Application of Surface Plasmon Resonance in Chemical/Biological Sensors

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In recent years, SPR (Surface Plasmon Resonance) was more and more used in the field of chemical and biological sensing, and the interest switched from the traditional method of measuring the amplitude of the p-polarized wave resonance to monitoring its phase. By using quite a few numerical simulation results, we can see resonance phase near the optimum SPR conditions is step-like, where the step is around the reflectivity minimum. Since the resonant phase transition is several orders steeper than that of reflectance transition near the SPR optimum, it is promising to apply the phase based SPR to bio/chemical sensors. Here we also demonstrate some experimental results showing the inversion of phase “step” when the whole system passes through the optimum SPR conditions: antigen-antibody binding signal and the fringe of a phase-relevant 2-dimensional interference pattern.

1. Surface Plasmon Resonance

First, let’s consider the equation of motion of the free electrons in metal by an external time dependent electric field

\[
\frac{d \vec{p}}{dt} = -\frac{\vec{p}}{\tau} - e \vec{E}(t), \text{ where } \tau \text{ is the relaxation time of free electron.}
\]

Now, we seek a steady-state solution of the form: \( p(t) = \text{Re}(p(\omega)e^{-i\omega t}) \)

Put it into the previous equation, we will get

\[
-i\omega p(\omega) = \frac{p(\omega)}{\tau} - e \vec{E}(\omega)
\]

Since the current density \( j(t) \) is just \( j(t) = -ne \vec{p}/m \).

By using \( j(t) = \text{Re}(j(\omega)e^{-i\omega t}) \), we have

\[
j(\omega) = -\frac{ne}{m} \frac{p(\omega)}{\tau} = \frac{(ne^2/m)E(\omega)}{1/\tau - i\omega} = \sigma(\omega) \cdot \vec{E}(\omega)
\]

Where \( \sigma(\omega) = \frac{ne^2}{m} \frac{1}{1 - i\omega\tau} = \frac{\sigma_0}{1 - i\omega\tau} \) is the AC conductivity of the metal, we can see it is frequency-dependent, and is determined by the applied electrical field.
In the presence of a current density \( \vec{j}(t) \), and we assume the net charge density is zero and the medium is uniform, so we will have the following Maxwell’s Equations in CGS unit:

\[
\begin{align*}
\nabla \cdot \vec{E}(t) &= 0 \\
\nabla \times \vec{E}(t) &= -\frac{1}{c} \frac{\partial \vec{H}(t)}{\partial t} \\
\nabla \cdot \vec{H}(t) &= 0 \\
\nabla \times \vec{H}(t) &= \frac{4\pi}{c} \vec{j}(t) + \frac{1}{c} \frac{\partial \vec{E}(t)}{\partial t}
\end{align*}
\]

Now we are trying to find a solution with time dependence \( e^{-i\omega t} \).

So \( \nabla \times (\nabla \times \vec{E}(t)) = -\nabla^2 \vec{E} = \nabla \times \left( -\frac{1}{c} \frac{\partial \vec{H}(t)}{\partial t} \right) = \frac{i\omega}{c} \nabla \times \vec{H} = \frac{i\omega}{c} \frac{4\pi}{c} \vec{j}(t) \)

Then \( -\nabla^2 \vec{E} = \frac{\omega^2}{c^2} \left( 1 + \frac{i4\pi\sigma}{\omega} \right) \vec{E} = \frac{\omega^2}{c^2} \epsilon(\omega) \vec{E} \)

Here, we can see a complex dielectric coefficient given by

\[
\epsilon(\omega) = 1 + \frac{i4\pi\sigma}{\omega} = 1 + \frac{i4\pi}{\omega} \frac{\sigma_0}{1 - i\omega\tau} = 1 + \frac{i4\pi\sigma_0 (1 + i\omega\tau)}{\omega(1 + (\omega\tau)^2)}
\]

