In-situ study of molecular dynamics in a water environment by using imaging ellipsometry

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Abstract

We report on the dynamics of bio molecules and a high polymer in a water environment by using imaging ellipsometry (IE). The morphology of collapsed films of arachidic acid (AA) and poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) Langmuir monolayers in a liquid solution is investigated. The IE images clearly show that the multilayer domains and thickness of the collapsed region change sensitively depending on Langmuir compression. Also, the adsorption of bovine serum albumin is observed by using total internal reflection resonance IE (TIRIE), which has the advantage of IE and surface plasmon resonance. We believe that IE is a powerful technique for analysis and applications of bio materials.

Keywords: imaging ellipsometry, Langmuir method, total internal reflection resonance imaging ellipsometry

Classification numbers: 2.04, 5.08

1. Introduction

Spectroscopic ellipsometry (SE) is a non-contact and non-destructive optical technique for the characterization of thin films and bulk materials [1]. It is an excellent technique for measuring ($\varepsilon$) spectra, since these spectra can be obtained without the need of Kramers–Kronig transformation [2, 3]. Generally, SE was successfully employed to investigate several semiconductor alloy systems [3–6]. Nano scale investigations of the structure of devices and the dynamics of molecules have been of great importance for recent applications. However, conventional SE is not the appropriate technique for the detection of fine structure, because its spatial resolution is about $\sim$mm. In this work, we used imaging ellipsometry (IE) which can magnify the sample image and measure optical properties of spatial parts to observe the structures of a sample. The main advantage of IE is that every point on a surface is measured at the same time generally with a high lateral resolution of a few micrometers. IE overcomes the spatial resolution limitation of a conventional SE by using a charge-coupled device (CCD) detector, which can construct images over an extended area of the sample [7]. Because of this benefit, we can study not only solid materials but also the reactions of bio materials by using IE.

The Langmuir method (LM) involves sweeping a barrier over the water surface causing the molecules to come closer together and eventually to form a compressed, ordered monolayer. The film produced by such a method is known as a Langmuir film. Langmuir films consist of surface active agents or surfactants trapped at the interface between two dissimilar phases, either liquid–liquid or liquid–gas [8]. Langmuir films of surfactant molecules have been studied extensively by various experimental techniques, including surface pressure/area measurement (to get $\pi$–A isotherm) [8], electron microscopy [9], ellipsometry [10, 11], x-ray reflectivity and diffraction [12–14], Brewster-angle microscopy (BAM) [15–17], atomic force microscopy (AFM) [16, 17] and nonlinear optical spectroscopy [18, 19]. These measuring techniques can provide only two-dimensional images of Langmuir films. However,
IE yields information about the third dimension of the individual domains, namely their thicknesses [20]. Here, we used in-situ IE for arachidic acid (AA) and poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) made by the Langmuir method. AA is a fatty acid composed of a carboxyl head group and a long alkyl chain tail group. We used the LM and in-situ IE to monitor the morphology of collapsed films of AA molecular layers in CaCl₂ solution. The IE image showed that the collapsed film was very inhomogeneous, and clear domains of collapsed regions were easily noticeable. MEH-PPV is one of the conducting polymers that has hole carrier mobility to be applied for a p–n junction device and also has an easily soluble property for relatively simple fabrication processes. Also, we used the LM and in-situ IE to find the phase change of MEH-PPV films under a pressure change.

Total internal reflection resonance imaging ellipsometry (TIRIE) combines the analytic ability of IE with the high surface sensitivity of surface plasmon resonance (SPR). SPR and quartz crystal microbalance [21] can monitor the adsorption and interaction of target bio-molecules sensitively, whereas they have a lack of measuring parameters for analyzing the physical properties of the target. We used TIRIE for the investigation of the adsorption of bovine serum albumin (BSA). BSA is widely used for forming a protein complex with biotin–streptavidin affinity because BSA can attach to a glass slide or the surface of a wafer easily. To study the adsorption dynamics of BSA, we carried out TIRIE while the BSA diluted solution was injected into liquid cell. As a result, we could confirm remarkable changes in the TIRIE image and that these changes are due to the adsorption of BSA molecules onto the surface of the TIRIE sensor.

