Dip-pen nanolithography and SERRS as synergic techniques†

Robert J. Stokes, Jennifer A. Dougan and Duncan Graham*

Received (in Cambridge, UK) 31st July 2008, Accepted 22nd August 2008
First published as an Advance Article on the web 30th September 2008
DOI: 10.1039/b813249f

We demonstrate the powerful combination of dip-pen nanolithography (DPN) performed on non-flat plasmonic gold surfaces and subsequent detection by surface enhanced resonance Raman scattering (SERRS).

Dip-pen nanolithography (DPN),[1] is a versatile technique in which a scanning probe microscope tip can be used to deliver a material (''ink'') to a surface *via* a water meniscus. DPN allows controlled deposition of a wide range of materials such as (but not limited to) alkyl thiols,[1] silazanes,[2] Au(III) complexes[3] and nanoparticles.[4,5] The size of the feature being written is related to a complex interaction between the tip, ink, meniscus and surface.[6] Development of the technique has allowed Mirkin and co-workers to report direct[7] and indirect[8] writing of biological materials onto suitable surfaces to form highly structured arrays. Because the feature sizes produced by DPN are so small (potentially down to 15 nm), detection of biological interactions is often achieved by atomic force microscopy (AFM) or, as is most common in the case of DPN-directed DNA arrays, fluorescence.[9] However, surface enhanced resonance Raman scattering (SERRS) potentially offers a number of significant advantages over conventional fluorescence detection.

SERRS is a highly sensitive spectroscopic technique that has been used in an increasing number of applications in biodiagnostics including novel gene probes,[10,11] and DNA detection.[12] The technique is flexible and with controlled chemistry can be performed using longer, biologically compatible, wavelengths of excitation (λ_{ex}) either in solution,[13] or on nanostructured plasmonic gold surfaces.[14] When considering the simultaneous detection of multiple targets, it is significant to note that a large proportion of the overall enhancement derives from the additional 'resonance' with the molecular chromophore. This is a major advantage of the technique, when applied in a real assay, as a number of characteristic bands within each dye class are enhanced to a greater extent than other materials in the matrix.[15,16] The characteristic narrow SERRS lines ($\sim$0.5 nm width) have the potential to form the basis of a highly effective multiplexed analysis from a single excitation source. A further advantage of SERRS is that the excitation wavelength can be selected anywhere in the optical range and wavelength selectivity can be observed using combinations of reporter dyes.[17] A number of effective SE(R)RS substrates have been reported in recent years, including those fabricated by nanosphere lithography,[18] silver metal island films[19] and nanostructured gold surfaces.[20,21] Herein, we demonstrate for the first time the powerful combination of DPN on non-flat surfaces and SERRS, highlighting the potential to generate high density biosensor arrays.

The method presented is synergic in that the high specificity, sensitivity and speed of the SERRS technique is complemented by the high spatial precision and scalability of DPN onto high density three-dimensional micro- and nanostructured plasmonic gold surfaces. The fact that the SERRS spectrum from each reporter dye is so distinct and retains a quantitative response when overlaid means that active features can potentially be placed in high spatial (sub-diffraction limit) proximity by DPN, thus maximising the array density. Using simple, cheap optics this is an ability of SERRS that is very difficult to replicate using molecular fluorescence or quantum dots.

Oligonucleotides modified with 5′-dye labels, previously reported to be strongly SERRS active,[13,14] were directly patterned onto nanostructured gold surfaces (Fig. 1A) by DPN. 3′-Thiol or thiotic acid and 5′-dye modified oligonucleotides were delivered to the surface using diving board type probes (max $k = 0.041$ N m^{-1}) a carrier fluid and a microfluidic inkwell system.† The carrier fluid method is important as it allows a wide variety of biological materials to be patterned that would otherwise be difficult to deposit using DPN tips. High density writing was achieved by rastering at 0.5 Hz in an area 0.6 × 0.6 μm centred in single microwell of width 1.3 μm (Fig. 1B). The SERRS spectra and map obtained are shown below in Fig. 1C–D. The spectra of each dye label are identical matches to those reported previously using silver and gold nanoparticles as the enhancing surface.[13] Typically between 600–2000 counts per second were obtained at 632.8 using short accumulation times (1 s) and low laser power ($\sim$0.6 μW at 0.75 NA) resulting in an ''active'' to ''blank'' pixel ratio of about 70 : 1. The intensity of the response depends, to some extent, on the degree of interaction between the molecular chromophore and the surface plasmon modes resonant in the red and near infrared (NIR).[14,21] Similar signal strengths were obtained when arrays were patterned by means of fast piezo drops to the centre of each well with minimum dwell times at the surface. The limits of detection in each case were found to be similar to or better than those reported previously where 1×10^{-11} M solutions of Cy7 labelled target strands could be recorded using 785 nm λ_{ex}.[14] This optimal enhancement in the near infrared is understandable as this type of surface has a broad plasmon resonance in this region.[21] Parallel multi-pen alignment and

