Demonstration of Superior Printing Accuracy by the BioOdyssey™ Calligrapher™ MiniArrayer

Theresa Redila-Flores, Jamie Wibbenmeyer, and Luis Ugozzoli, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

Introduction

The BioOdyssey Calligrapher miniarrayer is a benchtop robotic arrayer designed for printing DNA, proteins, or other biological material on slides, on membranes, and into 96-well plates. Designed with the same robotic precision demanded of larger arrayers, with 1 µm motion increment capability and 8 µm print repeatability, the instrument has an eight-pin printhead and can simultaneously print up to 16 slides, up to two 96-well plates, or up to two membranes.

A consistent printing process is required to ensure regular microarray spot morphology and good slide-to-slide reproducibility. Microarrays with poor spot morphology and poor slide-to-slide reproducibility can lead to results that are difficult to interpret. To understand the potential sources of error that might arise from printing with the BioOdyssey Calligrapher, we performed a critical evaluation of its accuracy and homogeneity. We first examined pin performance by measuring signal intensity across multiple slides. Misfiring of arrayer pins can cause inaccurate or even a total lack of sample deposition, resulting in significant variations in signal intensities. In addition to comparing signal consistency, we examined the positional accuracy of the printer by measuring sample placement on the substrate in a variety of dimensions. Positional accuracy is especially critical in downstream image analysis, where image analysis is facilitated by well-structured arrays.

Methods

A BioOdyssey Calligrapher miniarrayer equipped with a flow-through wash station, a vacuum-dry station, and quill pins was used in each assay. A 0.25 µM solution containing a 45-mer oligonucleotide prelabeled with Cy3 dye, Cy5 dye, or both, in 1x printing buffer was printed onto UltraGAPS slides (Corning). The slides were then scanned and the data analyzed with VersArray® analyzer 5.0 software.

For the pin performance assay, eight quill pins were placed into the printhead and 12 x 16 grids were printed with a Cy5-labeled oligonucleotide, for a total of 192 spots/pin. Signal intensity values for spots on multiple slides were extracted and averaged, and the % coefficient of variation (CV) was calculated. For the spot morphology test, duplicate 8 x 6 grids of a Cy3-labeled oligonucleotide were printed on two slides, and the spots were assessed for shape and area using VersArray analyzer 5.0. For distance regularity assays between spots and grids, the intended parameters for the printed arrays were programmed as described in the text and then measured for comparison.

For the superimposition test, four quill pins were used to first print a Cy3-labeled oligonucleotide. The slide was allowed to dry for 30 min, and the Cy5-labeled oligonucleotide was then printed using the same printing program. The slide was scanned first at 532 nm to excite Cy3 and subsequently at 635 nm to excite Cy5, and the scanned images were superimposed without further manipulation by VersArray analyzer 5.0.

Results and Discussion

To determine the printing accuracy of the BioOdyssey Calligrapher miniarrayer, several criteria were used. First, pin performance was examined by comparing signal intensities of spots across multiple slides. Next, spot morphology was assessed by measuring shape and density. Microarray regularity, or the position of array features on the slide in comparison to the parameters used to generate the printing program, was assessed in several dimensions. Measurements were made of distances from spot to spot, row to row, and column to column. In a final qualitative test, Cy3- and Cy5-labeled oligonucleotides were printed and then examined for superimposition.

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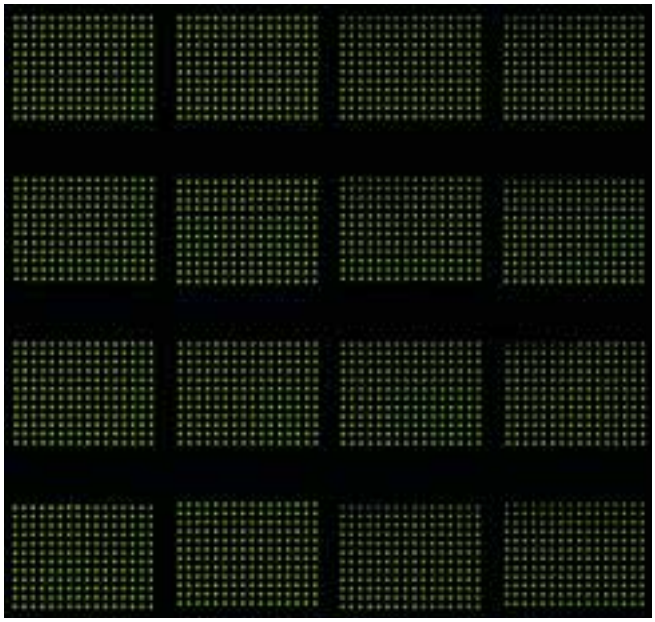

Fig. 1. A representative image of an array spotted using the BioOdyssey Calligrapher miniarrayer for the pin performance assay.

