The Affect of Hybridization Conditions on Fluorescent Signal Intensities Recovered from Microarrays.

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ABSTRACT

The intensity of fluorescent signals recovered from labelled targets hybridized to cDNA, oligonucleotide, protein or small molecule microarrays is influenced by a number of factors. These factors include target labelling efficiency and concentration, slide-bound probe concentration and the hybridization parameters [pre-hybridization blocking buffer and hybridization buffer pH and composition, hybridization stringency, wash stringency, background fluorescence and substrate autofluorescence]. In addition, different printing buffers, pre-hybridization blocking buffers, blocking and washing procedures influence the degree of background fluorescence and ultimately the overall hybridization sensitivity of the microarray. The affect of these factors on the fluorescent signal intensities recovered from oligonucleotide microarrays was investigated on six different NoAb BioDiscoveries activated, covalent binding slide surfaces.

INTRODUCTION

NoAb slide surface chemistry is based upon active covalent coupling of capture molecules to the slide surface through accessible primary amino (~NH_2), hydroxyl (~OH) or thiol (~SH) groups on the capture molecule. As the capture molecules are printed on the slide surface, their accessible primary amino, hydroxyl or thiol groups react with the surface aldehyde, epoxy or NHS ester groups on the slide surface and form secondary amine, ether or thioether linkages. These reactions occur only where the capture molecules are printed. All other regions of the surface remain reactive and capable of binding other molecules. Failure to block these reactive groups may result in binding of the labelled target molecules by both the printed and unprinted regions of the slide surface. This potential covalent coupling of the labelled target is the first component of the non-specific background problem. The second component is the weak adhesive (hydrophobic or electrostatic) association of the labelled target with the slide surface. Labelled target fluorescent signal intensities can be maximized and non-specific background fluorescence signals minimized by using a combination of appropriate target labelling, probe printing and microarray pre-hybridization, hybridization and wash conditions.

SUMMARY OF RESULTS

1. Hybridization buffers with pH values of 6.0-7.0 produce a consistent level of flourescent signal recovery from microarrays printed on active slide surfaces.

2. Printing buffers with pH values below 9.0 produce consistent spot diameters and morphology. Print buffer composition- and probe concentration-dependent spot spreading is observed on some of the active slide surfaces.

3. Pre-hybridization blocking buffers with pH values below 9.0 that contain molecules with free primary and secondary amine groups are most effective at reducing background fluorescence on active slide surfaces.

4. NoAb activated slide surfaces are appropriate substrates for both 5'-modified and unmodified oligonucleotide, cDNA, gDNA, protein, peptide and small molecule microarray applications.

