Development of a Portable Surface Plasmon Resonance Sensor with Multi-Sensing Points Based on the Linear CCD Sensor

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A portable-type surface plasmon resonance (SPR) sensor, composed from a new optical system for multi-sensing, has been developed to apply to environment analysis, clinical diagnosis etc., where many samples are desired to be analyzed at high throughput. The optical system of the sensor consists of a light-emitting diode, a pair of cylindrical lenses, a pair of collimator lenses, a correction lens, a prism, a polarizer and a linear CCD sensor with 2048 pixels. Reflected light from a sensor chip of the width of 6 mm at a certain incident angle was detected by ca. 618 pixels of the linear CCD sensor as an SPR sensor signal. An SPR sensor signal at a specified incident angle is controllable for optimization by adjusting the position of the CCD sensor. A sensor chip having a 30-stripe linear pattern (100 μm width/stripe) was prepared. The spatial resolution as well as the performance of the sensor were evaluated by using sucrose solutions. As a result, the acquisition of SPR sensor signals from 30 sensing points was successfully achieved with a spatial resolution of 100 μm (distance between 2 sensing points). A lower detection limit of ca. 3.2 – 5.5 × 10⁻⁵ RIU with a standard deviation of ±4.5% was obtained by averaging the signals from 6 – 7 pixels of the CCD sensor per one sensing stripe.

Keywords SPR sensor, multi-sensing points, linear CCD sensor

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Introduction

Since SPR sensors detect changes in the refractive index near the surface of the sensor chip sensitively, the SRP sensors have been widely used as a highly sensitive and selective analytical tool in the fields of clinical diagnosis, environmental monitoring and so on, when the surface of the sensor chip is appropriately modified with a specific receptor, which can recognize a target molecule. The most significant advantages of the SPR sensors are that label-free detection and real-time monitoring are possible. Indeed, many kinds of the SPR sensors have been widely applied to the detection of infection diseases, such as influenza,† HIV-1 protease,‡ and Ebola hemorrhagic fever in clinical diagnosis,§ as well as endocrine-disrupting compounds in water, such as atrazine, 2,4-dichlorophenoxyacetic acid, and 4-nonylphenol in the field of environmental monitoring. An SPR sensor system and (2) the prism-coupled based SPR system SPR sensor systems based on (1) the grating-coupled SPR sensor, composed from a new optical system for multi-sensing, has been developed to apply to environment analysis, clinical diagnosis etc., where many samples are desired to be analyzed at high throughput. The optical system of the sensor consists of a light-emitting diode, a pair of cylindrical lenses, a pair of collimator lenses, a correction lens, a prism, a polarizer and a linear CCD sensor with 2048 pixels. Reflected light from a sensor chip of the width of 6 mm at a certain incident angle was detected by ca. 618 pixels of the linear CCD sensor as an SPR sensor signal. An SPR sensor signal at a specified incident angle is controllable for optimization by adjusting the position of the CCD sensor. A sensor chip having a 30-stripe linear pattern (100 μm width/stripe) was prepared. The spatial resolution as well as the performance of the sensor were evaluated by using sucrose solutions. As a result, the acquisition of SPR sensor signals from 30 sensing points was successfully achieved with a spatial resolution of 100 μm (distance between 2 sensing points). A lower detection limit of ca. 3.2 – 5.5 × 10⁻⁵ RIU with a standard deviation of ±4.5% was obtained by averaging the signals from 6 – 7 pixels of the CCD sensor per one sensing stripe.

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SPR sensor chip with multi-sensing points. In their reports, an interference pattern generated from two coherent lights was used to prepare a master grating; its pattern was replicated to an elastomer stamp. The elastomer stamp was then placed on a polymer-coated glass to form a replica. The sensor chip was prepared by depositing Au on the replica. Finally, a 216-grating array sensor chip was prepared for high-throughput SPR bio-sensing. One of the advantages of this type of SPR sensors is that the optical system can be easily miniaturized by using the grating instead of a prism, although the preparation of the grating costs much for routine use, and is much more complicated, compared with a conventional sensor chip prepared by just the deposition of gold on a glass plate.

