimental results show that the optimum Ag film thickness is 56nm. The best resolution could reach a value of 6.9×10^{-9}RIU.

Keywords: surface plasmon resonance (SPR), heterodyne interferometry (HI), biosensor

摘要

為提高表面電漿共振感測器之靈敏度，本文提出在稜鏡鍍上銀膜與二氧化矽膜並採用共光程外差干涉的方法擷取相位。雖然銀膜之靈敏度比金膜要高，但對生物而言有其毒性與會氧化之問題，故我們在銀膜上加鍍一層薄薄的二氧化矽膜，除了可降低成本且此材料容易取得外，又可防止待測物體直接碰觸銀膜。本文中經模擬與實驗均已證實，銀膜之最佳厚度為 56 奈米，其感測器之最佳解析度為 6.9×10^{-9} 單位折射率(RIU)。

關鍵詞：表面電漿共振，外差干涉術，生物感測器

I. INTRODUCTION

SPR biosensors have become useful for biological measurements in recent years [1-6]. There are film combinations, such as, Ti/Au, Au/Ag, Cr/Au, etc. In 2001, Xinglong et al [2] proposed that the sensitivity of Ag is higher than that of Au and Ag film has a smaller resonant angle but with toxicity. To avoid its toxicity, in 2004, Wu et al [5] proposed the combination of Au and Ag films. The resolution would be improved to a value of 5.5×10^{-8} RIU, but the cost of Au coating is high. To solve this situation, we propose a new combination of Ag and SiO_2 films. The angle of resonance was near at 70° for the same refractive index measurement range and the best resolution could reach a value of 6.9×10^{-9}RIU.

II. PRINCIPLES

1. The phase shift due to SPR effects

In Naraoka's and Kajikawa's paper [6], the phase sensitivity of Kretchmann's configuration under the SPR condition is very high. For example, in the four-layer biosensor shown in Figure 1, the surface plasmon waves are excited when the incident angle α is equal to the resonant angle α_{sp}.

From Fresnel's equations [7, 8], the reflective coefficients of p- and s-polarizations can be expressed as

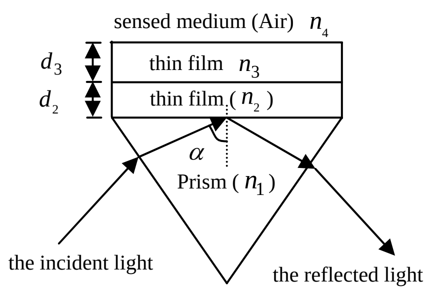

Fig. 1 The four-layer SPR biosensor ayers

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$$r_{1234}^t = \frac{r_{12}^t + r_{234}^t e^{i2k_{z2}d_2}}{1 + r_{12}^t r_{234}^t e^{i2k_{z2}d_2}} \quad (1)$$

$$r_{234}^t = \frac{r_{23}^t + r_{34}^t e^{i2k_{z3}d_3}}{1 + r_{23}^t r_{34}^t e^{i2k_{z3}d_3}} \quad (2)$$

where $r_{ij}^t = \frac{E_i^t - E_j^t}{E_i^t + E_j^t}$, d_2 and d_3 are the thicknesses of medium 2 and medium 3, respectively, and the amplitudes of the electric field intensities of p- and s- polarizations are expressed as

$$E_I^t = \begin{cases} k_{zI} / n_I^2 & t = p \\ k_{zI} & t = s \end{cases}, \quad I = i, j; i, j = 1,2,3,4 \quad (3)$$

In Equation (3), $k_{zi(j)}$ is the component of the wave vector in medium $i(j)$ in the z direction and it is given as

$$k_{zi(j)} = k_0 (n_{i(j)}^2 - n_1^2 \sin^2 \alpha) \quad (4)$$

where the values of n_1, n_2, n_3, and n_4 are the refractive indices of prism, Ag, SiO_2, and the tested medium, respectively, and k_0 is the wave vector in vacuum. Using SiO_2 film increases the adhesive force between the Ag metal and prism and avoids the toxicity and oxidation of Ag film.

If the amplitude reflective coefficients r_{1234}^p and r_{1234}^s are written as

$$r_{1234}^p = \left| r_{1234}^p \right| e^{i\phi_p}, \quad r_{1234}^s = \left| r_{1234}^s \right| e^{i\phi_s} \quad (5)$$

the phase difference δ between p and s polarization components is given as

$$\delta = \phi_p - \phi_s \quad (6)$$

And the reflectivities $R_p = \left| r_{1234}^p \right|^2$ and $R_s = \left| r_{1234}^s \right|^2$ are that of the p and s polarizations, respectively.

