Demonstration of Superior Printing Accuracy by the VersArray ChipWriter™ Pro System

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Introduction

With any laboratory technique, it is important to understand the potential sources of error that can impact research results. Those errors related to spot uniformity and morphology most often start with problems introduced during printing.

Printing problems can arise from pins or the arrayer. When pins misfire, inaccurate amounts of sample are deposited onto substrates such as slides. The positional accuracy of the arrayer is also critical to a successful experiment — especially in downstream image analysis. Printing inconsistencies can cause a lack of both spot homogeneity and slide-to-slide reproducibility. Significant variations in signal intensities from uneven target DNA depositions can affect hybridization and yield data of poor quality or results that are difficult to interpret.

Here we present data from a critical evaluation of the printing accuracy and homogeneity of the VersArray ChipWriter Pro system through a variety of assays.

Methods

The VersArray ChipWriter Pro system and quill pins were used in each assay. A solution of 0.25 µM labeled Cy3 oligonucleotide (45-mer), Cy5 oligonucleotide (45-mer), or both prepared in 1x printing buffer was printed onto Corning UltraGAPS slides. The slides were then scanned and data analyzed with VersArray® analyzer 5.0 image analysis software.

To determine the printing accuracy of the arrayer and the pins, several criteria were used. First, the slide-to-slide variability of each pin print was calculated. To determine volume consistency and shape regularity, spot morphology was examined. To assess microarray regularity, spot-to-spot as well as row-to-row distances were evaluated. In a final qualitative test, Cy-labeled oligonucleotides were printed in an overlay, then examined for superimposition.

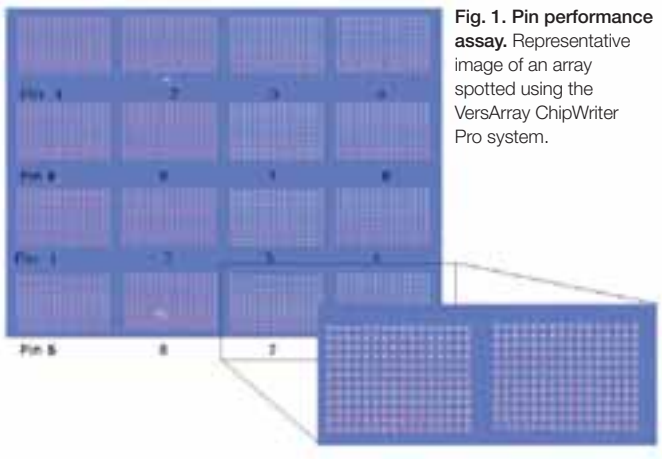

Fig. 1. Pin performance assay. Representative image of an array spotted using the VersArray ChipWriter Pro system.

Results and Discussion

Pin Performance Assay

To determine the slide-to-slide variability of each pin — a critical factor in the accuracy and reproducibility of results — eight pins were placed into the printhead and 12 x 16 grids printed for a total of 192 spots/pin (Figure 1). Signal intensities from four slides were collected. The background-corrected signal intensity from each spot on the slide was averaged and the coefficient of variation (CV) determined. The CV values ranged from 4.6 to 6.1%, with an overall average of 5.5% (Table 1).

Table 1. Pin performance of the VersArray ChipWriter Pro. Signal intensity values for each pin on four representative slides are shown.

Pin #	Slide 1	Slide 3	Slide 5	Slide 9
1	8,280.22	8,652.41	9,538.03	11,131.16
2	8,684.90	8,407.41	9,737.09	11,006.18
3	8,906.19	8,832.41	9,951.36	11,542.92
4	8,956.83	9,138.21	9,890.58	11,647.76
5	8,197.61	8,327.77	9,250.83	10,628.33
6	9,332.95	9,037.49	10,045.44	12,049.24
7	9,741.02	9,483.71	11,128.57	12,773.12
8	8,567.02	8,492.91	9,691.14	10,934.39
Average	8,833.34	8,796.54	9,904.13	11,464.14
%CV	**5.89**	**4.57**	**5.60**	**6.08**

The commonly accepted error rate for this parameter is 10%, although a competitor identifies an acceptable pen-to-pen variation in spotting reproducibility to be as much as 25%, with acceptable CV values of 11.6–14.4%. Values obtained from printing with the VersArray ChipWriter Pro and quill pins were well below these error rates.

Spot Morphology Assay

Irregularities in spot morphology demonstrate a lack of uniformity in material deposited onto slides and result in data that can be inaccurate or difficult to quantitate. Spot morphology was determined by calculating shape regularity and spot area using VersArray analyzer 5.0 software. Shape regularity indicates the spot shape, such that averaged values close to or equal to 1 have the most circular spots. Spot area indicates whether consistent volume was used to print the slides.

