Optimisation workflow for the production and processing of high quality microarrays
Applications in DNA, peptide, antibody, and carbohydrate microarraying

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Introduction

Task

Preparation and processing of microarrays for DNA, peptide, antibody, and carbohydrate applications

Problem

To obtain data of highest quality and meaningfulness a careful optimisation of protocols is necessary. Experimental errors need to be reduced as much as possible.

Solution

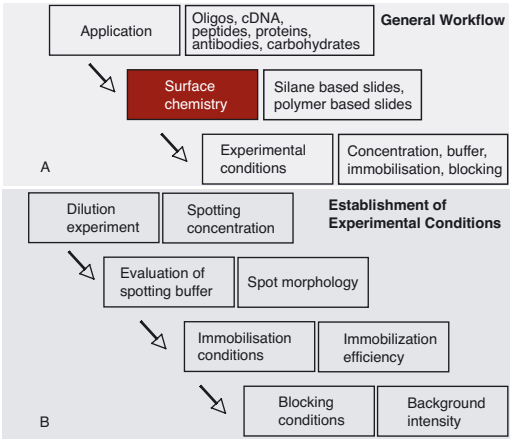
We have developed a workflow for optimisation of array preparation and for processing protocols. Only 4 independent model experiments are needed to determine the relevant parameter: Spotting concentration and solvent, immobilisation and blocking conditions. Optimal conditions for high quality microarray experiments were determined and used for array production and slide processing at the FGCZ.

Experimental

Slides used: E2D (epoxysilane, type **A** in Surface Chemistry), PEG (PEG coated, type **B**), E3D (polymer coated, not crosslinked, type **C**), all from Scienion, Berlin; CDL (CodeLink, Amersham; polymer coated, crosslinked gel, type **C**), PLL slides were home made. Model compounds: Oligos, HPLC or PAGE purified (Microsynth, CH), peptide (Jerini, Berlin) 5(6)-Carboxyfluorescein-KTKESLGRKIQIQRSG-NH_2. Chemicals were of highest available purity (Fluka, Sigma, Aldrich). Spotting: Non-contact piezo spotter (sciflexarrayer, Scienion, Berlin; Piezorray, PerkinElmer). Automated hybridisation station HS4800, confocal laser scanner LS400 (both Tecan, Austria).

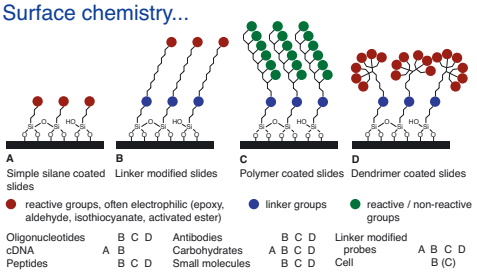
Workflow for Optimisation of Processing Protocols

The basis idea is the use of dye-labeled model compounds in the 4 experiments. Most important is the choice of a proper surface chemistry which determines the experimental protocols.

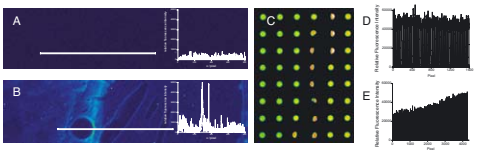


Choice of a suitable slide

Surface chemistry...



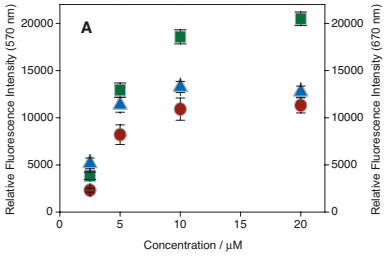
... and slide quality



Scan at highest laser intensity and amplification as a slide quality test (**A**, **B**) and effect of deposits on the slide surface on spot morphology (**C**). Line scan through a number of replicate spots (**D**, **E**). Inhomogeneous coating (**E**) results in a gradient in spot intensity.

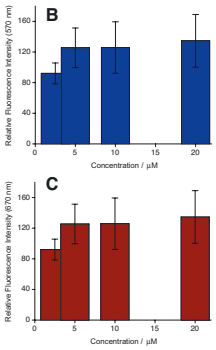
Spotting Concentration

- Spotting dilution series of dye-labeled model compound(s)
- Origin of plateau is found to be the optimal concentration (blue, 10 µM)
- Hybridisation (red) confirms that probe is accessible by the target



CY3-labeled 13mer oligo in 3xSSC spotted to an epoxysilane coated slide. Squares: fluorescence intensity after spotting. Triangles: after washing the slide. Circles: after hybridisation with complementary CY5-labeled oligonucleotide

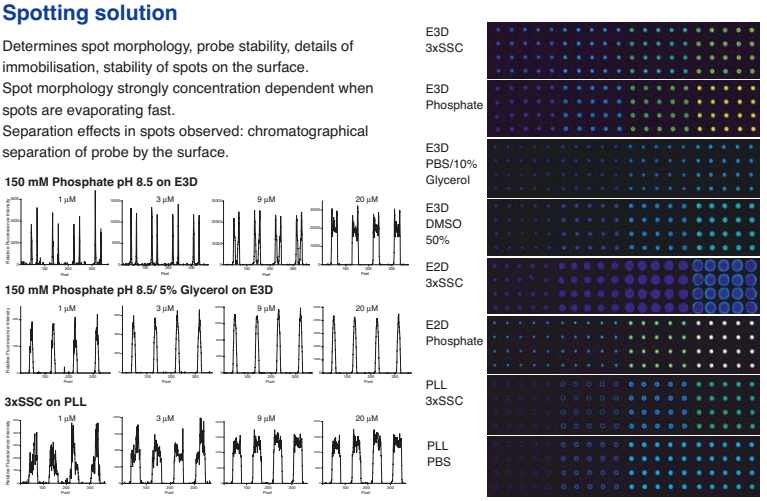
Optimal spotting concentration as determined by the above method: Oligonucleotides: 10 - 20 µM cDNA: 0.2 - 0.4 g/L Peptides: 3 - 5 mM Proteins: ca. 1 g/L, Antibodies: 0.65 - 1.3 g/L, Oligosaccharides: 2 - 4 mM



