High-throughput compound library screening from Arrayjet

Optimising high-density small molecule microarray printing with DMSO printing buffer

Introduction

This application note highlights the optimal printing conditions of DMSO mixtures for the application of compound library screening. Arrayjet tested different environmental conditions and epoxysilane-coated slides to provide evidence of the consistency and reproducibility that can be achieved when printing small molecules in DMSO using Arrayjet inkjet microarray printers.

Combinatorial chemistry has given us the means of creating large libraries of diverse compounds which can be screened for drug leads. For example, GSK have the world's largest commercial library which contains 4 billion unique molecules (Clark et al. 2009) but even most top-level universities possess libraries with tens or hundreds of thousands of molecules. Screening these libraries for hits (a confirmed interaction between the compound and a target) can be challenging and expensive. One method uses high-throughput screening (HTS) robots but they are expensive to set up and maintain.

An alternative approach uses small molecule microarrays (SMM), which were developed by Stuart Schreiber's group at Harvard (Macbeath et al. 1999). Small molecule microarrays are a cost-effective alternative for identifying hits in high-throughput, and the setup costs for an Arrayjet microarray printer can be as much as tenfold lower than for a high-spec HTS robot. Furthermore, Arrayjet's contract research and manufacturing service (Arrayjet Advance™) enables customers to access this technology without a large CapEx outlay.

Experimental design

Sample preparation

Two test buffers were prepared to a final volume of 505 µL.

Buffer 1: 50% DMSO dissolved 1:1 in water

Buffer 2: 100% DMSO

Buffers were spiked with 5 µL of 1 mg/mL Rhodamine B solution to permit fluorescence detection.

Each buffer was spiked with 5 µL of 1 mg/mL Rhodamine B solution to permit fluorescence detection.

Substrates

Based on earlier successful projects, Schott 2D-Epoxy and PolyAn 3D-Epoxy slides were evaluated.

Microarray printing

Inkjet printing was performed on an Arrayjet Ultra Marathon II in a Class 10k clean room. Each drop dispensed by Array printers is 100 pL. Deposition volumes of 200 pL and 400 pL were tested at printing temperatures of 16 °C, 20 °C, and 24 °C. Relative humidity was maintained between at 50 %.

Image and data acquisition

Printed slides were imaged in real-time using the Iris™ Optical QC System (Figure 1).

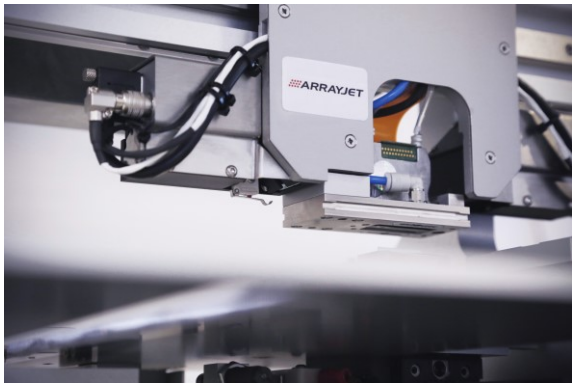

Figure 1: Twin cameras sit either side of the print head and comprise the Iris™ Optical QC System which captures images of printed slides within 300 ms of sample deposition.

Post-printing, slides were scanned using GenePix® 4000B (Molecular Devices) in the 532 channel at 230 PMT. Data were then extracted using Genepix® Pro 6.0.

Results

Figures 2 – 9 show images captured by Iris™ Optical QC System (left) and acquired images from GenePix® 4000B scanner (right).

