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Label-free detection of hybridization of oligonucleotides by oblique-incidence reflectivity difference method

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The microarrays of 20-base oligonucleotide with different concentrations are detected before and after hybridization by the oblique-incidence reflectivity difference (OI-RD) method. The experimental results prove that OI-RD is a label-free method which can not only distinguish the concentration difference of oligonucleotides before and after the hybridization but also detect the hybridization of short oligonucleotides. At present the OI-RD method can detect 0.39 μmol/L 20-base oligonucleotide or less. These results suggest that the OI-RD method is a promising and potential technique for label-free detection of biological microarrays.

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

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1 Introduction

Biological microarrays have been widely used in life sciences since microarray technology appeared at the end of the 20th century. It is developing rapidly and plays an important role in the modern biological research, such as gene expression, DNA replication, cancer, cell style, infectious disease, protein trafficking, glycomics and proteomics. The wide application of the microarray technique requires the development of detection techniques [1–3]. Labeling methods that use fluorescence and radioactivity to detect the biological microarrays remain the cornerstone of most biological research protocols, and for good reasons, too. Nowadays, fluorescence analysis becomes the most widely used microarray detection method because of its high sensitivity and high-throughput. However, the whole detection procedures of labeling methods are very time-consuming and the cost is high. Furthermore, the labeling procedures often cause some damage or modifications in the structure and biological activity of biological samples [4]. Some label-free detection methods such as surface plasmon resonance (SPR) and mass spectrometry (MS) have been applied to microarray detections [5]. Up to now, it is still a big challenge to obtain a label-free and high throughput detection method for biological microarrays.

In our previous work, we applied the oblique-incidence reflectivity difference (OI-RD) method to monitoring the growing process of oxide thin films in real time. It has been demonstrated that the OI-RD is a label-free detection method, and the detection sensitivity can reach 1×10^{-5} or higher [6–8]. We also applied this method to microarray detections [9]. Landry et al. [10,11] have reported the label-free detection of 60-base oligonucleotides by OI-RD. In this paper, we detected the hybridization of 20-base oligonucleotides with different concentrations using OI-RD. Mi-

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croRNAs (miRNAs), known as a kind of single-strand noncoding RNAs of about 21–23 nucleotides in length, play important roles in regulating gene expression, and the research on miRNAs has become a rapidly emerging field in life science in recent years [12]. In this work, we compare the detection results of the fluorescence scanner and the OI-RD method and the results prove that OI-RD can be used as a label-free detection method for detecting concentration and hybridization of miRNAs.

2 Experiment

Figure 1 shows a typical OI-RD setup for the detection of oligonucleotide microarrays. It is similar to that used in the oxide film detection [6–8]. A p-polarized He-Ne laser with 632.8 nm wavelength is used as the probe light. The initial p-polarized laser beam is modulated by a photoelastic modulator, which causes the laser beam to oscillate at a frequency of $\Omega = 50$ kHz between the p- and s-polarization. The laser beam passes through a phase shifter that introduces a variable phase Φ_0 between the p- and s-polarized components. Then the light beam is focused on the microarray surface at an incidence angle of 60°. The reflecting beam passes through a polarization analyzer and then the beam is detected by a Silicon-photodiode. The photocurrent of photodiode is detected by two lock-in amplifiers, and the first and the second harmonic amplitudes of the photocurrent, $I_{(\Omega)}$ 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 surface, 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 oligonucleotides, respectively. Define $\Delta_p = (r_p - r_{p0})/r_{p0}$, $\Delta_s = (r_s - r_{s0})/r_{s0}$. The difference in reflectivity change is $\Delta_p - \Delta_s$. It has already been given that $I_{(\Omega)} \sim \mathrm{Im}\{\Delta_p - \Delta_s\}$ and $I_{(2\Omega)} \sim \mathrm{Re}\{\Delta_p - \Delta_s\}$ in ref. [6]. The expression for "$\Delta_p - \Delta_s$" given in ref. [6] indicates that it depends on the incidence angle (θ), the dielectric constants of the ambient (ε_0), the oligonucleotides (ε_d), and the substrate of microarray (ε_s), as well as the scale of oligonucleotide (d), respectively. The oligonucleotide microarray is mounted on a motorized stage, which is driven by a computer-controlled stepping motor and it can scan in both x and y directions.

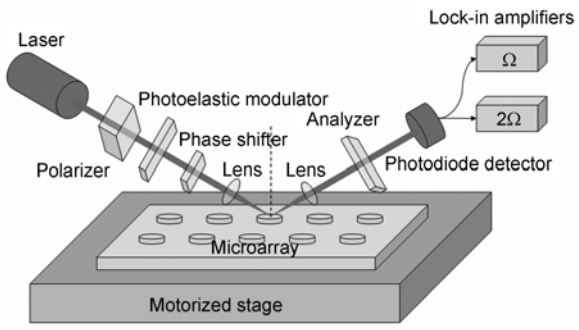

Figure 1 The layout of the OI-RD system for the detection of oligonucleotide microarrays. Laser: a He-Ne polarized laser of 7 mW. The microarray is mounted on a motorized stage that can be driven along the x and y directions, respectively.

