Label-free and real-time detections of the interactions of swine IgG with goat anti-swine IgG by oblique-incidence reflectivity difference technique

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Label-free and real-time detections of the interactions of swine IgG with goat anti-swine IgG by oblique-incidence reflectivity difference technique

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With the oblique-incidence reflectivity difference (OIRD) technique, we successfully label-free detected the interaction of swine IgG with different concentrations of 0.125, 0.25, 0.5, 1 mg/ml, and 5 μg/ml goat anti-swine IgG, and real-time detected the reaction dynamic processes of 0.5 mg/ml swine IgG and goat anti-swine IgG with different concentration of 5, 10, and 20 μg/ml, respectively. The interaction times are about 2043, 1828, and 1347 s for the reactions of 0.5 mg/ml swine IgG and goat anti-swine IgG of 5, 10, and 20 μg/ml, respectively. By fitting the reaction dynamic curves, we obtained that the association constant of swine IgG and goat anti-swine IgG is 1620.77 $M^{-1}\cdot S^{-1}$ at temperature about 22 °C. The experimental results demonstrate that the OIRD is a promising and competing method for label-free and real time detecting the biomolecular interactions and achieving the quantitative information of reaction kinetics. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4754469]

I. INTRODUCTION

Biomolecular interactions are widely studied with fluorescence-based detection method.[1–3] Fluorescence-based detection is well-known for the high sensitivity and high-throughput. However, adding the fluorescence materials may change the innate property of the biomolecular.[4,5] In addition, the fluorescence-based detection on the microarray can hardly give the association constant of the biomolecular reaction, which is very important to determine the reaction velocity and the affinity of one molecular with another molecular. The association constant is also very important in drug screening because it is used to determine which drug is the most effective to the disease. Although there are some other methods to detect the dynamic progress, for example, surface plasmon resonance (SPR). Because SPR needs valuable slide coated with gold and the throughput is not high enough, the SPR is limited to be used in some fields. It is still a big challenge to obtain label-free and high throughput detection method for biological microarrays, and the development of highly sensitive and reliable label-free detection techniques would bring about revolutionary impact on the biomolecular science.[6–8] Oblique-incidence reflectivity difference method (OIRD), with the characteristics of label-free and high-sensitivity, has been applied to detect the biological microarrays.[9,10] In our previous works, we have label-free detected the protein and oligonucleotide microarrays by OIRD method.[11–17] In this paper, we report the label-free and real-time detections of the interactions and the reaction dynamic processes of swine IgG with goat anti-swine IgG.

II. EXPERIMENTS

In Fig. 1, we show the scheme of the OIRD system for the label-free and real-time detection of biomolecule interaction. As mentioned in our previous works,[11,17] we choose a p-polarized He-Ne laser with a wavelength of $\lambda = 632.8$ nm as the detection light beam. The light beam passes through a photoelastic modulator (PEM) such that the polarization of the light beam oscillates from p- to s- polarization at the frequency of $\Omega = 50$ kHz. Then the light beam passes though a phase shifter (PS) which would introduce a variable phase ϕ_{ps} between the p- and s-polarized components. The light beam is focused by an optical lens into the microarray surface near the Brewster angle $\theta = 57.6°$. The reflected beam then passes though an analyzer (A) with its transmission axis set at θ_A from s-polarization. At last, the beam is detected by a photodiode (PD). We measure the first harmonic and the second harmonic signals by two lock-in amplifiers. Briefly, let $r_{p0} = |r_{p0}|\exp(i\varphi_{p0})$ and $r_{s0} = |r_{s0}|\exp(i\varphi_{s0})$ be the respective reflectivity of the bare microarray surface for p and s-polarized components, and $r_p = |r_p|\exp(i\varphi_p)$ and $r_s = |r_s|\exp(i\varphi_s)$ be the respective reflectivity of the biomolecule layer on the microarray surface, respectively. We define $\Delta_p = (r_p - r_{p0})/r_{p0}$ and $\Delta_s = (r_s - r_{s0})/r_{s0}$ as the fractional change of reflectivity, according to the classical three-layer model,[11]

$$\Delta_p - \Delta_s = -i\left[\frac{4\pi\varepsilon_s(\tan\theta_{inc})^2\cos\theta_{inc}}{\varepsilon_0^{\frac{1}{2}}(\varepsilon_s-\varepsilon_0)\left(\frac{\varepsilon_s}{\varepsilon_0}-(\tan\theta_{inc})^2\right)}\right] \times \frac{(\varepsilon_d-\varepsilon_s)(\varepsilon_d-\varepsilon_0)}{\varepsilon_d}\left(\frac{d_{aver}}{\lambda}\right), \quad (1)$$

where ε_d, ε_s, and ε_0 are the optical dielectric constants of the bio-sample, microarray, and the air, respectively, and d_{aver} is

