Arrayjet Microarray Technology - An Assessment of Printing Reproducibility

Analysis of the Printing Consistency of Protein Microarrays using Arrayjet Marathon Microarrayers

Introduction

This application note provides evidence of feature reproducibility and consistency across slide batches printed using Arrayjet Marathon microarrayers. It highlights the successful batch-to-batch printing of fluorescent tagged Bovine Serum Albumin (BSA) onto Schott Nexterion® epoxysilane slides to produce high quality spots.

Experimental Design

Sample preparation

Bovine Serum Albumin (BSA-IgG free, 1mg/mL, Sigma) was diluted in a 47% glycerol buffer containing 1µg/mL Rhodamine B. A sample volume of 20µL was deposited in 36 wells of a 384 well microplate.

Substrates

Arrays were printed onto Schott Nexterion® Epoxysilane slides.

Inkjet Printing

Each drop dispensed by an Arrayjet Marathon microarrayer is 100pL. One drop per spot was printed per sample, in triplicate across 48 miniarrays. To print a batch of 100 slides, 3 sample redraws were performed with 1.3µl of sample aspirated each time .The temperature and humidity (RH) were maintained between the ranges 15-20°C and 40-60% RH respectively.

Image Acquisition and Analysis

Slides at different tray positions were scanned using GenePix® 4000B scanner (Molecular Devices). Images were acquired under a wavelength of 532nm with 100% power gain and a PMT of 230.

Data Analysis

Data was acquired with GenePix® Pro 6.0 4000B. Mean intensity values of the median F 532 (minus background) of all replicate spots were used for signal calculation. The mean diameter of all features was recorded.

Results

Intra batch analysis

The printing reproducibility of the Arrayjet Marathon microarrayer was demonstrated within one batch of 100 slides. A good spot morphology was observed across all slides with consistent spot positioning (Figure 1). The intraslide %CV values (Table 1) were observed to be low thereby indicating minimum variability within a single slide. The mean intrabatch CV value was calculated to be 4.14% representing consistent signal intensity from all spots printed within a 100 slide batch. The spot size within a batch was in range of 110µm to 115µm.

Inter batch analysis

Two batches of 100 slides were printed on the same day and then again over two separate days to demonstrate inter batch printing consistency over time. Table 1 represents the results obtained following data acquisition and analysis. The mean signal intensity obtained from 2 separate batches over two different days showed a low %CV value of 4.36%, indicating consistency in print quality and inter batch reproducibility over time. The mean CV value for printing performed on the same day was 5.08% demonstrating printing reproducibility between sequential batch runs. The spot size throughout all batches was in the range of 108µm to 113µm.

Print Head Nozzle Performance

Arrayjet printing technology makes use of the Xaar™ print head to deposit samples onto substrates. A low volume 12 JetSpyder docks with the print head to simultaneously load 12

samples into different nozzle sets of the print head (Figures 2A and B). The individual nozzle performance was analysed by calculating the mean %CV of all spots printed with the same nozzle number (Figure 3). The mean %CV values from different nozzles were calculated to be in the range of 3- 4% highlighting spot precision and printing accuracy using Arrayjet microarrayers.

Conclusion

Results obtained following intra batch and inter batch slide analysis represent good printing reproducibility (~5% CV values) and spot size consistency using Arrayjet Marathon microarrayers. When a protein sample such as BSA was printed onto Schott Nexterion® epoxysilane slides, high quality reproducible spots were produced with minimum spot to spot, slide to slide and batch to batch variability. Furthermore, the accurate nozzle performance of the print head combined with rapid on the fly printing makes Arrayjet non-contact technology ideal for manufacturing multiple slide batches over time.

Figure 1: **Representative image showing 3 replicates of BSA sample across 4 miniarrays.**
Good spot morphology and consistent spot positioning can be observed across all arrays.

References

McWilliam, I., Chong Kwan, M., and Hall, D. (2011).Inkjet Printing for the Production of Protein Microarrays. In: Protein Microarrays: Methods and Protocols. (U. Korf, ed) Humana Press, New York

