Electronic supplementary information

Imaging and spectroscopic comparison of multi-step methods to form DNA arrays based on the biotin-streptavidin system

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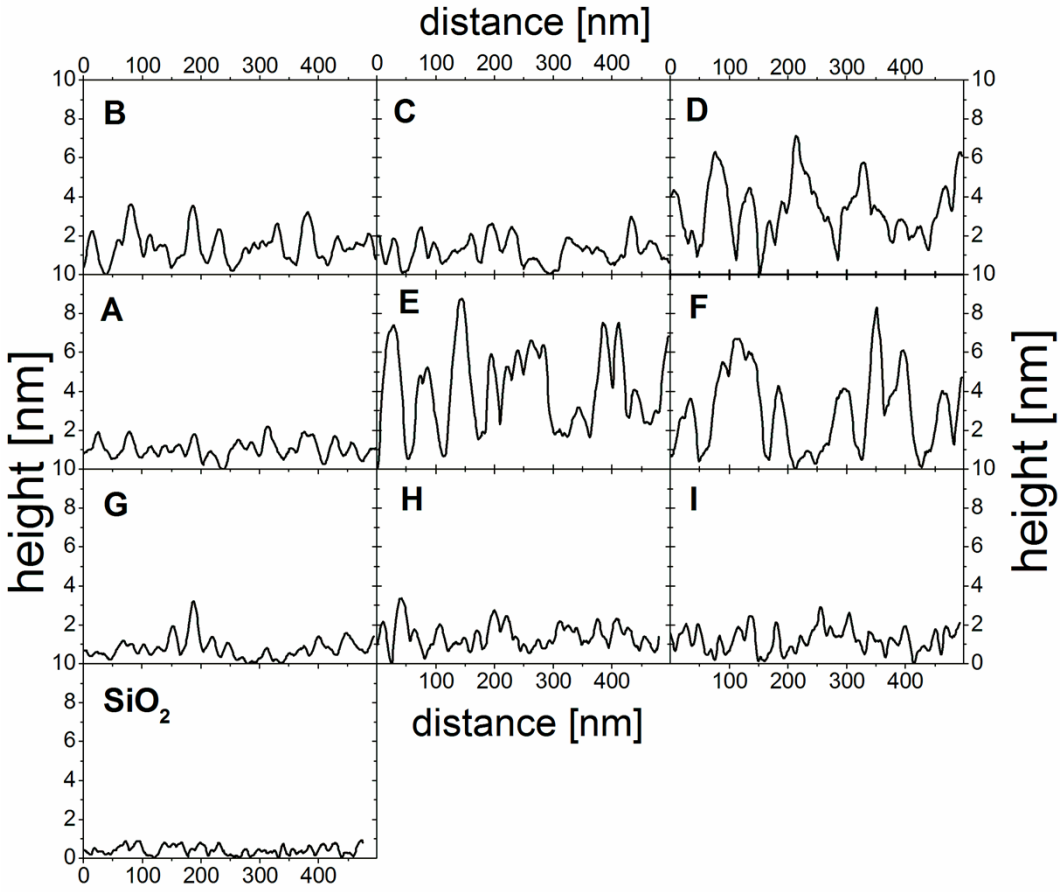


Figure ESI-1. The cross sectional analysis of topographic AFM micrographs (*cf.* Fig.2), recorded from epoxy-silanized SiO_2 surfaces after the successive steps of the different approaches followed for the immobilization of capture oligonucleotides in spots (*cf.* Fig.1 for used notation), and acquired from the bare SiO_2 substrate as the control (characterized by positive surface skewness, *RMS* = 0.22 nm and $\langle h \rangle$ = 0.43 nm).

