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Label-free and real-time detection of antigen-antibody interactions by Oblique-incidence Reflectivity Difference (OIRD) method

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We label-free and real-time detected three interaction processes of antigen-antibodies, Human Immunoglobulin G (IgG), Rabbit IgG, and Mouse IgG as the targets, and Goat Anti-human IgG, Goat Anti-rabbit IgG, and Goat Anti-mouse IgG as the probe, by the Oblique-incidence Reflectivity Difference (OIRD) method. The interaction dynamic curves of the OIRD signal, corresponding to the interaction processes of antigen-antibodies, are generated. The reaction times from beginning to equilibrium state are about 1800, 900, and 1200 s for Human IgG, Rabbit IgG, and Mouse IgG, respectively. The experimental results demonstrate that the OIRD method not only can distinguish biomolecular interactions, but also can be used in real-time detection of interactions and dynamic processes of biomolecules.

Oblique-incidence Reflectivity Difference (OIRD), label-free and real-time detection, dynamic process of biomolecular interaction

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1 Introduction

The investigation of biomolecular interaction process is very important to obtain the quantitative information for interaction kinetics. Till now, the label-based detection, such as fluorescent, chemiluminescent and radioactive labelling, is widely used in the monitoring of biomolecular interactions [1,2]. However, these labelling strategies are time-consuming and costly, but also often alter the surface characteristics and natural activities of the query molecule [3,4]. Currently, some label-free methods, such as surface plasma resonance, ellipsometry, carbon nanotubes and quantum dots [3–6], are used for the monitoring of biomolecular interactions. Therefore, the development of highly sensitive, reliable label-free detection techniques has been revolutionizing the areas of protein-protein interactions, pharmaceutical analysis, screening of potential drugs, cellular detection, biomolecular characterization, disease diagnostics and environmental monitoring. This is because label-free techniques avoid modifying reactants and measure an inherent property [4,7]. Oblique-incidence Reflectivity Difference (OIRD) method as a label-free and high-sensitivity detection method also has been used to detect biological microarrays [8,9]. We also reported label-free detections of biomolecular microarrays using the OIRD method [10–15]. In this work, we will report label-free and real-time detection of antigen–antibody interactions with Immunoglobulin G (IgG) microarrays by the OIRD method. The experimental results demonstrate that each antibody can be captured only by its corresponding antigen and the

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time taken to reach equilibrium for each antibody and antigen pair is different indicating that the OIRD is a promising candidate technique for studying the kinetic process of biomolecular interactions.

2 Experimental

We used label-free and real-time detection for three interaction processes of antigen-antibodies. We chose epoxy-functionalized glass slides (CEL Associates, TX) as the microarray substrates, Human IgG, Rabbit IgG, and Mouse IgG as the targets, and Goat Anti-human IgG, Goat Anti-rabbit IgG, and Goat Anti-mouse IgG as the probes (All the IgG was purchased from KPL Inc., USA). The protein microarrays we used were fabricated following the conventional procedure as described previously [16]. As shown in Figure 1, two sample spots of each IgG with the same concentration of 5 mg/mL were separately printed in duplicate along a line on the glass slides using a spotting robot microsystem (Personal Arrayer^{TM} 16, CapitalBio Corp., CA). The protein spots are generally circular with an average diameter of about 100 μm, the center to center separation between adjacent spots is about 500 μm. The prepared IgG microarrays were preserved at 4°C for 12 h so that the IgG molecules were well immobilized to the glass slides. The IgG microarray was mounted on a reaction chamber as shown in Figure 2(b). The biochip surface of the being printed IgG spots was then washed off with 1×PBS (Phosphate Buffered Saline) in order to remove the excess unbound IgG and the remnant salt precipitates. The biochip was then blocked with 1% glycine dissolved in 1×PBS for 1 h at room temperature to quench the intact epoxy groups on the surface. Finally, the reaction chamber and biochip were cleaned several times with deionized water.

The solution-phased Goat Anti-human IgG, Goat Anti-rabbit IgG, and Goat Anti-mouse IgG with a concentration of 0.02 mg/mL (diluted with 1% glycine dissolved in 1×PBS) were injected into the reaction chamber to react with the protein microarrays, respectively. We used label-free and real-time detection to the three interaction processes by the OIRD method.

Figure 2 shows the schematic diagram of the OIRD for label-free and real-time detection of antigen-antibody interactions. Figure 2(a) is the system sketch of optics and measurement. Figure 2(b) is the reaction chamber. A similar protocol was followed according to our previous work [10],

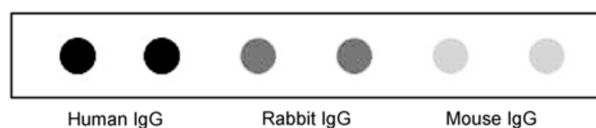

Figure 1 The arrangement of protein sample spots.

