Real-time biodetection using a smartphone-based dual-color surface plasmon resonance sensor

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Abstract. We proposed a compact and cost-effective red–green dual-color fiber optic surface plasmon resonance (SPR) sensor based on the smartphone. Inherent color selectivity of phone cameras was utilized for real-time monitoring of red and green color channels simultaneously, which can reduce the chance of false detection and improve the sensitivity. Because there are no external prisms, complex optical lenses, or diffraction grating, simple optical configuration is realized. It has a linear response in a refractive index range of 1.326 to 1.351 ($R^2 = 0.991$) with a resolution of 2.3×10^{-4} RIU. We apply it for immunoglobulin G (IgG) concentration measurement. Experimental results demonstrate that a linear SPR response was achieved for IgG concentrations varying from 0.02 to 0.30 mg/ml with good repeatability. It may find promising applications in the fields of public health and environment monitoring owing to its simple optics design and applicability in real-time, label-free biodetection. © 2018 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.23.4.047003]

1 Introduction

Cost-effective, portable, and rapid sensing systems play critical roles in bringing biodetection technologies from central laboratories to field applications.[1–3] Although laboratory-based benchtop instruments usually provide higher sensitivity and stability, they are usually expensive and bulky. Nowadays, there are intense interests in converting the smartphone into a low-cost and user-friendly sensing tool for different biochemical sensing applications. It is expected to be applied in situations where laboratory facilities are not available, such as point-of-care analysis in the home, clinic, or remote locations. Real-time communication and data sharing from any remote location are possible with the help of smartphones. Examples include smartphone-based microscopy,[4] enzyme-linked immunosorbent assay (ELISA),[5] polymerase chain reaction (PCR),[6] and surface plasmon resonance (SPR).[7,8] Unlike ELISA or PCR, SPR does not require a labeling procedure, which is a great advantage in field applications.[9–11] Utilizing high-performance complementary metal–oxide–semiconductor (CMOS)-based cameras on smartphones, many groups have designed spectrum- and intensity-based SPR biosensors on smartphone platforms. For spectrum-based assays, smartphones are usually converted to simple spectrometers. The changes of spectrum mean some reaction has happened or some analytes are present. Zhang et al.[12] demonstrated a grating-coupled SPR smartphone biosensor, which relies on a disposable sensor chip with Au diffraction grating and a compact disk as the spectra dispersive unit. Although it does not contain electronic components, the use of apertures, polarizers, and diffraction gratings increases the complexity of the optical configuration. A portable spectrum-based fiber optic glycerol smartphone SPR sensor was demonstrated by Bremer and Roth.[13] Silver-coating fiber SPR sensor eliminates the need

for external prisms, optical lenses, etc. for easier optical coupling and optical alignment. Spectrum-based analysis usually means more information and higher sensitivity, but it also means more complex image processing algorithms and greater computational complexity. Smartphones are only used to capture images, and subsequent analysis needs to be performed on the computer, which limited their actual diagnostic usefulness. Intensity-based assay has attracted much attention because of its low-cost, simplicity, and practicality. It provides real-time data analysis and is usually stable, inexpensive, and simple to perform. The optical intensity at fixed angle or wavelength is monitored by smartphones in real time. Preechaburana et al.[14] and Guner et al.[15] constructed angle- and wavelength-resolved intensity-based smartphone SPR sensors, respectively. Combined with fiber optic sensing technology, Liu et al.[7] proposed a fiber optic smartphone SPR sensor based on intensity integration method, which greatly simplified the optical configuration. In addition to the advantages of label-free, real-time analysis, it can be easily multiplexed to enable high-throughput screening in an array format.