Separate the real part and imaginary part, we have:

\[
\epsilon(\omega) = 1 - \frac{4\pi\sigma_0 \tau}{1 + \omega^2 \tau^2} + i \frac{4\pi\sigma_0}{\omega(1 + \omega^2 \tau^2)}
\]

Usually, the frequency is high enough to satisfy the condition \( \omega\tau >> 1 (\sigma_0 = ne^2\tau / m) \), finally we can get:

\[
\epsilon(\omega) = 1 - \frac{\omega_p^2}{\omega^2} + i \frac{1}{\omega\tau} \frac{\omega_p^2}{\omega^2} \quad \text{Where } \omega_p^2 = \frac{4\pi ne^2}{m}
\]

We also see that the imaginary part has a factor \( \frac{1}{\omega\tau} \ll 1 \), so the real part will be the dominant of the dielectric coefficient \( \epsilon(\omega) \). We call \( \omega_p \) as the plasma frequency. When \( \omega < \omega_p \), \( \epsilon \) is negative, \( k(\omega) = \frac{\omega}{c} \sqrt{\epsilon(\omega)} \) is imaginary, so the solution of electrical field will decay exponentially in the metal, and it is an evanescent wave, no radiation can propagate. When \( \omega > \omega_p \), \( \epsilon \) is positive, the electrical field become oscillatory, radiation can propagate through and the metal at this case is called transparent.

For the first case \( \omega < \omega_p \), when the light is incident on the surface of the metal, almost all the component will be reflected back. However, at some specific configuration, the
electromagnetic wave can induce the Surface Plasmon Polaritons, which will adsorb the radiation energy and reduce the reflection component. When the reflectance is minimized, we call it surface plasma resonance (SPR). As we know, only p-polarized wave (TM) can achieve the SPR, it has a propagation vector \( k_{sp} \), which lies in the plane of metal surface.

Now let’s consider the general case to produce the surface plasma polaritons. People usually use a prism to increase the light wave vector, so that the wave vector matching is possible.

In the following diagram, we show the Kretschmann configuration.

![Fig. 1](image)

In figure 1, medium 0 is a prism, medium 1 is a metal with thickness \( d_1 \), and medium 2 is a semi-infinity transparent medium, which could be gas or liquid. The field amplitude associated with surface plasma polaritons is maximum at the metal-dielectric interface and decay exponentially away from the interface. The dispersion relation of the surface plasmon at the metal-dielectric interface is given by

\[
k_{sp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}}
\]

And the parallel component of the photon’s wave vector in medium 0 \( k_x \) is:

\[
k_x = \frac{\omega}{c} \sqrt{\varepsilon_0 \sin \theta}
\]

When \( k_{sp} = k_x \), the momentum matching condition is satisfied and surface plasmons are created at the metal-air interface (assuming the semi-infinity medium 2 is air). The formation of SPR is most easily observed as a minimum of the reflectance versus the incident angle of the illuminated light. In visible light range, we always get negative dielectric coefficient for \( \varepsilon_1 \). For example, if the thin medium is gold, we have \( \varepsilon = \varepsilon_{gold} + i\varepsilon'_{gold} = -13.9 + 1.02 \cdot i \) at 670 nm.
2. Dependence of the reflectance on the angle of incident light

In the first part, we introduce a three-layer model to discuss about the surface plasmon generation. But when we want to apply this phenomenon to work as a chemical or biological sensor, we have to add a thin absorptive layer (layer 3) as biological or chemical receptors between the metal and semi-infinity medium (Fig.2), it can work as a sensor for the chemical or biological molecules in medium 2.

![Fig. 2](image)

(Compared with Fig.1, there is an additional absorption layer 3 between layer 1 and 2.)

Now, we are going to show some simulation results or experimental data to demonstrate how these different parameters in Kretschmann model will affect the reflectance curve.

First, let’s discuss about the three layer case. Figure.3 shows how the thickness of the metal layer affects the resonance angle; Figure.4 shows the different index of semi-infinity medium will also make the resonance angle shift; Figure.5 indicates that the reflectance is very sensitive to the incident wavelength.

