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Interferometric Detection of Prostate Specific Antigen Based on Enzyme Immunoassay

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Abstract

Interferometric detection of Prostate-specific antigen (PSA) based on enzyme immunoassay are investigated. Refractive index changes of substrate are measured for PSA detection. Michelson scheme of optical interferometer was used so as to be applicable to a disposable fluidic chip. When interferometer is used for the measurements of refractive index changes, the detection is over 8 times more sensitive than that of absorbance changes for the same amount of target protein.

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Keywords: Interferometry; Prostate specific antigen; Enzyme Immunoassay

1. Motivation

Some interferometric methods for the detection of target protein have been reported in the form of capture antibody immobilization on one arm of interferometer.[1, 2]. In order to use a disposable detection chip, separable detection schemes are needed so that it allows the separation of a reaction chip from a signal reader including interferometer. In the point of view, we suggest Michelson scheme of optical interferometer and a micro fluidic chip based on cyclo-olefin copolymer(COC) for Prostate specific antigen(PSA) detection.

Nomenclature

A	substrate (3,3',5,5'-tetramethylbenzidine, TMB)	E	enzyme (horseradish peroxidise, HRP)
P	product	K_M	Michaelis constant
K_{cat}	catalytic constant (turnover number)	K_i	reaction constants (i=1, 2)

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On the other hand, enzyme linked immunosorbent assay has been widely used for protein detection. In that case, absorbance change of substrate by enzyme reaction is measured in general. In this work, we measured the refractive index changes of 3,3',5,5'-tetramethylbenzidine(TMB) used as a substrate during enzyme reaction using Michelson interferometer. We found out that the detection of refractive index change is much more sensitive than that of absorbance change for the same amount of target protein.

2. Materials and Methods

First of all, optical properties of TMB used as a substrate were measured before and after enzyme reaction, respectively. TMB solution shows not only the absorption change around 650nm wavelength but also the drastic change of refractive index during enzyme reaction as in Fig. 1. Refractive index change of TMB was measured as large as up to about 1% by using surface plasmon resonance method at 1550 nm wavelength.

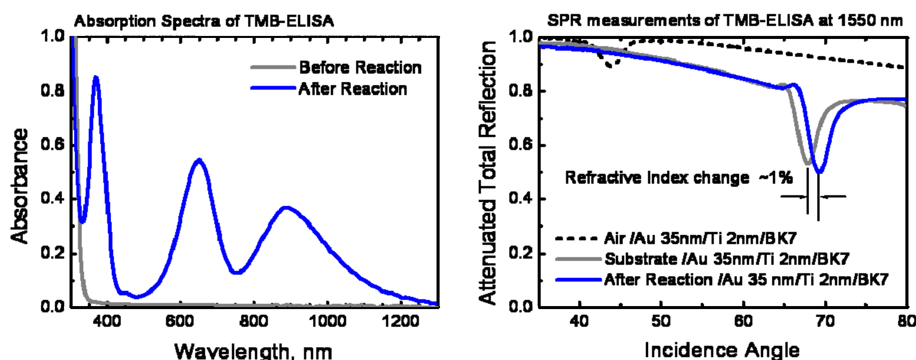

Fig. 1. Optical properties of TMB used as a substrate were measured before and after enzyme reaction respectively. TMB solution shows not only the absorption change around 650 nm wavelength (left) but also the drastic change of refractive index during enzyme reaction. Refractive index change of TMB was measured as large as up to about 1% by using surface Plasmon resonance method at 1550 nm wavelength. (right)

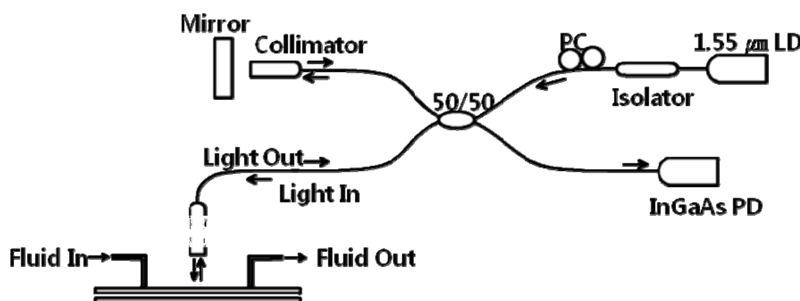

Fig. 2. Schematic diagram of an interferometric detection system is shown. Michelson interferometer was used to measure the refractive index changes of TMB and to obtain separable configuration of the interferometer as a signal reader with the disposable fluidic chip.