In this paper, we propose a smartphone-based dual-color fiber optic SPR sensor. Red and green color channels are monitored simultaneously based on the inherent color selectivity of phone cameras instead of using discrete lenses or diffraction gratings to get spectral data as those spectrum-based SPR sensors reported to date. Also, it does not require additionally external monochromatic light source or narrowband filter as those intensity-based single-color SPR sensors, which greatly simplify the system configuration and lower the cost. Ag/Au bilayer film was implemented to enhance sensitivity and color contrast. A naked-eye observable color change can be seen from the images corresponding to the different refractive index (RI). A schematic of the dual-color fiber optic SPR sensor developed here is shown in

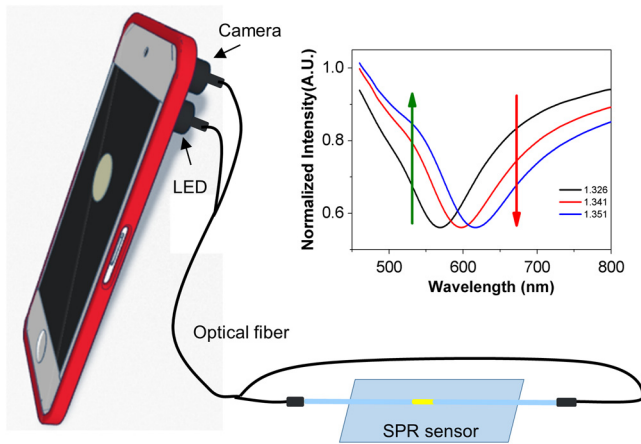*Address all correspondence to: Zhenguo Jing, E-mail: jingzg@dlut.edu.cn; Wei Peng, E-mail: wpeng@dlut.edu.cn

Fig. 1 Schematic diagram of smartphone-based dual-color fiber optic SPR sensor. (Inset) Normalized spectrums of the sensor in different RIs obtained by spectrometer.

Fig. 1. This method quantifies SPR signals from two color channels (one decreases, while the other increases as the surrounding RI changes) to enhance the accuracy and sensitivity of detection. An Android application software was developed to analyze the red-green-blue (RGB) image data in real time. SPR sensor was functionalized with staphylococcal protein A (protein A) for specific identification and binding of immunoglobulin G (IgG). Experimental results indicate that the proposed dual-color SPR sensor is effective for biosensor applications.

2 Red–Green Dual-Color Fiber Optic SPR Sensor

Au and Ag are the most widely used metal for the SPR experiments.[16] Ag metal-based SPR sensors usually have higher sensitivity and smaller line width of resonant dip compared with Au mental-based SPR sensors;[17–19] however, they are highly prone to oxidation, which greatly restricts their application. Coating Au film above the Ag film to eliminate the oxidation has been extensively studied.[20–22] In this work, we employed Ag/Au bilayer film to enhance sensitivity and color contrast. We used hard polymer cladding optical fiber (HPCF, HP 400/430-37/730E, YOFC Inc., Wuhan, China) to fabricate the

SPR sensor. Diameters of the fiber core and cladding were 400 and 430 μm, with numerical aperture 0.37. The fiber optic SPR sensor was 6-cm long, and in the middle of the fiber existed a 5-mm sensing region with cladding removed. The sensing region was cleaned by acetone and deionized water subsequently, and then coated with Ag/Au bilayer film (25-nm Ag layer and 15-nm Au layer). A magnetron sputtering coater (K575XD from E. M. Technologies Ltd., Kent) was used for metal layer coating. It features a rotating sample table that ensures even depositions. The rotation speed is about 6 r/min. To get a metal film deposited around on the fiber core surface uniformly, we used a set of rotation clamping mechanisms to make the fiber optic sensing part rotate uniformly in the vacuum chamber. Thickness monitoring was achieved by quartz crystal oscillation method.

A homemade wavelength interrogation fiber optic SPR system shown in Fig. 2(a) was utilized to demonstrate the performance of the fabricated SPR sensor. It consists of a halogen lamp (HL-2000-FHSA, Ocean Optics, Inc.) and a high-resolution spectrometer (HR4000, Ocean Optics, Inc.). The fabricated sensor was connected to the devices through SMA905 connectors. Figure 2(b) shows a clear SPR dip that shifts toward the red wavelength range for increasing RI of the surrounding liquid. We changed the liquid RIs using different sodium chloride solutions and calibrated the RI values by an Abbe refractometer (WAY-2S). Through linear fitting, the sensitivity of measuring RI of the sensor could reach 1839 nm/RIU ($R^2 = 0.992$).

