Antibody Microarrays using Arrayjet Non-Contact Inkjet Technology

Printing Antibodies onto Schott Nexterion® Epoxysilane Slides Using Arrayjet Jetstar™ Protein Printing Buffer C

Introduction

This application note highlights the successful printing of fluorescent tagged antibodies onto Schott Nexterion® epoxysilane slides using Arrayjet JetStar™ Protein Printing Buffer C to produce high quality spot morphology. It provides evidence of the consistency and reproducibility of antibody arrays manufactured on Arrayjet microarrayers.

Printing buffer plays a crucial part when designing a protein array experiment. To achieve high quality spot morphology, targets to be printed using Arrayjet Inkjet technology should have a viscosity between 4-20 centipoise (cP). At Arrayjet we have optimised a range of printing buffers to print different sample types. Initially each printing buffer should be evaluated for each type of protein to achieve good spot morphology without affecting the protein functionality. We achieved high quality spots when printing Immunoglobulin-G (IgG) using JetStar™ Protein Printing Buffer C.

The print head delivers 100 pL droplets with a maximum delivery volume of 10 nL achieved by delivering multiple drops per spot. One drop produces spots of approximately 100 µm diameter. By increasing the number of drops per spot during printing the spot diameter can be controlled. However, the spot diameter is also dependent on factors such as temperature, humidity, the printing buffer and the surface chemistry of the slide.

Experimental Design

Sample Preparation

Protein sample, antihuman IgG (Fc specific)-Cy3 fluorescent tagged , developed in goat (1mg/mL), (Sigma) was serially diluted 1:2 times to obtain 4 different concentrations of IgG samples using Arrayjet's JetStar™ Protein Printing Buffer C (as shown in Table 1).

Table 1: Dilution Series of Anti-human IgG-Cy3 samples

Sample number	IgG (µg/mL)
1	500
2	250
3	125
4	62.5

Inkjet Printing

Arrays were printed onto Schott Nexterion® epoxysilane slides using an Arrayjet Sprint microarrayer, with JetMosphere environmental control system. 10 replicate spots per IgG concentration were printed at 100, 200 and 300 pL delivery volume. The temperature and humidity (RH) were maintained between the ranges 15-20°C and 40-60% RH respectively.

Drying

The epoxysilane slides were incubated for 1 hour at 37°C to achieve high quality spot morphology.

Image acquisition

Slides were scanned using GenePix® 4000B scanner (Molecular Devices). Images were acquired at 532 nm wavelength and PMT gain 160.

Data acquisition and analysis

Data were acquired with GenePix® Pro 6.0 4000B. Local background corrected values were used. Mean values of the median F 532 of all replicate spots were used for signal calculation. All features were aligned with resized diameters. The successful print run was verified by measuring the CV % values for all IgG concentrations with different spot sizes.

Results

Results obtained following image acquisition showed high quality spot morphology as demonstrated in Figure 1. A consistent horizontal pitch was achieved, highlighting optimum placement accuracy throughout the print run. Figure 2 and Table 2 represent the results obtained following data acquisition and analysis. The signal represented was the mean average of the median spot intensity replicates after local background subtraction.

There was a linear correlation between protein concentration and signal intensity, and the variability between replicates is consistently low.

Conclusion

High quality spot morphology, reproducibility and placement accuracy, was observed when printing antibodies onto epoxysilane slides using Arrayjet's JetStar™ Protein Printing Buffer C. The spot diameter and signal intensity increased with higher IgG delivery volumes demonstrating the level of printing flexibility achieved with Arrayjet inkjet Microarrayer. Arrayjet microarrayers produce high quality spots with excellent printing consistency in a robust and reliable manner that satisfies customer-specific requirements.

