We describe a non-scan and real-time multichannel angular surface plasmon resonance (SPR) imaging method. We demonstrate experimentally, with multiple line shaped light illuminations to construct multi-imaging channels, that an image captured with an area detector can probe the surface plasmons with different field distributions. Thus, it provides a fixed optical-sensing module measuring the spatial variations of the refractive index in the flow system in real time without scanning. This technique has the advantages of high system stability and similar optical arrangement to the conventional parallel-channel SPR sensors and could have potential applications in multianalyte detection and reference-compensated biosensing. © 2014 Optical Society of America

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1. Introduction

Surface plasmon resonance (SPR) is a label-free optical sensing approach offering the advantage of high sensitivity [1]. The SPR sensors, combined with a flow system, can monitor the molecular interaction continuously, which has been widely applied for molecular analysis by biological and chemical researchers [2].

For robust measurement of the SPR sensors, the interfering background refractive index (RI) fluctuations should be discriminated from the signals. O’Brien et al. [3] proposed the parallel-channel SPR sensing method. In this approach, the detection elements and reference elements are arranged in a row and measured by a one-dimensional angular SPR imager. Fu et al. [4] reported the parallel-channel SPR imaging method based on the wavelength interrogation.

Recently, with the development of SPR affinity sensors, the traditional parallel-channel SPR sensor has been shown that it cannot, in general, allow discrimination of the sensor response to the surface process from bulk effects of the samples [5]. It is essential to develop the reference-compensation technique to improve the parallel channel-sensing method.

For parallel-channel assay capability and reference compensation, the SPR sensors with multi-independent sensing channels for multianalyte
In this paper, we propose a non-scan and real-time multichannel angular SPR imaging method. This method employs multiple wedge incident light beams to construct multi-imaging channels. This module can measure the RI changes in the flow system with different field distributions in real time without scanning. A laboratory-scale experimental system is presented. An imaging lens is designed to improve the system sensitivity. Then the symmetrical optical waveguide (SOW) sensing structure [12] with sharp resonance curve is applied to improve the system resolution.

2. Experimental Setup

Figure 1(a) shows the schematic of the experimental system. The light source (LS) employs a 25× objective lens to focus the light from a red light emitting diode with an electric power of 3 W on a pinhole. Then the light is collimated by the collimation lenses made of two convex lenses (L1, L3) and one concave lens (L2) before being filtered by a bandpass filter (BF, center wavelength 632.8 nm, bandwidth 10 nm, Thorlabs) and then p-polarized by a linear glass polarizer (P). A self-manufactured aperture (A) is used to acquire a rectangular light spot. After passing through a cylindrical lens array (CLA) constituted by three high-performance imaging cylindrical lenses (width 5 mm, height 10 mm, focus length 30 mm, Edmund Optics) with vertical axes of symmetry, the light is focused to three narrow lines (F1, F2, F3) on the sensing surface (SS) to construct three imaging channels for probing the samples with different field distributions in the fluidic channel (CH, 24 × 2 × 2 mm, 96 μl). The SPR module is configured in the Kretschmann manner with the attenuated total reflection method. A substrate (S, SF4 glass) coated with a 2 nm Cr adhesive layer and a 50 nm Au layer by magnetron sputtering is attached on the surface of a prism (PR, SF4 glass) using RI matching liquid (Cargille) to constitute the SPR module. A polymethyl methacrylate flow system (FS) is attached to the sensor chip for sample delivery to the sensing surface. The reflection light beams pass the imaging lenses (IL) and are recorded by a charge coupled device (CCD, TCC-6.1ICE, Tucsen), which is an astronomy camera with a sensing area size of 23.6 × 15.7 mm² (3032 × 2018 pixels, 7.8 μm pixel size), for further data analysis in a personal computer (PC).

To improve the system sensitivity, the IL consisting of the cylindrical convex lens CL1 with focus length of 100 mm and CL2 with focus length of 50 mm is employed. The optical path diagrams of the reflection light beams passing through the IL are shown in Fig. 1(b). The optical paths of three reflection light beams are marked in red, green, and blue. Owing to the reflection light beams separating from each other after the IL, the overlaps between the adjacent reflection light beams can be eliminated when the CCD sensing area is adjusted to an appropriate position. The reflection lights captured by the CCD without and with the IL in the system when the distilled water is measured are shown in Figs. 2(a) and 2(b), respectively. The plotted marked rows in Figs. 2(a) and 2(b) are shown in Figs. 2(c) and 2(d). The dips marked in red represent SPR angular spectra of the sensor spots belonging to different imaging channels. It can be seen that the interference among the imaging channels is eliminated. Besides, the sensing area of the CCD can be utilized more effectively so the system sensitivity is improved with the IL in this system. It could be seen from subgraphs in Fig. 2 that this multichannel SPR angular imaging system could be regarded as the integration of three parallel-channel SPR sensors with an independent channel. So it could be feasible to conduct a multianalyte detection in parallel with the current setup.

