Application of OLED integrated with BEF and giant birefringent optical (GBO) film in a SPR biosensor

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Abstract:

An organic light emitting diode (OLED) was integrated with a brightness enhancement film (BEF) and giant birefringent optical (GBO) film as a light source for a surface plasmon resonance (SPR) sensor system, to attain a portable SPR sensor design with further light source miniaturization that is simple to construct and cost-effective. It was found that by using OLED–BEF–GBO film based SPR, the quality of the signal is improved. A method for calculating SPR signal by summing the differences in optical intensity at two different wavelengths was employed to minimize the effect of the noise error particularly in portable SPR devices. The experimental results confirmed that the limit of detection (LOD) of the SPR using an Au sensing layer has an effective refractive index change of $7.8 \times 10^{-6}$ refractive index units (RIU). Furthermore, for the SPR system using Au/Ag sensing metal layers, an LOD of $3.2 \times 10^{-6}$ RIU was achieved due to a better sensitivity and resolution value. While for bio-affinity goat anti-mouse Immunoglobulin G (IgG) and target sample mouse IgG protein, the LOD is around 40.6 pg/ml.

Keywords:

organic light emitting diode (OLED), brightness enhancement film (BEF), GBO film, surface plasmon resonance (SPR), biosensor
1. Introduction

The surface plasmon resonance (SPR) sensor has been acknowledged as a promising sensor device for monitoring biomolecular activities due to its advantages of real-time monitoring, label-free assays, and high sensitivity. The SPR configuration based on the Kretschmann[1] prism coupler is the most popular SPR sensor, since SPR phenomena in this configuration are simple to build up and analyze. The principle of the SPR sensor is to excite the surface plasmon wave in the thin metal–analyte medium interface with $p$-polarized light at a specific angle through a high-refractive-index prism. Subsequently, the reflectivity dip profile is obtained, and the reflectivity dip will be shifted once the refractive index of the medium is changed[2]. The common commercial SPR sensor system to be introduced has large size, weight, high power consumption, and high cost[3–7]. Hence, the challenges in SPR sensor development are miniaturization, cost effectiveness, and portability of the sensor structure[8]. A portable SPR has the potential to be implemented either in a conventional laboratory, such as a common SPR sensor for biomolecular detection, or in the field, such as an SPR sensor for environmental monitoring, continuous monitoring water system, food safety, emergency vehicle tools[9–13].

Laser has been well known as the mature technology for biosensing light sources, either for conventional SPR sensor[2] or other optical sensor; such as recent
report in optical interferometer based optical waveguide light mode spectroscopy (OWLS) and grating based interferometer with Limit of Detection (LOD) around 0.2 µg/mL and $10^{-7}$ RIU respectively[14,15]. Several studies presented light-emitting diodes (LEDs) as a light source in SPR systems due to such advantages as low cost, low power, robustness, and small size[16]. Therefore, this light source can provide more miniaturized and portable SPR instrumentation compared to bulk light sources like halogen lamps or lasers. A white LED was reported for an SPR-based prism coupler[17] that achieved an LOD of $1.98 \times 10^{-4}$ RIU; an LED for an SPR-based optical waveguide achieved a better LOD of $2.3 \times 10^{-5}$ RIU[18]; and, using a phase analytical method, an LOD value of $7.9 \times 10^{-9}$ RIU was achieved[19]. The progressive improvement shown by these results has made the applications of LED light sources to SPR systems become a very important area of study in recent work on SPR system design.

