A NOVEL DETECTION PLATFORM FOR PARALLEL MONITORING OF DNA HYBRIDIZATION WITH HIGH SENSITIVITY AND SPECIFICITY

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ABSTRACT

We developed a high-sensitive platform to monior multiple hybridization events in real time. By creating a micro-optical array in a polymeric chip, the system combine the excellent discriminative power of supercritical angle fluorescence (SAF) microscopy with high-throughput capabilities of microarrays. The micro-optical array is easy to fabricate, and exhibits significantly improved analytical performance. It has a potential to become a basic tool for applications such as gene expression or single nucleotide polymorphism (SNP) detection.

KEYWORDS: Supercritical angle fluorescence; Micro-optics; DNA microarray; Real-time monitoring

INTRODUCTION

Despite the great popularity and potential of DNA microarrays, their use has been limited by the low sensitivity and non-specific binding. One efficient way to improve target discrimination is to monitor hybridization in real time [1]. Supercritical angle fluorescence (SAF) microscopy selectively measures target signals from the sensing surface and is particularly suitable for real-time detection [2]. However, applying SAF to microarray sensing has seldom been reported as the optical setup is bulky and not practical for multiplexed detection. Here, we developed a novel platform to couple the excellent discriminative power of SAF with high-throughput capabilities of microarrays. By creating miniaturized micro-optical structures on a polymeric chip, the platform allows real-time monitoring of parallel DNA hybridization with much enhanced sensitivity and specificity.

CONCEPT

Figure 1 illustrates the principle of operation of the micro-optical structure. For fluorophores situated at refractive-index discontinuities, the spatial emission pattern of the fluorescence is anisotropic. Significant portion of the radiation is emitted into the high refractive medium in the direction of critical angle, known as SAF. For any planar substrate, SAF is trapped in the substrate and cannot be collected. In order to detect the SAF emission, we innovatively structured cone-shaped micro-optical elements in cyclic polyolefin copolymer (COC). The structure has flat top surface and sloped side-wall whereby the SAF emission can be efficiently captured by undergoing total internal reflection at the side-wall.

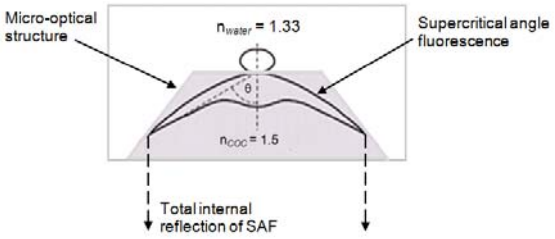

Figure 1: Principle of using the micro-optical structure for SAF emission detection.

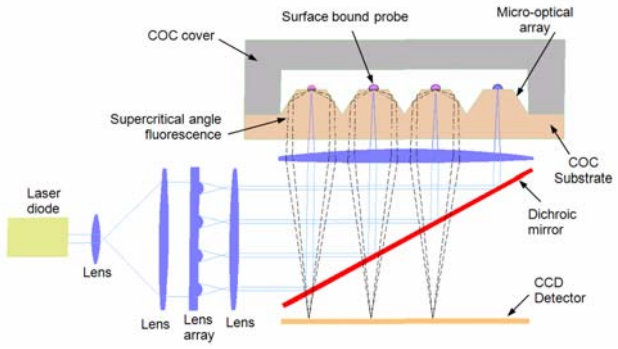

Figure 2. Schematic diagram of the detection platform.

The schematic diagram of the platform is shown in Figure 2. It consists of an array of micro-optical structures and components for optical detection. DNA probes are immobilized on top of the array. If a fluorescent-labeled target is complementary to the DNA probe, hybridization occurs and the surface-bound molecules are excited by a laser diode. SAF emission is then captured by the micro-optical array and directed to a CCD detector underneath. The surface signal is very weak if the target is unspecific to the probe.

EXPERIMENTAL

Fabrication of micro-optical array

The 8×8 micro-optical array was fabricated in cyclic polyolefin copolymer (COC) by injection molding (GuoSheng Precision Tool Co. Ltd, China) [3-6]. The master was made in hard aluminium using precision milling tool. Figure 3 shows SEM image of the micro-optical array. The inner and outer diameters of the individual element were 120 and 200

μm, respectively, and the height was 70 μm. The distance between each element was 400 μm. The surface roughness of the COC waveguides was measured to be 10 nm.

