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LABEL-FREE DETECTION OF PROTEIN MICROARRAY WITH HIGH THROUGHPUT SURFACE PLASMON RESONANCE IMAGING (SPRI)

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A surface plasmon resonance imaging (SPRI) system was developed for the discrimination of proteins on a gold surface. As a label-free and high-throughput technique, SPRI enables simultaneously monitoring of the biomolecular interactions at low concentrations. We used SPRI as a label-free and parallel method to detect different proteins based on protein microarray. Bovine Serum Albumin (BSA), Casein and Immunoglobulin G (IgG) were immobilized onto the Au surface of a gold-coated glass chip as spots forming a 6×6 matrix. These proteins can be discriminated directly by changing the incident angle of light. Excellent reproducibility for label-free detection of protein molecules was achieved. This SPRI platform represents a simple and robust method for performing high-sensitivity detection of protein microarray.

Keywords: Surface plasmon resonance imaging; protein microarray; label-free; high-throughput.

1. Introduction

Surface plasmon resonance (SPR) is a surface sensitive optical detection technique based on total reflection. It measures changes of the refractive index onto a sensing surface coated with a thin layer of gold or silver film.[1–2] The optimum thickness of metallic film is usually about 50 nm in order to provide better SPR coupling efficiency.[2] When resonance occurs, a fraction of the energy of incident light at a sharply defined angle can interact with the delocalized electrons in the metal film and reduce the reflected light intensity.[3–4] Since its possible use as a biosensor was demonstrated in 1983 by Lidberg *et al.*,[3] SPR has been developed into a label-free

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and versatile analytical tool and used to detect a number of different biomolecular interactions in real-time.[2,5–7] and to follow the kinetics of antibody-antigen reactions.[8]

Surface plasmon resonance imaging (SPRI), which is based on the traditional surface plasmon resonance principle,[1] is a label-free and high-throughput technique to study biomolecular interactions.[9–11] It involves measurement of the difference in reflected light intensity near the resonance response at a fixed-angle, due to differences in refractive index (RI), on the metal surface by using a charge-coupled device (CCD) camera through an imaging lens.[12] The advantages of SPRI furnish us with a tool for simultaneously monitoring of the biomolecular interactions at extremely low concentrations.

In this paper, we used SPRI as a label-free and parallel method to detect different protein molecules. *Bovine Serum Albumin* (*BSA*), *Casein* and *Immunoglobulin G* (*IgG*) were immobilized as a 6×6 matrix of spots on the Au surface of a gold-coated glass chip. Our self-built multifunctional SPR system allowed the measurement of biomolecular interactions in three modes, angular interrogation, wavelength interrogation and intensity measurement. Three kinds of protein molecules can be discriminated directly by various reflected intensity or changing the incident angle of light. Instrument sensitivity was also demonstrated. Excellent reproducibility for label-free detection of protein molecules was achieved.

2. Materials and Methods

2.1. *Materials*

Bovine Serum Albumin (BSA, MW 66,200), *Casein* (MW 24,000), and *Immunoglobulin G* (IgG, MW 150,000) were received from Sigma. 11-mercaptoundecanoic acid (MUA) and $1,1'$-carbonyldiimidazole (CDI) were purchased from Aldrich (Milwaukee, USA). Other chemicals used in this study were obtained commercially as the reagent grade, and unless mentioned otherwise, were used as received. Aqueous solutions were prepared in doubly distilled water. All experiments were performed at room temperature unless stated otherwise.

2.2. *Surface chemistry of protein microarray*

Figure 1(a) shows the procedure of surface chemistry. Thin gold films (47 nm) with a 3-nm underlayer of chromium were deposited onto BK7 glass slides. The gold chips were then immersed in 1 mM ethanolic MUA solution overnight to form a well-packed self-assembled monolayer. The MUA monolayer was then reacted overnight with CDI in acetone solution (10 g/L).