On the other hand, the organic light-emitting diode (OLED) has been developed and gradually improved for use in various display industries, in recent years. In addition, an OLED with a large area substrate as an integrated heat sink is one option that is a promising candidate for a portable SPR light source due to its good heat dissipation performance. However, there are only a few studies that have reported the application of OLED as a optical sensor light source[20–22].
An SPR sensor design that employs an OLED light source attached to the prism has been reported to enhance miniaturization of the sensing system and to simplify the assembly of the sensor, particularly in the light source portion of the design, and while attaining an LOD of $6 \times 10^{-4}$ RIU[21]. Nevertheless, there are obstacles to using an OLED attached to an SPR system as the light source. First, compared to a laser, an OLED emits light over a wide angle; thus, determining how to reduce the output angle of the light to be mainly in the direction perpendicular to the face of the prism plays an important role in enhancing the surface plasmon wave. Second, a complicated configuration of the polarizer is needed to get pure $p$-polarized light to be incident on the SPR system. A conventional polarizer such as a polarizing beam splitter (PBS) has a quite large cubic size (~1 cm$^3$) compared to the OLED thickness (~2 mm); therefore on an SPR system with an OLED, the polarizer is utilized on the path along which the light is reflected[21]. Using this polarizer configuration, the sensing metal layer is coupled by non-polarized light instead of by pure $p$-polarized light. A recent study showed that SPR can be enhanced by both $s$-polarized and $p$-polarized light respectively by insertion of an anisotropic thin film below the metal[23]. However, for SPR sensor design using a conventional noble metal film, such as Au or Ag, only $p$-polarized light is able to enhance the surface plasmon resonance [1,8,24].
In this article, an SPR sensor based on an OLED using a brightness enhancement film (BEF) and a giant birefringent optical (GBO) film is presented to provide the correct polarizer configuration for an SPR system without any significant change in the light source size. Polychromatic light sources composed of OLEDs, with a center wavelength of 620 nm for the Au sensing layer and 530 nm for the Au/Ag sensing layer were presented in this study respectively. The BEF is integrated with the OLED to improve the light intensity and decrease the output angle of the OLED light significantly[25,26]. Furthermore, the GBO film is employed as a reflective polarizer[27–32] next to the BEF film to obtain p-polarized light and couple the surface plasmon wave. The integrated light source using an OLED–BEF–GBO film contributes to cost-effectiveness and a simple optical construction, while inducing an optical enhancement of the SPR system. Moreover, a sum of differential-intensity-based measurements is utilized to analyze the SPR reflectivity due to its advantage of being able to reduce the noise error of SPR measurements.

Finally, in this study, the sensitive and portable SPR sensor based OLED with integration BEF and GBO film for optical enhancement is presented. The detection of mouse IgG protein using our portable SPR biosensor is demonstrated achieved LOD 40.6 pg/ml.

*(Figure 1 is preferred at this position)*
2. Materials and Methods

2.1 Integrated SPR system

The propagation constant of the surface plasmon wave $k_{SP}$ can be described by the equation,

$$k_{SP} = \frac{2\pi}{\lambda} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$  

while the wavevector of the incident light on the prism (Fig. 1(a)) can be described as,

$$k_x = \frac{2\pi}{\lambda} n_p \sin \theta$$

where $\varepsilon_m$ and $\varepsilon_d$ are the dielectric constants of the metal and the analyte medium, respectively; $n_p$ is the refractive index of the prism; and $\lambda$ and $\theta$ are the wavelength and incident angle of the light, respectively. The SPR will occur when $k_x = k_{SP}$.

From Eqs. (1) and (2), the incident angle of the light on the SPR system can be calculated from the equation,

$$\sin \theta = \frac{1}{n_p} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$

where, in this study, the incident angle is calculated to be 75°. The integrated portable SPR system in our experimental studies is illustrated in Fig. 1(a). A BK7 trapezoidal prism with an angle of $\theta$=75° corresponding to the incident angle of the light and a
refractive index \((n)\) of 1.515 is employed. The smaller angle of the prism is identical with the incident angle of the light on the SPR system since the flat substrate light source is attached to one of the angled sides of the trapezoid [21]. In our study, OLEDs (e-Ray Optoelectronics Tech. Co., Ltd., Taiwan) were used to apply polychromatic red and green light with peak wavelengths of 620 nm and 573 nm (Fig. 1(b)); the OLED substrates were coated by an optically clear adhesive (OCA) film (3M, USA) prior to attachment of the BEF microfilm (Efun Tech. Corp., Taiwan). Subsequently, the OCA was attached to the BEF surface, and the GBO film (Vikuiti™ 3M DBEF-D400, USA) film was attached to it. Later, the homemade integrated light source was attached to the prism by index-matching oil \((n=1.515)\) (Nikon, Japan). Next to the gold metal film is located a polydimethylsiloxane (PDMS) flow cell, and analyte solution is streamed through the inlet and outlet pipes, which have diameters on the scale of millimeters. A collimator is used to capture the reflected light and to limit the optimum incidence angle light, afterward a fiber optic waveguide transmits the light to a spectrometer (Ocean Optics, USA). Finally, the SPR reflectivity data are stored on a computer for analysis. The active part of integration system of this SPR is palm-sized (at around 5 cm \(\times\) 10 cm \(\times\) 10 cm).

