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Detection of hybridization of protein microarrays using an oblique-incidence reflectivity difference method

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Mouse-Immunoglobulin G (mouse-IgG) with different concentrations in a range from 1000 to 0.0128 μg/mL and a specific hybridization with goat anti-mouse IgG were detected successfully by using an oblique-incidence reflectivity difference (OI-RD) method. Two detection signals, consisting of an imaginary part ($\mathrm{Im}\{\Delta_p-\Delta_s\}$) and a real part ($\mathrm{Re}\{\Delta_p-\Delta_s\}$) of OI-RD, were obtained simultaneously. The detection results of hybridization by OI-RD were in accord with that of traditional fluorescent scans. In particular, we label-freely detected the washed mouse-IgG microarray with a series of concentrations and acquired a linear correlation between OI-RD intensities and the protein concentrations in logarithmic coordinates. The detection sensitivity of OI-RD can reach 14 fg. These experimental results suggest that the OI-RD method has potential applications in proteomics and clinical diagnosis.

protein microarrays, oblique-incidence reflectivity difference (OI-RD), label-free detection

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1 Introduction

Protein microarray technologies, allowing the simultaneous analysis of multiple biochemical binding processes within a single experiment, provide the effective means to investigate the full spectrum of protein attributes in a highly parallel, miniaturized, and automated fashion such as protein expression profiling and protein-protein interactions [1–3]. The high-throughput identification, assay and qualifications of biomolecules from protein samples have propelled the rapid development of detection methods. In most cases, fluorescence-labeling is widely employed for biochemical reactions in microarray format due to its inherent high sensitivity and large dynamic range [4]. However, the extrinsic fluorescent tag may unpredictably affect the biological activity of the compounds under investigation. In addition, the influence of labeling efficiency is often not known a priori. In order to circumvent these obstacles in label-based detection, there is a tendency to develop label-free schemes for the detections of microarrays in a high-throughput fashion.

The Oblique-Incidence Reflectivity Difference (OI-RD) method can be used to detect biological microarrays due to its high sensitivity and label-free detection. OI-RD technique was previously first developed in our group to monitor the growing process of oxide thin films in real time [5,6]. In principle, the OI-RD method measures the fractional difference between the reflectivity of p- and s-polarized light at oblique incidence, termed as "$\Delta_p-\Delta_s$," and can acquire two signals consisting of an imaginary part $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and a real part $\mathrm{Re}\{\Delta_p-\Delta_s\}$ simultaneously. The intensities of

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$\{\Delta_p-\Delta_s\}$ depend on the optical dielectric constant and roughness of the associated surface. Similar to the detection of oxide films, OI-RD can be applied to detect the protein microarrays in a label-free fashion because the various proteins or proteins with different concentrations could lead to changes in the optical dielectric constant and the surface roughness. Zhu et al. reported the detections of 60-base oligonucleotides [7] and protein microarrays [8] by an $\mathrm{Im}\{\Delta_p-\Delta_s\}$ signal. We have successfully detected 21-base oligonucleotides [9] and printed protein microarrays [10]. In the present paper, we will report the detections of the mouse-IgG microarray with different concentrations and the hybridizations between the antibody and antigen by the $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ simultaneously.

2 Experiment

A typical OI-RD setup for the detection of protein microarrays is shown in Figure 1. Similar to that employed for monitoring the oxide film growth [5], a p-polarized He-Ne laser beam with wavelength of λ=632.8 nm passes through a photoelastic modulator that induces the laser beam to oscillate between p- and s-polarization at a frequency of Ω=50 kHz. A phase difference between p- and s-polarized components is introduced by a phase shifter. Then the light beam is focused on the microarray surface at an incidence angle of 60°, which is near the Brewster angle for maximizing signal intensity [11]. The reflection beam passes through a polarization analyzer and is detected by a Silicon-photodiode. Afterward, $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ are measured by two digital lock-in amplifiers. At the beginning, in order to enhance the signal-to-noise ratio, the laser beam is focused on the bare slide surface of the protein microarray so that $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ are zeroed by adjusting the phase shifter and the analyzer respectively. Then the intensities of both $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ are recorded when the microarray is scanned in two-dimensions controlled by a computer-assisted stepping motor.

