

Compact multi-channel surface plasmon resonance sensor for real-time multi-analyte biosensing

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Abstract: A compact multi-channel surface plasmon resonance (SPR) biosensor is demonstrated based on a tablet as the measurement platform. The SPR biosensor employs a bundle of fiber-optic SPR sensors as the multiplexed sensing elements that are illuminated by a light-emitting diode (LED) plane light source and detected by a cordless camera. The multi-channel SPR biosensor was based on optical fiber components for precise, label-free and high-throughput detection without the use of complex, specialized or fragile instrumentation that would require optical calibration. The reference and control channels compensated for the fluctuation of the LED light source and the bulk refractive index, increasing the accuracy and reliability of the biosensor. The multi-channel SPR biosensor was applied for multi-analyte biosensing of immunoglobulin G (IgG) and concanavalin A (Con A). The channels functionalized with staphylococcal protein A (SPA) and ribonuclease B (RNase B) only showed relative intensity responses to their corresponding analytes. Moreover, the multi-channel SPR sensors responded to the specific detection of IgG and Con A with an approximately linear relative intensity response to the analyte concentration. Hence, multiple analytes were simultaneously and quantitatively detected with the multi-channel SPR biosensor. This compact, cost-effective multi-channel SPR biosensor is adapted for point-of-care tests, which are important in healthcare and environmental monitoring and for biomolecular interaction analysis.

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1. Introduction

Surface plasmon resonance (SPR) sensors have distinct advantages in biosensing, such as their high sensitivity, label-free detection, high-throughput capacity, real-time monitoring and non-destructive measurements [1–5]. In addition to the common molecular biology applications, SPR sensors are useful for food safety applications, disease diagnostics, drug testing, and health and environmental monitoring. These applications often require rapid detection near the point of sample collection [6–9]. However, traditional SPR devices are often limited to applications in centralized laboratories due to the high costs of instrumentation, maintenance and repairs; the complex mechanics, electronics and optics; the requirement of professionally trained personnel; and the bulky instruments that limit the applications to a laboratory setting [10]. To address the limitations of traditional SPR devices, miniaturized, portable and intelligent SPR sensors have attracted much attention recently, as demonstrated by the many studies that have reported small SPR devices [11–16]. However, the multiplexing capability of these small SPR devices is often limited to only a few channels, and high throughput is not possible. Although the current small SPR devices that have reduced the volume and cost and simplified the operation process have been applied for some point-of-measurement applications, a further reduction in cost and size and an increased multiplexity are required for their more widespread use.

In recent years, the rapid development of mobile technology, in combination with Personal Digital Assistant (PDA), E-commerce, multimedia and entertainment, and mobile devices such as smartphones and tablet computers, has led to the rapid spread of the availability of these devices in all geographical areas. Mobile electronic devices are now equipped with sensitive cameras, powerful computing hardware, and simple operating systems. Thus, the integration of mobile electronic devices is now accessible to several bioanalytical detection technologies [17–19].

Herein, we report a compact, low-cost, and wirelessly connected SPR sensing platform for sensitive protein biosensing for use outside the laboratory, which could ultimately include personal use by patients or medical doctors. The multi-channel SPR biosensor was integrated with the existing platform of a tablet computer, benefiting from the optoelectronic components and the existing operating system (in this case based on the Android OS) for biochemical analysis. Specifically, the multi-channel SPR device was based on narrow-band illumination using a light-emitting diode (LED) plane light source at 625 nm and on the quantification of light intensity changes of fiber arrays of SPR sensors. Due to the changes in the local refractive index of specifically adsorbed molecules on the resonance conditions of the surface plasmon waves, we demonstrated sensitive real-time multi-analyte biosensing of immunoglobulin G (IgG) and concanavalin A (Con A). In this multi-channel SPR, fiber-optic SPR sensors were used as the sensing elements instead of prism SPR sensors, which are suited for highly parallel implementation in a multiplexed sensor. As a result, optical calibration and multi-channel arraying were easily achieved without an expensive high-precision mechanical control system. The concept of a light, low-cost, and compact wireless biosensor was based on a popular type of personal electronic tablet with the aim of increasing the accessibility of bioanalytical sensors for personal use.

