Scanning Microarrays with the Sapphire FL Biomolecular Imager

Introduction

DNA microarrays allow high throughput testing of hundreds or thousands of targets in parallel. Microarray analysis is a hybridization method which investigates whether something in a sample is able to bind to an immobilized target. Microarrays have been used extensively to study gene expression and to carry out genotyping and have a broad range of research and clinical applications. Essential to the use of microarrays in any application is the ability to image and collect quantitative data from each of the targets arranged as tiny dots on the array.

A DNA microarray can be thought of as a miniaturized dot blot which can determine the presence or absence, or estimate the relative abundance of, a nucleotide sequence in a sample. The microarray consists of a large number of immobilized nucleotide probes. Nucleotides from a sample are labeled and incubated with the array, allowing any complementary nucleotides within the sample to hybridize to the immobilized targets. Unbound sample is washed away and any bound nucleotides from the sample are detected. Most often, the sample is fluorescently labeled since fluorescent detection provides good sensitivity and a sufficient linear dynamic range (Bumgarner). In addition, fluorescent detection allows two or more samples to be hybridized to the array in a single experiment by labeling each sample with a different fluorophore. Co-hybridization allows the two samples to be compared within a single experiment, so an experimental sample can be directly compared to a standard or control sample while avoiding potential complications that might arise from array-to-array variations.

To study gene expression, DNA fragments that correlate to expressed genes are spotted on the array. RNA extracted from a sample is converted into fluorescently labeled cDNA or cRNA and hybridized to the slide. Expressed genes will result in fluorescence of complementary sequences on the array. For genotyping, the DNA fragments spotted on the array are designed to hybridize to specific single nucleotide polymorphisms (SNPs) or variants.

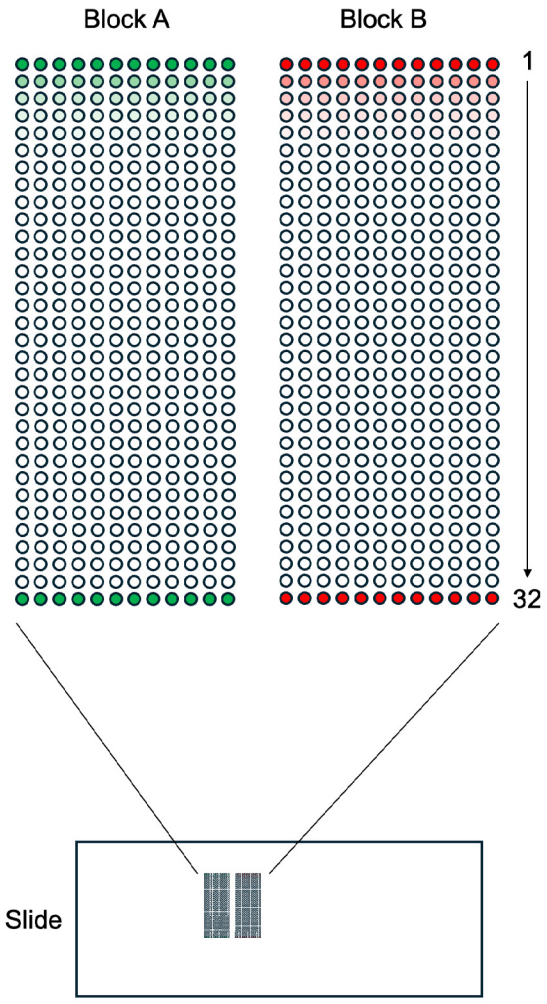


Figure 1. The layout of samples on the microarray imaged in this study. The array contains serial dilutions of Cy3 in block A and Cy5 in block B. Each concentration is repeated 12 times.

Microarrays may also be used to carry out comparative genomic hybridization (CGH), also known as chromosomal microarray analysis (CMA), a genome-wide screening method for detecting copy number variations in genomic DNA (Theisen). Array CGH (aCGH) is carried out by fluorescently labeling the genomic DNA from two samples and hybridizing the DNA to an array of probes representing the genomic DNA sequence. A change in the relative fluorescence intensity of the two bound samples indicates the presence of copy number changes, whether deletions or amplifications, even in small segments of DNA (Theisen).

