ScanArray G_X PLUS/ProScanArray Competitive Performance Analysis Using a Commercial Test Slide

APPLICATION NOTE

ARRAY SYSTEMS

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Introduction

Protein and DNA microarrays are widely used in academic and commercial laboratory settings. The density or number of spots on these arrays continues to increase to the point where spot numbers in the hundreds of thousands are typical, requiring a microarray scanner that can consistently deliver high sensitivity and resolution. The attributes of a good scanner are many, but a few differentiate the scanners produced by different manufacturers: dynamic range, limits of detection, limits of quantitation, and crosstalk between fluorescent probes. The dynamic range of an analytical assay is the range in concentration of analyte over which the assay yields a response over background that is proportional to the analyte concentration. A wide dynamic range allows a greater concentration range of sample to be measured by an instrument using the same setting. The limits of detection (LD) and quantitation (LOQ) are similar, yet differ in an important aspect. LD quantifies the lowest concentration the scanner can detect above the background signal. The LOQ is typically the lowest concentration value on a standard curve that is at least 5X above the blank measurement and reproducible within a precision of 20%. The fact that a spot falls within the LD does not imply that it can be used in a quantitative assay –

to be quantitatively useful, it must also fall within the stricter LOQ. Crosstalk arises if the excitation and emission wavelengths of the different fluorescent probes overlap slightly, as is the case with the most commonly used fluorescent probe molecules Cyanine-3 and Cyanine-5. The ability to discriminate between these molecules and ensure that the entire signal collected is from a specific probe relies upon the discrimination of the excitation and emission filters used in the scanner. Filters with a wider passband, i.e. wider range of wavelengths, admit more light but allow for more crosstalk whereas a narrow passband filter minimizes crosstalk but also cuts out light.

The following experiments compare the attributes described above for the PerkinElmer ScanArray G_x PLUS/ProScanArray™ family of scanners and a leading competitor's sequential scanner.

Materials and methods:

Slide:

- The Full Moon Biosystems All Purpose Scanner Evaluation Slide (Prod # AV 05) was used to perform these experiments. The slide contains four separate blocks of arrays. The top two blocks are used for the general evaluation, verification, and demonstration of scanners. The bottom two blocks are used for quantitative analysis of a scanner's performance. Each block consists of 28 sets of spots with two-fold dilutions of a Cyanine-3 or Cyanine-5 solution. Each block also contains 3 sets of blanks and one set of position markers, and each column contains 12 repeats of each solution.

Scanners:

- PerkinElmer microarray scanner (ScanArray G_x PLUS) with two lasers (red and green) and three standard ScanArray emission filters per channel: 560 nm, 570 nm, 578 nm, 660 nm, 670 nm and 694 nm all with a 10 nm FWHM passband.
- A competitor's scanner with two lasers (red and green) and 16-position filter wheel.

Each scanner was turned on and allowed to warm up for 30 minutes. The slide was scanned in both green and red channels at various PMT and laser power settings. For each scanner, the automated PMT and laser power settings were used for the initial scans but these settings were then manually optimized for each subsequent experiment. The same settings that were determined in the first scan of each instrument were also used on the last scan to control for the effects of any possible photobleaching. The resulting images were saved and processed using ScanArray Express 3.0 microarray analysis software. When analyzing images for comparison, data was used from each channel that was equal in signal strength at the given PMT setting to allow a direct comparison. The resulting data was exported to GraphPad Prism® for further analysis and graphing.

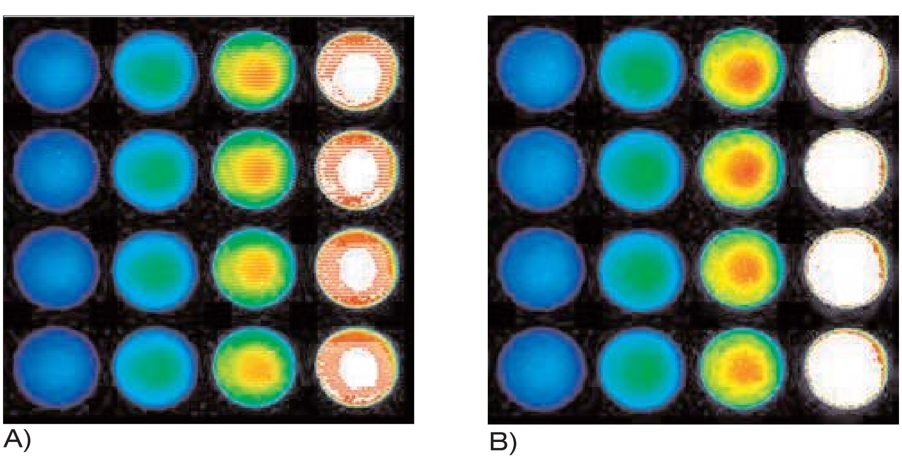

Figure 1. A) Competitive scanner image from a Cyanine-3 scan of the calibration slide. B) PerkinElmer microarray scanner image from a Cyanine-3 scan of the calibration slide. Note that both images have similar spot intensities and that the same area of the test slide was scanned for each image.