Centre for Molecular Nanometrology, WestCHEM,, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, UK. E-mail: Duncan.Graham@strath.ac.uk; Tel: +44(0)141 548 4701

† Electronic supplementary information (ESI) available: DPN and spectroscopy methodology. See DOI: 10.1039/b813249f

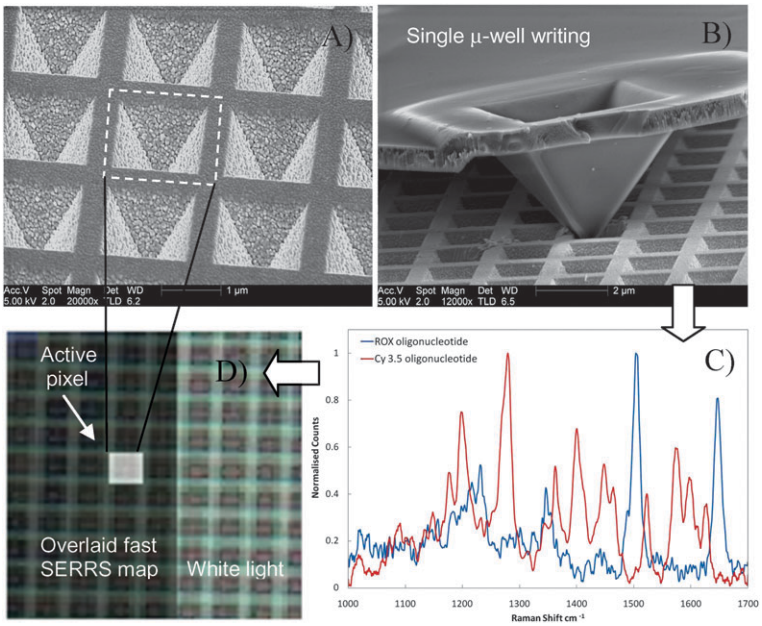

Fig. 1 SEM micrographs of (A) Klarite™ nanostructured gold surface (25 M μwells cm^{-2}), (B) AFM tip interaction with surface. (C) SERRS spectra obtained from ROX and Cy3.5™ dye-labelled oligonucleotide sequences written (1 × 1 μm area) into single well [1 × 1 s, λ_{ex} = 632.8 nm, 0.6 μW]. (D) SERRS map of single Cy3.5 oligonucleotide pixel (false colour map over white light image), showing signal (at 1280 cm^{-1}) from only one pixel (1.3 μm per map pixel).

writing was achieved by contact mode AFM imaging at tips at either end of the pen array, thus alignment could be performed by rotation relative to the very regular surface features.

The DPN technique has been shown to be massively scalable with the recent report of 55 000 pens arranged in a 2D-array.[22,23] Therefore, any complementary spectroscopic technique would need to be performed both with sufficient sensitivity and speed in order to read the large number of small array pixels within a reasonable timescale. Spectra in this case were obtained using a Streamline™ mapping stage, charge coupled device (CCD) and an *InVia* Raman microscope system (Renishaw, UK). The line mapping system operates by line focussing the laser and rastering vertically across the sample, simultaneously collecting multiple spectra across the CCD area. Using a 42 CCD line setup it was possible to acquire spectra at ~16 Hz from the surface of the nanostructured gold surface with sufficient sensitivity to identify the target dye. The laser power in SE(R)RS point mapping or confocal scanning is often attenuated to avoid sample damage to the monolayer or photo-degradation of the reporter dye. However, DPN and readout by fast line mapping allows the laser to be deployed more efficiently, illuminating a wider area (30–60 times as many active pixels) with a correspondingly lower surface power density.

