ToF-SIMS imaging to characterize DNA microarray spots

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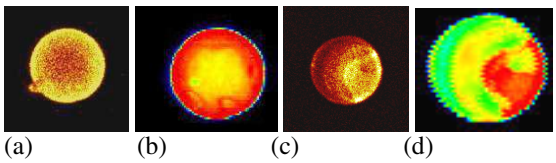
Statement of Purpose: Commercial DNA array slides are often made by microprinting techniques which involves putting nanoliter droplet of DNA solution on coated glass substrate. The droplet evaporates within seconds. It is believed that this fast drying leads to heterogeneous spots which can lead to inconsistent and misleading results. DNA arrays are most commonly analyzed by fluorescence imaging but only a small percentage of the DNA is fluorescently tagged. X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging provide chemical information for these spots. Multivariate analysis help extract as much information as possible from spectra and images[1,2]. Maximum Autocorrelation Factor (MAF) image analysis, a technique independent of the scaling of the raw data, was used to analyze the ToF-SIMS images. The added chemical information can help to better understand that heterogeneities within the DNA microarrays.

Methods: Amino terminated single stranded 40mer DNA probes were spotted onto amine reactive commercial microarray slides using a non-contact printer. The spotting solution concentration varied from 10 – 40 µM with varying amounts of fluorescent Cy3 labeled probes. ToF-SIMS imaging data was acquired on an IONTOF TOF.SIMS 5-100 instrument using Bi^+, Bi_3^+, Bi_3^{++} and C60 cluster ion sources. Multivariate analysis techniques are applied to spectra and images.

Results: Both ToF-SIMS and fluorescence imaging show that changing the spotting solution concentration affects the distribution of immobilized DNA. ToF-SIMS data of the PO_2^- fragment from the DNA backbone indicates the distribution of DNA in the spots (Figure 1 a & c). The increase concentration of DNA in a spot correlated very well with the increase fluorescence within the same spot (Figure 1 b & d) suggesting that there is an even distribution of the Cy3 label within the spot. These results indicate that the spot non-uniformity is not due to unbound Cy3 label.



(a) (b) (c) (d)

Figure 1. ToF-SIMS PO_2^- (a & c) and fluorescence images (b & d) for 100 % Cy3 labeled probe in a 20 µM (a & b) and 40 µM (c & d) spotting solutions.

MAF analysis of the ToF-SIMS image (Figure 2) shows that high fluorescence area of the spot are related to higher concentrations (positive scores) of PO_2^-, PO_3^-, SO_4^-, HSO_4^- and DNA base peaks. Sulfate containing peaks are likely due to sodium dodecyl sulfate (SDS), a component of the cleaning solution, which is uniform over the slide (SIMS image not shown).

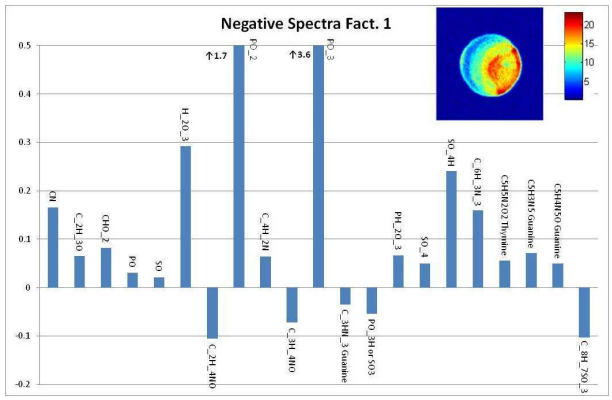


Figure 2. MAF analysis of 40 µM spot with 100% of Cy3 label. For more clarity only the scores ≥ 0.011 are shown. Both PO_2 and PO_3 signals are above the scale.

Spotted arrays with 0, 25, 75, and 100% Cy3 label incorporated into the spotted DNA were also imaged with ToF-SIMS and analyzed by MAF. These results indicate that the apparent distribution of DNA in the microarrays spots also changes with different amounts of Cy3 label.

Conclusions: The concentration of the spotting solution has a significant effect on the uniformity of the spot as well as the amount of fluorescent label incorporated into the DNA. The chemical information extracted from the ToF-SIMS images and MAF analysis of the data were able to show that the non-uniformity of the fluorescence within a spot is coming from a non-uniformity in the DNA coverage within this spot. This non-uniformity in the DNA coverage is probably due to the very rapid drying of the micro droplet deposited on the slide during the printing process of the micro array, but appears to depend not only on the initial DNA spotting concentration, but also the amount of Cy3 incorporation into the spotted DNA. The chemical information, provided by the MAF analysis, helped to better understand the micro array chemistry.

References:

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2. Tyler B. J., et al. Biomaterials 2007; 28: (15), 2412-2423.

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