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# Parallel detection and quantitative analysis of specific binding of proteins by oblique-incidence reflectivity difference technique in label-free format

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In this work, we parallelly detected the specific binding between microarray targets including 12 different kinds of proteins and the probe solution containing five corresponding antibodies and quantitatively analyzed the interactions between CDH13 and solution phase anti-CDH13 at six different probe concentrations by oblique-incidence reflectivity difference (OIRD) method in label-free format. The detection sensitivity reached 10 ng/mL. The experimental results indicate that the OIRD method is a promising and competing technique not only in research work but also in clinic.

**oblique-incidence reflectivity difference (OIRD), protein microarray, label-free detection, quantitative analysis**

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## 1 Introduction

Biomolecular interactions have been widely studied with the label-based detection method [1–3]. However, it is still a big challenge to obtain label-free and high throughput detection method in life sciences because the labeling agents may change the innate property and structure of the biomolecules [4,5]. So the development of highly sensitive and reliable label-free detection techniques would bring about revolutionary impact on the molecular biology [6–8]. Oblique-incidence reflectivity difference (OIRD) method, with the characteristics of label-free and high-sensitivity, has been used to detect the biological microarrays [9,10]. In our previous work, we successfully detected the interactions of protein-protein [11–14], protein-DNA [15], DNA hybridization [10,16], as well as the dynamic processes of biomolecular interactions in the label-free format [17–19] by the OIRD method. In this paper, according to the technical need of parallel detection and quantitative analysis in the label-free format in clinical application such as disease diagnosis, we report the label-free, parallel detection and quantitative analysis of specific binding of proteins by the OIRD method.

## 2 Experiments

Figure 1 shows the schematic of the OIRD system used in the work. As mentioned in our previous work [11,18], the

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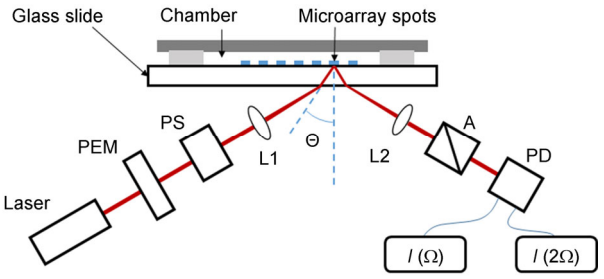


**Figure 1** (Color online) Schematic of OIRD system. The microarray attached with a fluid chamber was mounted on the two-dimensional scanning stage of the OIRD system. PEM: photoelastic modulator; PS: phase shifter; L1, L2: lens; A: polarization analyzer; PD: silicon photodiode.

OIRD technique measures the fractional difference between the reflectivities for the p- and s-polarized lights from a detected surface under the condition of oblique incidence, i.e., $\Delta_p-\Delta_s$. The imaginary and real parts of $\Delta_p-\Delta_s$, $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$, are determined by the first and second harmonics of the light intensity at the modulation frequency and measured by a silicon photodiode and two digital lock-in amplifiers respectively. We are able to obtain the OIRD signals caused by the difference or change in the optical dielectric constant and the equivalent thickness of the detected surface layer.

In this paper, we tested the capability of the OIRD method for the parallel and quantitative detection of specific binding of protein microarrays. We chose self-control polymer brush functionalized slides as the substrates due to their outstanding characteristics of high protein loading capacity and low nonspecific binding. The preparation of polymer brush substrate followed the processes reported in ref. [20].

