

Development of Biosensor based on Imaging Ellipsometry and its Applications

Gang Jin^a

^a NML, Institute of Mechanics, Chinese Academy of Sciences, #15 Bei-si-huan west Rd., Beijing 100190, P.R. China

ABSTRACT

We have reviewed the development of the biosensor based on imaging ellipsometry including its principle, methodology and general engineering model structure, mainly compared experimental setups between the previous one and the recently developed one. It's obvious that the sensitivity and the signal to noise ratio has been improved by a various spectroscopic light source, the optimization of polarized components setting and a cool CCD, especially the contribution of the CCD, which makes the biosensor available in more and more biomedical applications.

Keywords: Biosensor, imaging ellipsometry, protein interaction, protein microarray.

1. INTRODUCTION

The concept of biosensor based on imaging ellipsometry was proposed 15 years ago [1]. The prototype of experimental setup and the evaluation on the biosensor application was successively reported [2]. It has been developed into an automatic analysis technique for protein detection with merits of label-free, quantitative, high throughput and real-time analysis for protein interaction process, etc. Protein delivery and interaction is performed with a micro-fluidic reactor array. Imaging ellipsometry is used to visualize the molecule surface concentration distribution of protein microarray on a solid substrate. The protein microarray is designed upon the demand of target detection, such as the target molecule type, concentration, numbers and detection conditions. The biosensor is based on biomolecule specific affinity that one reactant as a ligand is immobilized to a surface to form a monolayer as a bioprobe to recognize its receptor. With the visualization of imaging ellipsometry which has a high spatial resolution in the order of 0.1 nm in vertical and micron in lateral, the complex of ligand-receptor can be determined, and in this way, the existence of the specific biomolecule to the ligand can be verified. Many bioprobes arrayed in matrix that is a protein microarray are used for high throughput protein detection. Its principle, methodology, related technique units, biosensor system and previous works are reviewed in Ref. [3].

With the development of its engineering model and applications, especially biomedical applications, further demand on the sensitivity and flexibility of the biosensor is more important. The flexibility mainly depends on the mechanical, electrical, informatics and biological control. To the requirement in biomedical applications, its operational mechanism and the signal to noise ratio (SNR) could not be fitted, especially for cases in the real-time analysis of protein interaction and protein weak interaction, so the biosensor in total internal reflection (TIR) mode of imaging ellipsometry [4] has been developed. It combines the microfluidic reactor array with imaging ellipsometer for an in-situ system. Considering the dispersive effect of the microarray, the related experimental condition to the sensitivity and SNR, we have introduced a spectroscopic light source, optimization settings of polarization components and a low noise CCD imaging which brings an obvious improvement in the sensitivity and SNR. It makes specifications of the biosensor more fitted to practical requirements, the sensitivity for immunoglobulin molecule in 1ng/mL, the consumption of ligand in ng and specimen in the order of 10 μ l, the throughput of 48 independent channels, and the time resolution of 0.04s as well as a continues variable spectrum in visible region. These parameters do not reach to their limitations and could be further improved upon requirements.

In order to show an overview of the biosensor, we look back the principle, methodology and the experimental setup; especially pay attention to the imaging ellipsometer and its improvement in this report.

2. PRINCIPLE AND METHOD [3]

Imaging ellipsometry is used to visualize the molecule mass surface concentration distribution of protein layers attached on a patterned surface as shown in Figure 1. A ligand and its receptor such as an antibody and its corresponding antigen could assemble into bio-complexes due to their specific affinity. The optical biosensor is based on that each reactant as a ligand is immobilized to a surface to form a monolayer as a bioprobe with its bioactivity. The other reactant as the analyte (or receptor) existed in a solution. The bioprobe is exposed to the solution containing analyte. When the analyte in the solution interacts with its corresponding ligand on the bioprobe and assembled into complex upon their affinity. The molecule mass surface concentration on the surface where the interaction takes place becomes higher than before exposure to the analyte solution. A significant increase of the surface concentration indicates that the solution contains receptor against the ligand on the surface. With the visualization of imaging ellipsometry which has a high spatial resolution in the order of 0.1 nm in vertical and micron in lateral, the increase could be determined, and in this way, the existence of the analyte in the solution can be verified. Many bioprobes arrayed in matrix to form a protein microarray is used for high throughput detection [5-7].

