Surface Chemical Analysis of DNA Microarrays

Application of XPS and ToF-SIMS for Surface Chemical Imaging on the µm Scale

The chemical composition of the functional surfaces of substrates used for microarrays is one of the important parameters that determine the quality of a microarray experiment. In addition to the commonly used contact angle measurements to characterise the wettability of functionalised supports, XPS and ToF-SIMS are more specific methods to elucidate details about the chemical surface constitution. Their application on printed DNA microarrays provides impressive chemical images down to the µm scale and can be utilised for label-free spot detection and characterisation.

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Quality Standards of Microarrays

Microarrays have caused tremendous interest in research being applicable for high throughput analysis in gene discovery, clinical diagnostics and pharmacogenomics. [1] They are among the fastest growing areas in biomedicine depending on precision surface technology. [2, 3] A high degree of reliability and reproducibility is required to meet the standards for their potential application in clinical diagnostics. Therefore, e.g. FDA's National Center for Toxicological Research initialised the "MicroArray Quality Control (MAQC)" project. A result of MAQC is that careful experimental design and appropriate data transformation and analysis indeed generate reproducible and comparable microarrays, irrespective of the sample labelling format. However, it was also emphasised that microarrays "have a long way to go before they can be used to support regulatory decision-making or accurate and consistent prediction of patient outcomes in the clinic". [4, 5] So intra- and inter-platform variability is still discussed in the recent literature and factors influencing this are suggested to be batch fluctuation of arrays and reagents, differences in settings of the scanner, operator and laser detection systems. [6] There are also activities on establishing standards for data quality, management, annotation and exchange and on the design of reference materials and test standards. These activities are driven, amongst others, by the Microarray and Gene Expression Data (MGED) Society and the American National Institute of Standards and Technology (NIST). [7]

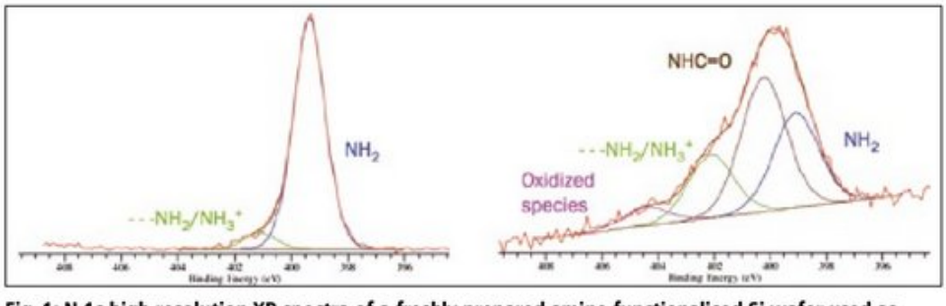

Fig. 1: N 1s high resolution XP spectra of a freshly prepared amino functionalised Si wafer used as reference for an ideal surface (left) and a comparable, commercially available slide (right) which is more characteristic for field samples used for microarrays.

Despite the great number of projects for improving the quality of microarray applications, a detailed chemical characterisation of the functional supports used for microarrays has not been taken into account very often up to now.

XPS and ToF-SIMS

The powerful techniques X-ray Photoelectron Spectroscopy (XPS), also called Electron Spectroscopy for Chemical Analysis (ESCA), and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) can be applied to analyse the chemical composition of functional surfaces. Spectral and imaging data about the chemistry and morphology of the surfaces, obtained from the chemical shifts in XPS spectra and the key fragment ions in ToF-SIMS mass spectra, can provide important information about the performance of surface functionalised materials in biomedical applications. It should also be mentioned that the Food and Drug Administration (FDA) routinely calls for XPS data to qualify medical devices. [6]

XPS was developed in the 1960s by Prof. Siegbahn's group at Uppsala University. Information about the chemical composition of a surface and the chemical species present can be obtained quantitatively by this technique. It is based on energy analysis of the photoelectrons emitted from a surface illuminated by X-rays. Since the mean free path of electrons in solids is very small, the majority of the detected electrons originate from the top few atomic layers, resulting in an information depth of around 10 nm. Spectra are displayed as a plot of the number of detected electrons per energy interval versus their kinetic energy. These kinetic energies can be easily converted into the binding energy of the atomic orbitals from which the electrons originate.

