On Chip Quality Control Method for Deposition of Capture Elements

G. M. Kresbach, G. Balciunaite, C. Calonder, J. Tränkle, M. Ehrat
Zeptosens - A Division of Bayer (Schweiz) AG, Benkenstrasse 254, 4108 Witterswil, Switzerland, info@zeptosens.com

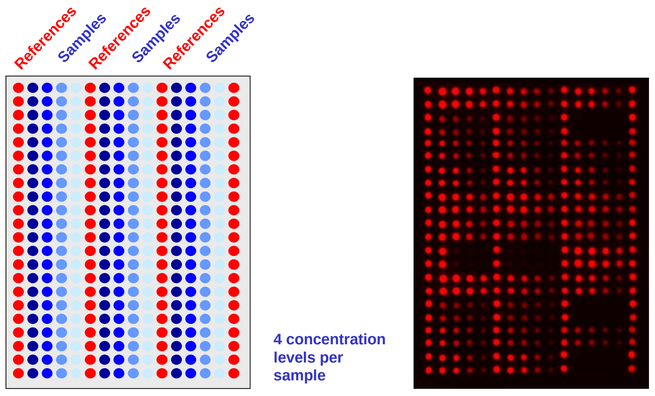
Introduction

For large scale production of antibody based capture microarrays as well as for optimization of surfaces and immobilization protocols we are developing an efficient and highly reproducible quality control method. Major requirements for this method are
- high precision determination of the amount of deposited capture proteins
- independent from assay to be performed on the arrays
- fast time to result in order to obtain immediate feedback on quality of arrays after manufacturing
- low cost reagents to keep expenses for quality control as low as possible
- independent from biological assay protocols
- measurement of protein amounts of as little as 100'000 antibody molecules on 100 µm spot size.

	Detection area (mm^2)	# of Antibodies per spot
96 well plate	40	4×10^{12}
1536 well plate	2.5	2×10^{11}
150 µm spot microarray	1.7×10^{-2}	2×10^{9}
15 µm spot microarray	1.7×10^{-4}	2×10^{7}
1.5 µm spot microarray	1.7×10^{-6}	9×10^{5}

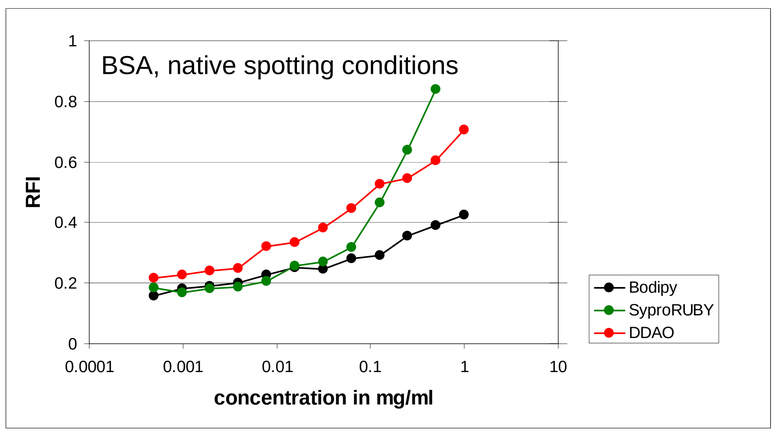
Experimental

Chip support: ZeptoMARK planar waveguide chips
Surface chemistry: Dodecylphosphate
Excitation: red (635 nm) for BODIPY and DDAO fluorescence dyes
green (532 nm) for SYPRO fluorescence dye
Readout: ZeptoREADER F3000
Array layout:



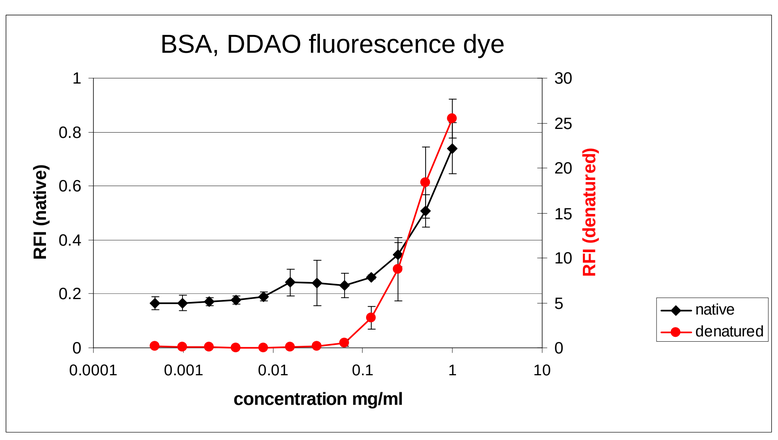
Evaluation of Dyes

Two amine reactive dyes (BODIPY®650/665x-SE [6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl) styryloxy)acetyl)aminohexanoic acid, succinimidyl ester] and CellTrace™ Far Red DDAO-SE [7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one, succinimidyl ester]) as well as a non-covalent acting dye (SYPRO® Ruby protein blot stain [molecular formula not disclosed]) have been evaluated. Under the given experimental conditions and considering the relatively large dynamic range DDAO proved to be the optimal candidate for further evaluation.



