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Ellipsometer Surface Plasma Resonance

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***Abstract*— In this dissertation, a multi-functional optical biochip system was presented. This biosensor is developed by integrating ellipsometry and SPR detection techniques, which adopted a paraboloidal mirror and a spherical mirror to vary the incident angle, a LCD phase modulator to control incident light beam retardance, a reference optical path to calculate the phase modulated by LCD, and a flow-injection system to drive bio-samples to biochip. The other important function of this biosensor is to observe SPR phenomenon by using the ellipsometric configuration. By using this method, the SPR resolution could be significantly enhanced, and the characteristics of ellipsometry give detailed information for bio-tech analysis. Information such as film thickness, physical parameters, concentration, mass, density, kinetic constants, binding specificity, etc. can all be retrieved. This biosensor was named ESPR which is an acronym of Ellipsometry-SPR. In summary, ESPR could serve both as a research and development tool as well as a manufacturing tool in biomedical area.**

I. INTRODUCTION

Bio-technology industry is regarded as one of the most potential industries in 21st century. This research work is expected to establish a Bio-chip measuring system. By using different operating softwares, the system has two different optical scanning functions in the same optical path.

The Ellipsometry has the ability to measure the optical parameter of thin film because of its property of high sensitivity to surface properties.. It also has the advantages of non-invasive measuring, high-speed and high accuracy to measure bio-molecules. In 1978, Cuypers detected the cells or the Bio-molecules between the solid and the liquid in use of Ellipsometry. In 1985, Nygren and Stenberg also utilized Ellipsometry to confer the binding ability of antibody and solid surface in ELISA (Enzyme-Linked Immunosorbent Assays) interaction [1]. In 1999, Ellipsometer is first used in Bio-technology, Brian Trotter, Garret Moddel,Rachel Ostroff, and Gregory R.Bogart published the results of measuring optical immunoassay applying Fixed-polarizer Ellipsometry, and the resolution is up to 4 pg/ml; the electrochemiluminescence system built up by IGEN(H. Yang, J. K. Leland, D. Yost, and R. J. Massey, 1994) has the resolution range between 10 pg/ml to 5 ng/ml. Therefore, the Ellipsometry is a potential application in Bio-chip from previous researches [2].

The surface plasma resonance technology is often applied in detecting small change of surface or interface, and it has become a very important and sensitive optical probe (Hass, et. al., 1970) in non-invasive detection that its resolution is reach to ng/ml level (B. Liedberg, C.Nylander, and I. Lundstrom, 1983). Compared with ELISA, affinity chromatography and equilibrium dialysis, SPR has two advantages. First, it can monitor the reaction of biological molecules in real time. Second, the biological molecules have no need to be labeled. In the whole view, the layout of SPR is simpler than Ellipsometer, but the Ellipsometer has better performance in resolution and sensitivity. Both the technologies were integrated to make the system has more exact analysis and more wide application.

II. DESIGN AND PRINCIPLE

In traditional application, Ellipsometry is employed in measuring the coating in semiconductor and optical coating. SPR is employed in measuring the chemical reaction. This study work combines these two technologies to focus on the Bio-technology measurement.

For design of Bio-chip, we combine the ELISA and BIA (Biomolecular Interaction Analysis) methods to measure the open and quantitative reagent living beings reaction in ELISA. Beside that, the pump cooperated with the flow channel can help us to measure the dynamic reaction with BIA method. ESPR can measure the bio-chip by Ellipsometry and SPR side by side. To sum up, the changing of optical parameter, the bio-properties of biological member's dynamic energy and surface absorption the reaction between antibody and antigen can be measured by index change.

