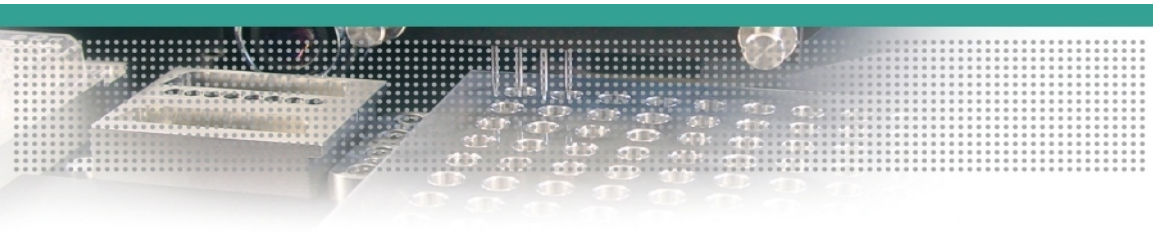

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sciFLEXARRAYER Application Note No. 08001

Comprehensive comparison of protein microarray supports using contact and non-contact systems

Reliable production of biochips depends on many parameters. In the DNA world, glass supports with different coupling chemistries are well established, whereas Nitrocellulose Membranes (NC) are the favoured support for protein biochips. The quality of the biochips also depends on the deposition technology. Contact printers are historically wide spread and used for DNA applications. But for semi-quantitative assays it is important to have the full control of the deposited volume as well as to have an unaffected surface after the printing process, which is possible with non-contact dispensers, generating free flying droplets.

Materials and methods

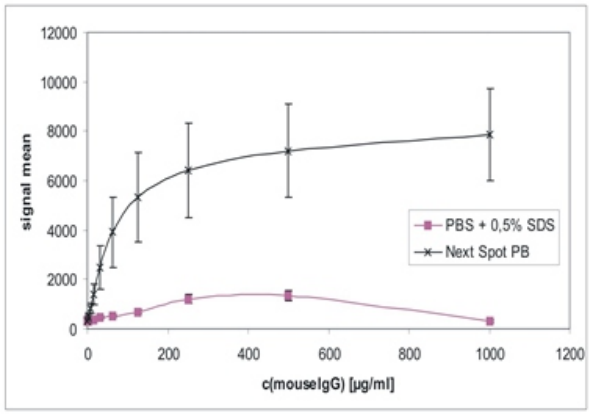
Mouse IgG (1:2 serial dilutions from 1000 to 0,5 µg/ml; n=10 spots) was used in different spotting buffers (PBS + 0.5% trehalose, PBS + 0.5% SDS, 200 mM sodium phosphate + 0.1 mg/ml BSA, Next Spot PB (SCHOTT Nexterion) and spotted onto Sartorius white (SCHOTT Nexterion Slides NC-W*), Sartorius black (SCHOTT Nexterion Slides NC-D*) as well as on slides of other commercially available NC slides.

The probe deposition was performed using a split-pin spotter (Genetix Qarray mini), pin and ring spotter (Affymetrix 417 Arrayer) and a non-contact spotter (sciFlex Arrayer S3). After spotting, assays were performed using Alexa Fluor 555 anti-mouse IgG. Signals were detected with an Axon 4000B scanner.
For protein binding capacity studies mouse IgG was spotted and bound proteins were detected by SyproRuby staining.

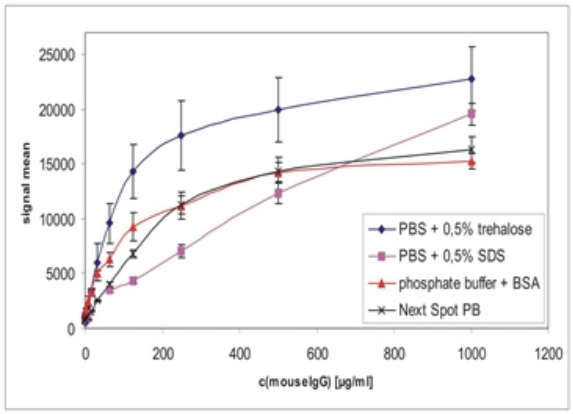
Results and discussion

Protein binding capacity (Fig.2), linearity of dilution series (Fig. 1), comparison of different buffer compositions (Fig.1), background (data not shown) and spot morphology (Fig.3) were investigated.

a)



b)



c)

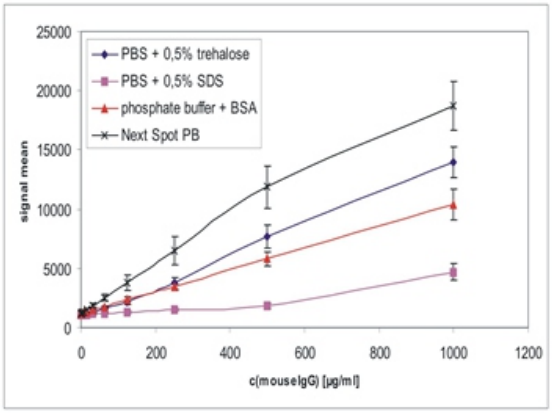


Fig. 1 Dilution series spotted with split pin (a), pin and ring (b) and non-contact (c). Linear regression only given using the non-contact system.

