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2011 Chinese Phys. B 20 010704

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Real-time and label-free detection of biomolecular interactions by oblique-incidence reflectivity difference method*

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(Received 2 February 2010; revised manuscript received 26 February 2010)

We successfully conduct the label-free and real-time detection of the interactions between epoxy groups and rabbit IgG and 5' CTT CAG GTC ATG AGC CTG AT 3' oligonucleotide, and between the hybridization of 5' CTT CAG GTC ATG AGC CTG AT 3' and its complementary 3' GAA GTC CAG TAC TCG GAC TA 5' oligonucleotide, by the oblique-incidence reflectivity difference (OI-RD) method. The dynamic curves of OI-RD signals, corresponding to the kinetic processes of biomolecular combination or hybridization, are acquired. In our case, the combination of epoxy groups with rabbit IgG and 5' CTT CAG GTC ATG AGC CTG AT 3' oligonucleotide need almost one and a half hours and about two hundred seconds, respectively; and the hybridization of the two oligonucleotides needs about five hundred seconds. The experimental results show that the OI-RD is a promising method for the real-time and label-free detection of biomolecular interactions.

Keywords: label-free detection, OI-RD, biomolecular reactions

PACS: 07.60.Fs, 81.70.Fy, 87.80Dj

DOI: 10.1088/1674-1056/20/1/010704

1. Introduction

Microarray is a powerful tool for *in vitro* analysis of multiple biochemical reactions between surface-immobilized macromolecules in a single experiment.^[1] Development of biomolecular microarrays has increased tremendously over the past decades.^[2] Correspondingly, the study of kinetics of biomolecular interaction on microarray becomes an important aspect of biological exploration. By the real-time measuring of the interactions, one can gauge the physiological relevance of observed interactions and gain an insight into the interplay of biomolecules in a complex environment.^[3] Some detection systems for kinetics are based on the direct detection of the tags/labels such as nanoparticles and fluorescence.^[4–6] So far the fluorescence detection has become a method that is most widely used due to its superior sensitivity and low background. Though the fluorescence detec-

tion is generally very sensitive, fluorescence quenching prevents it from being easily and continuously monitored.^[7] Besides, the labeling procedures may often cause some modifications to the biological activity of the samples.^[8] For these reasons, real-time and label-free detection represents an attractive alternative approach to microarray detection.

Currently, few methods are used for the real-time and label-free monitoring of kinetics in biomolecular interactions. Ellipsometry and surface plasma resonance have already been used for the real-time and label-free detection of microarrays because the optical techniques have the advantages of non-destructive characteristic and simple equipments.^[9] In the present paper, we apply the optical oblique-incidence reflectivity difference (OI-RD) method, which has recently been proved to have a similar sensitivity to the surface plasmon resonance,^[10] to the real-time and label-free detection of biomolecular interactions.

*Project supported by the National Basic Research Program in China (Grant No. 2007CB935700).

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2. Experiment

Early studies have demonstrated that the OI-RD can be used for the real-time *in situ* monitoring of the layer by layer growth of oxide films^[11,12] and for the imaging of the biomolecules on microarrays,^[13–17] proving that the OI-RD is a highly sensitive detection technique. The experimental setup of OI-RD for the real-time and label-free detection of biomolecular interactions is shown in Fig. 1. The probe beam from a He–Ne laser is initially p polarized. It first passes through a photoelastic modulator (PEM) that causes the resulting beam to oscillate between p- and s- polarizations with the modulated frequency $\Omega = 50$ kHz. An adjustable phase difference between p- and s- polarized components can be introduced by a phase shifter. Subsequently, the laser beam is focused on the surface of microarray at an incident angle of 60° . In this experiment, the biomolecule solution is added to a reaction chamber. The glass slide (substrate) is covered on a cell and the functionalized surface of the glass slide is in contact with the sample solution. We detect the intensity of the reflected beam from the functionalized surface by a silicon photodiode with a time resolution of one second.

