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A sensitive bithiophene-based biosensor for interferon-gamma characterization and analysis

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ABSTRACT

Real-time, label-free monitoring offers a wide range of applications to the biotech field. We developed an integrated bio-sensing platform which adapts a circular polarization interferometry configuration and a phase modulated ellipsometer to improve the performance of biomolecular measurements. An bithiophene-based conductive linker-5′-(mercapto)-[2, 2′-bithiophene]-5-carboxylic acid was developed for detecting antibody–antigen interactions. In this study, the biomarker interferon-gamma (IFN-γ), one of the most important indicators of tuberculosis, was chosen to test and verify the sensitivity of our measurement system. Our experimental results showed that an increase in the concentration of the IFN-γ (64 pM to 1 μM) was usually accompanied by a phase increase. This result is good evidence that our biosensing system can be useful for analyzing biomolecular interactions.

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1. Introduction

With the development of optical and electrochemistry-based biometrology techniques, biological determination methods have progressively improved. In recent years, miniaturized devices have advanced quickly to meet the trend towards point-of-care devices. Obtaining biomolecular interactions that are label-free and in real-time is highly sought after.

Optical metrology possesses advantages such as non-invasive probing and label-free detection in biomolecular interaction measurements. Both surface plasmon resonance (SPR) and ellipsometry systems have been previously studied in detail to analyze surfaces with an attempt to measure the phase shift of reflected light beams for wide measurement ranges. As biomolecular detection typically starts out with an assembly of a thin film on a flat substrate, it is particularly suitable for biomolecular ellipsometry measurements. A typical ellipsometer adopts either a rotating-polarizer or a rotating-analyzer configuration to obtain the ellipsometric parameters which generally takes time to obtain and cannot be measured in real-time. Chao and Han installed an ellipsometer equipped with a photoelastic modulator to measure the Ψ and Δ ellipsometric parameters [1]. Although the measurement was in real-time, it still required time for the modulation of the phase retardation due to the control of the photoelastic modulator. The works published in 2009 by Han et al. [2] detail an ellipsometer configuration with a polarizer azimuth angle set at 45° while recording the sampling beam intensity respectively when the analyzer was rotated to 0°, 60°, and 120° azimuth angles respectively. The parameters Δ and Ψ were calculated by using simple equations in the configuration. We chose three specific rotating angles of the analyzer (e.g. splitting the beam into three and then analyzed them using the three rotated angle analyzers) so that the parameters Δ and Ψ could be calculated easily. Han et al. [3] further demonstrated in 2011 that obtaining an ellipsometric parameter Δ in real-time can provide enough information to analyze the sample variation in situ. The physical meaning of Δ is the phase difference between the *p*-polarized and *s*-polarized incident beams. It is similar to the phase difference acquired by a common path phase-shift interferometry technique. The sensitivity enhancement can be attributed to a physical transduction mechanism and to the system configuration [4]. Conceptually, the common-path configuration provides a reference beam denoted by *s*-polarization for retrieving the exact phase of *p*-polarized light and which enhances the relative phase difference detection precision [5,6]. For point-wise measurements, phase-interrogation has advantages such as optimal sensitivity, long-term stability, and high resolution. All of this makes it suitable for measuring real-time changes in label-free molecular binding processes [7].

Interferon-gamma (IFN-γ) is a type of endogenously cytokine that is secreted in human peripheral blood lymphocytes [8]. Typically, this biomarker can measure whether a person is at risk for tuberculosis and to indicate the existence of an inflammation [9–11]. SPR technology and electrochemical immunosensors have been used to monitor the

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equilibrium and kinetic constants for neutralizing anti-IFN-γ monoclonal antibodies or IFN-γ binding reactions [12–15]. In recent years, RNA and DNA aptamers have been used to detect IFN-γ using SPR, electrochemical impedance spectroscopy, and quartz crystal microbalance [16–19]. The results show that the detection limit of IFN-γ is 100 fM with the RNA aptamer and 1 pM with the DNA aptamer [16].

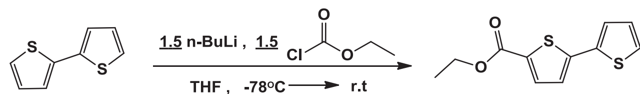
Previously, we constructed a system to monitor real-time biomolecular interaction using a common-path configuration sensor [20]. It included a light source, a SPR coupler, a flexible and precise incident angle varying system, as well as a quadrature interferometer detection configuration. One configuration of this system is an ellipsometer. No matter whether it acts as a SPR or as an ellipsometer, the sensitivity of the system depends on accurately controlling the incidence angle of the probing light beam [21]. Since an ellipsometric system is an optical technique that can be used to analyze surfaces, different reactions associated with biomolecular recognition leads to changes in film thickness. More specifically, different molecular bindings can result in different optical phase constants that can be detected by an ellipsometer since it can measure the phase of reflected light and provide a larger measurement range. The aim of this work was to demonstrate the usefulness of a synthesized conductive linker CS20S (5′-(mercapto)-[2, 2′-bithiophene]-5-carboxylic acid) and to detect the concentration difference of IFN-γ by measuring the phase change in corresponding refractive indices. Using the synthesized conductive linker to form the self-assembled monolayer (SAM) on the gold chip surface, it acts as a platform to characterize the biochip surfaces by adopting ellipsometry, SPR signal changes and electrochemical impedance spectroscopy techniques. The main emphasis of this paper is on the applicability of integrating this conductive linker into an ellipsometer which we had previously incorporated into a previous developed system [20]. It is expected that the structural features of the CS20S-modified film surface will be suitable for inhomogeneous solid thin films with respect to the transport of electro-active species.