In Ellipsometry, we used PMSA setup (Fig.1), which P, M, S, A represent Polarizer, Modulator, Sample, and Analyzer respectively[3]. Comparison in multi-wavelength Ellipsometry, this method has less unknown variables and better results in calculation membrane parameters. In order to find out the ellipsometric parameter ψ and Δ, the nematic liquid crystal is a modulator to modulate the phase of incident light. It forms a phase modulated Ellipsometer. The optical signal phase (δ) is modulated into three terms, which are dc component term, $\sin(\delta)$ term, and $\cos(\delta)$ term. By change the phase term (δ), we can calculate the three coefficients, I_0, I_s, and I_c and evaluate the ellipsometric parameter ψ and Δ.

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$$I(\delta) = G(I_o + I_s \sin\delta + I_c \cos\delta) \quad (1)$$

$$\psi = \sin^{-1}\left(\frac{(GI_s)^2 + (GI_c)^2}{(GI_0)^2}\right) \Big/ 2 \quad (2)$$

$$\Delta = \tan^{-1}\left(\frac{GI_s}{GI_c}\right) \quad (3)$$

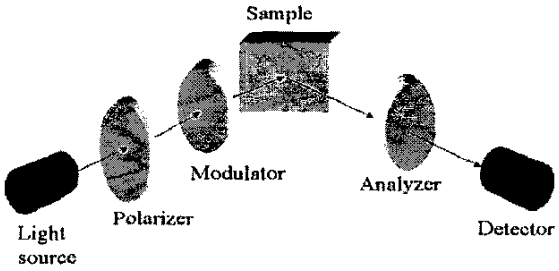


Fig.1 the optical design of Ellipsometer

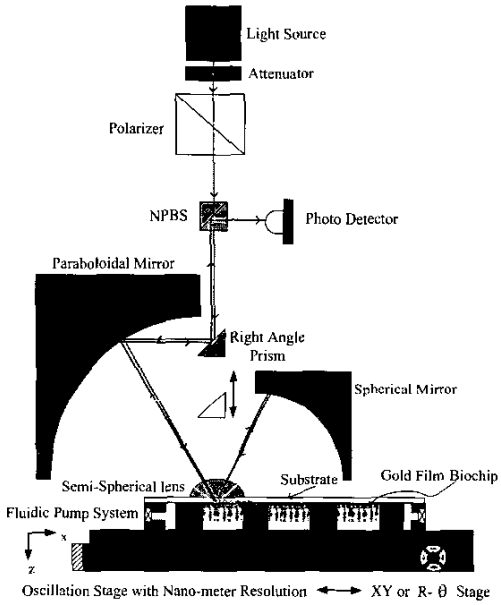


Fig.2 the SPR optical path design

For multi-layer, we need more ellipsometric parameters, ψ, Δ and gain to solve the unknowns by changing incident angle. In this structure, the triangle mirror and ellipsoidal mirror can not also achieve the working requests but reach high accuracy and small volume[4].

We use prism-coupler structure to excite surface plasma wave(SPW), there are two conditions must be met. First, the SPW interface is between two materials of dielectric constant with opposite sign. Hence the wave number of SPW, $k_{x,spw}$ can be defined by Eq.(4) [5].

$$k_{x,spw} = k_x = k_0 \sqrt{\frac{\varepsilon_d \varepsilon_m}{\varepsilon_d + \varepsilon_m}} = \frac{\omega}{c} \sqrt{\frac{N_d^2 N_m^2}{N_d^2 + N_m^2}} \quad (4)$$

Where k_0 is the wave vector in free space, ε_d is the dielectric constant in dielectric material, ε_m is the dielectric constant in metal. N_d is the refractive index of dielectric material, and N_m is the refractive index of metal.

Second, the wave number k_x of incident light must satisfied Eq.(4) and k_x will change by the variation of incident angle which is the SPR angle. If the concentration of sample changes, k_x must be changed to meet $k_{x,spw}$. This means that the SPW happens at different incident angles. Thus, we can find the bimolecular reaction from the change of incident angle[6].