References

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

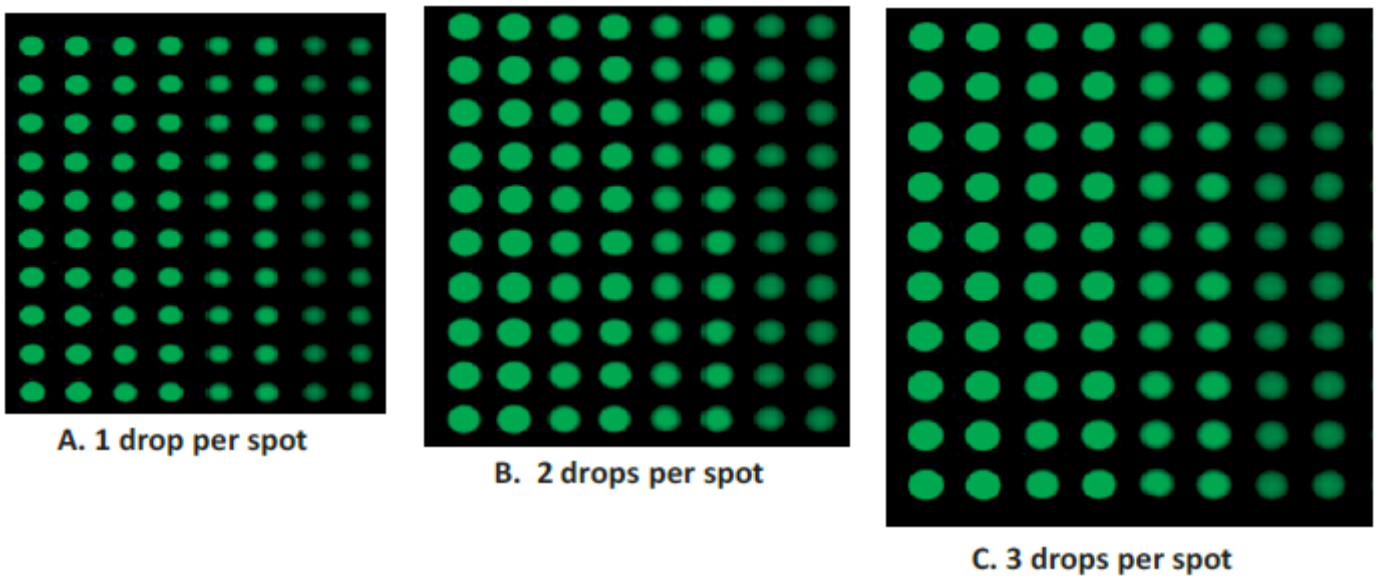

Figure 1: Image acquisition showing high quality spot morphology

A: 1 drop per spot of Anti-human IgG sample prepared in JetStar™ Protein Printing buffer C . The first two spots indicate 500µg/ml sample concentration following the pattern of 2 adjacent spots for each serial dilution (250µg/mL, 125µg/mL and 62.5µg/mL). 10 such repeats of fluorescent labelled anti-human IgG-Cy3 were printed to form one mini-array.

B & C: 2 and 3 drops per spot respectively indicate similar morphology and pattern as explained in A.

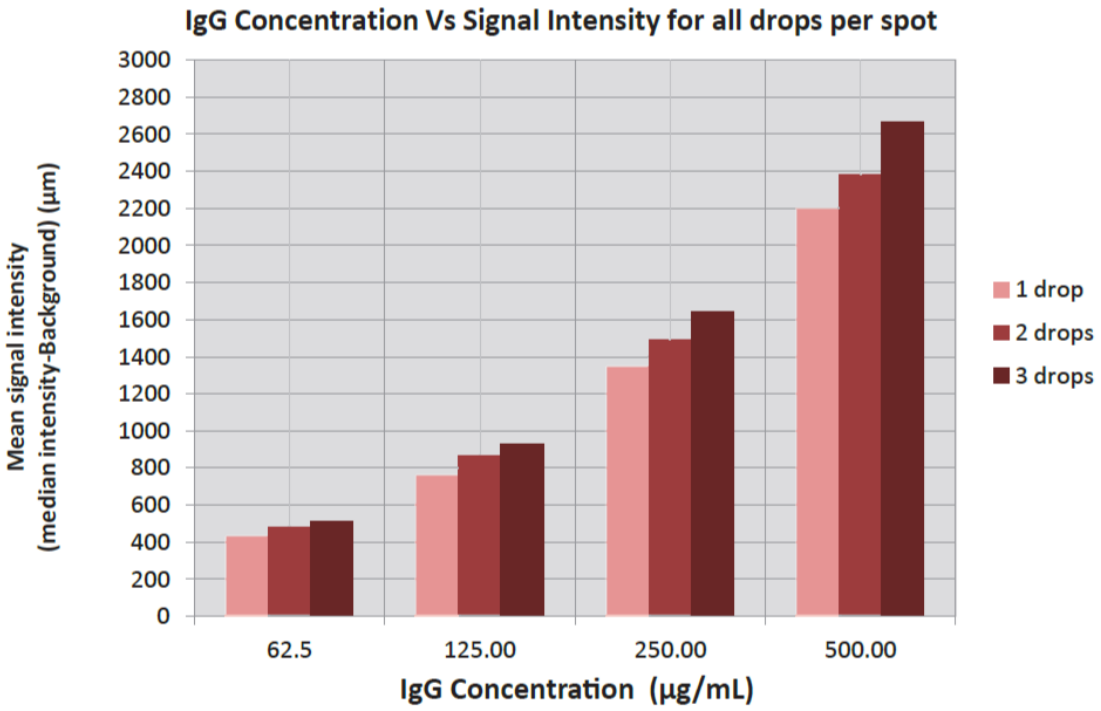

Figure 2: Graphical representation of signal intensity versus IgG concentration for different delivery volumes.
A linear increase in intensity with successive IgG concentrations for 1, 2 and 3 drops per spot.

Table 2: Quantitative data analysis of IgG samples.
A. Mean spot diameters calculated by averaging all replicates of serial dilutions for each drop per spot.
B. Mean signal intensity and CV% values of various IgG dilutions for different 1, 2 and 3 drops per spot.

A.

Mean spot diameter (µm)	Drops per spot		
	1	2	3
	145.9	198.16	240

B.

IgG concentration (µg/mL)	Drops per spot					
	1		2		3	
	Mean intensity	Mean CV %	Mean intensity	Mean CV %	Mean intensity	Mean CV %
500	2193.03	2.95	2379.1	1.2	2668.63	3.33
250	1345.92	9.74	1489.02	9.49	1643.81	5.95
125	758.7	8.5	866.78	4.62	925.78	4.44
62.5	430.43	3.91	482.72	4.62	512.61	5.35