2. Materials and methods

2.1. Fabrication of the SPR biosensor

The structure of the multi-channel SPR biosensor system is schematically illustrated in Fig. 1. The system consisted of four components including the LED plane light source, the sensing element packaged with the flow cell, a cordless camera and a mobile power supply. An LED plane light source was used to illuminate the fiber sensor bundle with a single light source [Fig. 1(a)]. A simple control circuitry was designed to adjust the light intensity of the LED plane light source and to supply power. The LED plane light source was placed in proximity to the fiber SPR sensors to ensure uniform illumination of all sensors [Fig. 1(a)]. The 625 nm emission wavelength of the LED plane light source was selected to match the region of 50% transmission in the SPR absorption spectrum to provide maximum sensitivity, which is similar to the common practice in imaging SPR instruments. The multi-channel SPR sensing elements were fabricated with nine SPR fiber sensors with uniform specifications (plastic cladding silica optical fiber, YOFC, core and cladding diameters 400/430 μm , numerical aperture 0.37, 5 cm length). The cladding of all multimode fibers was stripped to 5 mm in length in the center and coated with chromium/gold layers (Cr 5 nm, Au 50 nm) using a magnetron sputtering system (K575XD from E.M. Technologies Ltd. Ashford, Kent). All end faces of these fibers were polished with emery papers. Then, the fibers were packaged in a homemade flow cell. The flow cell was fabricated in polypropylene bottles via typical drilling and cutting processes. The top and bottom of the bottle were drilled with evenly spaced holes for the nine fiber SPR sensors to be fitted in a 3-by-3 sensor array. The holes for the inlet/outlet of the flow cell were drilled above and below the sensor array. A cordless camera equipped with a 1.3-megapixel detector was used to collect the transmitted light through SPR sensing elements. The cordless camera was equipped with a wireless receiver and web camera. Utilizing the wireless receiver of the cordless camera, the web camera captured the image of the sensor array and wirelessly transmitted the data to a mobile device (Honor X1

tablet; Huawei Technologies Co. Ltd.) via wireless fidelity (Wi-Fi). Because of the need for the miniaturization, integration and portability features of the SPR device, a mobile power supply powered the device rather than transformers or power adapters. A custom-designed Android application (app) that runs on personal electronic tablets was developed for data acquisition, data storage and data processing. The app showed the image collected by the camera in real time on the touch screen interface [Fig. 1(d)]. The brightness was extracted from the image data through a conversion of the colored image to grayscale images. As every image point has a grayscale value between 0% (white) and 100% (black) to show its brightness, the light intensity of the photograph can be calculated by integrating the grayscale value of the spots for every channel. The light intensity data for all channels were captured and processed every 0.5 seconds and were presented as intensity-time coordinates in real time. The recorded data for every channel each time were stored in the tablet memory to retrieve the data and perform further processing.

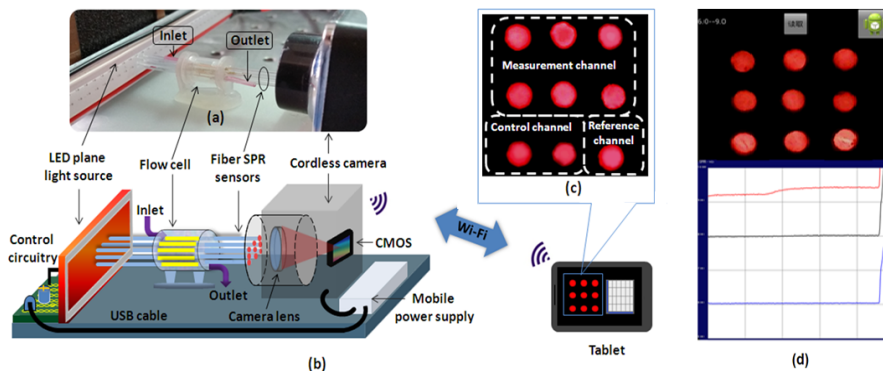


Fig. 1. Design of the multi-channel surface plasmon resonance biosensor. (a) Photograph of the sensing element; (b) schematic of the multi-channel SPR biosensor; (c) images of measurement, control and reference channels; (d) interface of the app that controls the SPR instrument.