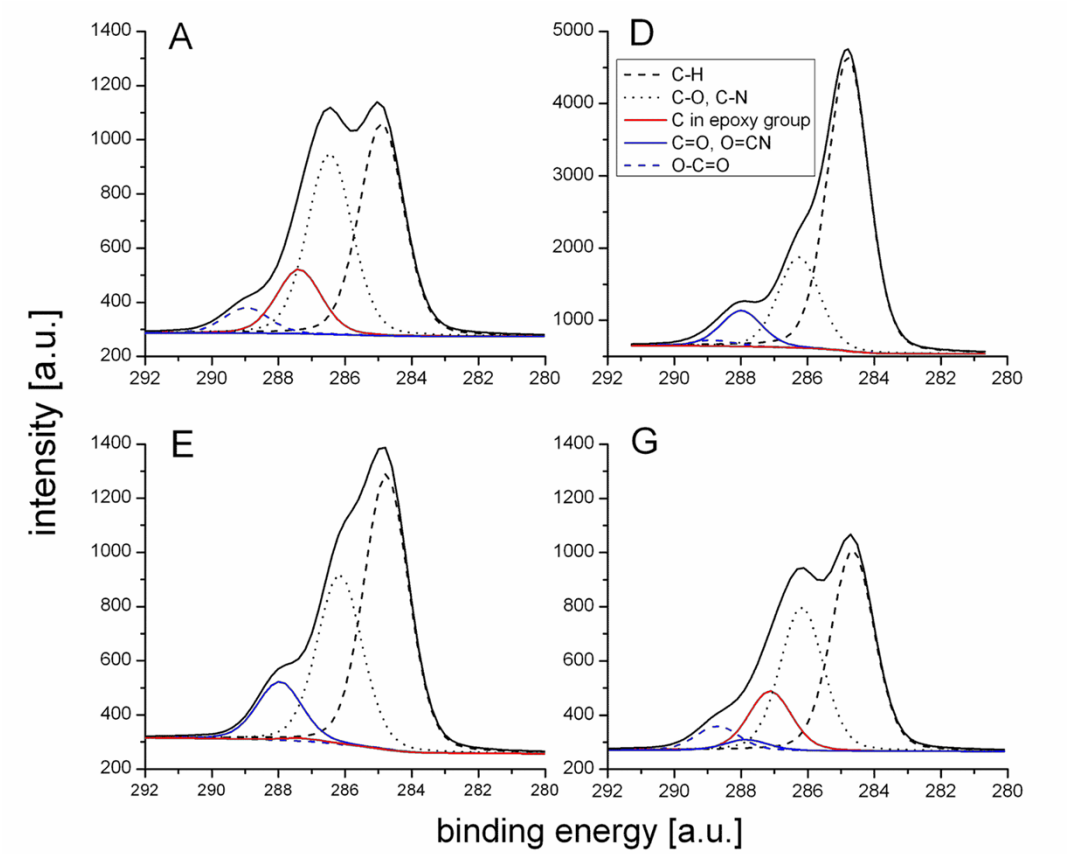


Figure ESI-2. High resolution XPS C1s core-level surface spectra recorded after characteristic steps of the three immobilization approaches (*cf.* Fig. 1). Contributions to the C1s envelope are shown, referred to various carbon environments (see legend). Note, that the intensities of signals from carbon in carbonyl O=C (and amide O=CN) groups, that are specific for biomolecules, and of the epoxy-groups of the silane layer (non-reacted) are anti-correlated.

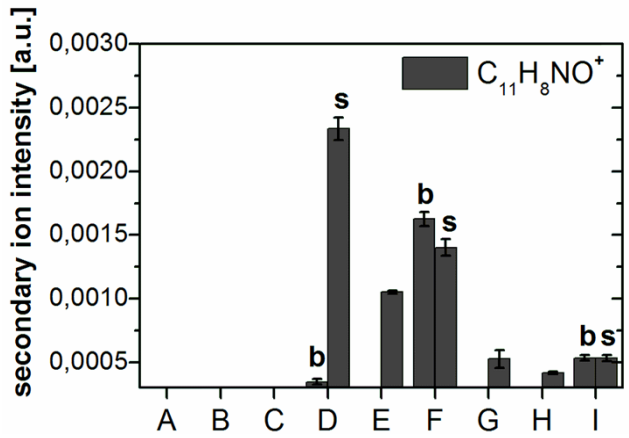
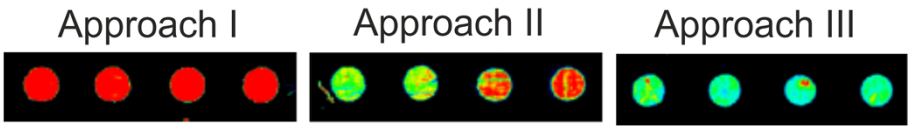