![Fig. 3](image)
Where the simulation is for $\lambda = 633$ nm, and a silver film: $\varepsilon_1 = -17 + 0.7i, \varepsilon_2 = 1$. curve 1 to 5 are corresponding to thickness of silver layer $d_1$ equal to 40, 45, 50, 55, 60 nm respectively.

Where the incident wavelengths are same for all the three curves, but the metal is gold film $\varepsilon_1 = -10 + 1.3i$ with a fixed thickness $d_1 = 46nm$. Now the dielectric coefficient of medium 2 is varied as: 1. $\varepsilon_2 = (1.31)^2$, 2. $\varepsilon_2 = (1.32)^2$, 3. $(1.33)^2$, 4. $(1.34)^2$, 5. $(1.35)^2$. From figure 4, we can clearly see that the optimum incident angle will become larger while the index of medium 2 increasing.
Where the simulation for silver layer with thickness $d_1 = 55 \text{nm}$, $\varepsilon_2 = 1$; curve 1, 2, 3 are corresponding to incident wavelength 653, 638, 623 nm, it tells us the incident wavelength is also a very important factor in SPR.

After getting some ideas about how the different parameters in 3-layer case affect the SPR reflectance, we will switch to 4-layer case by introducing the additional layer 3 (adsorption layer), we can see some difference in Fig. 6.

Where a HAS protein layer $\varepsilon_3 = 2.01 + 0.22i$ was put on a silver layer $\varepsilon_1 = -17 + 0.7 \cdot i$ with thickness $d_1 = 39 \text{nm}$, the semi-infinity medium is homogenous $\varepsilon_2 = (1.33)^2$; and the curve 1, 2, 3, 4, 5 are corresponding to thickness of adsorption layer of 6, 8, 10, 12, 14 nm.

From Figure 6, we can see that as the thickness of a protein receptor layer increases, the resonance contour of $R_p$ becomes wider, and the resonant angle will shift to larger values. So the protein layer has potential to be a biosensor. When this layer is binding with analyte molecules from ambient, the thickness is increasing, so the resonance contour will be modified.

Some paper discussed about people used this property to get the concentration of solution since there is an approximated linear relation between the shift of SPR incident angle $\Delta \theta$ and the concentration of test solution (Figure 7).
From Figure 7, we can see that the shift of incident angle increases linearly with the concentration of glucose or urea. Since the straight lines pass through the origin, it shows that even the low concentrations of these reagents can be measured with SPR. From the diagram, we can also see that the sensitivity observed for these films (the slopes of straight lines), are about $2.34 \times 10^{-3} \text{mM}^{-1}$ in glucose solution and $1.13 \times 10^{-3} \text{mM}^{-1}$ in a urea solution for Ag film; in a glucose solution, the sensitivity of the sensor with a thin gold film was slight higher than that with a thin silver film.

In the following plot, we will demonstrate several different situations:
For the first left curve, medium 2 $\varepsilon_2 \approx 1$ is air; for 2nd left curve the medium 2 is changed to water $\varepsilon_2 = (1.33)^2$; in 3rd left curve, where a monolayer of human anti-body $\gamma$-globulin (IgG) was attached on the metal, the index is around $\varepsilon_3 = (1.47)^2$, and estimated thickness is about 50 Å; the last one is when the antigen a-IgG is introduced into the solution, the IgG will bind it to form one more binding layer, which will lead the resonant angle shift even larger. So we can see the shifting of resonant angle of surface plasmon is kind of indicator of chemical or bio-binding, which can be used as bio/chemical sensor.

3. The Application of Phase Properties of SPR in (Bio)chemical Sensor

Besides the intensity measurement we discussed in the previous section, the phase measurement is an additional channel to acquire more data for a test system and usually it offers sensitivity higher by several orders than the traditional intensity measurement. Now, we will do some theoretical analysis of surface plasmon resonance phase peculiarities in the following.