2. Experimental set-up

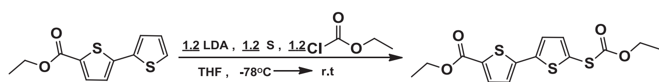
2.1. Linker and materials

The conductive linker CS20S, 5′-(mercapto)-[2, 2′-bithiophene]-5-carboxylic acid was synthesized by our team and dissolved in tetrahydrofuran (THF). The reaction formula of the CS20S molecular synthesis is detailed below:

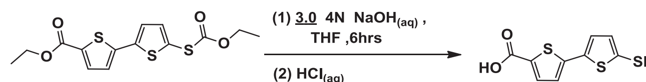
a. n-BuLi (7.5 mmol) was added in drops to a stirred solution of the corresponding 2,2′-bithiophene (5.0 mmol) in anhydrous THF (25 mL) under N_2 at −78 °C. Ethyl chloroformate (5.0 mmol) was added after one hour and the reaction was left to reach ambient temperature gradually, and then extracted with ethyl acetate/H_2O. After removal of the solvent by reducing the pressure, the reaction mixture was purified using chromatography.



b. Lithium diisopropylamide (6.0 mmol) was added drop by drop to a stirred solution of the corresponding substrate (5.0 mmol) in anhydrous THF (25 mL) under N_2 at −78 °C. Sulfur powder (5.0 mmol) and ethyl chloroformate (5.0 mmol) were added after one hour then left to reach ambient temperature, after which it was then extracted with ethyl acetate/H_2O. The solvent was removed using pressure, and the reaction mixture was purified using chromatography.



c. Sodium hydroxide (3.0 mmol) was added to a stirred solution of the corresponding substrate (5.0 mmol) in anhydrous THF (25 mL) under N_2 at room temperature for six hours. The reaction was quenched with hydrochloric acid. The crude reaction mixture was extracted with ethyl acetate/H_2O, the solvent removed by pressure and the reaction mixture purified by chromatography.



The recombinant human IFN-γ (carrier-free protein), anti-human IFN-γ Ab (monoclonal mouse IgG_{2A}) and mouse IgG_{2A} isotype control (monoclonal mouse IgG_{2A}) were obtained from R&D Systems (Minneapolis, MN, USA). The phosphate-buffered solution (PBS), EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride), NHS (*N*-hydroxysuccinimide) and Ethanolamine hydrochloric acid (ETA-HCl) were all obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Assembly of conductive linker on gold chip

A 1 nm Cr layer was deposited between the SF2 (refractive index: 1.64379 at 632.8 nm wavelength, Schott Inc., Duryea, PA, USA) substrate and a 50 nm Au thin film served as the intermediate layer for the bio-chip (Fig. 1(a)). The IFN-γ sensor was fabricated using this gold-coated bio-chip. The chips were cleaned in a detergent solution, then thoroughly ultrasonically cleaned with distilled H_2O and dried with nitrogen. The cleaned chip was then immersed in 5 mM CS20S solution for 12 h to modify the chip surface. Finally, the chips were rinsed with a THF solution, ethanol, and distilled H_2O. The SAM derived from the developed constructive linker on the gold chip surface was characterized by the SPR and ellipsometry signal changes.

2.3. SPR and ellipsometry systems

In our measurement system, a circularly polarized ellipsometer was integrated with a quadrature interferometer (Fig. 1(b)) [22]. The light source used was a 635 nm wavelength laser diode module (VHK™ Circular Beam Visible Laser Module, Edmund Optics Inc.) and driven by a 5 V DC power supply. Two incident light beams (*p*- and *s*-polarized) were adopted as the monochromatic light source propagating along a common path. The integrated miniaturized system named OBMorph (also known as Opto-Bio Morphin) adopted four photo-detectors to perform the differential phase measurement [20,21]. The sensitivity for the phase detection in the system was previously experimentally verified to be 8.2×10^{-6} (1/RIU). A FTA (fault tolerance algorithm) was integrated in the OBMorph System to compensate and to increase the system measurement reliability. By simulating the reflectivity and reflected phase of the SPR response, an estimated change of the *p*-polarized light was found to be within the linear region when the 50 nm Au/1 nm Cr was deposited on top of the SF2 substrate refractive index illuminated by a 635 nm wavelength incident light beam. (Fig. 1).

2.4. Analysis of CS20S assembly and IFN-γ binding

Experimental work was performed using the OBMorph platform [20,21]. The flow rate was set at 30 μL/min. A PBS buffer (10 mM PBS, 100 mM NaCl, 2 mM KCl, pH 7.4) was injected into the OBMorph to cleanse the prepared surface. The linker on the chip surface was activated with a 1:1 mixture of 0.4 M EDC and 0.1 M NHS for 10 min. For the mouse monoclonal anti-human IFN-γ Ab and IgG_{2A} immobilization, the antibodies were warmed to room temperature, and then dissolved in a PBS buffer in a 0.5 μM concentration. The antibody solution was injected and was allowed to interact with the linker for 20 min. 1 M ETA-HCl blocking solution (pH 8.5) was injected to reduce non-specific

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