The intensity at a fixed wavelength will change along with the SPR curve shifting caused by variations of RI. When monitoring the intensity at two wavelengths across the dips, it can be observed that the intensity for shorter wavelength increases and that for longer wavelength decreases. By taking the difference between the normalized intensity at two wavelengths, almost twofold improvement in sensitivity can be expected. A miniaturized optical waveguide SPR sensor based on dual light-emitting diodes as light sources has been demonstrated by Suzuki et al.[23] In this work, we used the inherent color selectivity of phone cameras to monitor red and green color channels simultaneously. CMOS digital cameras on the smartphone (HTC D816d) served as detector and the flashlight served as light source. Light intensities for red and green color channels were obtained simultaneously within the RGB passbands of the internal color filters of digital camera.[24]
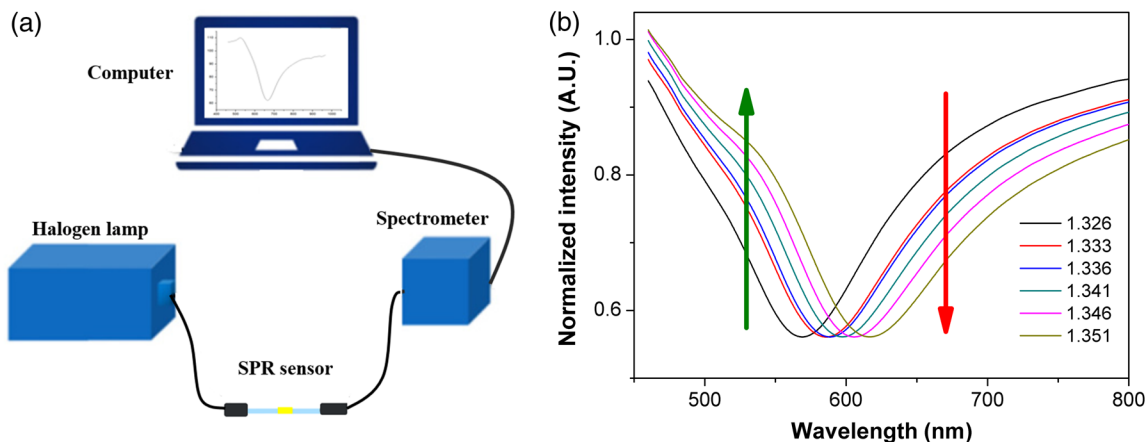

Fig. 2 Spectral characteristics of fiber optic SPR sensor: (a) wavelength interrogation system used to verify the fabricated SPR sensors and (b) normalized spectrums of the sensor in different RIs ranging from 1.326 to 1.351.

We developed an Android application for real-time image processing. There are 24 bits per pixel in color images, which include three 8-bit integers (0 to 255) that indicate the intensity of RGB colors. R values and G values of all the pixels in the region of interest (ROI) are averaged as the intensity of red channel and green channel, respectively. After capturing the required images with 640×480 resolution, the average intensities of red and green channels were calculated and displayed on the screen in real time. In this work, a rectangular area covering the bright image of optical fiber's end face was chosen as the ROI. Each recorded intensity was typically averaged over 2 s (10 averages) to reduce the noise.

Responses of red and green channels upon sequential injection of sodium chloride solutions at increasing RI (1.326 to 1.351) are presented in Fig. 3. Figure 3(a) shows the real-time color images when the sensor region was immersed in different RI solutions. A naked-eye observable color change can be seen from the images. The intensities of each channel are normalized with respect to the intensities when the sensor is exposed to the air, and the normalized intensity variations of green channel and red channel are shown in Fig. 3(b). To obtain reliable data, the test for each solution was performed three times. Error bars indicate the standard deviation (SD) of the measurements. As RI increases, the green channel intensity increases and red channel intensity decreases. In this work, we define the differential normalized intensity between green and red channels as dual-color sensor response.

