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Label-free microarray imaging for direct detection of DNA hybridization and single-nucleotide mismatches

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ABSTRACT

A novel method is proposed for direct detection of DNA hybridization on microarrays. Optical interferometry is used for label-free sensing of biomolecular accumulation on glass surfaces, enabling dynamic detection of interactions. Capabilities of the presented method are demonstrated by high-throughput sensing of solid-phase hybridization of oligonucleotides. Hybridization of surface immobilized probes with 20 base pair-long target oligonucleotides was detected by comparing the label-free microarray images taken before and after hybridization. Through dynamic data acquisition during denaturation by washing the sample with low ionic concentration buffer, melting of duplexes with a single-nucleotide mismatch was distinguished from perfectly matching duplexes with high confidence interval (>97%). The presented technique is simple, robust, and accurate, and eliminates the need of using labels or secondary reagents to monitor the oligonucleotide hybridization.

1. Introduction

DNA microarray technology is a powerful and versatile tool that is highly utilized in various fields of biology and medicine. The success of DNA microarrays stems from their capability of massive data generation and specific binding detection attributed to Watson–Crick base pairing. The impact of high-throughput platforms has been demonstrated by the utilization of DNA microarrays for many important applications including expression profiling and cancer research (Eisen et al., 1998; Golub et al., 1999; Schena et al., 1995; Wang, 2000). Microarrays are also gaining popularity in medical diagnostics as variations in the DNA sequences of individuals may affect how they develop diseases and respond to treatments (van't Veer et al., 2002). These variations are often observed as differences in a single nucleotide, or single nucleotide polymorphisms (SNP), and SNP detection is being utilized for diagnostic purposes such as evaluating an individual's risk for a certain disease and for genetic analysis in drug discovery (Galbiati et al., 2007; Syvanen, 2001). In order to take advantage of the microarray technology in SNP studies, it is imperative that the technology be capable of resolving single mismatched hybrids from perfect hybrids which have different binding energies. With conventional fluorescence based detection methods, it is often difficult to relate microarray fluorescence intensity to binding energies (Fish et al., 2007a,b). Thus, complicated procedures are required to accurately quantify the genetic expression levels based on fluorescence detection. As a result, the reliability and the reproducibility of the microarray data are questioned and significant effort is needed to achieve repeatable results such as maintaining uniform and consistent probe coverage for every spotted sample (Draghici et al., 2006; Ramdas et al., 2001; Stears et al., 2003).

Compared to fluorescence detection, label-free detection techniques offer quantitative measurement of interactions and eliminate the necessity of fluorescent labeling (Cooper, 2003; Ramachandran et al., 2005). We have recently introduced a label-free microarray imaging technique, spectral reflectance imaging biosensor (SRIB), which is amenable to high-throughput and dynamic detection of biomolecular interactions on glass surfaces (Ozkumur et al., 2008). SRIB measures the change in optical thickness of a biolayer as a result of mass accumulation and provides quantitative information about the interactions between analytes and immobilized probes (Fig. S1 of supporting information). In this study, we show that the SRIB system can specifically and quantitatively detect the hybridization of surface immobilized oligonucleotides with a target oligonucleotide. Single mismatch discrimination is also demonstrated by successfully distinguishing

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a perfectly matched duplex from a duplex containing a mismatch for only one base pair through the study of their denaturation kinetics. Presented technique reveals a very simple and accurate method for high-throughput oligonucleotide sensing and SNP studies.

2. Materials and methods

2.1. SRIB system and detection

Working principles of SRIB were explained elsewhere (Ozkumur et al., 2008). Shortly, a layered substrate of ~17 μm thermally grown SiO_2 on Si is used as the solid support for biomolecules and the illumination light goes through multiple reflections from the top surface and SiO_2–Si interface which creates an interference signature. The interference signature is characterized by illuminating the surface with a tunable laser and recording intensity images at different wavelengths by a CCD camera, forming a hyperspectral data cube. Part of the laser beam is directed to a single-cell photodetector through a fiber coupler to correct for random intensity fluctuations of the laser light (Fig. S2) (Ozkumur et al., 2009). Hyperspectral data is processed to find the total optical thickness between the reflecting interfaces for the whole surface in the field of view. The biolayer thickness of each spot is found using a custom software which calculates the average optical thickness included in a circle (oxide + biomaterial) and subtracts the average optical thickness included in an annulus surrounding this circle (oxide only) (Fig. S3).

Silicon wafers (Silicon-Valley Microelectronics), the tunable laser (NewFocus—TLB6300), the CCD camera (Q-Imaging—Rolera-XR), and the photodetector (Thorlabs—PDA65) were purchased from various vendors. Instruments were controlled by Labview (National Instruments) during data acquisition, and the data processing was done in Matlab (Mathworks) using custom-built algorithms.