To match the refractive index of the slide material with the hemisphere mirror which is made by glass SF2, the slide is also made by SF2 and its size is 20mm*20mm*1mm. The scratch in the slide surface is less than 20μm, and the digging hole in it must be smaller than 10μm. One surface of the slide is coated with 50 nm gold film and bound with 1.5nm bio-linker above the gold film so that it becomes the chip for bio-interactions. The chip is put on the hemisphere mirror of the opto-machanical system with the surface without the gold film of the chip adjacent to the mirror. The commercial refraction index matching liquid SERIES B produced by U.S.A. Cargille Laboratories Company, is filled between the chip and ball mirror to prevent from air gap existing. The gap will become the light waveguide at large angle if it exists. Use PMMA to build up a compress block with flow channel on the chip, as shows in Fig.3, the size of the flow channel is10mm*1mm*0.5mm, and the reacting area of biochemistry is 10mm*1mm. As a result, it needs 5μl of solution to fill-up the volume of the reaction area.

There is a fluid control pump to drive the react reagent and biological sample in steady speeds because this research needs to carry on the dynamic reacting amount of biochemistry.

The model FIAlab3500® fluid control pump shows as Fig.4, it has one syringe pump and a peristaltic pump. The stepper motor drives syringe pump and the peristaltic pump is using the elasticity of the pipeline and rotating speed of the motor to drive the fluid. After the sample enters the pipeline, switch over of six liquid valves to control the sample to enter the pipelines to connect with the chip.

Human immunoglobulin(human IgG) and human antigen immunoglobulin(humanAnti-IgG) are the bio-samples. The bio-linker(HS-(CH2)7COOH) activating reagent is made by NHS (N-Hydroxysuccinimide) mixed with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride). EA (1.0 M, Ethanolamine hydrochloride) is the blocker The bio-linker is developed by the group lead by professor Shih-Yuan Lee, Department of Chemistry, Tamkang University. The buffer liquid is phosphate buffer saline (PBS) of pH7.2 which is provided by the professor Shi-Ming Lin, National Taiwan University College of Medicine. The procedure of ELISA is shown as follow:

Step 1: Regard PBS solution as buffer liquid, 40μl, and wait for 100seconds.

Step 2: Add bio-linker (20mM, HS- (CH2) 7COOH), 40μl, and wait for 620 seconds.

Step 3: Put in activation (EDC/NHS) , 40μl, and wait for 350 seconds .

Step 4: Add the human immunoglobulin (Human IgG, 400μg/ml), 40μl, and wait for response time 433 seconds.

Step 5: Wash the reaction area with PBS in order to take away the human IgG that is not immobilized.

Step 6: Put in EA, 40μl, in order to disable the remained binding site of the bio-linkers and wait for the response time, 335 seconds.

Step 7: Wash out the un-immobilized EA with 40μl PBS and the response is 426 seconds.

Step 8: Add 40 μl of human antigen Immunoglobulin (Anti-IgG , 100μg/ml), and reaction time lasts 904 seconds for balance.

Step 9: Wash reaction region with 40μl PBS and wait for 280 seconds.

The bio-samples in the experiments performed with ELISA and BIA methods are the same. However, we use different concentration of Anti-IgG. First, IgG concentration is 400μg/ml, and interacts with different concentration of Anti-IgG, which are 50μg/ml, 100μg/ml, and 300μg/ml respectively. In another experiment the IgG concentration is 50μg/ml, and Anti-IgG concentration are 10μg/ml, 20μg/ml, 50μg/ml, 75μg/ml, and 100μg/ml respectively. The flow rate for all the reagents and buffer is 5 μl/min.

Step 1: Pump PBS, pH =7.2, into the channel and let it flow through the chip for more than 5 minutes.

Step 2: Add bio-linker (20mM, HS- (CH2) 7COOH), 65μl, 20mM, with constant flow rate passing the chip reaction region.

Step 3: Drive PBS, pH =7.2, into the chip no less than 2.5 minute.

Step 4: Put in EDC/NHS mixture for 65μl as activating reagent with fixed velocity of flow through chip to make bio-linker is activated.

Step 5: Let PBS, which pH value is 7.2), pass chip at least 2.5 minute with steady flow rate.