2. Experimental

The IE (Beaglehole Instruments, New Zealand) used in this work was composed of a quartz-halogen source, monochromator, polarizer, retarder, zoom lens, analyzer and CCD detector [20], whose schematic is shown in figure 1. The illumination assembly consisted of the lamp housing and the condenser. An aspheric lens in front of the lamp provided a parallel beam between these two parts in the illumination arm, so that the polarizer and the retarder could be placed there. A heat filter that cuts out the infrared radiation from the light bulb was placed before the polarizer to protect it from damage through overheating. The light from the lamp was polarized at an angle of 45° so that it consisted of equal in-phase amplitudes of s and p waves. It was then passed through the rotating retarder, which added a phase shift δ between the p and s waves under computer control. Following the retarder, a condensing lens converged the beam to provide suitable illumination of the surface. Light reflected from the sample was imaged through a zoom microscope objective onto the CCD detector. The CCD detector measured images of the ellipsometric signal over an extended area of the sample in real time. An analyzer was placed between the lens and the detector to convert the phase shift in the reflected light to intensity information that could be recorded by camera. The spectral range was from 400 to 800 nm and the maximum spatial resolution was about 3 μm.

The AA (CH₃(CH₂)₁₈COOH) was spread from a 3 : 1 mixture of chloroform-methanol solution onto 1 mM CaCl₂ solution and MEH-PPV was spread from a chloroform solution onto pure water in a home-built Langmuir trough, as shown in figure 1. The barrier of the Langmuir trough was pushed at a rate of 0.58 Å min⁻¹ per molecule. The barrier was controlled to maintain a constant surface pressure during the IE measurement. All of the IE experiments were carried out at room temperature (21 °C). When the barrier was moved, the IE incidence angle and the wavelength were set to 55° and 633 nm, respectively.

Figure 2 represents the schematic of the TIRIE experimental setup and the process of adsorption of proteins. Instead of depositing a metal layer directly on the surface of the prism, an Au-coated glass slide was used to allow convenient change of the sample. The Au layer with a thickness of 50 nm on the glass slide was deposited using physical vapor deposition. The prism and glass slide were made from BK7 glass and the index matching oil (n_d = 1.51) was used to minimize the reflection of light from the interface between the prism and the slide glass. The TIRIE liquid transfer system consisted of a liquid cell having a volume of 171 μl, a degasser of 2.5 ml to remove residual gas in liquid, and a syringe pump to control the liquid flow rate. Prior to the adsorption of the BSA protein, to remove the organic residue on the Au surface, 0.5 M KOH was injected into the cell for 5 min (200 μl min⁻¹). The T50 solution [1 M Tris-HCl, pH 8.0, 500 μl + 5 M NaCl 500 μl + DI water 49 ml] was used as a buffer in the TIRIE system. The T50 was injected to rinse the inner part of the cell for 10 min (200 μl min⁻¹).
Figure 3. \(\pi-A\) isotherm of the AA in 1 mM CaCl\(_2\) solution.

Later, the solution of BSA 80 \(\mu l\) (0.1 mg ml\(^{-1}\) in T50) was injected into the cell with 50 \(\mu l\) min\(^{-1}\), and T50 was injected to remove the surplus aggregations of BSA proteins until there was no change in the \(x\) and \(y\) values. We expect that these parameters would not change after the complete formation of a BSA monolayer. The TIRIE data were obtained at room temperature by using IE at an angle of incidence of 70\(^\circ\) and the wavelength was set to 750 nm.

