

**Analytical and Bioanalytical Chemistry**

**Electronic Supplementary Material**

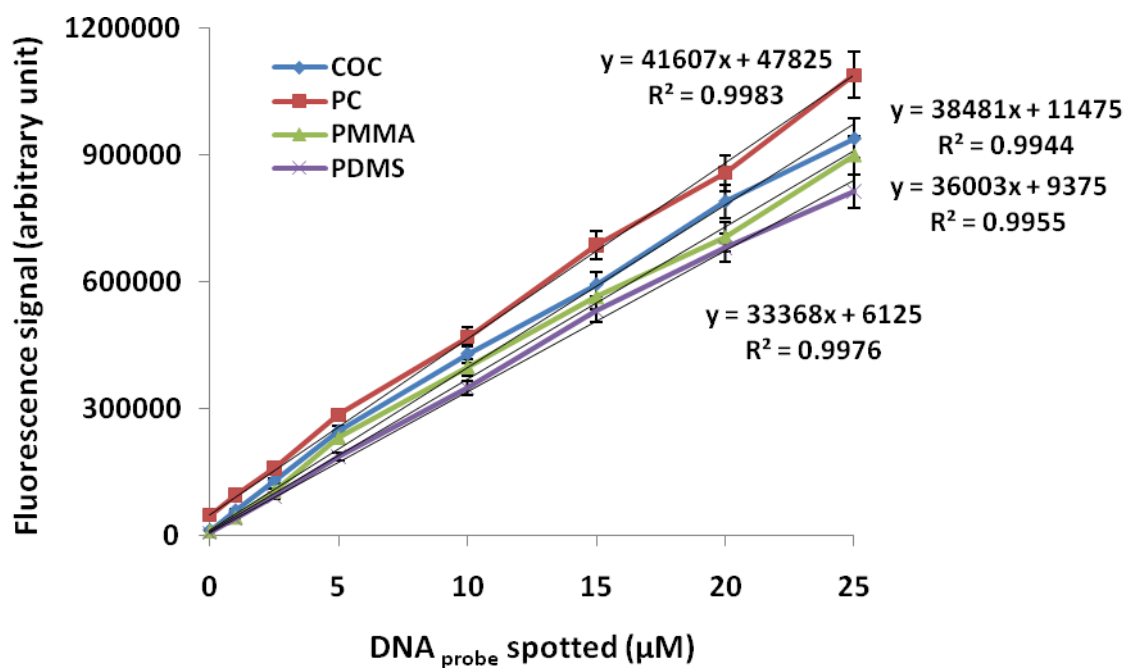
**Direct immobilization of DNA probes on non-modified plastics by UV irradiation and integration in microfluidic devices for rapid bioassay**

Yi Sun, Ivan Perch-Nielsen, Martin Dufva, David Sabourin, Dang Duong Bang, Jonas Høgberg, and Anders Wolff

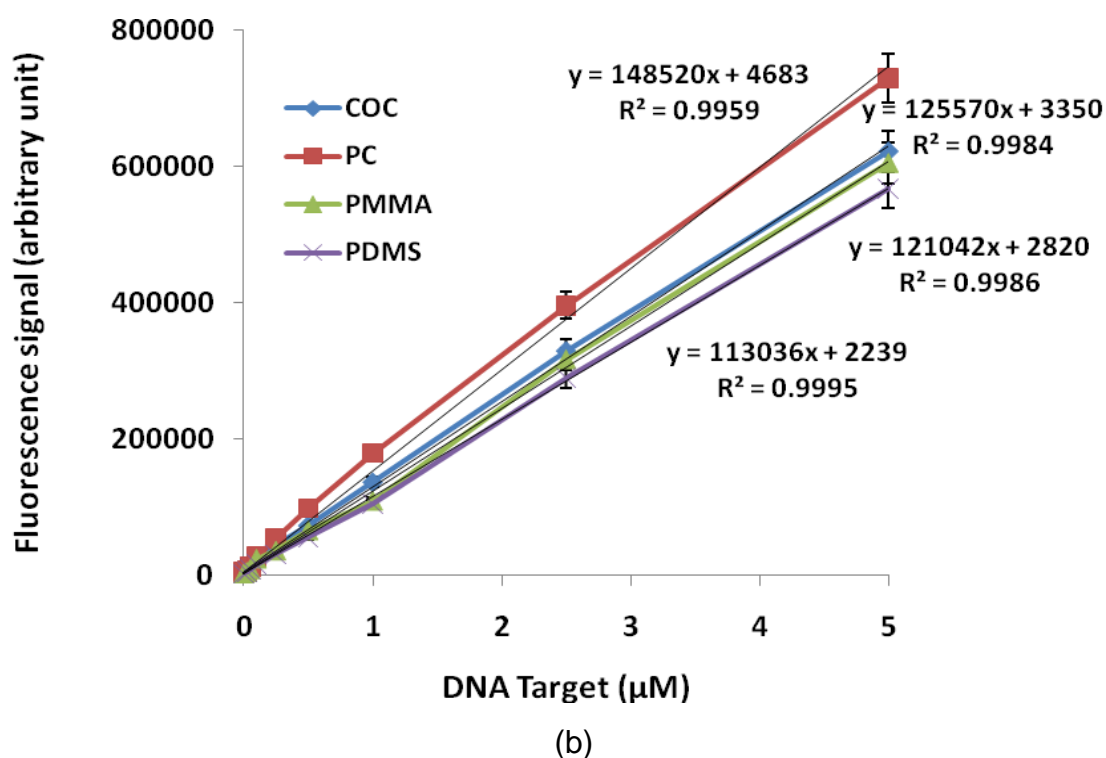
In this document, the standard curves for DNA probes and targets on the four plastic slides were presented. The quality of the microarray spots printed on the COC substrate was also investigated.

