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Detection of microarray protein biomolecules by oblique-incidence reflectivity difference technique without labelling agents*

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This paper reports that the detection to the protein in microarray format is carried out by oblique-incidence reflectivity difference (OI–RD) analysis without any labelling agents. The OI–RD intensities not only depend on the protein structure, but also vary with the protein concentration. The results indicate that this method should have potential application in detection of biochemical processes. The high throughout and in situ detection can be achieved by this method with further improving of the experimental system.

Keywords: detection, OI–RD, protein, microarray
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1. Introduction

In life science, physics techniques especially optical techniques play an important role.[1−9] Recently fluorescence-labelling is a main method to detect a single macromolecule and the protein and DNA in microarray format. However, there are many shortcomings for fluorescence-labelling, such as the fluorescent molecule or a quantum dot always changes the properties of a host macromolecule and bring photo-bleaching, the labelling needs extra preparation steps and cost and the samples vary in labelling step or during subsequent reactions. All these are often not known a priori. Nowadays surface plasmon resonance microscopy,[7,8] mass spectrometry [9] and oblique-incidence reflectivity difference (OI–RD) analysis are the hot spots for label-free optical techniques to detect microarray molecule.[10,11] The sensitivity of surface plasmon resonance microscopy derives from the sharp resonance of the plasmon surface polariton, so that this method requires microarrays to be fabricated on functionalized gold films. Mass spectrometry requires that the bio-molecules be fabricated upon a special matrix medium for laser-induced desorption and ionization. By comparison, the OI–RD method requires only optically flat substrates and can be used to measure a large quantity of samples rapidly.

The OI–RD is a more sensitive form of ellipsometry. It is a polarization-modulated nulling ellipsometry in which the harmonics of modulated photocurrents are measured under suitable nulling condition and are directly proportional to $\Delta\psi$ and $\Delta\delta$. This makes the measurement of OI–RD signals at least one order of magnitude more sensitive than the normal ellipsometer. The sensitivity is similar to that of surface plasmon resonance (SPR).

2. OI–RD

As an in situ probe method, OI–RD has been successfully applied to detect the small changes in the optical properties of thin films and the microarrays of biological molecules on glass.[12−15] At oblique incidence the reflectivities of p- and s-polarized light change disproportionately in response to the thickness and/or complex dielectric constant of a film change. Let $r_{\rm p0}$

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and r_{s0} be the reflectivities on a bare surface for p- and s-polarized light, respectively, r_p and r_s the reflectivities on the surface covered with a thin film, and $\Delta p = (r_p - r_{p0})/r_{p0}$ and $\Delta s = (r_s - r_{s0})/r_{s0}$. The OI–RD technique enables direct measurements of the real [$\mathrm{Re}(\Delta p - \Delta s)$] and imaginary [$\mathrm{Im}(\Delta p - \Delta s)$] parts of $\Delta p - \Delta s$. In terms of the ellipsometric ratio $\rho = r_p/r_s = \tan\psi \exp(i\delta)$, it is easily seen that $\Delta p - \Delta s \approx (\rho - \rho_0)/\rho_0$, so that $\mathrm{Im}(\Delta p - \Delta s) \approx \delta - \delta_0$ and $\mathrm{Re}(\Delta p - \Delta s) = 2\csc\psi_0(\psi - \psi_0)$. The equation for $\mathrm{Im}(\Delta p - \Delta s)$ given in Refs.[10–12] indicates that it depends on the incidence angle (θ) and the optical dielectric constants ε_0, ε_d and ε_s of the ambient medium, film, and substrate, respectively. In this paper we only recorded $\mathrm{Im}(\Delta p - \Delta s)$ of the sample.