2.2. Immobilization of antibodies for IgG and Con A

The top and middle rows of the fiber SPR sensor (Fig. 1, assigned as measurement channels) were immobilized with Staphylococcal Protein A (SPA), selective for immunoglobulin G (IgG) and ribonuclease B (RNase B), selective for concanavalin A (Con A), respectively, whereas the bottom row (Fig. 1, designated as control and reference channels) was not functionalized and was used as reference for the bulk refractive index and light source fluctuation compensation [Fig. 2(a)]. Two control channels were integrated with the SPR device to provide duplicate measurements. The light source fluctuation was compensated for by the measurement of the control channels and the reading on the reference channel, which was fabricated by a multimode fiber that was not subjected to the striping and coating process, and it only monitored the transmission of the light source. The bulk refractive index change was compensated for by the readings on the control channels. The immobilization process for SPA and RNase B was performed as depicted in Fig. 2(b). The six measurement channels were reacted in 1 mM 11-mercaptoundecanoic acid (MUA) in ethanol at room temperature for 12 hours to form a carboxyl surface, and they were then treated with a solution of 0.5 M N-hydroxysuccinimide (NHS) and 0.55 M 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC) in ultrapure water at 4°C for 30 minutes to convert the carboxyl surface to an activated ester. The sensors were rinsed with phosphate-buffered saline (PBS, pH = 7.4), and three of the six sensors were immersed in 0.1 mg/mL SPA, whereas the other three sensors were reacted with 0.1 mg/mL RNase B for 30 minutes. After the rinsing in PBS buffer, these sensors were immersed in 0.1 mg/mL bovine serum albumin (BSA) to deactivate the remaining activated esters and to prevent non-specific site binding during the

measurement. Finally, the sensors were mounted onto the flow cell for the measurements of IgG and Con A.

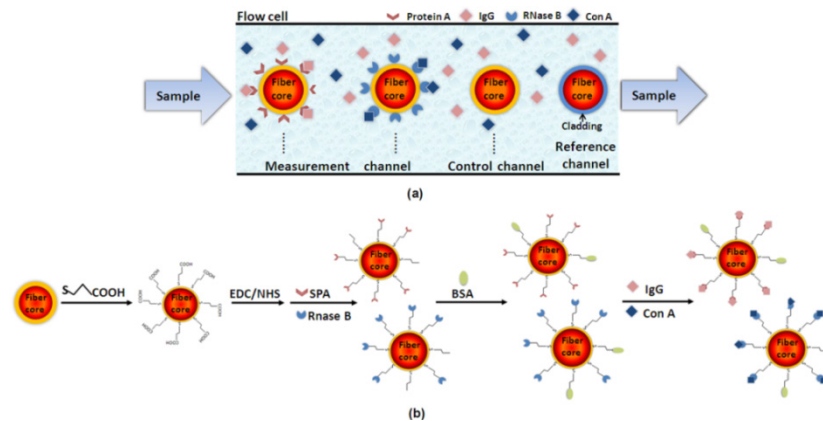


Fig. 2. (a) SPR biosensor representation for IgG and Con A detection and (b) the antibody immobilization protocol on the fiber surface.

2.3. Working principle

The multi-channel SPR biosensor was based on the intensity modulation and use of a Complementary Metal Oxide Semiconductor (CMOS) detector and LED light source. Bundles of fiber SPR sensors can be constructed as an array detector with each fiber SPR sensor illuminated simultaneously from a single light source and imaged within the active area of the camera CMOS imager chip. Due to the compact nature of the design, it is possible to construct a multi-channel SPR biosensor using a fiber SPR sensor array. Multiplexing could be further increased with denser arrays of fibers and/or fibers of a smaller core diameter.

The cordless camera and LED plane light source were aligned with the fiber SPR sensor array. The multi-channel SPR system relied on the light from the LED plane light source and transmitted the light from fiber SPR sensor to the camera [Fig. 3]. Due to the presence of the gold film on the core of the fiber, some propagation modes were in resonance with the gold film due to the SPR effect when light reached the sensing region of the fiber. Because the SPR absorption depends on the dielectric properties of the thin layer of the solution near the surface of the sensor region, the biomolecular interactions were analyzed with the SPR sensing. The quantification of the analytes is possible with the calibration of the SPR biosensor. The absorption spectra of the SPR sensor were recorded in solutions with RI of 1.328 and 1.338 [Fig. 3]. As expected, the absorption spectrum shifts to longer wavelengths for higher RI. By recording the SPR at a single wavelength (625 nm) and angle (dictated by the propagation modes of the optical fiber) couple, the power output will change with the change in RI.

