Using the Molecular Imager® PharosFX™ System to Image Arrays Printed With the BioOdyssey™ Calligrapher™ MiniArrayer

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Introduction

The BioOdyssey Calligrapher miniarrayer is designed to print arrays onto glass slides, into 96-well microplates, or onto membranes. Printing onto membranes or nitrocellulose-coated slides is particularly useful for proteomics research. Two-color laser scanners typically scan 1" x 3" slides and will not accommodate larger nitrocellulose formats. In this study, we investigated imaging both nitrocellulose membranes and FAST slides with the Molecular Imager PharosFX system.

The PharosFX system is designed specifically for complex imaging applications involving multiple fluorescence wavelengths in gels, blots, microplates, and macroarrays. It allows multicolor detection of a wide range of fluorophores via direct laser excitation, high excitation efficiency, and precise spectral assignment. The system's excitation/emission filter combinations are designed to maximize the signal-to-noise ratio (SNR) and thus increase sensitivity.

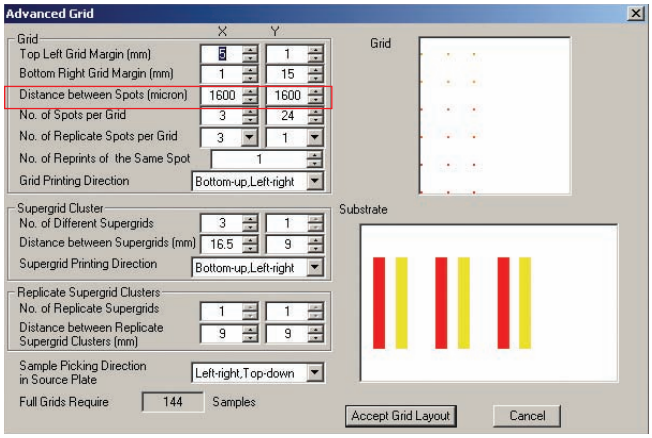

Fig. 1. Advanced Grid window displays the parameters used for printing nitrocellulose membranes using TeleChem SMP11 pins. The "Distance between Spots" has been set to 1,600 µm to account for wicking.

Here, we examine the ability of the PharosFX system to scan microarrays fabricated by the Calligrapher miniarrayer on nitrocellulose membranes and FAST slides, a glass-supported nitrocellulose surface. We demonstrate that the PharosFX system performs with the same sensitivity as a two-color microarray scanner when used with a nitrocellulose substrate arrayed with an adequate spot size.

Methods

For each test, arrays were printed with the BioOdyssey Calligrapher miniarrayer equipped with two pins, either Stealth SMP3 or SMP11 microarray spotting pins (TeleChem International, Inc.). Substrates included 0.2 µm pore nitrocellulose membranes (Whatman Inc.) cut to fit into an OmniTray (Nalge Nunc International) or FAST slides (Whatman Inc.). Oligonucleotides labeled with one of three dyes, Alexa Fluor 546 (Integrated DNA Technologies, Inc.), Quasar570 (BioSearch Technologies, Inc.), or Cy3 (TriLink BioTechnologies, Inc.), were arrayed individually on the substrates. The dye-labeled oligonucleotides were suspended at 50 µM in 1x Bio-Rad printing buffer containing 200 ng/µl carrier salmon sperm DNA. A series of 2-fold serial dilutions was carried out to yield a final oligonucleotide concentration of 0.095 nM. The dilution series was spotted onto each substrate at least in triplicate. To print the larger spot size, the spot distance for the grid was adjusted to 1,600 µm by accessing the Advanced Grid of the Define Array Parameters feature (Figure 1).

After arraying, the substrates were dried for 30 min and then imaged with the PharosFX system using 532 nm excitation and 605 nm emission filters, with the gain set at 35% for the membrane and at 55% for the FAST slides. For comparison, the printed arrays were also imaged using a two-color microarray scanner. To image nitrocellulose membranes in the microarray scanner, membranes were cut to fit onto a glass slide, and secured with thin pieces of tape. At an SNR (background-adjusted signal divided by the standard deviation of the background signal) of 3, the limit of detection (LOD) for each substrate with each instrument was determined in fluorophores/μm^2. This value was calculated as: (volume deposited) x (concentration of oligo) x Avogadro's number/surface area of the spot. For FAST slides, we assumed that the volume deposited was 0.7 nl for SMP3 pins and 4.4 nl for SMP11 pins. For membranes, we assumed that 50 nl was deposited, based on printing 3–4 spots/dip.