### Standard curves for DNA probe and DNA target

In order to quantify the fluorescence of the spots on the four plastic substrates after immobilization and hybridization, standard curves were made and the amount of immobilized and hybridized DNA was extracted from the respective calibration curves. The standard curves for DNA probes were prepared by diluting Cy5-labeled probes in the spotting buffer to a final concentration ranging from 1  $\mu\text{M}$  to 25  $\mu\text{M}$ . DNA microarrays were spotted using a non-contact array nano-plotter 2.1 that deposited 0.1 nl/spot on the substrates. Each dilution was spotted in 10 replicates on the four non-modified plastic slides. The fluorescent signals were plotted as functions of the amount of DNA molecules per spot (Fig. S1a). The same procedure was adopted for Cy3-labeled DNA targets while the range of dilutions for the DNA target was from 0.025  $\mu\text{M}$  to 5  $\mu\text{M}$  (Fig. S1b). For all of the four substrates, there are strong linear correlations between the spotted DNA concentration and obtained fluorescence in the standard curves.



(a)



**Fig. S1** (a) Standard curves for DNA probes by diluting Cy5-labeled probes in the spotting buffer to a final concentration ranging from 1  $\mu\text{M}$  to 25  $\mu\text{M}$ . The fluorescent signals were plotted as functions of the amount of DNA molecules per spot. (b) Standard curves for Cy3-labeled DNA targets while the range of dilutions for the DNA target was from 0.025  $\mu\text{M}$  to 5  $\mu\text{M}$ .

### Spot quality

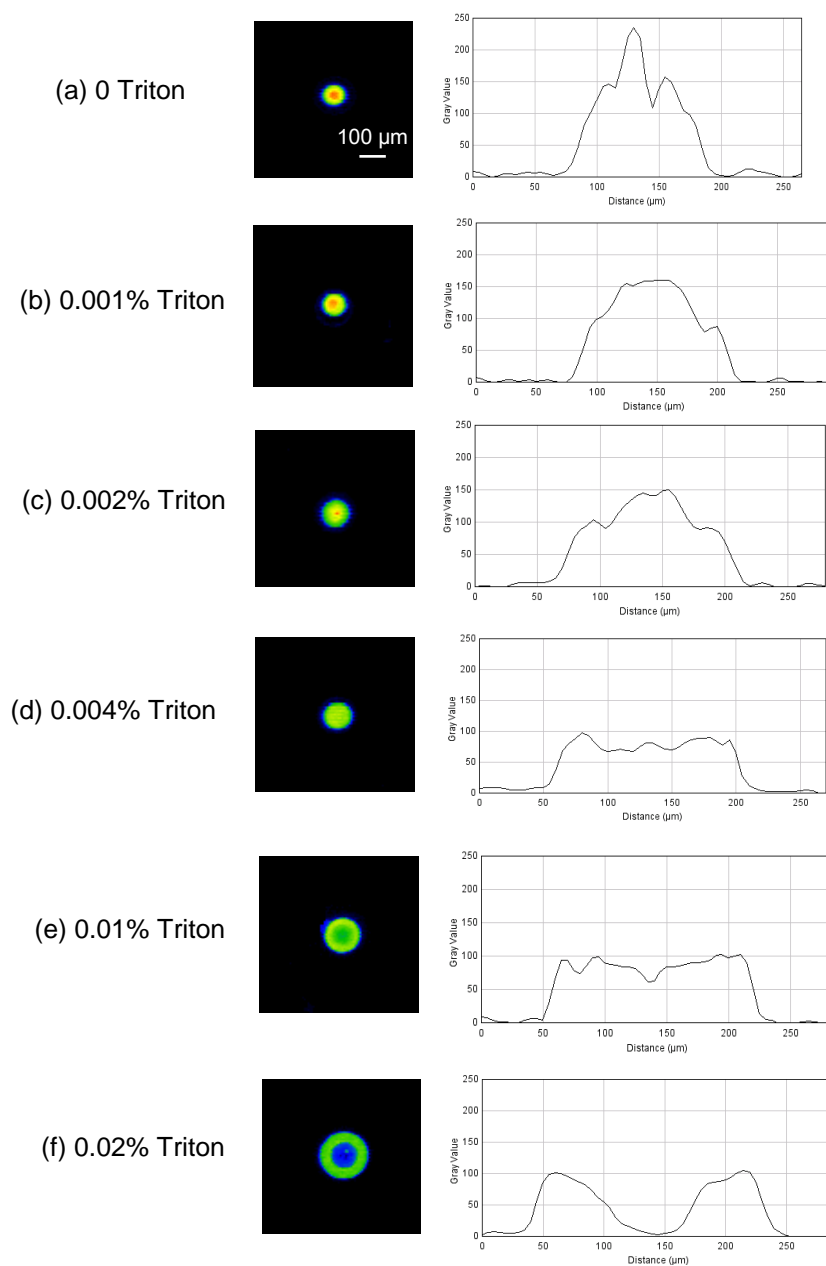
An important but often underestimated aspect of microarray analysis is the quality of the spots printed onto the microarray substrate. The intra- and inter-spot homogeneities are crucial to the reliability of microarray results. In this study, the morphology of spots deposited on COC substrate was investigated and uniform spots were achieved by adding Triton X to the PBS spotting buffer.