The Essential Macleod simulation tool (Thin Film Center Inc., USA) is employed to simulate the ideal design of SPR system using identical conditions with
our experiments. The refractive index model of gold film by Johnson and Christy[33] and the Hale model[34] for water are applied in the simulation database instead of the default model. The simulated SPR systems use polychromatic light, with various incident angles of light, a BK7 prism, a 47-nm gold sensing layer, and water as the medium. The \( p \)-polarized reflectivity profile is depicted in Fig. 1(c), where the white line represents the incident angle of the light (75° in our SPR system). It shows that for the optical spectrum of this SPR system, the SPR dip is detected at a wavelength position of around 615 nm.

2.2 Methodology

In our experiment, the SPR dip position was measured three times, then the standard deviation of the noise (\( \sigma \)) was calculated. The resolution of the SPR system can be given as \( 3\sigma \)[35]. The method for analyzing the SPR signal used the sum of the differential reflected light intensity 20 nm below and 20 nm above the SPR signal wavelength dip position, as shown in Fig. 2(a). The sum of the differential intensities A and B determines the SPR signal \( A+B \)[18]. This approach of summing the differences in intensity is able to reduce the limitations of light intensity fluctuations, mechanical deviations in component placement and thermal drift, and noise in the photo-detector and its electronics[18,36]. The intensity fluctuation from light sources around the wavelength of the SPR signal dip caused a deviation of the minimum
intensity position. Mechanical deviation is related to the component placement in the SPR system, particularly in portable SPR devices; it influences the noise of the incident angle of the light. Furthermore, thermal drift also induces deviations in the SPR measurement results due to the different thermal coefficients of the component materials. Afterward, the refractive index of the materials will be slightly shifted due to thermal effects. Another noise source in the SPR system comes from the dependence of the system on the spectrometer, where every spectrometer device has a different error tolerance, especially in a spectrometer system with a broad spectrum. Subsequently, the limit of detection (LOD) was calculated from the resolution and sensitivity ratio[35]. This methodology also benefits the asymmetric reflectivity dip of the SPR measurement; the asymmetric SPR signal commonly occurs in SPR systems using polychromatic light because the refractive index of the metal layer, the refractive index of the medium, and the propagation constant of the surface plasmon \( k_{SP} \) are all wavelength-dependent. Using the sum of the differential intensities before and after the SPR dip wavelength, both sides contribute to the calculated SPR signal.

(Figure 2 is preferred at this position)

(Table 1 is preferred at this position)

In order to confirm the methodology, a simulation result as theoretical justification of our methodology and conventional wavelength interrogation are
plotted in Table 1. In the range of refractive index change ($\Delta n$) $\leq 1 \times 10^{-5}$ RIU, the wavelength interrogation signal is not recognized theoretically; while, differential SPR signal A+B able to detect smaller $\Delta n$ compared to wavelength interrogation method.

2.3 Bio-affinity measurement preparation

The goat anti-mouse IgG and mouse IgG which were used as the capture antibody and target protein, respectively, were purchased from Sigma-Aldrich (St. Louis, MO, USA). In addition, 11-mercaptopoundecanoic acid (11-MUA), 3-mercapto-1-propanol (3-MOH) and phosphate buffer saline (PBS) tablets were also obtained from Sigma-Aldrich. The immobilization buffer (10 mM sodium acetate, pH 5.0) and an amine coupling kit containing N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and 1.0 M ethanolamine-HCl, pH 8.5 (ETH) were purchased from Biacore, Inc. Before the measurement,

Before bioaffinity measurement, the metal sensing was immersed in the self assembly monolayer (SAM), which is mixture of 9 mM 11-MUA and 1mM 3-MOH at ratio of 1:1 in the ethanol solution (20 hr); followed by immersion of 0.4 M EDC and 0.1 M NHS (10 min), and then washed with PBS buffer (3 min). Subsequently, the goat anti-mouse IgG is immobilized in the surface (30 min) prior to PBS buffer
washing (3 min). ETH was applied for blocking (15 min) and followed by PBS buffer washing (3 min).

3. Results and Discussion

3.1 BEF and GBO film for optical enhancement.

The BEF surface structure design consists of a periodic set of triangular prismatic strips which play an important role in decreasing the incident light angle, as illustrated in Fig. 3(a). The light will be refracted at a narrow angle (~70°); while, the wider angle or narrower angle of the output light will be refracted back into the film.

