Biosensor Based on Total Internal Reflection Imaging Ellipsometry

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Abstract: The principle of Biosensor based on total internal reflection of imaging ellipsometry, methodology, experimental setup and functions is reviewed, especially the settings of optimization condition, which has key importance for the biosensor sensitivity. The biosensor may be used for high throughput bio-interaction process detection between nucleic acid and protein, protein and protein, ligand and receptor, and even virus and ligand, etc. Some biomedical applications and an extension application of biological oxygen demand (BOD) detection are demonstrated.

Key-Words: Biosensor, imaging ellipsometry, biomedical applications.

1 Introduction

Various approaches of data acquisition for biosensor have been developed. Some labeling methods of fluorescence [1], Enzyme [2], radioactivity labeling [3], and even recently developed quantum dot [4], etc. play important roles in most biological research. They provide ultra-high throughput evaluation of molecular interactions, the approaches are well established and their shortcomings stick out a mile also. However, label-free methods, which rely on optical, acoustic, and other types of biosensors, notably Surface Plasmon Resonance (SPR) [5-8], Imaging ellipsometry [9], Revamping calorimetry [10], Quartz crystal resonators [11], Nanowires [12] and Ion Channel Switch (ICS) technology [13] and so on, improve on the conventional methods, although achieving high-throughput and sensitivity are still important challenges.

The biosensor based on total internal reflection imaging ellipsometry (BTIRIE) [14,15] being able to visualize protein monolayer has been presented for kinetic process analysis of biomolecule interactions several years ago. BTIRIE system is combined imaging ellipsometry performed in total internal reflection mode with a multichannel micro-fluidic system by an optical coupling. It has been described with functions of fast dada acquisition for a microarray, non-disturbance, and qualitative and quantitative detections with label-free, etc. Under the total internal reflection condition, the evanescent field appearing on a sensing surface is modified by biomolecule interactions, so that the polarized state of reflection changes. With the analysis of the polarized state of reflection, the information of biomolecule interactions can be deduced to realize a biosensor function. The use of evanescent field makes the biosensor have a very high sensitivity for a surface enhanced effect of evanescent wave and the optimization settings of the BTIRIE including the angle of incidence, the gold layer properties and azimuths of polarization elements, and it is not disturbed by the non-uniformity of solution refractive index also. We have developed it combining with a multichannel micro-fluidic system including an 8□3 cell array with 150 nl volume each cell, the function of microarray biosensor is realized, by which multiple biomolecule interactions can be detected simultaneously. Moreover, the sample consumption is minimized effectively to 15 μl or less [16].

2 Principle and experimental setup

Generally, a ligand and its receptor such as an antibody and its corresponding antigen could be assembled into a bio-complex due to their affinity. The optical biosensor is formed in a sense that each reactant as a ligand is immobilized to a surface to form a monolayer as a bioprobe with bioactivity. The other reactant as the analyte (or receptor) is in a solution. The bioprobe is exposed to the solution with the analyte. The analyte would interact with its corresponding ligand and assembled into a complex due to their specific bio-affinity. The molecule mass surface concentration on the surface where the interaction takes place would become higher than before exposure to the analyte solution. An increase of the mass surface concentration would indicate

that the solution contains some amount of the receptor against the ligand on the surface.

In the optical system, a coupling prism is used for the internal reflection. The incident angle of the BTIRIE is fixed and it is larger than the total internal reflection angle. An evanescent wave appears at the interface of the substrate and the tested solution. A micro-fluidic reactor array is combined with total internal reflection imaging ellipsometry [15]. It could be realized as an automatic analysis for protein interaction processes with real-time label-free method. The total internal reflection imaging ellipsometry system is performed in the internal reflection mode which the refractive index of the incident medium is larger than that of the reflecting medium and the incident angle is larger than the critical angle of total reflection. An evanescent wave field appears in the reflecting medium which is used to measure biomolecule interaction. The prism contacted with a glass slide is used to realize the internal reflection. The other side of the slide is coated with a thin gold film as a substrate for ligand immobilization and contacted a tested solution. The probe beam passes through a polarizer, compensator, perpendicularly into the window of glass prism and finally onto the gold film. Under the total internal reflection, the evanescent wave field appears on the substrate surface, which is modified by depositions of biomolecule layers on the surface, so that the polarization state of reflection is modified.