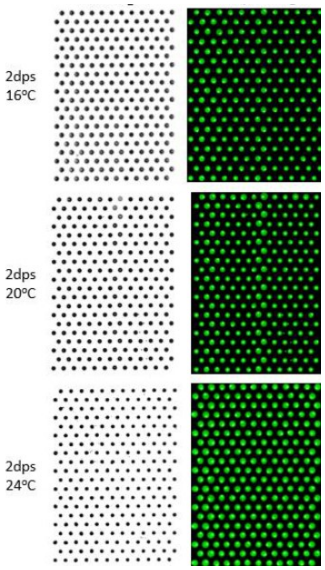

Figure 2: 50% DMSO samples printed at 200 pL on Schott 2D-Epoxy slides

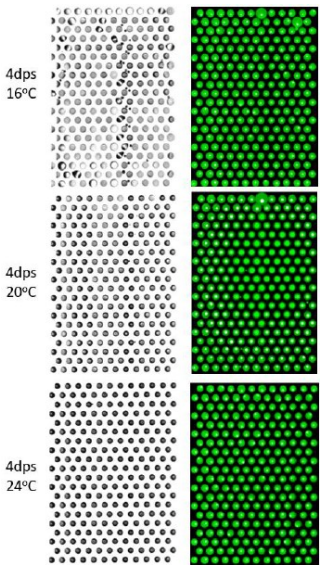

Figure 3: 50% DMSO samples printed at 400 pL on Schott 2D-Epoxy slides

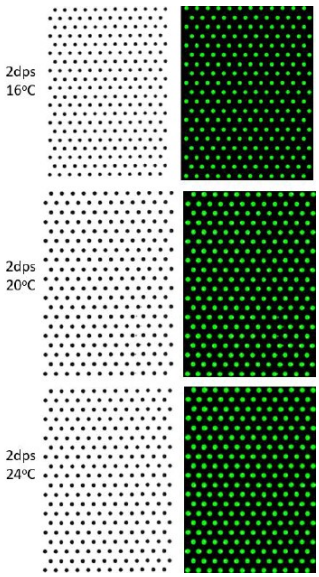

Figure 4: 50% DMSO samples printed at 200 pL on PolyAn 3D-Epoxy slides

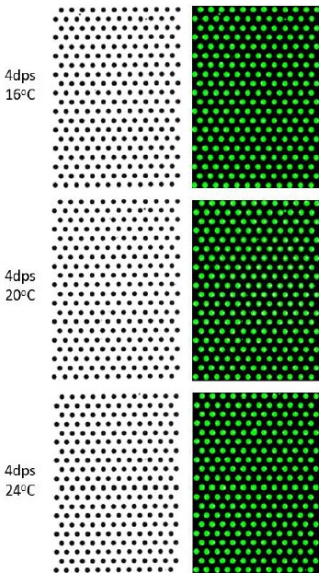

Figure 5: 50% DMSO samples printed at 400 pL on PolyAn 3D-Epoxy slides

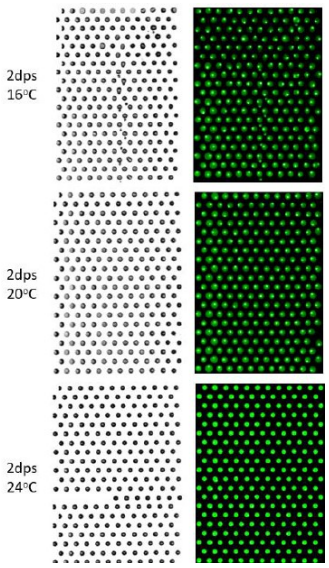

Figure 6: 100% DMSO samples printed at 200 pL on Schott 2D-Epoxy slides

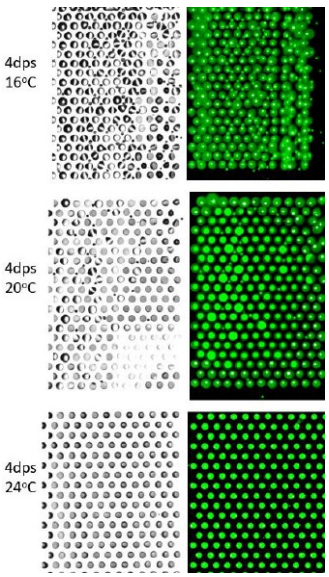

Figure 7: 100% DMSO samples printed at 400 pL on Schott 2D-Epoxy slides

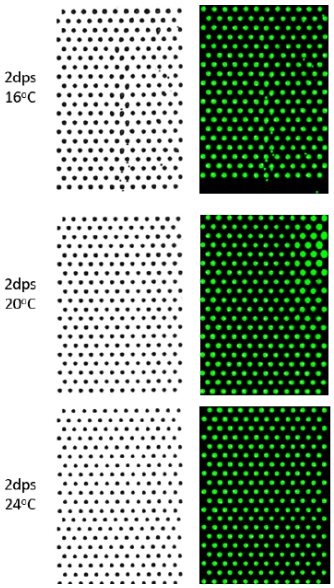

Figure 8: 100% DMSO samples printed at 200 pL on PolyAn 3D-Epoxy slides

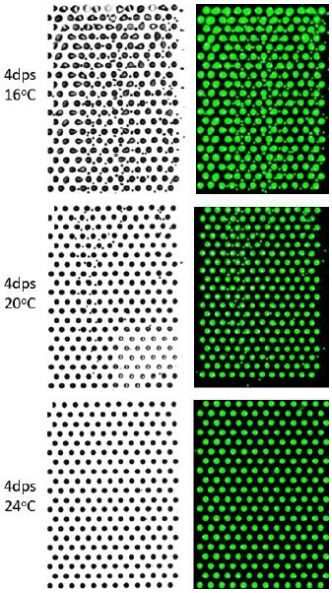

Figure 9: 100% DMSO samples printed at 400 pL on PolyAn 3D-Epoxy slides

Post-print images confirmed 100% of spots printed on all slides and with excellent morphology – particularly at the higher temperature of 24 °C. There were no artefacts or signs of dust contamination on the printed slides.

The best quality printing was achieved with 50% DMSO printed at 24 °C. Both deposition volumes printed with excellent morphology on both slides.

Printing with 100% DMSO was most consistent, and with fewest satellite spots at 24 °C and with a printing deposition volume of 200 pL.

Arrayjet consider spot morphology to be excellent when circularity is > 90% Overall, excellent printing was achieved on both slide types, but the 3D-epoxy coped better with the larger deposition volumes, presumably due to higher hydrophobicity resulting in smaller spots and less frequent merging (Table 1). These characteristics make 3D-epoxy more suitable for printing high density arrays of small molecules in DMSO.