Normalized intensity shifts of dual-color channel are shown in Fig. 4. Error bars correspond to the SD of triplicate measurements. It illustrates that the normalized intensity shift as a function of RI can be linear fitted in the RI range of 1.326 to 1.351, which can be used for many biochemical samples measurement.[25,26] In the RI range of 1.326 to 1.351, the system provides a sensitivity of 11.542/RIU. Analysis of the baseline noise was also performed. It was determined that the SD of noise in the normalized intensity shift is 2.6×10^{-3}; so, the RI resolution can be calculated to be 2.3×10^{-4} RIU.
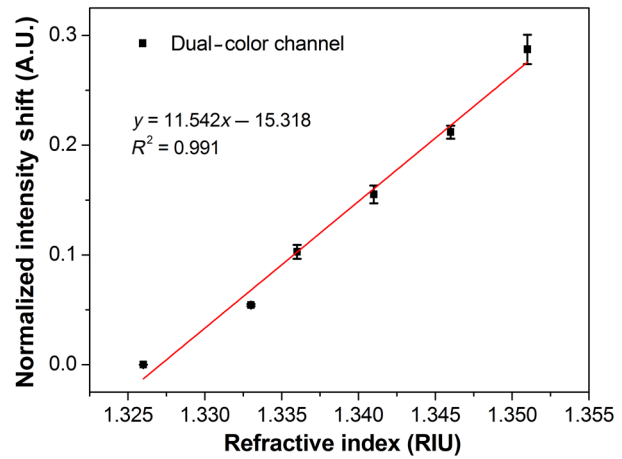
Fig. 4 Plots of normalized intensity shifts as a function of RI ranging from 1.326 to 1.351.

3 Real-Time IgG Detection

To confirm the feasibility of this dual-color SPR sensor in biosensing applications, the protein A and rabbit IgG interaction was monitored. Protein A interacts with the Fc region of human, rabbit, pig, and guinea pig IgG with high affinity and specificity.[27] In this work, we used protein A-modified SPR sensor for concentration detection of rabbit IgG.

Sensor functionalization and protein A–IgG binding procedure are shown in Fig. 5. An amine-coupling reaction was used to facilitate the attachment of protein A. The sensor was soaked in an ethanolic solution of 11-mercaptoundecanoic acid (1 mmol/L) at 25°C for 2 h to form a carboxyl surface, and then immersed into an aqueous solution containing 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (0.55 mol/L) and N-hydroxysuccinimide (0.5 mol/L) for 30 min at 4°C to convert the carboxyl surface to activated ester. The treated sensor was dipped in protein A [0.1 mg/mL in 0.01-mol/L phosphate-buffered solution (PBS; pH = 7.4)] for 30 min. Unreacted ester groups were blocked by bovine serum albumin (1 mg/mL in PBS) for 15 min.

