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Label-free high-throughput and real-time detections of protein interactions by oblique-incidence reflectivity difference method

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Selected Mouse IgG of 1 mg/mL as target was fabricated on microarray for 500 sample dots. Label-free and real-time reaction dynamic processes were detected between the microarrays with Goat Anti-mouse IgG of 0.02 mg/mL using the oblique-incidence reflectivity difference (OIRD) method. We obtained the reaction results and the reaction dynamic curves of 500 protein dots. In addition, we also used label-free detection of protein microarrays of 10080 sample dots, including BSA and different concentrations of Mouse IgG and Rabbit IgG, by OIRD. The obtained reaction results between the protein microarray with 1 mg/mL Goat Anti-mouse IgG and 1 mg/mL Goat Anti-rabbit IgG are reported herein. Experimental results show that OIRD can be not only label-free high-throughput detection method for biological microarrays but also label-free real-time detection in the interaction processes of biomolecules.

oblique-incidence reflectivity difference (OIRD), label-free, real-time, high-throughput

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1 Introduction

Obtaining label-free, high-throughput and real-time detection techniques for life science has a continuous research challenge. Currently label-based detection methods are widely applied in the monitoring of biomolecule interactions for high sensitivity and high-throughput [1,2]. However, for label-based detection methods, adding labeled materials may alter the innate properties of the biomolecules [3,4]. Moreover, label-based detection methods are difficult to monitor real-time dynamic process of biomolecule reactions, which are critical in determining the reaction velocity and the affinity of one molecule with another. Therefore, the development of label-free, high-throughput and real-time detection techniques is useful and may be revolutionary in life science [5–7]. Oblique-incidence reflectivity difference (OIRD), with the properties of label-free and high-sensitivity, has been applied in detecting biomolecule interactions.

We report herein the label-free detections of protein microarrays [8,9] and oligonucleotide microarrays [10,11], label-free and real-time detections of protein interactions [12–16], and the detection repeatability of protein microarrays [17], using OIRD. We detect the label-free detecting of protein microarray including 10080 sample dots and the label-free and real-time monitoring of protein interaction of 500 sample dots.

2 Experiments

Experiments are carried out on an OIRD system similar to that in our previous work [12,14]. Figure 1 shows the OIRD

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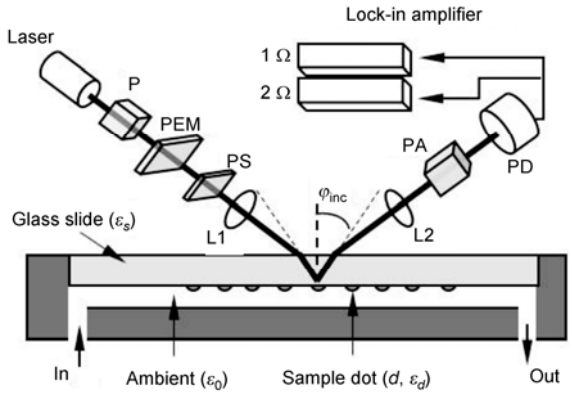

Figure 1 Schematic diagram of OIRD system for label-free, high-throughput and real-time detection of biomolecules interactions. Laser: a 17 mW He-Ne laser; P: polarizer; PEM: photo elastic modulator; PS: phase shifter (1/2 plate); L1 and L2: lens; PA: polarization analyzer; PD: photodiode detector.

system sketch for the Label-free, high-throughput and real-time detections of protein microarrays. As mentioned in our previous work, a p-polarized He-Ne laser with 632.8 nm wavelength passed through a photo elastic modulator which induced the probe beam to oscillate between p- and s-polarization in the frequency Ω=50 kHz. The modulated polarized light is then passed through a phase shifter (PS), which introduced a variable phase between p- and s-polarization components, thus giving an incident angle of 57° on the glass slide. The glass slide is mounted on the motorized stage and the reaction chamber is attached on the glass slide. The reflected beam passes through a polarization analyzer and the intensity is detected by a silicon photodiode and two lock-in amplifiers. Monitoring the changes of OIRD imaginary part Im$\{\Delta p-\Delta s\}$ was done.

In this work, we selected the epoxy-functionalized glass slides as the substrate of the microarrays. All of the biological samples were label-free and the protein microarrays were fabricated using the conventional procedures. The sample spots are effectively circular with an average diameter of about 80 μm, and the center to center separation between spots is about 200 μm. As shown in Figure 1, the reactions of protein molecules were in the reaction chamber.

