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Assembly of Supported Membranes Studied by Surface Plasmon Microscopy

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A home-made surface plasmon microscopy (SPM) was used in cooperation with surface plasmon resonance spectroscopy (SPR) to analyze the assembly process of the organized fatty acid LB films. The planar fatty acid multilayers were deposited on a support by piling up different layers of stearic acid and/or palmitic acid monolayers in different regions, which were possible to be imaged by SPM in the same vision field. A good contrast was obtained between different thickness regions and the numerical values of thickness for different regions were calculated according to the resonance angles.

INTRODUCTION

Surface plasmon microscopy (SPM) uses drives of surface plasmon resonance (SPR) as the contrast mechanism[1]. A typical experimental arrangement is the Kretschmann attenuated total reflection (ATR) configuration in which a p-polarized laser beam incident on a metal/dielectric interface is coupled through a prism. An advantage of this technique is its high vertical resolution (as small as 0.1~0.2 nm). Other advantages include no need for a vacuum or addition of probes, and no necessary mechanical contact. Moreover, by using the Kretschmann ATR configuration, the half space above the observed layer is left

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completely free, which make SPM very suitable for online nanometer or molecular level research of biological dynamic systems (especially the membrane system). In this work, a SPM set was developed on the basis of the extensive research done with SPR on biomolecular recognition. A supported multilayer was studied by imaging the assembly process step by step with the home-made SPM.

MATERIALS AND METHODS

SPM SETUP

The SPM setup is essentially the Kretschmann ATR configuration which is schematized in Fig.1. In order to get a better contract picture two polarizers were used to control the light density and make it match the camera sensitivity. A He-Ne laser served as the light source. A rotation table is used for accurate angular adjustment (angular increments: 1/60 degree). The substrate used was microscope cover slide attached to the prism (BK7 glass) by matching oil. A 50nm gold layer was sputtered on top of the substrate. All the LB films prepared were deposited on fresh gold films. The SPM images were recorded by a CCD camera connected with a video recorder and displayed on a monitor screen. Together with SPM image a home-made SPR spectroscopy was employed to measure resonance curves (data not shown). The measure was explained in detail by Liu et al.[2].

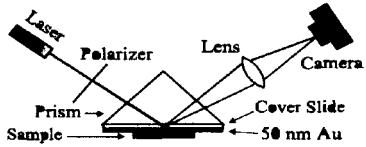


FIGURE 1 Schematic representation of the home-made SPM setup.

SAMPLE PREPARATION

The supported fatty acid multilayer was deposited by piling up different layers of stearic acid and/or palmitic acid monolayers by a LB tough so that regions of

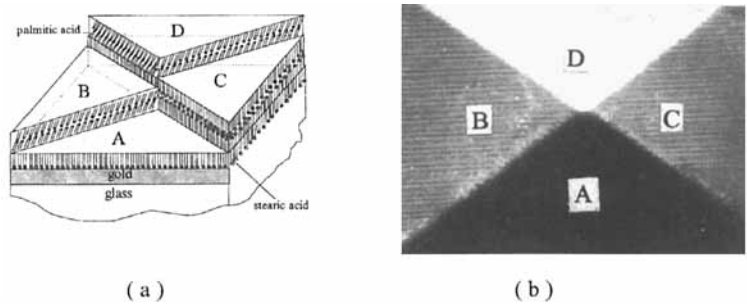
different thickness could be seen in the same vision field. Before deposition of fatty acid LB films, a 3mmol/l solution of stearic acid (from Baker Co.) or palmitic acid (from Sigma Co.) in 3:1 volume fraction chloroform/methanol was spread on a 1mmol/l $CdCl_2$ subphase. Then the monolayer at the air/water interface was compressed to the deposition pressure of 35mN/m.

RESULTS AND DISCUSSION

Multilayers of the fatty acid LB films with different compositions and thickness were deposited in different regions marked with A, B, C and D in the same field of vision, which are schematized in Fig.2(a). Their composition were A: one layer of stearic acid, B: one layer of stearic acid and two layers of palmitic acid, C: three layers of stearic acid, D: three layers of stearic acid and two layers of palmitic acid. The SPM images shown in Fig.2(b) were obtained by changing the incident angle. It can be seen from the pattern shown in Fig.2(a) that when the incident angle of the light source matched the resonance angle of the dielectric layer in a specific region, this region would be shown the darkest in the picture. When a region had a different resonance angle due to its different thickness of dielectric layer or different composition, its reflected light intensity would increase.

A former study showed that the shift in the resonance angle is related linearly to the thickness of the film[3]. The resonance angle of the free gold film was 44.00°. The resonance angles of regions A, B, C, and D were 44.40°, 44.93°, 45.22° and 45.75°, respectively. It could be calculated that in region A, the angle shift of a stearic acid monolayer was 0.44°; in region C, the angle shift of the three layers of stearic acid was 1.22°. By comparing the resonance angle of region A with that of region B the angle shift of two layers of stearic acid was obtained to be 0.82°. An average resonance angle shift of 0.41° was calculated for a stearic acid monolayer. Using the average resonance angle shift and the thickness of the

stearic acid monolayer (2.3nm), and assuming that both stearic acid and palmitic acid multilayers have the same optical index, the thickness of the different regions in the pattern would be A: 2.3nm, B: 5.2nm, C: 6.9nm and D: 10.0nm. All of these regions showed good contrast in SPM.



(a) (b)

FIGURE 2 The schematic representation of the formation of the multilayer (a), where A: one layer of stearic acid, B: one layer of stearic acid and two layers of palmitic acid, C: three layers of stearic acid, D: three layers of stearic acid and two layers of palmitic acid. A SPM picture of the organized supported mulitlayer (b), which was imaged at incident angles 44.40°, vision field: 400μm x 300μm. See Color Plate I at the back of this issue.

ACKNOWLEDGMENTS

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