Label-Free and High-Throughput Detection of Protein Microarrays by Oblique-Incidence Reflectivity Difference Method

2010 Chinese Phys. Lett. 27 107801

(http://iopscience.iop.org/0256-307X/27/10/107801)

CHIN. PHYS. LETT. Vol. 27, No. 10 (2010) 107801

Label-Free and High-Throughput Detection of Protein Microarrays by Oblique-Incidence Reflectivity Difference Method *

WANG Xu(王旭)[1,2], LU Heng(陆珩)[1], WEN Juan(温娟)[1], YUAN Kun(原昆)[1], LÜ Hui-Bin(吕惠宾)[1], JIN Kui-Juan(金奎娟)[1], ZHOU Yue-Liang(周岳亮)[1], YANG Guo-Zhen(杨国桢)[1,**]

[1]*Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190*

[2]*School of Science, Jiangnan University, Wuxi 214122*

(Received 25 January 2010)

We label-free detected the biological process of preparing a microarray that includes 400 spots of mouse immunoglobulin G (IgG) as well as the specific hybridization between mouse IgG and goat anti-mouse IgG by an oblique-incidence reflectivity difference (OI-RD) method. The detection results after each process including printing, washing, blocking, and hybridization, demonstrate that the OI-RD method can trace the preparation process of a microarray and detect the specific hybridization between antigens and antibodies. OI-RD is a promising method for label-free and high-throughput detection of biological microarrays.

PACS: 78. 20. Ci, 42. 79. Ls DOI: 10.1088/0256-307X/27/10/107801

In the past twenty years, microarrays[1] have been wildly used in biological experiments such as gene expression, DNA replication, protein trafficking, and proteomics etc.[2,3] Because of their high spot intensities, microarrays enable thousands of biochemical reactions to be investigated in parallel in a high-throughput fashion. Fluorescence analysis is by far the method most often applied to microarray detection in a variety of schemes. Though the fluorescence method is generally more sensitive than absorption measurement, the labeling procedures of fluorescence may often cause some modifications in the biological activity of the samples.[4] In addition, the whole procedures including labeling and detecting are very time-consuming and costly. Thus it is necessary to develop the label-free and high-throughput detection techniques in the field of life sciences. So far, only a few experimental results of high-throughput and label-free detection of microarrays have been reported, such as surface plasmon resonance (SPR),[5,6] reflection interference[7] and ellipsometry.[8] Oblique-incidence reflectivity difference (OI-RD) method as a label-free and high-sensitivity detection method has also been used to detect the biological microarrays.[9] In contrast to the SPR method that needs high-quality gold film coated on the substrate surface, OI-RD does not require a special substrate. Recently, we reported the label-free detections of protein and oligonucleotide microarrays using the OI-RD method.[10] However, it is still a big challenge to obtain a label-free and high-throughput detection method for biological microarrays. In this study, we detected the biological process of the preparation and hybridization with a mouse immunoglobulin G (IgG) microarray, including four steps: printing, washing, blocking and hybridization. The experimental results demonstrate that OI-RD is a feasible method to label-free detect microarrays in a high-throughput fashion.

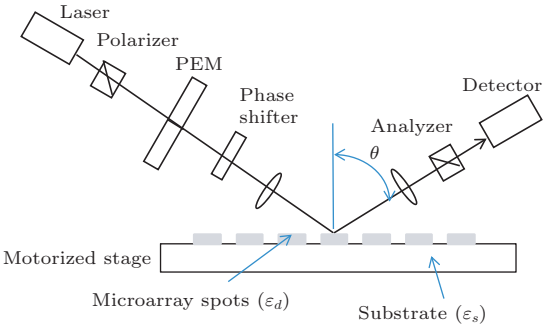

Fig. 1. Sketch of the OI-RD system for the detection of protein microarrays.

The experimental setup of OI-RD is shown in Fig. 1. As mentioned in our previous work,[10] a probe beam from a He-Ne laser at 632.8 nm is initially p polarized. The probe laser beam first passes through a photoelastic modulator (PEM) that induces the resultant laser beam to oscillate between p- and s-polarization with the modulated frequency $\Omega = 50$ kHz. An adjustable phase difference between p- and s- polarized components can be introduced by rotating a half-wave plate. Then the laser is focused on the surface of microarray at an incident angle of 60°. The microarray is lying on a two-dimensional motorized stage. The reflected beam passes through a polarization analyzer and the intensity is detected with a pho-

*Supported by the National Basic Research Program of China under Grant No 2007CB935701.

