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Detect the Hybridization of Single-Stranded DNA by Parallel Scan Spectral Surface Plasmon Resonance Imaging

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Abstract We investigate the ability of a parallel scan spectral surface plasmon resonance imaging system in analyzing hybridization of single-stranded DNA (ssDNA) on microarray. The ssDNA probes are modified by a thiol group and thereby can be immobilized onto the gold film. Hybridization experiments are carried out by using both complementary and noncomplementary sequence to confirm the specificity of interaction. We also demonstrate that the data analysis is very important in reducing the noise and improving the resolution by comparing polynomial fitting method with the combination of polynomial fitting and centroid method. Finally, the results demonstrate that the parallel scan spectral surface plasmon resonance imaging system can be used for high-throughput analysis of the hybridization of the ssDNA.

Keywords Surface plasmon resonance · Hybridization · Microarray

Introduction

In the past decade, DNA microarray has been utilized for various applications, such as DNA detection [1], diagnostics [2], monitoring gene expression [3], etc. To detect the DNA hybridization, radioactive, enzymatic, and luminescence labels have been used to label the probes. These labeled probes have the disadvantages of in radiation, storage, and disposal [4], while the probes used in surface plasmon resonance (SPR) systems do not need any labels. Thus, SPR has recently attracted the attention as a new approach for rapid detection and characterization of the hybridization process.

Since the phenomenon of surface plasmon resonance showing the possibility of being used in biosensors by Lidberg and Nylander firstly in 1983 [5], SPR biosensors have made great advances in both technique and applications [6–9]. Now, SPR has become one of the most advanced techniques, which can provide a high sensitivity without using any molecular labels in biomolecular interaction detections. It can also be applied in the high-throughput system for parallel measurement [10].

Surface plasmon is a charge density oscillation, excited at an interface of two media with dielectric constants of opposite sign, such as a metal and a dielectric. SPR sensors can be classified into four interrogations: angular, wavelength, intensity, and phase. For wavelength interrogation, a parallel p-polarized white light beam is directed to the surface of a thin metal film (~45 nm), such as gold or silver. A dip will appear in the spectrum of the reflected light. The position of this dip depends on the refractive index (RI) at the vicinity of the metal film. When the film is bound with analytes, the refractive index will change, which will result in a shift of the dip [11].

For the angular interrogation, surface plasmon is excited by a laser and then the reflectivity is measured at different incident angles. The RI resolution of the angular interrogation system is always higher than the wavelength interrogation system. However, the high coherence of laser will cause the speckle effect, which will reduce the imaging quality and is difficult to eliminate completely even by a rotating

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diffuser. For the intensity interrogation, the intensity of the reflected light is measured with fixed incident angle and wavelength. Although the setup is simple, it offers a narrow dynamic range and a low RI resolution. For the phase interrogation, the phase change of the reflective light is measured, which provides a high RI resolution. But the complicated interferometer setup and the instability limit its application. Compared with these three modes, the SPR imaging system based on wavelength interrogation can provide quantitative sensing with a larger dynamic range and without speckle effect by a simple setup.

In our previous work [12], we have developed a wavelength interrogation-based SPR imaging method—parallel scan surface plasmon resonance imaging system. The parallel imaging mode, compared with the traditional point-scanning mode, can provide a much higher detection throughput. In the point-scanning system, only the central areas of the DNA spots are analyzed [13], while the line-scanning mode provides more detailed information about the DNA arrays by moving the stage automatically and analyzing the whole area of the spots. The line-scanning system also takes less experiment time than point-scanning system. In this paper, we apply this system to study biomolecular interactions and demonstrate the potential ability of this approach in DNA characterization.