To simulate the effects in the different cases with or without SiO_2 film, we selected the wavelength at $\lambda = 632.8nm$ and the parameters for the four-layer biosensor (BK7 glass prism-Ag- SiO_2-air or liquid) as $d_2 = 57nm$, $d_3 = 0nm$ or $5nm$. The permittivities of the BK7 glass prism were selected as ($\varepsilon_1 = n_1{}^2$), Ag film ($\varepsilon_2 = n_2{}^2$), SiO_2 film ($\varepsilon_3 = n_3{}^2$), and air (or liquid) ($\varepsilon_4 = n_4{}^2$) as $\varepsilon_1 = (1.51509)^2$, $\varepsilon_2 = -18 + 0.47i$, $\varepsilon_3 = (1.457)^2$, and $\varepsilon_4(air) = (1.0003)^2$ or $\varepsilon_4(liquid) = (1.34)^2$, respectively. The simulation results are as shown in Figure 2(a) and (b), the plots of the reflectivity R_P and the phase difference δ versus the incident angle α for the tested medium of air and liquid, respectively. It is evident that the reflectivity and phase shift vary severely around the resonant angle (α_{sp}). The resonant angles shift from 42.822° to 43.236° for air (n_4=1.0003) and 68.775° to 69.345° for liquid (n_4=1.34) when a 5nm thick SiO_2 film coating is doped. Although the resonant angles are increased, they are still smaller than that for Au film.

2. The experimental setup

The experimental setup diagram was shown in Figure 3. The beam from a Zeeman laser (Agilent 5519A) with a wavelength of 632.8nm and a beat frequency of 2.5MHz

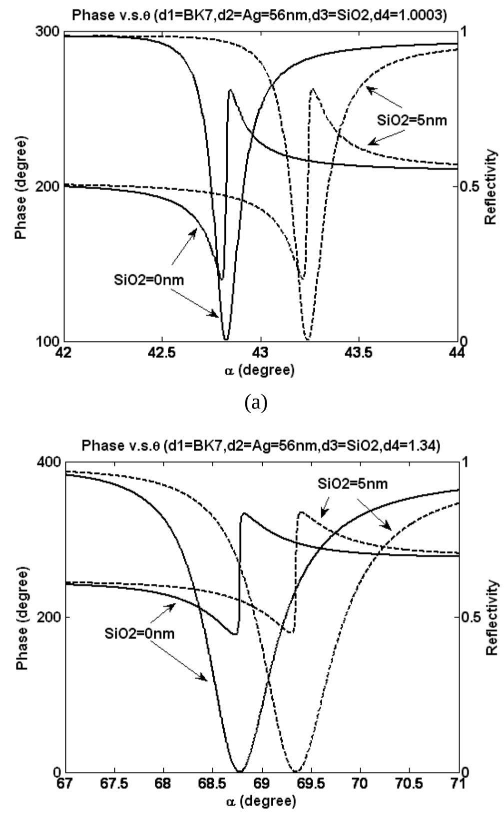


Fig. 2 Comparing the simulations of reflectivity and phase shift versus the incident angle α between with and without SiO_2 film: (a) in air (n_4=1.0003) measurement, (b) in liquid (n_4=1.34) measurement.

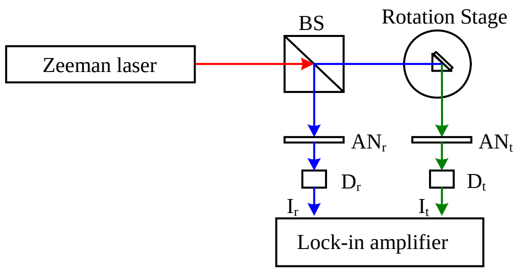


Fig. 3 The experimental setup diagram

was incident into a beam splitter (BS) and divided into the reflection and transmission beams. The reflected beam passed through an analyzer AN_r (Newport 10LP-VIS-B) whose transmission axis was at 45° with respect to the x axis, then entered a photodetector D_r (Thorlabs PDA55). In this method, the light measured by D_r is the reference signal (I_r). In addition, the transmitted beam (the test beam) entered a SPR biosensor, was reflected and then passed through an analyzer AN_t. It was then detected by another photodiode (D_t) (Thorlabs PDA55). The signal detected by D_t is called the test signals (I_t). To achieve the initial phase difference between two beams before the test beam is incident into the SPR biosensor, locating the analyzer AN_t and photodetector D_t in front of SPR biosensor and using a lock-in amplifier (Stanford Research SR844, 200MHz) to measure the initial phase difference (ϕ_0).