In this study, we selected labeled model miRNA (Cy5 at 5′), 5′-ATCAGGCTCATGACCTGAAG-3′, and label-free model miRNA, 5′-ATCAGGCTCATGACCTGAAG-3′, as the targets, and 5′-$(A)_{10}$CTTCAGGTCATGAGCCTGAT-3′ as the probe, to detect the hybridization between probes and targets with OI-RD method and fluorescence scanner. Two oligonucleotide microarrays, one named M1 which would be hybridized with labeled target, and the other named M2 which would be hybridized with unlabeled target, were fabricated with a spotting robot microsystem. The probes to be printed on the microarray were dissolved in 2×saline sodium citrate (SSC)/0.01% sodium dodecyl sulfate (SDS). As shown in Figure 2, for microarray M1, every column has seven spots with probe concentrations decreasing from top to bottom in a range from 40 to 0.625 μmol/L, and each row contains two spots with the same oligonucleotide concentration. The oligonucleotide was covalently bound to the epoxy glass surface. Each basically circular sample spot with an average diameter of about 150 μm contained about 1.1 nL oligonucleotide solution, and the center to center separation between sample spots was about 400 μm. The microarray was blocked with the solution of 1% glycine dissolved in 1×phosphate buffered saline (PBS) for one hour at room

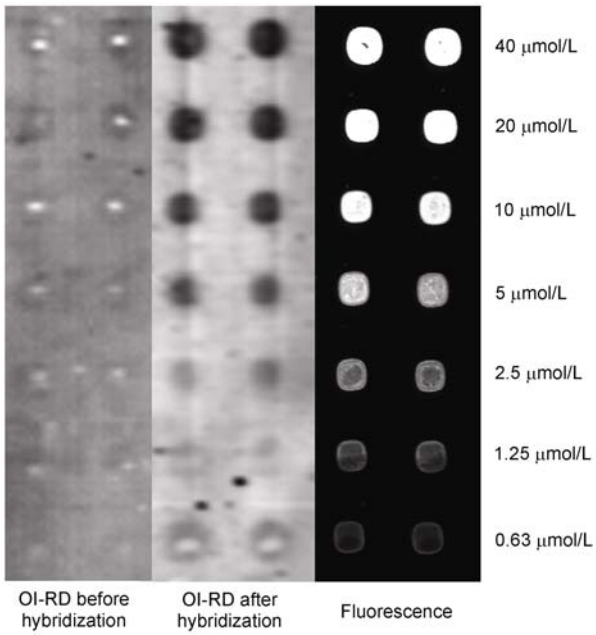

Figure 2 The 2D scan images of the OI-RD and fluorescence method for the microarray M1 hybridized with the labeled target. The left image is the intensities of OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ detected before hybridization, the middle image is the intensities of OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ detected after hybridization, and the right image is the intensities of fluorescence scan after hybridization.

temperature to lower the noise in the later fluorescence detection. Then the microarray was washed in PBST (1×PBS with 0.1% TWEEN20) for 5 minutes, in 1×PBS for 8 minutes and in double distilled water for 10 minutes. The microarray was hybridized with 10 nmol/L fluorescence labeled model miRNA target in hybridization solution (4×SSC/ 0.1% SDS) at 47°C for 17 hours. Finally, the microarray was washed in 2×SSC/0.025% SDS, 0.2×SSC, 0.1×SSC for 2, 2, 0.5 minutes, respectively. The preparation details of the oligonucleotide microarrays can be seen in ref. [13]. The preparation process of microarray M2 was the same as that of M1 except that M2 was hybridized with label-free model miRNA target. As shown in Figure 4, there are nine sample spots in each column of M2 with the concentrations decreasing from top to bottom in a range from 100 to 0.39 μmol/L, and each row contains three spots with the same oligonucleotide concentration.

The hybridized microarray M1 was detected using both methods of OI-RD and fluorescent scan before and after hybridization. For the microarray M2, we only detected it with OI-RD method because the fluorescence method cannot get the related information about the microarray M2 hybridized with label-free target.