Step 6: Inject 65μl of human IgG antibody that concentration is 400 μg/ml into the chip in constant velocity of flow.

Step 7: Wash away the human IgG which is not binding to bio-linker with pumping PBS through the chip at least 8 minutes.

Step 8: Add 65μl of EA (1.0 M, Ethanolamine hydrochloride) in order to disable the remained binding site of the bio-linkers.

Step 9: Wash out the un-immobilized EA with PBS at least 8 minute.

Step 10: Put in 65μl of human Anti-IgG (50μg/ml, 100μg/ml , 300μg/ml) with constant flow rate passing the chip reaction region.

Step 11: Pump PBS, pH =7.2, to pass the chip with stable velocity of flow for at least 3 minute.

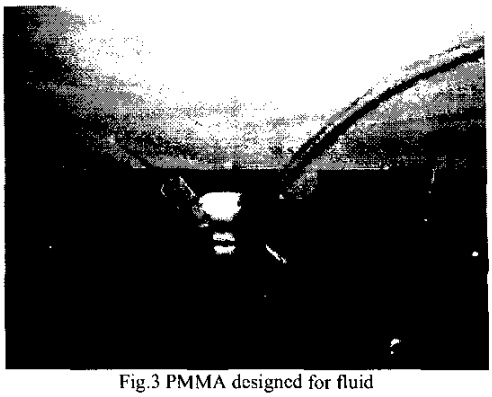


Fig.3 PMMA designed for fluid

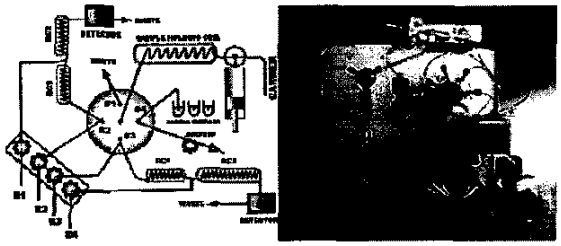


Fig.4 FIAlab3500® fluid control pump

III. ACHIEVEMENT AND DISCUSSION

The system is based on surface plasma resonance and ellipsometry in order to measure biological reactions. These two bio-reaction procedures are ELISA and BIA respectively. The reaction occurres between reactive reagent and sample in static state can be observed by performing ELISA, and the dynamic equilibrium can be inspected when BIA experiment is carried out with continuous fluid flow. Next, some results of two measurements will be described and discussed.

A. ELISA

First, the process is use thiol-linker of 8 carbon chains binding on the biochip with 50nm gold film. Then, we wash the chip surface with the PBS buffer. After that, the activation reagent (EDC/NHS) human immunoglobulin (human IgG, 400μg/ml), EA (1.0M, Ethanolamine hydro-chloride), human immunoglobulin antigen (human Anti-IgG, 100μg/ml) are added in sequence. Among every reagents, PBS of pH 7.2 is put in as buffers.. The detail experiment steps are described in previous section

The light intensity signals (I) is acquired by using DAQ card and LabVIEW [7]. program and the surface plasma resonance angle is picked up the program. From surface plasma resonance, the bio-reaction curve shows as following figures.

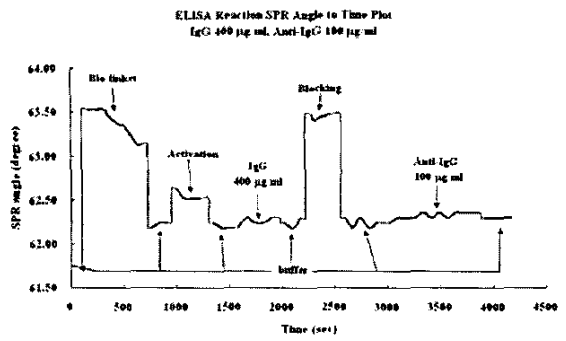
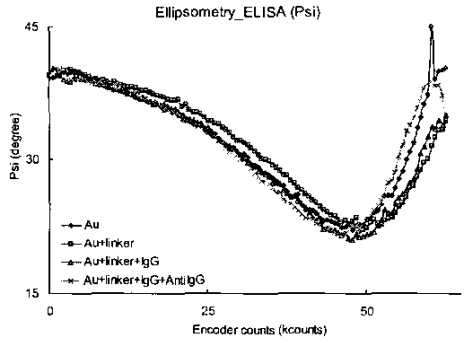