In the case of (2), the Kretschmann configuration is conventionally adopted for the SPR sensor due to its easy fabrication from the viewpoint of the optical system. There have been reported two types of optical systems based on the Kretschmann configuration for multi-analyte SPR sensors. The first type of SPR sensor is a so-called SPR imaging sensor, where a 2-dimensional CCD sensor is used for measuring reflected light from the sensor chip, on which a multi-channel or micro array was fabricated. For example, Pillari et al. reported a high-throughput SPR imaging sensor for nucleic acids, while identifying a specific bacteria pathogen. The sensitivity resolution of the sensor was 2 x 10^-6 RIU, and the detection of nucleic acid at the 10 pM level was achieved. In this case, 120 measuring areas were successfully fabricated on a sensor chip with 6 flow channels and 20 sensing points in each flow channel. In addition, Chinowsky et al. and Puttarugsa et al. also developed potable SPR imaging sensors based on a prism-coupled SPR sensor system with multi-detection areas. They applied the SPR imaging sensor to detect bio-compounds, such as viruses and microbes. The noise level of their SPR sensors was ca. 10^-6 RIU. The low contrast of the light intensity of the reflected light has been pointed out as being one of the drawbacks of the SPR imaging sensors. In order to overcome this drawback of low contrast, Bardin et al. reported a unique SPR imaging sensor combined with a grating for detecting a spectrum instead of a light intensity of reflected light. In this case, white light passed through a slit was introduced to a sensor chip fabricated on the prism; reflected light from the sensor chip was diffracted by the grating. The diffracted light was then monitored by a 2-dimensional CCD sensor. The sensitivity resolution of the SPR sensor was 3.5 x 10^-7 RIU, which was higher than that of the conventional SPR imaging sensors.

The second type of SPR sensor, based on the Kretschmann configuration, is that beam splitters or a cylindrical lens array can be used to divide a single mono-chromatic incident light beam into multi-beams. For example, Hemmi et al. developed this type SPR sensor by utilizing several cubic beam splitters to generate 9 beams for 9 sensing points. However, the incident light intensities of the beams were different due to the fact that beams passed through several beam splitters for the generation of the multi-beams. In addition, Mauriz et al. and Hooper et al. also developed similar multi-channel SPR sensors by using a single beam splitter and a cylindrical lens array for generating incident light beams. The intensities of the reflected light for the multi-beams were detected by a two-dimensional CCD sensor in both cases.

In our previous paper, we reported a Kretschmann-type SPR sensor with dual sensing channels, which is classified to as being the second type, where a single linear CCD sensor is used for detecting two SPR signals from the dual channels. One of the advantages of the SPR sensor is that the sensor signals as a resonance angle shift for both the sample and reference channels are detected simultaneously by a single linear CCD sensor. Therefore, the subtracted sensor signals for the sample channel with the reference channel have been improved significantly with respect to any drifts and temperature effects. The use of the linear CCD sensor for the SPR sensor is another advantage with respect to the cost for producing of portable SPR sensors for on-site analysis. However, there still remains necessary improvement of the SPR sensor to provide a function of multi-analysis. In this paper, we wish to report on an SPR sensor with a function of multi-analysis by improving the optical system of our previous SPR sensor along with the use of a multi-stripe patterned sensor chip.

**Experimental**

**Reagents and solutions**

Sucrose, Au film coated cover glasses (bare SPR sensor chips), and Cr shots were obtained from Kishida Chemical, Eliotec, and Kojundo Chemical Laboratory, respectively. A 100 mM sucrose stock solution was prepared by dissolving 1.7122 g of sucrose into 50 mL of pure water, which was provided by a Milli-Q Direct system. The standard sucrose solutions were prepared by diluting the stock solution into 3, 6, 8, 12, and 15 mM portions with the Milli-Q water. The 10.0 mM test solution for validating the present sensor was prepared by diluting another 100 mM stock solution, which was separately prepared in a similar manner to that of the standard stock solutions.

