Molecular Scale Origin of Surface Plasmon Resonance Biosensors

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Supporting Information

ABSTRACT: Surface plasmon resonance (SPR) has become an indispensable tool for label-free detection and quantification of molecular binding. Traditionally, the principle of SPR biosensors is described with a stratified medium model, in which discrete molecules are approximated with a uniform thin film. With the recent technical advances, SPR can now detect extremely low coverage of molecules, which raises the question of the validity of the traditional model. Here, we present combined theoretical, numerical and experimental analysis of SPR detection principle by considering the discrete nature of the molecules (particles). Our results show that the stratified medium model can provide reasonable description of SPR biosensors for relatively high coverage and weakly scattering samples. However, interference between the SPR images of individual particles needs to be considered for high spatial resolution images and for strong scattering samples at certain incident angles of light.

Surface plasmon resonance (SPR) has emerged as an icon of label-free detection technologies for quantification of molecular interactions because of its high sensitivity, throughput, simplicity and imaging capability. It measures the coverage of molecules bound on a sensor surface, and binding kinetics from the time profile of the coverage. Traditionally, the principle of SPR is described with a stratified medium model, in which molecules bound on the sensor surface are treated as a thin uniform film with an effective thickness and refractive index. This view may be reasonable when the coverage of the molecules is high such that the average distance between the molecules is much smaller than the wavelength (approximately a few hundred nanometers) and propagation length (a few microns for gold film) of the surface plasmonic waves.

With recent advances in SPR detection technology, commercially available SPR instruments can reach a detection limit of 0.1 RU or better (www.biosensingusa.com), corresponding to 0.001% coverage of a medium sized protein (~5 nm in diameter), or an average distance of 0.5–1 μm between two proteins. The stratified model of SPR is thus questionable, and one may need to consider the discrete nature of molecules in the interpretation of SPR data. This is especially the case when applying SPR to study long DNA molecules, nanoparticles, viruses, and cells. In addition, recent high-resolution SPR microscopy can image individual nanoscale objects, as distinct parabolic fringe patterns, which cannot be understood with the traditional stratified medium theory. These advances call for the development of a SPR theory that takes in account the discrete nature of molecules or other nano- and micron-scaled objects. Here, we present such a theory and compare it with the traditional stratified medium model of SPR. We also present simulations and experimental data in order to examine how the discrete nature of molecules, nanoparticles, viruses, and cells affects the accuracy of the traditional stratified medium model.

EXPERIMENTAL SECTION

Experimental Setup. The experiment was carried on an inverted microscope (Olympus IX-81) with a 60× high numerical aperture (NA 1.49) oil immersion objective. Wavelength 680 nm of light sources were used to excite surface plasmons and the plasmonic images were recorded by a CCD camera (AVT Pike F-032B) at a frame rate of 106 frames per second. All the images were processed by Matlab program.

Plasmonic Image Intensity of Nanoparticle. One hundred nanometer polystyrene nanoparticles were added into 150 mM phosphate buffer and the nanoparticles will bind onto the Au surface. The entire binding process was recorded and the background has been removed by subtracting the first frame. Nanoparticles’ intensity was averaged over a fixed area (20 by 40 pixel rectangular, and 123.3 nm per pixel).

Simulation of Particle Images. The simulation is performed in an area of 513 × 513 pixels, with a pixel size of 100 nm. The refractive indexes of polystyrene nanoparticles, water, gold and prism are set 1.0, 1.33, (0.163 + 3.4633i), and 1.515, respectively. The thickness of gold film is 50 nm. The propagation length of surface plasmons is 2.58 μm (calculated by 1/2κ).

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RESULTS AND DISCUSSION

We start with a brief summary of the main results of the stratified medium theory of SPR and then present the theory that includes the discrete nature of molecules, which will be referred to as particle-scattering theory. After presenting simulated SPR based on the theory and experimental SPR that validates the theory, we compare the predictions by the stratified medium theory and the particle scattering theory.

**Stratified Medium Model.** SPR can be excited optically with different configurations, but the most popular one is the Kretschmann configuration\(^2^5\) (Figure 1A). In the Kretschmann configuration, light is incident onto a metal thin film via a prism, and the reflected beam is detected with a photodiode or imaged with a CCD camera. When the incident angle is tuned to the so-called resonant angle, light energy is transferred into the surface plasmons, and the reflective light intensity drops to a minimum. Figure 1B shows a SPR reflectivity vs incident angle profile based on the stratified medium model, where the resonant angle is denoted as \(\theta_0\). Upon adsorption of molecules onto the metal film, the stratified medium model further predicts a shift in the resonant angle (or SPR reflectivity vs angle profile) to a higher angle.

