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Label-free detection repeatability of protein microarrays by oblique-incidence reflectivity difference method

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We examine the repeatabilities of oblique-incidence reflectivity difference (OIRD) method for label-free detecting biological molecular interaction using protein microarrays. The experimental results show that the repeatabilities are the same in a given microarray or microarray-microarray and are consistent, indicating that OIRD is a promising label-free detection technique for biological microarrays.

oblique-incidence reflectivity difference (OIRD), protein microarrays, specificity, repeatability

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1 Introduction

Label-free and high throughput detection has been required in life sciences, because currently, label-based detection methods are widely applied in the monitoring of bimolecular interactions [1–3]. In practice, however, not only is the label procedure costly and time-consuming, but also extrinsic labeling most probably impacts the biomolecule structure and natural activities [4,5]. Oblique-incidence reflectivity difference (OIRD) method, with the properties of label-free and high-sensitivity, has been applied to detect the biological microarrays [6–9]. In our previous work, we have successfully detected the reaction endpoint and the reaction processes of biological microarrays without labeling by the OIRD method [10–13]. The experimental results reveal that the OIRD method is usable for label-free and high throughput detecting the interactions of biological molecules. However, as a new detection method, the detection repeatability is critical. In this work, we report the detection repeatability of protein microarrays by OIRD method with the label-free property. The experimental results, including specificity and repeatability, show that the detection repeatability of OIRD method is acceptable.

2 Experimental

Figure 1 shows the schematic diagram of OIRD setup for the label-free detection of protein microarrays. As mentioned in our previous work [12–14], the probe light beam is a p-polarized He-Ne laser with 632.8 nm wavelength, which passed through a photoelastic modulator which induces the probe beam to oscillate between p- and s-polarization with the frequency Ω=50 kHz. The modulated polarized light then passed through a phase shifter (PS), which introduced a

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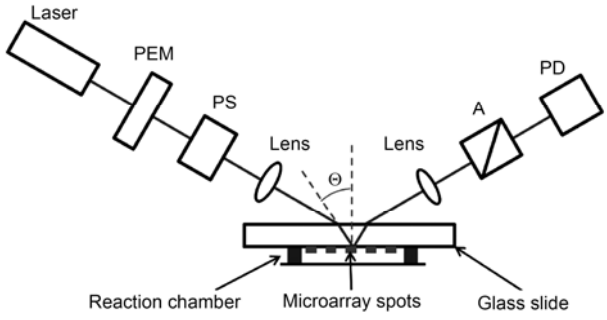

Figure 1 The layout of the OIRD system for the label-free detection of biological microarrays. PEM: photoelastic modulator; PS: phase shifter; A: analyzer; PD: photodiode.

variable phase between p- and s-polarization components, and was focused on the surface of protein microarray at an incident angle of 60°. The microarray and reaction chamber were mounted on a motorized stage. The reflected beam passed through a polarization analyzer and the intensity was detected by a silicon photodiode and two lock-in amplifiers. We monitored the second harmonics of the modulated frequency from the reflected beam intensity.

At oblique incidence, the surface reflectivities for p- and s-polarized light are different because of the difference of the thickness of the surface layer and/or the complex dielectric constant. Let us define r_{p0} and r_{s0} as the reflectivities for p- and s-polarized light from the bare microarray surface without protein molecules, and r_p and r_s be the reflectivities from the protein covered surface, respectively. The changes of p- and s-components in reflectivity are defined as $\Delta_p=|(r_p-r_{p0})/r_{p0}|$ and $\Delta_s=|(r_s-r_{s0})/r_{s0}|$, respectively. The difference in reflectivity change is $\Delta_p-\Delta_s$. In the experiment, we measured the changes of OIRD real parts $\mathrm{Re}\{\Delta_p-\Delta_s\}$ [12].