Results and discussion:

Image quality: In general, the images obtained from the PerkinElmer scanner were of better quality when compared to the competitive scanner (Figure 1). In many cases, the latter's images showed a series of lines of higher intensity going through the images on the Cyanine-3 channels. Data collected from these images could lead to higher amounts of error and subsequently inaccurate quantitation results. The PerkinElmer scanner showed no such lines in any scans.

Dynamic Range: The dynamic range of each instrument was measured by plotting the data resulting from the scan of the calibration slide scan on a log/log plot. The PMT and laser power settings were comparable when these experiments were performed resulting in very similar spot intensities and ratios. The x-axis units were expressed in fluorophores/μm^2 and the values were derived from the dilution series that was supplied by the manufacturer.

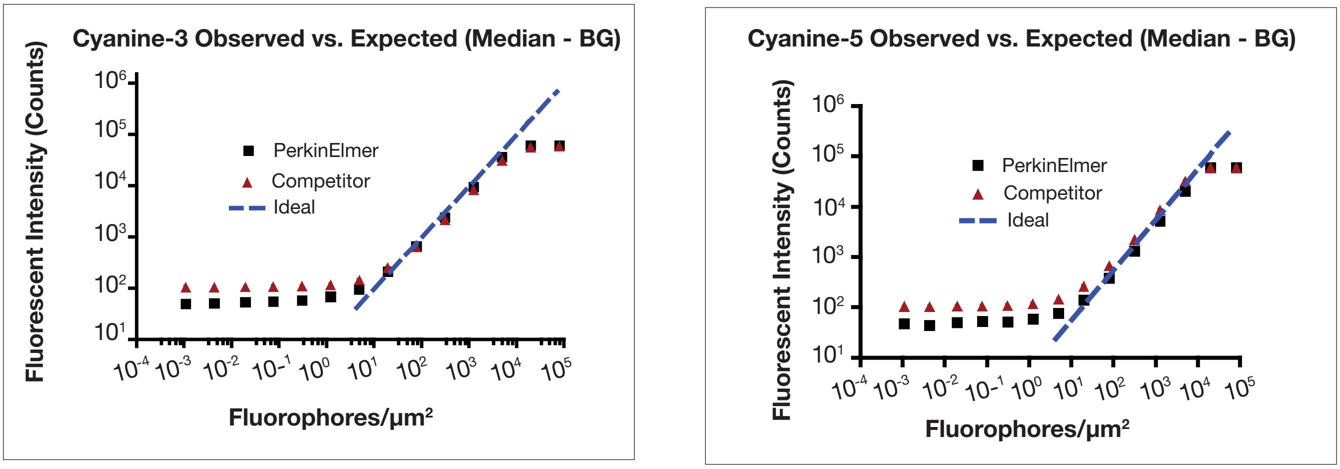

Figure 2. Comparison of dynamic range and linear response between the competitor's scanner (red triangles) and the PerkinElmer scanner (black square). Data is plotted in log/log format and is corrected for background. The linear response is estimated from the plot of the calculated ideal data (blue line).

The y-axis data was derived from processing images from both scanners using ScanArray Express 3.0 microarray analysis software. By fitting a straight line to the linear portion of the response curves, one can estimate the dynamic range of the curves. As seen in Figure 2, the dynamic range for both instruments is very similar in each channel – not surprising given the similar PMTs used in each instrument.

LD/LOQ: Using both scanners it was possible to determine the lowest dilution of each fluorescent dye that produced a spot falling at a statistically significant value above the background level. We have set the lowest limit of detection to 0.001 fluorophores/µm^2, which is the lowest dilution on the test slide. On average the 0.001 fluorophores/µm^2 spots were 2 – 2.5 times more intense than the blank or buffer only spots on the slide. To calculate the LOQ, we applied a filter that was twice the standard deviation of the signal taken from the most diluted nine spots on the curve. For both scanners although these spots can be visualized against the background, they are not statistically different from each other even though they contain increasing concentrations of dye. The PerkinElmer scanner performed better in all LOQ measurements (Mean, Median, Mean-BG, and Median-BG) as seen in Table 1. The dynamic range and measurements were similar in each instrument.