Immediately prior to arraying, the CDI-modified chips were rinsed in acetone and dried under a nitrogen stream. The chips were arrayed using a Microarrayer (CapitalBio, Beijing, China), equipped with a humidity control apparatus and ultrasonic wash station. During the array fabrication, the humidity was controlled at 40–55% and the platen temperature was set at 25°C. Following antibody

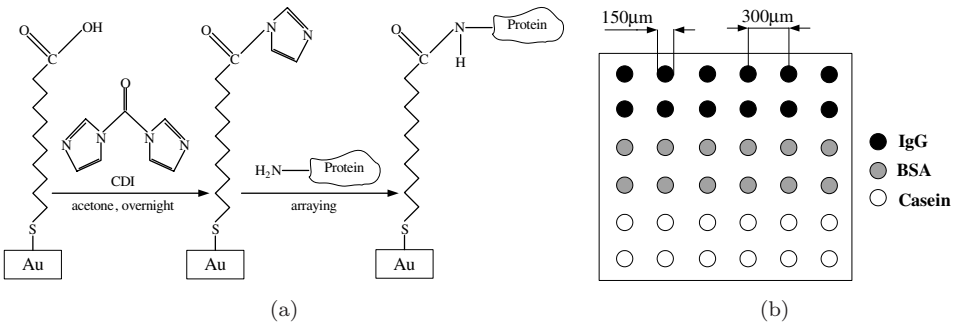

Fig. 1. (a) A schematic showing the covalent attachment of protein onto gold surface. The reaction of a MUA monolayer with CDI forms an imidazolide monolayer that couples with a lysine residue on a protein to create an amide linkage to the surface. (b) Protein microarray pattern. IgG, BSA and casein forms a 6×6 matrix. The concentration of each of the proteins was 1 mg/mL.

immobilization, the CDI-modified background was inactivated by washing thoroughly with water or buffer for 1 h before drying under a N_2 stream and storing at room temperature overnight.

2.3. *SPRI setup and procedure of imaging*

A self-built multifunctional surface plasmon resonance system based on the Kretschmann geometry of the attenuated total reflection (ATR) method.[13] was constructed to study biomolecular interaction. Different from former SPR sensors, this platform could undertake three modes of measurement, angular interrogation, wavelength interrogation and intensity measurement (i.e. SPRI). In this paper, we only used the intensity measurement mode, i.e. SPRI, to study protein microarray.

The incident light from a 10 mW light emission diode (532 nm) was expanded, collimated, and then polarized through a polarizer. After being reflected from the gold film via the glass prism, it was imaged by optical lens and collected by CCD camera. The collected image was treated and saved by self-developed software. The polarizer (GCL-050002, China Daheng Group, Inc.) was adjusted to produce p-polarized wave only. The glass prism was made of BK7 with $n = 1.517$. The slide with gold film was attached to the prism through index matching oil ($n = 1.51$). A 0.9 mm thick silicone gasket, with a 5 mm $\times$ 20 mm rectangular hollow in the middle, was mechanically pressed between polypropylene and the slide to form flow cell. During the experiment, the SPRI setup was placed in darkroom to avoid ambient light. All SPRI experiments of protein microarray were performed under equilibrium conditions.

3. Results and Discussion

As the molecular structures and molecular weights of IgG, BSA and casein are different, under identical conditions the reflectivity of the three different proteins