The ability of DPN to pattern features that complement the spectroscopic collection geometry allowed the lateral resolution and detection speed to be optimized. DPN patterning of broad lines, 3 μwells wide (Fig. 2A), enabled the rapid identification of adjacent targets by scanning orthogonally in a SERRS "bar-code". In this example scan rates of 16–32 Hz (lines per second) were obtained, although the high signal to background ratio means that significantly faster scanning rates could be achieved, potentially up to 350 Hz. An example SERRS false-colour image using Cy3.5 and Cy5 labelled oligonucleotides is shown in Fig. 2B. Patterning long, narrow lines also removes the need for complex sample alignment with the reader in a manner broadly analogous to a supermarket checkout scanner, further increasing the throughput of the combined technique.

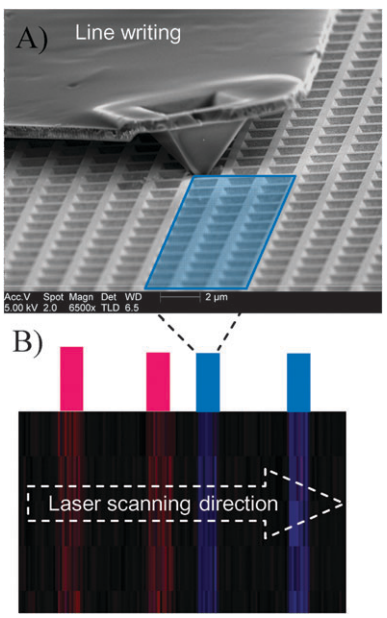

Fig. 2 (A) Representation of DPN line writing. (B) Dual overlaid Streamline™ mapping images recorded orthogonally to broad DPN lines drawn onto Klarite. Image consists of overlaid false colour maps with the intensity based on 1370 cm^{-1} (Cy3.5™, **Purple**) and 1600 cm^{-1} (Cy5™, **Blue**) integrated peak area to base line. Recorded and processed at 16 Hz (spectra per second).

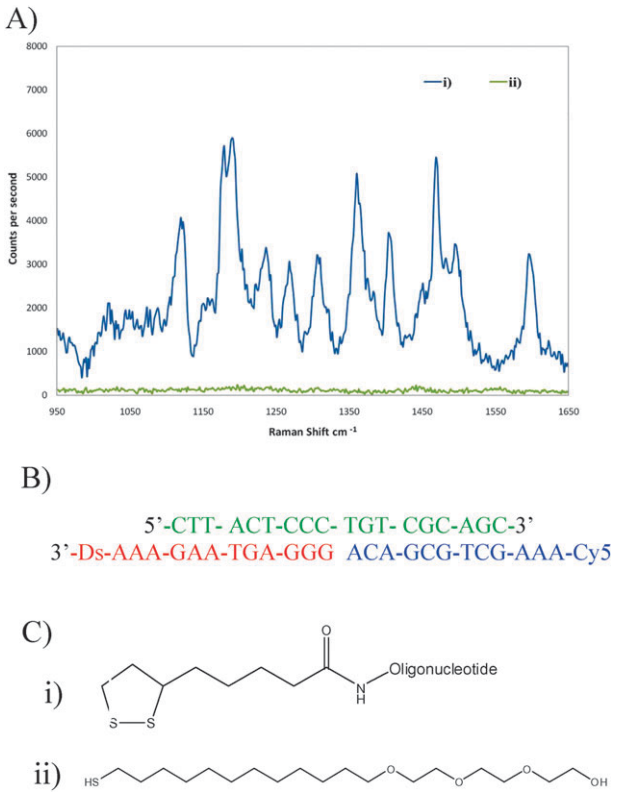

Fig. 3 (A) (i) SERRS spectra (4 × 4 microwells) from active area after hybridisation of disease target probe and dye labelled complement oligonucleotide (Cy5™). (ii) Spectra recorded from adjacent blank region. [1 × 1 s, λ_{ex} = 632.8 nm, 0.6 μW, 0.75 NA, levelled and set to zero]†. (B) Oligonucleotide sequences used. (C) (i) 3′ Disulfide modification to capture oligonucleotide. (ii) PEG-passivator.

To demonstrate the practicality of the combined DPN-SERRS method a DNA detection experiment using an 18 base region of a sequence coding for a *Chlamydia trachomatis* target probe was performed (Fig. 3). Two independently modified complements to the Chlamydia target were obtained, one is a 12mer modified at the 3′-end with a disulfide surface attachment moiety[14] and a triple "A" spacer, suitable for DPN. 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. 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 signal levels were $>3000:1$ and show good reproducibility from pixel to pixel (RSD $<5\%$, with 99 of data points falling within $\pm 1.96\ \sigma$ of the mean).† Significantly, the spectral contrast is very high with no trace of the reporter dye detectable in the "blank" regions that had also been exposed to identical hybridisation materials and conditions.