Material and Methods

Parallel Scan Spectral SPR Imaging Device

The parallel scan spectral surface plasmon resonance imaging system is based on the wavelength interrogation, with the schematic diagram shown in Fig. 1. The light from a white point source (GCI-0603 halogen lamp, China Daheng Group) is focused by an objective lens. Then, the light passes through a pin hole and turns to parallel light after being collimated by an achromatic convex lens (GCL-010630, f=50 mm, China Daheng Group). A linear polarizer (GCL-050003, extinction ratio >500, China Daheng Group) is used to generate p-polarized light. After that, the polarized light is focused into a narrow line shape by a cylindrical convex lens (L46-197, f=50 mm, Edmund Optics) to irradiate the SPR module, which is configured into a Kretschmann manner. The DNA microarray chip is prepared onto a BK7 glass sheet with 5-nm Cr film and 40-nm Au film coating. The glass sheet is stuck with a right angle prism (BK7 glass, refractive index=1.515) with the refractive index matching oil and fixed on a one-dimensional translation stage. Thus, the microarray chip can be scanned by the line-shaped light automatically. The incident line-shaped beam is focused onto the gold film with central incident angle of approximately 44.5°. The reflected beam enters the entrance slit of a spectrometer (SpectralPro 150, Acton research) with a 300 line/mm grating. The entrance slit is placed 10 cm away from the line-shaped light to allow only light whose incident angle is in a small range (44.5±0.005°) to enter the spectrometer. Finally, the light come out of the spectrometer is collected by a charge-coupled device (CCD, Q-imaging RETIGA EXi 1394).

Reagents and Sample

The buffer used in the experiment is as follows:

- Immobilization buffer: Tris (Sigma-Aldrich) 100 mM, NaCl 500 mM
- Hybridization buffer: Tris 10 mM, EDTA 1 mM, NaCl 1 M

Blocking thiol solution:

6-mercapto-1-hexanol (MCH) (Sigma-Aldrich)

5′Thiolated synthetic probes were purchased from Takara (Shenzhen, China). The sequences of the probes, synthetic targets, and noncomplementary strands are described below:

Thiolated probe VC-1:	SH-T18-GTCTCCGCATGATTCTCTG
Complementary sequence:	CAGAGAATCATGCGGAGAC
Noncomplementary sequence:	GCATCGGCATGGGCATGAG

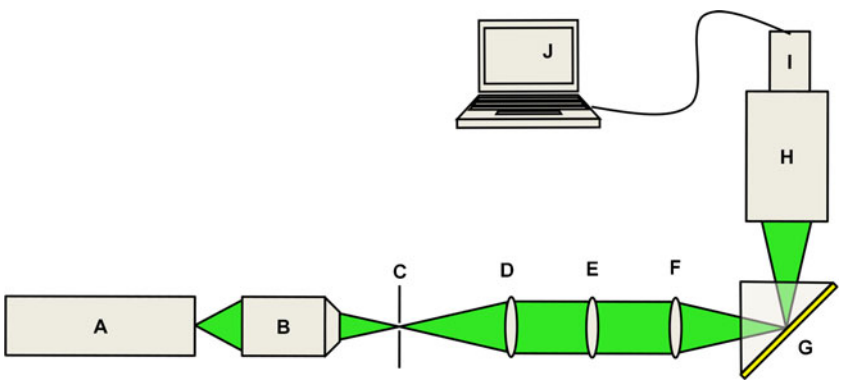

Fig. 1 The optical layout of the system. *A* white light point source, *B* objective lens, *C* pin hole, *D* achromatic convex lens, *E* polarizer, *F* cylindrical convex lens, *G* Kretschmann-type SPR module, *H* grating spectrometer, *I* CCD, *J* personal computer

Preparation of DNA Microarray

The surface of the gold film is pre-cleaned by a solution of H_2O_2 (30 %), NH_3 (30 %), and Milli-Q water in 1:1:5 ratios for 10 min [14] and then rinsed with Milli-Q water thoroughly.

Since sulfur bond in thiol group is very easy to bind with each other, Tris(2-carboxythyl)phosphine hydrochloride (100 mM) is added into the probe solution and incubated at 37 °C for 1 h to break the S–S bond. To avoid the noncomplementary hybridization, the single-stranded DNA (ssDNA) probes are denatured in 95 °C water bath for 5 min and then inserted into the ice immediately to keep the probes from rebinding. In order to increase the efficiency of the immobilization, high salt concentration buffer is used.

Probes are arranged as a 2×6 array (Fig. 3) with 0.4 μL for each spot on the microarray chip and incubated at 37 °C for 6 h. After the incubation, the chip is rinsed with the immobilization buffer and dried at room temperature. Before the hybridization, the chip is immersed into the blocking thiol solution (1 mM MCH) for 1 h and then rinsed thoroughly with Milli-Q water. The purpose of this procedure is to prevent the target sequence from binding with the gold film. Then, the target solution (0.5 μL) is dripped right at the position of probes for hybridization reaction. The reaction is processed at 65 °C for 30 min in a water bath. After that, the chip is washed by Milli-Q water thoroughly to remove the unbound target sequences and then stuck to the prism with the matching oil for scanning.

