International Journal of Nanotechnology (IJNT)

Volume 4 - Issue 1/2 - 2007

Special Issue on Nanotechnology in China

Guest Editors: Jianguo Hou, Enge Wang and Shihe Yang

Table of Contents

Editorial

» Objectives
» Readership
» Contents
» Subject Coverage
» Editorial Board
» Specific Notes for Authors
» Sample issue

Browse Published Issues:

» 2007 Vol.4 No. 1/2
» 2006 Vol.3 No. 4
» 2006 Vol.3 No. 2/3
» 2006 Vol.3 No. 1
» 2005 Vol.2 No. 4
» 2005 Vol.2 No. 3
» 2005 Vol.2 No. 1/2
» 2004 Vol.1 No. 4
» 2004 Vol.1 No. 3
» 2004 Vol.1 No. 1/2

Biosensor with total internal reflection imaging ellipsometry

Y-Y. Chen

Institute of Mechanics, Chinese Academy of Sciences,
#15, Bei-si-huan West Rd.,
Beijing 100080, P.R. China

Graduate School of Chinese Academy of Sciences.
#19, Yu-quan Rd, Shi-jing-shan District,
Beijing 100039, P.R. China
E-mail: yychen@imech.ac.cn

Z-H. Wang

Institute of Mechanics, Chinese Academy of Sciences,
#15, Bei-si-huan West Rd.,
Beijing 100080, P.R. China
E-mail: wangzh@mit.edu

Y-H. Meng

Institute of Mechanics, Chinese Academy of Sciences,
#15, Bei-si-huan West Rd.,
Beijing 100080, P.R. China

Graduate School of Chinese Academy of Sciences.
#19, Yu-quan Rd, Shi-jing-shan District,
Beijing 100039, P.R. China
E-mail: yhong@imech.ac.cn

G. Jin*

Institute of Mechanics, Chinese Academy of Sciences,
#15, Bei-si-huan West Rd.,
Beijing 100080, P.R. China
Fax: +86-10-62631816 E-mail: gajin@imech.ac.cn
*Corresponding author

Abstract: In order to monitor multiple protein reaction processes simultaneously, a biosensor based on imaging ellipsometry operated in the total internal reflection mode is proposed. It could be realised as an automatic analysis for protein interaction processes with real-time label-free method. Its principle and methodology as well as a demonstration for its applications are presented.

Keywords: biosensor; imaging ellipsometry; total internal reflection; micro-array.

Reference to this paper should be made as follows: Chen, Y-Y., Wang, Z-H., Meng, Y-H. and Jin, G. (2007) 'Biosensor with total internal reflection imaging ellipsometry', *Int. J. Nanotechnology*, Vol. 4, Nos. 1/2, pp.171–178.

Biographical notes: Yanyan Chen, PhD candidate in the Institute of Mechanics, Chinese Academy of Sciences, Bachelor Degree of Science 1999 and Master of Science 2002 in Northeast Normal University, Changchun, China, major research in characterisation of biomaterials with ellipsometry.

Z-H. Wang received PhD in Institute of Mechanics, Chinese Academy of Sciences 2004. He is Assistant Researcher in the Institute of Mechanics, mainly on microarray biosensors and the modification on the surface.

Y-H. Meng is an Engineer in Institute of Mechanics, Chinese Academy of Sciences. His research interest focuses on nanometre measurement methods and instruments, especially on ellipsometry.

Gang Jin, PhD in Physics of Pierre and Marie Curie Univ. of Paris France 1993, Associate Researcher in CNRS Bellevue France 1992–1994, Visiting Scientist in Linkoping Univ. Sweden 1994–1996, Visiting Professor in Lisbon Univ. since 1997, Membership in Asian Institute of NanobioSci. and NanoTech., Pusan, South Korea 2003–2005, Professor and Principal Scientist for Nanobiotechnology in Chinese Academy of Sciences at present, mainly on optical microarray biosensors and optical characterisation of biomaterials.

1 Introduction

The biosensor concept based on imaging ellipsometry was first reported in 1995 [1,2]. By solving a series of problems, such as ligand immobilisation, protein and tested sample delivery, biomolecule affinity presentation on substrate, specific interaction between biomolecule, unspecific binding influence, sensitivity, sample consumption, in-situ sampling and calibration for quantitative detection, etc., it has become an automatic technique for protein analysis with properties of label-free, multi-protein detection, real-time analysis for protein interaction processes and quantitative analysis, etc. [3].