Pin Performance

To determine the performance of each pin, 12 x 16 grids were printed on nine slides using eight pins, for a total of 192 spots/pin (Figure 1). The signal intensities, which are an indication of the amount of sample deposited, were collected from four of the slides. The background-corrected signal intensity from each spot on the slide was averaged and the CV determined for each slide. The CVs ranged from 3.8 to 9.6%, with an overall average CV of 6.7% (Table 1).

A commonly accepted CV for the amount of sample deposited is 10%, and at least one other supplier advises that an acceptable rate is as high as 25%. In fact, one supplier shows acceptable CV values to be between 11.6 and 14.4% (Table 2). Note that values obtained from printing with the BioOdyssey Calligrapher in the pin performance assay (Table 1) are well below those reported in Table 2 (though the exact means of determining amount of sample differ, the CV values are directly comparable).

Table 1. Average signal intensity and %CV obtained in a pin performance assay using the BioOdyssey Calligrapher. Average signal intensity and %CV calculated for all eight pins are shown for spots on selected slides.

	Signal Intensity Values (Pixels)			
Pin	**Slide 1**	**Slide 3**	**Slide 5**	**Slide 9**
Average	7,204.69	7,549.60	7,883.43	8,757.72
%CV	3.78	5.54	8.02	9.56

Table 2. Pin performance data from another supplier. Data are expressed as average volume deposited and %CV.

	Volume (x 10^7)			
Pin	**Slide 1**	**Slide 3**	**Slide 5**	**Slide 9**
Average	10.50	10.00	11.40	9.69
%CV	14.39	11.57	13.86	12.94

Spot Morphology

The second criterion examined, spot morphology, was determined by using the shape regularity and spot area algorithms available in VersArray analyzer 5.0 software. The shape regularity value indicates the spot shape: the closer the average value is to 1, the more circular the spot. The spot area value in pixels indicates whether consistent volume was used to print the slides.

For the spot morphology test, duplicate 8 x 6 grids were printed on two slides. As shown in Figure 2, morphology of the spots was highly consistent. The measured average shape regularity was between 0.84 and 0.85, and spot area values were between 207.7 and 217.15. All CVs were <7% (Table 3).

These data demonstrate that the BioOdyssey Calligrapher printed a consistent amount of material with the input parameters. Although spot deposition is highly dependent on pin type, other important factors are the approach speed, dwell time, and height of the printhead. The low CVs obtained in this assay demonstrate the highly consistent performance of the BioOdyssey Calligrapher miniarrayer.

Table 3. Average values of data extracted from the spot morphology assay. SR, shape regularity; SA, spot area.

	Slide 1				Slide 2			
	Grid 1		Grid 2		Grid 1		Grid 2	
	SR	SA	SR	SA	SR	SA	SR	SA
Average	0.84	214.35	0.85	207.7	0.85	215.83	0.85	217.15
%CV	3.11	3.08	5.87	6.78	2.09	1.97	2.09	2.69

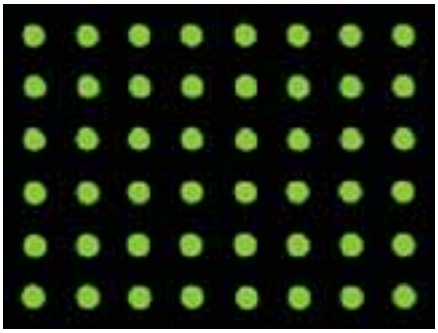

Fig. 2. Representative image of an array printed by the BioOdyssey Calligrapher miniarrayer for the spot morphology assay.

Spot-to-Spot Regularity

A test was designed to evaluate the positional precision of individual spots as well as rows and columns of spots across a defined grid. The spot-to-spot measurement was determined by calculating the deviations in the center-to-center distance (CCD) of each spot. The CCD between adjacent spots was measured and compared to the intended CCD parameters of 175 µm (grid A), 275 µm (B), 400 µm (C), and 500 µm (D) used for printing the slide (Figure 3).