Binding of samples to other types of probes besides nucleotides has been analyzed using arrays, including peptides, carbohydrates, and lipids. In addition to their high throughput nature, advantages of the microarray approach include a relatively simple workflow and rapid results (Roberts). A disadvantage is that the assays require some prior knowledge for slide design; a microarray can only test binding to the targets that are included on the array.

While high-throughput sequencing (RNA-Seq) has largely replaced microarrays for genotyping and gene expression studies, microarrays remain important for applications including aCGH as well as studies of the microbiome, of DNA-protein interactions such as mapping transcription factor binding sites, and of DNA modifications such as methylation of CpG islands (Roberts). In addition, microarrays are used in numerous clinical and diagnostic applications such as characterizing tumor biomarkers, genotyping cancers to select treatment, and genotyping tissues before organ transplant (Roberts).

Imaging microarrays requires a scanner with sufficient resolution, appropriate light sources, a wide dynamic range, and the ability to reproducibly position and focus on a microarray slide. Dedicated microarray scanning systems exist but may not be the optimal choice for a lab that frequently conducts other types of imaging. A multifaceted imaging system such as the Sapphire FL Biomolecular Imager provides the ability to scan microarrays among its large catalog of compatible applications.

This application note will demonstrate the ability to capture high-resolution images of a two-color microarray with the Sapphire FL Biomolecular Imager.

Methods

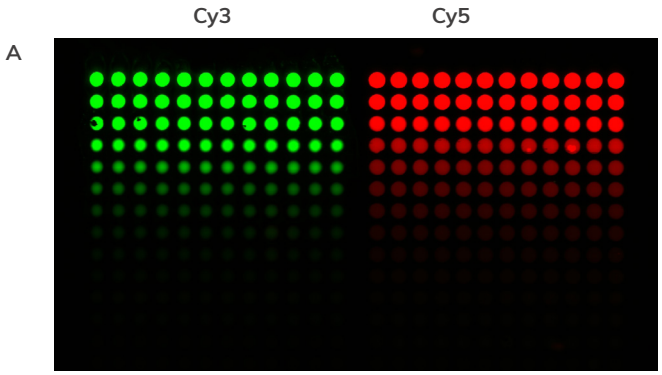
A Microarray Scanner Calibration Slide (Full Moon BioSystems part #DS 01) was scanned using the Sapphire FL Biomolecular Imager. The slide contains two arrays of spots containing dilution series of either Cy™3 or Cy™5 dye. The layout of the slide is shown in Figure 1. The spot to center distance of the array was 350 µm.

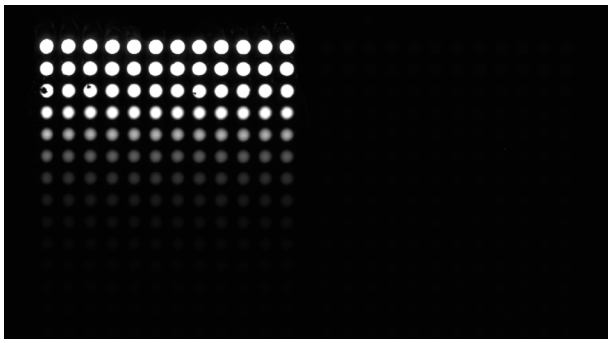
For scanning on the Sapphire FL Biomolecular Imager, the slide was placed face down in the slide holder accessory (Azure Biosystems product IS4053). Scan parameters included Slide (+1.00 mm) for Focus Type, 5-micron pixel size (resolution), and the highest Scan Speed. Cy3 was imaged using the 532 Standard Optical Module with laser intensity L4 and Cy5 was imaged using the 638 Standard Optical Module with laser intensity 6.