**To whom correspondence should be addressed. Email: yanggz@aphy.iphy.ac.cn

todiode. For different surface, at oblique incidence, the surface reflectivity of p- and s-polarized light is different as the thickness and/or complex dielectric constant of the surface layer is different. Briefly, let r_{p0} and r_{s0} denote the respective reflectivity from the bare functionalized glass slide surface without biomolecules for p- and s-polarized light, and r_p and r_s be the respective reflectivity from biomolecules on glass slide surface. The changes of reflectivity are defined as $\Delta_p = |(r_p - r_{p0})/r_{p0}|$ and $\Delta_s = |(r_s - r_{s0})/r_{s0}|$. The difference of fractional reflectivity change is $\Delta_p - \Delta_s$. In the experiment, we measure the changes of the imaginary part $\mathrm{Im}\{\Delta_p - \Delta_s\}$. The entire OI-RD detection system is mounted on an optical table and covered by a black box to minimize the effects of stray light, dust, and airflow. The detecting experiments were all performed in an ultraclean room.

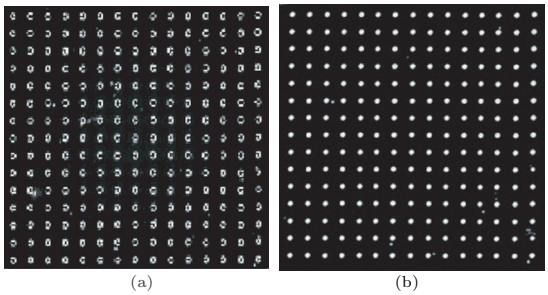

Fig. 2. Scan images of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ and fluorescence for a mouse IgG protein microarray with 15×15 spots after printing. The left one is $\mathrm{Im}\{\Delta_p - \Delta_s\}$ image, and the right one is fluorescence image.

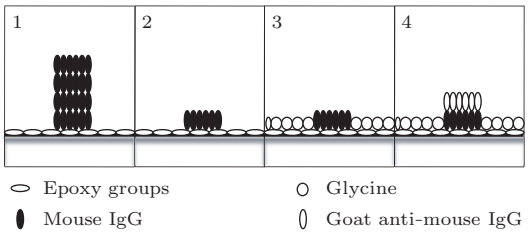

Fig. 3. Sketch map of the biological process for the hybridization of a protein microarray that includes (1) printing, (2) washing, (3) blocking and (4) hybridization.

First, we used OI-RD and fluorescence methods to detect a same labeled protein microarray and compared the experimental results. The sample printed on this microarray was mouse IgG with fluorescence tags mixed in a 1× phosphate buffered saline (PBS) solution with concentration of 1 mg/ml by a contact-printing robot. There are 15×15 spots with a diameter of about 100 μm in the microarray. The space between mouse IgG sports is 300 μm. The left and right images in Fig. 2 show the detected results of OI-RD and fluorescence scanner (LuxScan 10 K, CapitalBio Corporation, China), respectively. From Fig. 2, one can see that the result of OI-RD is consistent with the fluorescence scan. However, the mouse IgG sports in OI-RD image shows a doughnut-like profile. There might be two reasons to make the IgG sports different in OI-RD image: (a) the sample sports just printed in the microarray include mouse IgG and PBS solution, i.e. the sample sports is not a monolayer mouse IgG. Thus the sample sports would have different shapes due to the hydrophobicity of the mouse IgG solution and the effect from the solid printing pin. (b) As mentioned above, the OI-RD signal arises from the thickness and complex dielectric constant of the surface layer. The experimental result implies that OI-RD image can present more information than that of the fluorescence scan.

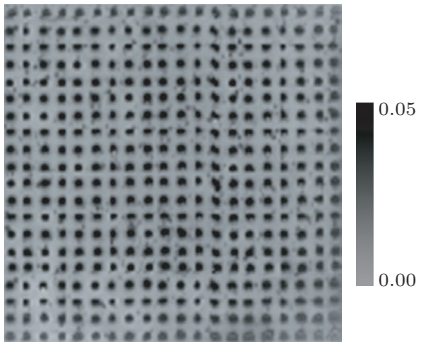

Fig. 4. $\mathrm{Im}\{\Delta_p - \Delta_s\}$ image of a 400-spot mouse IgG protein microarray after printing.