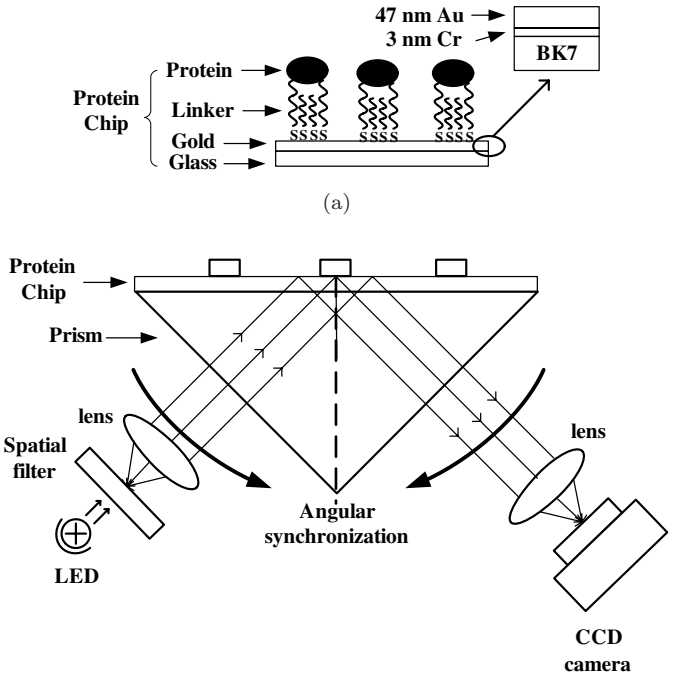

Fig. 2. Structure of protein chip (a) and scheme of SPRI system (b).

should be different. Each of the proteins was spotted and immobilized on the same sensitive surface (pre-coated with MUA monolayer and CDI monolayer). While changing incident angle of light, the reflected light intensity of proteins would be very weak and accordingly should exhibit dark spots when the spotted proteins are in the state of surface plasmon resonance.

As shown in Fig. 3(a), most of the incident light falling onto the protein spots was absorbed, so each of the protein spots appeared dark, indicating they were in the state of resonance. However, the degree of darkness was different for each protein. Casein exhibited much darker protein spots as it absorbed light more intensively, whereas the least absorption (or strongest reflected intensity) occurred on the BSA spots. The intensity of light absorption by IgG was intermediate. Figure 3(b) shows the line profile that illustrates the quantitative relationship of reflected light among these three kinds of protein spots. We can see that the reflected intensities of two rows of same protein were similar, while the reflected intensity differed slightly with various proteins on the same sensitive surface, and thus made label-free and high-throughput discrimination of protein realizable.

As noted above, the proteins appeared in dark spots due to the degree of surface plasmon resonance. However, the resonance signals can also be displayed by

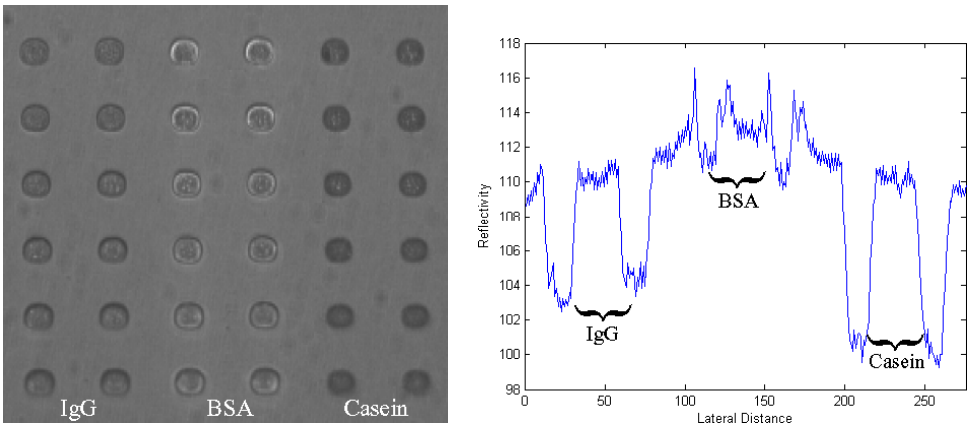

Fig. 3. *In situ* SPRI of protein microarray at resonance angle (a) and the corresponding line profile (b). In (a), from left to right, IgG (line 1–2), BSA (line 3–4) and casein (line 5–6). In (b), the absorption peak represents average reflected intensity of each indicated protein.

adjusting the position of the incident angle of light, causing the resonance condition be completely reversed. Thus under certain condition, the background would be in the state of surface plasmon resonance, while the spotted proteins would appear as bright spots because of stronger light reflectivity than the background.