3. Results and Discussion

The distilled water and a set of glucose solutions with different refractive indices are measured in a serial. The distilled water is used to create the baseline because its RI is free of evaporation effects. The
measurement of the water and glucose solutions is used to test the sensor response to the RI change. The sample is pumped in and out of the fluidic channel with a peristaltic pump. The relationship between RI $n$ and concentration $C$ of the glucose solution is $n = 1.325 + 1.515 \times 10^{-4} \times C$, where $C$ is concentration in grams per liter.

The output of an angular SPR sensor is the resonance angle represented by the corresponding pixel position affording the minimum intensity in the captured image. Figures 3(a) and 3(b) show two images captured by CCD when the distilled water and glucose solutions with concentrations of 6 g/L are measured, respectively. The plotted marked rows in Figs. 3(a) and 3(b) are shown in Fig 3(c) in blue and red, respectively. The imaging channels are marked with channels 1, 2, and 3, which correspond to the first area, second area, and last area in the fluidic channel that the samples flow through. It shows the SPR dips of three sensor spots all have position shifts when the RI of the sample changes. The 10-order polynomial curve-fitting method is applied to reduce the noise. The resonance angle shifts of channel 1, 2, and 3 are 9.63, 12.27, and 11.25 pixels, respectively. The difference among the sensitivities of three imaging channels can be due to the optical aberration.

The RI resolution of a sensor ($\sigma_{RI}$) is related to the standard deviation of the sensor output ($\sigma_{SO}$) and sensitivity factor (SF) according to [13]:

$$\sigma_{RI} = \frac{\sigma_{SO}}{SF}, \quad SF = \frac{\partial \theta}{\partial n},$$

where $\theta$ is the incident angle and $n$ is the RI of sample. The distilled water and glucose solutions with 1, 2, 3, 4, 5, and 6 g/L are pumped to the sensing

Fig. 2. (a), (b) Captured images without and with the imaging lenses in the system with the distilled water measured. (c), (d) Plotted marked rows in (a), (b). Three dips marked in red are the angular spectra of sensor spots belonging to three imaging channels respectively.

Fig. 3. (a) Captured image with the distilled water measured and (b) the glucose solution with the concentration of 6 g/L measured. (c) Plotted marked rows in (a) and (b) are shown in blue and red.
surface serially. Thirty images are captured with the exposure time of 8 ms every 15 seconds when each solution is measured. The measured resonant angles of imaging channel 1, 2, and 3 as functions of time are shown in Figs. 4(a), 4(b), and 4(c), respectively. The corresponding averaged measured resonance angles as the functions of the refractive indices are shown in Fig. 4(d), 4(e), and 4(f). It can be seen that the output response is linear for the RI in all three imaging channels and the sensitivity factors are $1.06 \times 10^4$, $1.36 \times 10^4$, and $1.31 \times 10^4$ pixels per RI unit (RIU) respectively. The standard deviations of baselines created by the drilled water are 0.29, 0.16, and 0.30 pixels, so the RI resolutions of channel 1, 2, and 3 can be due to the CCD pixel response nonuniformity. The lower resolutions of channel 1 and 3 compared with channel 2 should be caused by the uneven coated gold film thickness, which has been studied in our previous work [14].

The RI resolution of an angular SPR sensor can be improved if the whole resonance curve can be observed [15]. But the whole resonance curve of the SPR sensing structure constructed by a thin gold film cannot be observed because it is too wide for this system. So the RI resolution of this multichannel angular SPR imager can be improved if a sensing structure with a sharper resonance curve is employed, which is one advantage of the plasmon waveguide resonance sensor [16]. In this study, the recently reported SOW sensing structure is used instead of the SPR sensing structure.