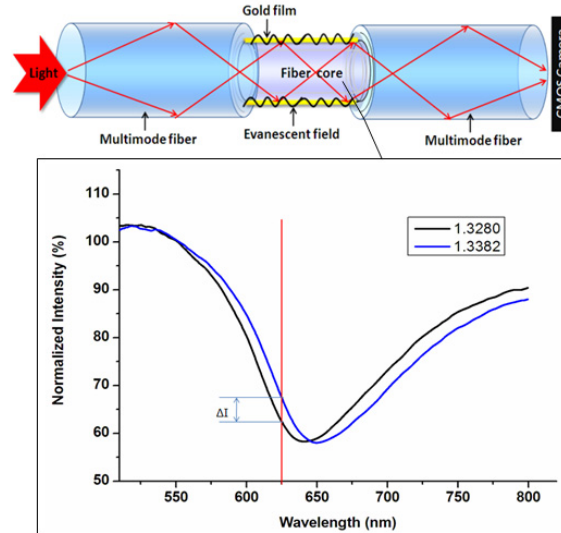


Fig. 3. Principle of the multi-channel SPR biosensor.

Because the end faces of each channel that were captured by the camera were displayed as light spots, the light intensities of these light spots could be integrated and processed separately. Due to the impact of the bulk refractive index change and the light intensity fluctuations of the LED plane light source on the test results, the reference channels were used to compensate for this effect, and the relative intensities were calculated for the measurement channels to effectively eliminate these errors. The relative intensity was expressed as $I_R = (I_m - I_c)/I_r$, where I_m , I_c and I_r are the average intensity values of the measurement, control and reference channels, respectively. By calculating the relative intensity as the output signal, we provide a simple and feasible method to exclude the impact of the surrounding factors.

2.4. Sensing system

The sensing platform was tested to establish the performance of the multi-channel SPR biosensor. To minimize the interference of stray light, the multi-channel SPR biosensor was covered by a black cover to create a completely dark environment. The experimental setup for the real-time biosensing of the multi-channel SPR biosensor is shown in Fig. 4. The black arrow indicates the electrical information transfer path, and the green line represents the solution flow path. The inlet and outlet of the flow cell were connected to a peristaltic pump (Longer BT100-2J) and waste container via silicone tubing. The solution was delivered by the pump at a controlled flow rate (1.2 mL/min). Each channel of the sensing element was immersed in the solution. The solution was then dispensed from the outlet to a waste container. Before injecting the sample, PBS solution was delivered to the flow cell, and light intensities from all channels were constantly recorded. After reaching baseline stability, the solution containing the IgG and/or Con A samples was introduced while the light intensity change due to the specific binding was continuously recorded. The IgG and Con A mixed sample solutions of various concentrations were prepared for the validation of the multi-channel SPR sensor.

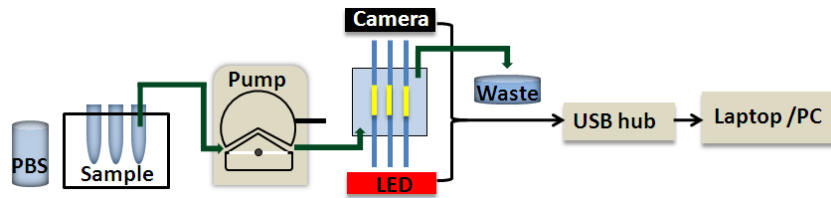


Fig. 4. Diagram of the real-time-sensing experimental setup for multi-analyte detection.

3. Results and discussion

The response of the multi-channel SPR biosensor was recorded during the injection of several samples with different refractive indices. The RIs of the sodium chloride solutions (calibration samples) were calibrated with an Abbe refractometer (WAY-2S). With an increase of the refractive index value of the sample in the flow cell, a typical light intensity change of one channel was observed, as shown in Fig. 5. Because all the channels shared one flow cell, each channel responded simultaneously. The sensitivity and resolution of the SPR biosensor were extracted from the calibration curve. Figure 5(b) illustrates the linear relationship of the relative intensity to the refractive index in the range of 1.3282-1.3641. This range of RI is in accordance with biosensing for the most common biofluids. By calculating the slope of the calibration curve, we determined that the system provides a sensitivity of 486%/RIU and a resolution better than 6.1×10^{-4} RIU, considering that the signal-to-noise ratio of the camera is better than 48 dB with a 0.39% noise level. The sensitivity and resolution of the multi-channel SPR biosensor are a result of the high-performance camera, sensitive wavelength response and narrow full width at half maximum. This design is thus effective for multi-channel SPR biosensing.

