An engineered nano-plasmonic biosensing surface for colorimetric and SERS detection of DNA-hybridization events

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ABSTRACT

We report a versatile nanophotonic biosensing platform that enables both colorimetric detection and enhanced Raman spectroscopy detection of molecular binding events. Through the integration of electron-beam lithography, dip-pen-nanolithography and molecular self-assembly, we demonstrate plasmonic nanostructures which change geometry and plasmonic properties in response to molecularly-mediated nanoparticle binding events. These biologically-active nanostructured surfaces hold considerable potential for use as multiplexed sensor platforms for point-of-care diagnostics, and as scaffolds for a new generation of molecularly dynamic metamaterials.

Keywords: Plasmonics, Nanoparticle, DNA, Biosensor, Colorimetric sensing, Dip-pen nanolithography, Electron-beam lithography, SERS

Introduction

Acting as antennae for light radiation, the plasmonic resonances inherent to metallic nanoparticles allow photonic manipulation beyond the diffraction limit, elegantly linking far-field optics to nanoscale light confinement.[1-3] Key to the advancement of plasmonic systems are reliable strategies for nanoscale assembly of particles into functional devices. Localised plasmon resonances take place when photons interact with the free electron cloud of a metallic nanostructure. When the energies of these two particles are matched, resonant enhancement of the plasmon results in confinement and amplification of the light field around the nanostructure, as well as determining what frequencies of light are absorbed and scattered from the structure.[4] As a result, assembly of nanoparticles in a controlled manner allows one to manipulate the photonic properties of assembled system; engineering change at the sub-wavelength near-field which can be read as a far-field photonic output. Although molecular assembly is perhaps the most promising of these strategies, and is prominent in solution based applications,[5-7] directing the molecular assembly of nanoparticle networks on surfaces, and their interaction with existing surface features, is sufficiently challenging that its use has been limited. The realization of such surfaces would lead to the creation of complex metasurfaces with non-natural optical properties which can be controlled in real-time by biomolecular events, a technology which would impact not only biosensing, but the wider fields of materials engineering and nano-optics.

Here we present a further step toward metasurfaces with multiplexed biological properties. Our approach combines electron-beam-lithography, dip-pen-nanolithography (DPN), and molecular self-assembly to create hybrid nano-optical surfaces whose optical and plasmonic properties can be altered molecularly-mediated nanoparticle binding events. We have previously demonstrated that nanoplasmonic surfaces can be patterned with biomolecular patches so small that only a single nanoparticle can bind to them, and with a positional resolution of approximately 5-10 nm, thus allowing fine-scale geometrical and plasmonic tuning of arbitrary nanostructures for high-sensitivity colorimetric and SERS studies.[8-10] Using electron-beam lithography and DPN in tandem brings with it several key advantages over our previous, single nanoparticle localization demonstrations. Using wet DPN to deposit the biomolecules onto the plasmonic surface allows the user to deposit multiple biomolecule targets onto the surface simultaneously, something not possible with the

electron-beam mask deposition technique demonstrated previously.[8-10] Coupling DPN wet-spot patterning of DNA with surface nanostructures, via thiol terminated sequences, also significantly increases the effective resolution of the DPN patterning, confining it to the nanoscale areas occupied by the surface nanostructures. Through the use of these patterning techniques we demonstrate that we can detect DNA sequence hybridization at high sensitivities through both colorimetric observation and SERS.

Methods

Figure 1 shows an illustration of the fabrication and molecular patterning process, while **Figure 2** shows SEM images taken of the plasmonic structures before and after nanoparticle binding. Arrays of silver nanostructures were fabricated using a standard electron-beam lithography lift-off process. A monolayer of (3-Aminopropyl)triethoxysilane (Sigma-Aldrich) was used as a silver adhesion promoter (attached to the surface by incubating the sample in a 5% solution, in ethanol, for 4 hours). Diamond shapes with dimensions 85 x 140 nm (x and y-axis dimensions in **Fig 2.**), and a height of 25 nm were chosen as the base plasmonic structure due to their high frequency polarization dependent resonances, and the areas of relatively high curvature seen at the "tips" of their geometry (a feature which focuses plasmonic field enhancement, further maximizing the coupled field amplification experienced by nanoparticles which bind to this region). The structures were fabricated as 800 μm square arrays on 500 μm thick Pyrex glass using a Vistec VB6 UHR EWF electron-beam lithography tool. The periodicity of the array was 400 nm.