Results and Discussion

Data Analysis Method

The key point in data analysis is how to determine the minimum position of the reflective spectrum precisely. There are several data-processing algorithms used at present, including quadratic curve fits [15], centroid method [16], and optimal linear data analysis [17]. But the most widely used methods are polynomial fitting method and centroid method.

The polynomial fitting method is a process of using polynomial to construct a curve, which has the best fit to the experimental data. Then, the minimum position can be obtained from the best fitting polynomial. Figure 4a shows the fourth and tenth order polynomial fitting which indicates that the tenth order curve fits the experimental data better than the fourth order. However, as Fig. 4b shows, the smaller the order of the polynomial fitting is, the less the standard deviation will be. Thus, usually the tenth order polynomial fitting is used to find out the position of minimum roughly, and then, the fifth order polynomial fitting is applied to calculate the minimum position with smaller standard deviation around the position found by tenth order polynomial fitting. This method is called "double polyfit" in contrast with the single polyfit method.

Centroid method is a simple method for determining the minimum position of SPR curve. It finds out the geometric center of the resonance minimum by calculating the pixels with values below a threshold value. This threshold value is a very important parameter which affects the noise level of the centroid output. Although the geometric center and the minimum position do not always locate at the same point, most SPR applications are relative measurements, so the offset of geometric center does not affect the final measurements [18]. However, this fixed threshold value centroid method has a disadvantage that is susceptible to the fluctuation of the light source. Thus, a dynamic threshold value has been used. First, the position of the minimum is determined by polynomial fitting method. Then, the more precise minimum position is found out by applying centroid method between certain widths of pixels around the position found by polynomial fitting method. This method is called "polyfit + centroid."

We study the effect of the width on noise degree of both methods. The results are shown in Fig. 4c. It is shown in Fig. 4c that the wider the better for the double polyfit method and the polyfit + centroid method has a best width around 300 pixels. According to the width of our CCD, 600 pixels are chosen for the double polyfit method, and 300 pixels are chosen for the polyfit + centroid method. Figure 4d shows the noise degree of the three methods: single tenth order polyfit, double polyfit, and polyfit + centroid. The results in Fig. 4d show that the polyfit +

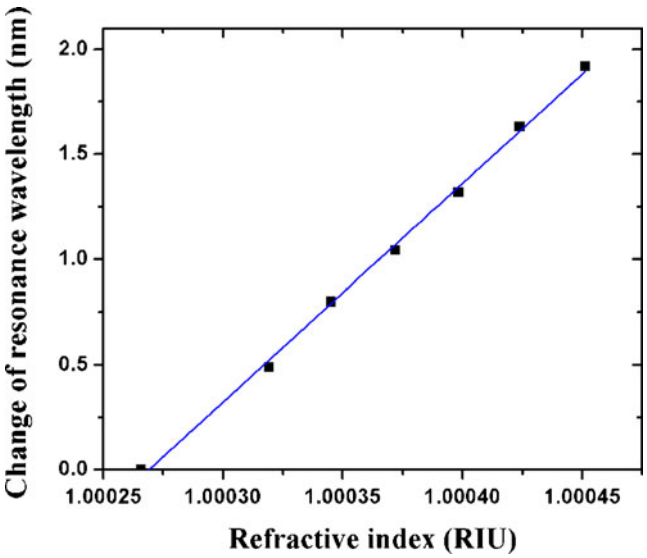

Fig. 2 The dependence of the change of resonance wavelength on the refractive index of air at different pressure

centroid method has the best noise degree of 0.09 pixels, which is 1.1×10^{-2}nm converted into wavelength.

The Refractive Index Resolution

The calculation of refractive index resolution is shown as follows [19]:

$$\delta n = \sigma/Sn \quad (1)$$

where δn is the RI resolution, σ is the standard deviation of output, and Sn is the sensitivity of the system. First, the sensitivity is studied by measuring the air around the bare gold film under different pressure. We stick a sealed gas chamber on the bare gold film and change the pressure step by step, so that the RI of air in the chamber varies with the changing of pressure. Then, the shift of the minimum of the SPR dip (the resonance wavelength) is recorded to determine the sensitivity. As shown in Fig. 2, the sensitivity is 1.03×10^{4}nm/refractive index unit (RIU). Then, the fluctuation of the resonance wavelength is studied and a standard deviation of 1.1×10^{-2}nm is obtained. According to Eq. (1), the RI resolution is 1.1×10^{-6}RIU.

