Label-Free and Real-Time Detection of Antigen-Antibody Capture Processes Using the Oblique-Incidence Reflectivity Difference Technique *

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We successfully label-free and real-time detect the capture processes of human immunoglobulin G (IgG)/goat anti-human IgG and mouse IgG/goat anti-mouse IgG antigen-antibody pairs with different concentrations using the oblique-incidence reflectivity difference (OIRD) method, and obtain the interaction kinetics curves and the interaction times. The experimental results prove that the OIRD method is a promising technique for label-free and real-time detection of the biomolecular interaction processes and achieving the quantitative information of interaction kinetics.

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Research of biomolecule interaction is very important to obtain the molecular characteristics and the information of interaction kinetics. To date, the label-based detection methods, such as radioactive labeling, chemiluminescence, and fluorescence, are widely applied in the monitoring of biomolecular interactions.[1,2] In practice, the label procedure is not only very costly and time-consuming, but also the extrinsic label probably impacts the biomolecule characteristics and the natural activities.[3,4] Some label-free detection technologies, such as surface plasma resonance, ellipsometry, carbon nanotubes, and quantum dots, etc.[3–6] can be used to investigate biomolecular interactions. Generally, these label-free detection methods have not been considered to be high-throughput methods. Therefore, it is still a big challenge to obtain a label-free and high throughput detection method for biological microarrays. Furthermore, only a few methods are adaptable to real-time detection of biomolecular interactions. A label-free and real-time detection technique is useful to detect biomolecular interaction and acquire kinetic information. Hence, development of highly sensitive and reliable label-free detection techniques would have a revolutionary impact in biomolecular science, including proteomics, pharmaceutical analysis, screening of potential drugs, disease diagnostics, cellular detection and biomolecular characterization, even environmental monitoring.[4–7] The oblique-incidence reflectivity difference method (OIRD), with the characteristics of label-free and high-sensitivity, has been applied to detect the biological microarrays.[8,9] In our previous works, we have label-free detected the biological microarrays by the OIRD method.[10–15] In this Letter, we report our label-free and real-time detection of antigen-antibody capture processes with the OIRD method.

Figure 1 shows the OIRD system sketch for the label-free and real-time detection of antigen-antibody capture processes. The system is similar to that depicted in our previous work,[10] except that a reaction chamber is added and the biological microarray is lying in reverse on the chamber. The chamber is mounted on a two-dimensional motorized stage. The probe light beam from a p-polarized He-Ne laser at $\lambda = 632.8\,\mathrm{nm}$ passes through a photoelastic modulator (PEM), which causes the output beam to oscillate between p- and s-polarizations with a modulated frequency $\Omega = 50\,\mathrm{kHz}$, and is focused on the surface of the microarray at an incident angle of 60°. A phase shifter (PS) is adopted to introduce an adjustable phase difference between p- and s-polarized components. In the situation of an oblique incidence, the surface reflectivities for p- and s-polarized components change disproportionately corresponding to a surface change. Briefly, let r_{p0} and r_{s0} represent the respective reflectivities for p- and s-polarized light reflected from the bare slide surface without biomolecules, and r_{p} and r_{s} be the respective reflectivities from the biomolecule covered areas, and define $\Delta_{\mathrm{p}} = |(r_{\mathrm{p}} - r_{\mathrm{p0}})/r_{\mathrm{p0}}|$ and $\Delta_{\mathrm{s}} = |(r_{\mathrm{s}} - r_{\mathrm{s0}})/r_{\mathrm{s0}}|$. In the experiment, $\mathrm{Im}\{\Delta_{\mathrm{p}} - \Delta_{\mathrm{s}}\}$ corresponding to the fractional reflectivity changes on the biomolecules is directly measured. From a classical three-layer model,[10] the optical response of OIRD from a surface layer can be expressed as

$$\mathrm{Im}\{\Delta_{\mathrm{p}} - \Delta_{\mathrm{s}}\} = -\left[\frac{4\pi\varepsilon_{\mathrm{s}}(\tan\theta_{\mathrm{inc}})^2\cos\theta_{\mathrm{inc}}}{\varepsilon_0^{1/2}(\varepsilon_{\mathrm{s}}-\varepsilon_0)\left(\varepsilon_{\mathrm{s}}/\varepsilon_0-(\tan\theta_{\mathrm{inc}})^2\right)}\right] \times \frac{(\varepsilon_d-\varepsilon_{\mathrm{s}})(\varepsilon_d-\varepsilon_0)}{\varepsilon_d}\left(\frac{d_{\mathrm{eff}}}{\lambda}\right), \quad (1)$$