The development of ToF-SIMS to an analytical tool can be traced back to the 1940s but the substantial contributions were accomplished 40 years later by Prof. Benninghoven's group at Münster University. When high energy primary ions collide with a solid surface, some of the primary ions can be backscattered but most of them transfer their kinetic energy to the surface lattice. As a result secondary ions (atomic, fragment or molecular ions to be analysed by the ToF mass spectrometer), but also electrons, photons, neutral particles and excited clusters are emitted. The information depth of ToF-SIMS is 3 nm, i.e. the secondary ions comprising the mass spectrum originate only from the very first atomic layers. Interpretation of ToF-SIMS spectra is, as usual in mass spectrometry, based on abundances of key fragment ions or patterns, and, when possible, molecular ions as well.

Application of XPS and ToF-SIMS in Surface Chemical Analysis of DNA Microarrays

The control of surface parameters as the nature, density and homogeneous distribution of specific functional groups is essential for a reliable immobilisation of DNA probes, following the printing step in the fabrication of microarrays. In addition to the analysis of the chemical constitution of functional surfaces by XPS and ToF-SIMS these methods can be applied to obtain a laterally resolved analysis of microarrays which results in chemical images of the single spots without using fluorescence markers. In this way also the uniformity of the spots based on their chemical constitution can be made visible. A laterally resolution of 10 µm is achievable for XPS, 0.1 µm for ToF-SIMS. Another application of imaging XPS focused on the determination of the DNA hybridisation efficiency was published by Castner *et al.* [8] Therein the authors used the P 2p photoemission intensity to determine directly the amount of hybridised DNA avoiding the requirement of fluorescent labels.

We have analysed aminopropylsiloxane layers prepared by silanisation of silicon supports as reference in comparison

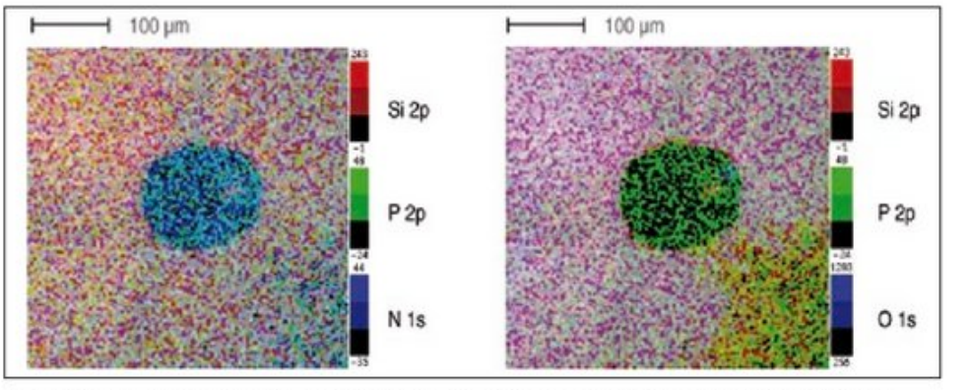

Fig. 2: XPS images of a spot of an *E. coli* microarray printed on an amino functionalised slide as an overlay of N 1s, Si 2p and P 2p (left) and Si 2p, O 1s and P 2p images (right), respectively.

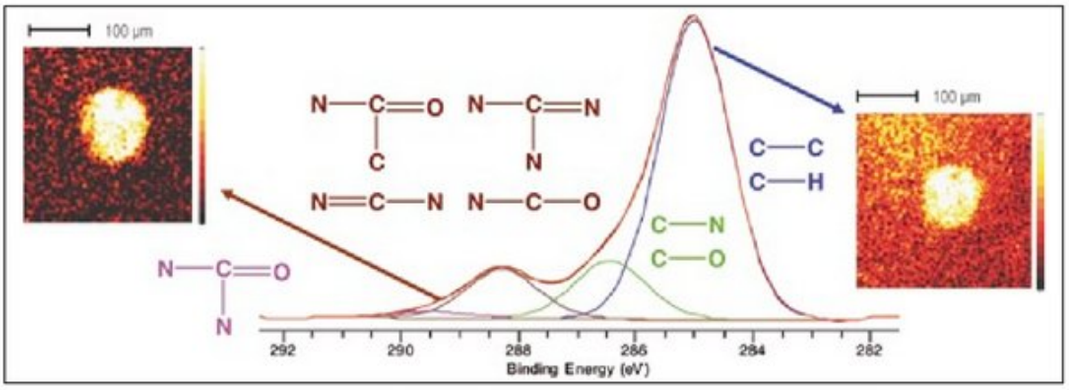

Fig. 3: C 1s high resolution XPS spectrum of a spot of an *E. coli* microarray printed on an amino functionalised slide showing the chemical species on the surface. In the left image the spot appears to be brighter, because of the DNA specific carbon species detected at ~ 288 eV compared to the spot based on the signal at 285 eV (right image).

