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Detection of protein microarrays by oblique-incidence reflectivity difference technique

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Biological microarrays with different proteins and different protein concentrations are detected without external labeling by an oblique-incidence reflectivity difference (OIRD) technique. The initial experiment results reveal that the intensities of OIRD signals can distinguish the different proteins and concentrations of protein. The OIRD technique promises feasible applications to life sciences for label-free and high-throughput detection.

oblique-incidence reflectivity difference, protein microarray, label-free detection

1 Introduction

The investigation of biomolecule interactions is very important to life sciences. The microarray platform as an important technique, has been widely used in modern biological research. Microarrays by definition are micrometer-scale spots of immobilized biological molecules arranged in a regular pattern upon a solid substrate that allows the specific binding of genes or protein molecules. One microarray can contain hundreds of thousands of target spots that simultaneously react with corresponding probes in hybridization solution. Microarrays, much used in scientific research, are one of the most important complements to the numerous genome projects and high throughput assays of protein expression [1,2]. The high-throughput detection is a key step in the process of microarray analysis. Fluorescence analysis is by far the method most widely used in microarray detection. Since most proteins do not fluoresce in visible spectrum, the protein labeling with an extrinsic fluorescent dye is a necessary part of the detection method. In addition, the fluorescence signal in microarray needs to be identified by special instruments like laser confocal scanner. The whole detection procedures, very time-consuming and costly for one thing, often cause some damage or modifications for another to the structure and biological activity of proteins [3,4]. So it is necessary to develop the label-free and high-throughput detection techniques in the field of life sciences.

Oblique-incidence reflectivity difference (OIRD) method is just such a label-free and sensitive detection technique. We have applied the OIRD technique to real-time monitoring of the oxide thin films growth and observed OIRD oscillation signals corresponding with the epitaxial growth of molecular layer [5–8]. The study has shown that the difference in relative reflectivity change $\Delta R/R$ between *s*- and *p*-polarized light is about 1×10^{-5} or below and that this technique can be used in a real-time monitoring of thin film growth at the level of a single atomic layer. Recently, the OIRD technique has been used to detect the biological microarrays [9–11]. This paper reports the label-free detection of the protein microarrays by the OIRD technique.

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2 Results and discussion

Figure 1 shows the typical OIRD setup for the detection of protein microarrays. The initial *p*-polarized laser beam from a 7 mW linearly polarized HeNe laser with λ = 632.8 nm is modulated by a photoelastic modulator, which causes the beam to oscillate at a frequency of Ω = 50 kHz between *p* and *s* polarization. Then the beam passes through a phase shifter that introduces a variable phase Φ_0 between the *p*- and *s*-polarized components. The resultant beam is incident on the microarray surface at an oblique angle θ = 72°. The reflected beam passes through an analyzer, and is finally detected by a silicon photodiode whose photocurrent is detected by two lock-in amplifiers at the same time. The light intensity consists of harmonics of polarization-modulation frequency Ω, and the first and second harmonic amplitudes, $I(\Omega)$ and $I(2\Omega)$ are measured. Let $r_{p0} = |r_{p0}|\exp(i\Phi_{p0})$ and $r_{s0} = |r_{s0}|\exp(i\Phi_{s0})$ be the reflectivities for the *p*- and *s*-polarized light from the bare microarray substrate, respectively, and let $r_p=|r_p|\exp(i\Phi_p)$ and $r_s=|r_s|\exp(i\Phi_s)$ be the reflectivities for the *p*- and *s*-polarized light from the surface of proteins, respectively, and define $\Delta_p=(r_p-r_{p0})/r_{p0}$, $\Delta_s=(r_s-r_{s0})/r_{s0}$. The difference in reflectivity change is $\Delta_p-\Delta_s$. The given condition is $I(\Omega)\sim\mathrm{Im}\{\Delta_p-\Delta_s\}$ and $I(2\Omega)\sim\mathrm{Re}\{\Delta_p-\Delta_s\}$ [4]. The expression for "$\Delta_p-\Delta_s$" given in ref. [4] indicates that "$\Delta_p-\Delta_s$" depends on the incidence angle (θ) and the dielectric constants of the ambient (ε_0), protein (ε_d), and substrate of microarray (ε_s), respectively.