3. Application of OI–RD to detect bimolecule on microarray format

The layout of our OI–RD system is shown in Fig.1. A p-polarized He–Ne laser beam passes through a polarization modulator (modulation frequency $\Omega = 50\,\mathrm{kHz}$). The modulator causes the output beam to oscillate between p- and s-polarizations with elliptically polarized intermediate states. The polarization-modulated beam then passes through a half wave slide that introduces an adjustable phase ϕ_0 between the s- and p-polarized components. The resultant beam is focused to a $50\,\mu$m spot on the protein surface at an incidence angle $\theta = 70°$. After reflection and recollimation, the beam passes through a rotatable analyser. The intensity of the transmitted beam $I_R(t)$, which consists of various harmonics of the modulation frequency, is detected with a photodiode and Fourier analysed with digital lock-in amplifiers. We detect the first and second harmonic amplitudes, $I(\Omega)$ and $I(2\Omega)$. Initially the sample is mounted in a position where the incident beam reflects off the bare surface of the glass substrate. We adjust the analyser to zero $I(2\Omega)$ and then adjust the phase (with the $\lambda/2$) to zero $I(\Omega)$. The proportionality constants are measured separately so that $\Delta p - \Delta s$ is determined absolutely. In this paper we only monitor the behaviour of the intensity of $\mathrm{Im}(\Delta p - \Delta s)$, which changes according to the biomolecular characteristics. In this configuration, a microarray-covered glass slide is mounted on a XY-axis crossed-roller translation stage (KS202-70) underneath fixed illumination and detection optics. The stage is driven by computer-controlled stepping motors controller.

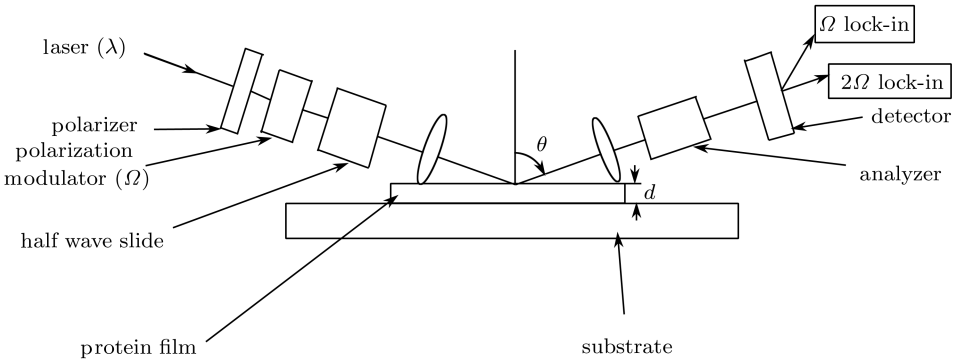

Fig.1. Optical layout of OI–RD system.

4. Results and discussion

In order to validate the potential of the OI–RD for the detection of biomolecule minded in Ref.[10], the experiment is done for the Mask sample and the microarrays samples respectively with the 10^{-4} background noise.

4.1. The mask biomolecular detection by OI–RD method

The experiment is done on three glass slides that are labelled A, B and C respectively, where A covers biotin-anti-IgG(immunoglobulin-G), B covers biotin-anti-IgG adding avidin and C covers biotin-anti-IgG

adding streptavidin. The sample is prepared as spots about 18 mm×18 mm in size on glass slides. The laser is incident at the centre of the spot in all measurements.

The detections are done on the samples area in the clips A, B and C at the same condition during a fixed period. The measurement is carried with the laser spot on the same position of the sample. The result is given in the Fig.2.

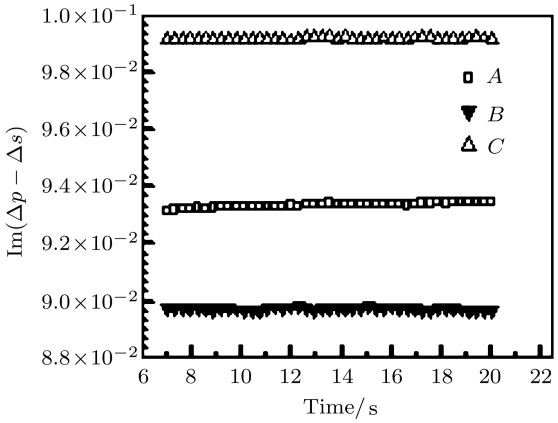

Fig.2. Im($\Delta p - \Delta s$) of OI–RD for the three protein samples. Open squares: A slide; Solid triangles: B slide; Open triangles: C slide.