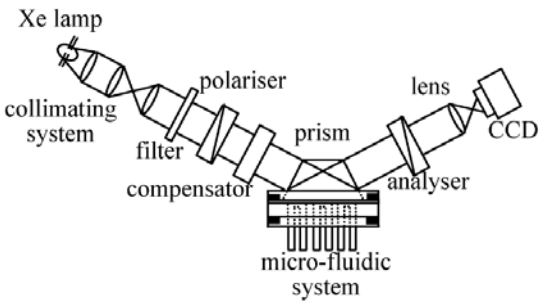

Fig.1 Schematic diagram of the total internal reflection imaging ellipsometry biosensor. The Xe lamp and various filters are used as a spectroscopic light source. The light is guided with a fiber to the optical passage of the polarizer and compensator, and perpendicularly propagates to the prism and finally onto the sensing surface. The micro-fluidic reactor array contacts the gold surface immobilized with ligand for biomolecule interaction. When the incident angle is larger than the critical angle, the evanescent wave appears at the sensing surface for detect biomolecule interaction. The reflection light passes the analyzer to be imaged by CCD camera. [15]

With the analysis of the polarized state of reflection, the information of these biomolecule layers could be deduced to realize a biosensor function. The use of evanescent wave field makes measurements not disturbed by the refractive index, non-uniformity and flow of solution. With a micro-fluidic system including an 8×3 cell array with 100 nl volume each cell, the function of microarray biosensor is realized, by which multiple bio-molecule interaction process could be measured in a real time experiment model.

3 SOME APPLICATIONS

The purpose of the optimization is to find out the optimal setting for the analyte to be detected with a high sensitivity and a wide detection range of target biomolecule concentration. An experiment is performed to verify the performance improvement of the biosensor. It concerns the quantitative detection of Hepatitis B surface antigen (HBsAg).

The incident angle is 59°. The wavelength is 600nm. The azimuths of the polarizer and the analyzer are 122° and 44°, respectively, according to the optimization setting. The temperature of the CCD during the detection is -70°. The pre-gain of the CCD is 2 and the dynamic range in grayscale is from 0 to 65535.

The SF10 glass slide prepared by the evaporation of 2 nm chromium on the surface and 30 nm gold layer is used as the substrate. The gold slide is immersed into MUA- ethanol solution for at least 18 hours, followed by a thorough rinsing with both ethanol and de-ionized water. The MUA monolayer with carboxyl group is self-assembled on the gold surface. The gold surface with carboxyl group on is activated with NHS and EDC then rinsed with PBS buffer. The Hepatitis B surface antibody (HBsAb) is used as the ligand and covalently immobilized on the gold surface with carboxyl group for 10 min. The surface is rinsed for about 6 min. to remove unbound molecules. Then it is blocked with blocking buffer for 10min., and rinsed with PBS buffer for 6 min. The HBsAg is diluted with PBS buffer into various concentrations from 0 to 250 ng/ml and incubated with ligand for interactions upon their affinity for 15 min. The whole dynamic process of the interaction between HBsAb and HBsAg is shown in Fig.2, where is shown an

average result over each sensing area at least 100 pixels in each cell in the array. The curves in Fig.2 are zoomed into “region 7” and “region 8” to show the dynamic process of the interaction in detail on the surface. Curves from “a” to “g” correspond to HBsAg concentrations of 0, 8, 16, 32, 63, 125, 250ng/ml. The data in “region 8” is averaged over the last 600 s, as indicated in the Fig.2.

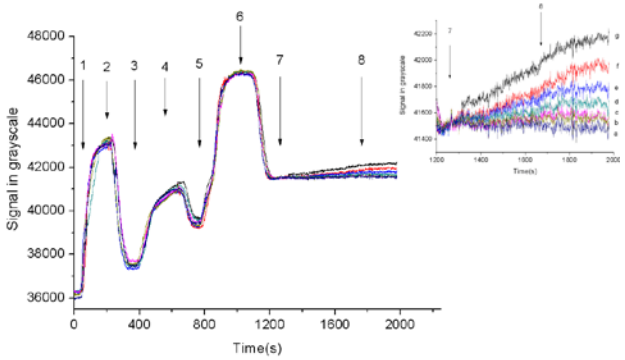

Fig.2 Real-time curves of the dynamic process for the Hepatitis B virus surface antigen binding with Hepatitis B surface antibody in various concentrations. The 1st region is the baseline; the 2nd region - NHS-EDC activation of carboxyl group assembled on the gold substrate; 3rd - PBS rising; 4th - HBsAb immobilization on the gold-coated substrate surface; 5th - PBS rinsing, 6th - blocking , 7th - PBS rinsing and 8th - HBsAg binding with its antibody. Curves a-h are for Hepatitis B virus surface antigen concentrations of 0,8, 16, 32, 63, 125, 250ng/ml [18].