The major task of SPR biosensors based on the traditional stratified medium model is to detect the shift in the resonant angle. A simple and widely used method to detect the resonant angle shift is to measure the reflectivity change associated with the shift in the SPR reflectivity profile at a fixed incident angle near the resonant angle. The change in the reflectivity, \(\Delta R\), is related to the shift in the resonant angle, \(\Delta \theta\), by\(^1^2^,1^3\)

\[
\Delta R(\theta) = -\frac{dR(\theta)}{d\theta} \Delta \theta
\]

where \((dR(\theta)/d\theta)\) is the slope of the reflectivity vs angle profile, thus the sensitivity of SPR (Figure 1C). The basic predictions of the stratified medium SPR model are as follows: (1) When the angle is smaller than the resonant angle, the slope is negative, such that the measured reflectivity increases with the coverage of molecules on the SPR sensor surface. In contrast, when the angle is greater than the resonant angle, the slope is positive, such that the measured reflectivity decreases. At the resonant angle, the slope is zero, and the reflectivity changes little. (2) There exist maximum negative and positive slopes at certain incident angles below and above the resonant angle, respectively. (3) The resonant angle or reflectivity changes smoothly and linearly with the coverage of adsorbed particles. Figure 1D plots the reflectivity change vs coverage for a 5 nm-diameter protein, showing a linear dependence. Note that in the stratified medium model, the effective thickness of the protein layer is taken to be \(d_{ef} = (N/N_{max})d\), where \(N\) is the coverage, \(N_{max}\) is the full monolayer coverage, and \(d\) is the diameter of the molecule. For a given size of the molecule, the reflectivity change is proportional to the number of molecules per unit area. These predictions are the working principle of SPR sensors, which have been used to quantify molecular binding.

**Particle-Scattering Model.** How will these basic predictions change when the discrete nature of molecules is considered? In order to address this question, we consider a theory that includes individual particles to model discrete molecules, nanostructures, viruses or cells. As shown in Figure 2A, the main point of the theory is that the SPR image contrast of a particle is originated from the scattering of the plasmonic wave propagating along the metal film by the particle. More exactly, \(p\)-polarized incident light is partially reflected \((E_r)\) at the interface of prism/gold and partially transmitted into the gold film as an evanescent wave, which excites surface plasmons \((E_p)\). The total reflected light intensity recorded by the camera \((I)\) is given by the superposition of the partially reflected field and scattered plasmonic field in the direction of reflection\(^1^1^,1^5^,1^6^,2^6^,2^7\)

\[
I = |E_r + \beta E_p|^2
\]

where \(\beta\) is a constant that describes the fraction of the scattered plasmonic wave in the direction of the reflected light. Note that \(E_r\) can be precisely calculated at different incident angles with
Fresnel’s equations based on the stratified medium model producing a SPR image with uniform intensity. However, the second term in eq 2 describes the particle on the surface, which results in a distinct parabolic pattern in SPR image, shown in Figure 2B, based on the following considerations.

Surface plasmon scattering by a particle can be described by the elastic scattering theory. When the particle is much smaller than the surface plasmon wavelength, we may express the scattered field by a decaying cylindrical plasmonic wave,

\[ E_s(r, r') = aE_{sp}(r')e^{-\kappa |r - r'|}e^{-ikr'} \]  

where \( r' \) is the location of particle, \( a \) is a scattering coefficient related to the polarizability of each nanoparticle, \( E_{sp}(r') \) is the surface plasmon field at the location of the particle, \( \kappa \) is the decaying constant of surface plasmons, and \( k \) is the wave-number of surface plasmons. The total surface plasmon field \( E_{sp}(r, r') \) based on the Born Approximation is given by

\[ E_{sp}(r, r') = E_{sp}^0(r') + aE_{sp}^0(r')e^{-\kappa |r - r'|}e^{-ikr'} \]  

where \( E_{sp}^0(r) \) is the surface plasmon field in the absence of the particle. The SPR image contrast of the particle is described by

\[ I(r, r') = |E_s(r) + E_{sp}^0(r') + aE_{sp}^0(r')e^{-\kappa |r - r'|}e^{-ikr'}|^2 - |E_s(r) + E_{sp}^0(r')|^2 \]  

(5)

Note that the background image in the absence of the particle is subtracted out in eq 5. In the case of multiple particles, eq 5 can be generalized as

\[ I(r) = |E_s(r) + E_{sp}^0(r) + \sum_{i=1}^{N} a_iE_{sp}^0(r_i)e^{-\kappa |r - r'|}e^{-ikr'}|^2 - |E_s(r) + E_{sp}^0(r')|^2 \]  

(6)

where \( i \) stands for \( i \)th particle and \( N \) is the total number of particles.