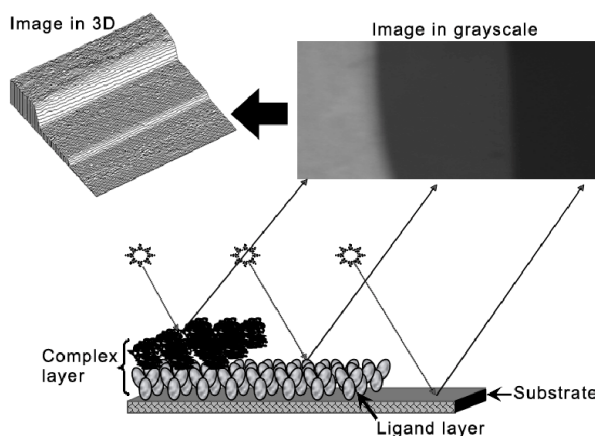


Figure 1 Model of bioprobe based on imaging ellipsometry [3]. The incident wave of polarized light as probe beam irradiates the sample and is modified, which makes the reflective beam carry sample information, such as protein surface concentration. The sample includes three parts: the bare substrate, the ligand layer and complex layer on the substrate, which could be seen in ellipsometric image with the value of reflection intensity I in grayscale. The variation of the protein surface concentration causes the value in grayscale of the image change. The surface concentration distribution of protein layer could be easily seen in three dimensions.

3. GENERAL STRUCTURE OF THE BIOSENSOR AND EXPERIMENTAL SETUP

Figure 2 gives the schematic diagram of a general structure of the biosensor based on imaging ellipsometry.

The biosensor is combined with several parts that are the control system, the microfluidic reactor array, the imaging ellipsometer as a reader and the biosensor database as well as software.

The control system has functions of hardware motion control and image data acquisition, played crucial role for performance and operation of the biosensor as an automatic system. A PC computer is used as the operation platform and data processor. Under the operation of homemade special software, a motion control card inserted in the computer produced motion signal for motor driver. The mechanical motion parts in microfluidic reactor array and imaging ellipsometer powered by the motor is driven automatically when needed. An image board inserted in the computer is used to transfer the image signal captured with imaging ellipsometer to a digital format.

The microfluidic reactor array is designed for protein array fabrication, solution delivery and biomolecule interaction. A microfluidic array containing many channels is used for fluid transferring to the sensing surface and forming a protein

array [8]. Each channel has independent inlet and outlet, which can be used individually to deliver solutions to each cell of the array and covalently immobilize ligands on surface simultaneously. The fluid such as protein solution, purified water, rinsing fluid in each test tube is transferred to the microfluidic array by a peristaltic pump successively.

A protein array prepared by the microfluidic reactor is used for a parallel detection of protein interaction, and then, the protein microarray would be visualized by the imaging ellipsometer for protein detection. The imaging ellipsometer is based on a configuration of Polarizer-Compensator-Specimen-Analyzer (PCSA). The probe light beam with single wavelength is expended and collimated to ensure that each point in the view field of the protein array has the same angle of incidence. In order to carry out the quantitative measurement, off-null ellipsometry sampling method [2] is used due to its fast speed in the order of sub-second. The protein array is imaged by a lens into a 2D imaging sensor which transfers the image signal into computer and forms a digital image for further analysis. Under the operation of control system, the imaging ellipsometer system featured some automatic functions including the auto-focusing, the setting of polarizer, analyzer, compensator and the variation of the angle of incidence, etc.