Probes and targets

A 20-mer oligonucleotide of Matrix gene of Avian Influenza virus (AIV) was synthesised and used as probe. The probe was modified at the 5' end with a poly (T)10-poly (C)10 tail to facilitate the attachment to the plastic substrates as previously described [7]. The sequence of the oligonucleotide was 5´-TTTTTTTTTTCCCCCCCCCC TCA GGC CCC CTC AAA GCC GA. Cy3-labeled -3´. The probe was used for sensitivity test. Two targets, complementary and non-complementary, were hybridezed to the probe. The non-complementary target has the sequence of 5´- GGC CAG TAT TAG AAA CAA CA -3', which has 30% sequence homology as the specific target. Both targets were labelled with Cy3 at the 5' end for visualization. All the oligonucleotide probes and targets were synthesized at DNA Technology A/S, Denmark.

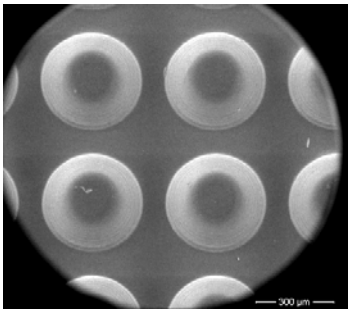

Figure 3. SEM of the micro-optical array in the COC chip.

Probe immobilization

A simple UV cross-linking technique was used to directly immobilize the poly(T)poly(C)-tagged DNA oligonucleotide probes on native COC without any surface modification [7]. The probe was diluted in 150 mM sodium phosphate buffer (pH 8.5) containing 0.004% Triton X to a final concentration of 2-20 μM. Spotting was performed using a non-contact array nano-plotter 2.1 (GeSim, Dresden, Germany). The Picoliter pin ejected 100 pl drops of DNA probe on top of the micro-optical elements and the spot size was around 90 μm. The spots were allowed to dry and then exposed to UV irradiation at 254 nm with power of 3 mW/cm^2 for 10 min (Stratalinker 2400, Stragtagene, CA, USA). Subsequently, the array was washed in 0.1× standard saline citrate (SSC) with 0.1% (w/v) sodium dodecyl sulfate (SDS) (Promega, WI, USA) solution, then rinsed in deionized water and dried by nitrogen.

Hybridization

For hybridization, Cy3-labeled complementary strand of the probe was used as target DNA and was diluted to a final concentration ranging of 1 nM in Perfect Hybridization Buffer (Sigma-Aldrich, MO, USA). 1 nM Cy3-labeled non-complementary DNA target was also used to test unspecific hybridization. 20 μl samples were loaded directly on the micro-optical array. Hybridization was carried out at room temperature. For each experiment condition, hybridization was performed on ten replica spots and each experiment was repeated five times.

Image acquisition and data analysis

In the present study, the optics was optimized for fluorescent dye Cy3 and a 550 nm laser diode was used as a light source. Two achromatic lens (f = 50 mm, and f = 100 mm, Thorlab, USA) was positioned in the input path of the light to make a parallel beam. A lens array (p = 150μm, f = 6.7mm, Thorlab, USA) was used to redistribute the laser light to produce multiple laser dots with focused laser energy, so that only the area with DNA probes was illuminated. This not only increased the utilization efficiency of the laser energy, but also reduced background noise. The interference filters were chosen for detection fluorescence around 570 nm upon excitation at 550 nm. Two achromatic lens (f = 60 mm, and f = 75 mm) were used to focus the signal onto the CCD camera. 16-bit greyscale images were generated on the CCD camera. Image acquisition and data analysis were done using a self-developed programs written in LabVIEW (Austin TX, USA). To monitor the real-time hybridization process, images were acquired at 2 minutes interval. Quantitative analysis was carried out by calculating the fluorescent intensity of the spots. The background was measured in the circular area surrounding the spot. The fluorescence intensity used for analysis was background-subtracted and averaged across all the replicas.

