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Sensors and Actuators B 121 (2007) 490–495
SENSORS AND ACTUATORS B CHEMICAL
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Post flight analysis of the surface plasmon resonance enhanced photoelastic modulated ellipsometry

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Received 6 September 2005; received in revised form 12 April 2006; accepted 13 April 2006
Available online 30 May 2006

Abstract

A total internal reflection ellipsometer equipped with a photoelastic modulator (PEM) is installed to investigate the chemical activation and antibody immobilization process on thin gold films. We set up two detection channels in this configuration: one for real-time monitoring with a data rate of 1 set per second, and the other for post flight analysis with a data rate of 25,000 sets per second. More detailed information has been obtained through the post flight analysis technique during the chemical activation process. This surface plasmon resonance enhanced PEM ellipsometry provides higher sensitivity and temporal resolution. It is feasible to resolve a faster reaction such as chemical reaction or protein folding by the post flight analysis technique.

Keywords: Ellipsometry; Surface plasmon resonance; Photoelastic modulator

1. Introduction

In the past decades, there has been a dramatic proliferation of research concerned with optical biosensors. A large variety of optical methods have been used in chemical and biological sensors which include interferometry, ellipsometry and surface plasmon resonance (SPR) [1]. The trend of these optical sensors is to provide a highly sensitive technique for monitoring the dynamic property of a biological event in real-time without any labeling. Ellipsometry is a well-developed optical technique in solid-state industry for measuring the thickness of thin film layers. Recently, this non-destructive measuring technique has been established [2] to monitor the etching/deposition processes in situ and real-time [3]. In addition to the solid-state industry, some groups pursued the interfacial investigations through the internal reflection of this ellipsometric technique to study liquid crystals [4]. They employed the total internal reflection to study the dynamic and anisotropic properties of liquid crystal layers [5] by using a prism to avoid the reflection from the glass substrate. In the bio-sensing technique, this prism-coupled ellipsometry has been further developed by introducing a metal film to form a SPR-enhanced ellipsometer. Westphal and Bornman [6] installed a SPR cell in a rotating-analyzer ellipsometer (RAE) to measure the slow process such as antigen–antibody interaction and DNA hybridization. But, most biological reactions monitored by SPR or quartz crystal microbalance (QCM) techniques [7] are in the time regime of seconds. Furthermore, many biological reactions such as protein folding, enzyme catalysis and protein isomerization are in the regime between milliseconds to microseconds [8]. It is our interest to conquer this slow mechanical RAE by the SPR-enhanced photoelastic modulation ellipsometry to measure the dynamics of those biological reactions in the time regime of milliseconds.

Our group has developed a multiple harmonic intensity ratio (MHIR) technique to calibrate the photoelastic modulator by discriminating the oscilloscope waveforms of the signals [9]. In this technique, a data acquisition (DAQ) system was used to obtain the digitized waveform. Its harmonic signals can be used to determine not only the azimuth position of the analyzer and polarizer but also the ellipsometric parameters. Based on this concept, we installed a second channel for post flight analysis in addition to the one for real-time measurement. Because the response time of twisted nematic liquid crystals (TN-LC) is in the millisecond regime, we use the transmitted PEM-DAQ ellipsometry to analyze the variation of its ellipsometric parameters under a square wave. Then, a Kretschmann configuration

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doi:10.1016/j.snb.2006.04.073

for the attenuated total internal reflection setup is used in this polarization-modulated ellipsometer, and its incident angle is set near the resonant angle of SPR to enhance the ellipsometric signals. In this paper, we will demonstrate the enhancement by using two incident angles, one is at the resonant incident angle and the other is an offset from the resonant angle by 2°, to measure the refractive index changes under various concentrations of glycerin–water mixtures. Only the change in phase or amplitude is considered in studying the protein-protein interaction by SPR technique, but not both of them [10,11]. Since multiple data measurements offer better signal to noise figures, we like to measure both the ellipsometric parameters (Ψ and Δ) in this SPR-enhanced PEM-ellipsometer to increase its capability in biosensing.