A mini pump and a homemade flow cell were used for sample injection. The flow rate is about 250 μL/min. Real-time responses of IgG detection are shown in Fig. 6. Figure 6(a) shows the responses of green and red channels, and Fig. 6(b) shows the calibrated responses of dual-color channel. PBS buffer was pumped into the flow cell at the very beginning to obtain a stable baseline. When the baseline was stable, we started the real-time biodetection of different IgG samples. Multiple measurements on one sensor were possible using regeneration solutions. Acetic acid solution (pH 2.0) was pumped into the flow cell at a flow rate of 250 μL/min for 5 min for surface regeneration. After regeneration, the baseline returned to its initial value. There was no clear deterioration (SD of baseline difference = 1.6×10^{-3}) during 10 binding and regeneration cycles. Figure 6(c) shows real-time binding process of five different IgG concentrations, and IgG samples with various concentrations ranging from 0.02 to 0.30 mg/mL (0.02, 0.05, 0.10, 0.15, and 0.30 mg/mL) were pumped into the flow cell for 8 min. After the specific recognition and binding stage, PBS buffer was used to wash away the unbound IgG from the surface. Signals from the 20th minute to the 22nd minute are averaged as the sensor response corresponding to the specific concentration. To get reliable data, the tests were performed
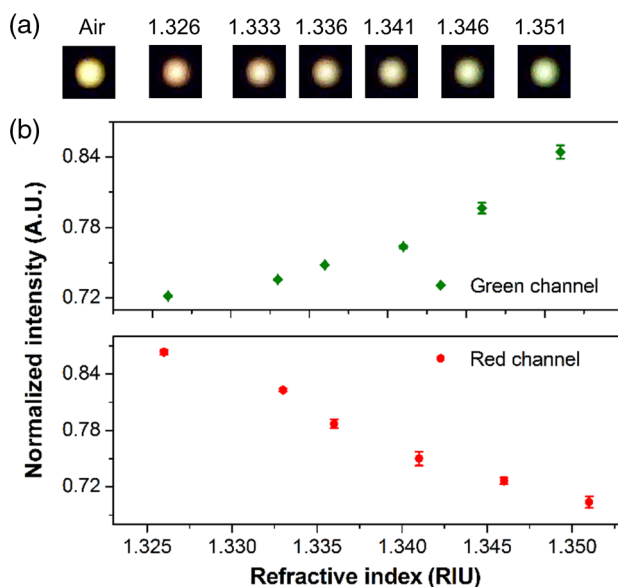

Fig. 3 Responses of red and green channels upon sequential injection of sodium chloride solutions at increasing RI (1.326 to 1.351): (a) real-time images and (b) normalized intensities of each channel.
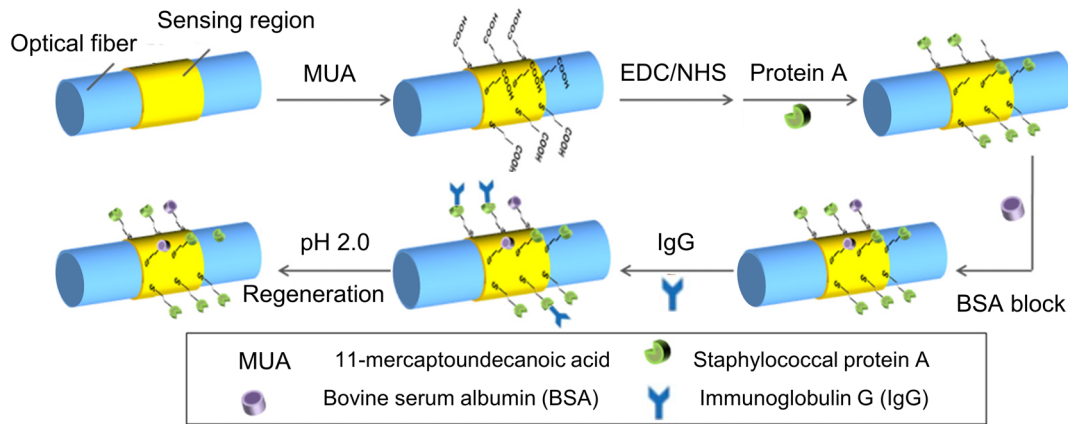
Fig. 5 Schematic illustration of functionalization and protein A–IgG binding procedure of the SPR sensor.
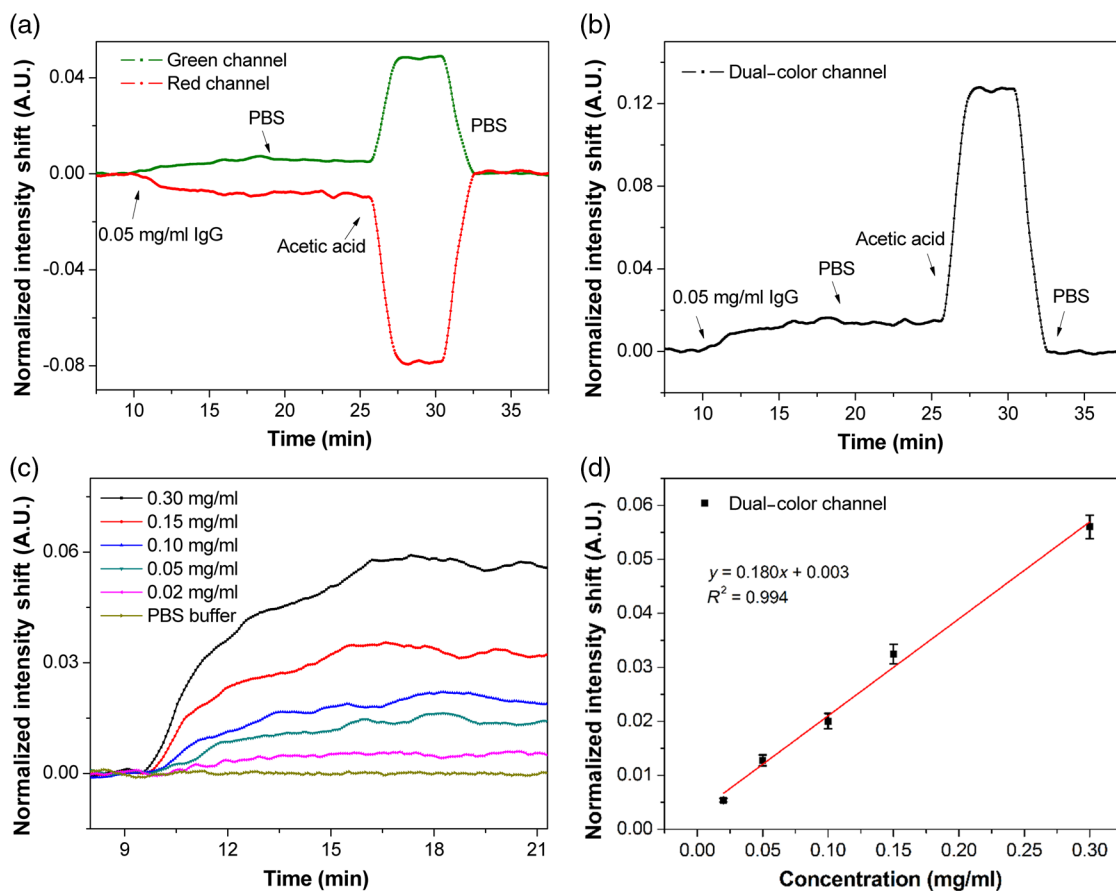