Molecular modification of the sample took place by incubating the sample at room temperature, for 12 hours, with a thoilated oligonucleotide (sequence 5' TCTCAACTGTA HEG-HEG-HEG-THIOL) suspended in a 10mM PBS, 0.3M NaCl buffer (all DNA was suspended in this binding buffer unless stated otherwise). This was applied either as a 2 μl spot, which covered the entire sensor area, or using the NanoInk DPN5000 DPN tool in conjunction with a 12 tipped pen (in which case the oligonucleotide solution was further mixed with glycerol, which prevents evaporation of the micron-scale spots, in a 10:1 ratio). The spots created by DPN ranged from 7 μm to 40 μm in diameter, **Fig. 4**. Samples were then washed for 5 minutes using 20mM PBS, 0.6M NaCl buffer before being spotted with a second, target oligonucleotide (sequence 5' TACGAGTTGAGAATCCTGAATGCG), half of which is complimentary to the first, thiolated sequence. Hybridisation took place over 6 hours at room temperature. Samples were washed again using the same protocol as before, and then hybridised with a nanoparticle labelled oligonucleotide complimentary to the other half of the target strand (sequence 3' ATGCTCAACTC-Nanoparticle). Hybridisation took place over 12 hours at room temperature. The label used was a 40 nm gold particle, modified 50:50 with DNA and the Raman reporter dye Malachite Green. Samples were then washed for 5 minutes, in turn, with the binding buffer plus 0.05% Tween20, the binding buffer, PBS, and DI water. After all of the steps had been performed, each silver nanostructure modified with the initial thiolated probe sequence is decorated with 40 nm Au particles bound to it through DNA hybridisation (**Fig. 2**).

Plasmonic characterisation of the sensor surfaces was performed using a Shimadzu UV3101PC absorption spectrometer. Dark-field colorimetric images were taken using a Zeiss Axio Imager A1 in conjunction with a 10x 0.25NA, a 50x 0.5NA lens and a dark-field condenser**.** SERS measurements were taken using a Horiba Jobin Yvon LabRam INV spectrometer in conjunction with an Olympus IX70 microscope, a 100x 0.75NA lens and a 0.5 mW 633 nm HeNe laser (power measured at objective).

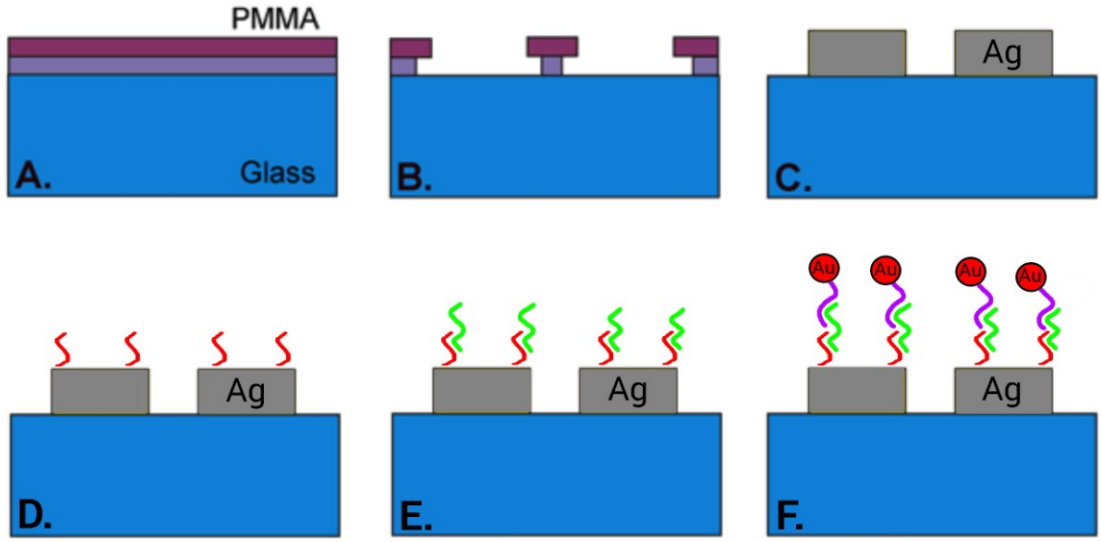

Figure 1. A schematic of the fabrication process. **A-** A bi-layer of Poly(methyl methacrylate) resist was spun onto the glass substrate at 5000 rpm, creating a resist thickness of ~50nm. A bi-layer of different molecular weights of PMMA was used in order to create a lift-off (undercut) profile for easier resist removal later in the process flow. **B-** After exposure to the electron-beam, the resist was developed in a 2.5:1 mixture of IPA:MIBK (Methyl isobutyl ketone) for 45 seconds before rinsing in IPA. **C-** The glass areas exposed after development of the resist were modified with an amino-silane adhesion monolayer. A custom made resistive-heating metal evaporator was used to deposit 25 nm of silver onto substrate. The excess resist and silver was removed by acetone lift-off. **D-** The silver structures were modified with a thiolated probe oligonucleotide (using either selective area DPN patterning or full array modification). **E-** The target oligonucleotide was hybridized to the probe oligonucleotide. **F-** The 40 nm Au nanoparticle-labelled oligonucleotide was hybridized to the target sequence.