**Optical system and system integration of present SPR sensor**

An optical system of the SPR sensor developed in this work is shown in Fig. 1. The optical system is based on the Kretschmann configuration, which consists of a light-emitting diode (LED, 770 nm, Hitachi, HE7601SG) as the light source, a pair of collimator lenses, a pair of cylindrical lenses, a collimator used as a correction lens, a semi-cylindrical prism (BK7), a polarizer, a linear CCD sensor (Sony, ILX551B, 2048 pixels) and 2 reflection mirrors. The size of one pixel of the linear CCD sensor is 14 μm square. A point light source emitting from the LED (1 in Fig. 1) spread into a corn-shaped light beam was collimated by the collimator lens (2) into a parallel beam. The beam was reflected by a reflection mirror (3), and then converted into a linear wedge light beam by the cylindrical lens (4). The wedge light was focused onto the backside of the sensor chip linearly under the total-reflection condition through a prism (5), as shown in a 3-D view of the prism in Fig. 1. The reflected light passed through a cylindrical lens (6), and was converted into a parallel beam, and was then polarized to p-polarized light by a polarizer (7). The p-polarized light was focused by a collimator lens (8) onto a reflection mirror (9). At last, the reflected light by the reflection mirror passed through a diffusion correction lens (10), and finally was projected to the center of the linear CCD sensor on pixel numbers between 461 and 1320 (11), as shown in a 3-D view of the linear CCD sensor in Fig. 1. The cross sections of the light beam between two optical elements are shown in the top view in Fig. 1. The position of the linear CCD sensor for detecting SPR signals was appropriately adjusted by turning a micrometer-head (12) equipped with a linear CCD sensor between a scale of 2 and 12 (10 mm distance), which corresponds to an incident angle of between 65° and 75°. Therefore, the position of the linear CCD sensor is indicated by the scale of the micrometer-head. Since each scale of the micrometer is divided into 10 minor graduations, the resolution of the incident angle was calculated.
to be 0.1°. The signals of the present SPR sensor are the light intensities of the reflected light at a certain incident angle. The incident light angle is adjusted appropriately by the position of the linear CCD sensor, which is fixed on an x-stage. Therefore, the conventional SPR response, in which the intensity of the reflected light is shown as a function of the incident angle, can be also obtained by changing the position of the micrometer-head (Fig. S1). The reflected light, which came from the sensor chip, was detected by the CCD sensor between pixel numbers 461 and 1320. The intensities of the reflected light of each pixel were converted with a 12 bit AD converter (resolution of light intensity: 4096 digits), and saved on the hard disk of a personal computer, and displayed on its monitor at the same time as a function of the pixel number of the CCD sensor at every 5 s.

The size and weight of the developed SPR sensor are 170 (W) × 150 (D) × 110 mm (H) and 1.5 kg, respectively. Photographs of the inside, an outline of the developed SPR sensor, and a part of the CCD sensor are shown in Figs. S2(a), (b) and (c), respectively.

Fabrication of the sensor chip with a 30-stripe pattern on the Au thin film coated cover glass

In order to fabricate a sensor chip having a 30-stripe pattern (100 µm in width and 8 mm in length) on a bare sensor chip, 29 stripes of Cr with 100 µm in width and 8 mm in length were prepared onto a bare sensor chip (Cr: 5 nm/Au: 45 nm coated on cover glass (16 × 16 × 0.15 mm³)) by vapor deposition of Cr through a stainless-steel mask. Before the vapor deposition of Cr, the bare sensor chip was cleaned by dipping into a piranha solution (H₂SO₄: H₂O₂ = 3:1) at 90°C for 30 min, and then rinsed with the Milli-Q water for 2 min and ethanol for 1 min (this process was named as piranha treatment in hereafter). The stainless-steel mask (16 mm × 16 mm × 50 µm³, shown in Fig. S3, Fuji Seimitsu Industries, Japan) was placed onto the bare sensor chip, and Cr was deposited in the chamber of an evaporative apparatus (VPC-410, ULVAC, Japan). The thickness of the Cr film deposited was ca. 50 nm.

Evaluation of the basic performance of the present SPR sensor

The SPR response as the intensity of the reflected light detected with linear CCD sensor as a function of pixels to water and sucrose solutions using a bare sensor chip: A piranha-treated bare sensor chip was set up to the flow-cell, as shown in Fig. S4(c1). The resulting flow-cell was then placed on a prism after dropping an aliquot of 30 µL matching oil (Immersion Oil Type-F, nᵣ: 1.518, Olympus, Japan). The Milli-Q water was used as the carrier liquid, which was flowed onto the sensor-chip by a syringe pump (Next-advance, SP300) at a flow rate of 40 µL/min. An aliquot of 120 µL of 8 and 15 mM sucrose solutions was injected via an injector (Rheodyne 9725, USA) into a Mill-Q water stream, which was lead to the sensor-chip at the same flow rate of 40 µL/min. The SPR sensor response as the intensities of the reflected light was recorded as a function of the pixel number from 461 to 1320 at every 5 s. In this case, the position of the micrometer-head was fixed at a scale of 3.12, and the applied current of the LED was 120 mA.

