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Real-time detection of DNA hybridization on microarray using a CCD-based imaging system equipped with a rotated microlens array disk☆

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ABSTRACT

This work describes a novel charge-coupled device (CCD)-based imaging system (MB Biochip Reader™) for real-time detection of DNA hybridization to DNA microarrays. The MB Biochip Reader™ consisted of a laser light source (532 nm), a microlens array for generation of a multi-beam laser, and a CCD for 2-D signal imaging. The MB Biochip Reader™ with a rotated microlens array, allowed large-field imaging (6.2 mm × 7.6 mm with 6.45 μm resolution) with fast time-resolution at 0.2 s without speckle noise. Furthermore, real-time detection of DNA hybridization, which is sufficient to obtain accurate data from tens of thousands of array element per field, was successfully performed without the need for laser scanning. The performance of the MB Biochip Reader™ for DNA microarray imaging was similar to the commercially available photomultiplier tube (PMT)-based microarray scanner, ScanArray Lite. The system potentially could be applied toward real-time analysis in many other fluorescent techniques in addition to real-time DNA microarray analysis.

1. Introduction

DNA microarrays have been widely used as a high-throughput tool to obtain large amounts of information in medical science and biotechnology, such as gene expression analysis, single nucleotide polymorphism detection or pathogenic microbial DNA detection (Anantharaman and Chew, 2009; Izutsu et al., 2008; Laubinger et al., 2008; Schena et al., 1995; Shen et al., 2007; Weile and Knabbe, 2009). In general, a large number of DNA probes (several thousands to several tens of thousands) are spotted and arrayed on a DNA microarray platform to perform high-throughput gene analysis based on DNA–DNA hybridization. Reaction times for hybridization to DNA microarrays are quite long to complete all the hybridization reactions in a large-field area. One of the main reasons for this is the low hybridization rates between free DNA and probe DNA immobilized on a solid support (Gao et al., 2006). Based on this understanding, the hybridization (or dissociation) kinetics on a solid support was intensively studied to better understand this issue and to improve the rate of DNA hybridization to DNA on a solid support (Ng and Liu, 2005; Sorokin et al., 2006). To obtain detailed information on the hybridization behaviors on solid supports, real-time detection of DNA hybridization in large-field area was required.

PMT-based scanning systems have been adopted for use in most commercialized DNA microarray detectors due to the high gain potential (Voss, 2000). However, a laser scanning system is required for two-dimensional (2-D) imaging of fluorescent DNA spots, so performance is limited in time-resolution. Alternatively, 2-D detectors (i.e., charge-coupled device (CCD) arrays) can be used for 2-D imaging to improve time-resolution and perform real-time imaging. In the case of CCD-based DNA microarray imaging, laser light has not been used as an excitation source because of the speckle noise generated by interference between lasers (Shin et al., 2006). Our research group has developed a CCD-based fluorescent reader system, MB Biochip Reader™, which is applied to the confocal scanner technology for noiseless fluorescent imaging with a rotated microlens array containing approximately 20,000 microlenses (Tanaami et al., 2002). The MB Biochip Reader™ consists of a laser light source, a microlens array disk, optical filters, and a CCD chip. The reduction of speckle noise can be accomplished by the generation of a splitting laser beam using a microlens array disk. A single laser beam is split by a rotated microlens array disk at high speed, resulting in the generation of a uniform multi-laser beam, and reduction of speckle noise.

In this study, the MB Biochip Reader™ was applied to real-time detection of a DNA microarray based on DNA hybridization. In particular, the effect of a microlens array disk on large-field fluorescent imaging was investigated. Furthermore, real-time DNA hybridization between the molecular beacon and probe DNA immobilized

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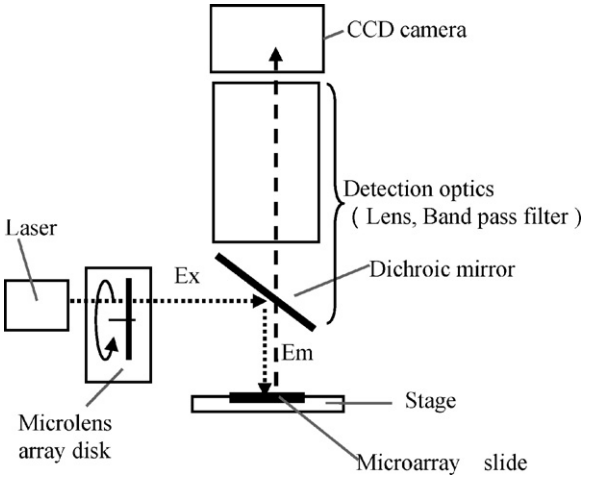

Fig. 1. Schematic diagram of optical components in the MB Biochip Reader™.

on a solid support was analyzed using the MB Biochip Reader™ with microlens array disk. Our proposed system will expand the application range of DNA microarray analysis.

