A Compact Imaging Ellipsometer for Label-free Biosensor

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Abstract — **A compact model of imaging ellipsometer is used for the data acquisition and analysis of label free protein microarray. Its principle, methodology, and experimental setup as well as sampling conditions and a demonstration result are presented here. Furthermore, results of protein adsorption and protein interaction in the microarray may be deduced. This shows that the compact model is effectively performed as a reader for the protein microarray sampling and provides a potential for applications of label free protein microarrays on site of care.**

Keywords — **imaging ellipsometer, compact model, label free protein microarray.**

I. INTRODUCTION

The biomolecule adsorption and biomolecule interactions are common interests in biology and medicine. A label-free protein microarray biosensor based on imaging ellipsometry [1] has been developed, which combines the imaging ellipsometer with protein microarray and has properties of label free, high throughput and fast test [2, 3, 4]. Its principle is that each ligand as a sensing probe individually immobilized on each unit of a patterned surface in a microarray, specifically bind corresponding receptors with their affinity resulting in an increase of biomolecule mass surface concentration. The increase can be measured with an imaging ellipsometer to realize a protein test [5]. It has been used for the biomedical applications including tumor marker detection, hepatitis B test, virus detection and so on [6, 7]. At present, the need for the biosensor to be easily deployed in the field and practical environment is undiminished. However, such kinds of imaging ellipsometer are cumbersome, complex and costly, which limit their applications in field monitor and on site of care testing, a compact imaging ellipsometer with low cost and high accuracy is required. Here, we present a compact model as an effective instrument for protein microarray screening.

II. EXPERIMENTAL DESIGN

Several steps of experiment are designed for the evaluation of a compact model of imaging ellipsometer used as a reader for protein microarray.

(1) Protein monolayer measurement: the thickness of protein saturated adsorption monolayer usually ranges in several nanometers, which is transparent in visible range and could be hardly seen by optical microscope [4]. The compact model should visualize the layer thickness distribution with an image in grayscale and the value in grayscale corresponds to the thickness.

(2) Protein interaction measurement: Ligand as a sensing probe on a solid substrate is incubated in a protein solution, the ligand and the receptor in the solution would bind into bio-complex, if there were its receptor in the solution, which would result in the biomolecule mass surface concentration on the substrate increase. The increase should be measured within a dynamic range and a quantitative layer thickness variation from the biomolecule interaction should be obtained.

(3) Measurement in a large area: the protein microarray is a high throughput technique with a large area pattern of multi-ligand in array around square centimeter for multi-target testing, so a performance for a large area visualization in square centimeter is required.

III. COMPACT MODEL OF IMAGING ELLIPSOMETER

The principle of imaging ellipsometer is that an expended parallel light beam illustrates a sample which modulates the polarized state of the reflection and is imaged by a CCD, the sample's properties, such as the thickness or refractive index, will be deduced with the image analysis.

Fig.1 gives the schematic diagram of a compact imaging ellipsometer which is based on the Polarizer – Compensator – Specimen – Analyzer (PCSA) configuration. A 530 nm LED with width 40 nm is used to provide a stable light source. The angle of incidence is set at 75° close to the quasi-Brewster angle of Si substrate. With an optimal azimuth setting of polarization elements P, C and A for a high contrast of imaging, the detected intensity is approximately linearly proportional to the thickness corresponding to the surface concentration [8], which provides a thickness resolution in a 0.1 nm order and a lateral resolution in micron. The field of view reaches to several square centimeters. The image captured by CCD is converted to digital and stored in 8 bit, $0-255$ grayscale, the value in grayscale is proportional to the layer thickness

on Si substrate. The digital image may be converted to the layer thickness distribution in 3 dimensions. For an image with over 10^5 pixels, the capture speed is about 25 frame/S. In practice, the average over more frames may improve the ratio of signal to noise. The volume of the compact model is 370×230×110 (mm3).The autocollimator is used simply to locate the sample for test.

Table 1: schematic layout of the protein microarray

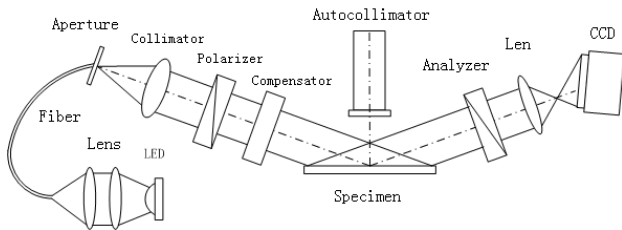|	A	B	C
1	Blank control	IgG	Fib
2	Biotin	IgG-AntiIgG	Fib-AntiFib
3	Biotin	IgG-AntiIgG	Fib-AntiFib

Fig. 1: schematic of a compact imaging ellipsometer

IV. FABRICATION OF PROTEIN MICROARRAY

We use a micro fluidic system of protein microarray [9] to fabricate a sample with 8 protein units in microarray. The fabrication process is as the following:

(1) Silicon wafer preparation

Polished silicon wafer is cleaned with a mixture of 30% H2O2 and concentrated H2SO4 (1:3 v/v) and placed to a rotate platform for 30 minutes. After thoroughly rinsed with deionized water and pure ethanol for 3 times each, it is treated with an ethanol solution of APTES (5% APTES and 95% pure ethanol) and the incubation lasts 2 hours at room temperature. Following by intensively rinsing with pure ethanol, the silicon wafer is reacted with over-saturated succinic anhydride in ethanol on a rotate platform overnight. Finally, the silicon wafer should be stored in pure ethanol after rinsing enough.