2. Theoretical background

The most prevalent technique to measure the refraction indices or layer thickness may be ellipsometry, which is based on analyzing changes in polarization state of the light reflected by the surface. It is usually depicted by the ratio of complex Fresnel reflection coefficients ρ, which can be expressed by the ellipsometric parameters Ψ and Δ. They are defined as follows:

$$\rho = \frac{r_p}{r_s} = \tan\Psi e^{i\Delta} \tag{1}$$

where r_p and r_s are the Fresnel reflection coefficients for the polarized light parallel or perpendicular to the plane of incidence, respectively. The system configuration is in a polarizer–compensator–sample–analyzer (PCSA) configuration; however, the compensator is substituted by a photoelastic modulator (PEM). The final polarization state can be expressed by the operation of their corresponding Mueller matrices, i.e.

$$S_f = M_A(A)R_{SPR}(\Psi, \Delta)M_{PEM}(\Delta_P)S_P, \tag{2}$$

where the Stokes vector S_f is the final polarization state of a polarized light, and S_P is the initial linear polarized state. M_{PEM} (Δ_P) represents the Mueller matrix of PEM with a modulated phase Δ_P when its optical axis is zero with respect to the incident plane, R_{SPR} (Ψ, Δ) represents the Mueller matrix of the sample with ellipsometric parameters Ψ and Δ through the SPR sensing device, and M_A (A) is the Mueller matrix of an analyzer with its transmission axis at A. Since the full information about the polarization changes of the reflected light is described by two ellipsometric parameters [12], one can substitute the Mueller matrix of any sample R_s (Ψ, Δ) into Eq. (2). If the azimuth angle of the linear polarized light is set at −45° and the azimuth angle of the analyzer is positioned at 45°, the intensity of the reflected light is given by:

$$I(t) = 0.5I_0\left[\frac{\tan^2\psi + 1}{2} - \tan\psi\,\cos(\Delta - \Delta_p)\right] \tag{3}$$

where I_0 is the normalized intensity of the system. The DAQ system can digitize the analog oscilloscope waveform, which can be saved for post flight analysis. If a twisted nematic liquid crystal is considered as a sample under a transmitted mode, known as polarimetry, one can illustrate the time regime of this PEM-ellipsometry in Eq. (3). Since the TN-LC becomes an isotropic medium under an external electrical field, then the ellipsometric parameters $\Psi = 45°$ and $\Delta = 0°$. By substituting these into Eq. (3), one can obtain the temporal response of the intensity as:

$$I(t) = I_0[1 - \cos(\delta_0 \sin(\omega t))]. \tag{4}$$

This waveform can be Fourier expended into multiple harmonic components. One can either access the components by lock-in amplifiers in real-time or save the waveform for post flight analysis, which can reach the time limit of the system, i.e. 40 μs.

The phase retardation of the PEM is modulated as $\Delta_P = \delta_0 \sin(\omega t)$, by substituting the Fourier expansion of the harmonic functions [9] into Eq. (3), and then one can obtain the temporal intensity distribution as:

$$I(t) = I_o\left\{\frac{\tan^2\Psi + 1}{2} + J_0(\delta_0)\tan\Psi\cos\Delta - 2J_1(\delta_0)\tan\Psi\sin\Delta\sin(\omega t) - 2J_2(\delta_0)\tan\Psi\cos\Delta\cos 2(\omega t) + \cdots\right\} \tag{5}$$

where δ_0 is 2.405 radian, $J_0(\delta_0) = 0$ and I_{DC} becomes a constant, i.e.

$$I_{dc} = I_0\left(\tan^2\Psi + \frac{1}{2}\right) \tag{6}$$

The other corresponding harmonic components are expressed as:

$$I_{(2m-1)f} = -I_o[J_{2m-1}(\delta_0)\tan\Psi\,\sin\Delta]\sin(2m-1)(\omega t);$$
$$I_{2mf} = -I_o[J_{2m}(\delta_0)\tan\Psi\,\cos\Delta]\cos 2m(\omega t),$$
$$\text{where } m = 1, 2\ldots \tag{7}$$