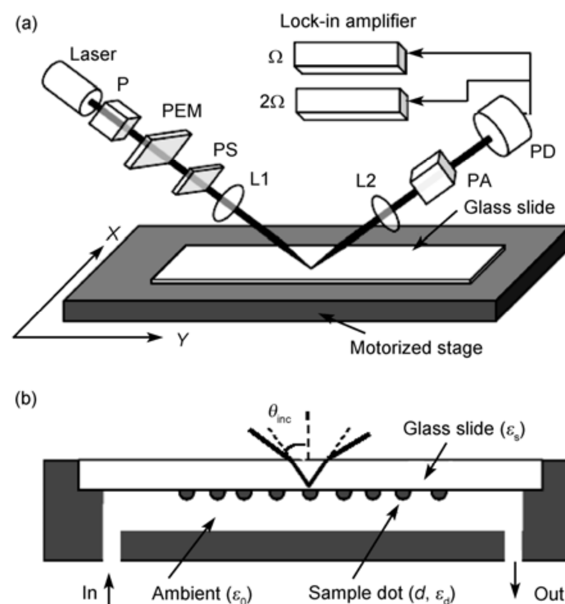

Figure 2 The schematic diagram of the OIRD for label-free and real-time detection of antigen-antibody interactions. (a) System sketch of optics and measurement. (b) Reaction chamber. Laser: a 7 mW He-Ne laser; P: polarizer; PEM: photoelastic modulator; PS: phase shifter~1/4 plate; L1 and L2: lens; PA: polarization analyzer; PD: photodiode detector.

except that a reaction chamber was added and the biological microarray was lying in reverse on the chamber. The reaction chamber was mounted on a two-dimensional motorized stage. Imaging of the biomolecular microarrays followed standard procedure [10]. The probe beam from a p-polarized He-Ne laser passed through a photoelastic modulator (PEM) and focused on the surface of microarray at an incident angle of 60°. The PEM causes the output beam to oscillate between p- and s-polarization with a modulated frequency Ω=50 kHz. An adjustable phase difference between p- and s-polarized components was introduced by a phase shifter (PS). In the case of oblique incidence, the surface reflectivity of p- and s-polarized light changes disproportionately in response to a surface change. Briefly, let r_{p0} and r_{s0} denote the respective reflectivity from the functionalized glass surface for p- and s-polarized light, and r_p and r_s be the respective reflectivity from biomolecules on the slide surface. The changes of reflectivity are defined as $\Delta_p=|(r_p-r_{p0})/r_{p0}|$ and $\Delta_s=|(r_s-r_{s0})/r_{s0}|$. We directly measure the fractional reflectivity changes of $\mathrm{Im}\{\Delta_p-\Delta_s\}$.

The IgG microarray was mounted on a motorized stage that can be driven along the *X* and *Y* directions, respectively. When the reaction solution was injected into the reaction chamber, the scanning program was carried out. The detection light-spot moved back and forth along the centerline of the six sample spots. We directly measure the ac component $I(\Omega)$ of the reflected beam intensity at the modulation frequency, which is proportional to $\mathrm{Im}\{\Delta_p-\Delta_s\}$.

3 Results and discussion

From a classical three-layer model [10], the optical response of the OIRD from a surface layer can be expressed as eq. (1):

$$\mathrm{Im}\{\Delta_p - \Delta_s\} = -\left[\frac{4\pi\varepsilon_s(\tan\theta_{inc})^2\cos\theta_{inc}}{\varepsilon_0^{1/2}(\varepsilon_s-\varepsilon_0)\left(\varepsilon_s/\varepsilon_0-(\tan\theta_{inc})^2\right)}\right] \times \frac{(\varepsilon_d-\varepsilon_s)(\varepsilon_d-\varepsilon_0)}{\varepsilon_d}\left(\frac{d_{eff}}{\lambda}\right). \quad (1)$$

In this case, θ_{inc} is the incidence angle; ε_0, ε_d and ε_s are the optical dielectric constants of the ambient, biomolecular layer and glass slide, respectively; d_{eff} is the the average thickness of biomolecular layer; λ is the wavelength of incident laser. For real-time detection of antigen-antibody reaction, d_{eff} would change when antigens are combing the antibody. From eq. (1), it can be easily deduced that as the reaction continues the d_{eff} changes which leads to an optical signal. When the reaction ends, d_{eff} and the OIRD signal tend to be an equilibrium value. Thus real-time detection of the reaction process of antigen-antibody by OIRD method can be accomplished.

Figure 3 presents the label-free and real-time detection results of the OIRD for the three interaction processes of antigen-antibody. Figures 3(a)–(c) show the variations of OIRD $\mathrm{Im}\{\Delta_p–\Delta_s\}$ signal with time, which corresponded to the reaction processes of the protein microarray with Goat Anti-human IgG, Goat Anti-rabbit IgG, and Goat Anti-mouse IgG, respectively. The real-time OIRD data reveal that the kinetic process and the curves H, R, and M correspond to Human IgG, Rabbit IgG, and Mouse IgG, respectively. In order to remove the environment interference and systematic error varying with time as far as possible, we take the difference between the average of sample spots and the average of the background near the corresponding spots as the net value varying of $\mathrm{Im}\{\Delta_p–\Delta_s\}$ with time in Figure 3.