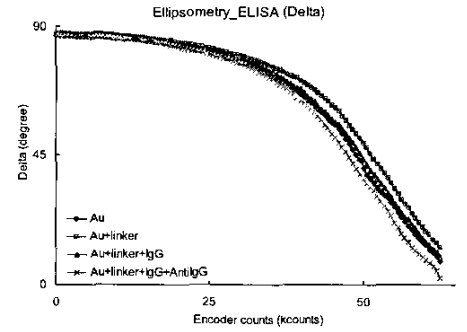
Fig.5 the SPR angle detected by ELISA in solid-liquid phase

In Fig.5, the signal would drop slowly from extremely high value by adding reagent in the beginning, then it would rise slightly to reach constant value because the reagent does not supply continuously. The result depends on the decreasing concentration of reagent, the increasing thickness and optical properties of film on biochip. Therefore, the response curve would not be rising slowly to stable state. Besides that, the SPR resonance angle is changed slightly with different bio-molecular-link reactions. As the figure shown, the SPR resonance angle after the reaction is greater than before. It reveals that the signals caused by the ELISA reaction can be measured feasibly by this technology.

With the function of ellipsometry, the reaction curve which is resulted from the reaction steps in the same biochemistry reaction is measured as the following figure:



(a) Ellipsometry coefficient ψ varied with encoder counts

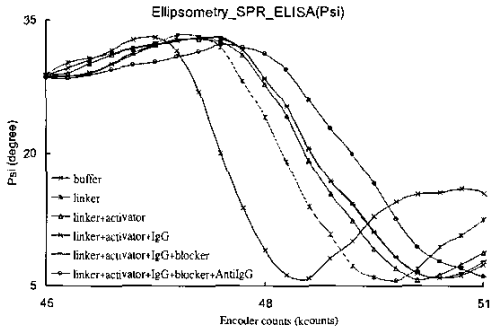


(b) Ellipsometry coefficient Δ varied with encoder counts

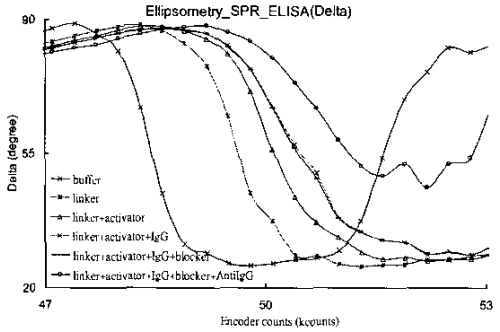
Fig.6 The application towards biomolecular membrane fixation of ellipsometry

The result is shown as Fig.6. Ellipsometry parameters ψ and Δ will have some change in every reaction step. The existing of the biochemistry reactions can be proved by signal changes. It would provide further reference on BIA biological reaction in the micro-fluid system. The sample requires to be dry to get fixed biology membrane through ellipsometry measurement is the main reasons for error happening. The whole light intensity and signals-noise ratio decrease probably because of this procedure.

The innovative application of ESPR system is that observing the SPR phenomenon occurred on the biomedical chip with ellipsometry function. By Fig.7 shown, the SPR resonance angle increased as the biology reaction occurred in the same ELISA process. The ellipsometry parameter, psi is provided better sensitivity than delta. In addition, the ellipsometry parameter, psi also changes obviously at the incident angle of 64° which is observed both in the experiment result and numerical simulation.