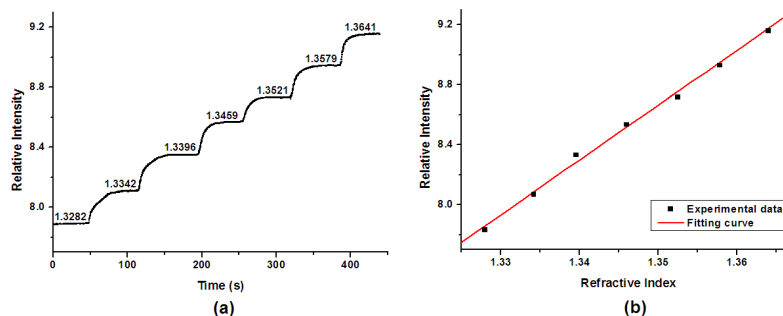


Fig. 5. RI calibration results from the multi-channel SPR biosensor. (a) Time response of the SPR sensor for solutions with different RI; (b) steady-state SPR response vs. refractive index units (RIU) from which the sensitivity and resolution were calculated.

To test the multi-analyte biosensing of the multi-channel SPR biosensor, the functionalized SPR biosensor was tested for real-time detection of IgG, Con A and their mixed samples. First, PBS buffer was pumped into the flow cell for 5 min to obtain a stable baseline signal. The response of the sensor was investigated for the specific binding of IgG and Con A that were diluted with PBS. The relative intensity increased due to the IgG or Con A specific binding to SPA or RNase B, respectively, in the sensing region [Fig. 6]. Then, PBS was pumped into the flow cell to remove the unbound IgG/Con A molecules. Urea solution (8.0 M) was then used to strip the surface-bound IgG/Con A and to effectively regenerate the sensing region after the sensing element was rinsed with PBS. Figures 6(a)–6(b) shows the real-time relative intensity response of the functionalized biosensors to the introduction of IgG (0.15 mg/mL) and Con A (0.15 mg/mL) solutions, respectively. As shown in Fig. 6(a) and 6(b), with IgG or Con A sample pumped into the flow cell, only the SPA- or RNase B-immobilized channels of the multi-channel sensors exhibited specific responses to their

corresponding analytes, and there was very little relative intensity change caused by the nonspecific binding in other channels.

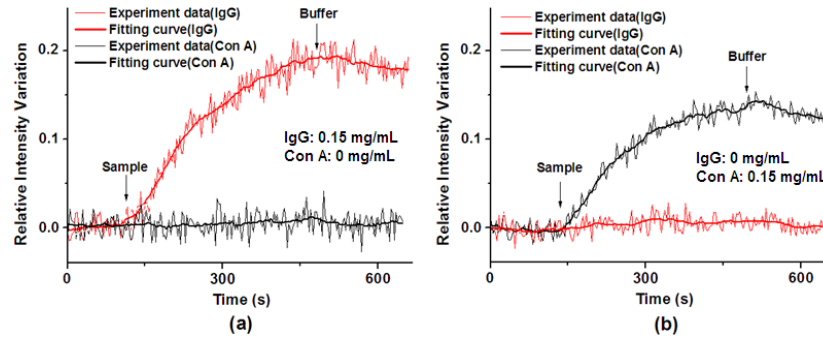


Fig. 6. Relative intensity response of the anti-IgG and anti-Con A-immobilized sensor. The response to (a) IgG sample (0.15 mg/mL), (b) Con A sample (0.15 mg/mL).

