Model for measurement of water layer thickness under lipid bilayers by surface plasmon resonance

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Abstract: A multilayered-substrate model is proposed for surface plasmon resonance (SPR)-based measurement of the thickness of a water layer sandwiched between a lipid bilayer and an underlying support. To calculate sensitivity, a 473 nm-wavelength excitation source and a silver layer are used for the SPR sensor. It is theoretically shown that the multilayered substrate design achieves sufficient sensitivity for such measurements and that sensitivity is enhanced with a SiO$_2$ layer of appropriate thickness and a buffer solution of high refractive index.

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References and links

1. K. Morigaki, T. Baumgart, A. Offenhäusser, and W. Knoll, “Patterning solid-supported lipid bilayer membranes by lithographic polymerization of a diacetylene lipid,” Angew. Chem. Int. Ed. Engl. 40(1), 172–174 (2001).
2. M. Tanaka and E. Sackmann, “Polymer-supported membranes as models of the cell surface,” Nature 437(7059), 656–663 (2005).
3. K. Watanabe, M. Ryosuke, G. Terakado, T. Okazaki, K. Morigaki, and H. Kano, “High resolution imaging of patterned model biological membranes by localized surface plasmon microscopy,” Appl. Opt. 49(5), 887–891 (2010).
4. W. C. Lin, C. D. Blanchette, T. V. Ratto, and M. L. Longo, “Lipid asymmetry in DLPC/DSPC-supported lipid bilayers: a combined AFM and fluorescence microscopy study,” Biophys. J. 90(1), 228–237 (2006).
5. E. Sackmann, “Supported membranes: scientific and practical applications,” Science 271(5245), 43–48 (1996).
6. B. W. Koenig, H. H. Strey, and K. Gawrisch, “Membrane lateral compressibility determined by NMR and x-ray diffraction: effect of acyl chain polyunsaturation,” Biophys. J. 73(4), 1954–1966 (1997).
7. B. Rothenhäusler, C. Duschl, and W. Knoll, “Plasmon surface polariton fields for the characterization of thin films,” Thin Solid Films 159(1-2), 323–330 (1988).
8. W. Hickel and W. Knoll, “Surface plasmon microscopy of lipid layers,” Thin Solid Films 187(2), 349–356 (1990).
9. E. Kretschmann and H. Raether, “Radiative decay of nonradiative surface plasmons excited by light,” Z. Naturforsch. Teil A 23A, 2135–2136 (1968).
10. C. Nylander, B. Liedberg, and T. Lind, “Gas detection by means of surface plasmon resonance,” Sens. Actuators 3, 79–88 (1982).
11. B. Liedberg, C. Nylander, and I. Lunström, “Surface plasmon resonance for gas detection and biosensing,” Sens. Actuators 4, 299–304 (1983).
12. L. S. Jung, C. T. Campbell, T. M. Chinowsky, M. N. Mar, and S. S. Yee, “Quantitative interpretation of the response of surface plasmon resonance sensors to adsorbed films,” Langmuir 14(19), 5636–5648 (1998).
13. J. S. Shumaker-Parry and C. T. Campbell, “Quantitative methods for spatially resolved adsorption/desorption measurements in real time by surface plasmon resonance microscopy,” Anal. Chem. 76(4), 907–917 (2004).
14. H. Raether, Surface Plasmons on Smooth and Rough Surfaces and on Gratings (Springer, 1988).
15. K. Tawa and K. Morigaki, “Substrate-supported phospholipid membranes studied by surface plasmon resonance and surface plasmon fluorescence spectroscopy,” Biophys. J. 89(4), 2755–2758 (2005).
16. J. F. Nagle and S. Tristram-Nagle, “Structure of lipid bilayers,” Biochim. Biophys. Acta 1469(3), 159–195 (2000).
17. C. Huang and T. E. Thompson, “Properties of lipid bilayer membranes separating two aqueous phases: determination of membrane thickness,” J. Mol. Biol. 13(1), 183–193 (1965).
1. Introduction

Lipids are amphiphilic molecules: they consist of both hydrophobic and hydrophilic sections. For this reason, they have the unique ability to self-assemble into bilayers on the surface of polar solvents such that the hydrophilic end is exposed to the solvent and substrate and the hydrophobic end is hidden. The cell membrane is, most famously, a lipid bilayer that plays an important role as a selective barrier for concentrating nutrients and excreting waste, as well as, in supporting receptor proteins that process biological signals from the environment. The membrane is thus a key chemical system in maintaining homeostasis.

A common approach to the study of the cell membrane is to use the bilayer form as a model to explore membrane functionality and behavior on a substrate. Such substrate-supported planar lipid bilayers find use in a wide range of biotechnology applications and scientific investigations [1–3].