(a) ellipsometry coefficient ψ varied with encoder counts



(b) ellipsometry coefficient Δ varied with encoder counts

Fig.7 Ellipsometry measure the surface plasma resonance in ELISA

B. BIA

In the BIA reaction process, the reagent is injected to the channel continuously with the constant flow rate, 5μl/min. After that, the buffer and sample are passed through the reaction region of the biochip. The detail

procedure is described in pervious paragraphs. In the experiment, four different concentrations of the anti-IgG antigen are chosen which are 75μg/ml, 50μg/ml, 20μg/ml and 10μg/ml respectively to distinguish the reaction response. The response of the solid-liquid phase kinetics of the biological reaction can be detected by SPR technology.

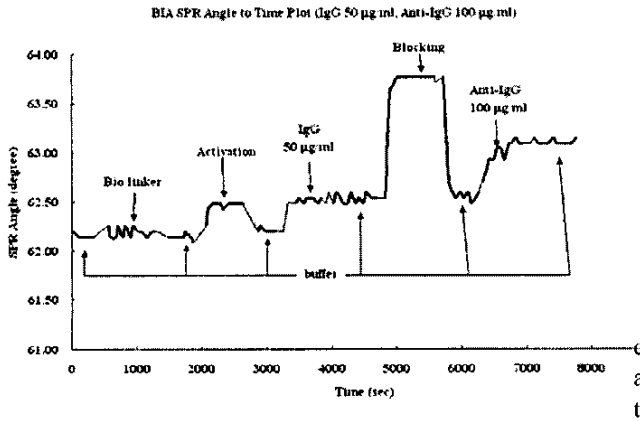


Fig.8 (a) IgG (50μg/ml) and Anti-IgG (100μg/ml) reacting relation

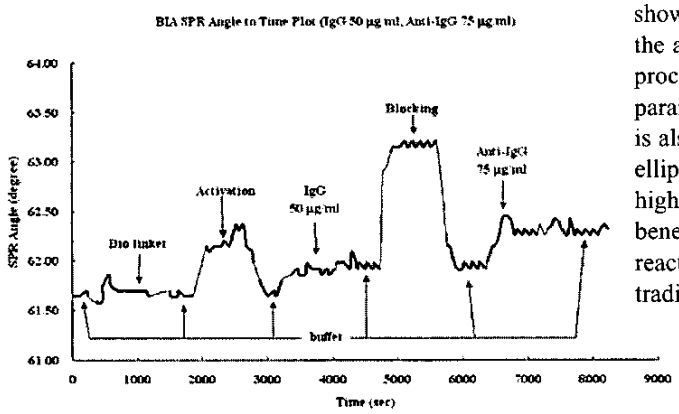


Fig.8 (b) IgG (50μg/ml) and Anti-IgG (75μg/ml) reacting relation

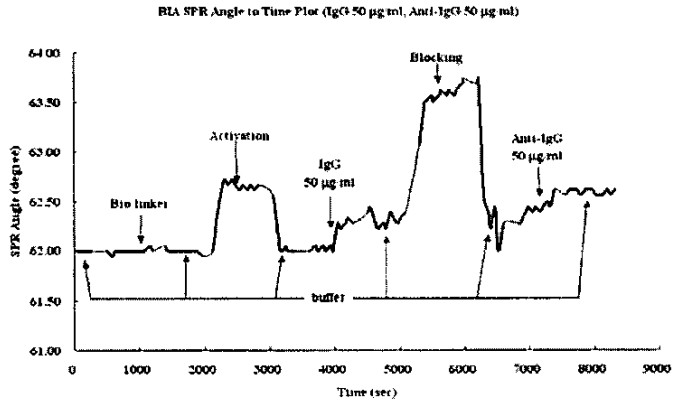


Fig.8 (c) IgG (50μg/ml) and Anti-IgG (50μg/ml) reacting relation

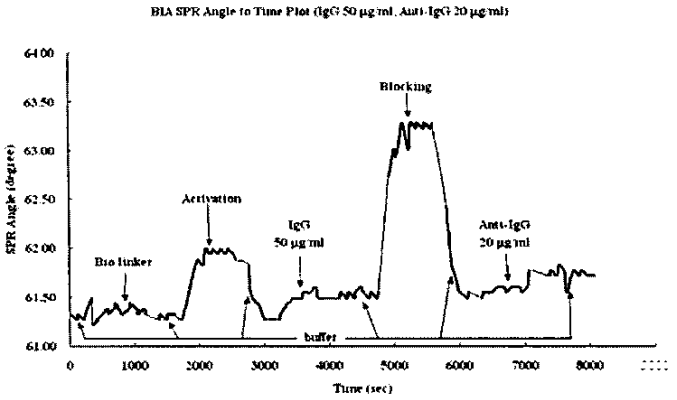
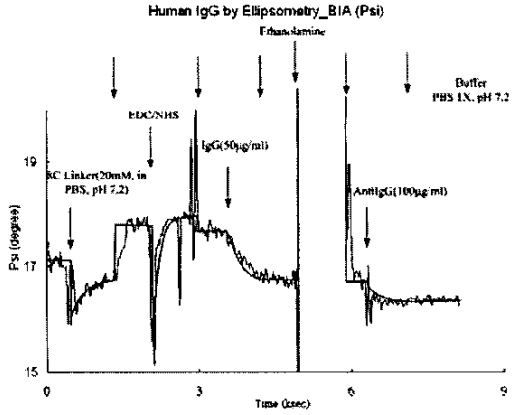
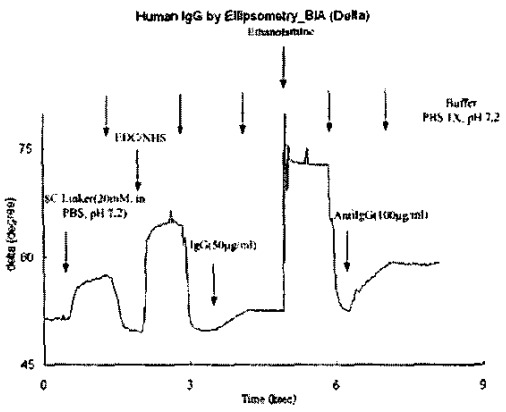


Fig.8 (d) IgG (50μg/ml) and Anti-IgG (20μg/ml) reacting relation

Comparing the response curves of the four anti-IgG concentrations, it can be found that the angle shift before and after the IgG and Anti-IgG reactions is proportional to the concentration. Furthermore, the higher the concentration is, the shorter the reaction time is. Fig.9 shows the result of the measured ellipsometry parameters at the angle near the SPR resonance angle while the the BIA procedure is adapted. The result shows that the