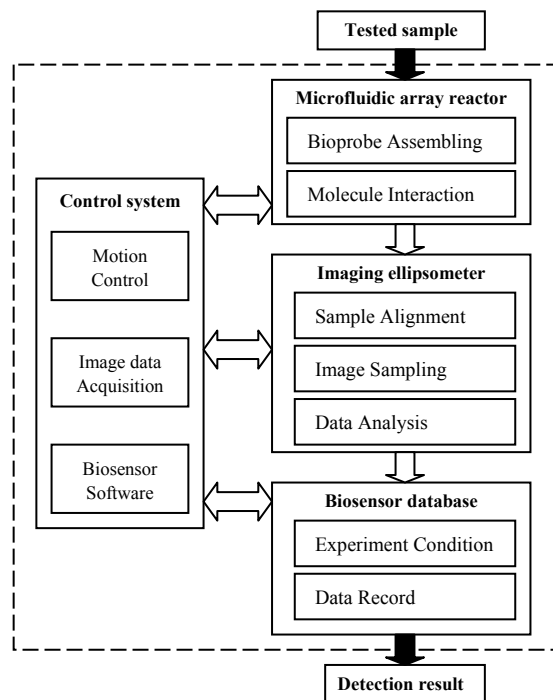


Figure 2 Schematic of Biosensor based on imaging ellipsometry [3]

The biosensor database is very important for the biosensor operation and applications. It has several functions: (1) Reference for experimental conditions setting from previous experiments; (2) Record and analysis of measurement conditions in microfluidic reactor array and imaging ellipsometer, such as measurement procedure and parameters, such as the fluid speed, reaction time, and polarizing components (polarizer, analyzer and compensator) setting, etc. (3) Record of the data of analyte, such as the bio-specificity, the molecule mass, concentrations, and corresponding ligands, (4) Record of detection condition, experiment data and analysis results, etc. All these are needed for users and potential applications.

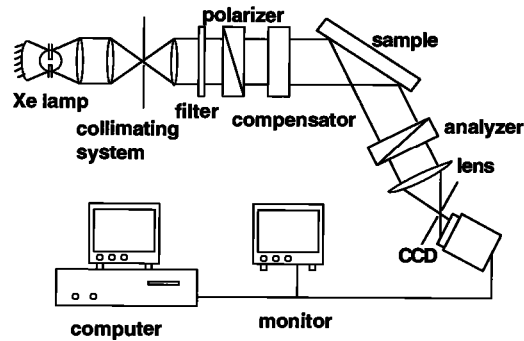


Figure 3 Schematic diagram of an imaging ellipsometer with an expanded beam, fixed polarizing components, and a CCD camera [1].

A general experimental set-up is shown in Figure 3 [1]. It is based on a PCSA ellipsometer with a collimated expanded beam and a CCD camera as a detector. In the design of the optical system, special consideration should be taken of the following.

Source intensity and detector linearity: operation in both null and off-null ellipsometric modes is required. Null ellipsometry is independent of detector linearity and source intensity fluctuations. However, off-null ellipsometry is not and therefore the stability of the light source and the linearity of the sensor elements in the CCD camera is important.

Parallel light: The expanded probe beam must have parallel light to ensure that the angle of incidence is the same over the entire test surface. This is essential for the quantification of the surface thickness distribution.

Beam uniformity: The intensity over the cross section of the probe beam must be constant, because the intensity distribution over the beam area after reflection is a measure of the thickness distribution of the layer under study. A non-uniformity of the probe beam intensity leads to errors in the thickness distribution.