Results and Discussion

We tested three dyes with similar spectral properties, Cy3, Alexa Fluor 546, and Quasar570, and found that each performed similarly (data not shown). All results shown in this report were obtained with Cy3. The rainbow images collected from the PharosFX are shown in Figure 2.

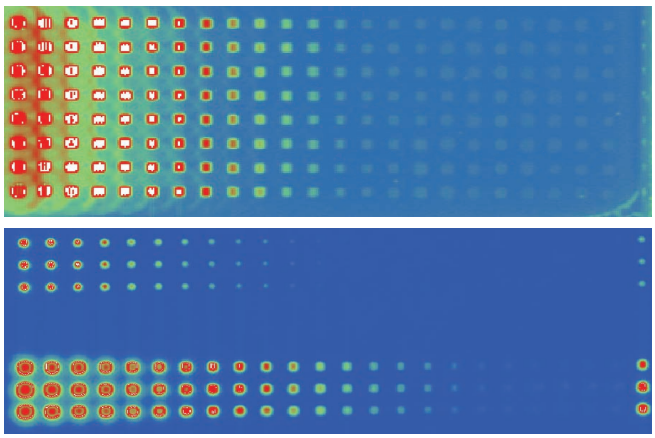

Fig. 2. Rainbow images of substrates containing Cy3-labeled oligonucleotide dilutions imaged with the PharosFX scanner. Top, FAST slide with 400 µm spots; bottom, nitrocellulose membrane (upper rows are 400 µm spots, lower rows are 800 µm). Spots were arrayed as described in the text.

In general, for an imaging device to detect a spot, the spot must be composed of at least 50–100 pixels. Since the resolution of the PharosFX is 50 µm, it was expected that a spot size of at least 400 µm would be required to reliably detect the spots. This spot diameter was achieved on FAST slides using the SMP11 pin, and on nitrocellulose membranes using the SMP3 pin. The SMP11 pin printed an 800 µm spot diameter on nitrocellulose membrane. The diameters printed on nitrocellulose membrane were greater than on FAST slides due to sample wicking.

Figure 3 displays the signal intensity and SNR obtained with the PharosFX or the microarray scanner for each fluorophore concentration and each spot diameter on nitrocellulose membranes and FAST slides. We attribute the higher error rates seen with the nitrocellulose to its physical properties.

Equivalent signal intensity was obtained with either the PharosFX or the microarray scanner when imaging FAST slides printed with SMP11 pins. The larger spot sizes (400 µm) produced by the SMP11 pin yielded adequate data for the PharosFX to capture.

Likewise, equivalent signal intensity was obtained with either the PharosFX or the microarray scanner when analyzing imaged array data from nitrocellulose membranes printed with either the SMP3 or SMP11 pin. Data were obtained over approximately 5 orders of magnitude using either instrument for both substrates. However, larger spot sizes (800 µm) were necessary to yield acceptable data from both the PharosFX system and the laser scanner.

The SNR results showed similar trends for each instrument and each substrate (Figure 3B, D). The higher the SNR, the more reliably the instrument can differentiate a signal from background. The commonly accepted SNR value of 3 was used to determine the LOD data shown in Table 1. The larger spot diameter (800 µm) on the membrane was required to obtain an acceptable LOD of 6–10 fluorophores/μm^2.

Table 1. Comparison of limit of detection (LOD) obtained with different array substrates and imaging instruments at two spot diameters.