Optimization of the present SPR sensor with 30-stripe patterned sensor chip

Position of the CCD sensor for the detection of the SPR sensor signals: As described in the previous section, the intensities of the reflected light depend on the position of the CCD sensor (Fig. S1), i.e. the scale of the micrometer-head. When the refractive index of a sample solution on the sensor chip increases, the resonance angle (the incident angle at the minimum intensity of the reflected light) shifts to the direction of a higher incident angle. The intensity of the reflected light at a certain scale (incident angle) increases with an increase in the concentration of the sample solution, when the CCD sensor is fixed at a lower scale (incident angle) than the resonance angle. However, the intensity of the reflected light decreases with an increase in the concentration of the sample solution, when the CCD sensor is fixed at a scale higher (incident angle) than the resonance angle, as shown in Fig. S6.

The 30-stripe patterned sensor chip was first treated by an O₂
plasma for 12 min, and then set up to the flow-cell, as shown in Fig. S4(c2). In this case, the stripes were oriented perpendicular to the flow direction. The resulting flow-cell was then placed on a prism, as described previously. An aliquot of 120 μL of 6, 12 and 15 mM sucrose solutions were injected into a Mill-Q water stream flowing into a flow-cell at the flow rate of 40 μL/min. In order to optimize the position of the CCD sensor, the scale of the micrometer-head was varied at 3.12, 3.40, 4.00, and 4.20. In this experiment, a current of 120 mA was applied to the LED. The intensities of the reflected light were measured by the CCD sensor at pixels between 875 and 878.

**Calibration curve for sucrose solution and validation of the present SPR sensor**

The sensitivity of the SPR sensor with the 30-stripe patterned sensor chip was evaluated by measuring the intensities of the reflected light at pixels between numbers 565 and 1180 for the sucrose solutions (3, 6, 8, 12 and 15 mM) under the same condition as described previously. The measurements for all the sucrose solutions were repeated 5 times so as to evaluate the repeatability. A 10 mM test sucrose solution, prepared separately, was injected into the Milli-Q water stream, and we measured the intensities of the reflected light for the 30 stripes on the sensor chip so as to validate the present SPR sensor.

**Results and Discussion**

**Evaluation of basic performance of the present SPR sensor using a bare sensor chip**

As will be described in the next section, a refractive-index change on the 30-stripe patterned sensor chip prepared within a 6.0 mm total width was observed at the pixels of 562 – 1180 of the CCD sensor. Therefore, a space of 6 mm on the sensor chip corresponds to 618 pixels on the CCD sensor. This means that a change in the reflective index on the sensor chip of ca. 10 μm in width can be detected by one pixel of the CCD sensor. In an ideal situation, where two 10 μm sample spots are placed on the sensor chip separated by 10 μm, sensor signals from the two spots can be detected by two pixels on the CCD sensor. However, such an ideal spatial resolution may not be achieved. This section describes the basic performance of the present SPR sensor, including the practical spatial resolution, which was examined by using the bare sensor chip experimentally. When water and 8 and 15 mM sucrose solutions were flowed onto the bare sensor chip, the intensities of the reflected light at pixel numbers 461 – 1320 were taken as a snapshot. The resulting light intensities are shown in Fig. 2. The distribution of the intensities of the reflected light against the pixel number is convex. Namely, the intensity of the reflected light at around the center pixels is stronger than that at the outer pixels. This is due to the fact that the light intensity projected to the CCD sensor is strong at around the center, and weak at outside, as expected, the shape and distribution of the light intensity, as shown in the top view in Fig. 1. This is because the incident light from the LED (point light source) was spread as a cornshape. The intensities of the reflected light at all pixels increased with an increase in the concentrations of sucrose solutions, as shown in Fig. 2. Although the distribution of the intensities of the reflected light is convex against the pixel number, the distribution of the intensities for the sucrose solutions from that for water is proportional to the concentration of the sucrose solutions, as shown in Fig. S5. As described in the Experimental section, the incident angle can be fixed by adjusting the scale of the micrometer-head for the present SPR sensor. In this case, since the scale of the micrometer-head is fixed at 3.12, the intensity of the reflected light increases with an increase in the concentration of the sucrose solutions, in the same way as expected from Fig. S6(a).