The Cy5-labeled 20-base M gene probe with a TC tag was diluted in spotting buffer to a concentration of 20  $\mu\text{M}$  and spotted on untreated COC slides using a non-contact array spotter. Immobilization was performed by UV irradiation at 3  $\text{mW}/\text{cm}^2$  for 10 min. After washing the unbound probes away, microarrays on the plastic surface were scanned. PBS was initially used as a conventional buffered solution. However, as the four polymer materials are highly hydrophobic, printing PBS on the plastic surfaces led to shrinkage of the droplets during evaporation (Fig. S2a). With the decreasing contact area, the oligo probe in solution was confined to the drying droplet, resulting in a small spike at the center of the initial drop area. Moreover, irregular array alignment was also observed due to droplet contraction, which will make it more difficult for the data processing software to extract and interpret the signals.

To improve the spotting conditions with respect to our technology, Triton X was added to the PBS buffer. The surfactant can lower the initial contact angle of the deposited solution, thus influencing

the evaporation behavior of the droplets. Fig. S2 (b-f) illustrates how Triton composition affected the spot pattern on the COC substrate. With the increase of Triton concentration in the spotting buffer, the initial contact angle was strongly modified and decreased from  $86^\circ$  for PBS solution to  $21^\circ$  for the mixture of PBS with 0.02% Triton, resulting in spots with increased diameter. The spike at the center tended to disappear and more homogeneous repartitioning of the immobilized probe molecules was observed inside the spot. However, for solutions containing Triton concentration higher than 0.004%, the coffee ring pattern started to occur, indicating more solutes were brought to the edge of the spot.

These findings could be explained by the droplet evaporation modes on solid surfaces. On hydrophobic plastic surfaces, the evaporation is dominated by constant contact angle mode where probes molecules are subject to pull-back forces at the perimeter of the droplet. The addition of surfactant to the spotting buffer modifies liquid surface tension and the interfacial forces, thus shifting the droplet evaporation behavior towards a constant contact area mode where the rim of the droplet is pinned to the surface and molecules are subject to convection flow from the center of the droplet to the periphery. When the Triton concentration reached 0.004%, the convection flow is balanced with the pull-back forces, producing homogeneous deposition of oligo probe molecules. With higher Triton concentration, the convection flow dominates and rain stains were then obtained when the droplet flattens in constant area regime. Therefore, PBS with 0.004% Triton was used as the optimized spotting buffer in order to achieve uniform distribution of immobilized oligo probes.



**Fig. S2.** Effect of Triton X on the spot morphology of DNA probes immobilized on the native COC surface. The Cy5-labeled M probe was attached with a poly(T)10-poly(C)10 tag at the 5' end. The probe was diluted to 20  $\mu\text{M}$  in 150 mM PBS buffer with different concentrations of Triton X and spotted on the COC slide using a non-contact array spotter. After exposing to UV irradiation for 10 min, the slide was washed and scanned using laser scanner. Image profiles were obtained by measuring the grayscale of pixels across the center lines.