Electronic Supplementary Material for Chemical Communications

Dip-pen Nanolithography and SERRS as synergic techniques

Electronic supplementary information

1) DPN and DPN of Direct-write sequences and labels:
All dye-labelled oligonucleotide sequences were purchased from ATDbio (UK) on 0.2 µmol scale with HPLC purification and stored at 4 °C. DPN was performed using a Nanoink (Skokie, IL, USA) NScriptor device, using A, C, D and M type probes. Ink was delivered to the individual cantilevers using Just Add DNA microfluidic inkwell and solutions (Nanoink). Dye labels suitable for SERRS detection in this case included ROX, Cy3.5, Cy5, Cy5.5, Bodipy650, IR700 and Cy7.

2) Clamydia probe sequences used in hybridisation study:

5'-CTT-ACT-CCC-TGT-CGC-AGC-3'
3'-Ds-AAA-GAA-TGA-GGG ACA-GCG-TCG-AAA-Cy5-5'

The capture sequence was concentrated to 97.1 µM *in vacuo*.

3) Hybridisation of Chlamydia Target and SERRS Active Complement
Two independently modified complements to the Chlamydia target were obtained, one is a 12mer modified at the 3'-end with a disulfide surface attachment moeity[14] and a triple „A" spacer. The remaining 9 bases are complementary to half of the target gene sequence. The other probe sequence is also a 12-mer with 9 bases complementary to the other half of the target gene sequence, followed by a triple „A" spacer and is 5'-modified with a Cy5™ dye. In order to capture the Chlamydia target on the nanostructured gold substrate, the 3'-thioctic acid modified complement was used. A triple "A" region was added to the 3'-end of the sequence as a spacer to remove the active probe region from the gold surface. After lithography of the capture strand and removal of the "Just Add DNA" carrier fluid, the surface was passivated with triethylene glycol mono-11-mercaptoundecyl ether to prevent nonspecific binding of the disease target sequence or the dye-labelled complement. The dye-labelled complement was modified at the 5'-end with Cy5™ dye again separated from the hybridising region by a triple "A" segment. Only in the presence of the *Chlamydia* target, immobilised by the capture sequence, would the dye labelled complement hybridise and thus provide a reporter for SERRS detection. The Chlamydia target (20 µl, 47.1 µM) and probe 2 (10 µl, 96.2 µM) were mixed together with NovaTaq Hot Start Buffer (670 mM Tris-HCl, 160 mM $(NH_4)_2SO_4$ and 0.1 % TWEEN-20, 70 µl). Aliquots of the sample mixture, 20 µl, were added to the DPN modified Klarite™ surface at room temperature and left to incubate for 1 h in a moistened hybridisation chamber. The sample plates were then washed with water (0.5 ml, distilled) prior to analysis.

4) Raman (SERRS) acquisition conditions
SERRS spectra were recorded using a Renishaw *System 2000* Raman microscope (Renishaw, UK). Point spectra were recorded at 632.8, 785 and 830 nm using a number of optical elements (0.12 to 0.9 NA) to tightly focus on the surface. The minimum spot size S can be estimated by $S = 0.61 \times \lambda / NA$. An optimal combination of laser power, spot size and collection efficiency was found to be 0.6 µW at 0.75 NA. Streamline mapping was performed with a triple wavelength inverted *InVia* Raman microscope and a Streamline™ mapping system. The beam was line focussed at 0º to and rastered across the surface to achieve a scan rate of ~1000 spectra per minute. Effective accumulation time per pixel ranged from 1.23 s (632.8 nm λ_{ex}) to 2 s (785 and 830 nm λ_{ex}). SERRS was collected 180º to the surface using the same objective (ranging from 5x/0.1 to 100x/0.95). The exact illumination area, accumulation time and rate in each case were determined by the NA of the objective, stage translation speed and the vertical area of the CCD being used for collection. This could be tuned to maximise speed i.e. 10 minutes was sufficient to scan the entire slide (4 mm^2) at 50 µm pixel size. Typically 2 µm pixel sizes were chosen to match the pitch of the array wells. Klarite nanostructured surfaces were purchased from D3 Technologies (Glasgow, UK). At the standard concentration of target lithography fluid, multiple areas (24 x 24 µm) patterned by multiple pen arrays resulted in a relative standard deviation in signal intensity of 4.6%. Spectra were analysed as the area between 1547 cm^{-1} and 1614 cm^{-1}. The spectra were recorded at a resolution of one spectra per surface well (pitch 2 µm) at a rate of 16 Hz. Statistical analysis found that >99% of the data points in these cases were found to fall within 1.96σ of the mean.