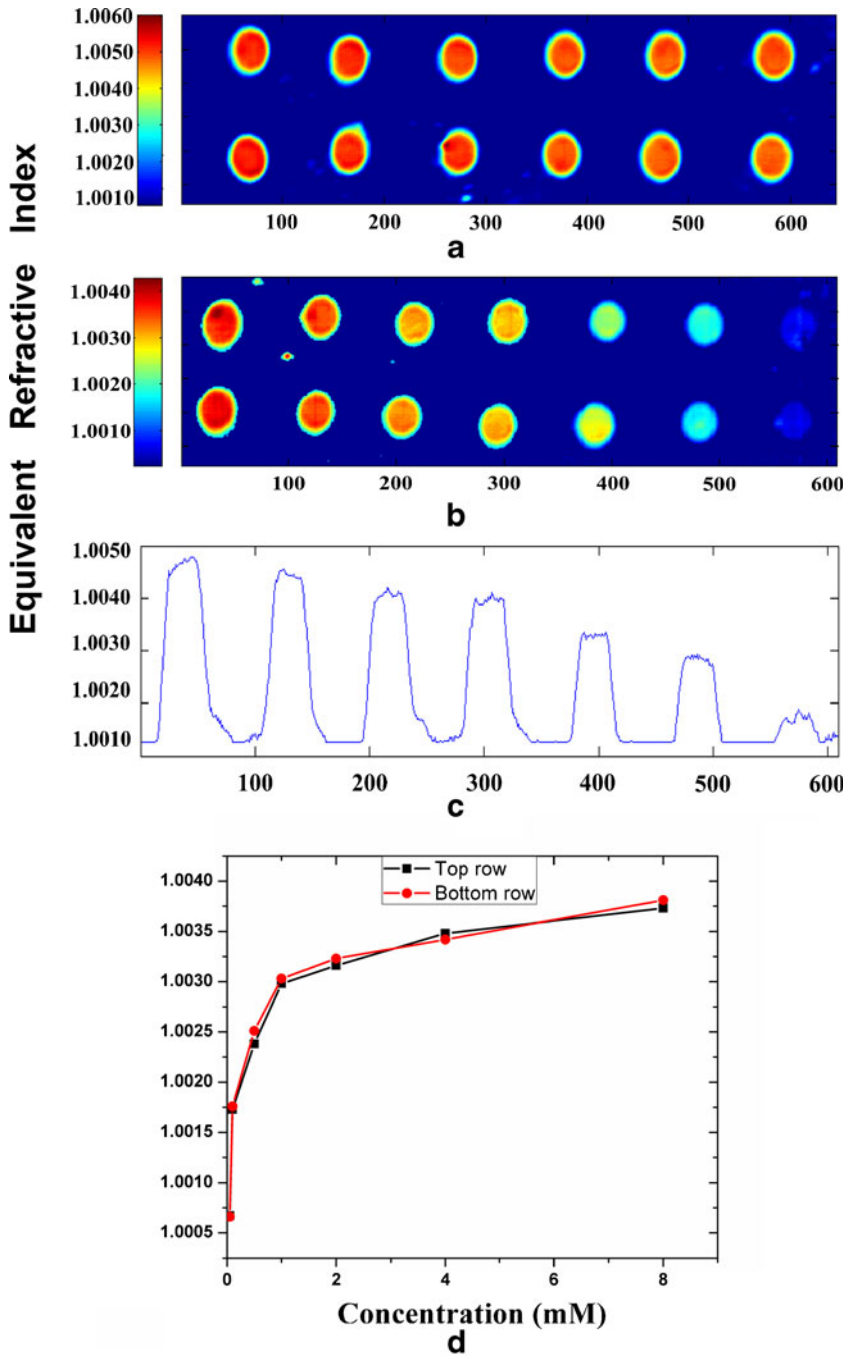

Fig. 3 The results of immobilization. **a** The detection result of a 2×6 DNA microarray by the system. All the DNA probes immobilized with concentration of 10 μM, and each spot is prepared with 0.4 μl probe solution. **b** The detection result of a 2×7 DNA microarray by the system. Two rows have the same distribution: the DNA probes immobilized with concentration of 8, 4, 2, 1, 0.5, 0.1, and 0.05 μM from left to right. Each probe spot is prepared with 0.4 μl probe solution. **c** The plotted row in (**b**). **d** The dependence of the change of refractive index on the probe concentrations derived from (**b**). Each data is calculated by averaging one probe spot between an upper and a lower limit

