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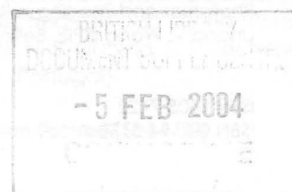
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Direct monitoring of antigen-antibody interactions by optical fiber bioprobe

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ABSTRACT

An optical fiber bioprobe is introduced as a biosensor to monitor the interaction of antigens and antibodies in immunoreactions. The detection is based on the theory of multiple-reflection principles in white-light interferometry. Incident light is partially reflected at the two interface of the biolayer, because of the interference resulted in the interface spectrum. The change of optical thickness can be calculated by testing the reflected spectrum phase shifting. The setup allows the consequent observation of changes in optical pathlength on a fractional nanometer scale with subsecond repetition times. The system has high sensitivity, high precision, cost effective and working on a high reliability. The testing process is nondestructive for the biolayer. The bioprobe is easily integrated as a BIAcore. The system and the experimental results on the reaction of rabbit-IgG with anti-rabbit-IgG are described in this paper.

Keywords: optical fiber, bioprobe, biosensor, transducer, interference

1. INTRODUCTION

Since the introduction of a commercial testing system for direct monitoring affinity reactions by biosensor, biomolecular interaction analysis (BIA) has become a valuable tool for many areas in fundamental and applied research ¹⁻³. Of all the optical transducer methods, direct methods do not require labeling of analytes with fluorescent compounds are of interest due to the relative assay simplicity and ability to study the interaction of small molecules and proteins that are not readily labeled. At present time, direct optical methods include surface plasmon resonance (SPR) ⁴, grating couplers ⁵, ellipsometry, evanescent wave devices, and reflectometric interference spectroscopy (Rifs) ⁶.

Reflectometric interference spectroscopy is a successful label-free method ⁷. This technique is based on the spectral distribution of white-light reflectance from transparent thin layers. Now, we introduced an optical biofiber for reflectometric interference spectroscopy for immunoassay. The advantage of this method is used the optical fiber as a biosensor. It is simple structure, cheaper, high sensitivity and high reliability and detecting automatically. The single bioprobe can be easily integrated as a BIAcore for direct real time monitor of the parallel immunoassay.

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2. FUNDAMENTAL

Fabrication of the bioprobe and schematic of the light path in the Rifs bioprobe are illustrated in Fig 1a and Fig 1b

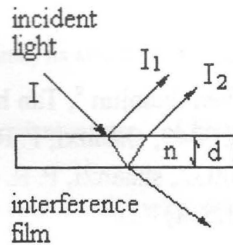


Fig 1a Schematic of the light path in the bioprobe

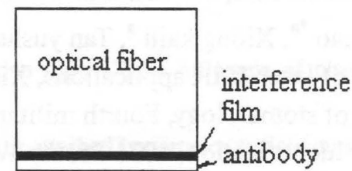


Fig 1b Structure of the bioprobe

During the manufacturing process of bioprobe, interference layers (10~20nm Ta_2O_5 /550~600nm special material) in front of fiber ($\Phi 0.6\text{mm}$) were deposited by standard vacuum plated techniques. For biomolecular interaction analysis, bioprobe with an interference layer of special material is used as transducer. Here, binding events are observed by detection of changes of optical thickness in real time. When incident white-light enters the thin film, white-light is partially reflected between upper and down surface of the film. The reflected partial beams can result in an interference pattern, depending on the angle of incident light, the wavelength of incident light, the physical thickness of the film and its refractive index. For perpendicular incidence, according to Fresnel's law, the reflectance I_R can be illustrated as follows^{3,8,9}

$$I_R = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(4\pi n d / \lambda) \quad (1)$$

where I_1 and I_2 denote the Fresnel reflectance at the two interface of the thin film. Sign d denotes the physical thickness of the film. Sign n denotes refractive index of the film. Sign λ denotes the wavelength of incident light.

Formula (1) shows that there is a similar cosine relationship between reflectance (normalized to reference) I_R and the wavelength of incident light λ . With the increase of λ , the cycle of the signal becomes longer. A typical computer simulation of interference pattern with λ is given in Fig2.

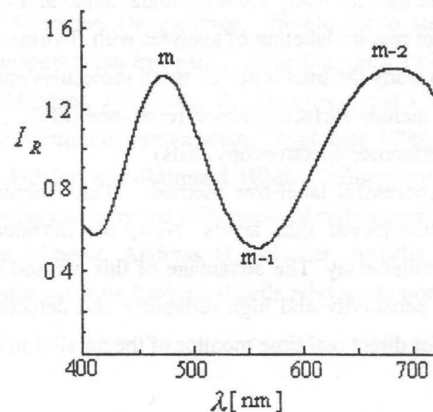


Fig 2 A typical computer simulation of reflectance I_R and λ

The optical thickness $n d$ can be determined by the position of an extremum of reflected spectrum with a given order value m as follows

$$n d = \frac{m\lambda}{2} \quad (2)$$

For biomolecular interaction analysis, transparent thin film of a bioprobe with an interference layer of special material can serve as transducer for real time monitor of immunoassay⁸

During bioproteins binding at the surface of interference layer, the phase of spectrum will be moved due to the increase of optical thickness of the film. The phase shift of spectrum can be detected by Rifs setup in real time. According to the changes of spectrum and the speed of phase shifting of spectrum, we can directly determine the concentration of bioproteins in sample.