Elucidation of the properties and behavior of a lipid bilayer on a substrate, such as the thickness, distribution, and fluidity of the lipid molecules, constitutes a crucial part of basic biophysical research because the functionality of the bilayer determines protein behavior [4]. In fluidity measurements, in particular, the thickness of the water layer between the substrate and bilayer is an especially important parameter because it indicates the fluid retention capacity of native cellular membranes [5]. In previous studies, the thickness of the water layer localized at the hydrocarbon region was estimated using the combined techniques of neutron and X-ray scattering and NMR measurement [6]. Although this approach provided the estimated water layer thickness and structural information of lipids, it is unfavorable for the further evaluation of lipid fluidity on the substrate because the measurement time is too long.

A successful method to observe the water layer thickness on the substrate requires the following: (a) sufficient sensitivity for film thicknesses on the order of a few nanometers; (b) a noncontact measurement technique, given that the water layer is sandwiched between the bilayer and substrate; (c) the capability for use in an aqueous environment with real-time measurement. Surface plasmon resonance (SPR) sensors satisfy all of these requirements and have even been used to detect lipid layer properties such as distribution and thickness [7,8]. This makes SPR sensors good candidates for application to practical systems to measure the water layer thickness.

Here, we calculate the measurement sensitivity achievable with a multilayered-substrate model to identify the optimal model parameters for SPR-based measurement of the thickness of a water layer sandwiched between a lipid bilayer and an underlying support. We also discuss methods to control the appropriate SiO$_2$ thickness and the refractive index of the buffer solution to maximize measurement sensitivity.

2. SPR sensor

2.1. Configuration and measurement principle

SPR sensors are typically based on the Kretschmann configuration [9] and consist of a glass prism, metallic thin film, and dielectric medium, as shown in Fig. 1(a). In this configuration, surface plasmons are excited when the metal surface is illuminated at an excitation angle $\theta_{sp}$ with p-polarized light that has been passed through the prism. Here, $\theta_{sp}$ is the angle of minimum reflection and maximum absorption of incident light because the energy of incident light is used to excite surface plasmons, as shown in Fig. 1(b). In the present calculations, we assume excitation at 632.8 nm, a refractive index of 1.72314 for glass, a 44-nm-thick gold layer with a complex refractive index of 0.300 + 3.089i, and a refractive index of 1.3300 for the dielectric medium. From curve A in Fig. 1(b), the value of $\theta_{sp}$ is 58.8°.
\[ \theta_{sp} \text{ is strongly dependent on the refractive index at the metallic surface. Therefore, even small changes in the refractive index can be detected as shifts } (\delta \theta_{sp}) \text{ in the excitation angle. These shifts are large enough to detect the adsorption of gases and proteins and to characterize thin film samples } [10,11]. \text{ As shown by curve B in Fig. 1(b), an increase in the refractive index of the dielectric medium by just } 5.6 \times 10^{-3}, \text{ consistent with the refractive index of a lipid bilayer } [3], \text{ produces a clear shift } \delta \theta_{sp}. \text{ The relationship between } \theta_{sp} \text{ and the effective refractive index } n_{eff} \text{ } [12,13] \text{ can be approximately expressed by the following equation:}

\[
\sin \theta_{sp} \approx \text{Real} \left( \frac{1}{n_g} \left( \frac{n_g^2 n_{eff}^2}{n_m^2 + n_{eff}^2} \right)^{1/2} \right),
\]

where \( n_g \) and \( n_m \) denote the refractive index of glass and the complex refractive index of the metal, respectively [14]. The effective refractive index can be described as a weighted average of \( n_a \) plus \( n_b \) where \( n_a \) and \( n_b \) are the refractive indices within and above a thinly adsorbed film [12]. The weighting factor, which is the sensitivity at distance \( d \) from the metallic surface, decreases exponentially with increasing \( d \).

3. Methodology

The measurement model for lipid bilayers deposited on a substrate can be assumed to consist of six layers: glass substrate, Ag film, SiO₂, the target water layer, lipid bilayer, and buffer solution (Fig. 2). The model is based on the experimental configuration where the lipid bilayers are deposited on the substrate [15]. The SiO₂ layer is necessary to preserve the hydrophilic nature of the lipids. In this multilayered model, all layers are extremely thin relative to measurement depth, and the obtained \( n_{eff} \) value is an average across the four layers from the Ag surface. The thickness of the water layer surrounded by the lipid bilayer is on the order of a few nanometers [16]. Therefore, any thickness changes reflected in \( n_{eff} \) would be too small to detect. Thus, it is necessary to design a substrate to achieve the greatest possible sensitivity.