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where $\theta_{\rm inc}$ denotes the incidence angle; ε_0, ε_d and $\varepsilon_{\rm s}$ are the optical dielectric constants corresponding to the ambient, biomolecular layer and the slide, respectively; λ is the wavelength of incident laser and $d_{\rm eff}$ is the average thickness of the biomolecular layer. For the real-time detection of antigen-antibody capture process, $d_{\rm eff}$ would change as the antigens are gradually captured by the antibodies. At the end of the reaction, $d_{\rm eff}$ would tend to equilibrium. Therefore, we can real-time monitor the antigen-antibody capture processes by the OIRD method.

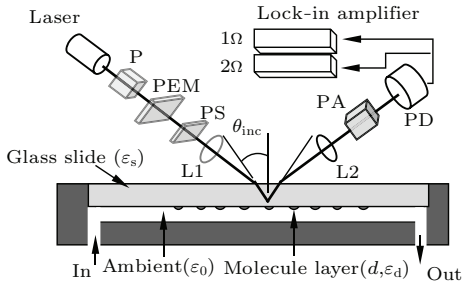

Fig. 1. The schematic diagram of OIRD for label-free and real-time detection of antigen–antibody capture processes. Laser: a 7 mW He-Ne laser; P: polarizer; PEM: photoelastic modulator; PS: 1/2 plate; L1 and L2: lens; PA: polarization analyzer; PD: photodiode detector.

In the experiments, we chose the epoxy-functionalized glass slide (CEL Associates, TX) as the substrates, human immunoglobulin G (IgG)/goat anti-human IgG and mouse IgG/goat anti-mouse IgG as the antigen-antibody pairs (all the IgG was purchased from KPL Inc., USA). We label-free and real-time detected the antigen-antibody capture processes between human IgG and goat anti-human IgG, and mouse IgG and goat anti-mouse IgG using the OIRD method, respectively.

The experiment consists of four steps. Firstly, as shown in Fig. 2, two microarrays were prepared following the conventional procedure described in Ref. [16]. Human IgG and mouse IgG with three different concentrations 1.25 mg/mL, 2.50 mg/mL, 5.00 mg/mL were respectively printed in duplicate on two glass slides using a spotting robot microsystem (PersonalArrayer$^{\rm TM}$ 16, CapitalBio corporation CA). Simultaneously, we printed two buffer spots (0 mg/mL) on each glass slide as the reference. The IgG spots were basically circular with an average diameter of about 100 μm and the center-center separation between two adjacent spots was 500 μm. Then we preserved the IgG microarrays at 4℃ for 12 h so that the IgG molecules were well immobilized to the glass slides. Secondly, the microarrays were respectively mounted on the chamber as shown in Fig. 1, and the surface with IgG spots was sealed in the chamber. After that, the surface printed with IgG spots was washed off with 1×phosphate buffered saline (PBS) and deionized water to remove the remnant unbound IgG and salt precipitates. Thirdly, the chamber was filled with 1% glycine dissolved in 1×PBS for one hour blocking at room temperature so that the intact epoxy groups on the surface were quenched. Later, the chamber was cleaned with deionized water. Finally, 0.02 mg/ml goat anti-human IgG and goat anti-mouse IgG solution were respectively injected into the chamber to react with the immobilized IgG molecules. Simultaneously, the scanning program was carried out with the probe light spot scanning to and fro along the centerline of the eight sample spots. The reflective light intensity proportionately corresponding to $\mathrm{Im}\{\Delta_{\rm p} - \Delta_{\rm s}\}$ was directly detected.

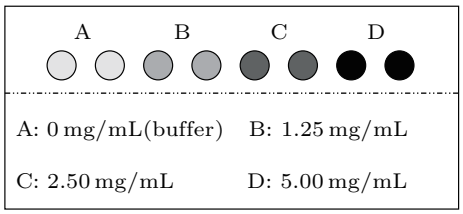

Fig. 2. Sketch map of the IgG microarrays. There are duplicate spots for every IgG concentration.