3. TESTING SYSTEM AND EXPERIMENTS

3.1 Reflectometric interference spectroscopy (Rifs)

The Rifs setup (Fig 3) includes a 12W tungsten halogen lamp with fiber in coupling optics, a "Y" type polymer fiber coupler ($\Phi 0.6$ mm optical fiber, 50/50 coupling ratio), a reflect spectrometer and a bioprobe. All sections connected with standard SMA905 connector. The system shows a high mechanical and thermal stability due to no moving parts were used.

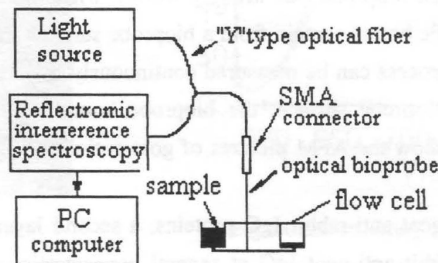


Fig 3 Sketch map of the Rifs testing system

In the testing system, white light from a tungsten light source is guided to the bioprobe using "Y" type fiber coupler. The light is partially reflected on the interfaces from the transparent interference layer in front of the bioprobe (Fig 1) and results in an interference spectrum. The phase of interference spectrum will be shifted when the optical thickness changes from the binding of bioprotein to the surface of the bioprobe. Data acquisition is performed by self-written software running under windows on a personal computer. The raw spectrum is corrected for the dark current of the spectrometer by subtraction and normalized to the reflectance spectrum from a bioprobe without an interference layer. The position of an interference extremum of spectrum at 700nm is determined by the sensitivity of testing system and the need of data processing. Optical thickness is calculated according to Eq (2). The evaluation of optical thickness is performed on line.

by software. Real time monitor of binding is obtained by plotting the wavelength position of an extremum of the reflectance spectrum versus time. The bioprobe is mounted in a flow cell of approximately 180 μ l volume. Sample handling was carried out by peristaltic pump from Lange inc. in china.

A compact Rifs device is Ocean Optics'USB2000, distributed by Ocean Optics inc. in U S A. An entrance slit between "Y" type optical fiber output and reflectance spectrometer is 50 μ m width and 100 μ m highness. The density of reflected grating is 2400 strip/mm. high-performance 2048-element linear CCD-array detector.

3.2 Bioprobe surface activation chemistry

The sandwich method was adopted in our experiments. For the bioprobe to be functional, a receptor protein, which is Rabbit-IgG molecules in our experiments, must be immobilized on its surface. The binding of complementary protein in a test sample is unimpeded by the bioprobe surface. Such receptor includes peptides, proteins and so on.

Before the immobilization of rabbit-IgG molecules, the bioprobe must be activated with freshly prepared piranha solution (concentrated H_2SO_4 70%/30% H_2O_2 (v/v)) ¹⁰ over 2h and then washed thoroughly with bidistilled water and drying in hot air. The activated surface were silanized with 3% 3-aminopropyl-triethoxysilane (china) in dry acetone for 3 min, followed by rinsing with dry acetone, dried in air, and washed in tridistilled water.

3.3 Binding and interaction experiments

After activating the bioprobe with amine, a functional linker molecule is attached to the bioprobe sensitivity surface. And then, 180ml of goat anti-rabbit-IgG solution in PBS at 50 μ g/ml concentration was added to the activated bioprobe surface with flow cell at 37 $^{\circ}$ C for 45 min. Next, exchange the reagent with BSA (Bovine Serum Albumin) and block nonspecific binding point and then the bioprobe was strongly washed with PBS solution (Phosphate Buffer Saline) three times so that wash off the nonspecific bound protein from a bioprobe surface. Fig 4 show a typical reflectance spectrum during coating stages. All coating process can be measured continuously by Rifs system. After the goat anti-rabbit-IgG proteins were immobilized on a bioprobe surface, the bioprobe bound readily to rabbit anti-goat-IgG protein for immunoassay. Fig 5a and Fig 5b show the AFM pictures of goat anti-rabbit IgG proteins bound onto the surface of a bioprobe.

After a bioprobe was coated with goat anti-rabbit IgG proteins, a second layer of protein was bound to the bioprobe surface through the addition of rabbit anti-goat IgG at several concentrations (1,10,100ng/ml, volume=180ml). The shifting of reflectance spectrum was continuously monitored as each solution was allowed to incubate on the bioprobe for 40 min. Fig 6 show the extremum of reflectance spectrum shifted to greater wavelength at the end of 100ng/ml incubation period. The curve for kinetic binding experiments indicates that a rapid increase was observed before 20min, followed by a very slowly increase in phase shifting of reflectance spectrum. This type of reaction profile was observed for all the kinetic binding experiments.