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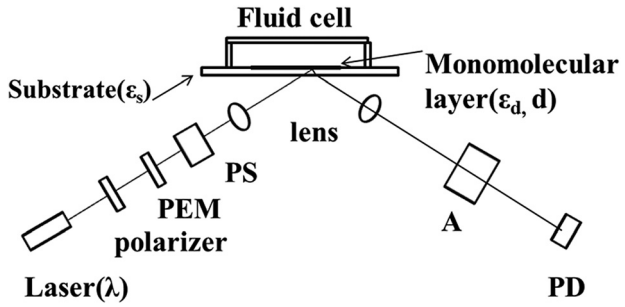

FIG. 1. Sketch of OIRD system for label-free and real-time detection of biomolecular interaction. Laser, a 7 mW He-Ne laser; PEM, photoelastic modulator; PS, variable phase shifter (half-wave plate); A, polarization analyzer; PD, photodiode detector. The microarray is fixed on a reaction chamber in a two-dimensional scanning stage of OIRD system.

the average thickness of the biomolecular layer. For the real-time detection of biomolecular interaction process, d_{aver} would change as the biomolecules are gradually reacted. At the end of the reaction, d_{aver} would tend to converge to a definite count. Therefore, we can label-free detect the reaction results and real-time monitor the interaction processes by OIRD method.

In the experiment, we selected homemade polymer brush functionalized glass slide as the microarray; the making procedure is reported by Hu *et al.*,[18] and selected swine IgG as the target, goat anti-swine IgG as the probe. As shown in Fig. 2, the microarrays consist of four swine IgG concentrations and two rows of negative control-bovine serum albumin (BSA). (The swine IgG and BSA were purchased from Cali-Bio, Inc., and goat anti-swine IgG was purchased from KPL, Inc., USA.) Every concentration of swine IgG has ten spots. The swine IgG was dissolved in a solution of 1×PBS (phosphate buffered saline) with 40% (v/v) glycerol, and goat anti-swine IgG was dissolved in a solution of 1×PBS (all the

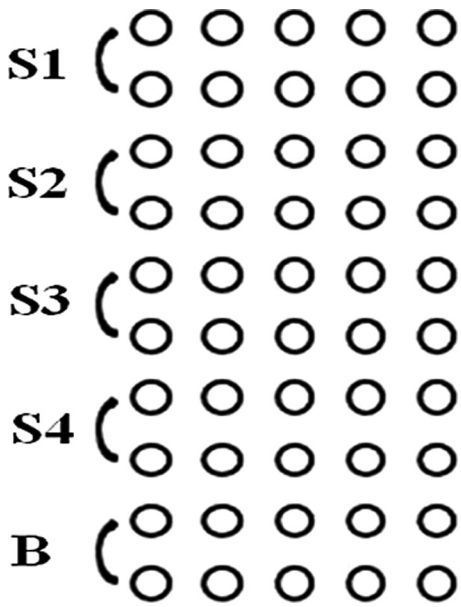

FIG. 2. The sketch of swine IgG microarray. There are the same ten spots for every swine IgG concentration and BSA. S1: 1 mg/ml swine IgG; S2: 0.5 mg/ml swine IgG; S3: 0.25 mg/ml swine IgG; S4: 0.125 mg/ml swine IgG, B: BSA.

phosphate buffer saline, *p*H 7.4) with 50% (v/v) glycerol. We used a robot arrayer (PersonalArrayer^{TM} 16, CapitalBio Corporation CA) to transfer the targets to the slide. The diameter of the sample spots is about 120 μm and the distance of the nearest spots (center to center) is about 500 μm. In order to lower the contamination, we first transferred the BSA to the slide and then the 0.125, 0.25, 0.5, and 1 mg/ml swine IgG to the slide. In order to make the swine IgG and BSA bind covalently to epoxy groups on the functionalized glass surface, the prepared microarray was preserved in a drying container at room temperature for 16 h. After that, the microarray was washed in PBST (1×PBS, *p*H 7.4 with 1% twain) for 5 min, 1×PBS for 5 min, deionized water for 5 min, respectively, and dried with a slow nitrogen flow.