At first, we label-freely and parallelly measured the specific binding between different proteins pairs in a single experiment. We selected 12 different kinds of proteins: p53, Toxoplasma, Zinc finger E-box-binding homeobox 1 (ZEB1), B-cell lymphoma 2 (Bcl2), H-cadherin (CDH13), cyclin-dependent kinase inhibitor 2A (p16), human immunodeficiency virus antigen (HIVAg), hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B surface antibody (HBsAb) and hepatitis B e antibody (HBeAb) as the targets and bovine serum albumin (BSA) as negative control to fabricate microarray I as shown in Figure 2(a) (Toxoplasma and HIVAg were purchased from Meridian Life Science, Inc.; ZEB1, p53, Bcl2, CDH13 and p16 were purchased from Proteintech Group, Inc.; HBeAg, HBeAb, HBsAg and HBsAb were purchased from Yemin Biotech). All these targets were dissolved in the solution of 1×Phosphate Buffered Saline (PBS) with 15% glycerol and printed on the polymer brush functionalized slide by use of a microarray spotter (Personal Arrayer16, CapitalBio Corp.). There were six duplicated spots for each sample. The separation of the adjacent spots was 500 μm and the diameter of the printed spot was around 100 μm. In fact, the spot diameter was different due to different surface intension of the protein solution. Microarray I was dried overnight so that the protein samples could covalently bind to the surface of polymer brush slide and followed by intensive washing. After that, microarray I attached with a fluid chamber was mounted on the two-dimensional (2D) scanning stage of the OIRD system. Before reaction, a 1×PBS solution with 1 mg/mL BSA and 10% serum in it (solution 1) was added into the chamber. The scanning program of the OIRD system was carried out and the OIRD images of Im{Δp−Δs} and Re{Δp−Δs} signal intensities were taken by 2D scanning. Then the solution was changed by the mixture of five antibodies including 137 ng/mL anti-ZEB1, 5 μg/mL anti-Bcl2, 335 ng/mL anti-CDH13, 365 ng/mL anti-p16 (Proteintech Group, Inc.) and 650 ng/mL HBe antibody (Yemin Biotech) dissolved in 1×PBS solution with 10% serum. After one hour's reaction the solution was changed back with solution 1 and the OIRD 2D images after reaction were taken again.

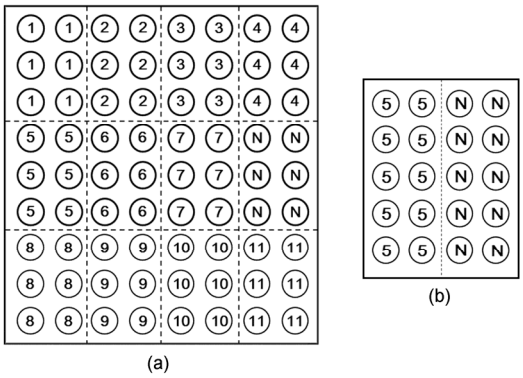


**Figure 2** Schematic of microarrays I (a) and II (b). 1: p53, 75 μg/mL; 2: Toxoplasma, 1 mg/mL; 3: ZEB1, 125 μg/mL; 4: Bcl2, 100 μg/mL; 5: CDH13, 150 μg/mL; 6: p16, 75 μg/mL; 7: HIV antigen, 1 mg/mL; 8: HBsAg, 625 μg/mL; 9: HBeAg, 1.38 mg/mL; 10: HBsAb, 625 μg/mL; 11: HBeAb, 1.38 mg/mL; N: BSA, 1 mg/mL.

As shown in Figure 2(b), we selected 150 μg/mL CDH13 as the target, 1 mg/mL BSA as negative control and six replicas of microarray II were fabricated. There are 10 duplicated CDH13 spots and 10 duplicated BSA spots in each replica of microarray II. The preparation and detection procedures of microarray II are similar to those of microarray I. In order to quantitatively detect the specific binding of proteins, anti-CDH13 solutions at six different concentrations of 10 ng/mL, 50 ng/mL, 100 ng/mL, 0.5 μg/mL, 1 μg/mL and 5 μg/mL in 1×PBS were selected as the probe solutions. We obtained the 2D images of the six replica of microarray II before and after the reactions with six different concentrations of anti-CDH13 solution by OIRD, respectively.

## 3 Results and discussion

Figure 3(a) shows the OIRD 2D image of $\mathrm{Re}\{\Delta_p-\Delta_s\}$ signal

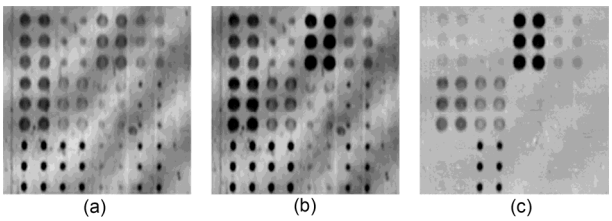


**Figure 3** 2D images of OIRD Re{Δp–Δs} for the specific binding between microarray I with the mixture of anti-ZEB1, anti-Bcl2, anti-CDH13, anti-p16 and HBe antibody. (a) Before reaction; (b) after reaction; (c) differential image by subtracting (a) from (b).