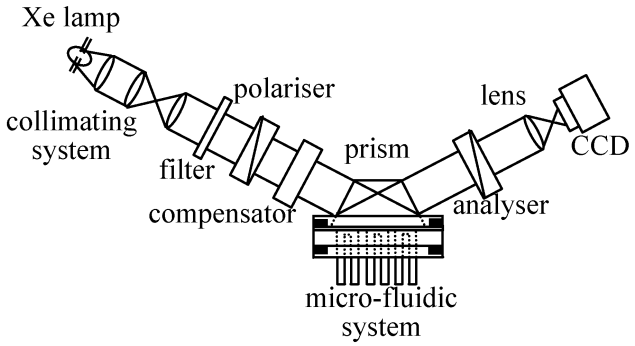
In previous performance, imaging ellipsometry with an ability to visualise biomolecule monolayers is operated in the external reflection mode. Actually, there are two modes of imaging ellipsometry, which can be performed – the external reflection mode and the internal reflection mode. For the former, the probe light is reflected at an interface where the refractive index of the incident medium is less than that of the reflecting medium. Under the mode, the real time measurement requires a cell to hold the liquid. A common design is an open glass cell with a typical cell volume of 1–2 ml [4]. For biological applications, it is preferable to use a significantly smaller sample volume. Moreover, when the protein solution is injected into the cell, the solution concentration is disturbed, which influences the imaging quality. The fact that only one kind of protein solution is detected in the cell, limits the application for more sample measurements at the same time. Therefore, the internal reflection mode is proposed, which is performed in the situation that the refractive index of the incident medium is larger than that of the

reflecting medium. So far ellipsometry operated in the internal reflection mode has been investigated by several groups. A very high sensitivity is seen to the deposition of thin layers on metal films from a liquid under the surface plasmon resonance (SPR) condition [5]. Especially for very small biomolecules, it may be a better detection method than conventional ellipsometry or SPR [6,7]. In this paper, the imaging ellipsometry operated in the total internal reflection mode, called total internal reflection imaging ellipsometry (TIRIE) here, is proposed to realise a biosensor for multi-protein interaction processes detection at the same time. A full picture of the biosensor and methodology as well as some experiment results is presented.

2 Methodology

Figure 1 shows the schematic of the TIRIE system, which is based on an imaging ellipsometer described previously [1,2], but equipped with a glass prism optically contacting with a glass slide by an index-matching liquid to realise the internal reflection. The other side of the slide is coated with a thin gold film as a substrate for ligand immobilisation and contacted test solutions, and a micro-fluidic system is designed for microarray biosensor functions in liquids. The probe beam passes through a polariser, a compensator (a quarter wave plate), perpendicularly into the glass prism and finally onto the gold film. When the incident angle is larger than the critical angle of total reflection, no transmission propagates through the gold film into the solution, and total internal reflection phenomena appear, at the same time only an evanescent field appears on the surface of the gold film. The depositions of some biomolecule layers on the surface modify the evanescent field, so that the polarised state of reflection is changed with the biomolecule layers. In this case, the analysis of the polarised state of reflection will provide information of the biomolecule layers to realise a biosensor function. Moreover, the fact that the decay distance of evanescent field propagating into the layers on gold substrate surface is about 100 nm or so [7], makes the solution concentration disturbance have almost no influence on the image. The reflection beam passes an analyser, an imaging lens to a CCD camera, and a series of images with information of biomolecule layers interaction process is recorded by the TIRIE system. The principle and technical details are presented in what follows.

Figure 1 Schematic view of the TIRIE system



A. Principle of biosensor

Biosensor based on TIRIE is described as given above. Here we introduce the principle of the biosensor. A ligand and its receptor such as an antibody and its corresponding antigen can assemble into complexes due to their affinity. The optical biosensor is based on the fact that each reactant as a ligand is immobilised to a surface to form a monolayer as a bioprobe with its bioactivity. The other reactant as the analyte (or receptor) exists in a solution. The bioprobe is exposed to the solution containing the analyte. When the analyte in the solution interacts with its corresponding ligand on the bioprobe and assembles into complex upon their affinity. The molecule concentration on the surface where the interaction takes place becomes higher than before exposure to the analyte solution. A significant increase of the layer thickness (surface concentration) with time indicates a kinetic process of the interaction. The process of the interaction can be visualised with TIRIE which has a high spatial resolution in the order of 0.1 nanometre in the vertical and a micron in the lateral, and in this way, the existence of the analyte in the solution can be verified, and then kinetic data can be deduced. Many bioprobes arrayed in the matrix on the slide surface are used for the detection of multi-protein interaction processes.