In the previous experimental setups [1, 9], a 75 W xenon arc lamp and a collimating system are used as a light source to provide an expanded parallel probe beam with a diameter of 25 mm. The xenon lamp is mounted in a standard housing (60010 lamp housing, ORIEL, U.S.A.) and driven by a stabilized power supply (68805 universal power supply, ORIEL, U.S.A.) under constant current conditions. The collimating system consists of an optical condenser with a short focal distance lens (45 mm) combined with a double convergent lens collimator with a pinhole. The former is set close to the lamp in order to collect as much light as possible. Its focal point is located in the emitting region of the lamp. The off-focal light and the light reflected from the bulb wall introduce a nonparallel part in the beam, which is eliminated with the double convergent lens collimator. The back focal plane of the first convergent lens is coincident with the front focal plane of the second convergent lens and the pinhole is located in the coincident plane of the two focal planes. When the quasi-parallel beam from the condenser passes through the first lens, the parallel part of the beam will focus at the center of the focal plane and the nonparallel part will focus in the off-center region. The pinhole blocks the off-center part. There is a trade-off between intensity on one hand and parallelism and uniformity on the other hand. A high parallelism and uniformity requires that the diameter of the pinhole should be as small as possible; however, a small diameter results in a low intensity. In our setup, a pinhole diameter of 0.6 mm is used, which results in a non-parallelism and non-uniformity of the probe beam of less than 0.3° and 2%, respectively.

To increase the ellipsometric contrast, a wavelength selective filter (632.8 nm) with a bandwidth of 10 nm is placed in the incident optical path. The light then passes through a dichroic polarizer and a compensator with a quarter-wave-plate at the wavelength of 632.8 nm. The monochromatic and polarized beam is directed onto the sample at an angle of incidence of 76° . The reflected beam passes through another dichroic polarizer (called the analyzer) and a focusing lens. The lens projects the ellipsometric image of the sample on the sensor area of a CCD camera. The aperture of the lens should be larger than the illuminated region. In Figure 3 it is shown that the photosensitive chip of the CCD camera is set obliquely to the optical axis, in order to get a clear image of the entire sample surface. The reason for this is that the sample surface as an objective plane is located obliquely at the angle of incidence of the beam, and consequently the corresponding image plane is located non-perpendicularly to the optical axis of the system. The angle between the

normal of the CCD chip and the optical axis depends on the incident angle of the probe beam and the objective distance, that is, the distance from the sample center to the imaging lens.

An achromatic lens is used to image the specimen onto a scientific B/W video CCD camera (SONY XC-ST30CE, Japan), with a spectral range of 400 to 900 nm, sensitive area of 768 x 576 pixels on 6.0 mm x 4.96 mm, and single pixel size of 6.5 μ m x 6.25 μ m. The electronic gain can be fixed for quantitative measurements, the output signal speed is 30 frames per second at maximum, and the nonlinearity of the CCD camera is better than 1%. The image signal is transferred to an image board (Meter-II, Matrox, Canada) inserted in the computer for A/D converting. The ellipsometric images are recorded on an 8 bit (0~255) or 10 bit (0-1023) grayscale format. A monitor is connected to the video camera for sample positioning and null ellipsometry adjustments.

In normal operation, the compensator is fixed at an azimuth of 45° to the plane of incidence. The analyzer and the polarizer are adjusted so that the ellipsometric null conditions are fulfilled on a bare silicon substrate. The absolute beam intensity and the gain of the CCD camera are not necessary to determine. It is sufficient to measure the relative intensity distribution over the beam after reflection to obtain the thickness distribution of a layer, as long as a reference sample with known layer thickness is used for an absolute calibration. Thickness calibration measurements are done with a null ellipsometer. The refractive index used for SiO₂ is 1.457. The refractive index for proteins is in the range 1.48 – 1.57. Here we have used 1.5 throughout. Simple calculations using the exact ellipsometric expressions show that if the index is wrong, e.g. ± 0.02 , the thickness error is less than 2%.

4. RECENT IMPROVEMENT

Considering the dispersive effect of the protein microarray, the related experimental condition to the sensitivity and SNR, we have introduced a spectroscopic light source, optimization settings of polarization components [10] and a low noise CCD imaging [11].