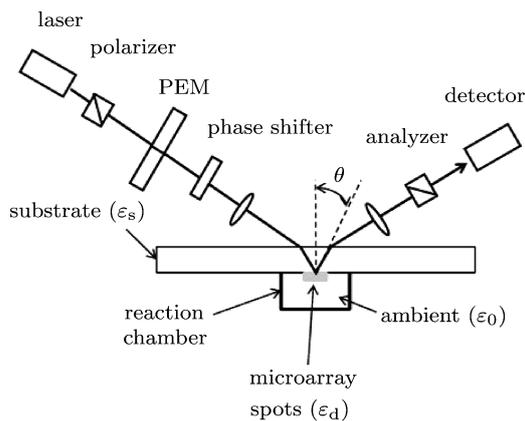


Fig. 1. Sketch of OI-RD system for real-time and label-free detection of biomolecular interactions.

At oblique incidence, the reflections for p- and s-polarized light change disproportionately in response to a surface change. Briefly, let r_{p0} and r_{s0} denote the respective reflectivities from the functionalized glass surface for p- and s- polarized light, and r_p and r_s be the respective reflectivities from biomolecules on the slide surface respectively. The changes in reflectivity are defined as $\Delta_p = |(r_p - r_{p0})/r_{p0}|$ and $\Delta_s = |(r_s - r_{s0})/r_{s0}|$. The intensity of the reflected beam that we directly measure in OI-RD experiments

is proportional to the difference in imaginary part of the fractional reflectivity change between p-polarized light and s-polarized light, i.e. $\text{Im}\{\Delta_p - \Delta_s\}$. The experimental system is mounted on an optical table and covered with a black box to minimize the effects of stray light, dust, and airflow.

From a classical three-layer model,^[18] the optical response of OI-RD from a surface layer can be expressed as

$$\text{Im}\{\Delta_p - \Delta_s\} = - \left[\frac{4\pi\epsilon_s(\tan\theta_{\text{inc}})^2 \cos\theta_{\text{inc}}}{\epsilon_0^{1/2}(\epsilon_s - \epsilon_0)(\epsilon_s/\epsilon_0 - (\tan\theta_{\text{inc}})^2)} \right] \times \frac{(\epsilon_d - \epsilon_s)(\epsilon_d - \epsilon_0)}{\epsilon_d} \left(\frac{d_{\text{eff}}}{\lambda} \right), \quad (1)$$

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_{eff} is the the average thickness of biomolecular layer; λ is the wavelength of incident laser. For the real-time detection of reaction, ϵ_d and d_{eff} would change when biomolecules are combined to the slide surface. From Eq. (1), it can be easily deduced that as the reaction goes on the parameters in Eq. (1) change, which leads to an optical signal. When the reaction ends, ϵ_d , d_{eff} and the OI-RD signal each tend to be an equilibrium value. So we can obtain dynamic curves by the real-time detecting of the interaction process of biomolecules through the OI-RD method.

We real-time detected three interaction processes of biomolecules in this work. The biomolecular samples under study are listed in Table 1. The biomolecular interactions take place between sample A and sample B. Sample A is initially immobilized on the slide, then sample B solution is added to the reaction chamber to induce the reactions.

Table 1. Samples for OI-RD detection.

reactions	A	B
1	epoxy groups	rabbit IgG
2	epoxy groups	oligonucleotide I
3	oligonucleotide I	oligonucleotide II

Oligonucleotide I: 5' CTT CAG GTC ATG AGC CTG AT 3'
 Oligonucleotide II: 3' GAA GTC CAG TAC TCG GAC TA 5'

In experiment 1, we real-time detected rabbit IgG combining with epoxy groups. In order to exclude the influence of noises from slide-solution interface and obtain signals from the real combination process, we only blocked a half surface of the epoxy functionalized slide by immersing in a 2% solution of IgG-free

glycine in $1\times$ phosphate buffered saline (PBS) for one hour (glycine would react with epoxy groups), and another half of the slide was not blocked but covered with the epoxy groups. Then we inserted the glass slide into the reaction chamber and injected rabbit IgG solution dissolved in $1\times$ PBS with 40% glycerol at a concentration of $6.7\ \mu\text{M}$ (10^{-6} mol/l), and let the half blocked and half not blocked surfaces contact the sample solution in the reaction chamber at the same time. Since the area covered with glycine would not react with rabbit IgG, only the half surface where epoxy groups remained could react with the rabbit IgG. We simultaneously detected the values of $\text{Im}\{\Delta_p - \Delta_s\}$ from blocked area and unblocked area by making the slide (on a motorized stage) back and forth. So we could obtain the actual information about the combination process of rabbit IgG and epoxy groups by subtracting such two intensities of $\text{Im}\{\Delta_p - \Delta_s\}$.