2. Materials and methods

2.1. Fluorescence reader system, MB Biochip Reader™

Fig. 1 shows the optical components of the prototype of MB Biochip Reader™. The MB Biochip Reader™ consists of a laser light source for wavelength at 532 nm (Compass™ 215 M, COHERENT, Santa Clara, CA), a microlens array, a dichroic mirror, a band-pass filter and a custom-designed CCD camera. The microlens array was used for generation of a multi-beam laser. The MB Biochip Reader™ requires no scanning components in the *X* and *Y* direction because multi-beam excitation by the microlens array and subsequent detection with the CCD camera allows us to obtain a 2-D image of fluorescent dots in 6.45 mm × 8.8 mm as a maximum area. The CCD chip (Sony, Japan, ICX285AL) was operated at 2 °C for visualizing 2-D signal images. Data obtained by the CCD camera was stored at 16-bit gray scale levels, and fluorescence data was analyzed using ImagePro software (Media Cybernetics, Bethesda, MD). To evaluate the performance of the MB Biochip Reader™, the 2-D image of a ready-made microarray (Scanner Calibration Slide, Full Moon BioSystems, Sunnyvale, CA) was obtained at 0.2-s exposure time under dry conditions. The Scanner Calibration Slide consists of 28 sets of 2-fold dilutions of Cy3 (lane 1–9: 574–147,000 molecules/μm^2), coupled with 12 repeats of each sample (Fig. 3A).

2.2. Preparation of hand-made DNA microarray

N-hydroxysuccinimide (NHS) ester-activated glass slides (Geneslide, TOYO KOHAN, Japan) were used for the immobilization of amine-labeled oligonucleotides. The amine-labeled probe oligonucleotides (1–10 μM, 25 μL) (Table 1) were spotted onto a glass slide, Geneslide (TOYO KOHAN, Japan) using synQUAD™ Dispensers (Cartesian Technologies, Inc., Irvine, CA). Dimethyl sulfoxide (DMSO) was used as spotting solution. The glass slides were incubated at 80 °C for 60 min, and washed with 2 × SSC containing 0.2% SDS for 15 min. Subsequently, unreacted NHS ester on the glass slides was inactivated by heat treatment at 90 °C for 5 min, rinsed with ultra pure water for 1 min, and dried with a blower at room temperature.

Table 1
Molecular beacon target and probe oligonucleotide sequences. Underlining indicates the stem sequence of the molecular beacon.

Name	Sequence (5′-3′)	Modification
MB-T	CGCTCCCTTTTTTTTTTTCCGAGCG	5′-Cy3, 3′-BHQ2
Probe A	GGAAAAAAAAAAAGG	5′-C6-NH2
Probe T	GGTTTTTTTTTTGG	5′-C6-NH2

2.3. Real-time detection of DNA hybridization on microarray

The molecular beacon (25-mer; Cy3-5′-CGCTCCCTTT-TTTTTTTTCCGAGCG-3′-BHQ2), which contained Cy3 at the 3′ end and Black Hole Quencher 2 (BHQ2) at the 5′ end, was used as a target (Table 1). The sequence of molecular beacons was designed as described previously (Bonnet et al., 1999). The molecular beacons are in a closed state at 25 °C. The Cy3 and the BHQ2 are maintained in close contact by the hairpin structure, without fluorescence due to quenching of the fluorophore with fluorescence resonance energy transfer (FRET). The target molecular beacon (10 nM) in hybridization buffer was introduced onto the DNA microarray under a hybridization chamber (10 mm × 10 mm × 0.25 mm) and incubated at 25 °C. In this assay format, fluorescence occurs when the target molecular beacon was hybridized with the probe oligonucleotide on the glass slide. The glass slides were introduced into the MB Biochip Reader™. The fluorescence intensities were monitored by the MB Biochip Reader™ after the addition of the target molecular beacon. Image processing was performed using a fluorescence reader system every 0.5–30 min.

2.4. Comparison between MB Biochip Reader™ and PMT-based scanner

The target molecular beacon was hybridized with the probe oligonucleotide on the hand-made array slide for 2 hours in the same condition as the above-mentioned. The microarray slide were washed with 2 × SSC containing 0.2% SDS for 15 min. Subsequently, the microarrays were rinsed with 0.05 × SSC and ultra pure water, and then dried with a centrifuge at 1000 rpm. Microarray images were obtained under dry conditions by using the MB Biochip Reader™ or a commercially available PMT-based microarray scanner, ScanArray Lite (PerkinElmer, Waltham, MA) as a comparison.

3. Results and discussion

3.1. Evaluation of a large-field imaging using the MB Biochip Reader™

For a preliminary performance comparison, fluorescent images of a flat glass slide surface were obtained by the MB Biochip Reader™ with and without the microlens array. A speckle noise was observed on the entire imaging area without the microlens array (Fig. 2A), while a uniform bright-image, originating from reflection of the excitation light, was obtained by the MB Biochip Reader™ equipped with the microlens array (Fig. 2B). The entire size of the uniform bright-image was 6.2 mm × 7.6 mm at 6.45-μm resolution. These results suggested that the MB Biochip Reader™ with microlens array could be applied to the large-field imaging of DNA microarray. Fig. 3B shows a fluorescent image of the Scanner Calibration Slide obtained by using the MB Biochip Reader™ at 0.2 s exposure time. The fluorescent spots of Cy3 were clearly and uniformly visualized on the entire area. The line profiles on the spots of Fig. 3B over 5 mm are presented in Fig. 3C. The fluorescent intensities were proportional to the immobilization density of Cy3 with high linearity ($r^2 = 0.993$). The fluorescence detection sensitivity of the MB Biochip Reader™ was evaluated with the Scanner