Here, the calculation procedure to determine the sensitivity for measuring the water layer thickness is shown. The procedure can be divided into four steps: (1) calculation of the reflected light intensity as a function of the incident angle for the model shown in Fig. 2 without the water layer; (2) estimation of \( n_{eff} \) from the excitation angle, using Eq. (1); (3) incorporation of a 0.2-nm-thick water layer into the model; and (4) repetition of steps (1)-(3) until the water layer thickness is 2.0 nm. The detected effective refractive index is normalized by the refractive index of water (1.33821).
Moreover, in order to investigate the influence of the SiO$_2$ layer thickness and the refractive index of the buffer solution, the procedure is extended to include changes in the SiO$_2$ thickness and refractive index as well.

4. Results and discussion

4.1. Sensitivity for water layer thickness by controlling SiO$_2$ thickness

Using the method described above, the variation in $n_{\text{eff}}/\text{nm}$ as a function of SiO$_2$ thickness is calculated. In the calculation, changes in the SiO$_2$ thickness were measured in 1 nm steps from 0 to 80 nm. The excitation wavelength is assumed to be 473 nm, and the refractive indices of Ag, SiO$_2$, and water are set to $0.13464 + 2.68475i$, 1.55062, and 1.33821, respectively. The thickness of the Ag film is assumed to be 42 nm. The lipid bilayer is assumed to be made of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and have a thickness of 4.78 nm [16] and a refractive index of 1.49 [17]. As shown in Fig. 3, the sensitivity is maximal at 14 nm, and $n_{\text{eff}}$ becomes asymptotic at 0 as the SiO$_2$ thickness increases. The result shows that with an appropriate SiO$_2$ thickness, the sensitivity is 13% higher than without a SiO$_2$ layer.

Fig. 3. Plot of $n_{\text{eff}}/\text{nm}$ as a function of SiO$_2$ thickness.

To better understand the sensitivity enhancement mechanism in relation to the refractive index differences between the target layer and other layers, the three-layer model shown in inset of Fig. 4 is assumed. The sensitivity, $|n_{\text{eff}}/\text{nm}|$, for detecting the second layer is calculated by controlling the refractive indices of the first and third layers. These layers are assumed to...
have the same refractive index of $n_1$, whereas the refractive index of the second layer is assumed to be 1.338. The thickness of first layer is assumed to be 10 or 20 nm. Figure 4 shows the calculated plots. The result shows that the sensitivity increases as the difference in the refractive indices, $|n_1 - 1.338|$, increases. In addition, an increase in the thickness of first layer leads to lower sensitivity for detecting second layer because of the increased distance from the metal surface.

From these results, the plots in Fig. 3 can be understood to show that increasing the thickness of the SiO$_2$ layer increases the difference between the effective refractive index of the target water layer and the sum of the effective refractive indices of the SiO$_2$ layer, lipid bilayer, and buffer solution, whereas a large increase in SiO$_2$ thickness increases the distance from the metal surface and leads to lower sensitivity.

Fig. 4. Plots of $n_{\text{eff}}/\text{nm}$ as a function of $n_1$.

4.2. Sensitivity for water layer thickness by controlling refractive index of buffer solution

We plotted $n_{\text{eff}}/\text{nm}$ as a function of SiO$_2$ thickness for buffer solutions of different refractive indices. The SiO$_2$ thickness is increased in 1-nm steps from 0 to 50 nm, and the refractive index of the buffer solution in steps of $1.0 \times 10^{-3}$ from 1.334210 to 1.343210. As shown in Fig. 5, the optimal SiO$_2$ thickness decreases with an increase in the refractive index of the

Fig. 5. Calculated plots of $n_{\text{eff}}/\text{nm}$ as a function of SiO$_2$ thickness for buffer solutions of various refractive indices increased in steps of $1.0 \times 10^{-3}$. 
buffer solution. In addition, the sensitivity to detect water layer thickness increases with the refractive index of the buffer solution. Consequently, it is better to have a buffer solution of high refractive index and the thinnest possible SiO$_2$ layer.

It should be noted that the lipid bilayers on the substrate are difficult to control, even under the same protocol and conditions. In order to achieve more reliable sensitivity, statistical analyses of lipid bilayers would be required to determine thicknesses and refractive indices with standard deviations. Considering this variability would allow us to better evaluate the sensitivity.

5. Conclusion

It is theoretically revealed that the sensitivity for the detection of water layer thickness is enhanced by increasing the refractive index of the buffer solution and selecting an appropriate thickness for the SiO$_2$ layer.

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