In order to measure the reaction result of the microarray with the different concentration swine and goat anti-swine IgG, the microarray was fixed on a reaction chamber in the two dimensional (2D) scanning stage of the OIRD system. We detected the $\mathrm{Im}\{\Delta_p\text{-}\Delta_s\}$ signals and obtained the 2D scanning images of the microarray when the scanning stage made a 2D scanning. Before reaction, we filled the reaction chamber with 1×PBS and label-free measured the 2D scanning image of the swine IgG microarray with different concentrations. Then, 1×PBS was substituted with 5 μg/ml goat anti-swine IgG in the reaction chamber, and we measured the 2D scanning images at the reaction end.

In order to acquire the association constant between swine IgG and goat anti-swine IgG, we prepared the same three microarrays; there are two spots of 0.5 mg/ml swine IgG and two spots of BSA in a row for the every microarray. We label-free and real-time detected the reaction dynamic processes of the microarrays with 20, 10, and 5 μg/ml goat anti-swine IgG, respectively. The preparation and experimental processes of the microarrays are the same as mentioned above. The microarrays were, respectively, fixed on the reaction chamber, and 20, 10, and 5 μg/ml goat anti-swine IgG were, respectively, injected into the chamber to react with 0.5 mg/ml swine IgG. Simultaneously, the scanning program was carried out and the probe light spots scanning to and fro along the centerline of the five sample spots in the microarray surface. The reflective light intensity proportionately corresponding to $\mathrm{Im}\{\Delta_p\text{-}\Delta_s\}$ was directly detected; we obtained reaction kinetics curves of the swine IgG microarrays with the three different concentrations of goat anti-swine IgG, respectively.

III. RESULTS AND DISCUSSION

Figures 3(a) and 3(c) show the 2D scanning images of $\mathrm{Im}\{\Delta_p\text{-}\Delta_s\}$ signal intensities of before and after reaction. However, the interaction result of swine IgG and goat anti-swine IgG is not very obvious; even we can see the BSA spots which should not bind with goat anti-swine IgG. In order to acquire the clear result, we made the difference of $\mathrm{Im}\{\Delta_p\text{-}\Delta_s\}$ signals between before and after reaction, i.e., subtracted (a) from (c), as shown in Fig. 3(e). Figs. 3(b), 3(d), and 3(f) are the intensity profiles taken along the yellow lines in Figs. 3(a), 3(c), and 3(e). The swine IgG concentration from top to bottom decreases sequentially from 1 to 0.5,