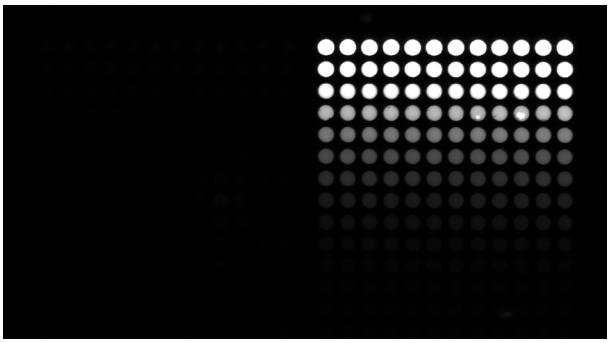
Results and Discussion

Images of microarrays scanned on the Sapphire FL Biomolecular Imager are shown in Figure 2. The microarray that was scanned contains two arrays of spots, one containing two-fold serial dilutions of Cy3 and the other containing two-fold serial dilutions of Cy5. Cy3 was imaged using the 532 Standard Optical Module of the Sapphire FL. The raw image is shown in Panel B of Figure 2. Cy5 was imaged using the 638 Standard Optical Module and the raw image is shown in Panel C of Figure 2. Panel A shows the two channels merged into a single image, with the Cy3 channel shown in green and the Cy5 channel in red. The images were captured with 5-micron resolution, providing clear images of the spots on the array. The spot-to-spot distance on the array was 350 µm.

These images demonstrate the ability of the Sapphire FL to scan microarrays and generate high-resolution images ready for downstream analysis. The Sapphire FL simplifies slide imaging to make obtaining clear images of slides, including microarray slides, simple and straightforward. When using the slide holder accessory, the Sapphire FL's Slide focus setting







D

Row	Fluorophores/µm²	Row	Fluorophores/µm²
1	1.47 x10⁵	8	1.15 x10³
2	7.35 x10⁴	9	5.74 x10²
3	3.68 x 10⁴	10	2.87 x10²
4	1.84 x 10⁴	11	1.44 x10²
5	9.19 x 10³	12	7.18 x10¹
6	4.59 x10³	13	3.59 x10¹
7	2.30 x10³	14	1.79 x10¹

Figure 2. Microarray scanned on the Sapphire FL Biomolecular Imager with 5-micron resolution. A. Merged image containing the scans in the 532 channel (green) and 638 channel (red). B. Scan using the 532 Standard Optical Module. C. Scan using the 638 Standard Optical Module. Images were cropped to include rows 1 through 14 of each array block. D. Concentration of fluorophores per dot by row shown in parts A, B, and C.

provides the ideal, or very close to ideal, focal plane. If additional focal optimization is required, the focal plane can be adjusted by as little as 0.01 mm. The slide holder accessory holds the slide off the glass surface of the imaging system, eliminating Newton's rings interference patterns. In addition, the accessory positions up to 15 slides for scanning at one time for increased throughput and efficiency.

The AzureSpot Pro software includes multiple analysis modes for different sample types, including an Array mode. Within this software, it is easy to define wells or spots for multi-well plates or arrayed samples including microarrays and dot blots. The Sapphire FL has standard optical modules for many common fluorescent dyes, including Cy3 and Cy5. In addition, the instrument is fully customizable with user-changeable laser and filter modules that can be mixed and matched to work with almost any fluorophore. Learn more about the Sapphire FL Biomolecular Imager at https://azurebiosystems.com/products/sapphirefl/.

References

1. Bumgarner R. DNA microarrays: types, applications and their future. Curr Protoc Mol Bio. 2013;Chapter 22, Unit 22.1. doi: 10.1002/0471142727.mb2201s101
2. Theisen A. Microarray-based comparative genomic hybridization (aCGH). Nat Educat. 2008;1(1):45. https://www.nature.com/scitable/topicpage/microarray-based-comparative-genomic-hybridization-acgh-45432/
3. Roberts JP. Microarrays still have their uses and fans. Biocompare. Posted Oct 10, 2019. https://www.biocompare.com/Editorial-Articles/518117-Microarrays-Still-Have-Their-Uses-and-Fans/

MM-0172 R1