As shown in Fig. S5(a), the light intensities among the pixels are scattered at the snapshot. The standard deviations of the light intensities among the pixels for the sucrose solutions are about 30 digits. In order to evaluate the noise level (fluctuation) of the SPR sensor against the elapse time, the intensities of the reflected light for water at pixel number 961 - 1060 were measured for 130 s. Namely, the standard deviation of the light intensities for the time scale at selected pixels was evaluated. The intensity of the reflected light at 1 pixel (number 961) is plotted in Fig. 3(a). The standard deviation (SD) was calculated to be 22 digits. While, the averaged light intensities among 4 pixels (number 961 - 964) are plotted in Fig. 3(b), the SD decreased to 11 digits. The SD decreased from 7.8 digits to 3.5 digits when the number of pixels used for averaging was increased from 10 to 100. As can be seen from Fig. 3, the SD became smaller when the number of pixels used for averaging became larger. These results suggest that averaging the light intensities using at least 4 pixels may be allowed for obtaining a detection limit of ca. 3.2 × 10⁻⁹ RIU (0.9 mM sucrose solution), judging from Fig. S5(b).

**Basic performance of the present SPR sensor using the 30-stripe patterned sensor chip**

Numbers of pixels needed to obtain SPR sensor signal from one stripe: The flow-cell with a 30-stripe patterned sensor chip (Fig. S4(c2)) was placed on the prism of the SPR sensor, and Milli-Q water was flowed into the flow-cell. The SPR sensor response (intensities of the reflected light plotted against the pixel number) to water as a snapshot is shown in Fig. 4(a). The SPR phenomenon, which occurs on the 30-stripe patterned sensor chip with a 200-μm period, i.e. the widths of one stripe and the gap of neighboring stripes are 100 μm and the pattern is prepared within 6 mm in width, is detected by the 618 pixels at pixel numbers 562 - 1180. Namely, the SPR sensor response on 1 stripe (100 μm width) on the sensor chip is detected by about 10 pixels on the CCD sensor, as described in the previous section. The intensities of the reflected light observed at pixel numbers 461 - 562 and at 1180 - 1320 were ca. 4000 digits. This is due to the fact that the incident light so as totally
reflected at the sensor chip, where Cr was deposited. As can be seen from Fig. 4(a), 30 downward valley-shaped signals were observed. This indicates that the SPR sensor response to water occurs on the Au stripe of the sensor chip, because the intensities of the reflected light decrease via the SPR phenomenon. The intensity of the reflected light at the valley bottoms along with the pixel number is convex, just the same as that observed in the case of the bare sensor chip, as shown in Fig. 2. In order to show the SPR response at one Au stripe, the light intensities observed at pixel numbers 720 – 750 are shown by the black line in Fig. 4(b), where two Cr deposited stripes are separated by one Au stripe of 100 μm in width. When a 12 mM sucrose solution was flowed into the flow-cell, the SPR sensor response was shifted to the direction of higher intensity of the reflected light at pixel numbers 730 – 740, which corresponds to the Au stripe with 100 μm in width, as shown by the red line in Fig. 4(b). However, constant changes in the intensity of the reflected light from water to the sucrose solution were observed only at pixel numbers 733 – 737, indicating that the SPR signal at these pixels can be adoptable for measurements of the sucrose solution. Similar changes in the intensities of the reflected light were observed for all other stripes on the sensor chip. This indicates that the effective width of the stripe is narrower than that expected from the width of the stainless-steel mask (100 μm). This may possibly be explained by the fact that Cr vapor is penetrated into the neighboring stripes through a gap between the stainless-steel mask and the bare sensor chip during the deposition of Cr, as estimated in Figs. 4(c) and 4(d). This reduces the effective width of the Au stripe from 100 μm to a narrower width. In other words, only 6 – 7 pixels among 10 pixels indicate the normal SPR sensor response for a sample on one Au stripe of the sensor chip. A spatial resolution of 100 μm was achieved by using a 30-stripe patterned sensor chip under the condition.