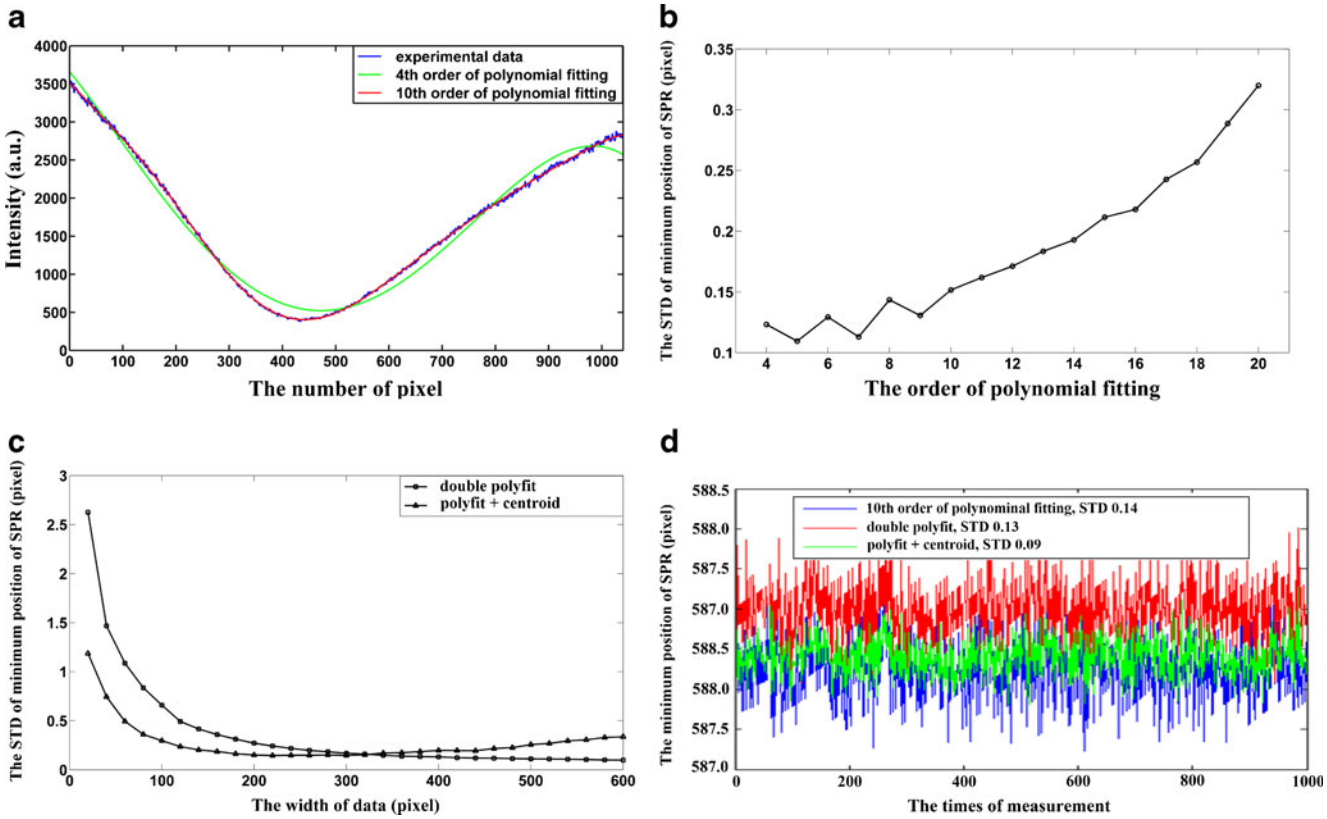

Fig. 4 The data analysis method for reduction of noise. **a** The comparison of the experimental data with the fourth and tenth order polynomial fitting. **b** The standard deviation of several orders of polynomial fitting. **c** The function of the width of pixels with the standard deviation for both quadratic polynomial fitting method and polynomial fitting and centroid method. **d** The comparison of noise of three methods

