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Precision measurement of the refractive index and thickness of GOx thin film at different pH levels

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ABSTRACT

Glucose oxidase (GOx) is widely used in optical biosensors for glucose concentration measurement. There are many studies on the electrochemical properties of GOx, there is relatively little information about the refractive index (RI) and thickness of GOx thin film at different pH levels. This study develops a precision circular heterodyne interferometer (PCHI) to measure RI and thickness of GOx immobilized on the glass substrate with covalent bonding method at different pH levels. The results of this study reveal the non-linear dependence of RI on the pH levels of GOx, but the thickness is independent on pH levels of GOx. In addition, the comparison results are provided by commercial ellipsometer for measuring both RI and thickness, multimode fiber interferometer for measuring relative difference of RI, and field emission scanning electron microscope (FE-SEM) for the inspection of thickness. Based on these results, there is no significant difference between the proposed method and comparison methods. We believe that the valuable information of GOx can be helpful for the glucose sensor development.

1. Introduction

Optical glucose sensors have attracted much attention over the past few decades. Many sensors [1–6] integrate the glucose oxidase (GOx) enzyme to determine the glucose concentration caused by GOx selectivity. Therefore, the real-time optical signals, such as the change of the absorption property and the refractive index (RI) of the analytic solution, make it possible to distinguish between the GOx and analytic solution as the chemical reaction progresses. However, the performance of those sensors is highly influenced by the sensor structure, such as RI and the thickness of the GOx. Therefore, these essential parameters must be adequate to create a useful glucose sensor.

In the past decades, many studies [7–11] have been conducted on the measurement of the refractive index or thickness or both. Lee et al. [10] proposed a polarization Fizeau interferometer to extract temporal phase change of the reflection coefficients reflected by the thin film surface. In their method, they can have complete information about the reflection coefficient, refractive index, and thickness of the growing film in real time. Lin [7] proposed a sensitivity-tunable total internal reflection (TIR) heterodyne interferometer for the refractive index measurement. His findings showed high sensitivity and lower error in refractive index measurement under a specific measurement condition. Lee and Tsai [9] proposed a method for measuring the refractive index change based on surface plasmon resonance (SPR) using a phase quadrature interferometer. In their results, the resolution can be reached 6×10^{-6}. Chen [8] developed a single apparatus which integrated with heterodyne interferometer for measuring the wavelength shift of a laser, refractive index variation of solution, and the small angle variation. In previous work [11], we proposed a method for measuring RI and thickness of thin metal film with circular heterodyne interferometer. Based on the measurement condition, in which the incident angle and azimuth angle of analyzer were arbitrarily chosen, the errors in RI and thickness can be improved with precision measurement conditions.

In this study, we improve the performance of the previous work [11] with precision measurement conditions for determining the RI and thickness of GOx at different pH levels. The phase error could be close to 0 by selecting an appropriate incident angle and azimuth angle of analyzer. The phase error could be estimated at better than 1×10^{-30} and cause the errors in RI and thickness for each sample was better than 1×10^{-4} and 0.01 nm under precision measurement conditions. Based on the proposed method, this is the first time to determine the RI and thickness of GOx at different pH levels. To verify the results, we also measured the RI and thickness of GOx with commercial ellipsometer, multimode fiber interferometer and field emission scanning electron microscope (FE-SEM). Based on these results, there is no significant difference between the proposed method and comparison methods. Therefore, we believe that the determination of the essential parameters of GOx can be useful to those novel glucose sensor developments.

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2. Principle

2.1. Phase measurement with circular heterodyne interferometer

Fig. 1 shows the schematic diagram of the precision circular heterodyne interferometer (PCHI). The circular heterodyne light source was incident onto the glucose sensor with the incident angle θ, which is at the interface between the GOx and air. Multiple reflections occur at the multilayer structure (air/GOx/glass) which is shown in the inset of Fig. 1. The testing light will be reflected and passed through the analyzer with azimuth angle α and detected by the photodetector D_t. Based on the Jones calculation [11,12], the testing signal can be written as

$$I_t = |E_t|^2 = I_0[1+\gamma\cos(\omega t+\phi)], \tag{1}$$

where I_0 and γ are the bias intensity and the visibility of the interference signal, respectively, and ϕ is the phase difference between the p- and s-polarizations coming from the multiple reflection of the GOx thin film that can be presented as

$$\phi(n_1,d,\alpha) = \tan^{-1}\left[\frac{2(r_p r_s)\sin\alpha\cos\alpha}{|r_p|^2\cos^2\alpha - |r_s|^2\sin^2\alpha}\right]. \tag{2}$$