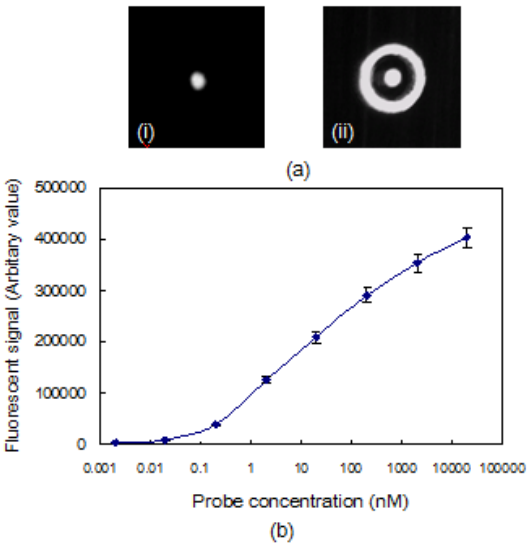

Figure 4. (a) Fluorescent images of DNA probe spotted on the micro-optical array. (b) Sensitivity test for the platform.

RESULTS AND DISCUSSION

To test the ability for SAF collection, 10 μM Cy3-labeled DNA probe was spotted on the micro-optical array. After immobilization, water was applied on top of the array. Fluorescent images were taken from both the water (top) and the COC (bottom) side. When taking directly from the top, only a small dot was seen (Figure 4a(i)); while for the image taken from bottom, a brighter dot surrounded by a light ring was observed (Figure 4a (ii)) and the signal increased 16-fold. The light-ring corresponded to the SAF emission reflected at the side-wall of the micro-optical element.

To determine the sensitivity of the system, Cy3-labeled probe was measured at concentrations ranging from 2 pM to 20 µM on the micro-optical array, as shown in Figure 4b. The detection limit was 20 pM, corresponding to 0.15 fluorophores per μm^2. The low detection limit clearly indicated the high sensitivity of the system.

The capability of the system for real-time measurements of biomolecular binding reactions was demonstrated by hybridizing the probe with 1 nM Cy3-labeled complementary and non-complementary oligonucleotide targets. As illustrated in Figure 5, for perfect match target, the binding of labelled analyte to the surface was visible as a substantial increase in signal intensity to a maximum level within 10 min. For hybridizing with the mismatched target, despite of the high concentration of free fluorophores in the bulk solution, only faint fluorescence signals were observed as an effect of non-specific adsorption of fluorophores. The mismatched target caused no further increase in the surface intensity. The results indicated that SAF detection using the micro-optical array had high surface selectivity and only imaged fluorophores in the near field. This feature allows continuous measurement of signals from the sensing surface without removing the bulk sample solution.

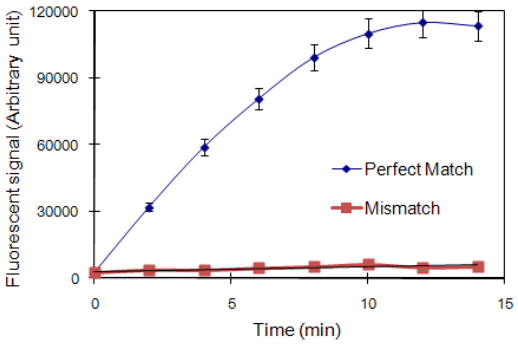

Figure 5. Real-time monitoring of target-probe hybridizations.

CONCLUSION

We have developed a novel system that utilized a micro-optical array to achieve SAF detection for multiple hybridization events in real time. Compared to conventional microarray systems, it provides enhanced fluorescence collection efficiency, high-surface selectivity and the ability of real-time measurements of hybridization. The platform will greatly expand the use and benefits of microarray technology.

ACKNOWLEDGEMENT

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REFERENCES

[1] M. Dufva, J. Petersen, L. Poulsen, "Increasing the specificity and function of DNA microarrays by processing arrays at different stringencies," Analytical and Bioanalytical Chemistry, **395**, 669-677 (2009).

[2] A. Krieg, S. Laib, T. Ruckstuhl, S. Seeger, "Fast detection of single nucleotide polymorphisms (SNPs) by primer elongation with monitoring of supercritical-angle fluorescence," ChemBioChem, **5**, 1680-1685 (2004).

[3] Patent 201210101101.9 "An Integrated Microlens Array Device".

[4] Patent 201220145566.X "An Integrated Microlens Array Device".

[5] Patent 201210104693.X "An Integrated Micro-optical Structure Array Device".

[6] Patent 201220150052.3 "An Integrated Micro-optical Structure Array Device".

[7] Y. Sun, I. R. Perch-Nielsen, M. Dufva, D. Sabourin, D. D. Bang, J. Høgberg and A. Wolff, "Direct immobilization of DNA probes on non-modified plastics by UV irradiation and integration in microfluidic devices for rapid bioassay," Analytical and Bioanalytical Chemistry, **402**, 741-748 (2012).

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