Let’s start from most general case: a plane monochromatic wave incident on one of the boundaries of an arbitrary layered system. So the reflection coefficient from this boundary is related to a complex term:

$$ r = |r| \exp(i \cdot \delta) $$

Here, $\delta$ is the phase shift of the electric field of the wave due to the reflection. It is a smooth function of $r$, which is well defined in the whole complex plane, except at a singularity point of $r = 0$.

$$ \delta = \arg(r) = \arctan(\text{Im}(r)/\text{Re}(r)) + m\pi, $$

where $m=0, +1, -1$.

Let $r = r(t)$ be such a smooth function of an arbitrary variable $t$, so the value $R = |r|^2 \equiv r \cdot r^*$ (reflectance) possesses a minimum. Here, for the Kretschmann SPR scheme, $t$ is usually the incident angle; and $r$ also varies with another parameter $q$, where $q$ can be several different quantities, such as metal film thickness, the ambient refraction index or the illuminated wavelength, which we have discussed in the previous section.

At the point of minimum:

$$(r \cdot r^*)' = 0 \quad \text{and} \quad (r \cdot r^*)'' > 0$$

Take the first derivative of the phase term, we can get $\delta' = \text{Im}(\frac{r'}{r})$. From $(r \cdot r^*)' = 0$, we can get $r'/r = -r^*/r^*$, which shows that the ratio $r'/r$ at the point of minimum is always imaginary $\frac{r'}{r} = (\pm i) \frac{|r'|}{|r|}$. So if we consider the 2nd derivative $(r \cdot r^*)'' \equiv r'' r^* + r \cdot r'^* + 2|r|^2$ at
the point of the minimum remains greater than a prescribed positive value, then we can see that $|r^p|$ is also greater than some fixed positive value. From the equation $\delta' = \text{Im}(r^p)$, we can conclude that when the minimum of $R$ approaches zero, the term $\delta'$ turns out to be infinity. Besides, if $r = r(t, q)$ is a smooth function of both $t$ and $q$, $\delta'$ changes its sign when $r$ goes through the point $r=0$ in the complex plane. This is the case when the parameter $q$ passes through its optimum value. When we consider the in the region of " $\delta'$ vs. $r$", and $q$ approaches its optimum value, $\delta'$ will switch the sign when it passes through this value.

Below, we will give the simulation results due to the phase peculiarity for several different situations, which are corresponding to the different cases we have discussed previously.

Figure 9 has a same situation as Figure 3, which demonstrates how the thickness of metal layer affects the difference in the phase shifts of p- and s- polarizations: $\Delta$ (Since the phase of s-polarization just changes slightly, so actually, $\Delta$ indicates the change of p-component near the SPR optimum region.) Here, we can see in the vicinity of the point of $R_p$ minimum, $\Delta$ undergoes the most abrupt changes. When the thickness of metal grows up to its optimum (curve 1-3), the steepness of the $\Delta$ slope increases dramatically; when it grows further, the steepness of slope will switch the sign and decrease. We can say that near the optimum the dependence of $\Delta$ is step-like, the position of the 'step' is at the point of the $R_p$ minimum. So compared with Figure 3, the phase change is a much strong indicator near the optimum position.

Figure 10 give us a similar picture, but here the q-parameter becomes the index of ambient medium; and where the physical conditions are identical to those in figure 4.
As we know, Fig. 9 and Fig. 10 show the 3-layer model; Figure 11 demonstrates the four-layer system, where the situation is same as that of Figure 6. So we can see the thickness of the absorption protein layer $d_3$ influences on SPR characteristics in a similar way, it contributes to the effective refractive index of the layered medium neighbor to the metal.

So the analysis up to now shows that we can substantially increase sensitivity and resolution of SPR-based sensors by recording the phase of a reflected wave rather than its intensity. However, we have to figure out a feasible method to measure the phase shift.