(Figure 3 is preferred at this position)

In addition, the main mechanism of the GBO film as a reflective polarizer can be described by noting that inside the film, $s$-polarized light is reflected back, while $p$-polarized light is transmitted. The mechanism of polarization can be explained based on the Fresnel derivative of the transmission and reflection of light through different refractive index materials[32]. Giant birefringent optical polymer material inside the microstructure is utilized to split the $s$- and $p$-polarized light using the biaxial refractive index, while the reflection of polarized light can be adjusted by different combinations of refractive indices of polymer material in the stack direction ($z$-axis).
relative to the in-plane directions of the microstructure[32]. For a specific polarization axis and a specific incident angle of light where s-polarized light is perpendicular with the plane of incidence, s-polarized light is slightly reflected, while almost all of the p-polarized light is transmitted[32]. Consequently, all s-polarized light is reflected gradually, and after hundreds of repetitions, only p-polarized light output is obtained.

The polarizer in this SPR system used a GBO film, to acquire pure p-polarized incident light. The polarization light characteristics of the GBO film were measured by using the degree of polarization (DOP). The output light from the OLEDs at central wavelengths of 620 nm is compared in the presence and absence of the GBO film, as shown in Figs. 3(b) and (c). In our OLED, the DOP result in the absence of the GBO film is 3.3%; it is improved to 83.5% once the GBO film is coated on the OLED substrate.

A measurement showing the intensity improvement in the narrowness of the angle of light due to the BEF is plotted in Fig. 3(d). It shows that the incident angle of the light of the OLED has been significantly reduced by 50% and that the intensity is improved by around 25%. In addition, from the characteristics of the intensity and viewing angle of the light of the OLED using the BEF and GBO film, the results indicate that the combination of the BEF and GBO film enhances the optimum intensity and also enhances the sharpness of the incident angle at 0°.
3.2 SPR measurements using different optical configurations

In our experiment, the surface plasmon resonance with a different polarizer design with water as the analyte medium \((n=1.332)\) and a 47-nm Au metal film is shown in Fig. 4. It shows the characteristics of the original OLED and the effect of integrating it with SPR systems using different optical configurations: a BEF and a GBO film; the original OLED light shows a shallow SPR reflectivity signal with a full width at half maximum (FWHM) value of 47.6 nm. After the OLED was covered by the BEF, the SPR reflectivity signal dip was slightly improved (FWHM = 36.31 nm). Finally, the OLED covered by the BEF–GBO film microstructure reached the deepest reflectivity dip (FWHM=51.9 nm). The signal quality factors \((Q)\) were calculated and added to the legend in Fig. 4(a) to confirm the quality of the signals, where \(Q=\frac{\text{SPR signal dip}}{\text{FWHM}}\). The SPR reflectivity signal using the integrated BEF–GBO film shows the highest value of the normalized \(Q\).

3.3 SPR measurements and simulation using gold and gold-silver sensing layers

A sensing bimetallic Au/Ag layer and an OLED green light were employed in the second experimental SPR system based on the OLED–BEF–GBO film, and the SPR
reflectivity dip occurred at a wavelength of 580 nm when measured with water as the medium. The analyte medium was ramped from 0 to 2.5 wt% of sucrose water (sw) concentration with 0.5 wt% steps. The SPR signal A+B using a sensing metal was calculated and plotted in Fig. 5. In order to confirm the experimental result, the SPR simulations results are presented using both Au and Au/Ag as sensing metals. Considering that 1 wt% of sw corresponds to $\Delta n = 0.00143$ RIU, the achieved sensitivities are 5384.6 RIU$^{-1}$ and 4055.9 RIU$^{-1}$ for the simulation and experimental results respectively. The sensitivity of the experimental SPR system using an Au/Ag sensing layer is less than the theoretical result, which is obtained by simulation due to insufficient intensity at wavelength range of above 580 nm where the optimum SPR dip occurs and shifts to higher wavelength when the higher concentration of sucrose water is measured. The baseline error propagation value of the experimental SPR system using an Au/Ag sensing layer is calculated to be around 0.004, and the resulting LOD of this system is $3.2 \times 10^{-6}$ RIU.