For real-time monitoring, two lock-in amplifiers can be used to obtain the ellipsometric angles Ψ and Δ through the following relations:

$$\Psi = \frac{1}{2}\sin^{-1}\left\{\left[\frac{I_{1f}}{2J_1(\delta_0)I_{dc}}\right]^2 + \left[\frac{I_{2f}}{2J_2(\delta_0)I_{dc}}\right]^2\right\}^{1/2}$$
$$\Delta = \tan^{-1}\left[\frac{I_{1f}J_2(\delta_0)}{I_{2f}J_1(\delta_0)}\right] \tag{8}$$

3. Experiments

The transmission axis of the polarizer and analyzer (Melles Groit 03FPG015 sheet polarizer with an extinction ratio of 10^{-4}) and the strain axis of PEM (Hinds PEM 90 operated at an oscillating frequency around 50 kHz) were well aligned and calibrated according to the references [9,13]. First, we constructed a transmitted ellipsometer to measure the ellipsometric parameters of a TN-LC cell by applying a 5 Hz square wave with a step voltage of 5 V. A DAQ system (National instruments PCI-6111, 12-bits,

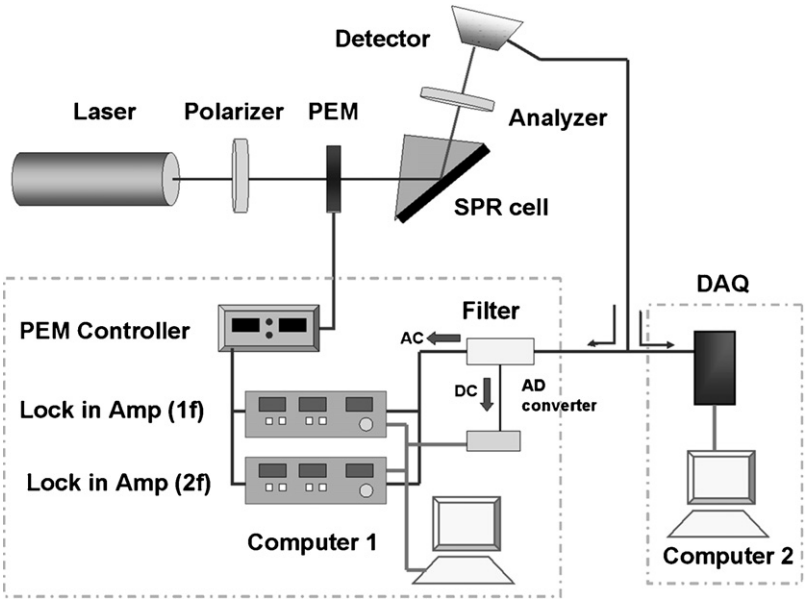

Fig. 1. Experimental setup of the SPR-enhanced PEM-ellipsometer. The signal was obtained by two lock-in amplifiers for real-time display and the data acquisition (DAQ) system for post flight analyses.