The experiments are focused on the label-free, and high-throughput detection of the biological process including the preparation and hybridization of the protein microarrays by OI-RD. Epoxy functionalized glass slides (CEL Associates) were chosen as the substrates, which means that the surface of the slide was all covered by epoxy groups initially. As shown in Fig. 3, the ordinary biological process of a protein microarray includes four steps: printing, washing, blocking and hybridization.[11] For the printing, unlabeled mouse IgG proteins mixed in a 1× PBS solution with concentration of 5 mg/ml were printed on the surface of the slides. In our case, the diameter of the protein spots is about 100 μm and the space between protein sports is 300 μm. The IgG was covalently bound to the epoxy groups on the surface as shown in Fig. 3 in step 1. For the washing, the excess of the printed proteins and solution were removed by washing in $\mathrm{ddH_2O}$ solution for 10 min, leaving only monolayer mouse IgG on the surface of the slide as shown in Fig. 3 in step 2. For the blocking, the rest of the slide surface was blocked by immersing in a 1% solution of IgG-free glycine in 1× PBS for 1 h. This step ensured that the surface of without mouse IgG sports was covered with glycine except step 3 as shown in Fig. 3. Hybridization: reaction was performed in a mixture of 1× PBS and 20 μg/ml goat anti-mouse IgG at 25°C for 1 h. Only

the spots covered with mouse IgG would react with goat anti-mouse IgG as shown in Figs. 3 in step 4.

We detected the biological process of the label-free protein microarray by OI-RD. Figures 4–7 show the $\mathrm{Im}\{\Delta_p - \Delta_s\}$ scan images of the microarray after printing, washing, blocking, and hybridization, respectively. The experimental results from Figs. 4–7 show that the OI-RD method can label-free detect the microarray in every step during the protein microarray process. The scan time of 400-spot protein microarray took about 20 min with steps of 20 μm. The maximum intensities of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ signals are 5×10^{-2}, 5×10^{-3}, 3.5×10^{-3} and 4.5×10^{-3} after printing, washing, blocking, and hybridization, respectively.

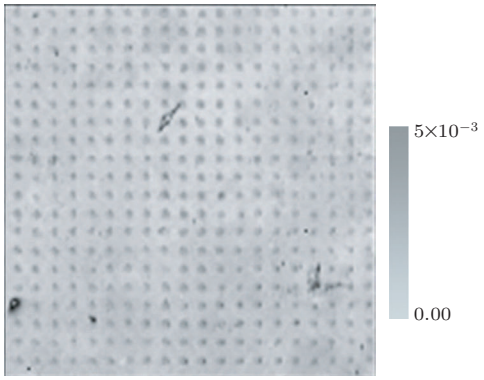

Fig. 5. $\mathrm{Im}\{\Delta_p - \Delta_s\}$ image of a 400-spot mouse IgG protein microarray after washing.

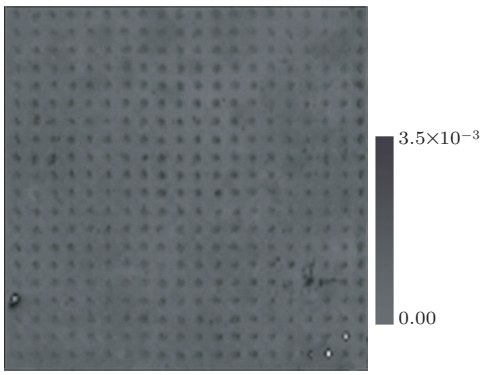

Fig. 6. $\mathrm{Im}\{\Delta_p - \Delta_s\}$ image of a 400-spot mouse IgG protein microarray after blocking.

The signal intensities in Figs. 4–7 change from step to step. Here we present a qualitative analysis about the $\mathrm{Im}\{\Delta_p - \Delta_s\}$ signals. From a classical three-layer model,[12] the optical response of OI-RD from a surface layer can be expressed as

$$\mathrm{Im}\{\Delta_p - \Delta_s\} = -\beta \frac{(\varepsilon_d - \varepsilon_s)(\varepsilon_d - \varepsilon_0)}{\varepsilon_d} d,$$
$$\beta = \frac{4\pi\varepsilon_s(\tan\theta_{\mathrm{inc}})^2 \cos\theta_{\mathrm{inc}}}{\varepsilon_0^{1/2}(\varepsilon_s - \varepsilon_0)(\varepsilon_s/\varepsilon_0 - (\tan\theta_{\mathrm{inc}})^2)\lambda}, \quad (1)$$

where θ_{inc} is the incidence angle; ε_0, ε_d and ε_s are the optical dielectric constants of the ambient, the biomolecular layer and the glass slide, respectively; d is now really its "effective thickness", i.e. average thickness; λ is the wavelength of incident laser.