The SOW sensing structure is shown as Fig. 5(a) I, which is realized with a substrate (BK7 glass) coated with a 505 nm MgF$_2$ layer, a 39 nm Au layer, and a 645 nm MgF$_2$ layer. It is different from a substrate coated with a gold layer serving as the SPR sensing structure, shown in Fig. 5(a) II. It has been demonstrated theoretically and experimentally that the SOW can allow the RI detection in a wide range [12]. Using multiple reflectance theory and Fresnel’s formula [17], the calculated resonance curves with water as an analyte of the SOW and SPR sensing structures are shown in Fig. 5(b). Figure 5(c) shows one image captured by CCD when the SOW sensing structure is used in this system. The plotted marked row is shown in Fig 5(d). The dips marked in red represent the angular spectra of the sensors that belong to three imaging channels. To account for the effect of SOW on the RI resolution of system, the figure of merit (FM) function is employed [18] and shown as

$$\text{FM} = \frac{R_{\text{max}} - R_{\text{min}}}{\text{FWHM}},$$

where $R_{\text{max}}$ and $R_{\text{min}}$ are the maximum and minimum normalized reflectivity and FWHM is the full width at half-maximum. This function reflects the accuracy of finding resonance angle position. The SF and FM for the SPR are 71° per RIU and 0.5° −1 and for SOW 62° per RIU and 8.9° −1 in this study. It can be seen that the SOW has lower SF and higher FM than the SPR. Thus, the RI resolution could be improved owing to the high accuracy of finding resonance angle position with the SOW structure.
To measure the system characteristics when the SOW sensing structure is applied, the distilled water and glucose solutions with 1, 2, 3, 4, 5, and 6 g/L are pumped to the sensing surface serially. Thirty images are captured with the exposure time of 6 ms every 15 seconds when each solution is measured. The measured resonant angles of imaging channel 1, 2, and 3 as functions of time are shown in Figs. 6(a), 6(b), and 6(c), respectively. The corresponding averaged measured resonance angles as the functions of the refractive indices are shown in Figs. 6(d), 6(e), and 6(f), respectively. It can be seen that the output response is linear for the RI in all three imaging channels, and the sensitivity factors are $7.86 \times 10^3$, $8.39 \times 10^3$, and $1.0 \times 10^4$ pixels RIU. The standard deviations of baselines created by the drilled water are 0.074, 0.033, and 0.066 pixel, so the RI resolutions of channel 1, 2, and 3 can be calculated to be $9.4 \times 10^{-6}$, $3.9 \times 10^{-6}$, and $6.6 \times 10^{-6}$ RIU, respectively. The difference among the RI resolutions of three imaging channels should be caused by the same reasons with the SPR sensing.
structure. It can be seen that the RI resolution of this system is substantially improved when the SOW sensing structure is applied owing to the lower standard deviation of the sensor output.

In the multichannel SPR imaging system, the RI resolution of $1.2 \times 10^{-5}$ RIU is achieved with the most sensitive imaging channel and is improved to $3.9 \times 10^{-6}$ RIU by using the SOW sensing structure. The sensing area of the CCD is divided into three zones for three independent imaging channels resulting in lower RI resolution than the conventional SPR and SOW imagers [9,12]. The RI resolution of this system could be improved by more efficient utilization of the sensing area of CCD with suitable lenses design, employment of high quality area detector, or application of new technique such as the polarization interferometry [9].

The dynamic range is of importance for the SPR sensor. The angular SPR sensor responds linearly to the RI changes in a wide range [9]. The dynamic range is limited by the angular range of the wedge incident light and sensing area of CCD. Using the focus length and width of the cylindrical lens and considering the refraction effect of the prism, the angular range of each wedge incident light beam is approximately 5.44°. To the dynamic ranges determined by the cylindrical lens, the angular range can be divided by the SFs, resulting in 0.077 RIU and 0.088 RIU with the SPR and SOW, respectively. It can be seen from Figs. 2(d) and 5(d) that the effective sensing area for one imaging channel has a width of about 800 pixels, consisting of an angular spectral analysis range of 300 pixels [19] and dynamic range of 500 pixels. The dynamic range determined by CCD converted to RI range is 0.036 RIU and 0.05 RIU with the SPR and SOW, respectively. In summary, the dynamic range is at least 0.036 RIU. As a sensitive detector, this dynamic range could allow the measurement without scanning the incident angle.

4. Conclusion
In this paper, we propose and demonstrate a non-scan and real-time angular SPR imaging method with multi-imaging channels. This method provides a low-cost and fixed module where surface plasmons with different field distributions can be measured in parallel and real time. The SPR imaging system has a RI resolution of better than $2.7 \times 10^{-5}$ RIU. When the SOW sensing structure is applied, the imaging system can achieve a RI resolution of better than $9.4 \times 10^{-6}$ RIU with the most sensitive imaging channel owing a RI resolution of $3.9 \times 10^{-6}$ RIU. This system has high stability and a similar optical arrangement to the conventional parallel channel SPR sensors; thus it provides a low cost and compatible approach to improve the common SPR sensors combined with the parallel channel assays. It could have potential to promote the application of multim analytic detection in reference-compensated biosensing.

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