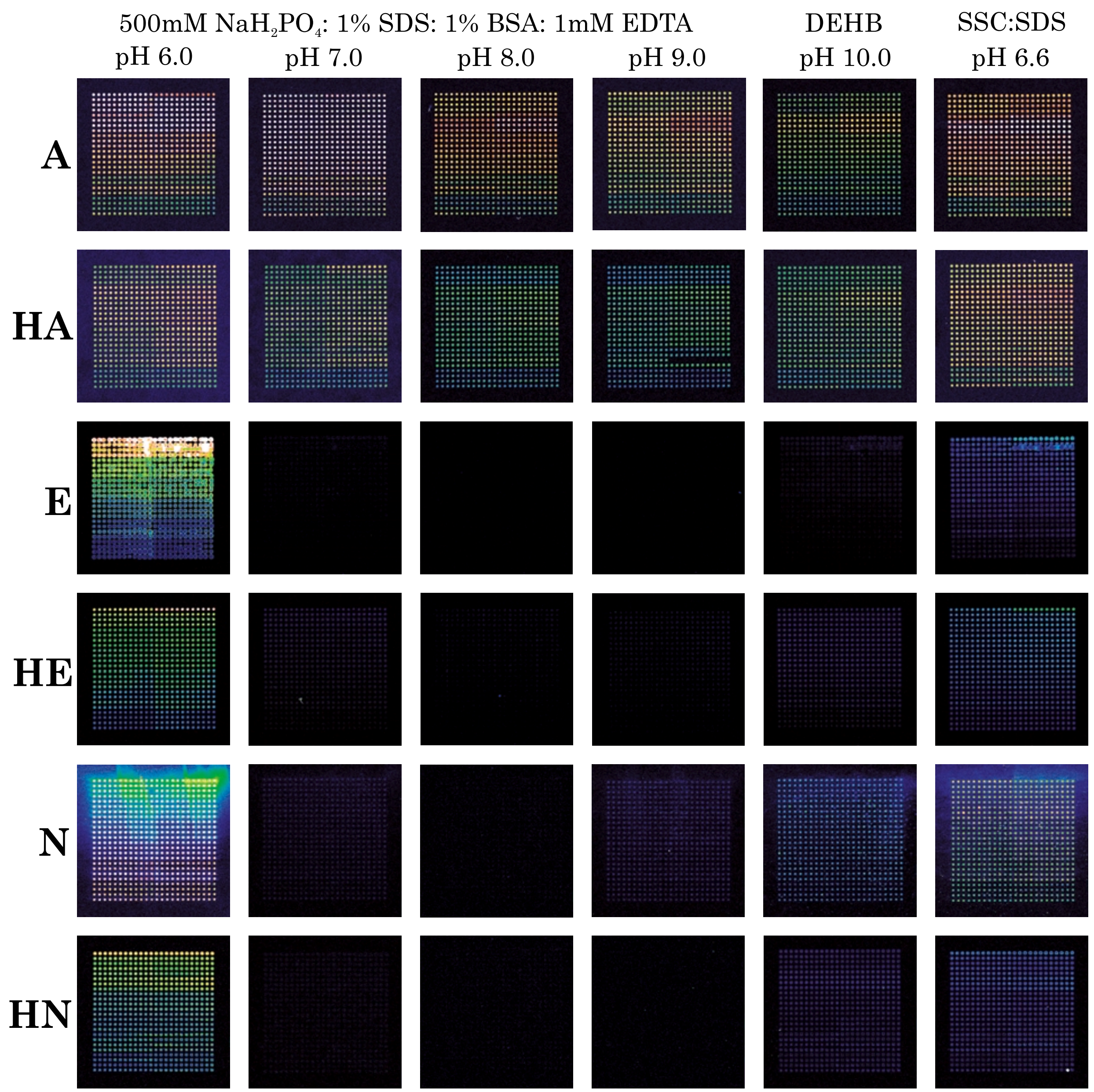

Figure 1. The influence of hybridization buffer pH on fluorescent target signal intensity.
A 2-fold dilution series (13, 6.5, 3.3, 1.7, 0.9 and 0.5fmole/spot) of unlabelled JG5 oligonucleotide (23mer) was printed in 150mM sodium phosphate pH8.5 buffer on NoAb activated slide surfaces. 96 replicates of each dilution were printed using a Virtek SDDC-2 arrayer and TeleChem SMP5 pins. Slides were air-dried in the arrayer cabinet. Slides were blocked in NPBB for 2h at RT and incubated with a Cy3-labelled complementary JG2 oligonucleotide (23mer, 25nM) in the indicated hybridization buffers for 18h at 45°C. Following hybridization the microarrays were washed for 2h in 0.1x SSC:0.1% SDS at 45°C, rinsed in 0.1xSSC, spin-dried at 1000rpm for 3 minutes and scanned at a resolution of 10um, a laser setting of 70 and PMT gain of 70 in a GSI Lumonics ScanArray Lite. ***Abbreviations***: A = Aldehyde slide surface, E = Epoxy slide surface, N = NHS Ester slide surface, HA = Hydrogel Aldehyde slide surface, HE = Hydrogel Epoxy slide surface, HN = Hydrogel NHS Ester slide surface, NPBB = NoAb Pre-Hybridization Blocking Buffer, DEHB = DIG Easy Hyb Buffer (# 1603558), SSC:SDS = 1x SSC:0.1% SDS.