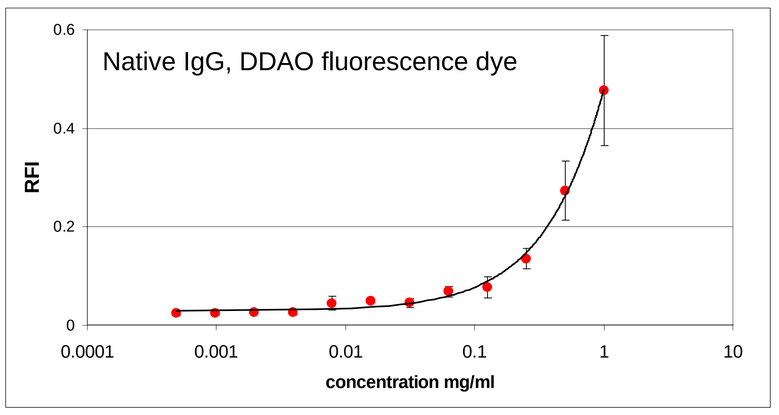
Impact of Spotting Conditions

The tertiary structure of the protein immobilized on the surface has a significant impact on the accessibility of reactive groups. A 30 fold increase in signal was observed for BSA spotted under denaturing conditions compared to BSA immobilized with non-denaturing buffers.



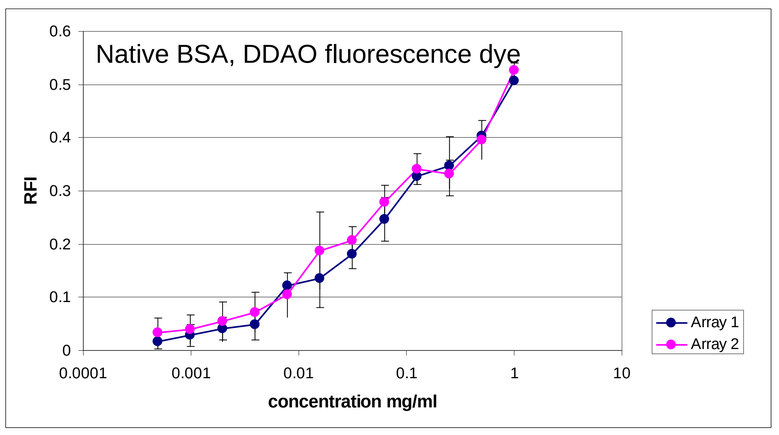
Determination of the Limit of Quantitation

With the approach developed so far a limit of quantification (LOQ) of about 0.05 mg/ml can be achieved. This LOQ is approximately 10 fold below the typical concentrations used for spotting capture as well as reverse arrays.



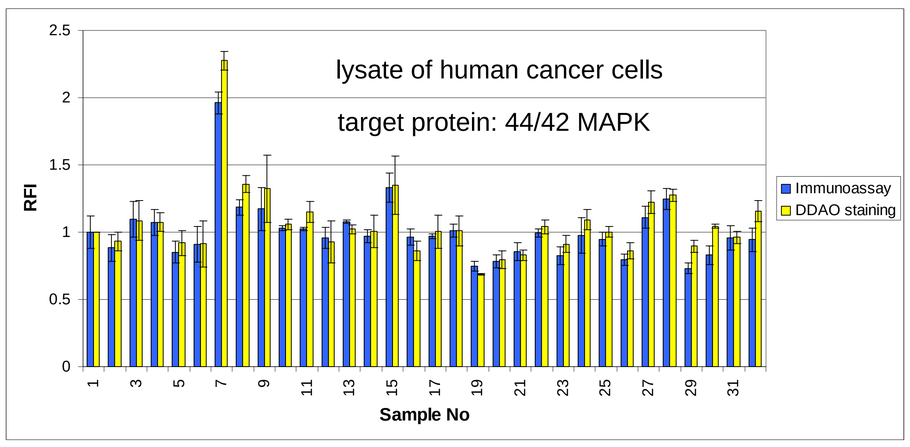
Comparison of Intra Chip Reproducibility

Six individual microarrays are spottet on a standard ZeptoMARK protein microarray. In this specific experiment arrays with a dilution series ranging from 0.004 to 1 mg/ml protein were spotted; the comparison of two adjacent arrays via fluorescence staining shows the high reproducibiltiy of the spotting process.



Comparison of Results Obtained by Immunoassay and Fluorescence Staining

The good correlation between the chemical and the immunochemical fluorescence signal could be demonstrated. Chemical staining of immobilized cell lysates was performed with DDAO and signals compared to the ones obtained by the binding of an antibody to a specific protein in the cell lysate samples. In this experiment total protein concentrations of spotted samples were between 0.1 and 0.8 mg/ml.



Summary and Outlook

We could demonstrate that quantification of the amount of spotted proteins with fluorescent dyes reacting with functional groups of the protein is feasible, that the signals obtained from dye staining correlate well with the signals obtained from antibody binding, and that the sensitivity (LOQ) proved to be sufficient for typical capture as well as reverse array settings. Further improvements in the process, however, are required to reach the targeted concentration precision of 5%. Adaptation of the composition of spotting buffers, stabilization or modification of the coupling chemistry as well as efficient ways to remove unbound dye will be necessary to reach this goal. Impact of the sample protein composition as well as chemical and physical properties of the proteins need to be further investigated.

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