The r_p and r_s are the multiple reflection coefficients which are the functions of the RI (n_1) and thickness (d) of GOx, respectively, the azimuth angle of analyzer (α), and the incident angle (θ). Based on Fresnel's equations [12], the multiple reflection coefficients can be expressed as

$$r_k = \frac{r_{lmk} + r_{lmk}e^{i2\beta}}{1+r_{lmk}r_{lmk}e^{i2\beta}} \quad (k=p,s;\ \ l,m=0,1,2;\ \ l\neq m), \tag{3}$$

where r_{lmk} is the amplitude reflection coefficient of p- and s-polarizations at the interface as the beam from medium l is incident on medium m. Subscripts l and m are any of 0 (air), 1 (thin GOx film), and 2 (glass substrate), whose refractive indices are n_0, n_1, and n_2, respectively. r_{lmp} and r_{lms} can be expressed as

$$r_{lmp} = \frac{n_l\cos\theta_m - n_m\cos\theta_l}{n_l\cos\theta_m + n_m\cos\theta_l}, \tag{4a}$$

$$r_{lms} = \frac{n_l\cos\theta_l - n_m\cos\theta_m}{n_l\cos\theta_l + n_m\cos\theta_m}, \tag{4b}$$

and

$$\beta = \frac{2\pi d\sqrt{n_1^2 - n_0^2\sin^2\theta_0}}{\lambda}, \tag{4c}$$

where θ_l and θ_m are the corresponding incident angle and refractive angle, λ is the wavelength of the light beam, and d is the thickness of the thin GOx film.

The reference signal comes from the function generator which can be $I_r = [1+\cos(\omega t)]/2$. Both I_r and I_t sent into the lock-in amplifier, and the phase difference ϕ can be measured instantaneously. In practice, the RI and thickness of GOx at a fixed wavelength can be obtained by changing α, θ, or both. To prevent the misalignment of the incident angle, changing the two azimuth angles of analyzer is an appropriate way to achieve precise measurement. Therefore, RI and thickness of GOx can be determined by solving a set of equations of $\phi(\alpha_1)$ and $\phi(\alpha_2)$.

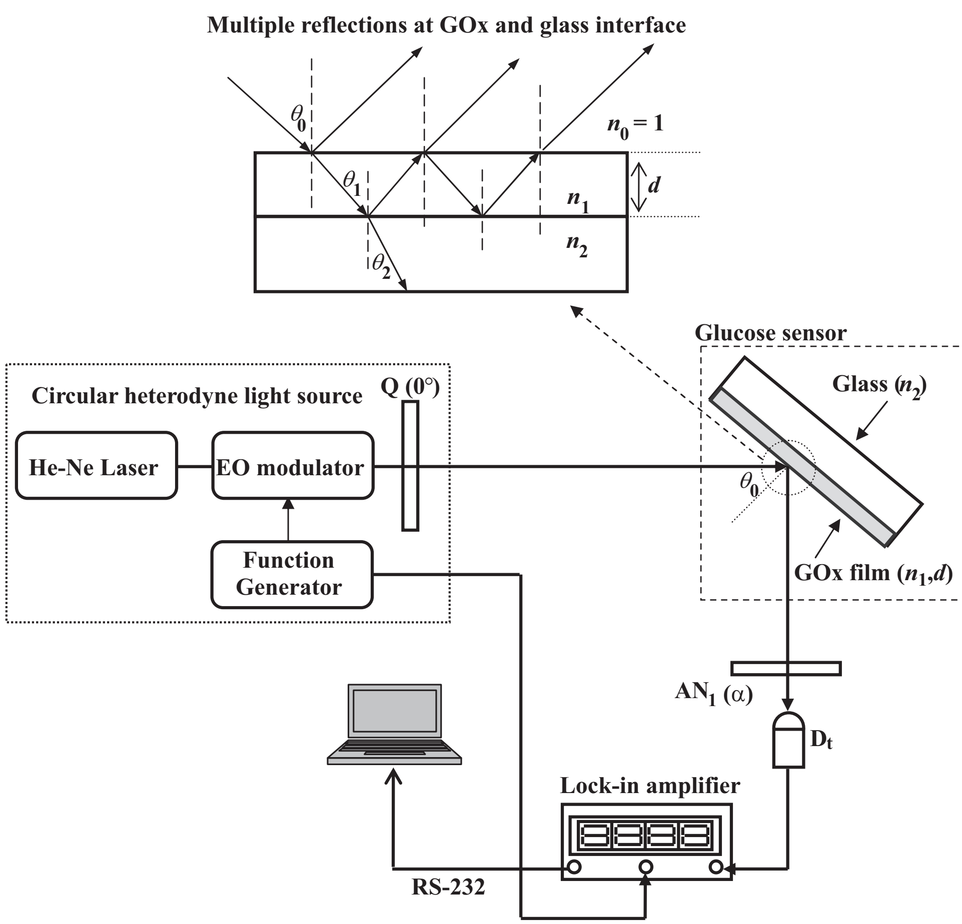

Fig. 1. Schematic diagram of the precision circular heterodyne interferometer (PCHI). AN: analyzer; Q: quarter-wave plate; D: photodetector.