The relative intensity responses were calibrated by the injection of IgG and Con A with concentrations ranging from 0.025 to 0.2 mg/mL. Figure 7 shows the relative intensity response of the functionalized SPR biosensor (SPA-immobilized and RNase B-immobilized) as a function of the IgG and Con A concentrations. The relative intensity change of the functionalized SPR biosensors was approximately linear in the range of 0.025–0.2 mg/mL, with sensitivities of 1.28 and 0.72 (mg/mL)⁻¹ for IgG and Con A, respectively. To further demonstrate the specificity, IgG and Con A were injected as a mixture in the flow cell [Fig. 8]. The SPA-immobilized channels and RNase B-immobilized channels responded accordingly with similar relative intensity profiles to the experiments with the single analytes. Figure 8 compares the relative intensity change of the multi-channels for the single and mixed samples. The relative intensity variations of the SPR biosensor were almost the same as the single analytes at equal concentration, which indicates that multiple analytes can be independently and reliably detected in real time with the multi-channel SPR biosensor. The current demonstration benefits from a triplicate measurement of the sensing channels and a duplicate measurement of the control channels. However, different configurations can be envisioned. With the current nine-channel SPR biosensor, if each reference signal and control signal occupy one channel, the remaining seven channels can be modified and functionalized for the detection of multiple components in a complex sample. Otherwise, fiber bundles with a greater number of fibers can be deployed in the fluidic cell, leading to a larger number of analyses in triplicate measurements. The number of fibers that can be simultaneously deployed depends on the resolution on the CMOS chip, the core diameter of the fiber and the size of the CMOS detector.

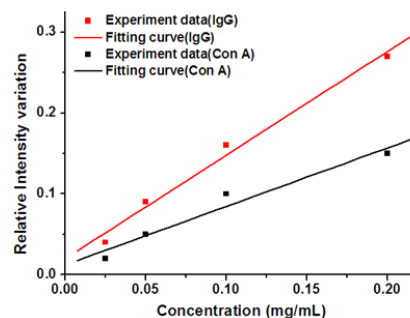


Fig. 7. The relative intensity variation of the sensors as a function of concentration of IgG–Con A sample (0.025–0.2 mg/mL).

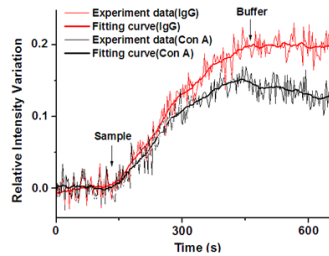


Fig. 8. The relative intensity variation of IgG and Con A sensors for single and mixed samples.

In addition to the performance optimization of the sensing element and the detector, the sensitivity of the SPR biosensors can be enhanced by utilizing secondary antibodies or nanoparticles, which are very adaptable for improving the SPR biosensor [20, 21]. This multi-channel SPR biosensor research has mainly focused on attaining a multi-channel biosensor using low-cost optoelectronic devices and a multi-channel structure to achieve simultaneous multi-analyte detection with the compensation of the bulk refractive index and light source fluctuation. In our future work, the multi-channel SPR biosensors based on the mobile device platform will be further optimized and demonstrated for accurate, simple and low-concentration detection with appropriate sensitivity enhancement methods.

4. Conclusions

A compact multi-channel SPR biosensor based on a mobile device as the measurement platform and its multi-analyte and biomolecular detection capabilities have been successfully demonstrated. The SPR biosensor employed a fiber-optic bundle configuration as the sensing elements, an LED plane light source and a cordless camera as the detector. The multi-channel sensing element was based on optical fiber components, which have high sensitivity and portability, leading to an SPR instrument that can perform precise, label-free and high-throughput detection without complex, dedicated, specialized and fragile light elements or sophisticated optical calibration. A reference channel and control channel were easily added to eliminate the fluctuation of the LED light source and bulk refractive index to increase the accuracy and reliability of the biosensor. The multi-channel SPR biosensor was applied for the multi-analyte biosensing of IgG and Con A with the immobilization of SPA and RNase B on six channels of the sensing element. To demonstrate the performance of the multi-channel SPR biosensor, IgG, Con A and their mixed sample within a range of 0.025–0.2 mg/mL were quantified. The test results showed that the SPA- and RNase B-immobilized channels were selective to their corresponding analytes. Moreover, the multi-channel SPR sensors detected the binding process with an approximate linear relative intensity response as a function of analyte concentration, which indicates that multiple analytes can be simultaneously and quantitatively detected by the multi-channel SPR biosensor. Because the sensing element can be modified for diverse SPR experiments and the mobile device can be connected to the Web, the cost-effective and multi-channel SPR biosensor can be useful for point-of-care tests, which are urgently needed in clinical diagnostics, and it is suitable for biomolecular interaction analysis. Additionally, all the optoelectronic components of the multi-channel SPR biosensor were inexpensive and the measurement platform was based on a common mobile device. This type of instrumentation is adequate for a portable device and promotes the application of the SPR technique in a more convenient, practical and effective manner.

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