First, we selected Mouse IgG (3T6937, KPL Inc. USA) of 1 mg/mL as the targets and the corresponding antibodies, Goat Anti-mouse IgG (090387, KPL Inc. USA) of 0.02 mg/mL, as the probe. As shown in Figure 2(a), we fabricated the protein microarray including 500 sample dots of Mouse IgG (20 lines, 25 columns). We not only measured the two dimensional (2D) scanning images of OIRD Im$\{\Delta p-\Delta s\}$ intensities before and after reaction, but also real-time monitored the reaction dynamic process of 500 protein dots.

Second, we selected Mouse IgG (3T6937, KPL Inc. USA) and Rabbit IgG (1O3245, KPL Inc. USA) as the targets and their corresponding antibodies, Goat Anti-mouse IgG (090387, KPL Inc. USA) and Goat Anti-rabbit IgG (110328, KPL Inc. USA), as probes. Bovine serum albumin (BSA) was used as negative control and comparison. We fabricated two identical protein microarrays. Each microarray consisted of 8 blocks and includes 10080 sample dots. Each block contained 45×28 sample dots corresponding to different proteins, 1: Mouse IgG of 0.25 mg/mL; 3: Mouse IgG of 1 mg/mL; 6: Mouse IgG of 0.5 mg/mL; 5: Rabbit IgG of 0.25 mg/mL; 2: Rabbit IgG of 0.5 mg/mL; 7: Rabbit IgG of 1 mg/mL; 4 and 8: BSA of 1 mg/mL. The two protein microarrays were used to react with Goat Anti-mouse IgG and Goat Anti-rabbit IgG of 0.02 mg/mL, respectively. We measured the 2D scanning images of OIRD Im$\{\Delta p-\Delta s\}$ intensities of the protein microarrays before and after each reaction, respectively, and obtained the differential images of OIRD Im$\{\Delta p-\Delta s\}$ signal, that is reaction results, by subtracting 2D scanning image of before reaction from the 2D scanning image after reaction.

3 Results and discussion

Figures 2(a) and (b) show the 2D images of OIRD Im$\{\Delta p-\Delta s\}$ intensities of before and after reaction between 1 mg/mL Mouse IgG and 0.02 mg/mL Goat Anti-mouse IgG. In order to acquire clear result, we made different Im$\{\Delta p-\Delta s\}$ signals between before and after reaction, that is, subtracted (a) from (b), as shown in Figure 2(c). In addition to obtaining reaction results, we also obtained the real-time scanning data and dynamic curves of the 500 protein dots. Figure 3 displays the dynamic curves of the 500 protein dots. OIRD signal rises rapidly initially and then slows down gradually and comes into equilibrium, thus showing the reaction rate and the time constant of reaction. From Figure 3, we can see that the time constant of reaction is approximately 10 min in this case. The experimental results indicate that the OIRD can be label-free and real-time detection method of the reaction processes of biomolecules.

Figure 4(a) shows the 2D image of OIRD Im$\{\Delta p-\Delta s\}$ intensities of the protein microarray including 10080 sample dots. All of the sample dots including BSA and the different concentrations Mouse IgG and Rabbit IgG are clear. Figure 4(b) and (c) display the 2D differential images after and before reacting of OIRD Im$\{\Delta p-\Delta s\}$ intensities, by subtracting 2D scanning image of before reaction from the 2D scanning image of after reaction, for the specific binding between the protein microarrays with Goat Anti-mouse IgG and Goat Anti-rabbit IgG of 0.02 mg/mL, respectively. From Figure 4(b), we can see that only the sample dots of mouse IgG in block 1, 3 and 6 have the specific binding with the corresponding Goat Anti-mouse IgG. In Figure 4(c), only the sample dots of rabbit IgG in block 2, 5 and 7 have the specific binding with the corresponding Goat Anti-rabbit IgG. Different grey levels indicate gradient of con-