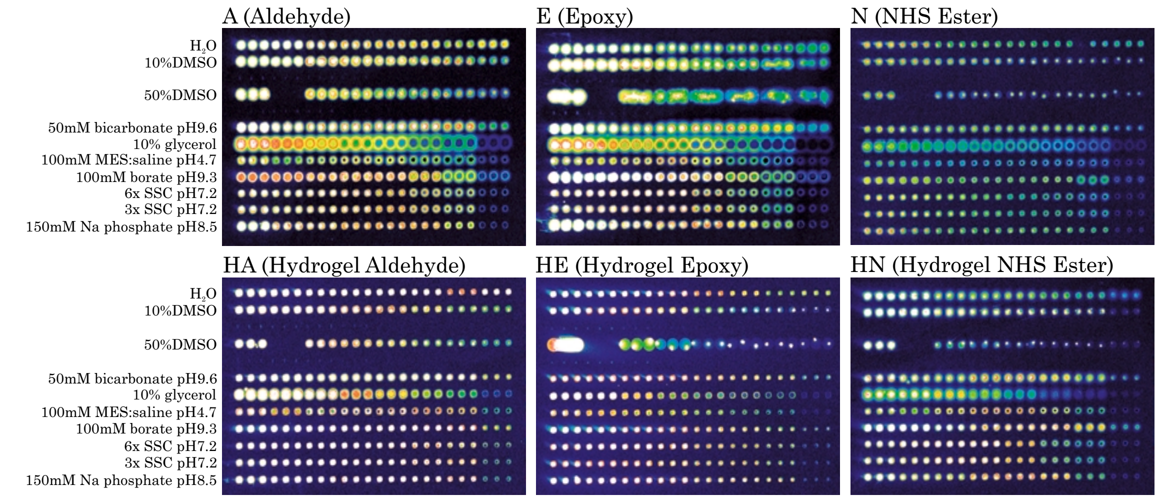

Figure 2. The influence of printing buffer on spot morphology.
A 4-fold dilution series of Biotin-PEO-amine (625, 156, 39, 9.8, 2.4, 0.6 and 0.15pg/spot) was printed, in triplicate, in the indicated printing buffers using a Virtek SDDC-2 arrayer with TeleChem SMP5 pins. Printed slides were air-dried in the arrayer cabinet after which they were blocked in NPBB for 2 hours at RT. Blocked slides were incubated with a 4ug/ml solution of Cy3-labelled Streptavidin for 1 hour at RT, washed in DI H_2O, spin-dried at 1000rpm for 3 minutes and then scanned at 10um resolution in a GSI Lumonics ScanArray Lite at a laser setting of 70 and PMT gain of 70.