In conclusion, we have shown how DPN performed on non-flat plasmonic gold surfaces can be used to selectively create SERRS active DNA array pixels in a relatively simple manner. Very high information density and depth can be obtained by embedding multiple reporter dyes within the same sub-diffraction limit area. Careful tuning of the surface chemistry and spectroscopic conditions enable the most sensitive results to be gained from this combined method. The generation of high efficiency SERRS from the surface translates directly into the fast acquisition times demonstrated in this work that will ultimately make sensitive reproducible nanoarrays a practical reality.

Notes and references

1 R. D. Piner, J. Zhu, F. Xu, S. Hong and C. A. Mirkin, *Science*, 1999, **283**, 661.
2 A. Ivanisevic and C. A. Mirkin, *J. Am. Chem. Soc.*, 2001, **123**, 7887–7889.
3 B. W. Maynor, Y. Li and J. Liu, *Langmuir*, 2001, **17**, 2575–2578.
4 M. B. Ali, T. Ondarçuhu, M. Brust and C. Joachim, *Langmuir*, 2002, **18**, 872–876.
5 D. Prime, S. Paul, C. Pearson, M. Green and M. C. Petty, *Mater. Sci. Eng., C*, 2005, **25**, 33–38.
6 S. Rozhok, R. Piner and C. A. Mirkin, *J. Phys. Chem. B*, 2003, **107**, 751–757.
7 L. M. Demers, D. S. Ginger, S.-J. Park, Z. Li, S.-W. Chung and C. A. Mirkin, *Science*, 2002, **296**, 1836.
8 L. M. Demers, S.-J. Park, T. A. Taton, Z. Li and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2001, **40**, 3071.
9 D. S. Ginger, H. Zhang and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2004, **43**, 30 (and references therein.).
10 T. Vo-Dinh, K. Houck and D. L. Stokes, *Anal. Chem.*, 1994, **66**(20), 3379–3383.
11 L. R. Allain and T. Vo-Dinh, *Anal. Chim. Acta*, 2002, **469**, 149–154.
12 Y. C. Cao, R. Jin and C. A. Mirkin, *Science*, 2002, **297**(5586), 1536–1540.
13 R. J. Stokes, A. Macaskill, P. J. Lundahl, K. Faulds, W. E. Smith and D. Graham, *Small*, 2007, **3**(9), 1593–1601.
14 R. J. Stokes, A. Macaskill, J. A. Dougan, P. G. Hargreaves, H. M. Stanford, W. E. Smith, K. Faulds and D. Graham, *Chem. Commun.*, 2007, 2811–2813.
15 (*a*) D. Graham, C. McLaughlin, G. McAnally, J. C. Jones, P. C. White and W. E. Smith, *Chem. Commun.*, 1998, 1187; (*b*) G. M. McAnally, C. McLaughlin, R. Brown, D. Robson, K. Faulds, D. R. Tackley, W. E. Smith and D. Graham, *Analyst*, 2002, **127**, 838.
16 R. J. Stokes, A. Ingram, J. Gallagher, D. Armstrong, W. E. Smith and D. Graham, *Chem. Commun.*, 2008, 567.
17 K. Faulds, F. McKenzie and D. Graham, *Angew. Chem., Int. Ed.*, 2007, **46**, 1829–1831.
18 A. J. Haes, C. L. Haynes, A. D. McFarland, S. Zou, G. C. Schatz and R. P. Van Duyne, *MRS Bull.*, 2005, **30**, 368.
19 R. Allain and T. Vo-Dinh, *Anal. Chim. Acta*, 2002, **469**, 149–154.
20 D. M. Kuncicky, B. G. Prevo and O. D. Velev, *J. Mater. Chem.*, 2006, **16**, 1207.
21 N. M. B. Perney, J. J. Baumberg, M. E. Zoorob, M. D. B. Charlton, S. Mahnkopf and C. M. Netti, *Opt. Express*, 2006, **14**(2), 847–857.
22 K. Saliata, S. W. Lee, X. Wang, L. Huang, T. M. Dellinger, Chang Liu and C. A. Mirkin, *Small*, 2005, **1**, 940.
23 K. Salaita, Y. Wang, J. Fragala, R. A. Vega, C. Liu and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2006, **45**, 1–4.