Im($\Delta p - \Delta s$) Intensity of OI–RD is different for those samples. The difference is over 10^{-3} that is much more than the background noise of the signal. Where, the difference of A and B is 3.0×10^{-3}, that of A and C is 6.6×10^{-3}, B and C is 9.5×10^{-3}. Figure 2 shows that the Im($\Delta p-\Delta s$) intensity of the protein is much larger than that of the background noise and the type of the biomolecule can be specified qualitatively by its Im($\Delta p - \Delta s$) intensity.

4.2. The microarrays biomolecular detection by OI–RD method

In our experiment, the microarrays samples are printed in one glass slide with functionalized surface. The slide is divided into two parts; where part 1 printed GST (glutathione-S-transferase) with the 1mg/ml concentration spots and part 2 printed the same material with the concentration vary from 1/512 mg/ml to 1mg/ml according to the row 1 to row 5. The space between two spots centre is 500μm and the scan area is 3500 μm × 3500 μm. The Im($\Delta p - \Delta s$) intensity of part 1 and part 2 are shown in Fig.3 and Fig.4 respectively.

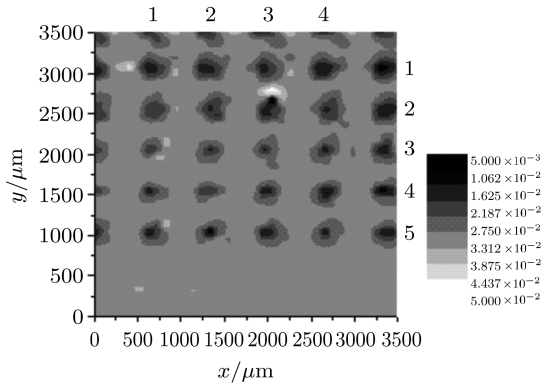

Fig.3. Im($\Delta p - \Delta s$) intensity of the same concentration microarrays biomolecule.

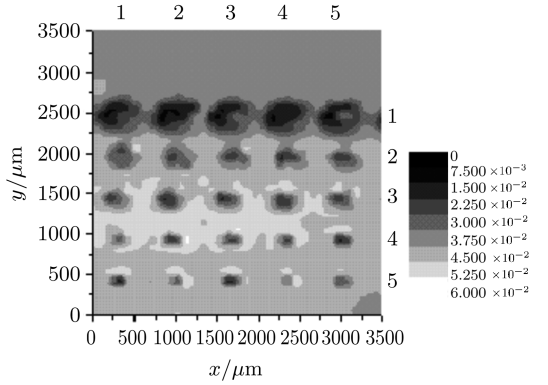

Fig.4. Im($\Delta p-\Delta s$) intensity of the varying concentration microarrays biomolecule.

Figure 3 indicates that an 5×4 array including 20 whole protein spots is got clearly, in which every spot is nearly equal. From Fig.4 we can see that a 5×5 array is given. The spots in one row have the same size and that of the different row are different. The spot becomes smaller gradually from row 1 to row 5.

These results indicate that the concentration of the biomolecule in microarray format can be specified qualitatively by its Im($\Delta p - \Delta s$) intensity.

5. Conclusion

In conclusion, we have demonstrated that the OI–RD technique can be used to detect biomolecule in microarrays format without any labelling agent. The type and concentration of the biomolecule can be specified qualitatively by its Im($\Delta p - \Delta s$) intensity. The present study proves that the OI–RD method can be affectively applied to detect biochemical processes. The high throughout and in situ detection for the biomolecule interaction in microarrays format can be achieved by OI–RD with the photoelectric diode arrays or CCD receiver.

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