Figures 4(a) and 4(c) show each of spotted proteins as bright spots, changing from absorption peak (Fig. 3) to pulse peak (Fig. 4). Here it can be seen that BSA exhibits the strongest reflected intensity and the casein the least. The incident angles of Figs. 4(a) and 4(c) were 45 degrees and 47 degrees, respectively. Though the change in angle is small, the reflected intensity of different proteins increased significantly. The reflected intensities of IgG and casein increased greater than BSA, demonstrating a good sensitivity. When incident angle is 47 degrees (Fig. 4(c)), the background is darker than at 45 degrees (Fig. 4(a)) because of a better SPR condition of background, and the signal values of two angles are close, so the signal-to-noise at 47 degrees is better than at 45 degrees. This is an advantage of SPRI, the background of which could be kept at much lower values than traditional microarray based on glass or plastic.

Figure 5 compares the signal-to-noise ratio that background is at resonant condition and protein is at resonant condition. We can see that the signal-to-noise ratio that background is at resonant is much larger than that protein is at resonant condition. However, the conditions shown in Fig. 5(a) are that reflected light of all proteins are absorbed, and one particular protein may be not in the optimal resonant situation. Thus, if only one kind of protein is target, we can expect a better signal-to-noise ratio at a given angle. In addition, the signal difference under diverse situations is also not the same. As shown in Fig. 5(c), when incident angle