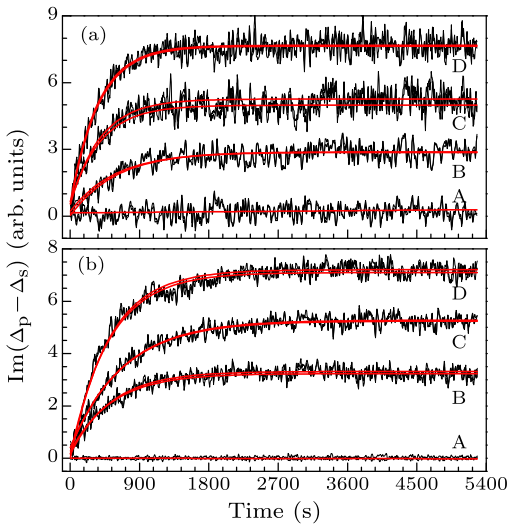

Fig. 3. The OIRD $\mathrm{Im}\{\Delta_{\rm p} - \Delta_{\rm s}\}$ signal versus time for two capture processes of antigen-antibody pairs. (a) Human IgG with goat anti-human IgG; (b) mouse IgG with goat anti-mouse IgG. A, B, C, and D denote the different concentrations of human IgG and mouse IgG. A: 0 mg/mL(buffer), B: 1.25 mg/mL, C: 2.50 mg/mL, D: 5.00 mg/mL.

Figures 3(a) and (b) respectively show the variations of OIRD $\mathrm{Im}\{\Delta_{\rm p} - \Delta_{\rm s}\}$ signals with time for the capture processes of human IgG/goat anti-human IgG and mouse IgG/goat anti-mouse IgG antigen-antibody pairs. The curves A, B, C and D correspond to the different IgG concentrations 0 mg/mL(buffer), 1.25 mg/mL, 2.50 mg/mL, and 5.00 mg/mL, respectively, and each of them contains two curves reflecting the duplicated sample spots. In order to remove the environment interference and to decrease the systematic error varying with time as far as possible, we deduct the background from the spots' signal. The thin curves are the measurement outcome and the thick curves are the fitting results corresponding to

the thin curves.

The changes of $\mathrm{Im}\{\Delta_\mathrm{p} - \Delta_\mathrm{s}\}$ signals present the dynamic process of antigen-antibody capture. From Figs. 3(a) and 3(b), we can see that the $\mathrm{Im}\{\Delta_\mathrm{p} - \Delta_\mathrm{s}\}$ signal intensities corresponding to the different IgG concentrations are different. Curves B, C and D correspond to two sample spots of different IgG concentrations 1.25 mg/mL, 2.50 mg/mL, and 5.00 mg/mL, respectively, and the curves overlap for the two sample spots with the same IgG concentration except curves C in Fig. 3(a) and curves B and D in Fig. 3(b) have a small dispersion, indicating that the measurement results for the two same sample spots are well consistent. The $\mathrm{Im}\{\Delta_\mathrm{p} - \Delta_\mathrm{s}\}$ signal intensities change with the time performing an exponential behavior, and the higher the IgG concentration, the greater the $\mathrm{Im}\{\Delta_\mathrm{p} - \Delta_\mathrm{s}\}$ signal intensity, indicating that goat anti-human IgG can be captured by human IgG, and goat anti-mouse IgG can be captured by mouse IgG. For curves A, the $\mathrm{Im}\{\Delta_\mathrm{p} - \Delta_\mathrm{s}\}$ signal intensities corresponding to the buffer spots approach to zero, indicating that there are no interactions between buffer and goat anti-human IgG or goat anti-mouse IgG. Curves B, C and D eventually tend to the equilibrium values, meaning that the interactions of antigen-antibody capture finish. Thus, in our case, the interaction times are about 1350 and 1800 s for human IgG/goat anti-human IgG and mouse IgG/goat anti-mouse IgG antigen-antibody pairs, respectively.

In summary, we have successfully label-free and real-time detected the capture processes of human IgG/goat anti-human IgG and mouse IgG/goat anti-mouse IgG antigen-antibody pairs using the OIRD method, and obtained the interaction kinetic curves and the interaction times. The experimental results prove that the OIRD method is a promising technique for label-free and real-time detection of the biomolecular interaction processes and achieves the quantitative information of interaction kinetics. Further investigations into kinetic processes of biomolecular interactions and quantitative analysis are being planned.

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