Proceedings of the 16th Conference on
Optoelectronics and
Optical Communications

ISSN 1738-2661

COOC 2009

제16회

광전자 및 광통신 학술회의 논문집

VOL.16 NO.1

일시 2009년 5월 13일(수)~5월 15일(금)

장소 대천 한화리조트

공동주최

한국광학회 [광자기술분과]

한국통신학회 [광통신연구회]

대한전자공학회 [광파 및 양자전자 연구회]

대한전기학회 [광전자 및 전자파 연구회]

http://www.cooc.org

학술발표 T2B 나노포토닉스I

10:50~12:20 좌장 : 송봉식(성균관대)

학술발표 T2C WDM-PON

10:50~12:20 좌장 : 윤호성(KT)

Detection of Avian Influenza-DNA Hybridization using a Wavelength Interrogation-based Surface Plasmon Resonance Biosensor

파장측정용 표면플라즈몬공명 센서를 이용한 조류독감 DNA의 혼성화 검출

Shin Ae Kim[1], Kyung Min Byun[2], Michael L. Shuler[3], and Sung June Kim[1]

[1]School of Electrical Engineering and Computer Science, Seoul National University, Korea

[2]Department of Biomedical Engineering, Kyung Hee University, Korea

[3]Department of Biomedical Engineering, Cornell University, USA

Abstract

A wavelength interrogation-based surface plasmon resonance (SPR) biosensor has been investigated for a detection of avian influenza DNA (AI-DNA). The immobilization of oligonucleotides and hybridization reaction of AI-DNA was monitored in real time without labeling. The results showed that SPR biosensor can provide linear detection suitable for quantification of AI-DNA. This study demonstrates the potential for a simple AI virus diagnosis. Since these viruses can be transmitted into humans and often are fatal, improved AI detection is important.

Since avain influenza viruses (AIVs) not only lead to economic losses but also cause a fatal disease in humans, a rapid screening of AIVs is required [1]. Various virological detection methods, involving virus isolation and RT-PCR, have been introduced for AIVs detection. However, they require relatively long measurement time, complex experimental sequences, and expensive huge facilities. To overcome these limitations, it is desirable to develop a rapid, label-free, and highly specific detection method to identify an AIV infection.

Surface plasmon resonance (SPR) is well-known as an effective technique without any labeling [2]. This method is also applicable *in situ* real time monitoring, because the plasmon waves can detect the DNA hybridization kinetics immediately. In this study, we employed a wavelength interrogation SPR method for a quantitative detection of AI-DNA hybridization.

1. SPR setup and materials

We used three different oligonucleotides, as listed in Table 1. Target probe was originated from A avian influenza (NY/73-63-6/00(H7N2)) hemagglutinin.

A gold film with a thickness of 45 nm was sputtered on a SF10 slide glass with a 5nm chromium adhesion layer. We used a custom-made Kretschmann-configuration optical setup to detect SPR signals as shown in Fig. 1. An incidence angle was fixed at 60° for wavelength scanning from 550 to 650 nm. Two PDMS microfluidic (working and reference) channels were placed on the SPR sensor chip and all SPR measurements were carried with a flow rate of 2 μl/min. The resonance wavelength was determined by fitting the reflectance curve to the second-order

polynomial equation. In addition, the reflectance characteristics of s-polarized light had been recorded to eliminate various noise components depending on the wavelength of light source.

Name	*Sequence (5'-3')*	*Description*
Capture probe (C_1)	Thiol - TTT TTT TTT TTT TTT ATT GGA CAC GAG ACG CAA TG	35-mer oligonucleotide with 15-mer Thymine (underlined) as a spacer.
Target probe (T_1)	CAT TGC GTC TCG TGT CCA AT	Complementary oligonucleotide
Mismatched target probe (T_2)	CAT TGC GTC TGC ACA GGT TA	Ten-base (underlined) mismatched oligonucleotide

Table 1. The sequence of used oligonucleotides

2. Results

To estimate the detection efficiency of our SPR system, the sensitivity was assessed by measuring the dependence of SPR signal on ethanol concentration. The minimum value of measurable refractive index variation was determined to be about 1.6×10^{-4} in refractive index unit (RIU).

Next, the hybridization process was performed by injecting of 1 μM T_1 and T_2 probes without additional labling, respectively. An obvious reaction was observed on the working channel where a solution of T_1 was injected, while no significant change was found for a solution of T_2 (Fig.2 inset). The result confirms the specificity of the assay procedure.

For wavelength interrogation SPR to be useful quantitatively, it is important that changes of resonance wavelength can be linearly proportional to changes of the concentration, and thus to the amount of bound analytes. Figure 2 shows the experimental result when T_1 was diluted in the hybridization buffer. The values of resonance wavelength were equal to 0.59 ± 0.16 nm, 1.75 ± 0.25 nm, 2.77 ± 0.34 nm, and 4.84 ± 0.24 nm to the T_1 concentrations of 1, 2, 3, and 5 μM, respectively. Using the linear function of $y = ax$, we found that the proportional constant (a) equals 0.9377 with $R^2 = 0.9835$. The results indicate that the wavelength interrogation SPR sensor has a highly linear dynamic range to be useful quantitative detection.

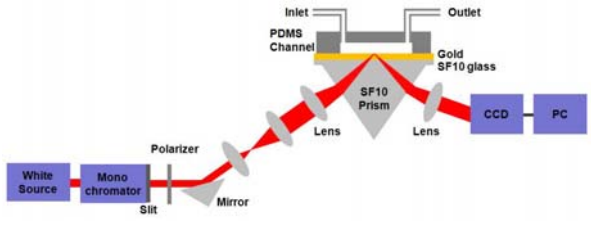

Fig.1. Schematic diagram of SPR measurement system

3. Conclusion

We have confirmed that a wavelength-scanning type SPR biosensor can provide a label-free, real-time, and specific detection of AI-DNA hybridization with a highly linear performance. This indicates that the wavelength interrogation SPR system is suitable for an application of AI-DNA hybridization detection for a simple AI virus diagnosis.

[1] J. Xu et al., "Detection of avian influenza virus using an interferometric biosensor", Anal. Bioanal. Chem. Vol. 389, pp. 1193-1199, 2007.

[2] A. J. Thiel, et al., "In situ surface plasmon resonance imaging detection of DNA hybridization to oligonucleotide arrays on gold surfaces," Anal. Chem. Vol. 69, pp. 4948-4956, 1997.

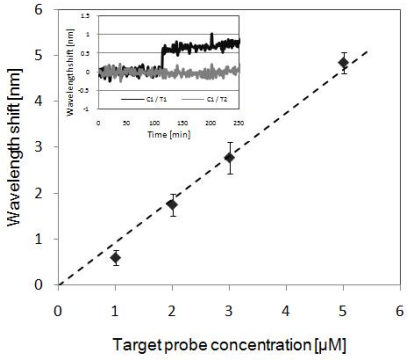

Fig.2. SPR wavelength shift at different target DNA concentrations