B. Micro-fluidic and bio-molecule interaction cell

The importance of protein arrays in biological research is presented in many reports [8–10], but most of them are analogous to DNA chips in terms of fabrication and detection. Proteins are labelled and the results are detected with fluorescence readers. Thomas Kodadek points out several problems of the protein labelling [8], such as the difficulty in quantitative analysis, the efficiency of labelling, protein denaturation, and a labour-intensive consumption, etc. To get quantitative and reliable results, the spots on a protein chip should have a homogeneous surface and the same size. It is difficult to get good enough spots by the spotting or printing techniques currently used in protein array fabrication [9].

Recently, micro-fluidic devices formed in poly(dimethylsiloxane) (PDMS) have received an increased amount of interest for a simple, rapid, and low-cost fabrication methodology [11,12]. The employment of a micro-fluidic system makes smaller sample volumes available. For the fabrication of a protein array and the reaction of a protein array with analyte solution, a micro-fluidic system in PDMS to fit the TIRIE has been developed in our laboratory [13], which includes an 8×6 cell array, and each cell volume is minimised to only 50 nl. When the 8×6 cell array of the micro-fluidic system is attached to the gold surface, 48 individual chambers are formed independently and the gold surface is patterned into 48 elliptic spots (each about 1 mm^2) in the array. By such a micro-fluidic system, many kinds of molecules can be delivered individually to each spot of the array and immobilised on the surface simultaneously. The homogeneous biomolecule layers in the spots of the array can be obtained by this solution delivery method, which is helpful of quantitative measurements. Moreover, the micro-fluidic system can minimise the sample consumption of biomolecule (15 μl) effectively and reduce the test time (normally within 30 minutes). Especially, it can be repeated used for real time measurement so that the cost of the array test is reduced.

C. Automatic system of TIRIE

The protein array biosensor based on TIRIE is a fast and convenient detection technique for the measurements of protein interaction processes. Multiple analytes can be detected simultaneously and samples can be measured directly without any labelling. Imaging ellipsometry combines the power of ellipsometry with microscopy. The one used in this study is an automatic system, and ellipsometric conditions can be controlled by auto-adjusting the polariser and the analyser; the angle of incidence is variable from 45° to 90° with a resolution of 0.05°; the magnification of image to object is modulated according to the dimension of the field of view, so that the lateral resolution of a micron can be reached; auto-focusing is realised with the standard of the Laplacian algorithm [14]. All the adjustments are carried out automatically with micro-stepping motors controlled by a computer with home-made software. The light source is a xenon lamp, and a specific collimating system is used to provide an expanded parallel probe beam with a diameter of about 25 mm. An optical filter at 633 nm wavelength is placed in the incident optical passage in order to obtain a high contrast in ellipsometric images. The beam passes through a polariser and a compensator, then perpendicularly into a SF10 glass prism and finally onto the gold film patterned with the protein array. The reflection beam passes through an analyser and an imaging lens with a spatial filter located at its focus plane, and then the ellipsometric image is focused onto the sensing area of the CCD camera. Here, the protein solutions are delivered by the micro-fluidic system, and protein interaction processes are automatically captured in a series of image in grey scale format (8 bits, 0–255 grey scale) and processed with the same software.

D. Quantitative detection

For images, the grey value corresponds to the reflection intensity of the protein layer, which is related to the protein surface concentration, so it is preferable to obtain the higher variation in grey value corresponding to a certain increase of the protein surface concentration on the gold substrate surface, that is a high sensitivity for the detection of protein binding process is needed, which can be realised by the optimisation of the TIRIE, such as the choice of incident angles, wavelengths, gold film thickness [7] and the azimuth angle settings of the polariser, compensator and analyser [15]. Under the optimal conditions, the relationship between the detected intensity ‘I’ and the protein surface concentration is known for a certain protein.

