Automated multi-layered multiplex assay production with inkjet technology

Susan Seaton, Duncan Hall & Howard Manning

Arrayjet Limited, Unit 5, McSence Business Park, 32 Sycamore Road, Mayfield, Dalkeith, EH22 5TA. www.arrayjet.co.uk

Abstract:

This work reports on the production and exact reproducible nature of the Aj120 Inkjet Microarray Spotter when printing multiple droplet microarrays. This has lead to successful collaborations between Arrayjet and a number of scientific groups furthering the use of this novel method of non-contact printing within the microarray field, a few of which are highlighted here: cell lysate microarrays to develop new approaches in the diagnosis of cancer, antibody microarrays to detect and purify antigens, and "biochip" microarrays to detect chemicals and biologicals for homeland defence.

Introduction:

Protein microarrays are tools that can be used in many different areas of research, including: proteomics, protein binding, antibody and reverse -assays. Arrayjet utilises modern inkjet technology combined with a proprietary liquid handling device to draw samples up from 96 or 384 well plates and print them in arrays ranging from a few spots in a low density format to slides containing 43,000 elements or more. Protein concentrations of 100µg/ml – 30mg/ml BSA in H_20 have been successfully printed.

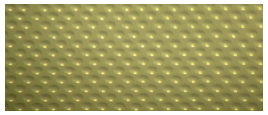
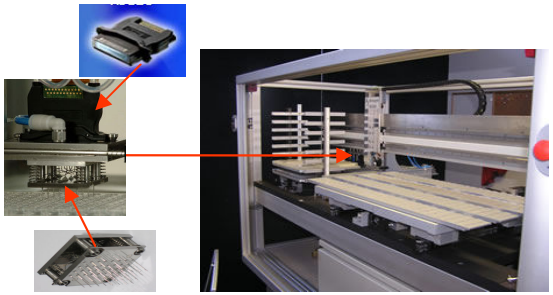


Figure 1: 100pl Aj buffer spots at 280µM pitch on GAPS II (43, 000 spots per slide).

The piezo-electric head is ideal for printing microarrays as samples are not heated during printing enabling arrays of DNA, proteins, carbohydrates, antibodies, serum, cells and cell lysates to be printed intact. Dispensing 'on the fly' spot on spot without contact enables Arrayjet instruments not only to produce very high quality microarrays, but also to produce them at speeds required for the throughput demands of diagnostics and compound screening. The implementation of print head horizontal orientation, unique to this application, enables precise spot-on-spot printing to be performed on-the-fly, not possible with any other technology. This extraordinary capability gives rise to the exciting possibility of studying protein-protein interactions on microarrays.



Figures 2a, 2b, 2c: & 2d: Arrayjet printer, close ups of the printhead and Jetspyder™ extracting samples from 384 well plate, and close ups of print head and 32 sample Jetspyder™.

Experiment 1 –
Materials & Methods

Printing of spot on spot Cy3 and Cy5 microarrays with Arrayjet120 was investigated. Microarray print buffer (Aj buffer) was optimised to the microfluidic optimum of the Aj120 print system, as well as to the biochemical requirements of coupling efficiency on both slide types (GAPS II and siltint). Ten droplets (100pl per drop, of Cy3 in Aj buffer) per spot were printed onto GAPS II and siltint slides. In the second section the procedure was repeated by printing Cy5 (100pl) onto wet Cy3 (100pl) spots. In the third section to confirm accuracy of wet on dry spotting 100pl droplets of Cy5 were printed on top of dry Cy3 spots.

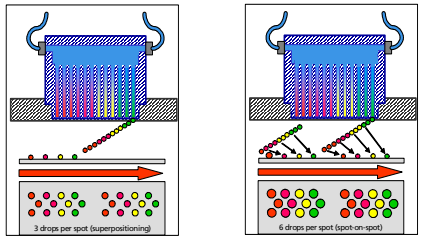


Figure 3: Illustration of superpositioning and Spot-on-Spot on-the-fly printing.