**Single Particle.** Using eqs 3 and 5, and Fresnel’s equations, we have simulated the SPR image of a single particle as a function of the incident angle. The image contrast is highly sensitive to the incident angle, as shown in Figure 2B. When the angle is smaller than the resonant angle, the image contrast is positive. When the angle is greater than the resonant angle, the image contrast is negative. At the resonant angle, the image contrast is smallest. The inversion of the SPR image contrast of the particle around the resonant angle is due to the phase inversion of the reflected field at the resonant angle.

To confirm the theoretical calculations based on the particle-scattering model, we have imaged 100 nm polystyrene nanoparticles with the high resolution SPR microscope. Figure 2C shows several experimental SPR images of the particle at angles lower than, near and higher than the resonant angle, which are in excellent agreement with the calculated SPR images of the particle at different incident angles.

The average reflectivity change vs incident angle was also obtained from the experimental SPR images, and plotted in Figure 2D. The experimental data are consistent with the calculated reflectivity change. Note that the error bars of the experimental data are the standard deviations from 50 individual particles. By comparing the calculated and experimental image contrasts, the polarizability, \( \alpha \), in the particle-scattering model for 100 nm polystyrene nanoparticle is about 0.027 (Supporting Information). The excellent agreement between the calculated and experimental images and reflectivity changes validates the particle-scattering model.

The basic findings of the particle-scattering model are qualitatively similar to those of the stratified medium model, except that the image contrast of the particle at the resonant angle is finite, instead of zero, due to the formation of the parabolic pattern. The average reflectivity change associated with binding of the particle vs incident angle (solid blue line) is compared with the reflectivity change associated with the binding of a molecular layer (dashed line) calculated with the stratified medium model in Figure 2D. The results from the particle-scattering model and stratified medium model are not identical both they do share similar characteristics, including the crossover from reflectivity increase to reflectivity decrease near the resonant angle, and maximum reflectivity increase and decrease at certain angles (marked by arrows) on both sides of the resonant angle.

**Two-Particle Interference.** Figure 2A and 2B show the distinct parabolic fringe pattern of the SPR image of a single particle originated from the scattering of the surface plasmon waves by the particle. When two particles bind to the sensor surface within a distance smaller than the surface plasmon propagation length, the overlap between the parabolic fringe...
patterns of the two particles leads to constructive and destructive interferences. Consequently, the total average reflectivity of the two particles fluctuates depending on the relative positions of the two particles. In other words, the reflectivity change based on the particle-scattering model is not necessarily proportional to the number of particles, which is different from the prediction of the stratified medium model.

To illustrate this point, we have calculated and measured SPR images of two particles at different locations (Figure 3A). The corresponding total average reflectivity as a function of the relative positions of the two particles is shown in Figure 3B, which reveals bright and dark fringes due to the interference discussed above. Note that the calculation was performed by fixing one particle at the center and moving the other to different locations. Figure 3B shows that fringes fade away when the two particles are separated with a distance greater than the propagation length of surface plasmons, indicating that we may neglect the interference effect when the coverage is dilute such that the average separation between particles are much larger than the propagation length of surface plasmons.

The reflectivity fluctuations can be quantified with the standard deviation (Supporting Information):

$$\sigma = \frac{|I - \langle I \rangle|}{\langle I \rangle + \langle T \rangle} = 2\alpha^2 \frac{|E_{i1}| |E_{i2}| \cos(\phi_i - \phi_j)}{\langle T \rangle + \langle T \rangle}$$

where $\langle I \rangle$ is the average reflectivity of two particles, $\langle T \rangle_1$ and $\langle T \rangle_2$ are the average reflectivity of each of the particles, $E_{i1}$ and $E_{i2}$ are the scattered fields of the two particles defined in eq 3 for $\alpha = 1$, and $\phi_i - \phi_j$ is the relative phase between the two particles, which depends on the relative locations of the two particles. Figures 3C and D show the oscillation of $\sigma$ with different distances between two particles along horizontal and vertical directions for different $\alpha$ at incident angle of $70^\circ$. The reflectivity change fluctuates with the relative positions of the two particles. Note also that the horizontal and vertical profiles are not identical because of the propagation of the surface plasmons in vertical direction.