(Figure 5 is preferred at this position)

On the other hand, for the SPR signal A+B using an Au sensing layer, the sensitivity of the simulation and the experimental result show a very good agreement. The slope of the SPR signal A+B from the simulation and the experimental SPR system using an Au sensing layer yields sensitivity values of 3510.5 RIU$^{-1}$ and 3426.5
RIU$^{-1}$ respectively. In addition, the error propagation calculation value of the SPR signal A+B is around 0.009. Consequently, the LOD of the SPR using an Au sensing layer achieved a value of $7.8 \times 10^{-6}$ RIU.

Biomolecule affinity measurement is performed for detection the mouse IgG was as a target protein and goat antimouse IgG as an antibody. Bimetallic Au/Ag sensing layer was immersed in the SAM solution for 20 hr, followed by activation of gold surface using NHS-EDC, antibody immobilization, and ETH blocking. Subsequently, series-dilution of mouse IgG protein in PBS solutions (0 pg/mL for negative control, 10 pg/mL, 100 pg/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL) were injected into the flow cell to interact with the immobilized capture antibody on the SPR chip to form the capture antibody/target protein immunocomplex (20 min), following with the PBS wash (3 min) before three repetition SPR signal measurement. The coefficient is correlation coefficient ($R^2$) is equal to 0.9965, and the error bar is standard deviation from three time repetition measurements. the LOD of bioaffinity measurement is the concentration at which the signal correlated to $3\sigma$ (three times standard deviation of the blank measurement without the target antigen) is calculated with the dose-response non linear curve. Accordingly, the LOD of the DBEF-BEF-based OLED-SPR biosensor was calculated to be 40.6 pg/ml of target sample.
4. Conclusion

The portable SPR sensor based on an OLED using a BEF and GBO film for optical enhancement has been presented. The optical integration is allowing to simple construction of reliable light source for portable SPR sensor. The BEF and GBO film is utilize to enhance the intensity and polarization of the incident light. An LOD of 7.8 × 10^{-6} RIU is achieved using Au sensing metal; the SPR system using Au/Ag sensing metal established an LOD of 3.2 × 10^{-6} RIU due to a higher sensitivity and better resolution value. For bio-affinity measurements by goat antimouse IgG and target sample mouse IgG protein, the LOD is around 40.6 pg/ml. This study has shown that the SPR system using an OLED–BEF–GBO film light source can provide an alternative option for SPR system light sources, which is the performance is comparable to other light source based SPR sensor. Using this simple construction optical allowing low cost fabrication of SPR sensor device and avoid movable mechanical part such as rotatable motor, without any drawback in the device performance.

Acknowledgments

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References:


Figures and captions
Fig. 1. (a) Top view of the integrated SPR sensor system (not to scale) using an OLED light source covered by BEF and GBO film. (b) Incident light spectra from the polychromatic OLED with center wavelengths of 630 nm and 530 nm, respectively. (c) Simulation result of the SPR reflectivity using 47 nm of gold sensing for various wavelengths and angles of incidence for the light. The white line represents the incident angle in the current SPR system.
Fig. 2. SPR measurement method based on summing of the differential intensity SPR signal.
Fig. 3. (Color online) (a) BEF structure cross section and mechanism by which the microstructure BEF acts as a wide-angle light filter. DOP measurements of (b) the unfiltered light of the OLED and (c) OLED light filtered by the GBO film. (d) Measured OLED intensity plotted against the incident angle with different combinations of BEF and GBO film; the grey area is the optimum angle for capturing of light by the collimator.
**Fig. 4.** (a) Experimentally measured SPR reflectivity signal with different optical configurations using water as the analyte medium. The BEF–GBO film, which has the highest normalized quality factor \((Q)\), achieves a significant improvement in the SPR reflectivity signal dip.
Fig. 5. (a) Simulation and experimental results for the SPR signal A+B based on using an OLED and metal films of Au/Ag and Au, respectively. The inset
figure depicts the noise for different configurations of the experimental SPR system. Baseline error propagation from three repetition measurements are \( \sigma_{\text{AU/AG}} = 0.004 \); \( \sigma_{\text{AU/AG}} = 0.009 \). (b) Measurement of bioaffinity capture antibody (goat antimouse IgG) and the antigen (mouse IgG) using Au/Ag sensing layer, \( (R^2 = 0.9965) \), resulting LOD \( \sim 40.6 \) pg/ml of mouse IgG concentration.
Table 1. The simulated signal comparison of SPR signal A+B with conventional wavelength interrogation in range refractive index change ($\Delta n$) $1\times10^{-9}$ to $1\times10^{-4}$ RIU.

<table>
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<th>SPR A+B (%)</th>
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