A series of images is captured in the kinetic process of protein interaction, which means the change in the protein surface concentration on the gold substrate surface over time can be obtained to form the binding curve. It provides a possibility further to deduce kinetic data with theoretical models. In order to determine the surface concentration corresponding to the grey value, some quantitative measurements should be performed by some auxiliary experiments. Ellipsometry is a common choice to do this, by which the parameters of the biomolecule layer, such as refractive index and layer thickness, can be deduced. Usually the refractive index of saturated protein layers in the visible light region is in the range of 1.4–1.5 [16], and the relative variation of layer thickness (th) is of interest, but not the absolute value of the thickness, so for simplicity the refractive indices of the protein layers are supposed the same, so that the thickness of the protein layer is obtained by conventional ellipsometry. The relationship between protein surface concentration and layer thickness is

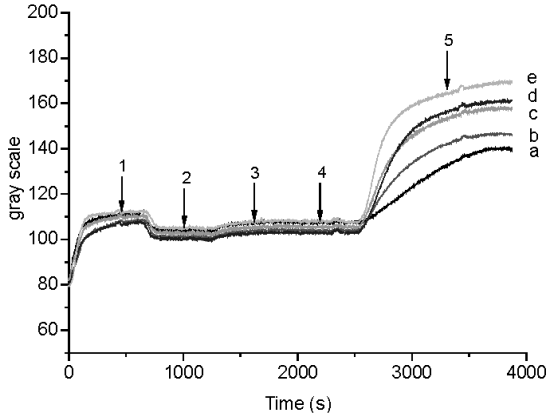
the surface concentration ($\mu g/cm^2$) ≈ *K*th (nm),

where $K \approx 0.12$ [17].

3 Applications

As a demonstration, the multiple protein interaction processes of hepatitis B virus surface antibody (HBsAb) adsorption on bare gold-coated substrate surface, PBS buffer (100 mM phosphate, 100 mM NaCl pH 7.4) rinsing, bovine serum albumin (BSA) blocking, and interactions between HBsAb and hepatitis B virus surface antigen (HBsAg) with five concentrations were detected simultaneously with the TIRIE. The gold-coated substrate was prepared by the evaporation of 2 nm of chromium on SF10 glass slides, and added by the evaporation of 35 nm of gold. The prism was made of SF10 glass with the incidence angle of 59º. After the prism and the micro-fluidic system were mounted on the sample holder of the imaging system, HBsAb solutions with the concentration of 0.1 mg/ml were first pumped into five individual micro-cells in an array patterned on gold surface by the micro-fluidic system at a flow rate of 10 µl/min. Following 10 minutes incubation, PBS buffer was used to rinse the cells for 10 minutes. BSA solution prepared in PBS buffer as 10 mg/ml was then injected into all micro-cells to block the gold surface for 10 minutes, and was replaced by PBS buffer thoroughly to rinse the cells for 10 minutes. HBsAg solutions diluted by PBS solution to the concentration of 80, 60, 36, 24 and 12 µg/ml were pumped into the micro-cells at the same flow rate to bind with the antigens, respectively, and a series of images of the array was captured at the same time with a rate of 1 s/frame. The whole binding processes were shown in Figure 2, which proved that multi-protein interaction processes could be detected simultaneously.

Figure 2 Five kinetic processes shown in grey scale vs. time, which obtained simultaneously by TIRIE. Curve a-e represented 5 micro bioprobe results in the array, and the region 1 was corresponding to the HBsAb (0.1 mg/ml) adsorption on gold-coated substrate surface, the 2- PBS rising, the 3- BSA blocking, the 4- PBS rising and the 5- HBsAg interacting with HBsAb, respectively. Curve a–e were related to different HBsAg concentrations of 80, 60, 36, 24 and 12 µg/ml during the interaction processes between HBsAb and HBsAg, respectively. The flow rates in the whole process were 10 µl/min



4 Conclusion

Imaging ellipsometry operated in the total internal reflection mode has provided a potential for biosensor applications, especially for kinetic process detection of biomolecule interaction. It is possible to obtain kinetic measurement of multiple biomolecule reactions simultaneously since imaging ellipsometry has function of fast imaging for a large field of view, non-disturbance, and qualitative and quantitative detections with label free, etc. A micro-fluidic system fitting to a high throughput manner integrates more functions of solution delivery, ligand immobilisation, rinsing, surface blocking, biomolecule interaction with less sample consumption, fast detection, and high sensitivity, etc. The biosensor with the TIRIE may avoid the effect of solution concentration disturbance on the image since the detection with evanescent field under the total internal reflection conditions.