The reflectivity fluctuations as described by $\sigma$ is dependent on both the incident angle $\theta$ and $\alpha$ (Supporting Information). Figure 4A shows $\sigma$ of two particles with a separation of 100 nm along the horizontal direction at different incident angles. When the incident angle is smaller than the resonant angle, $\sigma$ shows a sigmoidal dependence on $\alpha$, which saturates at both small and large $\alpha$. However, when the incident angle is greater than the resonant angle, the reflectivity fluctuation dependence on $\alpha$ is strikingly different. As shown in Figure 4B, for each incident angle, a singular point is observed in $\sigma$ at a specific $\alpha$.

The singular point occurs when the average reflectivity change by a single particle is zero, such that the average reflectivity change of two particles purely arises from the interference of SPR images of the two particles. The incident angle, $\theta_0$, at which the singularity occurs depends on $\alpha$ as shown in Figure 4C. For small $\alpha$, $\theta_0$ is close to the resonant angle, but it increases with $\alpha$ and reaches $0.9^\circ$ above the resonant angle at $\alpha = 0.1$. We thus conclude that two-particle interference can lead to an effect that is significantly different from the traditional stratified medium model, especially for particles with large $\alpha$ at angles where the singularity occurs.

**Multiple-Particle Interference.** When multiple particles bind to the sensor surface, interference between multiple particles needs to consider. For weak scattering particles ($\alpha$ is small), we can describe multiple ($N$) particle interference as a sum of $N(N - 1)/2$ pairs of two particle interference, and interference of three or more particles simultaneously is given by high order of $\alpha$ terms, which can be neglected. In this case, the standard deviation by N nanoparticles (Supporting Information) is

$$\sigma = \frac{|I - \sum_{i=1}^{N} \langle T \rangle_i|}{\sum_{i=1}^{N} \langle T \rangle_i} \approx \frac{2\alpha^2}{\sum_{i=1}^{N} \langle T \rangle_i} \sum_{i=1}^{N} \sum_{j=1}^{N} |E_{i1}| |E_{i2}| \cos(\phi_i - \phi_j)$$

where $E_{i1}$ and $E_{i2}$ are the scattered fields of two particles defined in eq 3, and $\phi_i - \phi_j$ is the relative phase difference between the two particles, which depends on the relative locations of the two particles. For randomly distributed particles, the phase

Figure 3. Two-particle interference in SPR imaging. (A) Comparison between simulated (i) and experimental (a) SPR images of two particles with different distances; (B) Distribution of average intensity of SPR image of two particles with different relative locations; (C) Horizontal cross sectional profile of fluctuation of SPR intensity in relation to the scattering constant; (D) Vertical cross sectional profile of fluctuation of SPR intensity in relation to the scattering constant.
Difference for each pair of the particles is random. Therefore, $\sigma$ should decrease with increasing $N_r$ which indicates the interference corrections to the prediction of the traditional stratified medium model diminish when the coverage is high.

The particle scattering and interference effect of surface plasmons play a great role in practical SPR imaging of biological samples, especially with high spatial resolution where individual objects (e.g., molecules, viruses and cells) are resolved. For example, we have successfully imaged single DNA molecules with high spatial resolution SPR. The image is highly dependent on the orientation of the DNA molecules relative to the prorogation direction of SPR, which cannot be described with the stratified medium model.\(^{18}\)

**CONCLUSIONS**

We have developed a theory of SPR biosensors by considering the discrete nature of particles (e.g., molecules, nanoparticles, viruses, and cells), and carried out experiments to validate the theory. The essence of the theory is that the SPR biosensors detect the scattering of the surface plasmonic waves by the individual particles. The interference of the scattered field in the direction of the reflection with the reflected field is detected or imaged by SPR biosensors. Using the theory, we have calculated SPR images of single and multiple particles as a function of the incident light angle. When the incident angle is below the resonant angle, the image contrast is positive. When the incident is above the resonant angle, the image contrast is negative. At the resonant angle, the image contrast is minimum. These results are in excellent agreement with the experimental SPR images of polystyrene particles and also match well with the prediction of the stratified medium model. Our analysis further show that for weak scattering samples, such as molecules, randomly adsorbed on the sensor surface, the measured reflectivity increases approximately proportionally with the coverage of the particles, which also validates the traditional stratified medium model. However, for relatively large particles, such as viruses and cells, interference between images of the individual particles leads to deviation of the linear relationship between the reflectivity change and coverage, especially at certain incident angles, because of the interference. The scattering model provides theoretical guidance to optimize the sensitivity and resolution of SPR sensing and imaging.

**REFERENCES**


**ASSOCIATED CONTENT**

Supporting Information

Estimation of scattering coefficient and analysis of intensity disturbance by interference. This material is available free of charge via the Internet at http://pubs.acs.org.

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