In this study, the aldehyde-coated glass slides are chosen as the microarray upholders. Mouse-IgG is selected as the target and Cy5-labeled goat anti-mouse IgG as the probe. Following the conventional procedure for the fabrication of protein microarrays [12], the mouse-IgG protein microarrays are printed, washed, blocked and hybridized with anti-mouse IgG in sequence. As shown in Figure 2, mouse-IgG is separately printed in triplicates with a concentration decreasing from top to bottom in a range from 1000 to 0.0128 μg/mL by a factor of 0.2 respectively. Each row contains three spots of mouse-IgG with the same concentration. The center to center separation between sample spots with an average diameter of 135 μm is around 500 μm. As shown in Figure 3, the mouse-IgG spots with 4 mg/mL are covalently coupled to the glass slide in duplicate. In order to compare with fluorescence detection, Cy5-labeled goat anti-mouse IgG, diluted with 1% glycine/1×PBS ranging from 1000 to 0.32 μg/mL, is hybridized with mouse-IgG at room temperature for 1 hour in a humidity chamber after blocking. The washed mouse-IgG microarray is detected by the OI-RD method. In addition, the hybridized microarray is detected using both OI-RD and fluorescence respectively.

3 Results and discussion

Figure 2 shows the detection results of OI-RD for the washed mouse-IgG microarray. The two-dimensional (2D) gray level-images of $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ are displayed on the right side, and the $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ intensities versus protein concentrations are plotted on the other side. The data in the curves are obtained from the 2D images by averaging the mean intensities from the three spots in each row with the same concentration for both $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ respectively. It can be observed that the gray levels for OI-RD signals attenuate gradually with the decrease of mouse-IgG concentrations. Meanwhile, the spatially averaged $\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $\mathrm{Re}\{\Delta_p-\Delta_s\}$ intensities appear linear to the logarithms of mouse-IgG concentration from 0.0128 μg/mL to 1000 μg/mL. In addition, the corresponding mass value of mouse-IgG immobilized on the

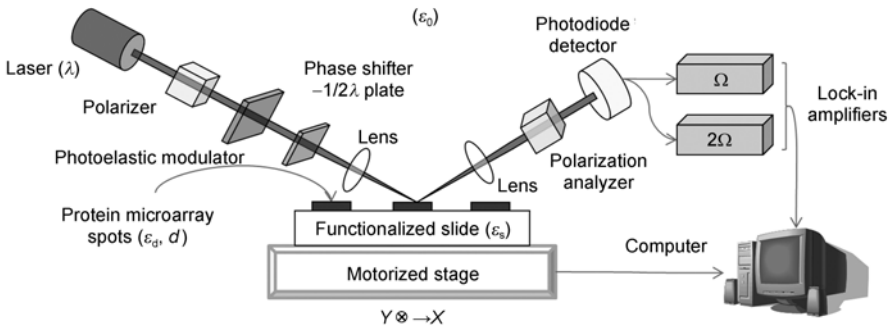

Figure 1 The layout of the OI-RD system for the detection of protein microarrays. Laser: a 7 mW He-Ne polarized laser. The microarray is mounted on a motorized stage that can be driven along the *X* and *Y* directions, respectively.

