Surface Analysis of DNA Microarrays

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Abstract and Background:

Surface-bound deoxyribonucleic acid (DNA)-based arrays have shown the potential to be powerful tools to diagnosis and analyze several different diseases.

	MEG SAM DATA			Previously Published Values (1)		DNA-Covered Spot	
XPS Line	BE - Ayo	Atomic %	% STDV	Atomic %	% STDV	Atomic %	% STDV
Au 4f	84	19	0.3	17.4	1.4	15.1	0.4
O 1s	533	19.4	0.6	15.1	1.6	20.6	0.9
N 1s	400	3.2	0.3	2.8	0.7	3.1	0.4
C 1s	286	57.3	0.7	63.8	0.4	60.1	0.9
S 2p	162	1.1	0.3	0.9	0.1	1.1	0.3
P 2p	na	na	na	na	na	na	na

Figure 1: XPS comparison of MEG-SAM atomic composition and DNA immobilized slide.

However, for microarrays to meet theoretical expectations for detection sensitivity and the potential for single base mutation detection, the current technologies need to be optimized. The purpose of this study was to analyze model DNA arrays in order to determine potential optimization area in the current methods. Surface analysis techniques such as surface plasmon resonance (SPR) and x-ray photo electron spectroscopy (XPS) were used to analyze the arrays. In addition, fluorescent imaging was used to evaluate individual spot uniformity within an array on a commercial substrate.

Methods:

Gold covered SPR slides were modified with maleimide-ethylene glycol disulfide (MEG) self-assembled monolayers (SAMs). The maleimide group specifically binds with thiol terminated single stranded DNA (HS-ssDNA). Monolayers were made using the previously reported method [1]. The MEG SAM coated slides were manually spotted with 40-mer-ssDNA. The spotted DNA had a 15 μM concentration in buffer (1.5 M sodium chloride and 0.015 M sodium citrate in water) and was treated with a reducing agent, 0.2 mM tris(2-carboxyethyl)phosphine. The slides were incubated at ~ 70% humidity for 1.5 hr before repeated rinsing in both buffer and water. The slides were blocked with 6-mercapto-1-hexanol (MCH) to inhibit the target DNA from binding to unreacted maleimide groups.

XPS provided an elemental analysis of what was on the top ~ 8 nm of the surface. XPS was performed on a MEG-SAM modified slide before and after the immobilization of DNA onto the slide.

A Plexera imaging SPR was used to compare hybridization efficiencies for three different spot sizes on the model DNA array surface. The Plexera imaging SPR works by shining monochromatic light through a prism at multiple angles to the slides. The light excites a plasmon wave at specific angles dependent on the interactions on the slide and solution [2]. When target DNA is flowed through the SPR flow-cell, there is a change in index of refraction caused by interactions on the slide. These results can be used to determine real time kinetic information of the spotted DNA. The SPR experiment was set up to run buffer, non-complementary DNA and then complementary DNA across the slide.

Fluorescence imaging was used to characterize individual spots on a set of commercial slides that were printed using an automated printer. The images were obtained by using a confocal laser scanning microscope with a 543 nm laser and a 610 nm filter.

Results and Discussions:

The XPS analysis of the MEG-SAM slide coating was compared to previously published XPS data [1] for the monolayer as seen in Figure 1. The atomic composition of the tested MEG-SAM slide was similar to previously published results, suggesting that the protocol used for the MEG-SAM slide coating worked. XPS was also used to determine if there was DNA immobilized onto the slide after spotting. If there was DNA immobilized onto the slide, it would be evident by the presence of phosphorous

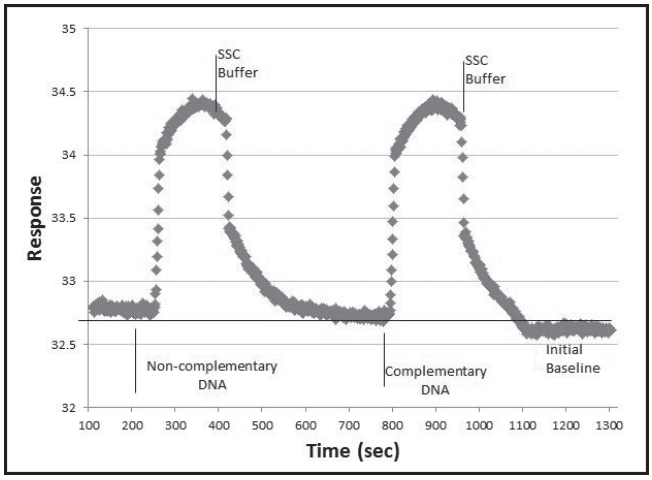

Figure 2: SPR analysis of hybridization.

in the atomic composition (from the DNA backbone) as well as attenuation of the gold signal and an increase in the nitrogen signal. As shown in Figure 1, phosphorous was not detected by XPS of the DNA immobilized slide. However, the Au signal was slightly attenuated and the nitrogen signal increased slightly indicating that there might be a very small addition of DNA to the surface.

The SPR results (Figure 2) show the spotted slide response to non-complementary DNA and complementary DNA. If hybridization occurred, it would be expected that after the addition of the complementary DNA the response would be higher than the baseline after the addition of the buffer. These SPR results indicated no detectable hybridization occurred. This result, in combination with the XPS data, indicated that there was little DNA bound to the surface after the spotting step. However, these results did indicate the DNA did not non-specifically bind, suggesting that the MCH blocking step was successful.

Fluorescence results of the commercial DNA array suggested that there may have been an issue with sample storage of this particular commercial substrate, correlating to a problem with spot reproducibility.

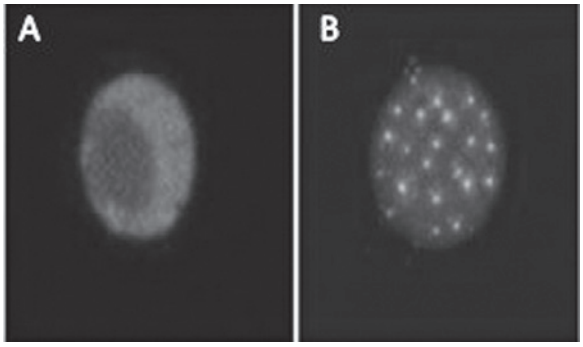

Figure 3: Comparison of two fluorescence images; a) image of general spot uniformity (left), b) image showing segregated spot taken months after hybridization (right).

Figure 3 compares two fluorescence images taken at different time-points. As can be seen with the image from the aged slide, there seems to be segregation within the individual spot.

Conclusions:

This study illustrates the importance of surface analysis in better understanding DNA microarrays and the usefulness of these methods for detecting previously unknown problems. Knowing these problems exist is the first step in being able to fix them and ultimately leading to a better microarray.

Future Work:

Further investigations must be done in order to optimize the surface chemistry used for the MEG-SAM DNA immobilization. This will be done by analyzing each step in the process to determine which step is working improperly. Once the surface chemistry is optimized, these slides can be used in model hybridization experiments characterized by SPR. Also, more fluorescence data needs to be obtained and compared to other supplementary methods in order to determine the cause of the segregation on the slide.

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