Substrate	Instrument	Pin Type	Spot Size (µm)	LOD
FAST slide	PharosFX system	SMP11	400	2–4 fluorophores/μm^2
	Microarray scanner	SMP11	400	2–4 fluorophores/μm^2
Nitrocellulose	PharosFX system	SMP3	400	300–600 fluorophores/μm^2
	Microarray scanner	SMP3	400	300–600 fluorophores/μm^2
	PharosFX system	SMP11	800	6–10 fluorophores/μm^2
	Microarray scanner	SMP11	800	6–10 fluorophores/μm^2

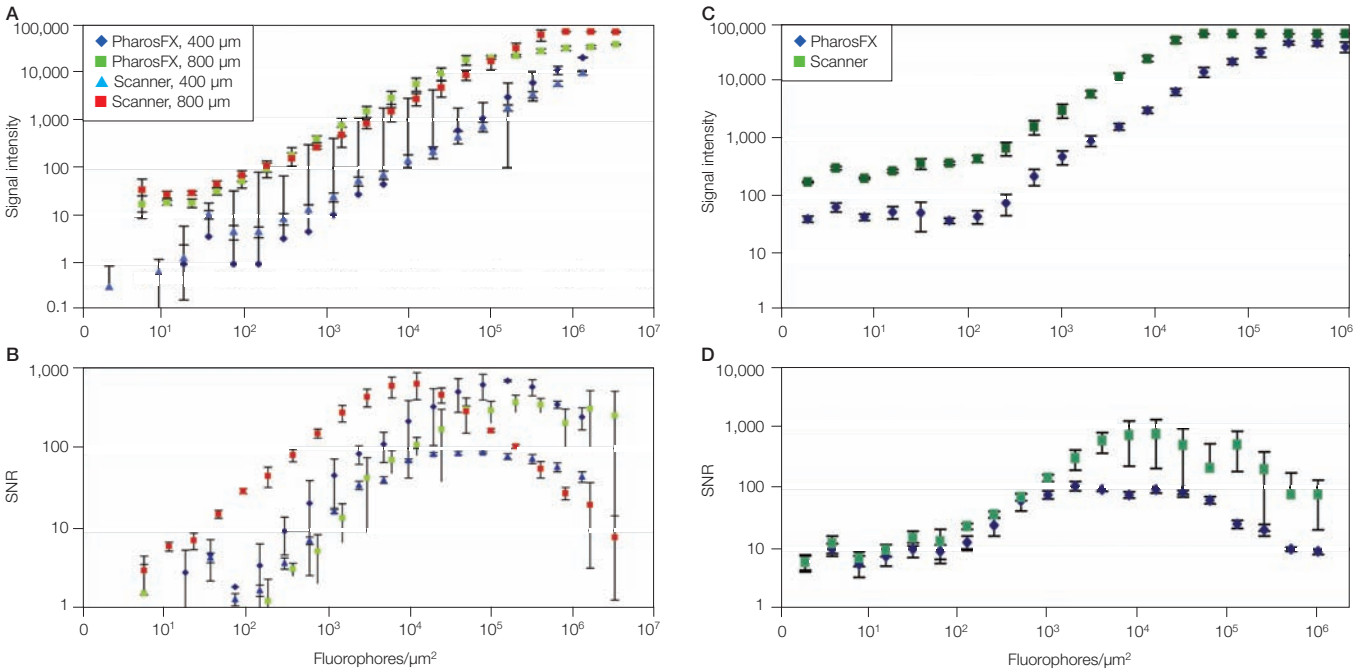

Fig. 3. Comparison of signal intensity and SNR of arrays measured using the PharosFX system and a two-color microarray scanner. A, signal intensity, and **B**, SNR for nitrocellulose membranes. Values are averages of three replicates. **C**, signal intensity, and **D**, SNR for FAST slides. Values are averages of eight replicates. SNR was calculated by dividing the background-corrected signal by the SD of the background. Error bars indicate SD. FAST slides show only one spot size, as described in text.

Conclusions

The BioOdyssey Calligrapher miniarrayer has the flexibility to print onto various substrates, including nitrocellulose membranes. We have demonstrated that the PharosFX system can accurately quantitate fluorescent arrays fabricated on nitrocellulose membranes or FAST slides when an appropriate spot size is printed. The values obtained with the PharosFX were consistent with those measured using a standard microarray scanner. While only data with a Cy3-labeled oligonucleotide are shown here, the PharosFX has multiple excitation lasers coupled with emission filters that allow detection of different fluorophores, a flexibility that allows scientists to perform various types of assays using arrays printed with the Calligrapher miniarrayer.

We have also demonstrated that imaging for the quantitation of arrays on nitrocellulose can be performed with a standard laser-based microarray scanner by simply affixing the membrane to a glass slide. These options should allow greater use of array technology in laboratories equipped with either type of scanner.

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