Experiment 2 is similar to experiment 1 except that the IgG solution was replaced with oligonucleotide I (shown in Table 1) solution mixed in $4\times$ saline sodium citrate (SSC) with 0.1% sodium dodecyl sulfate (SDS) at a concentration of $3\ \mu\text{M}$. We obtained the information about the combination process of oligonucleotide I molecules with also epoxy groups.

For experiment 3, we real-time detected the hybridization reaction between oligonucleotides I and II. First, we printed the oligonucleotide I (mixed in a $2\times$ SSC solution at $40\ \mu\text{M}$ concentration) on a surface of epoxy-functionalized glass slide as $400\ \mu\text{m}$ spots with a contact-printing robot. Then the slide was blocked with glycine. So the slide surface was divided into two areas: one is immobilized with glycine and the other is immobilized with oligonucleotide I molecules. After the injection of oligonucleotide II solution dissolved in $4\times$ SSC with 0.1% SDS at a concentration of $1\ \mu\text{M}$, the hybridization reaction of oligonucleotides I and II took place only where oligonucleotide I was printed, since oligonucleotide II was complementary to oligonucleotide I while glycine did not react with oligonucleotide I. Following the same detection procedure adopted in experiment I, we obtained the real information about the hybridization process between oligonucleotides I and II.

3. Results and discussion

Figure 2 shows the variation of $\text{Im}\{\Delta_p - \Delta_s\}$ signal with time for rabbit IgG to be bound to epoxy groups. We inserted the rabbit IgG solution into the

reaction chamber quickly and then recorded the optical signal in real-time. The real-time OI-RD data in Fig. 2 reveal the kinetic process of rabbit IgG binding with epoxy groups on a slide surface. The OI-RD signal shows that the adsorption of one monolayer of the rabbit IgG in this case takes almost one and a half hours. The glycerol is believed to be the main factor that slows down the whole binding reaction, since glycerol has always been used to be a stabilizer. This result is consistent with the experimental process for the microarray preparations in which the immobilization of IgG molecules on a slide generally takes one night.

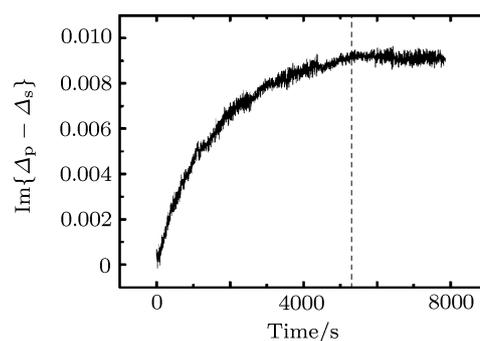


Fig. 2. Real-time $\text{Im}\{\Delta_p - \Delta_s\}$ measurement of rabbit IgG binding to epoxy groups on a slide surface.

Figure 3 shows the variation of $\text{Im}\{\Delta_p - \Delta_s\}$ signal with time for oligonucleotide I molecules to be bound to epoxy groups. The binding of monolayer oligonucleotide I molecules takes about two hundred seconds, much faster than the binding of monolayer rabbit IgG molecules. However the data in Fig. 3 are very similar to those in Fig. 2, which may be attributed to the same covalent adsorption process. Figures 2 and 3 show that the OI-RD can be used to measure protein and DNA– small-molecules binding reactions on glass slide surface in real time.

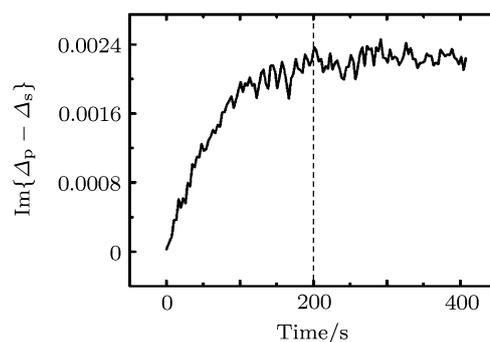


Fig. 3. Real-time $\text{Im}\{\Delta_p - \Delta_s\}$ measurement of oligonucleotide probe binding to epoxy groups on slide surface.

Figure 4 shows the variation of $\text{Im}\{\Delta_p - \Delta_s\}$ signal with time for the hybridization between oligonucleotides I and II. The hybridization took about five hundred seconds. The data in Fig. 4 proves that the OI-RD can also be used to real-time detect the process of hydrophobic interaction.