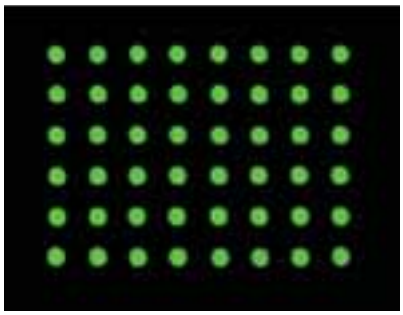

Fig. 2. Spot morphology assay. Representative image of an 8 x 6 array of a Cy3-labeled oligonucleotide printed by the VersArray ChipWriter Pro and used for the spot morphology assay.

Table 2. Average spot morphology values. SR = shape regularity, SA = spot area (µm).

	Slide 1				Slide 2			
	Grid 1		Grid 2		Grid 1		Grid 2	
	SR	SA	SR	SA	SR	SA	SR	SA
Average	0.86	220	0.86	216.8	0.87	226.04	0.89	226
%CV	2.5	2.6	5.3	4.9	5.0	3.5	3.3	1.9

Duplicate 8 x 6 grids (Figure 2) were printed on two slides. The morphology of the spots was highly consistent, displaying an average shape regularity of between 0.86 and 0.89. Spot area values were between 216.77 and 226.04. All CVs were <6% (Table 2).

These data demonstrate that the VersArray ChipWriter Pro prints a consistent amount of material with the input parameters. Although spot deposition is highly dependent on pin type, the approach speed, dwell time, and z-height of the robot all play important roles in this parameter. The low CV values obtained in this assay demonstrate the highly accurate performance of the VersArray ChipWriter Pro.

Microarray Spot-to-Spot Regularity Assay

The microarray spot-to-spot regularity assay was designed to evaluate the positional precision of the spots, as well as the rows and columns of spots across a defined grid. This experiment shows the precise positioning of the instrument and the ability of the VersArray ChipWriter Pro to print microarrays with great consistency — ensuring that research results are both reliable and reproducible.

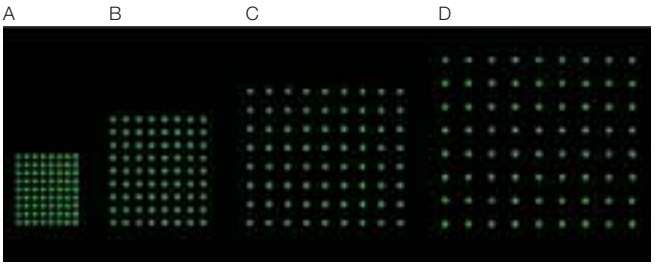

Fig. 3. Spot-to-spot regularity assay. Image of an array printed with the VersArray ChipWriter Pro with a Cy3-labeled oligonucleotide. Parameters were defined such that each grid (A, B, C, or D) had a distinct center-to-center spacing of each spot. Center-to-center distances were: A = 175 µm, B = 275 µm, C = 400 µm, D = 500 µm.

Spot-to-spot regularity was determined by calculating deviations in the center-to-center distance (CCD) of each spot. The CCD between adjacent spots was measured and compared to the parameters of 175 µm, 275 µm, 400 µm, and 500 µm used for printing the slide (Figure 3).

In each grid, 20 CCDs were randomly selected and measured in both the x- and y-directions. The CCDs in all grids were, in general, well within 10 µm of the entered parameters (Figure 4). The error rate for all measurements of spots printed with the VersArray ChipWriter Pro was <3% — far below the accepted limit of 10%.

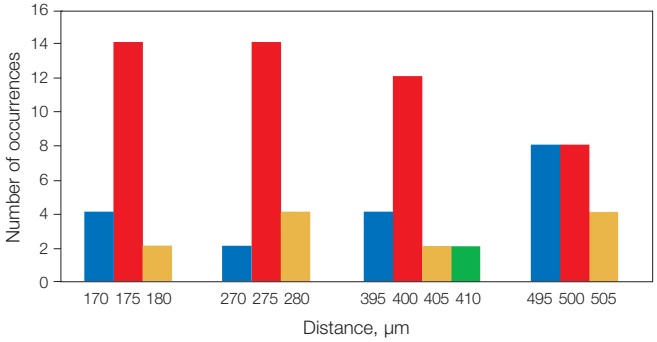

Fig. 4. Spot-to-spot regularity. Plotted is the CCD between adjacent spots vs. the number of spots at that distance for the four sets of grid distances shown in Figure 3.

Microarray Grid-to-Grid Regularity Assay

Positioning of the array on the substrate will accelerate the quantitation process by enabling rapid and precise gridding. The microarray grid-to-grid regularity test was designed to determine the error rate of the grid-to-grid distance in the x- and y-directions (GGX and GGY) generated by the instrument when printing. The distance measured was 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, in both the x- and y-directions (Figure 5).