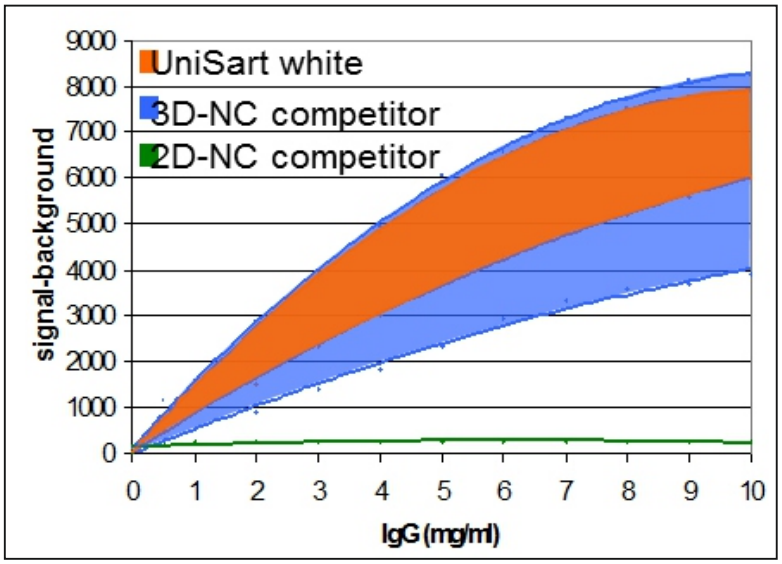
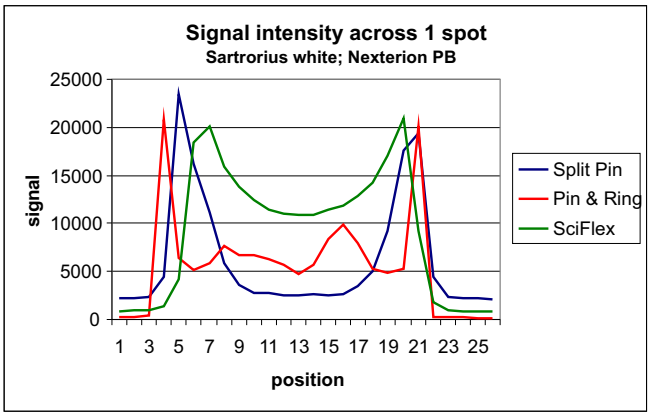


Fig. 2 Protein binding (lot-to-lot variation)

a)



b)

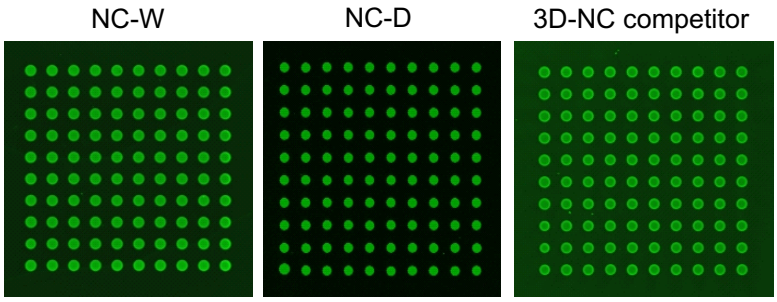


Fig. 3 "Coffee Ring" ("Donut") lowest using the non-contact system (green line) (a); typical array on Sartorius white, black and 3D-NC competitor (b).

There is an increasing request for the printing of protein microarrays in the academic as well the Dx market. The advantage of NC membranes for protein applications are shown and proven in other protein applications, such as Western Blotting.
Thus, the use of NC slides for protein microarrays are the favoured support for this application. The data obtained in this study clearly indicate that improvements of the support itself are still possible as well as the non-contact deposition of protein solutions gives more reliable and robust data with even superior spot morphologies. Furthermore, with the non-contact system lower volumes are needed which leads to lower overall costs of an array compared to contact printing technologies.

Courtesy of Dr. Uwe Andag / Sartorius Stedim Biotech, Germany.
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*These slides are commercially available under the SCHOTT Nexterion brand name

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