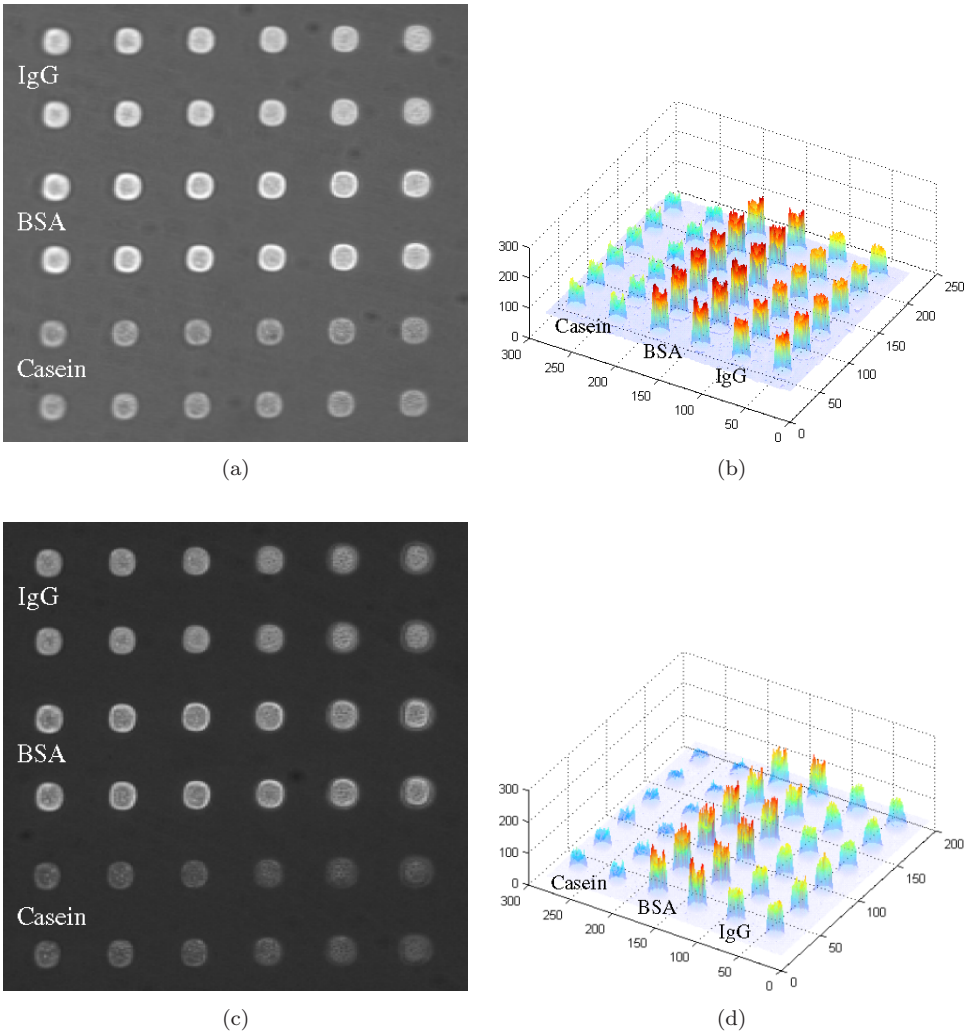

Fig. 4. *In situ* SPRI images of protein microarray at different incident angle (a), (c) and their corresponding three-dimension SPRI images (b), (d). The microarray consists of IgG, BSA, casein, and inactivated CDI-modified background elements with the same pattern shown in Fig. 1(b). In Figs. 4(b) and 4(d), the height of spots represents the degree of reflected intensity.

is 47 degrees, the difference between IgG and BSA is smaller than when incident angle is 52 degrees, while the signal of casein is much smaller at 47 degrees than at 52 degrees.

4. Conclusion

We have demonstrated a simple method for label-free and high-throughput detection of protein microarray by using SPRI. Discrimination of three kinds of proteins

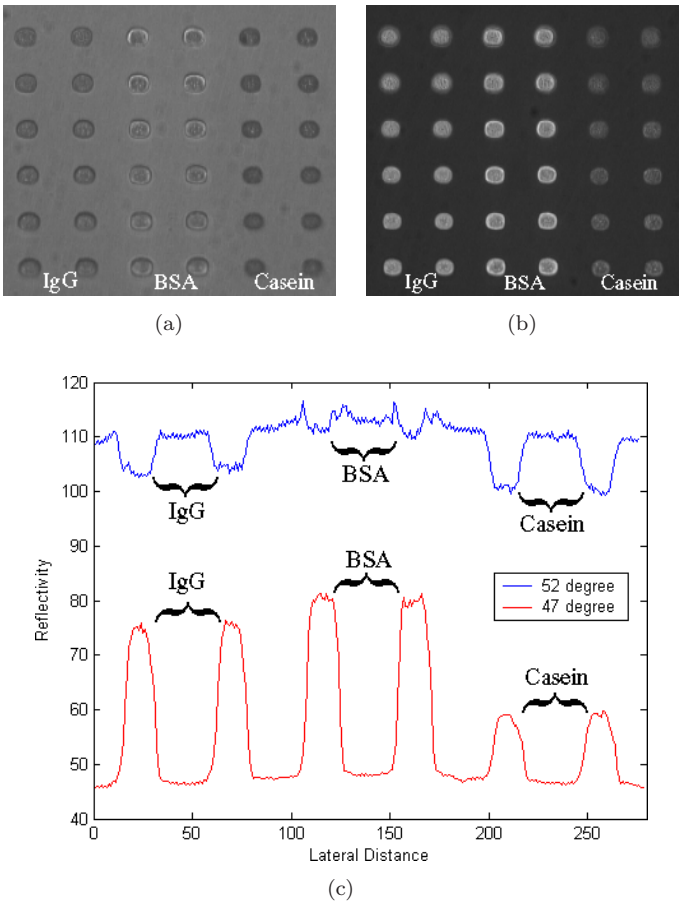

Fig. 5. Proteins are at resonant condition (a) and background is at resonant condition (b). (c) Corresponding profile under two conditions. The incident angle of (a) is 52 degrees, and (b) is 47 degrees.

was achieved by changing the incident angle of light. The relevance between different proteins and incident angular positions was established. Excellent reproducibility for label-free detection of protein molecules was achieved, where the values of 12 spots of one kind protein are close to each other, the relative standard deviation of IgG, BSA and Casein at 45 degrees is 2.4%, 1.6%, and 2.4%, respectively. This SPRI platform represents a simple and robust method for performing label-free, high-sensitivity, and high-throughput detection of protein microarray. Other kinds of molecules could also be discriminated using this technique.

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