5M samples) was used to record the digitized waveform for post flight analysis, a 40 μs of resolution can be achieved by using two cycles. Because of the memory limit of the DAQ system, we only recorded the waveform of 1 s every 12 s for post flight analysis. The Kretschmann configuration of SPR was installed into the PEM-ellipsometer for examining its ability in enhancement and resolving power in time, as shown in Fig. 1. A He-Ne laser with wavelength 632.8 nm and a broad bandwidth PIN silicon photodiode served as the light source and detector (Thorlabs PDA-55), respectively. Two sensor chips were used in this experiment: a SPR sensor chip from GenTelglod, which contained a gold film of thickness 47.5 nm and a chromium adhesion layer of 1 nm on the top of a glass slide for examining the SPR enhancement by glycerin/water mixtures; the other one was made by Biacore (CM-5), which not only contained a gold film (∼50 nm) but also was coated with a self-assembled monolayer (SAM) and a dextran layer. We used it to study the chemical linkage of biological molecules. The index-matching oil was used to couple the prism and sensor chip for avoiding the multiple reflections between interfaces. A reaction chamber outside the sensing region with a capacity of 0.3 ml was mounted on a motorized stage (step: 0.01°) to control its incident angle. This design is feasible to adjust the incident light to be near the resonance angle to achieve higher sensitivity. The concentration of glycerin mixed in water was from 0 to 4 wt% in the experiment to measure the corresponding ellipsometric parameters at an incident angle of 73° (the resonant angle) and 71°, respectively. According to the reference [14], the index of refraction changes from 1.333 to 1.338 under various concentrations of glycerin/water mixtures. The real-time measurements of Ψ and Δ were performed by two lock-in amplifiers. In addition, we added a DAQ system to form a two-channel sensing system for real-time monitoring and post flight analysis of the biological affinity interactions. This experiment was performed in a basic phosphate buffered saline solution (PBS) under an incident angle of 72.8°. The antibody immobilization process was operated in the following procedures [15]: (1) an *N*-ethyl-*N*′-dimethylaminopropyl carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) solution was injected with a flow rate of 0.2 ml/s to activate the sensor surface; (2) a PBS buffer was injected for replacing the EDC/NHS solution; (3) a the stock anti-mouse IgG solution (1 mg/ml) was diluted in an immobilization buffer (acetate pH 5.0), and the final concentration of the ligand solution of 80 μg/ml was filled into the reaction chamber and incubated for 10 min. For analyzing the process of the activation of the EDC/NHS solution to the dextran layer, we reduced the injection rate by half, and then analyzed the transaction by a new DAQ (National instruments PCI 6115, 64 M samples) card which had larger memories.

4. Results

The ellipsometric parameters were obtained by using Eqs. (6)–(8) through the intensity measurements of dc, 1*f* and 2*f*. The dynamic responses of the TN-LC cell were analyzed by the data obtained from the DAQ system; its rising time (7.5 ms) and fall time (19 ms) were measured. These results are comparable to those measured by the conventional transmittance measurements under the same condition. Its time-dependent ellipsometric parameters are plotted in Fig. 2 for illustrating the time resolving ability of this post flight analysis technique. The saturated value of Ψ and Δ indicate that the TN-LC becomes isotropic under the influence of the applied voltage. For studying the stability of the SPR enhancement in ellipsometry, we illustrated the measured ellipsometric parameters at the resonance angle in real-time, as shown in Fig. 3. Its step values are related to the increasing concentration of glycerin in water. We started this experiment by adding 0.1% of glycerin into pure water to test the minimum sensitivity of the system, and a 12.18° step was

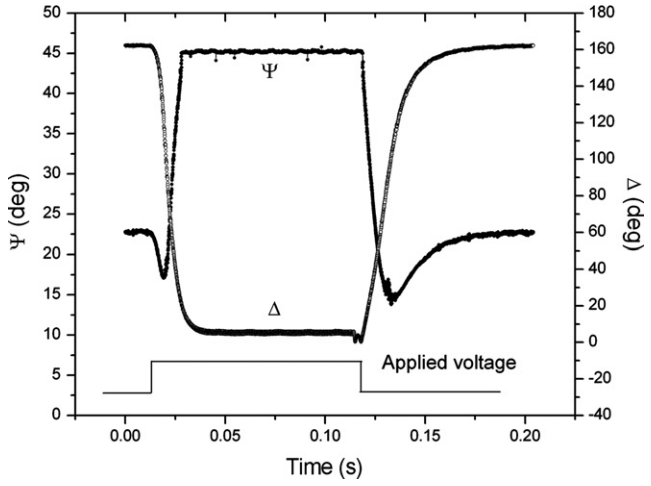

Fig. 2. Histograms of measured ellipsometric parameters for the twisted nematic liquid crystal cell (transmission ellipsometry, $\lambda = 632.8$ nm). A square wave of 5 Hz and strength 5 V was applied.

