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Fiber Optic Direct-Sensing Biosensor Applied In Detecting Biolayer Thickness of Nanometer Grade

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Abstract A fiber optic direct-sensing biosensor has been developed, which can be used to detect the thickness of nm level biolayers. The experimental process, experimental results and a method of improving signal/noise (S/N) ratio are provided, and further compared with the surface plasmon resonance (SPR) technology.

Key words: antigen, antibody, fiber optic, interference, direct sensing biosensor

0 Introduction

It is a common approach in research and medical diagnosis to detect whether a sample contains a specific biological substance. In the case of detecting whether a specific antibody is present in plasma, a specific antigen corresponding to the antibody can be used to detect it. There are some detection approaches that have been developed based on the foregoing mechanism, such as immunodiffusion, immunoelectrophoresis, immunofluorescence, and so on. The commonly used biosensors can be divided into different types, including sandwich fiber optic sensors, displacement fiber optic biosensors, SPR sensors, grating biosensors, and the like. The foregoing biosensors have large sizes and require a large amount of biological samples, or need to use certain toxic indicators (fluorescent indicators), and they can be used for only one test, and cannot be used for in vivo tests.

A direct sensing biosensor has been developed by us, which applies an approach of phase tracking approximation and uses a fiber optic probe as a biosensor. It can detect whether a substance is present in a solution by the following mechanism: it can detect whether the thickness of a biolayer on probe terminal surface changes based on changes in the spectral phase of the interference pattern. It has the following features: small size, nontoxic, capable of carrying out different tests at the same time, and capable of being used in in vivo tests.

1 Detection mechanism

The probe part of the fiber optic sensor is as shown in Figure 1.

Before conducting a detection on the thickness of a biolayer, the fiber optic probe end portion is covered by a siliconization film, and the thickness of the film is from 1 to 2 μm . In the case where the test target is an antigen, a corresponding specific antibody needs to be used. In this case, a specific antibody layer is applied on top of the siliconization film provided on the end of the fiber optic element. In this way, the presence of the specific antigen can be detected by virtue of the specific binding between the antigen and this layer of antibody. The refractive index of the siliconization film is very close to those of the antigen and antibody. As a result, the entire film layer (including the siliconization film, the antibody and the antigen) can be considered as a single layer from an optical perspective.

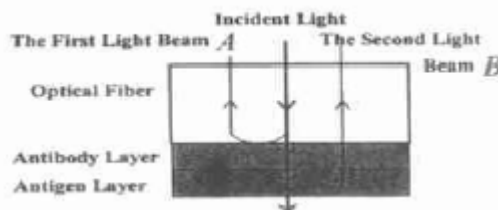


Figure 1 Light reflection at the bottom of a bioprobe

An incident light travels to one end of the probe along the fiber optic, when there is only one antibody layer, the incident light is first reflected on the interface between the optical fiber and the film layer (interface 1), a small part of light (a first light beam) is reflected back to the other end of the "Y"-shaped optical fiber, while a large part of the light continues on to enter the film layer, and has a second reflection on the interface between the film layer and air (interface 2), the light from the second reflection is transmitted through the interface 1, a majority of the reflected light then enters the optical fiber (a second light beam), and returns to the other end of the "Y"-shaped optical fiber.

When the refractive index of the film is relatively low, the reflection of the light within the thin film is quite weak, such that the condition of multiple reflections does not need to be considered, and the situation of multiple interfering light beams does not exist. As a result, only the case where a single reflection of light has

interference needs to be considered. Since the film has a certain thickness, there would be an optical path difference Δ between the first light beam and the second light beam, $\Delta = 2d\cos i$, where i is the angle of incidence of light, under the condition that the incident light is close to perpendicular, i is approximately zero degree. As a result, $\Delta = 2d\cos i = \Delta = 2d\cos(0) = 2d$. In the coherence length of white light, the existence of optical path difference may cause light interference between two reflected light beams. Let the light intensity of the first light beam be I_1 , the light intensity of the second light beam be I_2 , according to Fresnel's law, the intensity of the interference light is

$$I = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos(2\pi\Delta/\lambda) \quad (1)$$