(2) Protein microarray fabrication

The 8 units on a microarray are selected to immobilize different proteins on modified surface by the interaction between carboxyl group on the silicon surface and amido group in protein. Each unit is a 2 mm by 0.5mm rectangle, and the interval between 2 units nearby is 1 mm by 1 mm approximately. As shown in table 1, Biotin at the concentration of 2.5mg/mL, IgG and Fib are assembled on column A, B and C for 10 minutes, respectively. Afterward, Anti-IgG antibody and anti-Fib antibody were added to their corresponding antigen units for 10 minutes, in order to ensure the recognition between them and the formation of antigen-antibody complex.

V. RESULT AND DISCUSSION

Fig.2. presents the image of the protein microarray sample in grayscale and in 3 dimensions, which is obtained by the compact model. Table 2 gives the value in grayscale averaged over each unit of the protein microarray which corresponds to the layout, the roughness in each unit corresponding to the surface concentration of proteins.
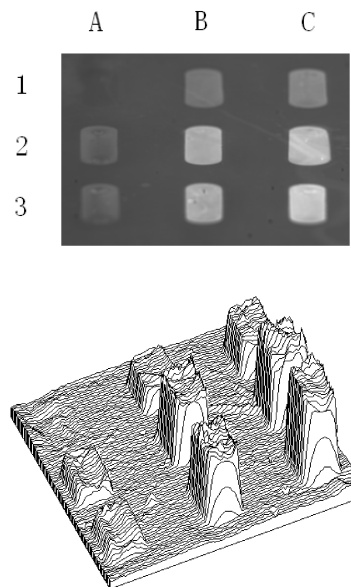

Fig. 2: the image of the protein microarray

Table 2: the values in grayscale upon units in the protein microarray
(relative error: ±2 , the substrate: 80)

	A	B	C
1	/	117	133
2	94	169	194
3	95	168	198

Due to the oblique incidence of the imaging ellipsometer, there is a difference of magnification between X and Y axis of image so that an image of each unit like a square corresponding to a rectangle unit in the protein microarray. We could see the image in good contrast and definition with obvious gradient in grayscale to visualize protein adsorption

layers in each units of the microarray, such as A2、A3 and B1、C1 units where there are several kinds of protein saturated adsorption layers, which reflect different protein mass surface concentrations. The Biotin molecule mass is only 244 D, the thickness about 1 nm calibrated with a conventional ellipsometer. The difference of values in grayscale between the Biotin layer and the substrate is around 15, which shows the thickness resolution of better than 0.1 nm, and is able to visualize small molecule layers.

Compared with adsorption monolayer on B1、C1 units，B2、B3 and C2、C3 with binding complex layers of antigen and antibody upon their affinity, so the larger mass surface concentration than monolayer and the higher in grayscale, which shows that it is possible to distinguish biomolecule interactions. To show the repeatability, we perform the same protein interaction in the lines 2 and 3 and get results in a good agreement. There are some dots with grayscale values much different from other neighbors', which means local pollution during the fabrication seen clearly. This is another advantage of the distinct power of the compact model.

Some more protein microarray specimens are screened with the compact system to verify the validity of the device used in the biosensor. The experiment results show that the mono-layer of Biotin even with a molecule weight of 244 D, and protein complexes due to protein interaction are all quantitatively acquired with a high resolution. Furthermore, the visual result of 48 areas in protein microarray shows that a variety of target molecules can be quickly measured at the same time with acquisition time less than 1 s, so it is demonstrated that the compact instrument is able effectively to screen the protein microarray with label-free, and has a promising potential for practical applications of the biosensor on site of care testing.

VI. Conclusion

This work shows that a compact model of imaging ellipsometer is possible to be used as a reader for protein microarray, which provides a potential in applications of the protein microarray biosensor based on imaging ellipsometry in test site-interested.

Acknowledgment

References

1. Jin G, Roger J, Arwin H. (1996) Imaging ellipsometry revisited: Developments for visualization of thin transparent layers on silicon substrates. Rev. Sci. Instrum, 67:2930-2936.
2. Jin G, Wang ZH. (2002) Micro-systems for optical protein-chip.Int. J. Nonlinear Sci. Number. Simul. 3:191-194
3. Jin G, Wang ZH, Meng YH. (1998) Imaging ellipsometry for the visualization of bio-molecular layers. in Proc. 20th Inernational Conference of the IEEE Engineering in Medicine and Biology Society. Hong Kong. IEEE, 581-584
4. Wang ZH, Jin G. (2003) A lable-free multisensing immunosensor based on imaging ellipsometry. Anal Chem, 75:6119-6123
5. Jin G, Wang ZH, Meng YH et al. (2001) Optical proteinchip as microarrays for protein interaction determination. in Proc. 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Istanbul, Turkey, p. 58-61
6. Zhang HG, Qi C Wang ZH et al. (2005) Evaluation of a new CA15-3 protein assay method: optical protein-chip system for clinical application. Clinical Chemistry, 51:1038-1040.
7. Qi C, Duan JZ, Wang ZH. (2006) Investigation of interactions between two neutralizing monoclonal antibodies and SARS virus using biosensor based on imaging ellipsometry. Biomed Micro-devices. 8:186-192.
8. Y-Y. Chen, Y-H. Meng, and G. Jin (2007) Optimization of off-null ellipsometry for air/solid interface [J]. Appl Opt 46:8475-8481
9. Zhan-hui Wang, Yong-hong Meng, Pei-qing Ying, Cai Qi, Gang Jin (2006) A label-free protein microfluidic array for immunoassays. Electrophoresis, 27:4078-4085