Conclusion

DMSO is a compatible printing buffer with Arrayjet technology. Arrayjet Advance™ scientists have designed and optimised protocols, and printed customer libraries of 7,680 unique small molecules samples. Consistent spot morphology and the capacity to print large batches of high-density arrays make Arrayjet an excellent choice for customers wishing to screen large compound libraries by microarray. Incorporating Iris™ Optical QC System and Command Centre Pro™ analysis software into the printing process ensures no missing spots, and the acquisition of full data sets.

Table 1: Summary data highlighting optimum conditions for printing DMSO buffer on epoxysilane slides

		50% DMSO				100% DMSO			
		Satellites	Circularity >90%	Deposition volume (pL)	Mean diameter (µm)	Satellites	Circularity >90%	Deposition volume (pL)	Mean diameter (µm)
Schott 2D-epoxy	16 °C	Y	N	200	200	Y	N	200	200
				400	250			400	250
	20 °C	Y	Y	200	200	Y	N	200	200
				400	250			400	250
	24 °C	N	Y	200	200	Y	Y	200	200
				400	250			400	250
PolyAn 3D-epoxy	16 °C	Y	N	200	150	Y	N	200	150
				400	190			400	200
	20 °C	Y	Y	200	150	Y	N	200	150
				400	190			400	200
	24 °C	N	Y	200	150	Y	Y	200	150
				400	190			400	200

References

Clark, M. A., Acharya, R. A., Arico-Muendel, C. C., Belyanskaya, S. L., Benjamin, D. R., Carlson, N. R., Morgan, B. A. (2009). Design, synthesis and selection of DNA-encoded small-molecule libraries. Nature Chemical Biology, 5 (9)

Macbeath, G., Koelher, A., and Shreiber, S. L. (1999). Printing small molecules as microarrays and detecting protein–ligand interactions *en masse*. Journal of the American Chemical Society, 121 (34)