In the foregoing formula, Δ is light pass, λ is the wavelength of incident light, because light intensity I_1 and I_2 are close to each other, which can be approximated as equal. Hence, let $I_1 = I_2 = I_R$, the foregoing formula can be simplified as

$I = 2I_R(1 + \cos(2\pi\Delta/\lambda))$ (2), for a given Δ , there is a cosine relationship between Δ/λ and I . When Δ/λ equals to 0.5, 1, 1.5 ... the extreme value of the reflected light intensity can be obtained. If the initial value of Δ at the μm level, in formula (2), the relationship between I and λ is a cosine-like relationship, that is, the shape is much like the cosine, but the period of the signal increases as the value of λ increases, as shown in Figure 2.

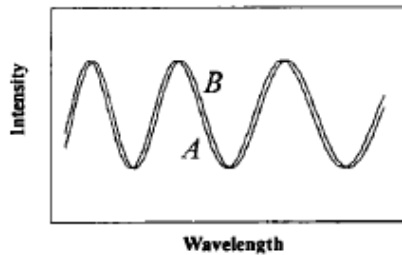


Figure 2, The relationship between wavelength (λ) and reflected light intensity (I), curve A indicates initial value of Δ , curve B indicates that Δ has increased

The order of each extreme value and the value of the optical path Δ can be calculated by using two or more extreme values. The wavelength values at the extreme points are first calculated a, b, c ..., let $k = 0.5$, when $\Delta/\lambda = k, 2k, 3k \dots$ they correspond to the extreme points of reflected light intensity. Because we do not know exactly in which order the extreme point taken by us are, we thus may set the orders at a, b, and c are $mk, mk-1, mk-2 \dots$. The order is decremented because $1/\lambda$ decreases as λ increases, so the order should gradually decrease. Thus, the equation set is made as follows

$$\Delta/a = mk \quad (3)$$

$$\Delta/b = mk - 1 \quad (4)$$

$$\Delta/c = mk - 2 \quad (5)$$

For given values of a, b, c, the values of the optical path Δ and the order m can be obtained by solving the above three equations in parallel.

At near normal incidence, for a given refractive index n and optical path Δ , the actual thickness d of the film is as follows:

$$d = \Delta / 2n \quad (6)$$

When $I_1 \gg I_2$ or $I_1 \ll I_2$, although it can still obtain a result, the contrast ratio (degree) of the image will decrease, and the accuracy of determining the extreme point will be reduced, which will increase the test error.

After the antigen and the antibody react, the film layer thickness Δ increases, which is represented as a phase shift on the spectrogram. Therefore, if the measured interference pattern has a phase shift, it can be inferred that the antibody reacts with the antigen, that is to say, it can be detected for the presence of antigen in the blood, so as to achieve the purpose of the test.

2 Detecting system structure

The detection system consists of a white light source, a "Y" shaped optical fiber, a fiber optic connector, a fiber optic probe, a spectrophotometer, and a computer. The fiber optic probe and the "Y" shaped optical fiber are coupled by lenses using a standard SMA connector.

The broadband white light emitted from a tungsten halogen lamp enters the "Y" shaped optical fiber through a coupling, and travels forward directly to the fiber optic coupler, and then enters the fiber optic probe. At the end of the probe, some of the light is returned by the fiber optic cross-section and then enters the spectrophotometer.

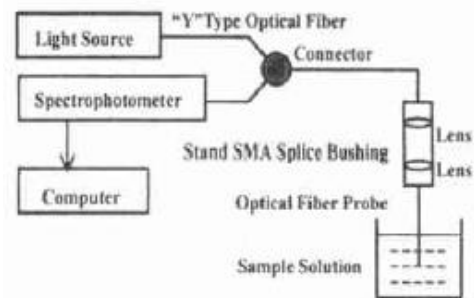


Figure 3 Detecting system structure

The spectrophotometer used in this study is an S2000 spectrometer from Oceanoptics Inc. of the United States. The instrument includes a spectroscopic optical path and an A/D conversion circuit. It is directly connected to a serial port of the computer. The optical path of the splitting light is a cross-symmetric horizontal imaging system, which is shown in Figure 4.