Background intensity and noise for data in **A**. **B** at 570 nm for the immobilised oligonucleotide, **C** at 670 nm for the hybridised oligonucleotide

Spotting solution

Determines spot morphology, probe stability, details of immobilisation, stability of spots on the surface.
Spot morphology strongly concentration dependent when spots are evaporating fast.
Separation effects in spots observed: chromatographical separation of probe by the surface.

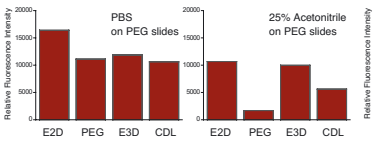


Immobilisation

Prerequisition: matching surface chemistry. Immobilisation via 1. thermal coupling (preferred) using a specific coupling chemistry 2. physical adsorption (large molecules) 3. irradiation (UV light) often leads to crosslinked probes (H. Y. Wang et al. 2003, Genome Biology **4**, R5)

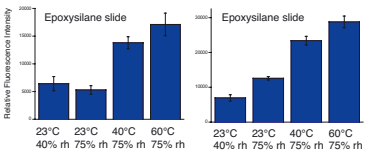
Immobilisation of a 15mer model peptide

FITC-labeled, immobilised at different surfaces



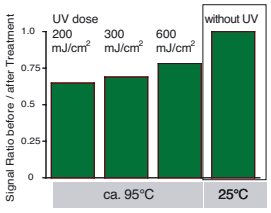
Immobilisation of a dye-labeled 13mer oligo

immobilised under different conditions (rh = rel. humidity)



Immobilisation of cDNA on epoxysilane slides

Influence of UV dose and high-temperature treatment for strand separation on immobilisation efficiency



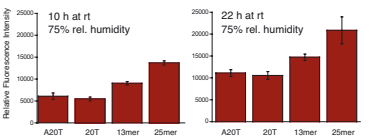
Dye-labeled cDNA in 3xSSC, immobilised. Up to 30% of cDNA is torn off the surface when (mis)treated at 95°C of 2 minutes.

Spotting cDNA: denaturing solvents recommended.

Immobilisation of model oligos on epoxysilane slides

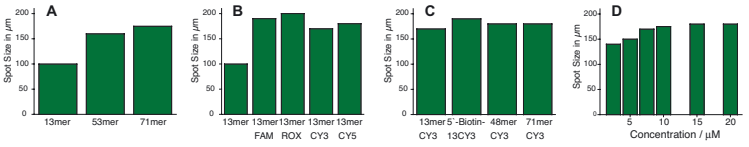
Influence of linker chemistry on immobilisation efficiency

A20T 3`-Amino-T_{20}-CY3
T20 3`-T_{20}-CY3
13mer 3`-Amino-TGC CAG CCA AAT A -CY3
25mer 3`-CAC CAG AGA AGA CCA CGA CG-CY3



20T immobilizes as good as A20T: no amino-linker required. Reactivity is low: A long time is needed for immobilisation.

Spot Size

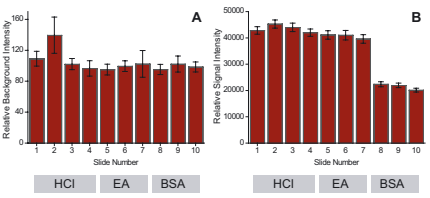


A - C: Oligonucleotides spotted in 3xSSC to epoxysilane coated surface (E2D), D: concentration dependence of spot size for a 13mer.

Blocking conditions

Depends on chemical nature of immobilised probe and surface chemistry.
E.g. acids, bases for epoxy groups
(Salmon sperm) DNA ("prehybridisation")
Proteins (BSA, TopBlock) for all types of slides

13mer oligonucleotides were spotted to 10 epoxysilane slides. Blocking HCl (1-5 mM, 15 min, r.t.), with ethanolamine (EA, 50 mM, 15 min, r.t., and BSA (3% 1h, r.t.).
A: Background, noise B: Signal of a matching 13mer oligo



Examples / Applications

Optimisation results:

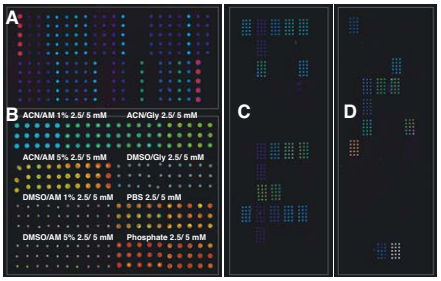
A: 19mer oligo array used for decoding a chemical library

(Melkko et al., Nature Biotech. 2004, **22**, 568)

B: Optimisation of a spotting solution for peptide microarrays. Spot morphology and immobilisation conditions were determined.

ACN acetonitrile, AM Hünigs Base, Gly glycerol

C, **D**: Antibody arrays. 20 anti-mouse and anti-rabbit antibodies were spotted in PBS (15 replicates) and probed with matching antibodies.



Acknowledgement

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