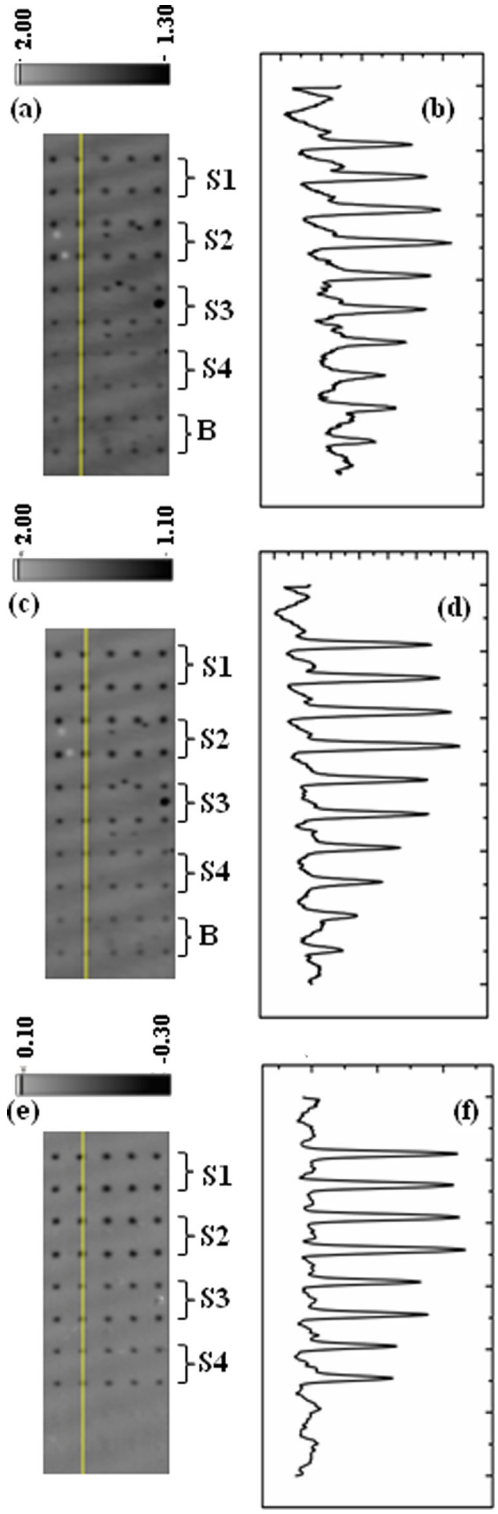

FIG. 3. OIRD detection results for the reaction of different concentrations swine IgG with goat anti-swine IgG. (a) and (c) show the 2D scanning images of Im{Δ_p-Δ_s} signal intensities before and after reaction; (e) is the difference image of Im{Δ_p-Δ_s} signals, subtracted (a) from (c); (b), (d), and (f) are the intensity profiles taken along the yellow lines in (a), (c), and (e).

0.25, 0.125, and 0 mg/ml in the microarray, and the gray scale in Fig. 3(e) and the signal amplitude in Fig. 3(f) show the concentration difference. From Figs. 3(e) and 3(f), we can see that the gray scale in Fig. 3(e) and the signal amplitude in Fig. 3(f) are nearly equal for the spots of 1 and 0.5 mg/ml swine IgG, indicating that the concentration of 0.5 mg/ml IgG has reached saturation for the reaction, and the gray scale in Fig. 3(e) and the signal amplitude in Fig. 3(f) are almost zero for all BSA spots, indicating that the BSA has no binding with goat anti-swine IgG. The experimental results proved that the OIRD method can be used to label-free detect not only the reaction of protein-protein but also the different concentrations. In addition, the good intensity profiles in Figs. 3(b), 3(d), and 3(f) demonstrate that it is possible to make the OIRD detection much faster using one-dimensional line scan instead of 2D scan as the spots of the biological sample in the microarray are uniform.

Figure 4 shows the variation of OIRD Im{Δ_p-Δ_s} signals with time for the reaction dynamic processes of 0.5 mg/ml swine IgG and 20, 10, and 5 μg/ml goat anti-swine IgG. In order to remove the environment interference and to decrease the systematic error varying with time as far as possible, we deducted the background from the spot signal and took an average of the three same swine IgG in the microarray. From Fig. 4, one can see that the Im{Δ_p-Δ_s} signal intensities change with the time performing a exponential behavior, and the changes of Im{Δ_p-Δ_s} signal intensities corresponding to the different concentrations of goat anti-swine IgG are different. The higher the goat anti-swine IgG concentration, the greater the Im{Δ_p-Δ_s} signal intensity, indicating that swine IgG can be captured by goat anti-swine IgG, and the higher the goat anti-swine IgG concentration, the faster the reaction. For the buffer spots of BSA, the Im{Δ_p-Δ_s} signal intensity approaches to zero, indicating that there is no binding between BSA and goat anti-swine IgG. The curves correspond to the different goat anti-swine IgG concentrations of 20, 10, and 5 μg/ml eventually tend to the equilibrium values, meaning that the reactions of swine IgG and goat anti-swine IgG finish. Thus, from Fig. 4, we can obtain that, in our case, the reaction times are about 2043, 1828, and 1347 s for the goat anti-swine IgG of 5, 10, and 20 μg/ml, respectively.

The association constant can be calculated from the OIRD real-time reaction dynamic curves of swine IgG obtained under different concentrations of goat anti-swine IgG. We employed the integrated rate analysis method[19] to

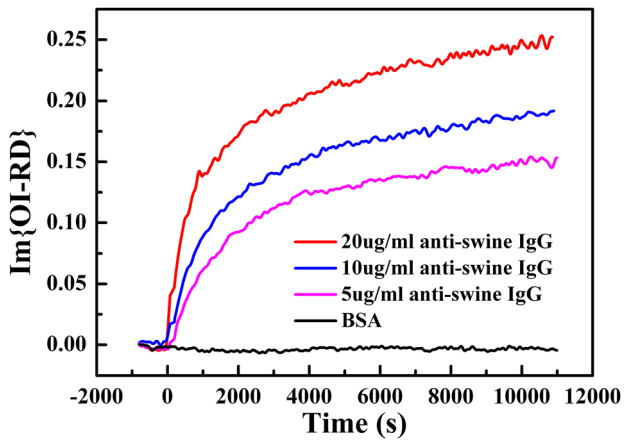

FIG. 4. The variation of OIRD Im{Δ_p-Δ_s} signals with time for the reaction dynamic processes of 0.5 mg/ml swine IgG and 20, 10, and 5 μg/ml goat anti-swine IgG.