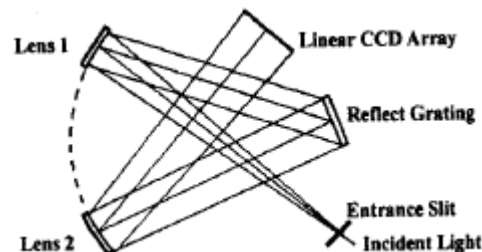


Figure 4 Spectrometer structure

The slit of the spectrophotometer is 25 μm in width (1,000 μm in height), the reflection grating density is 1,200 lp/mm, and the linear array CCD is Sony ILX511 2048-unit CCD. The sampling frequency of the A/D circuit is 500 kHz, and the resolution is 12 digits.

3 Testing process

3.1 Preparation

The end face of an optical fiber is treated by a siliconization method, in which a film of a polymer (the main component is polystyrene) is prepared on the end face of the optical fiber. This siliconization film is mainly for the purpose of increasing the thickness of the film layer and increasing the adhesion ability of the optical fiber to the antibody. The film thickness is at the level of μm , and the refractive index is about 1.509.

3.2 Fixation of the antibody layer

Immerse the siliconization-treated fiber optic probe in a purified goat antigen IgG antibody at a concentration of 100 $\mu\text{g/mL}$. After 1 hour, clean the optical fiber end face with triple distilled water to remove excess antibody. Thus, on the optical fiber end face, there is a layer of polymer film and an antibody layer. A set of data is then collected and saved.

3.3 Fixation of the antigen layer

The fiber optic probe is again immersed into a solution of human IgG (that is, an antigen). After 1 hour, the optical fiber end face is washed with triple distilled water to wash away the excess antigen. Because the antibody and the antigen undergo a biological reaction, there are three layers on the end face of the optical fiber: a polymer layer, an antibody layer and an antigen layer. The binding between the antibody and antigen is very strong, and the bound antigen will not be washed away. Similarly, we can consider the addition of the antigen layer simply as an increase in the film layer thickness. A new set of data is then collected and saved.

3.4 Discussion

The thickness of a single-molecule antibody layer as measured by ellipsometry is about 5 to 7.5 nm, and the antigen layer and the antibody layer have nearly similar size and chemical and biological properties. The thickness of the tested protein molecular layer is much smaller than the wavelength of the illuminating light. When the antigen and antibody react to increase the thickness of the protein layer, since the refractive indices of the protein layer and the siliconization film are very close, we can consider the addition of the protein layer as an increase in the thickness of the polymer film. The refractive index of the protein molecular layer is about 1.44 to 1.51.

4 Test results

The test results are shown in Figure 5. Figure 5(a) shows the processed data, including the two lines before and after the antigen and antibody reaction. Figure 5(b) shows the signal-processed data. It also includes two lines before and after antigen and antibody reaction. Figure 5(b) shows a significant phase shift.

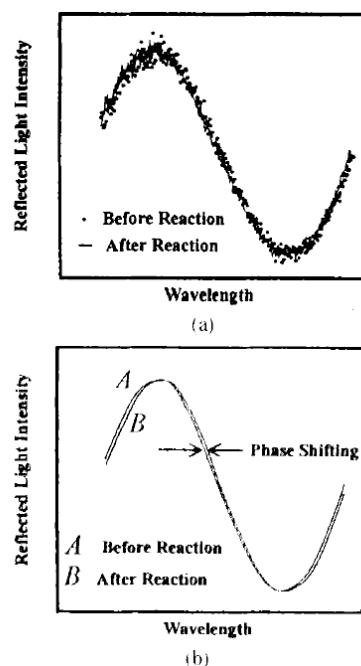


Figure 5 Testing results

According to the sampling data, the thickness of the siliconization film is about 0.987 μm (including the antibody layer and the antigen layer), and the increased thickness of the film (that is, the antigen layer) is about 7 nm. The test error is less than 1 nm.

In the following series of experiments, we also have conducted gradient experiments with different concentrations of antibodies and antigens. Experiments have shown that even if the concentration of antigen or antibody is very low (for example, 1 $\mu\text{g/mL}$), when the binding time is increased, the test purpose can still be reached.

5 Signal processing

The A/D sampling signal is sent to the computer through the serial port for data processing. Data processing mainly includes denoising, optical signal processing, calculation of extreme points, calculation of film thickness and calculation of refractive index.