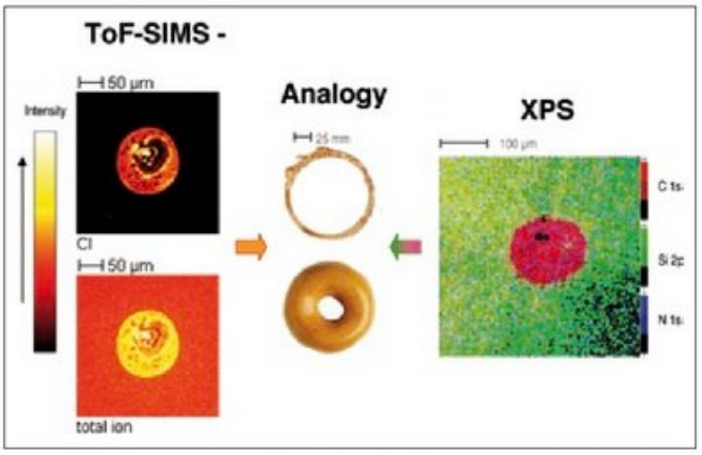

Fig. 4: XPS (right, overlay of C 1s, Si 2p and N 1s photoemission signals) and ToF-SIMS images (left, Cl- and total negative secondary ions) of *E. coli* microarray printed on an amino functionalised slide resolving even slight inhomogeneities of the spot morphology. Images of the eponyms for this effect, a real coffee ring and donut, are shown for analogy (middle).

to amino functionalised glass slides commonly used in microarray experiments. The XP N 1s spectra (fig. 1, left) indicate that the sample synthesised under controlled conditions [9] contains less different nitrogen species (hydrogen bonded/protonated amine and free amine) on the surface compared to a commercially available sample (fig. 1, right) where hydrogen bonded/protonated amine, free amine, amide and some oxidised species were found. This raises the question about the role of the primary amino groups since UV cross-linking is commonly applied to build covalent bonds in a nonspecific way between the functional surface and the biomolecules in the immobilisation step.

Exemplarily, DNA microarrays printed with a sciFlexarrayer (Scienion) were investigated with imaging XPS and ToF-SIMS. Obtained XPS images are displayed in figure 2. By overlaying images of Si 2p, N 1s and P 2p and Si 2p, P 2p and O 1s photoemission signals, respectively, the morphology of a DNA spot becomes visible. The elements contained amongst others in the amino functionalised glass substrate are Si, O, N and C. The DNA comprises the elements P, C, N and O. Si is unique to the substrate, P is unique to DNA, whereas N and O are contained in both systems. The resulting images show highest contents of P and N within the spot. By ToF-SIMS fragment ions like CN^-, CNO^-, PO_2^- and PO_3^- were identified within the spot (not shown).

Detailed information about the constitution of the spotted microarray can be obtained by C 1s high resolution XPS (fig. 3). The species found there can be imaged by selecting their specific binding energies. The carbon peak at 285 eV contains in general aliphatic and aromatic hydrocarbons which are not specific for DNA, resulting in an image with a lower contrast between substrate and spot. However the carbon peak at ~288 eV represents carbon species specific to the DNA bases [10], resulting in an image with a rather high contrast between spots and substrate.

A high uniformity of the spots of a microarray is one of the most important requirements for a successful analysis using fluorescence detection techniques. However, the DNA spots of microarrays often show nonuniformities as the well known donut effect. In addition to fluorescence imaging, detailed information on the substructure of DNA spots can be obtained by ToF-SIMS chemical imaging with rather high lateral resolution. Figure 4 displays an example where Cl^- secondary ions, or even the total secondary ions were used for image formation (Cl^- was selected because of being present only within the spot as a component of spotting solution). Spot uniformity of this sample can also be controlled by imaging XPS as revealed by overlaying images of C 1s, Si 2p and N 1s photoemission signals.

Conclusion

The quality of the functional coatings and the spot uniformity of printed microarrays can be investigated by recent methods of surface chemical analysis in their imaging modes. In this way the important question about the exact nature of the chemical species present on the surface can be answered. This might help to explain different degrees in the immobilisation step of probe DNA on supports formally claiming to have the same functionality. Functional homogeneity across the slide and uniformity within the spots are other important requirements. Imaging ToF-SIMS and XPS may also be applied to obtain information about the spot quality of the printed microarrays before the hybridisation step with target DNA, i.e. without using labelling techniques.

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