3. Results and discussion

3.1. Arachidic Acid (AA)

Figure 3 shows a \(\pi-A\) isotherm of the AA Langmuir layer in 1 mM CaCl\(_2\) solution measured by the Wilhelmy plate method. Our result is in good agreement with earlier reports [20, 22, 23]. Moreover, we confirmed the image of phase change of the AA layers. Figure 4 represents the \(y\) value image of the AA Langmuir layer at several different values of molecular areas, as shown in figure 3. The IE data are given in the form of \((x, y)\) instead of general parameters of \((\psi, \Delta)\) or \((\varepsilon_1, \varepsilon_2)\) [2], where \(x = 2 \tan \psi \cos \Delta/[1 + (\tan \psi)^2]\) and \(y = 2 \tan \psi \sin \Delta/[1 + (\tan \psi)^2]\). The \(x\) and \(y\) images are very similar, so in the remainder of the paper we consider only \(y\) images. The thickness values of the AA layers are monolayer (\(\sim 28 \text{ Å}^2\)), bilayer (\(\sim 50 \text{ Å}^2\)), and trilayer (\(\sim 75 \text{ Å}^2\), taken from the x-ray and neutron reflectivity [14, 20]. And the refractive index of the AA layers and 1 mM CaCl\(_2\) solution are 1.44 [22] and 1.33 [24] at 633 nm. Using the thickness value of the AA layers and each value of the refractive indices, we made a three-layer model simulation (air/AA/substrate). Through simulation, we achieved the \(y\) value according to the thickness of the AA layers (monolayer: \(\sim -0.0095\), bilayer: \(\sim -0.01699\), and trilayer: \(\sim -0.0253\)). At the starting point (at 25 \(\text{Å}^2\) region, figure 4(a)), the \(y\) value images are covered with light green, green and some blue. This shows that condition that the AA layer is composed of gas phase and partially monolayer (The \(y\) value of zero is 1 mM CaCl\(_2\) solution.). In the middle region (before collapse at the 19.5 \(\text{Å}^2\) region, figure 4(b)), the \(y\) value images are filled with green and some blue dots. We can see the AA layer composed of fully monolayer and some multilayer seeds. At the final region of \(\pi-A\) isotherms (after the collapse at 9.5 \(\text{Å}^2\) region, figure 4(c)), the \(y\) value shows many blue regions that tell us that the AA layers make a multilayer structure.

3.2. Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV)

Figure 5 shows the \(y\) value image of the MEH-PPV Langmuir layer on pure water. Figure 5 shows (a) the \(y\) value image at the starting point and (b) at the end point of the MEH-PPV.
Langmuir layer made by the LM. The refractive index and extinction coefficient of the MEH-PPV are ~1.86 and ~0.019 at 633 nm [25]. Also, we have simulated the \( y \) value according to the thickness of the MEH-PPV, (10 Å: \( y \sim 0.016 \), 20 Å: \( y \sim -0.031 \), 30 Å: \( y \sim -0.047 \), and 40 Å: \( y \sim -0.062 \)). In figure 5(a), we can see that the \( y \) value images are covered with yellow and dark yellow. This shows that the surface in figure 5(a) is water, MEH-PPV gas, and a MEH-PPV monolayer phase. On the other hand, the \( y \) value images in figure 5(b) are filled mostly with green and some blue. So, we can see the MEH-PPV layer composed multilayer structure. On comparing figure 5(a) with figure 5(b), we can distinguish clearly that the MEH-PPV layer goes to multilayer from the gas phase with increasing pressure.

### 3.3. Bovine serum albumin (BSA)

We carried out the TIRIE measurement to monitor the kinetics of the formation of a protein layer at 750 nm wavelength and the result is shown in figure 6 (In this measurement, the most sensitive region was 750 nm.). Figures 6(a) and (b) are the \( y \) value images of the Au surface under buffer ambient conditions before and after the injection of BSA into the TIRIE liquid cell, respectively. In figure 6(a), the \( y \) value image was covered mostly with red and green. However, after the injection of BSA, the green region was changed to yellow (the red region was unchanged), as shown in figure 6(b). We consider that this clear change in the \( y \) value was caused by the adsorption of BSA. The significant change in the TIRIE values results from the sensitive response of the light to the change of small difference in the sample surface at the incidence angle where surface plasmon resonance occurs. In addition to the change in the reflectance, parameters measured by TIRIE contain information about the phase change of the reflected light from that of the incident light, which was caused by the adsorbed protein on the sample surface, which also gives the structural change in the samples. Therefore, the TIRIE could maximize its sensitivity on the change in the sample surface. We expect that the fast observation of protein adsorption by TIRIE has opened up a strong application for the protein chip industry where multiple sampling is possible.

### 4. Conclusions

We performed an imaging ellipsometry (IE) study with the Langmuir method (LM) and surface plasmon resonance. In the arachidic acid (AA) experiment, we could clearly observe correct \( \pi \)-A isotherm data and the image change of the AA Langmuir layer by using *in-situ* IE with the LM. The AA Langmuir layer constructed monolayer and multilayer phase according to before and after collapse. Also, we confirmed the image change of poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) films by the LM. The image of the MEH-PPV Langmuir layer clearly showed formation of a multilayer with increasing pressure. In addition, we used total internal reflection resonance imaging ellipsometry (TIRIE) for bovine serum albumin (BSA). We could observe the image change before and after BSA adsorption. We believe that IE is a useful method in the study of bio materials. We also expect the combination of IE with a conventional bio method will be a strong technique for the analysis and applications of bio materials.

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