ellipsometry parameter, psi is more sensitive than delta, and its S/N ratio is also higher. For this reason, it can be illustrated that the ellipsometry parameter, the resolution of psi is 1 order higher than delta. By the measurement, the result of ESPR benefits obviously on the solution of real biomedical reaction signals, and has more superiority than ones by SPR traditionally.



(a) psi



(b) delta

Fig.9 Ellipsometry parameters measure the SPR in BIA

IV. CONCLUSION

In ESPR, using these two special optical components which are parabolic mirror and spherical mirror can vary the incident angle easily, enhance the angle accuracy, and reduce the system size. Besides these, it is also important to understand the relations between the optic parameters such as reflective rate, thickness etc., and the different biomedical reaction conditions such as the concentration. This is because the affinity of molecules in solid-liquid interface can be derived from the relations.

To sum up, this paper introduces a newly optic-biochemistry examination system which constitute with ellipsometry and surface plasma resonance technology. Through carrying on two kinds of biochemistry reaction methods, ELISA and BIA, ellipsometry which exactly measured the thickness and birefraction rate of thin film had the advantage of non-contact, rapid and high accuracy, and SPR function had advantage of measuring real-time biochemistry reaction and determining the affinity of molecular. In the future, the system will develop a multiple function biomedical examination facility successfully with more research fellows forward this part of the biomedical field.

V. REFERENCE

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