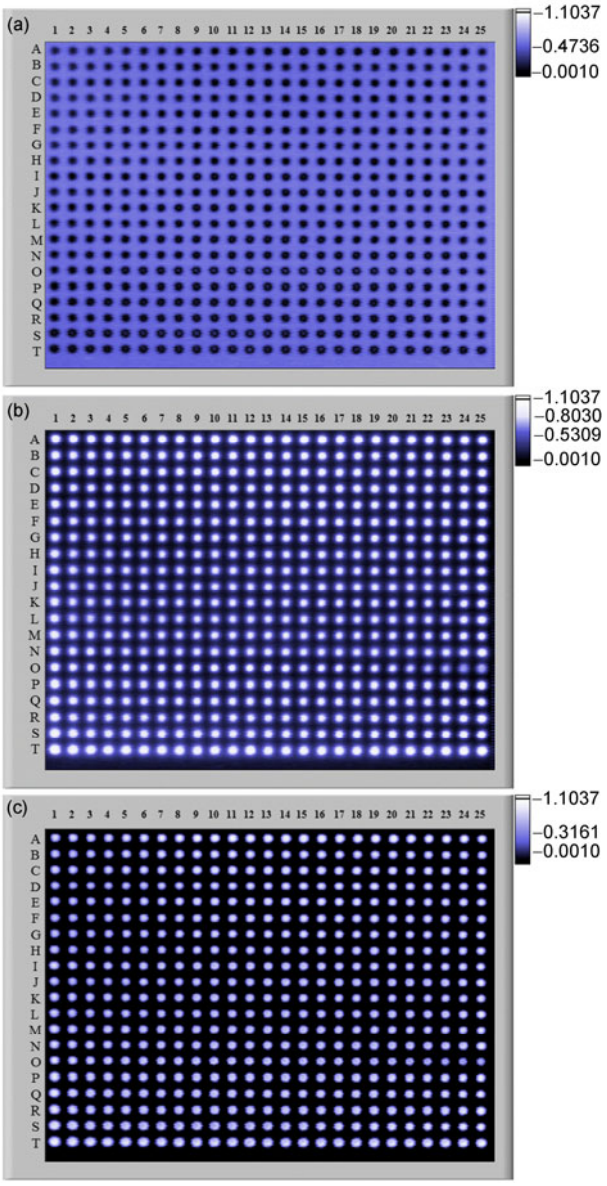

Figure 2 (Color online) Two-dimensional images of protein microarray of 500 sample dots. (a) Before reaction between the microarray with Goat Anti-mouse IgG; (b) after reaction between the microarray with Goat Anti-mouse IgG; (c) differential image of (a)–(b).

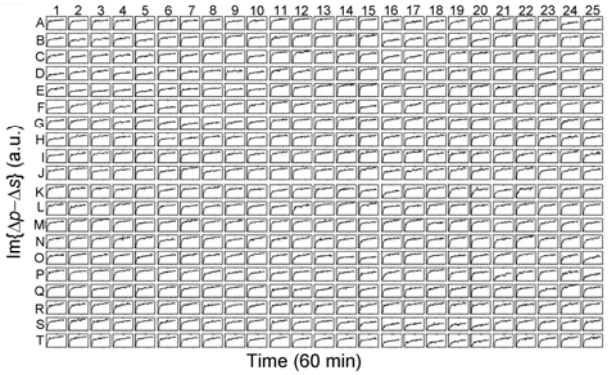

Figure 3 500 dynamic curves of reaction between Mouse IgG and Goat Anti-mouse IgG.

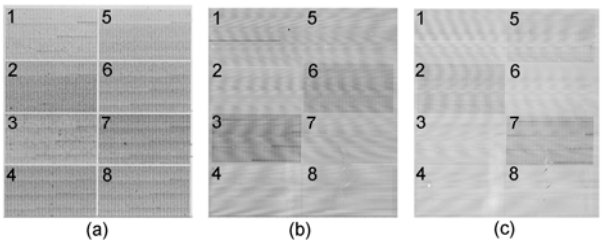

Figure 4 2D images of OIRD of the protein microarray including 10080 sample dots. (a) Before reaction of the microarray. (b) 2D differential images of OIRD after and before reaction between the protein microarray with Goat Anti-mouse IgG of mg/mL. (c) 2D differential images of OIRD after and before reaction between the protein microarray with Goat Anti-rabbit IgG of mg/mL.

centrations of the samples. All sample dots of BSA in block 4 and 8 do not react with Goat Anti-mouse IgG or Goat Anti-rabbit IgG. In addition, these 10080 sample dots occupy only 18 mm×23 mm in the slide. Seemingly, for the standard biological slide, 75 mm×25 mm, we can detect more than 30000 sample dots in a slide and one time. The experimental results show that the OIRD technique can be label-free and high-throughput detection of biological microarrays.

4 Conclusions

We have successfully label-free tracked and measured whole reaction processes of Mouse IgG microarray of 500 sample dots, including the before and after reaction as well as the dynamic processes of reaction. In addition reaction results of 500 dynamic curves. Concurrently, we also detected the protein microarrays of 10080 sample dots, including BSA and different concentrations of Mouse IgG and Rabbit IgG. The reaction results between the protein microarray for 0.02 mg/mL Goat Anti-mouse IgG or 0.02 mg/mL Goat Anti-rabbit IgG, respectively, were reported. The experimental results demonstrate that OIRD is a promising technique in life science for label-free high-throughput detection in biological microarrays and label-free real-time useful for understanding the interaction processes of biomolecules to acquire kinetic and thermodynamic parameters.

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