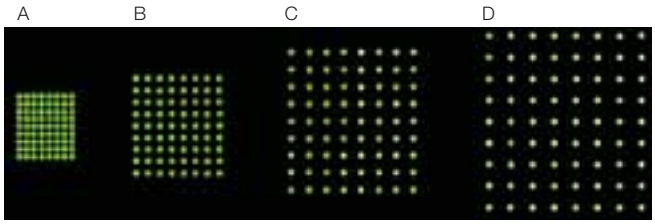

Fig. 3. Differentially spaced grids (A–D) printed to assess spot-to-spot regularity. Parameters were defined such that each grid had a distinct CCD for each spot: A, 175 µm; B, 275 µm; C, 400 µm; D, 500 µm.

In each grid, 20 CCD distances were measured in both the x and y direction from randomly selected spots. The measured CCDs were 170–180 µm (A), 270–280 µm (B), 390–410 µm (C), and 495–510 µm (D). The CCD measured in all grids was no greater than ±10 µm of the parameters used for generating the printing program (Figure 4).

This experiment shows the precise positioning of the BioOdyssey Calligrapher and its ability to print microarrays with great consistency. A 10% error rate is commonly accepted for CCD measurements (Schena 2003). The error rate for all the measurements of the spots printed with the BioOdyssey Calligrapher was <3%, far below the accepted limit.

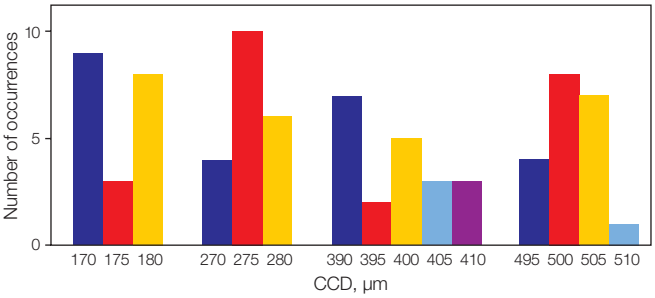

Fig. 4. Regularity of measured spot-to-spot distances. CCD between spots in each grid was plotted. Target CCDs for grids were: A, 175 µm; B, 275 µm; C, 400 µm; D, 500 µm.

Fig. 5. Representative image of an array printed to assess grid-to-grid regularity. A Cy3-labeled oligonucleotide was printed using parameters for an expected GGX distance of 2.25 mm (indicated by white line).

Grid-to-Grid Regularity

A test was designed to determine the error rate of the grid-to-grid distance in both the x and y direction (GGX and GGY, respectively) generated by the instrument when printing. The distance between a spot in the first row or column of a grid and a spot in the first row or column in the adjacent grid was measured in both the x and y direction (Figure 5).

Grids with expected spacings of 2.25, 5.0, and 8.62 mm were printed; the x and y distances measured by the software were very similar to the expected distances, with an error rate <3%. Of the 16 measurements made for the 2.25 mm grids, all but one was precisely placed in the x direction. The one that was positioned incorrectly was shifted by only 0.005 mm. For the 5.0 and 8.62 mm grids, the measured GGX values were between 4.980 and 5.000 and 8.61 and 8.625 mm, respectively (Figure 6A). The GGY measurements for the 2.25 mm grid range were 2.24–2.255 mm; for the 5 mm grid, 4.995–5.000 mm; and for the 8.62 mm, 8.615–8.625 mm (Figure 6B).

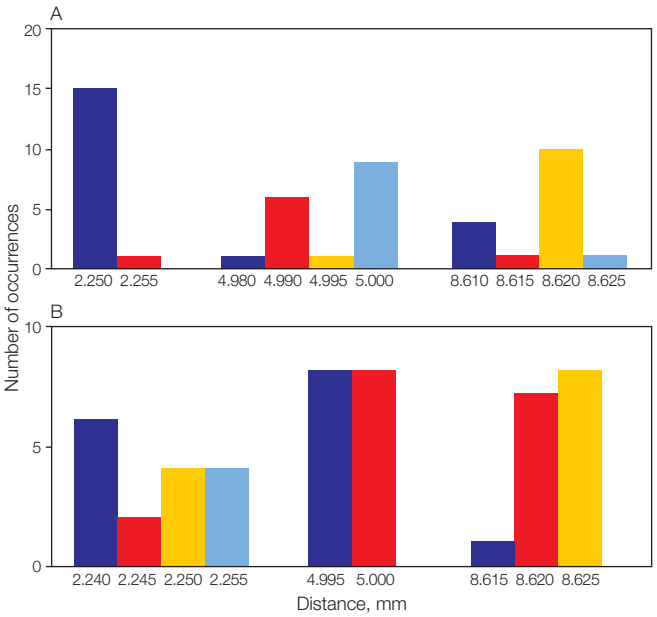

Fig. 6. Regularity of grid-to-grid distances. Grid-to-grid distance target values were 2.25, 5.0, and 8.62. A, GGX; B, GGY.