The systematic error mainly comes from the external light noise and the high frequency noise from the linear array CCD in the spectrophotometer. Thus, in order to improve the signal-to-noise ratio and improve the image quality, the noise must be removed. Firstly, the frequency domain average method is used, that is, for the same film, n sets of data are collected under the same experimental condition, and then perform frequency domain averaging, which can increase the S/N ratio by $\sqrt{n}$ times, wherein $n = 4$. Next, the secondary vector subtraction method is utilized, that is, when the light source is turned on, cover the end face of the bare fiber optic, and then measure the reflected spectrum, which would be the background noise data, and then subtract the background noise from the actual measured data, which can remove most of the light noise; and then use the 9th-order Butterworth low-pass filter to remove the high-frequency noise from the CCD. The image obtained at this stage has been removed of most of the system noise, and thus the image is smooth and can be used for analysis.

The method of calculating the extreme points is the same as described above.

6 Comparison with the surface plasmon resonance (SPR) technology

In recent years, biosensors have made quick progress. SPR sensors, grating biosensors, and Fabry-Perot biosensors can all be used to determine the thickness of biofilm layers. Among them, the employment of SPR sensors measuring the thickness of biofilms is currently the most commonly used technology. This type of sensor consists of a prism coated with a thin metal layer, the metal layer forming an interface between the prism and an insulator, as shown in Figure 6.

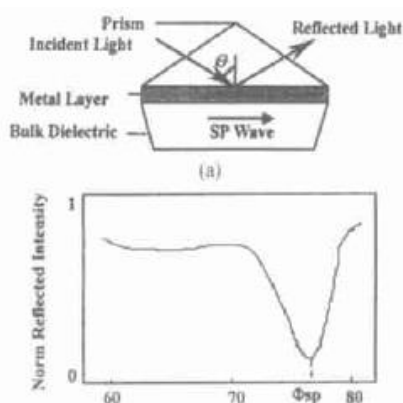


Figure 6 Surface plasmon resonance (SPR) sensor

A horizontal magnetized uni-directionally polarized light is the incident light reaching one face of the prism and is reflected by the metal layer to reach the other side of the prism. The intensity of the reflected beam can be measured to calculate the incident angle θ of the incident beam. The intensity of the reflection light sees a sudden drop at a specific angle of incidence Φ_{SP} , and at this specific angle, the energy of the incident light matches the surface plasmon resonance (or "SPR") generated by the metal-insulator interface excitation. A layer of film (such as a biofilm) is deposited on the metal layer, and the refractive

index of the insulating material changes. The refractive index depends on the thickness and density of the insulating material and the precipitated film. The value of the notch angle is measured, and the thickness and density of the precipitated film can be obtained. Its test sensitivity can reach nm level.

The SPR sensor is state-of-the-art. It does not need toxic indicators (such as fluorescent indicators) that are often used in biological tests. It can also be measured in real time. When our instrument is compared to this type of sensor, ours has the following advantages: 1) It uses a broadband white light source, whereas SPR sensors require polarized light as its light source and typically use a fixed incident wavelength light source. 2) Using disposable fiber optic probes makes the test easier and more convenient, while the SPR sensor cleaning is much more complicated, and additionally, the very thin metal film (around 50 nm) needs to be plated on the prism. These will increase the cost of the test.

In addition, the number of biological samples in medical testing is usually very small, and the SPR sensor has a relatively large cylindrical surface, which means that a larger volume of test sample is required. Conversely, the amount of sample required for our fiber optic probe is very small. Moreover, since the angle of the incident light needs to be adjusted, the SPR sensor is not suitable for in vivo testing, while our instrument is expected to be tested in vivo.

7 Summary

This paper presents a novel biosensor testing method for detecting the presence of an antigen (or antibody) by fiber optic bioprobe based on the mechanism of white light interference. This type of fiber optic biosensor has not been developed in China and abroad, it has small size and nontoxic features; it can achieve a variety of testing purposes at the same time, and the same method can be used in many other situations. It is very useful and has a promising market prospect.

The main idea of this paper was applied for a patent in the United States, and was granted the U.S. Patent No. 5,804,453 on September 8, 1998.

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