We develop a spectroscopic imaging ellipsometry (SIE) [9, 12] system for the biosensor. It has the lateral resolution of optical imaging ellipsometry with various wavelengths. A 150-W Xenon short arc lamp (OSRAM, Germany) operated by a supply (Newport, U.S.) with light ripple less than 0.5% regulation accuracy is used to provide a stable outgoing flux with continuous spectrum from IR(1700 nm) to UV (190 nm). In order to adequately utilize the light flux, an achromatic convergent lens set with a focal length of 200 mm and a diameter of 50 mm is used to converge the light beam into the entrance slit of a mechanical scanning grating monochromator (7-Star, China) with a focal length of 150 mm and an f-number of 1/4.5. The monochromator controlled by a computer provides a spectrum of 260–1600 nm with adjustable entrance and an exit for the control of light intensity and bandwidth, respectively. A glass optical fiber bound (Chunhui, China) with a spectrum of 380–1300 nm and a diameter of 1.0 mm is mounted flexibly to transfer the incident light from the exit of the monochromator to the focus point of the second achromatic convergent lens set. It collimates the single wavelength light into an expanded parallel beam with a diameter of 25 mm for imaging ellipsometry at an angle of incidence.

Two Glan–Taylor prisms (MELLES GRIOT, USA) with a spectral range of 250–2300 nm and an extinction ratio of 10–5 are used as the polarizer and the analyzer, respectively. A mica retardation plate (MELLES GRIOT, USA) with a spectral range of 400–700 nm is used as a compensator. Its phase retardation between fast and slow axis is near 90°, which is calibrated by a commercial V-VASE ellipsometer (J.A. Woollam, USA) and fitted by the Cauchy model. In order to adjust the azimuth with respect to the plane of incidence, the polarizer, compensator, and analyzer are fixed in hollow annular rotation stages driven by a servo motor (Newport, US) with a maximum speed of 40°/s and an absolute accuracy of 0.015°. An achromatic imaging lens set (Zeiss, Germany) with a focal length of 50 mm, f-1.4 is used to image the specimen onto a cool CCD camera (Andor Technology, DU937N-BV, read noise 10.3e@2.5 MHz, dark current 0.0005e-/pixel/sec @ -80°C). The camera features a corresponding spectrum range of 400–900 nm, 512×512 active pixels, 13×13 μ m pixel size, 6.6×6.6 mm image area and boasts 95% QEmax., high dynamic range and exceptionally low readout noise. It benefits from negligible dark current with thermoelectric cooling down to -100°C, enabling use of significantly longer exposure times. The image signal is inputted into an image grab board (Matrox, Canada) inserted in the computer for analog-to-digital (A/D) conversion and the digital image processing and storage. The ellipsometric images are recorded in 14-bit grayscale format. Image averaging method available is used to improve the signal-to-noise ratio [13].

The improvement in imaging system brings an obvious improvement in the sensitivity and SNR. It makes specifications of the biosensor more fitted to practical requirements, the sensitivity for immunoglobulin molecule in 1ng/mL and the time resolution of 0.04s, the consumption of ligand in ng and specimen in the order of 10 μ l, the throughput of 48 independent channels, as well as a continuous variable spectrum in visible region. For a protein pattern on a gold layer/glass substrate, the imaging resolution corresponding to the sensitivity increases about 4 times when the wavelength is chosen from 550 nm to 600 nm, which shows the various spectroscopic light source is helpful for the biosensor data acquisition. Comparing with the previous system with the CCD of Sony XC-30CE used in TIRIE [4], the sensitivity has increased about one order and the detection limit for Hepatitis B surface antigen (HBsAg) 50 times better than the previous one. The main contribution to the improvement of the sensitivity and the detection limit comes from the use of the cool CCD [11].

5. BIOMEDICAL APPLICATIONS

The biosensor technique is developed mainly to focus on biomedical applications, and it has been successfully used for the biomolecule interaction [6, 7], the cancer marker test [14], the clinic diagnosis for hepatitis B [15], the quantitative protein competitive adsorption [16], the kinetic detection for multi-protein interaction process [4, 11], and the virus detection [17, 18], etc. These biomedical applications just show some examples of the biosensor based on imaging ellipsometry in biomedical fields.

6. CONCLUSION

The improvement of the imaging system has shown a better sensitivity and SNR in the biosensor, and proved one order better than the previous one. It is helpful for protein interaction process detection in real-time. In this way, the high sensitivity makes it available of fast detection and protein weak interaction detection.

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