From Figure 3(a), it can be seen that the signal intensities of the OIRD $\mathrm{Im}\{\Delta_p–\Delta_s\}$ are changing with time for curve H, and overlapped and close to zero for curves of R and M. The results can be interpreted that the Goat Anti-human IgG can be captured only by Human IgG, and cannot be captured by Rabbit IgG or Mouse IgG.. Similar to the above, from Figures 3(b) and (c), one can see that the Goat Anti-rabbit IgG can be captured only by Rabbit IgG, and the Goat Anti-mouse IgG can be captured only by Mouse IgG. The experimental results and drawn curves, H in Figure 3(a), R in Figure 3(b), and M in Figure 3(c), show the reaction speed, indicating that the reaction processes of antigen–antibody are exponential. The reaction times from beginning to equilibrium state are about 1800, 900, and 1200 s for Human IgG, Rabbit IgG, and Mouse IgG, respectively.

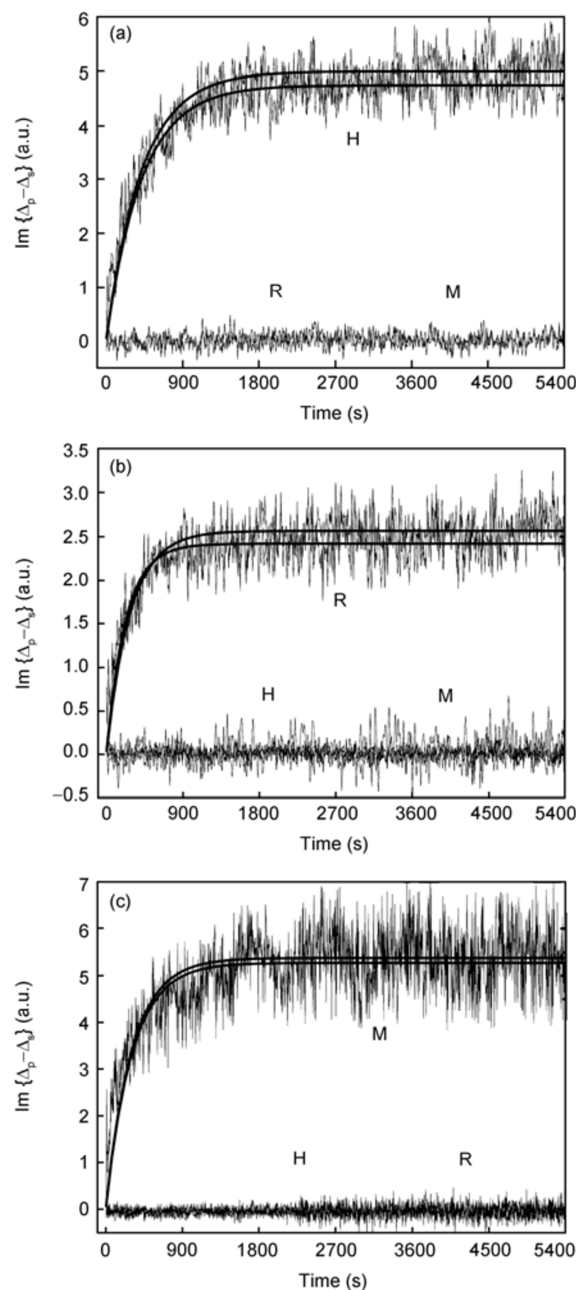

Figure 3 The variations of the OIRD $\mathrm{Im}\{\Delta_p–\Delta_s\}$ signal with time for three interaction processes of antigen–antibody. The protein microarray with Goat Anti-human IgG (a), Goat Anti-rabbit IgG (b), and Goat Anti-mouse IgG (c), respectively. H, R, and M correspond to Human IgG, Rabbit IgG, and Mouse IgG, respectively.

4 Conclusion

In summary, we label-free and real-time detected three interaction processes of antigen–antibody, including Human IgG/Goat Anti-human IgG, Rabbit IgG/Goat Anti-rabbit IgG and Mouse IgG/Goat Anti-mouse IgG, using the OIRD technique. We obtained the different curves for variation of the OIRD $\mathrm{Im}\{\Delta_p–\Delta_s\}$ signal with time, corresponding to the

kinetic processes of different antigen-antibodies. The experimental results demonstrate that the OIRD technique not only can distinguish the biomolecular interactions, but also can be used in real-time detection for the interaction processes of biomolecules. Further investigations on kinetic processes of biomolecular interactions and the analysis of quantitative data of kinetic process are being investigated for future work.

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