2.2. Surface functionalization

A recently introduced surface functionalization technique was used to coat the silicon substrates prior to oligonucleotide spotting. This method is explained in detail elsewhere (Cretich et al., 2004; Pirri et al., 2004). Shortly, as the first step, the polymer copoly(*N*,*N*-dimethylacrylamide (DMA)–acryloyloxysuccinimide (NAS)–3(trimethoxysilyl)-propyl methacrylate (MAPS)) is synthesized. In the second step, clean SiO_2 surfaces are treated for 30 min with 0.1 M NaOH for the introduction of OH^- groups on the surface, and washed in DI water for 10 min. The chips are then immersed in the polymer solution (1%, w/v polymer in a water solution of ammonium sulfate at a 20% saturation level) for 30 min, washed extensively with DI water, dried with argon, and baked in the 80 °C oven for 15 min. The polymer-coated chips are kept in the dessicator until use. The copoly(DMA–NAS–MAPS) prepared this way self-adsorbs to the surface and enables covalent attachment of amino-modified oligonucleotides.

2.3. Oligonucleotide probe design

All DNA sequences were purchased from Integrated DNA Technologies (IDT). The sequences of all the oligonucleotides used in this study are shown in Table 1. The oligonucleotides were designed to have minimum self-complementary interaction and approximately equal AT and GC content. The C–C mismatch was introduced in the middle of the sequence in case of the single mismatch probes and every third of the sequence in the case of the double mismatch probes to maximize duplex instability (Peyret et al., 1999). Thermodynamic parameters for the sequences were confirmed with the Oligo Analyzer provided by IDT. All probes were amino modified at the 5′ end to enable their covalent attachment to the polymer coating on the surface. One 20-mer target sequence was used. One base from each end of the 20-mer probe sequences was excluded for the 18-mer probes. 40-mer probe (40(−)) with no complementary region to the target was used as the negative control. Double stranded 20-mer (20ds), which was hybridized in solution prior to spotting, was also used as an additional control. Hybridization of the duplex was carried out with equimolar amounts of the 20-mer perfect match strands and the target strands in 2× SSC by heating the sample to 85 °C for 3 min and letting it cool slowly to room temperature.

2.4. Spotting and hybridization

Substrates with 17 μm oxide were used for spotting following the surface functionalization. All probes were spotted at 25 μM concentration in 150 mM potassium phosphate buffer (pH 8.5) with BioOdyssey™ Calligrapher™ MiniArrayer (Bio-Rad). The spotted arrays were left in a humid chamber overnight and washed the next day. The washing procedure consisted of four 10-min washes with 6× SSPE containing 0.01% Tween-20 at 40 °C with agitation. The arrays were dried with argon gas and scanned with SRIB, and/or stored in a dessicator for later use.

The spotted arrays were incubated with the hybridization solution containing 1 μM target DNA for 2 h at 40 °C. The hybridization buffer was composed of 100 mM MES, 1 M [Na^+], 20 mM EDTA and 0.01% Tween-20. At the end of the hybridization, the sample was washed with the same protocol that was used after spotting, then dried with argon gas for scanning.

3. Results

3.1. End-point detection of DNA hybridization

The DNA samples listed in Table 1 were spotted on a substrate that was functionalized with a novel polymeric coating developed for conventional glass microarray slides (Cretich et al., 2004; Pirri et al., 2004). It has been shown that this surface coating provides high functional probe density by significantly increasing its volume when immersed in buffer (Yalcin et al., 2009). Since the SRIB utilizes a SiO_2 surface for probe immobilization, the coating was readily applied.

The complete array was formed of 4 replicate arrays, containing a total of 40 replicate spots for each DNA sample (Fig. 1a). The spots are labeled according to their strand length (20 for 20-mers and 18 for 18-mers), and how they match the target sequence (PM for perfect match, MM for single mismatch, and DM for double mismatch). The sequence labeled as 40(−) is a random sequence used for the negative control. The 20ds sequence is a double-stranded oligonucleotide sequence formed by hybridizing the 20PM sequence with the target strand in solution, prior to spotting.

After probe immobilization and washing, the sample was scanned with SRIB and the biolayer thickness for each spot was found and visualized in a gray-scale image (Fig. 1a). After hybridization, the sample was scanned again, and the initial data was subtracted from the post-hybridization data to find the incremental mass changes on the spots (Fig. 1b). The specific binding of the target to the 20-mer and 18-mer single strands is clearly seen whereas there is no binding to the 40(−) and the 20ds. The faint spot outlines for 40(−) and the 20ds are seen in the difference image, Fig. 1b, because of the registration error between the pre-hybridization (Fig. 1a) and post-hybridization (data not shown) images. Reduced hybridization for the double mismatched spots of 20DM and 18DM are visible as weaker spot intensities in the difference image.