Figure ESI-3. TOF-SIMS microanalysis of streptavidin immobilized at the surface after the successive steps of deposition/reaction involved in the three immobilization approaches (see Fig.1 for the used notation). Secondary ion intensities (normalized per total ion intensity) characteristic for streptavidin ($C_{11}H_8NO^+$ from tryptophan) determined for uniform surfaces (samples A-C, E and G-H) as well as inside (s - spot) and outside (b - background) oligonucleotide spots (samples D, F and I). Error bars are standard deviation values determined from repetitive (3-8) measurements of the same surface.



Mean spot intensity (RFU) of 64 spots		
26680±1541	15406±1054	6797±601

Figure ESI-4. Fluorescence microscopy images of the spots of fluorescently-labelled oligonucleotides on glass slides, prepared according to protocols I, II, and III and the respective mean fluorescence intensity values ±SD obtained from 64 spots.

Cumulative material distribution and complementary molecular composition maps within DNA spots, obtained with TOF-SIMS from 1.0 mm x 1.0 mm areas

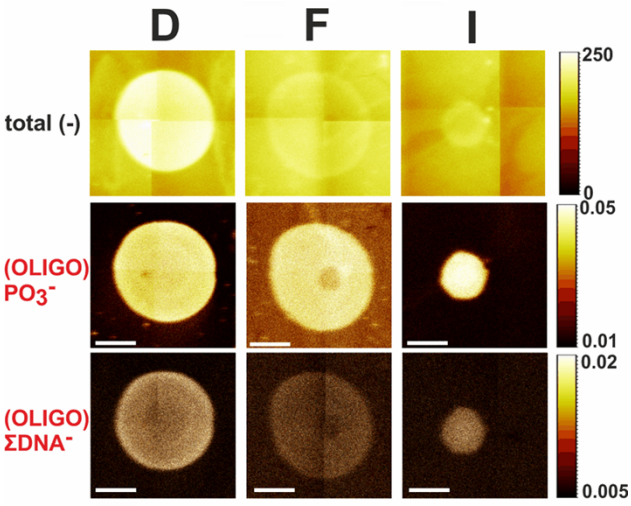


Figure ESI-5. Molecular distribution of the whole material (the row 'total (-)') and composition maps of oligonucleotide (the rows 'oligo', corresponding to signal of PO_3^- and nucleotide bases fragments SUM DNA^-) obtained by TOF-SIMS imaging of the oligonucleotide spots created following each one of the three immobilization protocols (columns D, F and I, respectively, *cf.* Fig. 1) on epoxy-silanized SiO_2 surfaces. The TOF-SIMS intensity maps of all negative ion fragments (corresponding to row 'total (-)') were used to normalize for each pixel the intensities of DNA-derived negative ions (shown after normalization as the rows 'oligo').