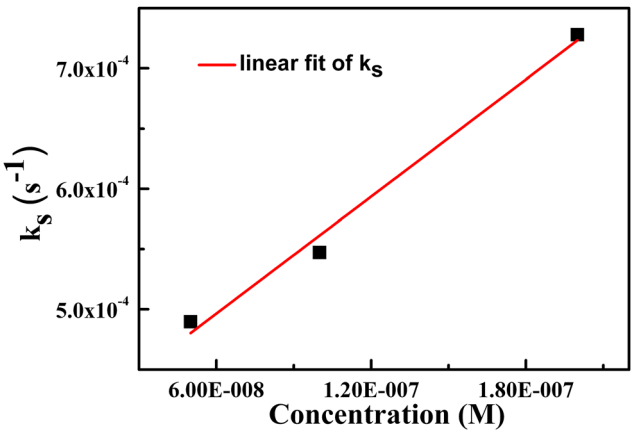

FIG. 5. The fitting of the dynamic constant k_a.

determine the association constant. Generally, for the biomolecular reaction, a simple and reversible reaction model is proposed

$$A + B \underset{k_d}{\overset{k_a}{\rightleftarrows}} AB,$$

in our case, A is the goat anti-swine IgG in the aqueous solution, B is the swine IgG on the surface of the microarrays, AB is the reaction product of swine IgG and goat anti-swine IgG, k_a is the association constant, and k_d is the dissociation constant. Thus, the association rate of complex AB is

$$\mathrm{d}[AB]/\mathrm{d}t = k_\mathrm{a}[A][B]_\mathrm{t} - k_\mathrm{d}[AB],$$

because

$$[B]_\mathrm{t} = [B]_0 - [AB].$$

Thus,

$$\mathrm{d}[AB]/\mathrm{d}t = k_\mathrm{a}[A]([B]_0 - [AB]) - k_\mathrm{d}[AB].$$

The bulk concentration of A is assumed to be constant in time due to the negligibly small change during interaction, and the OIRD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ signal R represents the concentration of complex AB on the microarray surface; therefore,

$$\mathrm{d}R_\mathrm{t}/\mathrm{d}t = k_\mathrm{a}CR_\mathrm{max} - (k_\mathrm{a}C + k_\mathrm{d})R_\mathrm{t}.$$

Thus,

$$R_\mathrm{t} = E^*(1 - e^{-kst}) + R_0, \qquad (2)$$

where C is the concentration of the goat anti-swine IgG, R_max is the maximal change of OIRD $\mathrm{Im}\{\Delta_p\text{-}\Delta_s\}$ signal in response to a certain concentration, R_0 is the response at $t = 0$, and

$$E^* = k_\mathrm{a}CR_\mathrm{max}/(k_\mathrm{a}C + k_\mathrm{d}), \quad k_\mathrm{s} = k_\mathrm{a}C + k_\mathrm{d}. \qquad (3)$$

On the basis of Eq. (2) and Fig. 4, we calculated the values of k_s by fitting the reaction dynamic curves of a set of concentrations of the goat anti-swine IgG. Based on Eq. (3), in Fig. 5, we show the linear fit of k_s. From the slope of the red fit line in Fig. 5, we obtained that the association constant k_a is about 1620.77 $M^{-1}\cdot S^{-1}$ for the reaction temperature at about 22 °C.

IV. CONCLUSION

In summary, we label-free detected the microarray interaction of different concentrations swine IgG with goat anti-swine IgG, and real-time monitored the reaction dynamic processes of swine IgG microarrays with different concentration goat anti-swine IgG, respectively. We obtained not only the reaction results but also the interaction times and association constant of swine IgG and goat anti-swine IgG. The experimental results prove that the OIRD method is a promising technique for label-free and real time detecting the biomolecular interaction processes and achieving the quantitative information of reaction kinetics.

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