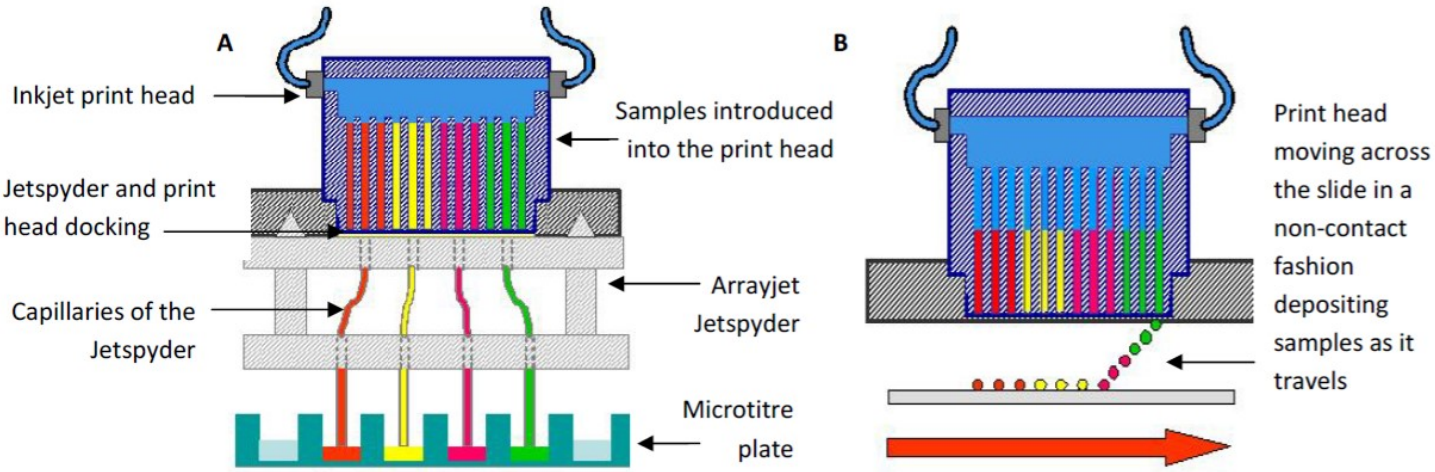

Figure 2A and B: **Arrayjet print head and JetSpyder™ operation.**
The JetSpyder™ is docked to the print head, and moved to the wells of the microplate containing samples to be arrayed. 12 samples are simultaneously drawn through the JetSpyder™ into the nozzles of the print head. The nozzles of the print head contain the samples to be printed. As the print head travels in a non-contact fashion across the slide, the samples are deposited in the form of spots on the slide.

Table 1: Intra batch and inter batch slide analysis

Arrayjet Marathon microarrayer demonstrating printing reproducibility and spot size consistency.
(Mean intensity = median 532- background 532)

	Slide position							
	25	50	75	100	Mean intensity	Mean standard deviation (SD)	Mean CV (%)	Mean diameter (µm)
	Intra-slide CV							
Batch 1	5.8	5.34	6.13	5.67				
	Intra-slide diameter (µm)							
Batch 1	113.01	110.89	115.97	112.76				
Intra-batch signal intensity (MFU)								
Batch 1	20509.18	21693.65	19704.73	21184.5	20773.02	861.72	4.14	113.15
Inter-batch analysis (2 days)								
Batch 1	20509.18	21693.65	19704.73	21184.5	20451.71	892.72	4.36	113.15
Batch 2	19469.52	21150.03	19249.51	20652.58				108.81
Inter-batch analysis (1 day)								
Batch 1	17994.8	20454.08	18874.19	20042.05	19175.98	974.92	5.08	111.37
Batch 2	17679.36	19192.64	19262.74	19907.98				111.71

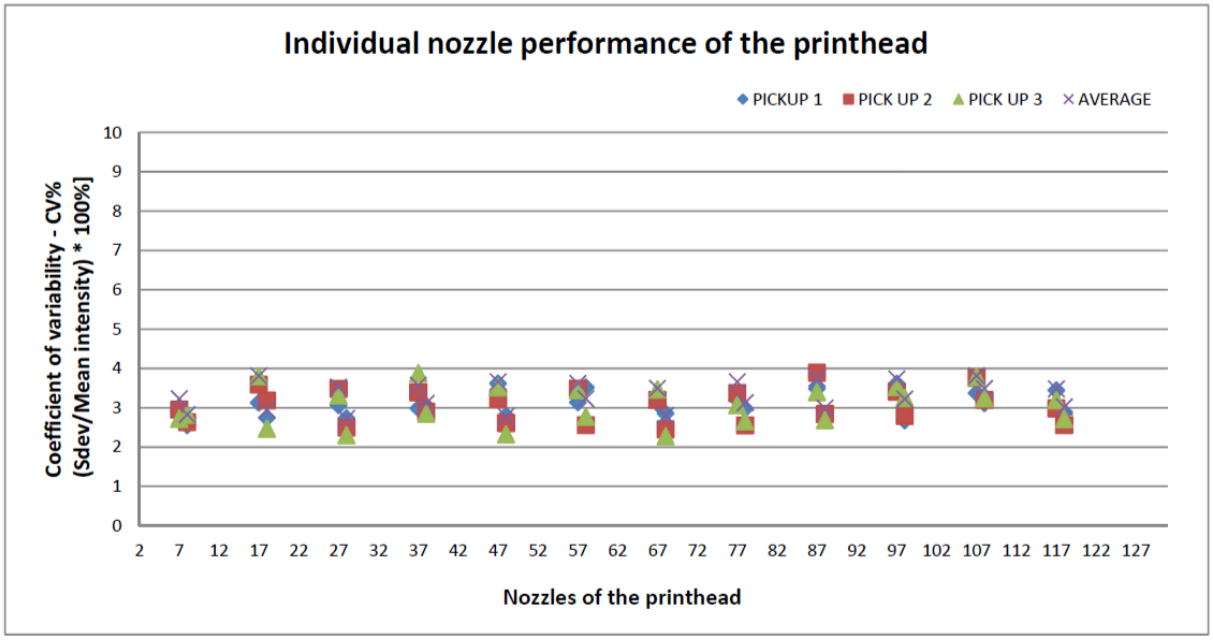

Figure 3: Individual nozzle performance for spots printed

The individual nozzle performance was analysed by calculating the mean coefficient of variability (%CV) for these positions. The CV
values were calculated to be 4% or lower indicating highly precise and consistent individual nozzle performance