Results

Multiple spotting was good on both slide types with well placed equal sized spots. This was reflected in both wet spots viewed under the microscope and in slides scanned following drying.

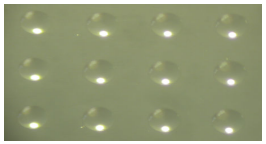


Figure 4: Multiple spotting of Cy3, 1nl spots on GAPS II viewed wet under the microscope.

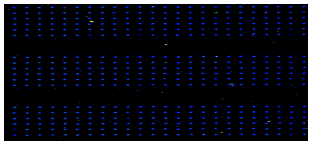


Figure 5: Multiple spotting of Cy3 on GAPS II.

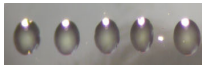


Figure 6: Wet on dry spotting of Cy5 on top of dry Cy3 on siltint slides viewed when wet by microscope.

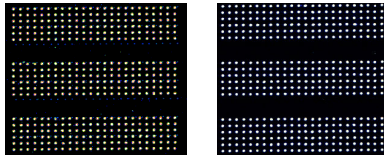


Figure 7a & b: Scan on Cy3 and Cy5 settings respectively. First section Cy3 on Cy3, wet on wet. Second section,Cy5 on wet Cy3, wet on wet. Third section Cy5 on Cy3 wet on dry.

Conclusion

'Precise and executed on-the-fly, spot on spot printing achieved high quality, reproducible multiple spotting microarrays.

Experiment 2 –
Materials & Methods

Four target antibodies (100µg/ml) were printed (10 drops per spot) onto PATH slides, dried at 37°C then rinsed in 1% BSA in PBS. Arrays were washed using standard kit wash buffer and incubated with recombinant protein (2.74-2000pg/ml) to generate a standard curve, then incubated in streptavidin Dylight 649. Slides were scanned and images scored using Arrayvision software.

Results

Multiple spotting achieved good spot morphology, reflected under the microscope and when scanned following processing.

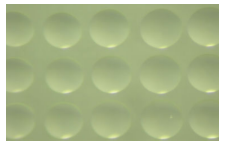


Figure 8: 1nl spots of IgG in Aj buffer printed onto PATH at 300um pitch.

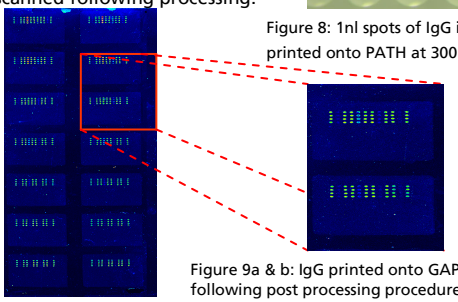
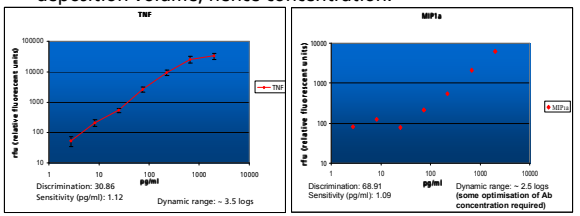


Figure 9a & b: IgG printed onto GAPS II following post processing procedures.

Analysis confirmed spot on spot printing achieved reproducible well placed spots, with consistent deposition volume, hence concentration.



Figures 10a & b: .IgG printed on PATH slides.

Conclusion

'On the fly' spot on spot printing achieves high quality antibody arrays on a number of slide surface chemistries. Additionally, antibodies printed with this method were shown to have retained their biological activity post-immobilisation, effectively demonstrating the utility of the system in producing high quality protein microarrays.

Acknowledgements

The majority of our work with companies remains confidential at this time and therefore whilst the protein microarray work was carried out with the assistance of a company, we will not disclose their identity at this time.