In our configuration, the protein microarray is mounted on a translation stage, which is driven by a computer-controlled stepping motor so that it can move in both *x* and *y* directions.

Two protein microarrays are fabricated with a spotting robot microsystem. As shown in Figure 2(a), Microarray A consists of a series of spots of human immunoglobulin-G (IgG) protein with a range of concentrations from 100 to 0.8 μg/mL. The IgG is covalently bound to the epoxy glass surface through primary amines from the exposed Lysine residues on the protein surface. The protein spots are basically circular with an average diameter of about 150 μm, and the center to center separation between spots is about 750 μm. Every column is printed with protein concentration decreasing from top to bottom, and each row contains triplicate spots. In Figure 2(b), Microarray B is composed of ubiquitin protein ligase E3A (UBE3A), bovine serum albumin (BSA), and homo sapiens platelet-derived growth factor receptor alpha polypeptide (PDGFRA). These proteins are covalently bound to the aldehyde surface through a Schiff base link established through nucleophilic attack of the surface aldehyde groups by the primary amines of Ly-

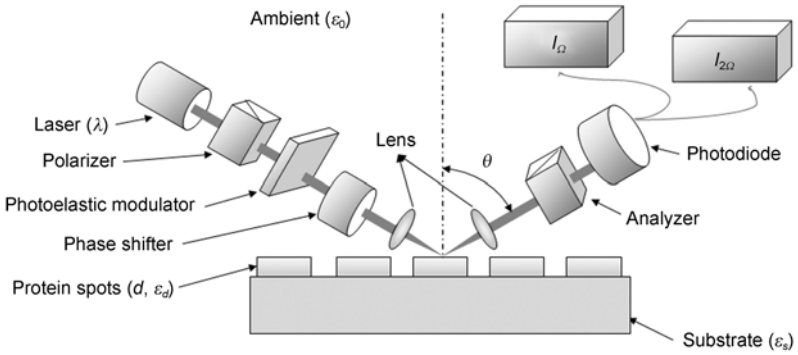

Figure 1 OIRD system for the detection of the protein microarray. The protein microarray is mounted on a translation stage that is movable along both the *x* and *y* directions.

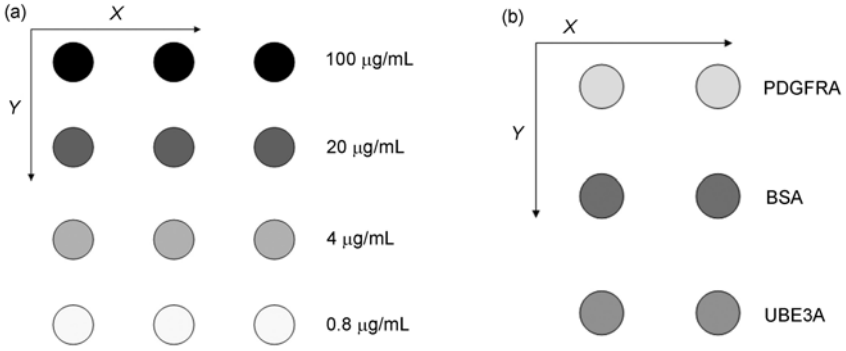

Figure 2 The arrangements of printed spots for (a) Microarray A and (b) Microarray B. In Microarray A, the IgG concentrations are indicated on the right side of the four rows, each of which contains triplicate spots. In Microarray B, each protein in a row is printed in duplicate spots.

sine residues on the surface of the protein molecules. In Microarray B, the diameter of the spots is about 400 μm, and the separation between neighboring spots is about 750 μm. Every row is marked with the same protein concentration 200 μg/mL. All the proteins in this study are dissolved in phosphate buffered saline (PBS, pH 7.4) with 40% glycerol. In Microarray B, each protein in a row is printed in duplicate spots.