Fig. 6 Real-time biodetection with various concentrations of IgG samples: (a) response curves of green and red channels for 0.05-mg/ml IgG sample, (b) calibrated response curves of dual-color channel for 0.05-mg/ml IgG sample, (c) real-time binding process of five different concentrations from 0.02 to 0.30 mg/ml, and (d) plot of normalized intensity shift versus the IgG concentrations.

three times for each IgG concentration. There is a linear relationship between IgG concentrations and the normalized intensity shift ($R^2 = 0.994$) as shown in Fig. 6(d). Error bars represent the SD of triplicate measurement. The relative SD was <7% for each concentration, which shows its good stability.

The results indicate that this dual-color SPR sensor is effective for biosensor applications. We can further enhance its sensitivity and resolution by optimizing the performance of SPR

sensor probe and utilizing secondary antibodies or nanoparticles to achieve signal amplification.[28]

4 Conclusion

A portable and low-cost dual-color SPR sensor has been successfully realized through simple optical configuration. In conjunction with inherent color selectivity of the smartphone camera, SPR signals from red and green channels were quantified in real time, which can reduce the chance of false detection

and improve the sensitivity. It shows a linear response to RI changes between 1.326 and 1.351 with a resolution of 2.3×10^{-4} RIU. We used the sensor for real-time biodetection by monitoring the binding of IgG and protein A. Experimental results demonstrate that a linear SPR response was achieved for IgG concentrations varying from 0.02 to 0.30 mg/ml with good repeatability. Considering the advantage of portability and simplicity, it is attractive for point-of-care and remote detection of biomedical and environmental targets.

Appendix

The code for the smartphone application can be found online at https://github.com/lqls/Dual_Color_SPR.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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