When the mouse IgG was first bound on the surface of the slide, $\mathrm{Im}\{\Delta_p - \Delta_s\}$ obtained a maximum value as 5×10^{-2} because the sample sports are not a monolayer mouse IgG but includes excessive mouse IgG and PBS solution. After the washing step, the sample sports become a monolayer mouse IgG on the surface as shown in Figs. 3 step 2, which makes the $\mathrm{Im}\{\Delta_p - \Delta_s\}$ decrease to 5×10^{-3}. Then glycine was added to the surface area without mouse IgG. At this time, the background signals no longer came from epoxy groups on the surface but from glycine molecules on the surface. By subtracting the signals from glycine, the intensity of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ in Fig. 6 after blocking decreased to 3.5×10^{-3}. In other words, the glycine caused a negative signal of 1.5×10^{-3} in $\mathrm{Im}\{\Delta_p - \Delta_s\}$. Finally, after the hybridization, more biomolecules were bound on the surface of the sample spots, which make the increase of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ signal in Fig. 7 comparable to Fig. 6.

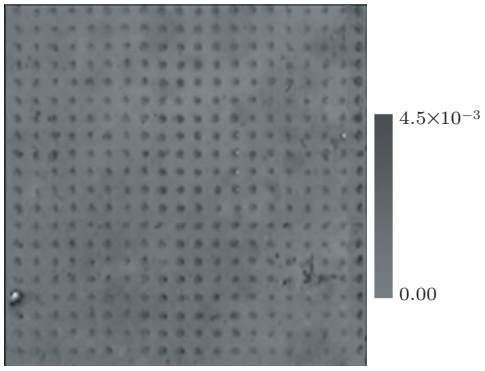

Fig. 7. $\mathrm{Im}\{\Delta_p - \Delta_s\}$ image of a 400-spot mouse IgG protein microarray after hybridization.

In conclusion, we have detected the biological process of a label-free protein microarray with 400 spots of mouse IgG in each step includes printing, washing, blocking, and hybridization, and obtained the $\mathrm{Im}\{\Delta_p - \Delta_s\}$ images after each step. The experimental results demonstrate that the OI-RD method can not only label-free detect the specific hybridization between the antigens and antibodies but also trace the preparation process of a microarray. It is noted that we only used a single photodiode in the present study. It will make the detection much faster if the single photodiode is replaced with a photodiode array. Further work is being planned.

References

[1] Schena M, Shalon D, Davis R W and Brown P O 1995 *Sci-*

ence **270** 467
[2] Schena M 1996 *Bioessays* **18** 427
[3] Ollila J, and Vihinen M 1998 *Biochem. Biology. Res. Commun.* **249** 475
[4] Zhu X D, Landry J P, Sun Y S, Gregg J P, Lam K S and Guo X W 2007 *Appl. Opt.* **46** 1890
[5] Thiel A J, Frutos A G, Jordan C E, Corn R M and Smith L M 1997 *Anal. Chem.* **69** 4948
[6] Nelson B P, Frutos A G, Brockman J M and Corn R M 1999 *Anal. Chem.* **71** 3928
[7] Piehler J, Brecht A and Gauglitz G 1996 *Anal. Chem.* **68** 139
[8] Wang Z H and Jin G 2003 *Anal. Chem.* **75** 6119
[9] Landry J P, Zhu X D and Gregg J P 2004 *Opt. Lett.* **29** 581
Landry J P, Gray, O"Toole M K and Zhu X D 2006 *Opt. Lett.* **31** 531
[10] Wang X, Yuan K, Lu H, Wen J, Lu H B, Jin K J, Zhou Y L, Yang G Z, Li W and Ruan K C 2010 *J. Appl. Phys.* **107** 063109
Lu H, Wen J, Wang X, Yuan K, Lu H B, Zhou Y L, Jin K J, Yang G Z, Li W and Ruan K C 2010 *Sci. Chin.-Phys. Mech. Astron.* **53** 1230
Yuan K, Wang X, Lu H, Wen J, Lu H B, Zhou Y L, Jin K J, Yang G Z, Li W and Ruan K C 2010 *Sci. Chin.-Phys. Mech. Astron.* **53** 1434
Wen J, Lu H, Wang X, Yuan K, Lu H B, Zhou Y L, Jin K J, Yang G Z, Li W and Yuan K C 2010 *Sci. Chin.-Phys. Mech. Astron.* **53** 306
Zhang H Y, Liang R Q, Jin K J, Lu H B, Zhu X D, Zhou Y L, Ruan K C and Yang G Z 2006 *Chin. Phys. Lett.* **23** 1032
[11] Li W and Ruan K C 2009 *Anal. Bioanal. Chem.* **394** 1117
[12] Zhu X D, Fei Y Y, Wang X, Lu H B and Yang G Z 2007 *Phys. Rev.* B **75** 245434