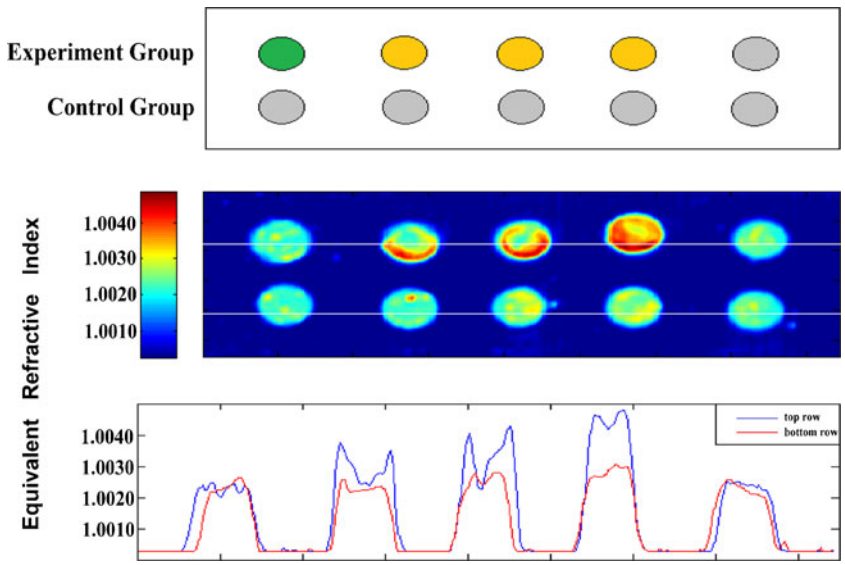

Fig. 5 The results of hybridization experiment. **a** The hybridization experiment model of a 2×5 DNA microarray. The DNA probes immobilized with concentration of 1.0 μM. Each probe spot is prepared with 0.4 μl probe solution. The *first column* is negative control group, the *next three* columns are hybridization groups, and the *last column* is the hybridization buffer group. The concentrations of hybridization solution and noncomplementary solution are both 3 μM. The *first row* is experiment row and the *second row* is the control row. **b** The detection result of the hybridization. **c** The plotted rows in (**b**)

The Detection Limit of the DNA Concentration

A 2×6 DNA microarray with the concentration of 10 μM was prepared in order to show the ability of our system for detecting the RI changes on the surface of the gold film. Figure 3a shows the results after the immobilization. It is very clear that the refractive indices of these spots are almost the same, though there is a slight difference between the left side and right side of the chip. The difference was related to the angle changes of incident light during the movement of the stage (Fig. 4).

To test the detection limit, a 2×7 DNA array was prepared. The concentrations of each spot are 8.00, 4.00, 2.00, 1.00, 0.50, 0.10, and 0.05 μM from left side and the two rows are the same. Figure 3b shows that the refractive index of the spots increases with the increase of the concentration. The minimum concentration that can be detected is 0.05 μM. Figure 3c, d shows the refractive index of the plotted row in Fig. 3b and the mean refractive index of the whole analyte spots of the two rows, respectively. The nonlinear curve in Fig. 3d shows that the refractive index increases fast with the concentration below 1.00 μM, but the trend slows down when the concentration continues to rise up.

Analysis of the ssDNA Hybridization by Parallel Scan Spectral SPR Imaging

In order to demonstrate the ability of our system in biomolecular interaction detecting, a confirmatory experiment is designed and carried out as shown in Fig. 5a. A 2×5 DNA array was prepared with probe concentration of 1.0 μM. The first column is the negative control group. The next three columns are hybridization groups and the last column is the hybridization buffer group. The concentrations of hybridization solution and noncomplementary solution are both 3 μM. Besides, the first row is experiment group and the second row is control group.

As shown in the Fig. 5b, c, the refractive indices of two spots of the first and last columns are almost the same, while the two spots of the other three columns have obvious differences. The refractive indices of the experiment groups are higher than the control groups due to the hybridization, though the hybridization is not uniformly distributed which may be caused by the less liquidity of the hybridization solution. Besides, the control groups also have a few differences due to the washing procedure. This experiment demonstrates that the proposed SPR system has the potential for detection of DNA hybridization.