Cumulative material distribution and complementary molecular composition maps within DNA spots, obtained with TOF-SIMS from 0.5 mm x 0.5 mm areas

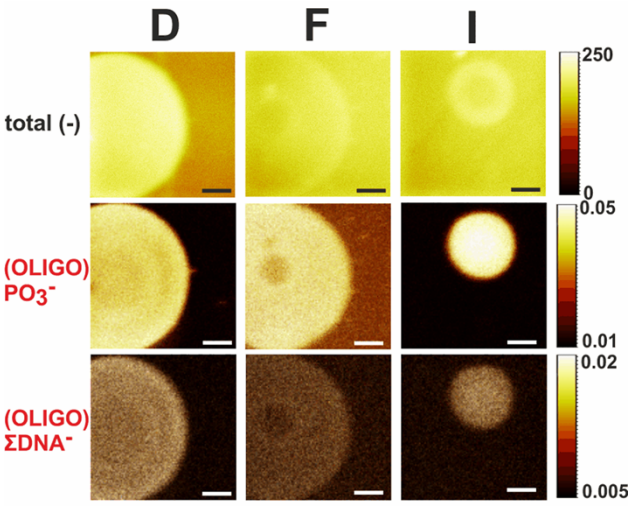


Figure ESI-6. Molecular distribution of the whole material (row 'total (-)') and composition maps (*cf.* Fig. 8) of oligonucleotide (rows 'oligo', corresponding to signal of ion PO_3^- and nucleotide bases fragments SUM DNA^-), obtained by TOF-SIMS imaging of the DNA spots created following each one of the three immobilization protocols (columns D, F and I, respectively, *cf.* Fig. 1) on epoxy-silanized SiO_2 surfaces. The TOF-SIMS intensity maps of all positive ion fragments (row 'total (+)') were used to normalize for each pixel the intensities of positive fragment ions characteristic for the different molecules (shown after normalization as the rows 'oligo').

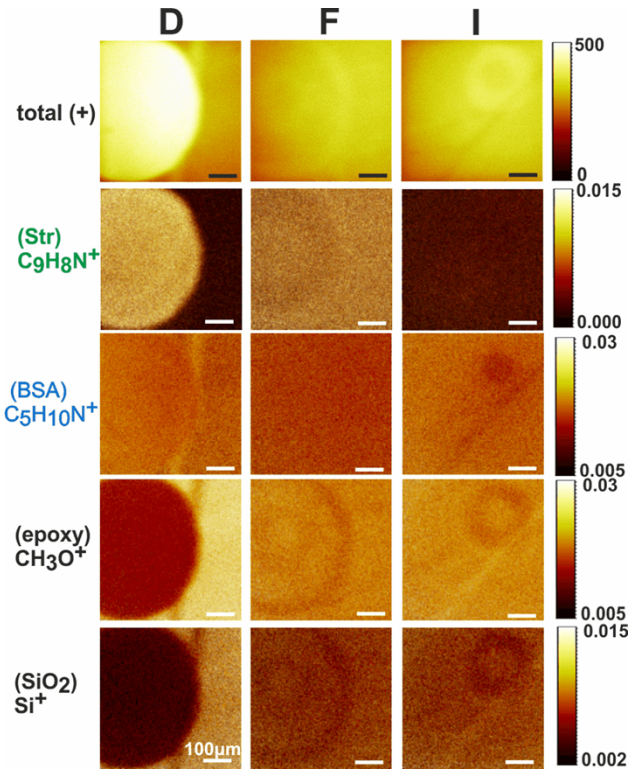


Figure ESI-7. Molecular distribution of the whole material (row 'total (+)') and complementary composition maps (*cf.* Fig. 9) of streptavidin (row 'Str', corresponding to signal of ion $C_9H_8N^+$ characteristic for tryptophan), BSA (row 'BSA', corresponding to signal of ion $C_5H_{10}N^+$ specific for lysine), epoxy-terminated silane GOPS (row 'epoxy', corresponding to signal of ion CH_3O^+) and

silicon substrate (row 'SiO_2', corresponding to signal of ion Si^+), obtained by TOF-SIMS imaging of the DNA spots created following each one of the three immobilization protocols (columns D, F and I, respectively, *cf.* Fig. 1). The TOF-SIMS intensity maps of all positive ion fragments (row 'total (+)') were used to normalize for each pixel the intensities of positive fragment ions characteristic for the different molecules (shown after normalization at the rest of the rows).