3 Results and discussion

Figure 2 shows the two-dimension (2D) scan images of OI-RD and fluorescence method for the microarray M1. The left image is the intensities of OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ detected before hybridization, the middle image is the intensities of OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ detected after hybridization, and the right image is the fluorescence scan after hybridization. As shown in Figure 3, we also plotted the curves of the oligonucleotide concentrations vs. the average values of OI-RD and fluorescence signal intensities of two sample spots in a row in Figure 2. From Figures 2 and 3, we can see

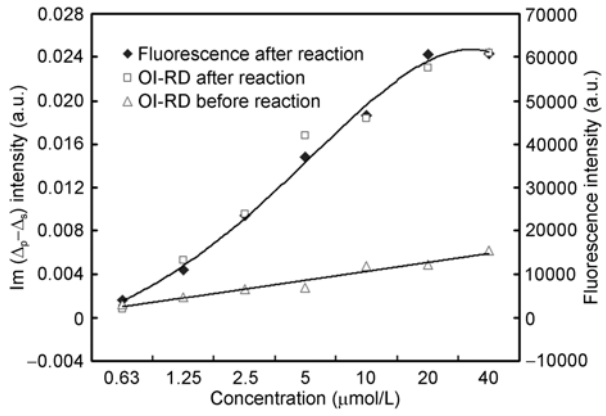

Figure 3 The signal intensities of OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ and fluorescence scan as a function of the oligonucleotide concentrations for the microarray M1.

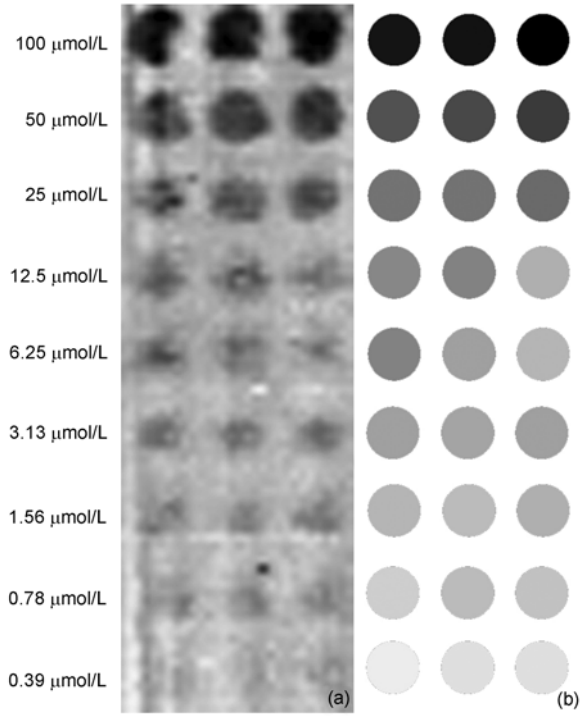

Figure 4 (a) The OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ 2D scan image of microarray M2 after hybridization with the label-free target. (b) Gray scale image drawn from the averaged values of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ intensities over every sample spot in Figure 4(a).

that: (1) the signal intensities of OI-RD are different before and after hybridization, indicating the hybridization happening. The results of OI-RD detection coincide with that of fluorescence method after hybridization, and both of the OI-RD and fluorescence signal intensities increase with the oligonucleotide concentrations. It proves that the OI-RD method can detect the hybridization of short oligonucleotides with different concentrations. (2) The OI-RD method can detect the concentration difference of oligonucleotide before hybridization, and the $\mathrm{Im}\{\Delta_p - \Delta_s\}$ intensities of OI-RD increase linearly with the oligonucleotide concentrations. This method has a great advantage compared with the fluorescence method to detect the concentration difference of oligonucleotides without labels.

Figure 4(a) shows the OI-RD $\mathrm{Im}\{\Delta_p - \Delta_s\}$ 2D scan image of microarray M2 after the hybridization with the label-free target. The result proves that OI-RD can be used as a label-free method to detect hybridization. The intensities of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ on the sample spots in Figure 4 go down with the decrease of oligonucleotide concentrations. From this result we can see that 20-base oligonucleotide with a concentration as low as 0.39 μmol/L can still be detected by OI-RD method. Though the gray scale of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ 2D image in Figure 4(a) is changing with the oligonucleotide concentrations, it is not clear enough. Figure 4(b) presents the gray scale image with the average of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ inten-

sities. The gray scale values in Figure 4(b) are the mean of $\mathrm{Im}\{\Delta_p - \Delta_s\}$ intensities in every sample spot. It is obvious that the image in Figure 4(b) is good enough by reasonable data treatment. The result indicates that OI-RD method can be used as a label-free detection method of oligonucleotide microarrays.

4 Conclusions

The hybridizations of 20-base oligonucleotides with different concentrations are detected by OI-RD method. The experimental results prove that the OI-RD method can be used as a label-free method not only to detect the hybridization of short oligonucleotide but also to distinguish the concentration difference of oligonucleotide before and after hybridization. The OI-RD method can detect 0.39 μmol/L 20-base oligonucleotide or less. As mentioned above, with the OI-RD method, we measure the difference between the reflectivities of p- and s-polarized light at oblique incidence. The signal intensity of OI-RD "$\Delta_p - \Delta_s$" is a function of the optical dielectric constant and the scale of oligonucleotides. It is well known that any biological molecular reaction or combination will change the optical dielectric constant and the scale of biomoleculer layer. The experimental results suggest that OI-RD is a promising label-free detection method for biological microarrays. Further investigations, especially, for label-free and high-throughput detections, are in progress.

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