



Comprehensive comparison of protein microarray supports using different spotting systems

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BACKGROUND

In high-performance protein microarray development the design and quality of the solid support plays a pivotal role. 2D substrates and robust contact pin printing techniques are very well established for DNA microarrays. 3-D substrates are favored for protein microarrays because of stabilization of active protein conformation and high protein binding capacity resulting in a higher dynamic range. Different types of printers are also available, mainly divided in non contact printers and contact printers that may cause damage of the surface.

A comparison of different nitrocellulose substrates as well as two different contact (pin and ring technology and split-pin technology) and one non-contact spotting systems will be presented in this study. Furthermore, the influence of the spotting buffer is investigated.

EXPERIMENTAL

The slides investigated in this work are Schott Nexterion Slides NC-W, Schott Nexterion Slides NC-D, FAST slides (Whatman), and PATH slides (GenTel).

Mouse IgG was spotted in 1:2 serial dilutions from 1000 to 0,5 µg/ml in ten replicates using a pin and ring printer (Affymetrix 417 Arrayer), a split-pin printer (Genetix Qarray mini), and a non-contact printer (SciFlex Arrayer S3). The slides were probed with Alexa Fluor 555 anti mouse IgG. For spotting four different buffer compositions were used, PBS + 0.5% trehalose, PBS + 0.5% SDS, 200 mM sodium phosphate + 0.1 mg/ml BSA, and Next Spot PB (Schott Nexterion).

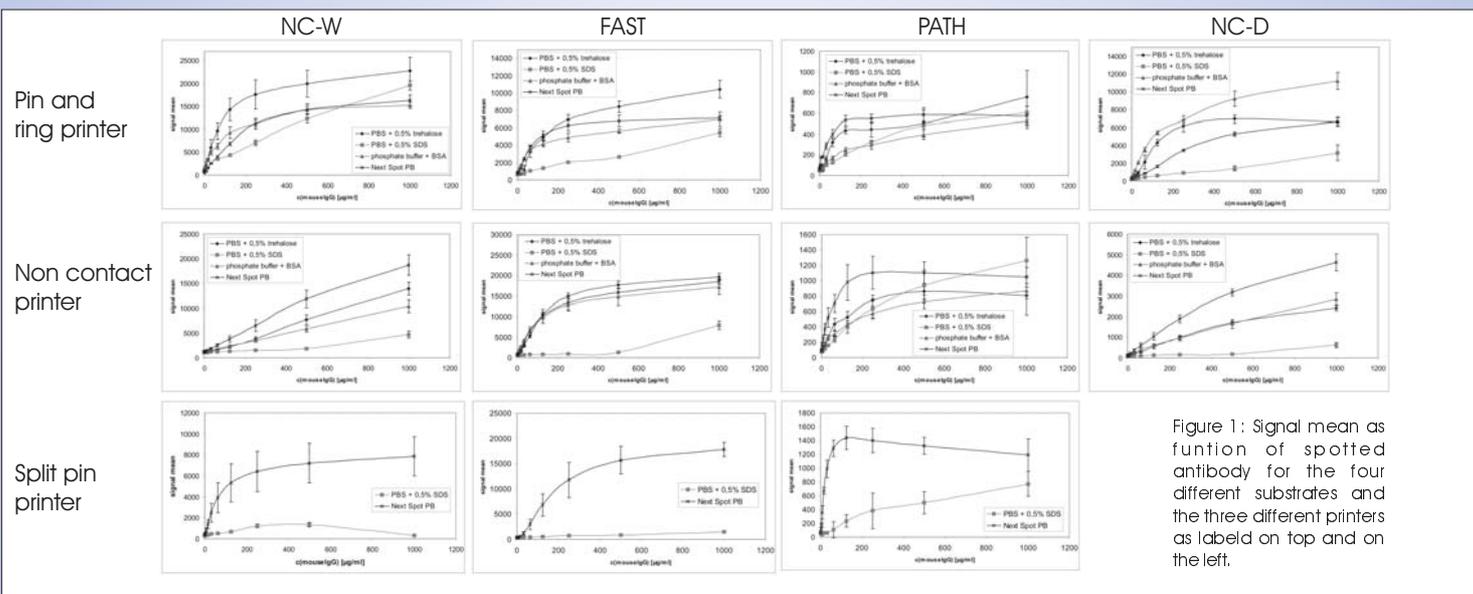


Figure 1: Signal mean as function of spotted antibody for the four different substrates and the three different printers as labeled on top and on the left.

	SNR max	LOD [µg/ml]	top of dynamic range [µg/ml]
pin and ring	777,8	0,5	250
split pin	361,7	0,5	125
non-contact	515,4	3,9	1000

Table 1: Influence of the printer with Next Spot PB on Schott Nexterion NC-W.

	SNR max	LOD [µg/ml]	top of dynamic range [µg/ml]
PBS + 0.5% trehalose	430,5	15,6	250
PBS + 0.5% SDS	781,8	0,5	1000
phosphate with BSA	516,2	0,5	125
Nexterion	777,8	0,5	250

Table 2: Influence of the buffer on Schott Nexterion NC-W with the pin and ring printer.

	SNR max	LOD [µg/ml]	top of dynamic range [µg/ml]
Schott Nexterion NC-W	777,8	0,5	250
Schott Nexterion NC-D	557,7	0,5	500
FAST slide	199,9	1,9	125
PATH slide	115,5	0,5	125

Table 3: Influence of the substrate with pin and ring printer and Next Spot PB.

RESULTS

The influences of the printer, the buffer and the substrate on SNR and LOD were investigated (figure 1). Among the different printing systems, the pin and ring printer shows the highest SNR values on Schott Nexterion NC-W (table 1). Utilizing this printer, PBS + 0.5% SDS and Next Spot PB results in highest SNR and lowest LOD (table 2). A comparison of the different nitrocellulose based surfaces was performed using Schott Nexterion NC-W. Within the slides, the highest signal intensities and SNR-values were achieved by the Schott Nexterion NC-W, the lowest by the PATH slide (table 3).

The non contact printer in combination with the Schott Nexterion NC yields in highest dynamic range with almost linear correlation (figure 1). Furthermore, the non contact printer results in good spot morphology (figure 2 and 3).

CONCLUSION

Many factors contribute to microarray quality. Individual optimization and fine tuning have to be performed empirically for each system.

In regard to dynamic range, best performance was achieved utilizing the non contact printer. Due to the damage-free spotting process, good spot morphologies were achieved. The non contact and the pin and ring printer enable the spotting of a well defined volume, while for the split pin printer the transferred volume depends on the contact time, especially when printing on 3-D substrates. Thus, the utilization of split pin printer results in high variation.



Figure 2: First five dilutions on a FAST slide with Next Spot PB, from left to right: pin and ring, split pin, non-contact printer.

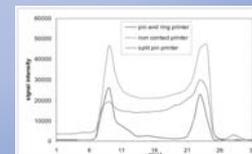


Figure 3: Intensity distribution across the spots on a FAST slide with Next Spot PB.