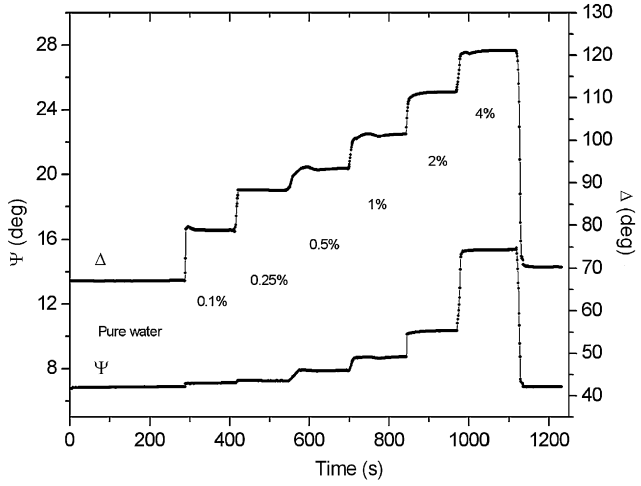

Fig. 3. Histograms of measured ellipsometric parameters: various weight ratios of glycerin–water mixtures mixtures at an incident angle of 73°.

obtained in Δ but very little difference in Ψ. Since the precision of this system is 0.02°, the sensitivity of this system is 2×10^{-7} RIU (refractive-index unit) at the resonant angle. Fig. 4 shows the ellipsometric parameters as a function of glycerin concentration in the glycerin/water mixtures. One can observe that the variation of Δ is drastically reduced outside the resonant angle, but the variation of Ψ is almost linear at both incident angles. It is our interest to explore the application of both ellipsometric

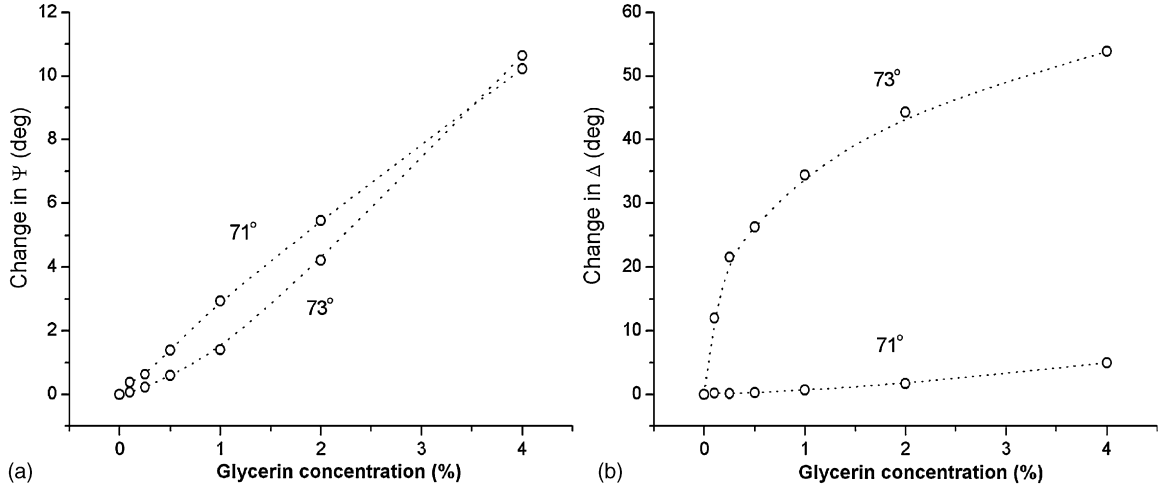

Fig. 4. Ellipsometric parameters, (a) Ψ and (b) Δ, as a function of glycerin weight percent of glycerin–water mixtures mixtures at incident angles of 71° and 73°.