Figures 3(a) and (b) show the respective three-dimension images of Im$\{\Delta_p-\Delta_s\}$ and Re$\{\Delta_p-\Delta_s\}$ signal intensities for a column IgG protein with different concentrations in Microarray A, acquired by the dual-axis mechanical scan. It can be seen that: (1) The intensities signals of Im$\{\Delta_p-\Delta_s\}$ and Re$\{\Delta_p-\Delta_s\}$ increase with the protein concentration from 0.8 to 100 μg/mL, indicating that the OIRD as a method can distinguish the different concentrations of IgG protein; (2) the protein spots with a concentration as low as 0.8 μg/mL can be detected by the OIRD technique. The actual amount contained in one spot for 0.8 μg/mL IgG is in pg order since the volume of every spot printed is only about 1 nL, again indicating that OIRD has a high detective sensitivity. The scan time used for Figures 3(a) and (b) is 5 min. Figures 3(c) and (d) show the typical curves of Re$\{\Delta_p-\Delta_s\}$ by single-axis mechanical scan along the *y* axis with the same sample as in Figure 3(b). From Figure 3(d), it can be seen that the intensity is linear to the logarithm of the human IgG concentration, consistent with the results in Figures 3(a) and (b). Note that the time needed to obtain the curve of Figures 3(c) or (d) is only 12 seconds. The experimental results demonstrate the possibility of high-through detection of biological microarrays by way of the OIRD technique of single-axis mechanical scan.

Figures 4(a) and (b) display the two-dimension and three-dimension images of Im$\{\Delta_p-\Delta_s\}$ signal intensities for PDGFRA, BSA and UBE3A proteins in Microarray B by the dual-axis mechanical scan, respectively. Relative to the background of microarray, PDGFRA and UBE3A are positive signs, while BSA is a negative sign. As mentioned earlier in the paper, the sign of OIRD, "$\Delta_p-\Delta_s$", depends on the incidence angle (θ) and the dielectric constants of the ambient (ε_0), protein (ε_d), and microarray substrate (ε_s). In this case, we can assume that the θ, ε_0, and ε_s are the same, and the different intensities of Im$\{\Delta_p-\Delta_s\}$ signals are ascribed to the differences of dielectric constants (ε_d) for PDGFRA, BSA, and UBE3A proteins. The intensities of Im$\{\Delta_p-\Delta_s\}$ signals are different for PDGFRA, BSA and UBE3A, indicating that the OIRD as a method can distinguish the different proteins.

3 Conclusion

In conclusion, we have detected the microarrays with different proteins and different protein concentrations by