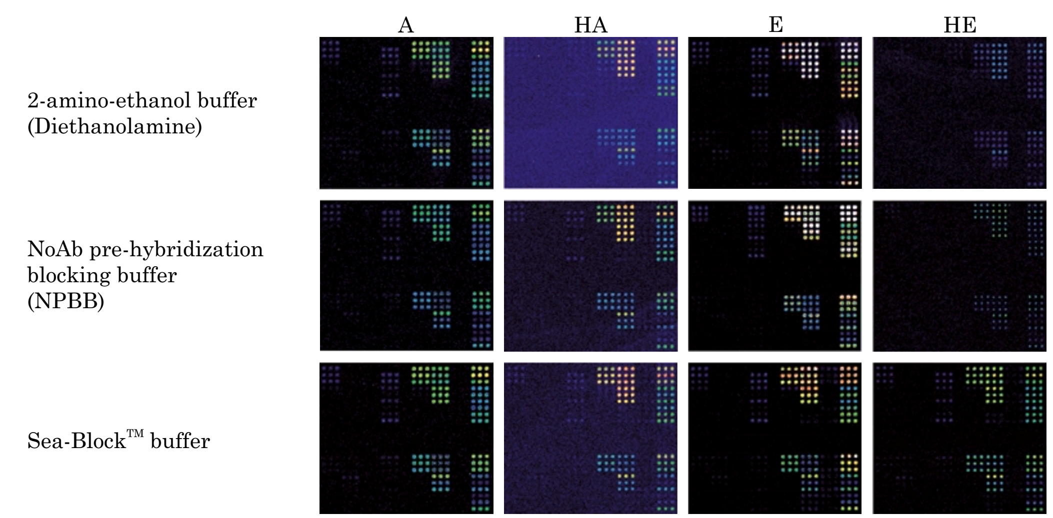

Figure 3. The influence of pre-hybridization blocking buffer on signal intensity.
A mixture of aminoallyl-dUTP-modified oligonucleotides (60mers) in 150mM sodium phosphate pH 8.5 buffer was printed into 4 subarrays using a Virtek SDDC-2 arrayer and TeleChem SMP-5 pins. Each subarray was printed at final element concentrations of 2.5fmole, 250 amole, 25 amole and 2.5 amole. The printed slides were air-dried for 4 hours in the arrayer cabinet after which they were incubated for 2 hours, at room temperature, in the indicated pre-hybridization blocking buffers. The blocked slides were then hybridized to a Cy3-labelled oligonucleotide (23mer) cocktail in NPBB for 2 hours at 37°C. Hybridized slides were then washed twice in 0.1x SSC:0.1% SDS, spin-dried at 1000 rpm for 3 minutes and scanned at 10um resolution in a GSI Lumonics ScanArray Lite at a laser setting of 70 and a PMT gain setting of 70.

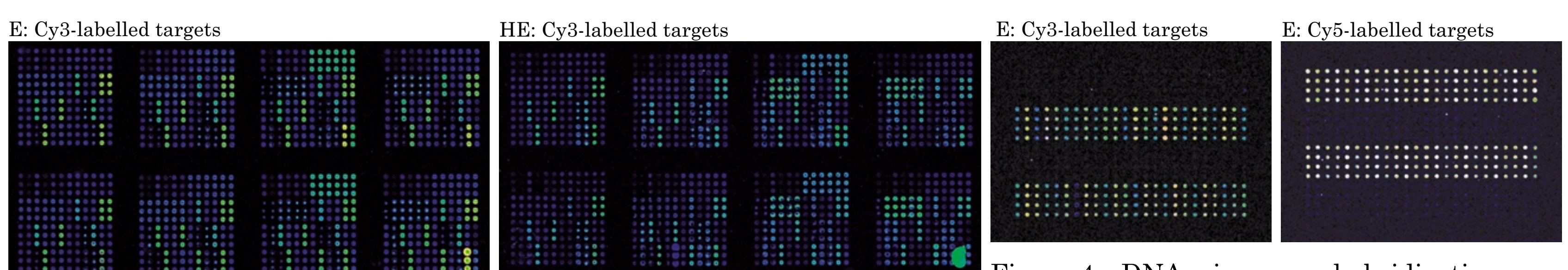

Figure 4. cDNA microarray hybridizations.

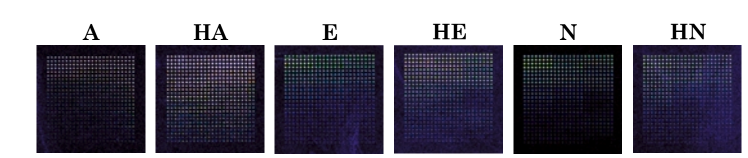

Figure 5. IgG microarray hybridizations.
A 2-fold dilution series of purified mouse IgG (33, 16, 8, 4, 2 and 1amole; 96 replicates of each dilution; ZyMed Laboratories Inc.) was arrayed in a 24x24 grid. The arrays were air-dried, blocked in NPBB for 2 hours at room temperature and incubated with Cy3-GAMIg (1:100; Zymed Laboratories Inc.) in PBS pH7.4 for 2 hours at 37°C. The arrays were washed twice in PBS pH7.4, "spin-dried" and scanned in a GSI Lumonics ScanArray Lite at a resolution of 10um, a laser setting of 70 and a PMT gain setting of 70.