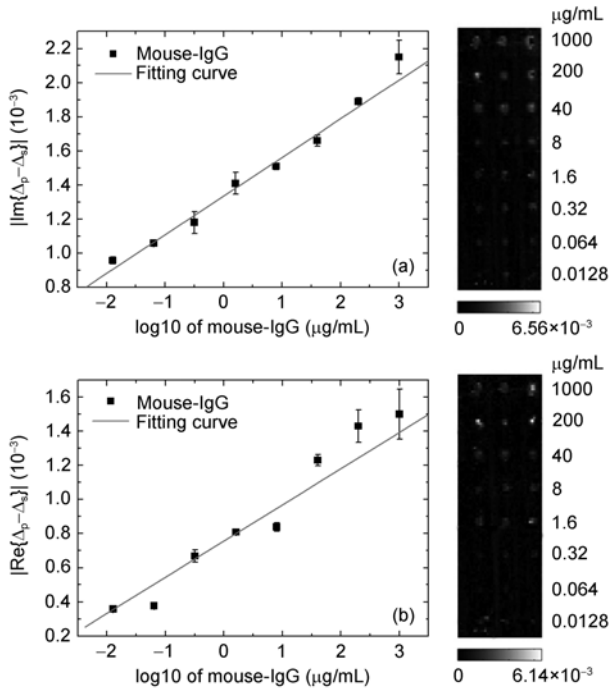

Figure 2 The results of the OI-RD two-dimensional (2D) scan for the washed mouse-IgG microarray. (a) The concentration dependence of Im{Δ_p–Δ_s} intensities. (b) The concentration dependence of Re{Δ_p–Δ_s} intensities.

slide are in a range from 14 fg to 1.1 ng because the volume of a solution droplet printed to each spot is around 1.1 nL. The dynamic range is approximately five orders of magnitude. In other words, the mouse-IgG spots printed with concentrations as low as 0.0128 μg/mL, which is equal to 14 fg, can be measured by Im{Δ_p–Δ_s} and Re{Δ_p–Δ_s}, respectively.

The experimental results demonstrate that the intensities of Δ_p–Δ_s can not only reflect the binding efficiency between the primary amino linkers on IgG and the aldehyde groups on the slide, but also distinguish the difference in concentration for mouse-IgG in a label-free fashion. Therefore, the OI-RD technique is much more advantageous than the fluorescence method in the aspect of label-free detection for different protein concentrations.

Figure 3 displays the detection results after hybridization between mouse-IgG and Cy5-labeled anti-mouse IgG for both OI-RD and a fluorescence scan. As shown on the right side of Figure 3, Figures 3(a) and (b) are the 2D images of Im{Δ_p–Δ_s} and Re{Δ_p–Δ_s} respectively, and Figure 3(c) is the 2D fluorescent scan for the same protein microarray. The curves on the left side of Figure 3 plot the concentration dependence of Im{Δ_p–Δ_s}, Re{Δ_p–Δ_s} and fluorescent intensity, respectively. Similar to the treatment in Figure 2, the data in the plots are obtained by spatially averaging the mean gray levels of the duplicate spots in each row with the same concentration for Im{Δ_p–Δ_s}, Re{Δ_p–Δ_s} and fluorescence respectively. It can be concluded from Figure 3(c)