The results of the experiment show the improvement of the sensitivity and the detection limit of the BTIRIE.

The normalized transduction signal versus the concentrations of HBsAg obtained from the previous biosensor system and the improved system is shown in Fig.3. The slope of the regressive curve is 6×10-6 and 5×10-5, respectively, which are taken as the sensitivity of the biosensor system. Obviously, the sensitivity is improved by almost one order.

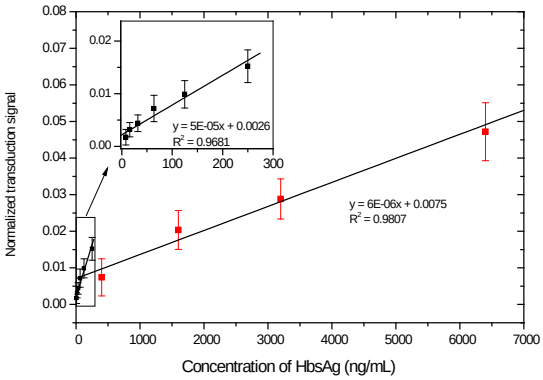

Fig.3 The normalized transduction signal versus the concentration of HBsAg. The zoomed curve represents the data obtained by the biosensor after optimization [18].

The kinetic results of HBsAb reacting with various concentrations of HBsAg obtained with the previous system and the improved system are compared. The previous biosensor system with the wavelength set as 633 nm and the imaging device is the video CCD camera (Sony XC-ST30 CCD B/W video camera). The detection limit of the previous biosensor is 400ng/ml, as being affected by the noise. The signal can hardly be distinguished when the concentration is lower than the detection limit. The performance of detection limit is improved from 400ng/ml to 8ng/ml by optimizing the setting of the wavelength, the azimuth setting of the polarizer and the analyzer as well as by using the cool CCD. The detection limit in the improved biosensor system is enhanced by about 50 times.

The applications is not limited to biomolecule interaction, but the mimic cell of giant lipid vesicles (GUVs) interacting with an adhesive surface coated with poly-l-lysine is investigated by the biosensor [19]. The adhesion of GUVs on the adhesive surface is regulated by the concentration of the poly-l-lysine coating, the pH of the vesicle suspension, and the flow rate of the suspending fluid. The biosensor detects very clearly and sensitively GUV adsorption, flattening, rupture, and de-adhesion, all these behaviours, which show that the setting of the BTIRIE biosensor, optimized for molecular sensing can be extended to GUV sensing. This work paves the way to develop a new sensitive high-throughput, low-consumption and low-cost biosensor technology for micron-size objects such as cells, capsules and liposomes.

A recent extension is a combination sensor composed of electrochemistry and total internal reflection imaging ellipsometry for dissolved oxygen (DO) detection. The performance may overcome the signal drift phenomenon in DO electrode sensor and both the optical and electrical signals of the combination sensor for DO detection are amplified with a poly-aniline modified gold surface.

4 Conclusion

With a recent improvement that is an optimization of wavelength and polarization settings as well as the use of a cool CCD, the sensitivity has increased almost one order and the detection limit for HBsAb and HBsAg 50 times better than before. It maintains several significant advantages: non-contact and label-free. Non-contact ensures the detection without disturbance to samples, while label-free guarantees the results with less false positive and less labor. It is demonstrated in image, so its detection quality can be directly observed and evaluated in real-time. A large field of view may perform multiple detections of numerous samples simultaneously. So far, it's achieved of the detected concentration of 1ng/ml for Immunoglobulin detection, the sensitivity basically meets the demand for most clinical purpose, so that it brings a bright potential for further biomedical applications. Its applications have been extended to mimic cell adsorption and dissolved oxygen detection.

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