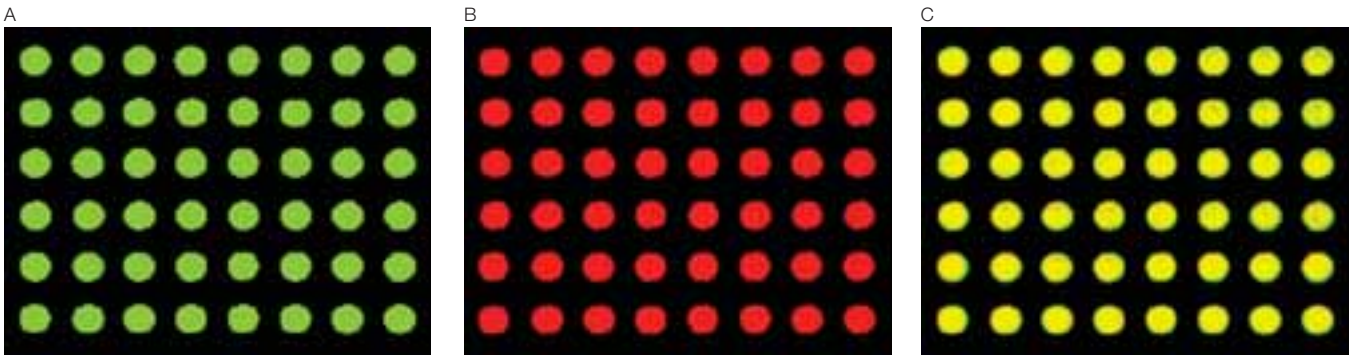

Fig. 7. Representative image of a grid printed with a single pin for the superimposed image assay using the BioOdyssey Calligrapher. A, a Cy3-labeled oligonucleotide was first printed; B, a Cy5-labeled oligonucleotide was printed using identical parameters; C, the two images were superimposed.

Results from both GGX and GGY tests show the precise positioning of the BioOdyssey Calligrapher for printing microarrays. In this test, the measured distances were almost identical to the distance parameters used for printing the slides.

Superimposed Image Assay

The final test was more qualitative. In this assay, grids were printed using a Cy3-labeled oligonucleotide and subsequently printed with a Cy5-labeled oligonucleotide using the same printing program. The slide was then scanned first at 532 nm and subsequently at 635 nm, and the scanned images were superimposed without further manipulation by VersArray analyzer 5.0. Figure 7 shows the scanned images of the Cy3-labeled oligonucleotide array (grid A), the Cy5-labeled oligonucleotide array (B), and the superimposed images (C). Images A and B were superimposed by VersArray analyzer 5.0 with no correction performed to overlay the images.

Superimposed equivalent amounts of Cy3- and Cy5-labeled oligonucleotides should yield yellow spots, with no red or green evident. In Figure 7C, the majority of the spots appear totally yellow, another indication of the precise printing of the BioOdyssey Calligrapher miniarrayer.

Conclusions

The BioOdyssey Calligrapher is a highly accurate benchtop miniarrayer. To demonstrate this precision, we examined several criteria. The performance of quill pins in concert with the miniarrayer demonstrated that the BioOdyssey Calligrapher performed as well as or better than larger arrayers, as evidenced by the low error rates observed. The advantage of this low error rate is cumulative; limiting errors at each step in the experiment improves accuracy in the final data set. In addition, the high positional accuracy of the BioOdyssey Calligrapher speeds the quantitation process by reducing time spent gridding the array image. Although many image analysis software programs, including VersArray analyzer 5.0, contain automatic spot finding and gridding algorithms, the more regular the spot and array, the more quickly the algorithm is performed. Finally, once a grid is created, high slide-to-slide reproducibility of the grid ensures rapid analysis.

Reference

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