Optimization of the position of the CCD sensor of the SPR sensor

As described in the previous section, the SPR sensor response of the present SPR sensor is dependent on the position of the CCD sensor. Indeed, the SPR sensor response to the sucrose solutions at 6, 12 and 15 mM were obtained by changing the position of the CCD sensor by setting the micrometer-head from...
was observed when the scale of the micrometer-head was set at 3.12 to 4.10, as shown in Fig. S6. The highest SPR response was observed when the scale of the micrometer-head was set at 3.12. Hence, in consequent experiments the position of the CCD sensor was set by adjusting the scale of the micrometer-head at 3.12.

Calibration curve for sucrose solution and validation of the present SPR sensor with a 30-stripe patterned sensor chip

A sensorgram and calibration curve obtained from a selected single stripe among the 30-stripe patterned sensor chip are shown in Figs. S7(a) and (b). The sensorogram was obtained by averaging the intensities of the reflected light of selected 6 pixels of the CCD sensor, which corresponds to the selected single stripe. Trapezoidal-shaped responses were observed for the sucrose solutions, as shown by the inset of Fig. S7(a). This is due to the fact that the volume of the injected sucrose solutions is sufficiently large so as not to be dispersed in the manifold including the flow cell. The drift of the baseline under this situation as the light intensities at this sensing stripe was as small as 23.8 digit/h, which was obtained by subtracting the average light intensities of the baseline from those of the plateau part of the peak top obtained by averaging for 90 s. The noise level of the baselines was calculated to be 7.2 digits (9.6 × 10⁻⁶ RIU) from the intensities of the reflected light for 150 s. A linear calibration curve for the sucrose solution was obtained with a correlation coefficient of 0.999 and relative standard deviations (RSDs) of 0.4 - 3.4% for the 5 times of repeated injection of the sucrose solutions. The detection limit (S/N = 3) for the sucrose solutions of the present sensor at this sensing stripe was calculated to be 1.6 mM, which corresponds to 5.5 × 10⁻⁵ RIU, as a reflective index unit. Almost the same performance was obtained for the other 29 sensing stripes. The sensorgrams and calibration curves obtained for all 30 stripes of the sensor chip are shown in Figs. 5(a) and 5(b). The absolute light intensities of the baseline were different among the sensor stripes for the same reason as discussed in the previous section. The drifts of the baseline were 15.0 - 23.8 digit/h, and the correlation coefficients for each calibration line were very close to 1.0 (0.998 - 0.999). The relative standard deviation (RSD) of the slope for the 30 calibration curves was 4.5%. The noise levels of the light intensities for the 30 stripes were 5.0 - 9.2 digit, which correspond to 1.0 - 2.0 × 10⁻⁵ RIU. A validation of the present SPR sensor was confirmed by measuring of the 10.0 mM test sucrose solution. As a result, an accuracy of 100 - 102% was obtained at the 30 stripes of the sensor chip by using each calibration curve, as shown in Table S1, indicating that the stripes were independent to each other, and not affected by the neighboring stripes.

Conclusions

In this paper, basic performance of an SPR sensor with multi-sensing points developed by using a linear CCD sensor has been described. Since a refractive-index change on the sensor chip within 6 mm distance can be detected by 618 pixels of the CCD sensor, the refractive change of a sample with a 10-μm spot size may be detected by a single pixel. However, the SD of the SPR signal obtained by a single pixel was as large as 22 digits. The SD of the SPR signals was found to be able to be reduced by increasing the number of pixels of the CCD sensor for averaging. The actual minimum number of pixels to maintain the detection limit of on the order of 10⁻⁵ RIU was ca. 4 pixels. This means that a sensing size of ca. 40 μm, which is larger than the ideal one, ca. 10 μm, is necessary at least for practical use. We then prepared a 30-stripe patterned sensor chip with 100 μm in width per one stripe for the present SPR sensor to detect a multi-point. As a result, the SPR sensor showed a detection limit of 3.2 × 10⁻⁵ - 5.5 × 10⁻⁵ RIU, which is comparable to that of conventional SPR sensors and our dual-type SPR sensor reported previously. For applying the present SPR sensor to the simultaneous detection of multi-antibodies, the immobilization of receptors on the stripes with 100 μm in width of the sensor chip will be the next issue of our research. By using a micro-spotter to immobilize the receptors, the present SPR sensor has a strong potential as a biosensor to detect a number of bio-makers, environmental pollutants and contaminates of food products by applying it to immunoassay.

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