When the test medium is on the sensor, the measurement is beginning. There are several steps must be done before measuring. First, the angle of resonance is searched by rotating the sensor and using an analyzer and a detector or power meter to detect the P-polarization output power until the power is at minimum. Second, the phase difference (ϕ') between two signals (I_t and I_r) is measured using a lock-in amplifier. Thirdly, calculating the phase shift difference ($\delta = \phi' - \phi_0$) due to the SPR effect. Finally, substituting the value of δ into Equations (1)~(4) to calculated the refractive index of the test medium using a numerical method. If there is a little change in the concentration of the test medium, the phase difference shift $\Delta\delta$ will be measured in real-time.

III. RESULTS AND DISCUSSIONS

For proving our inference and ensuring that the coating of Au/SiO_2 films is the best selection. It is necessary to compare their sensitivities and resolutions to the different coating with Ag (56nm), Au (47nm), Ag (56nm)/Au (5nm), and Ag (56nm)/ SiO_2 (5nm) films, respectively. In this paper, the sensitivity is defined as

$$S = \left|\frac{\partial \phi}{\partial n}\right| \tag{7}$$

and resolution is defined as

$$R = \Delta n_{\min} = \frac{1}{S}\Delta\phi_{\min} \tag{8}$$

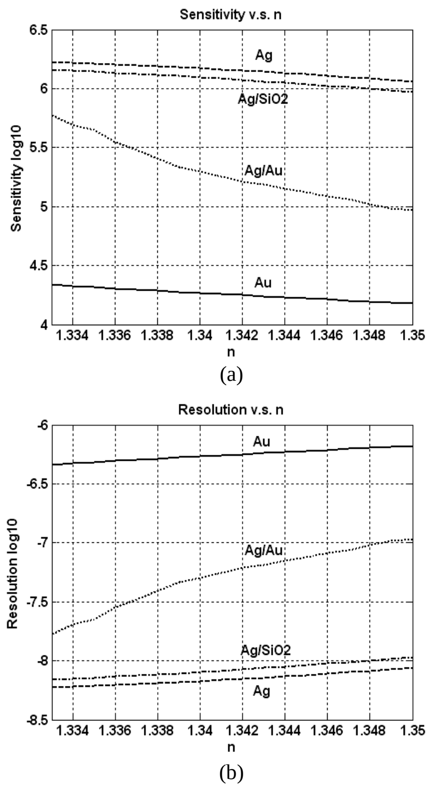
where the value of $\Delta\phi_{\min}$ is the minimum phase variation of measurement system and $\Delta n_{\min}$ is the minimum variation of refractive index. Because the optical system was a common-path heterodyne configuration, the interferences were stable enough, so the minimum phase difference $\Delta\phi_{\min}$ could reach to a value of 0.01°.

According to the mentions above, the simulation results have been shown as Figure 4(a), (b), and Table 1. It is clear that the best resolution is 5.9×10^{-9} RIU for the Ag film coating, but it is toxic and easily oxidized. The best resolution is 6.9×10^{-9} RIU for the Ag/SiO_2 film coating. This improves the sensitivity of Au film by two orders. The resonant angle is the smallest of all of the coatings.

The experimental setup is shown in Figure 3. A SPR biosensor with a right-angle prism (BK7) coated with the thin films of Ag and SiO_2, and the rotation stage and the lock-in amplifier with the resolutions of 0.001°and 0.01°, respectively, were used. To ensure that the film thicknesses

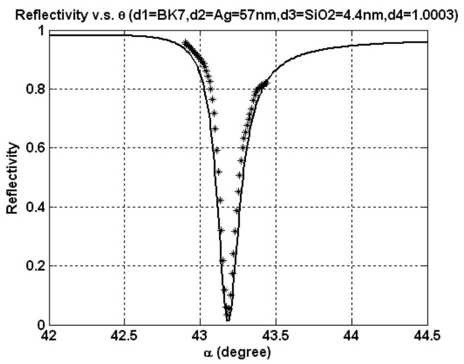
Table 1 The best sensitivities and resolutions for different coatings in the refractive index range from 1.332~1.35

item \ coating	Ag	Au	Ag/Au	Ag/SiO_2
Sensitivity (degree/RIU)	1.7×10^{6}	2.1×10^{4}	5.9×10^{5}	1.5×10^{6}
Resolution (RIU)	5.9×10^{-9}	4.6×10^{-7}	1.7×10^{-8}	6.9×10^{-9}
Resonant angle range (degree)	67.942-70.021	72.122-74.851	69.027-71.278	68.518-70.59

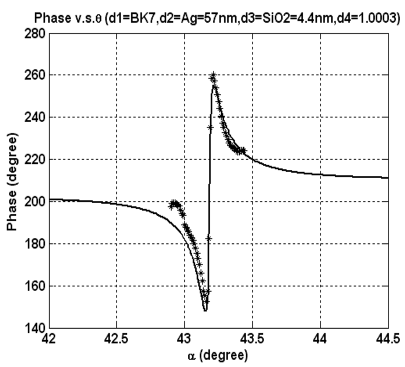


(a)
(b)