Sensitivity experiments and nonspecific binding experiments will be investigated during next stage. The results of the experiments will be discussed in another papers.

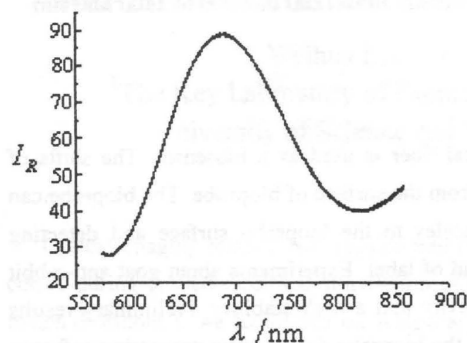


Fig 4 Obtained reflectance spectrum Of the bioprobe

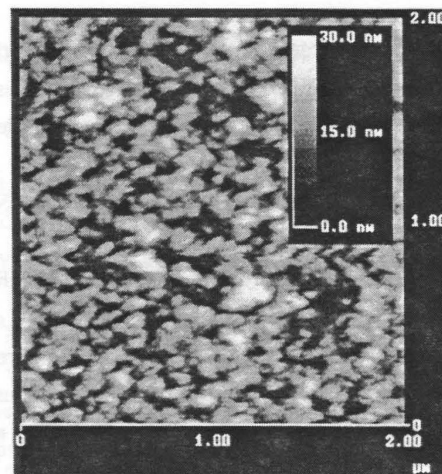


Fig 5a 2D AFM picture of protein bound onto the surface of a bioprobe

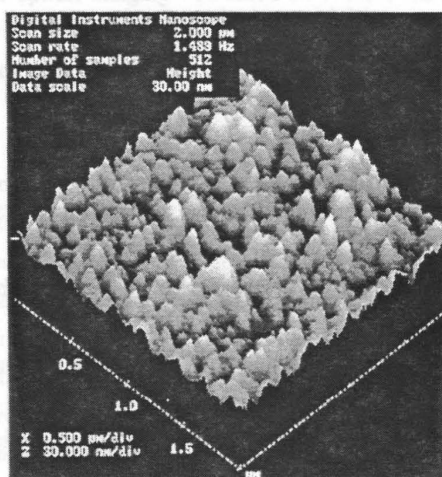


Fig 5b 3D AFM picture of protein bound onto the surface of the bioprobe

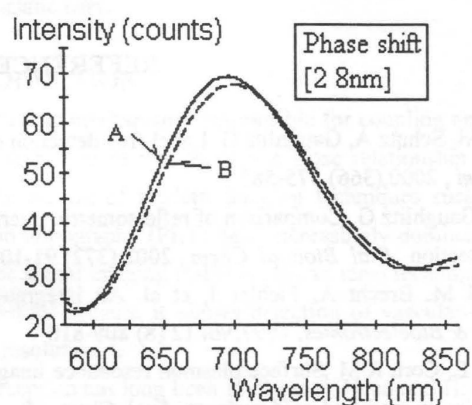


Fig 6 Caused shifting of the reflectance spectrum by the binding of protein

4. DISCUSSION

Rifs is a simple method for measuring protein binding on an optical surface. A dominant error of the testing system is from the influence of surrounding light and an electronic noise of CCD with high frequency. In order to obtain an accurate wavelength of extremum, the data of reflectance spectrum was processed as follows: Firstly, the signal average method in frequency scope is used in the spectrum data processing. Data of spectrum was acquired 10 times from Rifs setup and then processed by above method. The ratio of signal to noise will be increased $\sqrt{10}$ times. Secondly, the high frequency noises from CCD were erased by low pass filter. With the help of FFT method, the curve of each

spectrum is smoothed in order to find the accurate peaks position of reflectance spectrum

Sensitivity of the system is limited by slit width, grating density and resolution of CCD. Theoretically, the resolution of the testing system will be increased one time by changing reflected grating density from 1200 line/mm to 2400 line/mm

5. CONCLUSION

A novel approach for immunoassay was investigated in which an optical fiber is used as a biosensor. The shifts of reflected spectrum can be detected when bioprotein is added or removed from the surface of bioprobe. The bioprobe can be served as a biosensor for immunoassays by linking receptor molecules to the bioprobe surface and detecting complementary binding molecules in a sample without the use of any kind of label. Experiments about goat anti-rabbit IgG with rabbit anti-goat IgG show that the bioprobe has a high sensitivity and a high stability. Preliminary results indicate that detecting sensitivity of the system is superior to 1 ng/ml, and the bioprobe design, data processing software and activated surface efficiency have yet to be optimized.

In future work, a single bioprobe will be integrated as a BIAcore for real time monitor of parallel immunoassay. Future work will also demonstrate more detail experiments to quantify detecting sensitivity, particularly for the detection of small molecule interactions.

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