Table 1. Comparison of Lowest Limit of Quantitation

Cyanine-3 (Fluorophore/µm^2)

	Median	Mean	Median-Blank	Mean-Blank
PerkinElmer	0.4205	0.4205	0.4205	0.21
Competitor	0.8405	0.8405	0.8405	0.4205

Cyanine-5 (Fluorophore/µm^2)

PerkinElmer	0.8405	0.8405	0.8405	0.8405
Competitor	13.4	13.4	13.4	13.4

Both scanners can detect the lowest spot concentration on the slide with little difficulty. The LOQ can be different between the scanners because it is dependent on how the signal is processed or manipulated before it is presented in numerical form. Typically, one can predict the LOQ in rough terms by the graph. The point at which the graph makes a steady inflection upward usually is close to the LOQ. Figure 2 clearly shows that both curves from the PerkinElmer scanner (blue) start their upward inflection before the same point on the curve from the competitive scanner (red) – a clear advantage in sensitivity and ability to quantitate low level concentrations.

Crosstalk: The degree of optical crosstalk in the Cyanine-3 channel was measured by reading the signal from the Cyanine-3-labeled spots using the laser and optical filters for Cyanine-5. The reverse procedure was used to obtain the crosstalk in the Cyanine-5 channel. The Cyanine-5 crosstalk was similar in both instruments, whereas the crosstalk in the Cyanine-3 channel was somewhat less in the higher concentrations on the PerkinElmer scanner as seen in the overlay graph in Figure 3. In both cases the overall intensity of the crosstalk signal was higher with the competitive scanner. The degree of the crosstalk as well as the differences in the Cyanine-3 channel are most likely a result of the differences in the emission filters used in the two instruments. The filters in the competitive scanner have a 50 nm pass band in contrast to 10 nm in all PerkinElmer scanners. While the wider pass band in the competitive scanner does result in higher signal intensity, it doesn't translate into a higher sensitivity as shown in the discussion of LOQ above. It does however result in a greater degree of crosstalk among the channels by allowing for more of the emission spectrum overlap area to be detected by the PMT.

Conclusion

With the ever increasing reliance on DNA and protein microarrays in laboratories throughout the world, it is imperative that high quality scanners are used to collect the data from these important experiments. PerkinElmer's ScanArray G_x PLUS/ProScanArray line of microarray scanners has always set the bar for microarray scanning and data presented here shows that the PerkinElmer scanner continues to perform as well as or better than a popular competitor. Offering high image quality, wide dynamic range, low quantitation limits, and low crosstalk, the ScanArray G_xPLUS/ProScanArray family of scanners is the logical choice for all microarray scanning needs.

References

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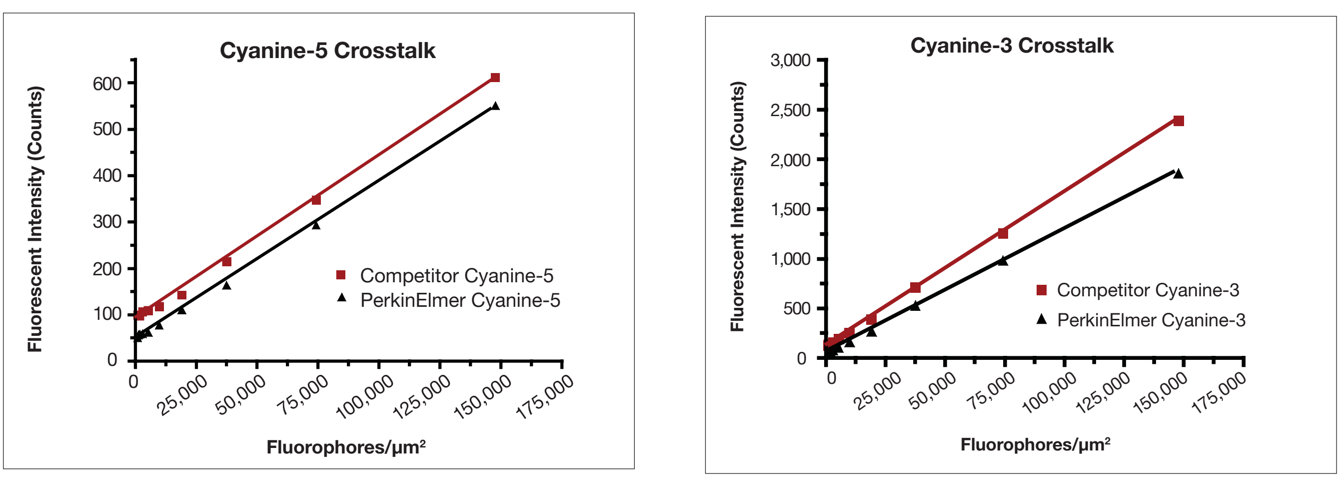

Figure 3. Comparison of Cyanine-3 and Cyanine-5 dyes for each of the scanners. Crosstalk was plotted for the competitive scanner (red) and the PerkinElmer scanner (black) for each fluorescent probe. Note the higher overall intensity of the crosstalk signal in the competitive scanner.

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