intensity for microarray I before reaction. All the sample spots are distinct, which proves that all the samples have been successfully bound to the substrate. This is an advantage of OIRD against labeling technique because the labeling detection is indirect and couldn't directly acquire the information about printing, such as the efficiency of binding. Figure 3(b) is the 2D image after reaction of microarray I. Figure 3(c) is the differential image by subtracting Figure 3(a) from (b). It is clear that Figure 3(c) shows the reaction result of microarray I with the mixture of five antibodies. As we expect, the antibodies, anti-ZEB1, anti-Bcl2, anti-CDH13, anti-p16 and HBe antibody have specifically bound to ZEB1, Bcl2, CDH13, p16 and HBeAg, respectively. The gray scales in the images are different, indicating the different optical dielectric constant and the equivalent thickness for the different protein samples. The experimental results demonstrate that OIRD can label-freely detect the specific binding of different proteins in the parallel format.

Figures 4(a) and (b) display the 2D images of microarray II before and after the reactions with 5 μg/mL anti-CDH13 solution, respectively. Figure 4(c) is the differential image by subtracting Figure 4(a) from (b). From Figure 4(c), we can see that anti-CDH13 only reacts with CDH13, not with BSA. The results are similar for the rest five replicas of microarray II. So, we only present the 2D differential images for the specific binding between CDH13 and anti-CDH13 at six different probe concentrations of 10 ng/mL, 50 ng/mL, 100 ng/mL, 0.5 μg/mL, 1 μg/mL and 5 μg/mL in Figures 5(a)–(f), respectively. From Figures 5(a)–(f), one can see that the gray level of the CDH13 sample spots increased with the increase of anti-CDH13 concentration. The higher

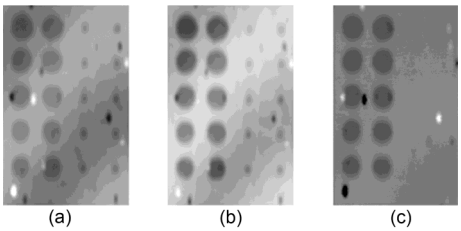


**Figure 4** 2D images of OIRD Re{Δp–Δs} for the specific binding between microarray II with 5 μg/mL anti-CDH13. (a) Before reaction; (b) after reaction; (c) differential image by subtracting (a) from (b).

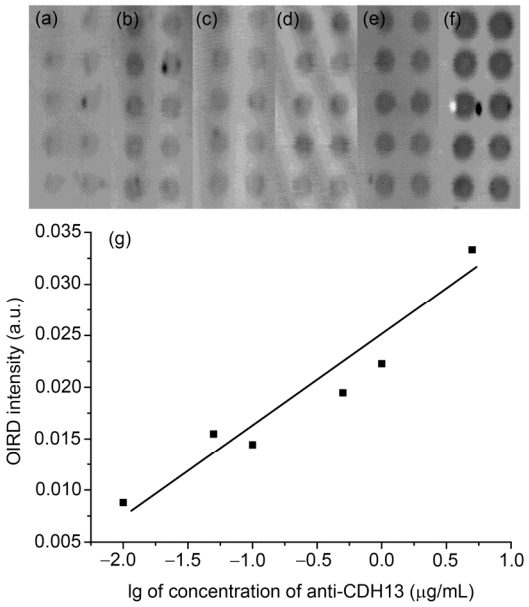


**Figure 5** 2D images of OIRD Re{Δp–Δs} for the specific binding between CDH13 and different concentration anti-CDH13. (a) 10 ng/mL anti-CDH13; (b) 50 ng/mL anti-CDH13; (c) 100 ng/mL anti-CDH13; (d) 0.5 μg/mL anti-CDH13; (e) 1 μg/mL anti-CDH13; (f) 5 μg/mL anti-CDH13; (g) OIRD signal intensity versus lg of anti-CDH13 concentration.

the anti-CDH13 concentration is, the darker the CDH13 sample spots are. The detection sensitivity is 10 ng/mL. Figure 5(g) shows the signal intensity of OIRD Re{Δp–Δs} versus the logarithm of concentration of anti-CDH13, and the results exhibit good linearity. The experimental results indicate that OIRD can label-freely and quantitatively detect the specific binding of proteins.

## 4 Conclusion

We successfully applied the OIRD method to detect the reactions between microarray targets and the five corresponding antibodies in parallel and label-free format. Also the specific bindings between CDH13 and anti-CDH13 at six different probe concentrations are quantitatively analyzed by OIRD. The experimental results reveal a good prospect of OIRD in the clinical applications such as disease diagnosis. The study of practical application of OIRD, using the clinical samples, is planned.

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