Schematic diagram of an interferometric detection system is shown in Fig. 2. Michelson interferometer was used to measure the refractive index changes of TMB and to obtain separable configuration of the interferometer as a

signal reader with the disposable fluidic chip. It was made using optical fibers with collimating lenses at the ends of arms. Nearly parallel beam with the diameter of 3mm is incident normally on the reaction region of the fluidic chip with a gold mirror at the bottom and the channel of 250-micron depth. Interference signals are measured during enzyme reaction in TMB solution at various concentrations of PSA. 1.55 μm wavelength laser diode (LD) and InGaAs photodiode (PD) are used as a light source and a detector, respectively, for the measurement. Assuming that enzyme reaction of TMB follows Sigmoid function of time, an initial reaction rate is calculated numerically using steady-state approximation with the enzyme parameters of horseradish peroxidase (HRP) such as a turnover number and a catalytic efficiency listed in Table 1.

Table 1. Reaction constants used to calculate an initial reaction rate. [3, 4]

Reaction constant	Value	Unit
K_M	0.15 @ PH 6.4	mM
K_{cat}	790 @ pH 6.4	S^{-1}
K_1	(1.0~) 2.0×10^7	$M^{-1}S^{-1}$

It is assumed as follows; A single molecule of enzyme (E) combines reversibly with a single molecule of substrate (A) to form an enzyme-substrate complex (X), which is transformed irreversibly to free enzyme and the products of reaction (P). And, the reversible part is much faster than the irreversible one, that is $K_{cat} << K_2$, where the reversible reaction constants are K_1 and K_2 for forward and reverse senses, respectively.

$$A + E \rightleftarrows X \rightarrow P + E \quad (1)$$

We measured an output light intensity using InGaAs PD during enzyme reaction in a reaction region of a fluidic chip with a concentration of PSA. Interference fringes or light output oscillations are measured due to a refractive index change in a fluidic chip in Fig.3. The shorter oscillation periods were measured, the higher concentration of PSA. A short oscillation period means a fast initial reaction rate based on the numerical calculation of enzyme reaction. We could plot the phase change or the number of fringes as a function of the concentration of PSA in our fluidic chips for the reaction time of 2 min.

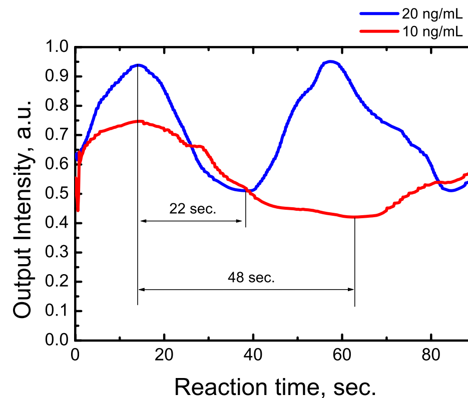

Fig. 3. Interference fringes are measured due to a refractive index change in a reaction region of a fluidic chip. The faster initial reaction rates are obtained, the higher concentration of PSA.

3. Results and discussion

Fig. 4 shows a number of interference fringes at each concentration of PSA that is phase change in the reaction region of the fluidic chip during the reaction of 2 min. When it is compared with the absorbance signal using TMB as a substrate, the detection is over 8 times more sensitive. And the longer the reaction time, the lower the limit of detection can be obtained if the signal fluctuation of an interferometer is suppressed additionally.

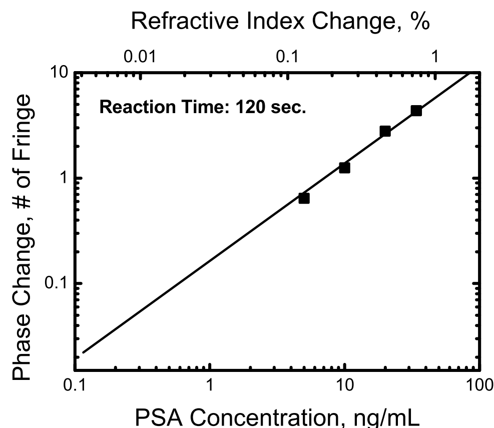

Fig. 4. Phase changes in the reaction regions of the fluidic chips are measured at various concentration of PSA. When it is compared with the absorbance signal using TMB as a substrate, the detection is 8.1 times more sensitive.

Acknowledgements

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