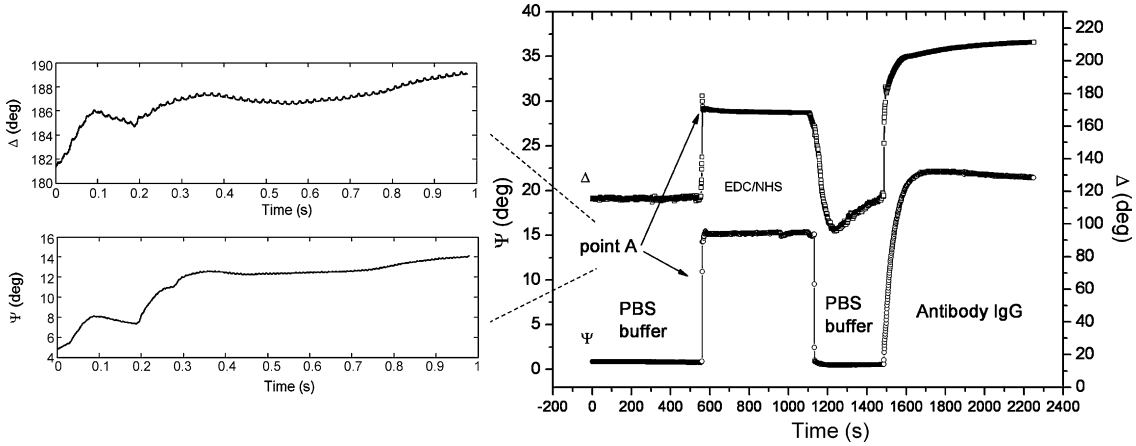

Fig. 5. Histograms of ellipsometric parameters under the activation process and immobilization of antibody IgG: (right) monitored by two lock-in amplifiers in real-time; (left) enlargement of the recorded data from 561 to 562 s.

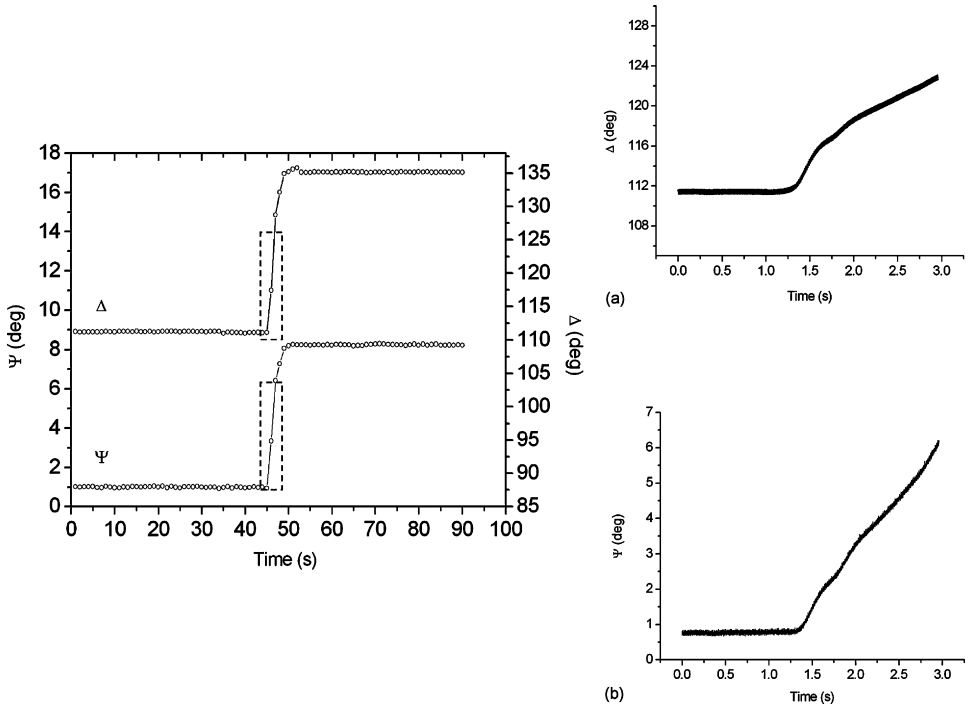

Fig. 6. Histograms of ellipsometric parameters during the activation at the surface of the SAM by injecting the EDC/NHS solution at a flow rate 0.1 ml/s, which is half of the flow rate in Fig. 5.