Conclusions

We have demonstrated the ability of the parallel scan spectral SPR imaging system by analyzing the hybridization of ssDNA on microarrays successfully. We also demonstrated that the data analysis is important in reducing noise and improving resolution. The refractive index resolution of this system is 1.1×10^{-6}RIU by applying the combination of polynomial fitting and centroid method.

In conclusion, the parallel scan spectral SPR imaging system can be used as a high-throughput analysis method for biomolecular interactions. In particular, the line-scanning mode is a very efficient method to analyze DNA microarrays with high throughput.

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References

1. Kimmel AR, Brian O (eds) (2006) Methods in enzymology, volume 410, DNA microarrays part A: array platforms and wet-bench protocols. Academic Press, San Diego
2. Zhao XJ, Tapec-Dytioco R, Tan WH (2003) Ultrasensitive DNA detection using highly fluorescent bioconjugated nanoparticles. J Am Chem Soc 125:11474–11475
3. Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. Science 270:467–470
4. Vikholm-Lundin I, Piskonen R, Albers WM (2007) Hybridisation of surface-immobilised single-stranded oligonucleotides and polymer monitored by surface plasmon resonance. Biosens Bioelectron 22:1323–1329
5. Liedberg B, Nylander C, Lundstrom I (1983) Surface-plasmon resonance for gas-detection and biosensing. Sensors and Actuators 4:299–304
6. Bolduc OR, Live LS, Masson JF (2009) High-resolution surface plasmon resonance sensors based on a dove prism. Talanta 77:1680–1687
7. Piliarik M, Parova L, Homola J (2009) High-throughput SPR sensor for food safety. Biosens Bioelectron 24:1399–1404
8. Bardin F, Bellemain A, Roger G, Canva M (2009) Surface plasmon resonance spectro-imaging sensor for biomolecular surface interaction characterization. Biosens Bioelectron 24:2100–2105
9. Gan QQ, Gao YK, Bartoli FJ (2009) Vertical plasmonic Mach-Zehnder interferometer for sensitive optical sensing. Opt Express 17:20747–20755
10. Yuk JS, Kim HS, Jung JW, Jung SH, Lee SJ, Kim WJ, Han JA, Kim YM, Ha KS (2006) Analysis of protein interactions on protein arrays by a novel spectral surface plasmon resonance imaging. Biosens Bioelectron 21:1521–1528
11. Homola J, Yee SS, Gauglitz G (1999) Surface plasmon resonance sensors: review. Sensors Actuators B Chem 54:3–15
12. Liu L, He Y, Zhang Y, Ma S, Ma H, Guo J (2008) Parallel scan spectral surface plasmon resonance imaging. Appl Opt 47:5616–5621
13. Yi SJ, Yuk JS, Jung SH, Zhavnerko GK, Kim YM, Ha KS (2003) Investigation of selective protein immobilization on charged

protein array by wavelength interrogation-based SPR sensor. Mol Cells 15:333–340
14. Wang RH, Tombelli S, Minunni M, Spiriti MM, Mascini M (2004) Immobilisation of DNA probes for the development of SPR-based sensing. Biosens Bioelectron 20:967–974
15. Stenberg E, Persson B, Roos H, Urbaniczky C (1991) Quantitative-determination of surface concentration of protein with surface-plasmon resonance using radiolabeled proteins. J Colloid Interface Sci 143:513–526
16. Kukanskis K, Elkind J, Melendez J, Murphy T, Miller G, Garner H (1999) Detection of DNA hybridization using the TISPR-1 surface plasmon resonance biosensor. Anal Biochem 274:7–17
17. Chinowsky TM, Jung LS, Yee SS (1999) Optimal linear data analysis for surface plasmon resonance biosensors. Sensors Actuators B Chem 54:89–97
18. Nenninger GG, Piliarik M, Homola J (2002) Data analysis for optical sensors based on spectroscopy of surface plasmons. Meas Sci Technol 13:2038–2046
19. Homola J (1997) On the sensitivity of surface plasmon resonance sensors with spectral interrogation. Sensors Actuators B Chem 41:207–211