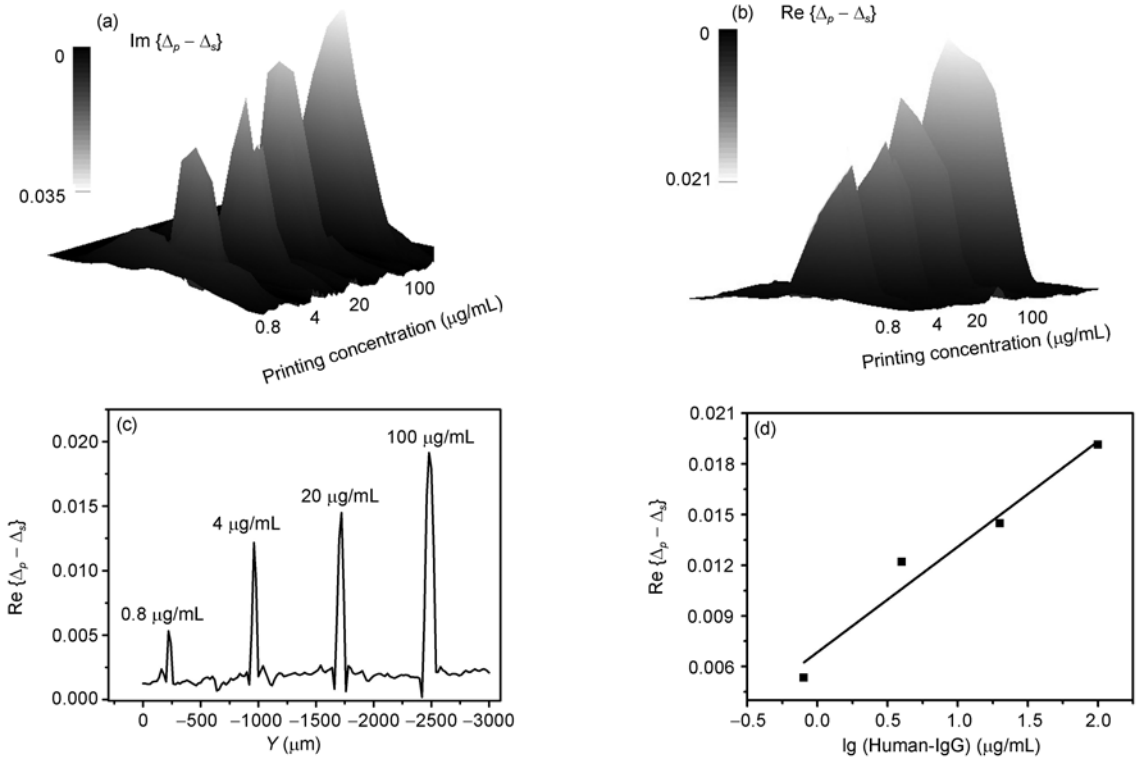

Figure 3 OIRD three-dimensional images of a column IgG protein with different concentrations in Microarray A. (a) Im$\{\Delta_p-\Delta_s\}$ signal intensity; (b) Re$\{\Delta_p-\Delta_s\}$ signal intensity; (c), (d) typical curves of the human IgG concentration dependence of the Re$\{\Delta_p-\Delta_s\}$ signal intensity by single-axis mechanical scan along the *y* axis with the same sample as in (b).

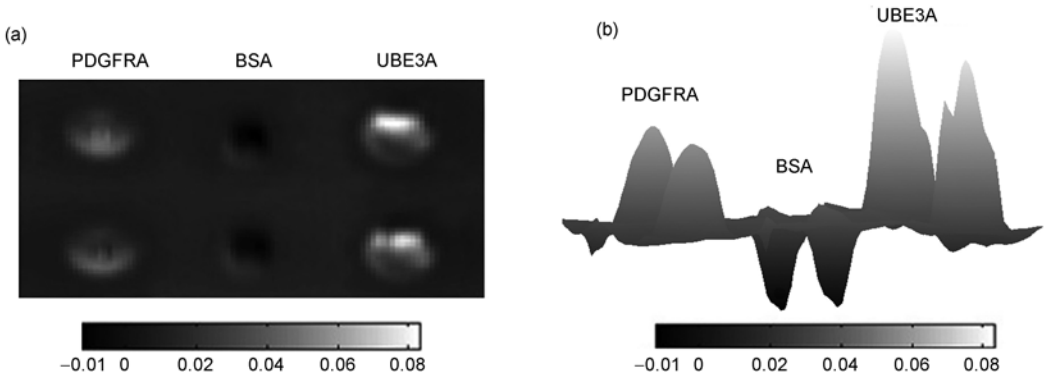

Figure 4 Images of Im{Δ_p–Δ_s} signal intensities of PDGFRA, BSA and UBE3A different proteins in Microarray B. (a) A two-dimensional image; (b) a three-dimensional image.

OIRD technique. The initial experimental results reveal that the OIRD technique can detect the different proteins and different protein concentrations without labeling. Further investigations, especially those on the label-free and high-throughput detections are well within our plan.

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