Acknowledgements

The national natural science foundation of China (No. 90206029) and Chinese Academy of Sciences are acknowledged for their supports.

References

1 Jin, G., Tengvall, P., Lundstršm, I. and Arwin, H. (1995) 'A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions', *Anal. Biochem.*, Vol. 232, pp.69–72.

2 Jin, G., Jansson, R. and Arwin, H. (1996) 'Imaging ellipsometry revisited: developments for visualization of thin transparent layers on silicon substrates', *Rev. Sci. Instrum.*, Vol. 67, pp.2930–2936.

3 Wang, Z.H. and Jin, G. (2003) 'A label-free multisensing immunosensor based on imaging ellipsometry', *Anal. Chem.*, Vol. 75, No. 22, pp.6119–6123.

4 Arwin, H. (1998) 'Spectroscopic ellipsometry and biology: recent developments and challenges', *Thin Solid Films*, Vols. 313–314, pp.764–774.

5 Thompson, N.L. and Lagerholm, B.C. (1997) 'Total internal reflection fluorescence: applications in cellular biophysics', *Curr. Opin. Biotechnol.*, February, Vol. 8, pp.58–64.

6 Westphal, P. and Bornmann, A. (2002) 'A. Biomolecular detection by surface plasmon enhanced ellipsometry', *Sens. Actuators, B*, Vol. 84, pp.278–282.

7 Arwin, H., Poksinski, M. and Johansen, K. (2004) 'Total internal refection ellipsometry: principles and applications', *Appl. Opt.*, Vol. 43, pp.3028–3036.

8 Kodadek, T. (2001) 'Protein microarray: prospects and problems', *Chem. Biol.*, Vol. 8, pp.105–115.

9 Talapatra, A., Rouse, R. and Hardiman, G. (2002) 'Protein microarrays: challenges and promises', *Pharmacogenomics J.*, Vol. 3, No. 4, pp.527–536.

10 MacBeath, G. and Schreiber, S.L. (2000) 'Printing proteins as microarrays for high-throughput function determination', *Science*, Vol. 289, pp.1760–1763.

11 Esch, M.B., Locascio, L.E., Tarlov, M.J. and Durst, R.A. (2001) 'Detection of viable cryptosporidium parvum using DNA probes and liposomes in a microfluidic chip', *Anal. Chem*., Vol. 73, pp.2952–2958.

12 Deng, T., Wu, H.K., Brittain, S.T. and Whitesides, G.M. (2000) 'Prototyping of masks, masters, and stamps/molds for soft lithography using an office printer and photographic reduction', *Anal. Chem.*, Vol. 72, pp.3176–3180.

13 Jin, G., Wang, Z.H., Qi, C., Zhao, Z.Y., Meng, Y.H., Ying, P.Q., Chen, S., Chen, Y.Y., Gao, B. and Xia, L.H. (2005) 'A microfluidic system for microarray fabrication and biomolecule interaction', *EMBEC'05, 3rd European Medical and Biological Engineering Conference*, Prague, Czech Republic, November 20–25.

14 Meng, Y. and Jin, G. (2000) 'The study for the autofocusing of imaging ellipsometry system', *J. Test and Measurement Technology*, Vol. 14, pp.220–225.

15 Wang, G.L., Arwin, H. and Jansson, R. (2003) 'Optimization of azimuth angle settings in polarizer-compensator-sample-analyzer off-null ellipsometry', *Appl. Opt.*, Vol. 42, pp.38–44.

16 Arwin, H. (1986) 'Optical properties of thin layers of Bovine serum albumin, γ-globulin, and hemoglobin', *Appl. Spectrosc.*, Vol. 40, No. 3, pp.313–318.

17 Stenberg, M. and Nygren, H. (1983) 'The use of the isoscope ellipsometer in the study of adsorbed proteins and biospecificbinding reactions', *J. Phys. Coll.*, Vol. 44, pp.83–86.