parameters for measuring the refractive index in a wider dynamic range. In the study of biological affinity interactions, we split the measurements into two channels: (1) real-time measurements by two lock-in amplifiers, shown on the right side of Fig. 5, and (2) the post flight measurements of the activation process of EDC/NHS at the 561st second enlarged on the left side of Fig. 5. Since there was a fluctuation observed in the activation process, we reduced the speed of injection by half and obtained a smooth result, as shown in Fig. 6. This gives us a bright future for analyzing the immobilization process.

5. Discussion and conclusion

In contrast to a conventional ellipsometer, which is usually operated an external reflection mode, the precision limitation in measuring the refractive index is about 0.002; the sensitivity of this SPR-enhanced ellipsometry in phase measurement is highly enhanced and a 0.00012 precision is achievable. Compared with the rotating element ellipsometer [6], this PEM-ellipsometer contains no mechanical moving parts, and thus the error caused by the beam deviation can be completely avoided. Furthermore, the phase retardation of PEM is electronically modulated in a frequency of 50 kHz, so that its time resolution can be improved to the order of 40 μs. The PEM-ellipsometer has already been used to monitor the plasma etching process by 10 pairs of ellipsometric parameters per second [16]. The ellipsometric parameters of this PEM ellipsometry are measured by the intensity ratio of its corresponding harmonics (such as I_{1f}/I_{2f} or I_{1f}/I_{dc}); under these circumstances the intensity stability of laser has no influence on the experiment. Although the temporal resolution of this PEM ellipsometry can reach 40 μs, the duration of measurement is limited by the sampling rate and the memory of the RAM in DAQ the card. The real-time monitoring system can be used to locate the interesting event in the biological system; the DAQ system can be used to analyze this event afterward. Recently, we were able to construct an imaging ellipsometer [17] by analyzing the digitized waveform, which could provide fast imaging ellipsometry.

Ellipsometry known as a standard technique for thin film measurement in semiconductor industry has been developed as spectrometry for analyzing all kinds of materials which includes anisotropic materials, such as liquid crystals, organic light-emitting diode (OLED), etc. The traditional SPR technique only measures the amplitude or phase changes near the resonant angle of incidence, while the ellipsometry can measure the polarization state changes, i.e. amplitude and phase, at any incident angle. Although Δ of this SPR PEM ellipsometry can provide a highly sensitive signal for resolving the glycerin/water mixture near the resonant angle of incidence (Fig. 4b), the linear property of Ψ (Fig. 4a), at which the incident angle is off the resonance, can be used to extend the measurement to higher concentrations. The advantage in time resolution of this technique can be observed in the biological affinity interactions, as shown in Fig. 5. After the surface-active solution (EDC/NHS) was substituted by a PBS solution, Ψ went back to zero but not Δ. The dextran layer was still in the active mode for immobilizing the anti mouse IgG protein. In the total internal reflection mode, the maximum of surface plasmon is located at the interface of its resonant angle of incidence. Thus, the field can be enhanced by two orders of magnitude in comparison with that of the conventional ellipsometry [10]. It is our interest to exploit the exact enhancement factor, and then it can be used to

analyze the structure of biological event, such as protein folding, surface chemical modification and immobilization rate with respect to the injection speed of the solution. The multilayer buildup process can be investigated more thoroughly by adding a quartz crystal microbalance with dissipation monitoring (QCM-D) system in this SPR-enhanced PEM ellipsometry.

Acknowledgement

This work was supported by the National Science Council of Taiwan under Grant No. 93-2215-E-009-056.

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