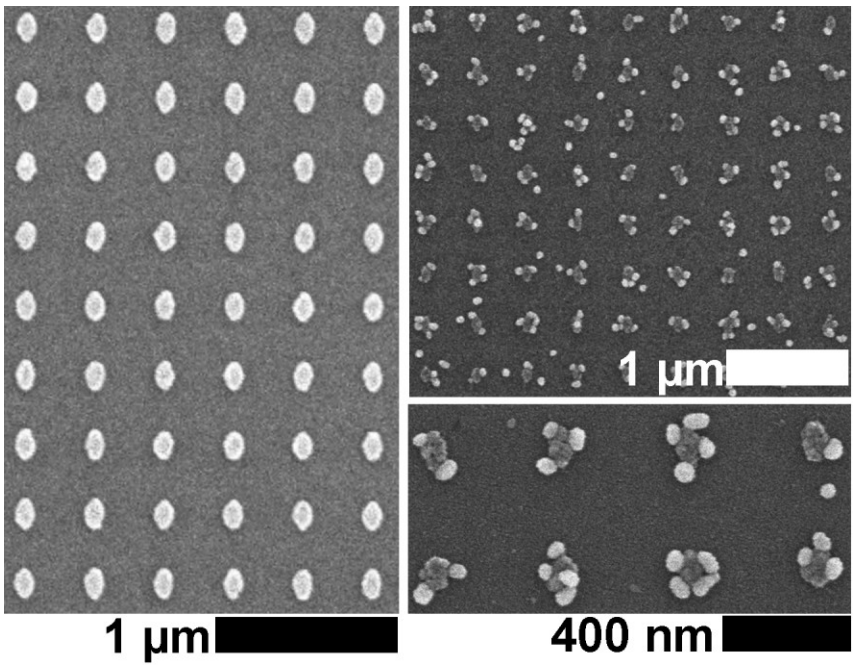

Figure 2. SEM images taken of the array before and after DNA-mediated nanoparticle binding. Left – Ag nano-diamond array before modification. Right – Ag nano-diamond array after successful sensing assay, with Au nanoparticles located around each Ag structure via DNA-hybridization.

Results and Discussion

Figure 2 shows we are able to capture the 40 nm Au nanoparticles on the surface of the plasmonic Ag surface features. 97% of the Ag structures have nanoparticles bound to them, with the majority of these having 3 nanoparticles attached to their surface (the percentage of structures with 1, 2, 3 and 4+ particles bound is 13%, 24%, 46% and 14%, respectively). Little non-specific binding apparent, with only 217 non-specifically bound nanoparticles in the 7.2 µm x 12 µm test area (84% of the observed nanoparticles within this area were specifically bound to the Ag nanostructures). Once bound, the plasmon of each nanoparticle couples to the plasmon of the silver diamond, shifting the peak resonance frequency to a lower energy. Silver was chosen as the material for the fabrication of the sensor surface because of its higher intrinsic plasmon frequency; a trait which allows the range of colors supported by the sensor to span the entire visible spectrum (colors tunable by varying the diamond dimensions). Diamond shapes were chosen because of the shapes tested thus far, these structures appear to experience a greater plasmonic shift when introduce to Au nanoparticles. Furthermore, their asymmetric geometry leads to polarization dependent resonances; they exhibit two different resonance peaks, and therefore two scattered color profiles, within the visible spectrum (a higher frequency resonance along their shorter x-axis, and a lower frequency resonance along their longer y-axis). In the context of the colorimetric sensors we demonstrate here, this feature is not strictly necessary (both resonances exhibit a visible shift upon DNA-hybridization). However, in the context of molecularly tuneable metasurfaces, and indeed multiplexed SERS sensors, the ability to support, and subsequently alter, two separate plasmonic resonances within a single structure would open up a variety of new applications.