Fig. 4 (a) The sensitivity simulations versus n_4 for the different coatings, (b) The resolution simulations versus n_4 for the different coatings were correct the sensor was first tested in air. The experimental results are shown in Figures 5(a) and (b). To achieve the exact thickness values of d2 and d3, the theoretical tracing curves must be aligned with the experimental data (as the symbol of *). The results are that d2=57nm and d3=4.4nm, respectively. From Figure 5, the phase variation is severe around the resonant angle. Furthermore, for aqueous media, e.g. the alcohol solution, the refractive indices in the range of $n_4 = 1.332 \sim 1.350$ were measured by this sensor, and the phase difference δ varied severely at $n_4 = 1.341$ while the angle of incidence was at 69.4°. That is to say, it is very convenient to observe the variation of refractive index around $n_4 = 1.341$ at its resonant angle. From Figure 6, these experimental results coincided with the simulation results (solid line), and the resolution could be reached to 3.7×10^{-8} RIU. The resolution is near the best resolution 7.4×10^{-9} RIU at $n_4 = 1.341$ as d2=56nm and d3=5nm. If the refractive index is changed, the resonant angle is changed. We could select any resonant angle to correspond to the refractive index needed to measure.



(a)



(b)

Fig. 5 (a) The biosensor tested results (as the symbol of *) by intensity measurement in air, (b) The biosensor tested results (as the symbol of *) by phase measurement in air

For real-time measurement, the experimental curve of phase variations versus time due to the different concentrations of alcohol from 0% to 2.5% is shown as in Figure 7. Clearly, it is very sensitive for little change in concentration. From Figure 8, using the relative curve of the phase difference versus the refractive index, simulated at the angle of resonance 68.412°, gives the refractive indices of the concentrations.

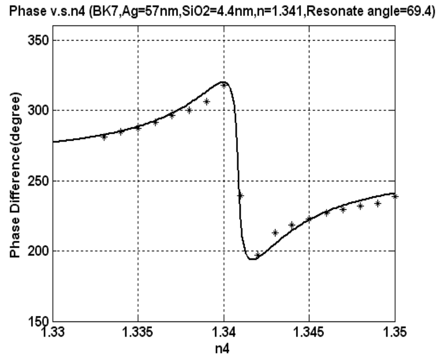


Fig. 6 Experimental results (as the symbol of *) of the phase difference versus the refractive index at the resonant angle 69.4° of n_4=1.341

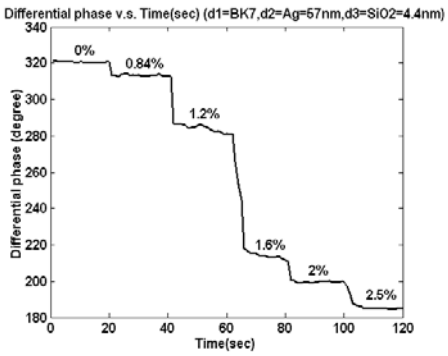


Fig. 7 Real-time experimental results of the phase difference versus time at the resonant angle 68.412° of water. Increasing the alcohol concentration from 0% to 2.5% causes the phase dropping evidently

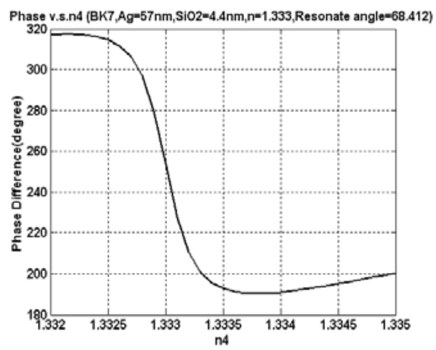


Fig. 8 The simulation curve for the phase difference versus the refractive index at the resonance angle of 68.412°

IV. CONCLUSIONS

In this study, Ag and SiO_2 film coatings were used instead of Au or Au/Ag films to improve SPR biosensor sensitivity by two orders. The feasibility of this configuration was demonstrated and verified. From the simulation results, the best resolution reached 6.9×10^{-9}RIU for the refractive index range of 1.332~1.350. From the experimental results, the refractive index resolution reached the value of 3.7×10^{-8} for $n_4 = 1.341$. Therefore, this high sensitive SPR biosensor could be used to detect or observe a small concentration variation in liquid or gas. It could also provide some useful applications in chemical or biological processes. It has some merits, such as, very high sensitivity, inexpensive, no toxicity and oxidization and real-time measurement.

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BIOGRAPHIES

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