In this study, we use homemade polymer brush functionalized glass slide as the substrate, following the procedure as reported by Hu et al. [15]. We selected seven proteins: tumor suppressor protein p53 (p53) of 0.15 mg/mL, zinc finger E-box binding homeobox 1 (ZEB1) of 0.25 mg/mL, B-cell lymphoma 2 (Bcl2) of 0.2 mg/mL, H-cadherin (CDH13) of 0.3 mg/mL, cyclin-dependent kinase inhibitor 2A (p16) of 0.15 mg/mL, Hepatitis B e Antigen (HBeAg) of 1.25 mg/mL, and Hepatitis B surface Antigen (HBsAg) 2.75 mg/mL as the antigen targets and their corresponding antibodies as the probes. Bovine serum albumin (BSA) of 1 mg/mL was used as negative control and comparison. All of the protein samples including targets, BSA and probes were label-free. The protein microarrays were fabricated using the conventional procedure [14,16]. The microarrays were stored overnight and washed with 1×Phosphate Buffered Saline (PBS) before reaction. We measured the two dimensional (2D) scanning images of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ intensities of the protein microarrays before and after each reaction, respectively, and obtained the differential images of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ signal, that is, reaction results, by subtracting 2D scanning image of before reaction from the 2D scanning image of after reaction.

Firstly, one protein microarray is used to react with the antibodies of ZEB1, CDH13, p16 and HBeAg in sequence, and we measured the specificity of protein microarray by OIRD. Secondly, we altered a protein microarray and added the mixed antibody solution of 137 ng/mL anti-ZEB1, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16 and 650 ng/mL anti-HBe to the chamber to react with a microarray, and test the repeatability of specific binding in one microarray. Lastly, we changed three protein microarrays and added the mixed antibody solution of 65 ng/mL anti-P53, 137 ng/mL anti-ZEB1, 5 μg/mL anti-Bcl2, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16, 650 ng/mL anti-HBe, 500 ng/mL anti-HBs to the chamber to react with the three microarrays, respectively, and tested the repeatability of microarray to microarray. All of protein microarrays used in the experiment were prepared under the identical condition.

3 Results and discussion

Figure 2(a) shows the pattern of the protein microarray and Figure 2(b) is a 2D scanning image of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for one microarray after washed (before reaction). As shown in Figure 2(b), twelve groups of protein samples including eleven groups of protein targets and one group BSA can be seen clearly indicating that all the proteins have well bonded to the surface of the polymer brush functionalized glass slide. Among these groups, p53, ZEB1, CDH13, and HBeAg are printed in two duplicated blocks and the remainders have one block. Each block contains six identical spots. The center to center separation of the adjacent spots is about 300 μm, and the diameter of the sample spots is about 100 μm.

Figures 3(a)–(d) display the 2D differential images after and before reacting of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for the specific binding between protein microarrays with 137 ng/mL anti-

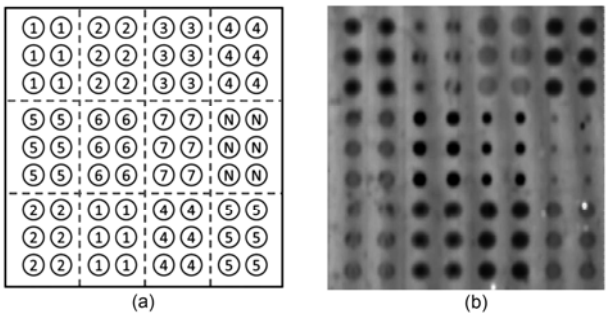

Figure 2 (a) Schematic of the protein microarray. 1: p53 0.15 mg/mL; 2: ZEB1 0.25 mg/mL; 3: Bcl2 0.2 mg/mL; 4: CDH13 0.3 mg/mL; 5: p16 0.15 mg/mL; 6: HBeAg 1.25 mg/mL; 7: HBs 2.75 mg/mL; N: BSA 1 mg/mL. (b) 2D scanning image of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for one microarray after washed (before reaction).