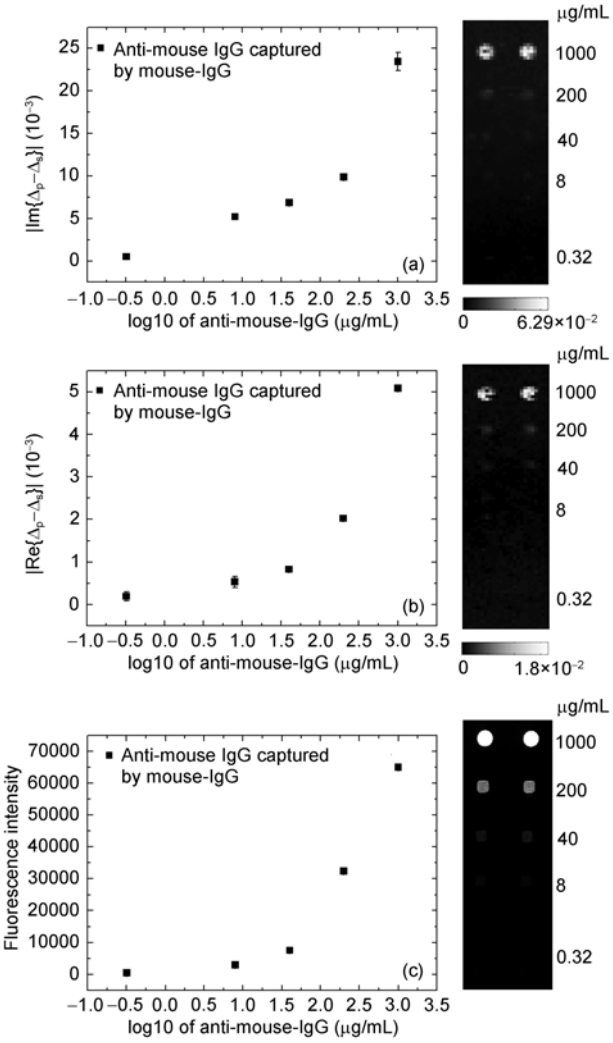

Figure 3 The results of the 2D scan of the OI-RD method and fluorescence scan after hybridization between the mouse-IgG and Cy5-labeled anti-mouse IgG with a set of concentrations. (a) The concentration dependence of Im{Δ_p–Δ_s} intensities. (b) The concentration dependence of Re{Δ_p–Δ_s} intensities. (c) The concentration dependence of the fluorescent intensities.

that the anti-mouse IgG has indeed been captured by their cognate targets. Comparing Figures 3(a) and (b) with (c), the results of Im{Δ_p–Δ_s} and Re{Δ_p–Δ_s} are consistent with that of fluorescence, indicating that the OI-RD method can detect the hybridization between an antibody and antigen. In addition, it is possible to generate an Re{Δ_p–Δ_s} signal from the absorption of Cy5 labeled in an anti goat mouse-IgG since the detection laser with 632.8 nm lies in the absorption band of Cy5, which will be studied in future.

4 Conclusions

The protein microarrays of mouse-IgG with different concentrations are detected successfully by Im{Δ_p–Δ_s} and Re{Δ_p–Δ_s} simultaneously. The experimental results dem-

onstrate that the OI-RD method is able to distinguish the difference in protein concentrations in a label-free fashion and detect the specific hybridization between the antigens and antibodies. In particular, we should notice that the OI-RD method not only employs a label-free format with high sensitivity but also does not require special holders for biomolecular microarrays. It is of great value for the high-throughput detection of protein microarrays based on the OI-RD method. Further investigations on the detection sensitivity and high-throughput assay are in progress.

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1 MacBeath G, Schreiber S L. Printing proteins as microarrays for high-throughput function determination. Science, 2000, 289: 1760–1763
2 Habb B B, Dunham M J, Brown P O. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol, 2001, 2: 1–13
3 Wilson D S, Nock S. Functional protein microarrays. Curr Opin Chem Biol, 2002, 6: 81–85
4 Thomas K, Eckenrode S, Gu Y R, et al. Protein microarrays to detect protein-protein interactions using red and green fluorescent proteins. Anal Biochem, 2002, 306: 50–54
5 Zhu X D, Lu H B, Yang G Z, et al. Epitaxial growth of $SrTiO_3$ on $SrTiO_3$(001) using an oblique-incidence reflectance-difference technique. Phys Rev B, 1998, 57: 2514–2519
6 Wang X, Jin K J, Lu H B, et al. Movement of oxygen vacancies in oxide film during annealing observed by an optical reflectivity difference technique. J Appl Phys, 2007, 102: 053107-1–5
7 Landry J P, Zhu X D, Gregg J P. Label-free detection of microarrays of biomolecules by oblique-incidence reflectivity difference microscopy. Opt Lett, 2004, 29: 581–583
8 Fei Y Y, Landry J P, Sun Y S, et al. A novel high-throughput scanning microscope for label-free detection of protein and small-molecule chemical microarrays. Rev Sci Instrum, 2008, 79: 013708-1–7
9 Wang X, Yuan K, Lu H, et al. Detection of specific hybridization on oligonucleotide microarrays by label-free Oblique-Incidence Reflectivity Difference method. J Appl Phys, 2010, 107: 063109-1–4
10 Wen J, Lu H, Wang X, et al. Detection of the protein microarrays by oblique-incidence reflectivity difference technique. Sci China Phys Mech Astron, 2010, 53: 306–309
11 Landry J P, Gray J, O'Toole M K, et al. Incidence-angle dependence of optical reflectivity difference from an ultrathin film on solid surface. Opt Lett, 2006, 31: 531–533
12 Liang R Q, Tan C Y, Ruan K C. Colorimetric detection of protein microarrays based on nanogold probe coupled with silver enhancement. J Imminol Methods, 2004, 285: 157–163