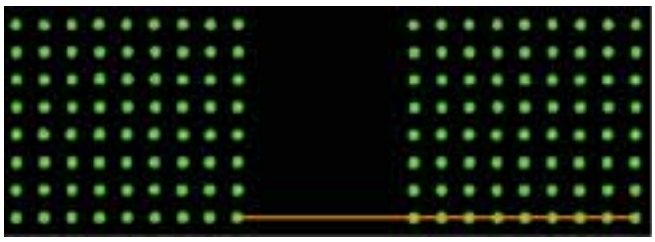

Fig. 5. Microarray grid-to-grid regularity assay. A Cy3-labeled oligonucleotide was printed using parameters such that the expected GGX and GGY distances were 5 and 8.62 mm, respectively. Orange line indicates CCD.

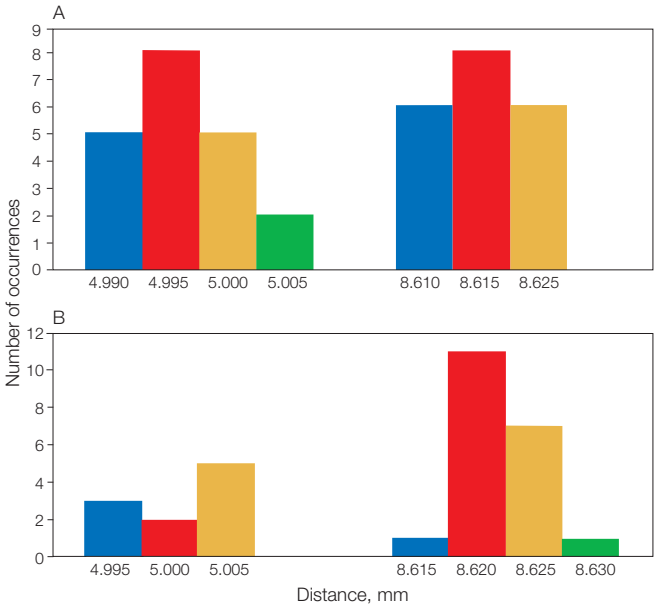

Fig. 6. Grid-to-grid regularity. Plotted are the measured grid-to-grid distances A, GGX; B, GGY values vs. the number of occurrences at each distance.

The distances as measured with the software were very similar to the expected parameter distances used for performing this experiment. For the 5 mm and 8.6 mm expected GGX distances, the measured values were within 4.99 and 5.0, and 8.6 and 8.6, respectively (Figure 6). The error rate for all measurements was <1%. The distances measured for the GGY direction were within the range of 4.99 and 5.0, and 8.61 and 8.63, respectively. The error rate was <1%.

Results from both GGX and GGY tests show the precise positioning of the VersArray ChipWriter Pro for printing microarrays. The measured distances were almost identical to the distance parameters used for printing the slides for this particular test.

Superimposed Image Assay

Though this final test is qualitative, it is integral in demonstrating both the accuracy and reproducibility of the printer. In the superimposed image assay, grids were printed using Cy3-labeled oligonucleotides and four split pins. After printing the Cy3-labeled oligonucleotide, the slide was allowed to dry for 30 min, then printed with a Cy5-labeled oligonucleotide using the same printing program. The slide was allowed to dry for 30 min and scanned first at 532 nm, then at 635 nm.

Superimposed equivalent amounts of Cy-labeled oligonucleotides should yield yellow spots, with no red or green evident. In Figure 7, the majority of the spots appear totally yellow, indicating a precise overlay of the printing pin. Any red perimeter may be attributed to the pin depositing a greater amount of Cy5- than Cy3-labeled oligonucleotides. Again, these data demonstrate the printing precision of the VersArray ChipWriter Pro.

Fig. 7. Superimposed image assay. A, scanned image of the Cy3-labeled oligonucleotide array; B, image of the Cy5-labeled oligonucleotide array; C, superimposed images of A and B generated by VersArray analyzer 5.0 software, with no correction performed to overlay the images.

Conclusions

The VersArray ChipWriter Pro arrayer is a highly accurate system capable of printing with 48 pins onto 126 slides. To demonstrate high printing accuracy, we examined the performance of split pins in concert with our arrayer. In all assays performed, the VersArray ChipWriter Pro delivered error rates as low as or lower than those commonly accepted. The primary advantage of this low error rate becomes evident as experimentation progresses; limiting errors early will improve the accuracy of results in the final data set. Accuracy is essential not only to ensure integrity of research results, but also to save time on routine tasks. The high positional accuracy of the printer speeds the quantitation process by reducing time spent gridding the array image.

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