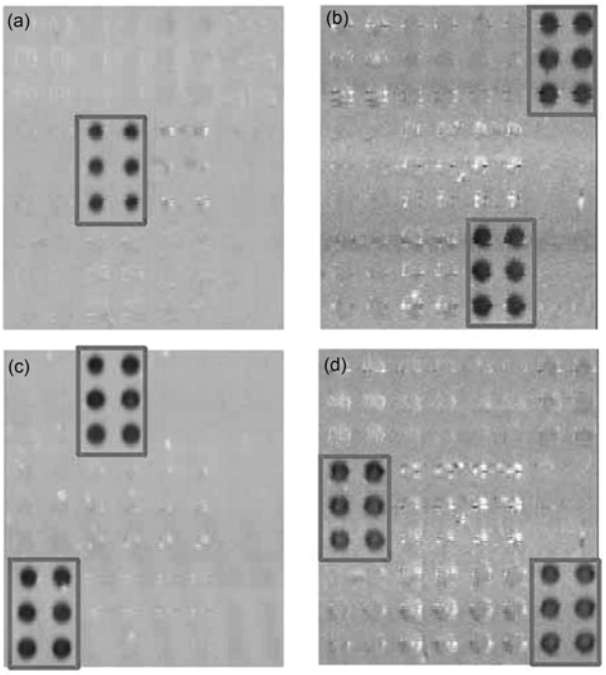

Figure 3 2D differential images after and before reacting of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for the specific binding between protein microarrays with (a) 137 ng/mL anti-ZEB1, (b) 335 ng/mL anti-CDH13, (c) 365 ng/mL anti-p16 and (d) 650 ng/mL anti-Hbe.

ZEB1, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16 and 650 ng/mL anti-HBe, respectively. The experimental results show good specificity of the protein microarrays, with only the corresponding antigen-antibody having specific binding. From Figures 3(b)–(d), one can also see that the repeatabilities for the same antigen-antibody, in different groups, are consistent in the same microarray.

Figure 4 shows the 2D differential image after and before reacting of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for the specific binding between the protein microarray with the mixed solution of 137 ng/mL anti-ZEB1, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16, and 650 ng/mL anti-HBe. It shows that the specific binding and repeatability can reproduced, even though the reaction was in the mixed solution of four antibodies.

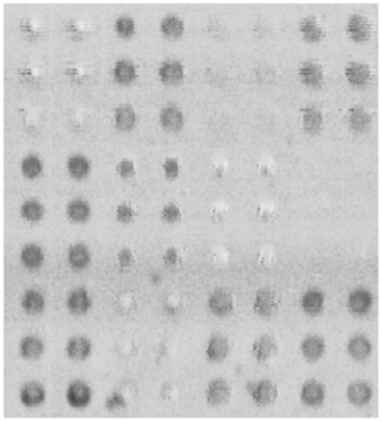

Figure 4 2D differential image after and before reacting of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ for the specific binding between the protein microarray with the mixed solution of 137 ng/mL anti-ZEB1, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16, and 650 ng/mL anti-HBe.

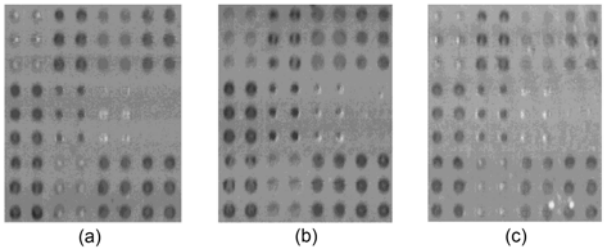

Figure 5 The reproducibility of microarray-microarray by OIRD detection using the same protein microarrays and the same mixed solution of the seven antibodies. (a)–(c) Differential images for the three identical microarrays.

Figures 5(a)–(c) are the 2D differential images of OIRD $\mathrm{Re}\{\Delta_p-\Delta_s\}$ after and before reacting for the specific binding of three protein microarrays with the mixed solution of the seven antibodies, respectively. The BSA spots are barely visible, meaning that BSA can not bind with the seven antibodies. From Figures 5(a)–(c), we can see that the gray levels corresponding to the same antigen-antibody groups are quite consistent in the three microarrays, indicating that the repeatability of microarray-microarray also is very well.

4 Conclusion

We tested the repeatabilities of OIRD label-free detection using protein microarrays. The experimental results show that the repeatabilities, including specificity and repeatability, are acceptable. This indicates that OIRD is a promising label-free detection technique in life sciences.

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