4. Imaging of a Surface Plasmon Resonance Phase and Its Application

Here, we introduce an experiment set-up is in the following diagram ---- Figure 12. Two orthogonal polarization components of the same divergent beam are allowed to interfere. The p-polarized radiation couples with surface plasmons and carries the information of interest. At the same time, the s-polarized is unaffected by SPR and serves as a reference.
In this Figure, 1 is a metal film, 2 is a Kretschmann prism, 3 is a birefringent crystal used to separate two orthogonal polarizations, 4 is a polarizer, and 5 is a screen displaying the interference pattern. Here, the laser is expanded beam and focused on the metal film.

Now the whole system is applied to biosensing. The antibodies for a specific binding were immobilized on the metal film deposit on the Kretschmann prism and placed it in the flow of antigen aqueous solution of different concentrations. Here the antibodies were taken from rabbit anti-2,4-d serum. The antigen is the pesticide solution. The rabbit anti-2, 4-d serum is for specific binding, if people want to check a non-specific binding, they can use anti-bodies extracted from normal rabbit serum.

Before apply it to bio-sensing, we can first check how the thickness of metal film affects the interference pattern. We can see the Figure 13 (a) and (b), where a gold-air interface is built in. The interference fringes demonstrate a well-marked bend, which corresponds to the ‘step’ in SPR phase dependence.

![Fig. 13]

We can see a dark zone which goes along the vertical direction across the fringes and intersects them at the bend, it is corresponding to the resonant minimal. However, the fringes are different for (a) and (b), since the thickness of gold film are different: (a) is less than the optimum value; (b) is larger than the optimum value. One may say that the inversion of phase was observed when the thickness of metal is changing from smaller case across the optimum value, to the larger value.

Now, the system (metal layer attached with anti-body) is applied to a binding reaction solution, where the conditions such as thickness of metal were preset to near the surface plasmon coupling optimum. Then, we can use either use different concentrations of antigen aqueous solution, or use a fixed concentration and measure the “binding reaction vs. time”. Here, we show the time evolution of the pattern accompanied by the inversion of the ‘step’, resulted from the binding of 2,4-d pesticide in a $1.1 \times 10^{-8} M$ aqueous solution to the rabbit anti-2,4-d serum.
The pattern of Figure 14(a) was recorded 30 s after introducing of the pesticide solution, when the binding is negligible. The sign of the ‘step’ slope here is negative, comparable with Fig. 13(a).

Here, in Fig. 14(b), the pattern was recorded at binding of coupling conditions were very close to the optimum. However, for each fringe, we can see the slopes at the ‘step’ are split in two, positive and negative. This is due to the fact the surface inhomogeneity, so the interference was contributed by the area with the antibody binding layer thickness both greater and less than the optimum.
Fig. 14(c) demonstrated the binding reaction resulted in the complete inversion of the ‘step’, the ‘step’ slope here is positive like in Figure 13(b).

The binding reaction can be applied in an aqueous solution with higher concentration, it will lead to the decrease in the time needed for the inversion.

The results of this kind of experiments shows that the SPR interferometry technique opens up the possibilities for application (bio)chemical sensors with ultra-high phase transition sensitivity and wide dynamic range, but the signals are not so obvious in those pictures, so people have to work hard to improve the interferometry technology in this kind of application.

There is another technique of phase-polarization contrast (PPC) which can be used to enhance the contrast of a surface plasmon resonance. This technique exploits the phase peculiarities of light and enables one to lower the resonant minimum of the reflected intensity near to zero, therefore, enhances the contrast. Since the restriction of the length of this paper, we are not going to discuss it further here.

5. Conclusion

In this paper, we discussed the fundamental of SPR, showed the theoretical simulation results of both the intensity and phase characteristics near the optimum conditions, meanwhile, we presented some experimental data. We see that there are abrupt changes in the SPR optimum region, which can be the p-polarized wave’s reflectance, the inversion of phase step or the interferometric signal with reference signal. These changes could work as bio-chemical sensor indicating the ambient condition, such as the concentration of certain molecules or dynamics. So I think the application of SPR phenomena in bio-chemical sensors is very promising, more relevant researches will be developed in near future. And we also expect to see more commercial products direct or indirect related to SPR application will show up very soon.
References