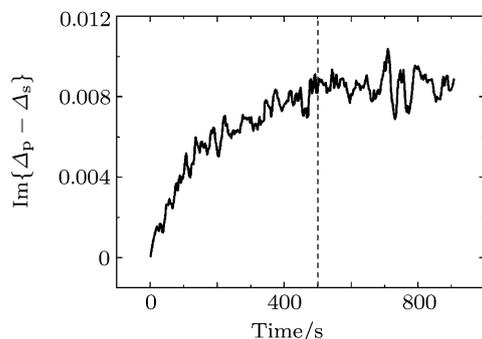


Fig. 4. $\text{Im}\{\Delta_p - \Delta_s\}$ binding curve at oligonucleotide I spot after the injection of the aqueous solution of oligonucleotide II.

The intensities of $\text{Im}\{\Delta_p - \Delta_s\}$ in Figs. 2, 3 and 4 are different. The main reason is that the binding reaction between small-molecule and protein or DNA is caused by covalent attachment while the hybridization between oligonucleotides I and II is caused by hydrophobic interactions, and the proteins and oligonu-

cleotides have different molecular weights and structures with different optical dielectric constants and sizes.

4. Conclusion

We successfully real-time detect the combination processes of epoxy groups with rabbit IgG and oligonucleotide I, and the hybridization process between oligonucleotides I and II molecules. We obtain the curves for variation of $\text{Im}\{\Delta_p - \Delta_s\}$ signal with time, corresponding to the kinetic process of biomolecular combination or hybridization. In our case, the combination processes of epoxy groups with rabbit IgG and oligonucleotide I need almost one and a half hours and about two hundred seconds, respectively; and the hybridization process between oligonucleotides I and II needs about five hundred seconds. The experimental results demonstrate that OI-RD is a versatile optical platform for the real-time and label-free detection of biomolecular interactions. Further investigations on the detection sensitivity and analysis of quantitative data of kinetic process are being planned.

References

- [1] Schena M, Shalon D, Davis R W and Brown P O 1995 *Science* **270** 467
- [2] Sassolas A, Leca-Bouvier B D and Blum L J 2008 *Chem. Rev.* **108** 109
- [3] Boutell J M, Hart D J, Godber B L, Kozlowski R Z and Blackburn J M 2004 *Proteomics* **4** 1950
- [4] Wu Z S, Jiang J H, Fu L, Shen G L and Yu R Q 2006 *Anal. Biochem.* **353** 22
- [5] Daniel M C and Astruc D 2004 *Chem. Rev.* **104** 293
- [6] Wang J, Liu G and Merkoci A 2003 *J. Am. Chem. Soc.* **125** 3214
- [7] Du H, Disney M D, Miller B L and Krauss T D 2003 *J. Am. Chem. Soc.* **125** 4012
- [8] Zhu X D, Landry J P, Sun Y S, Gregg J P, Lam K S and Guo X W 2007 *Appl. Opt.* **46** 1890
- [9] Wang Z H and Jin G 2003 *Anal. Chem.* **75** 6119
- [10] Zhu X D 2006 *Opt. Commun.* **259** 751
- [11] Wang X, Fei Y Y, Lu H B, Jin K J, Zhu X D, Chen Z H and Yang G Z 2007 *J. Phys.: Condens. Matter* **19** 26206
- [12] Wang X, Jin K J, Lu H B, Fei Y Y, Zhu X D and Yang G Z 2007 *J. Appl. Phys.* **102** 053107
- [13] 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
- [14] Zhang H Y, Lu H, Li W, Liang R Q, Jin K J, Zhou Y L, Ruan K C and Yang G Z 2008 *Chin. Phys. B* **17** 1674
- [15] Wen J, Lu H, Wang X, Yuan K, Lu H B, Zhou Y L, Jin K J, Yang G Z, Li W and Ruan K C 2010 *Science China-Physics Mechanics & Astronomy* **53** 306
- [16] 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 *Science China-Physics Mechanics & Astronomy* **53** 1434
- [17] 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 *Science China-Physics Mechanics & Astronomy* **53** 1230
- [18] Zhu X D, Fei Y Y, Wang X, Lu H B and Yang G Z 2007 *Phys. Rev. B* **75** 245434