Figure 3 shows absorption spectra taken for the diamond arrays before and after nanoparticle binding, as well as scattered plasmonic colors collected via a dark-field microscope. The differently colored resonances were excited by inserting a linear polarizer between the illuminating light source and the sensor. Although the shift experienced by each individual structure will be subtly different (given the inhomogeneous particle distribution), the overall effect on the plasmonic response of the array is to shift its peak wavelength by 5 nm, for the x-axis resonance, and 25 nm for the y-axis resonance. In addition to these peak shifts, the spectra are significantly broadened after nanoparticle binding. Both of these effects combine to alter the scattered colour profile of each resonance, allowing the Ag surfaces to act as effective colorimetric biosensors for DNA detection.

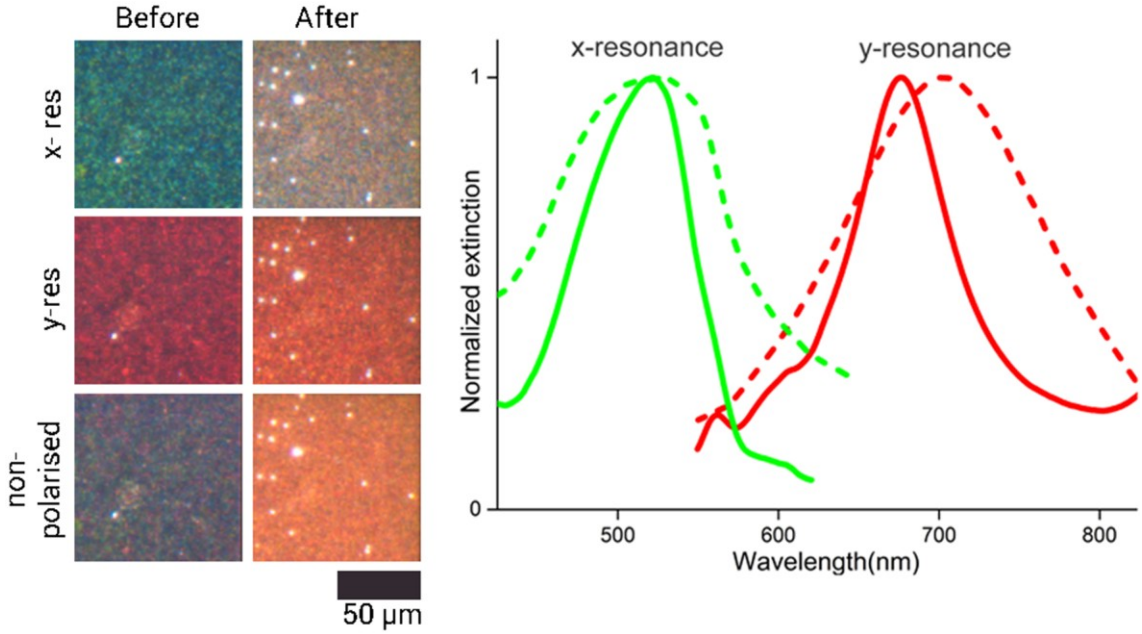

Figure 3. Plasmonic shift after DNA-nanoparticle binding. Left – Dark-field images of the scattered plasmonic colors produced when the structures are excited along their x (short) and y (long) axes (referred to as x-resonance and y-resonance, respectively). The scattered color from non-polarized excitation is also shown. **Right –** Plasmonic absorption spectra associated with each resonance before and after nanoparticle binding.

Figure 4 shows a dark-field image of DNA-nanoparticle binding for the case where the sensor has been selectively patterned with a DPN tool. Here we use this selective binding to demonstrate the use of these sensor surfaces in SERS applications. In addition to the far-field color shift shown in **Figure 3**, the plasmon coupling produced by the successful DNA binding assay leads to amplification of the localized electric near-field at the interface of the Ag structure and the Au particles. Within these nanoscale gaps, field strengths orders of magnitude greater than the driving electric-field (the incident light-field) are produced.[8,10-14] Since the Raman effect scales in efficiency with the 4th power of the localized electric-field, this leads to extraordinarily large Raman signals which have been shown capable of enabling single-molecule binding.[15-18] Exploiting this effect in our sensor allows us to operate at a sensitivity that is not readily compatible with low-magnification colorimetric sensing, where relatively large amounts of binding over relatively large areas are required. Using SERS lets us work with micron-scale molecular patterns and detect small amounts of individual binding events. As our nanoparticles are modified with both DNA and the Raman active dye malachite green, it is possible to monitor DNA hybridization via SERS. **Figure 4** shows the SERS response from two areas of the sensor surface; one which was modified with the initial thiolated strand, the other which was not. Both areas went through the same modification with the target sequence and nanoparticle-labelled sequence, but only those structures modified with the initial probe strand show any particle binding, and therefore only these areas exhibit a SERS response.

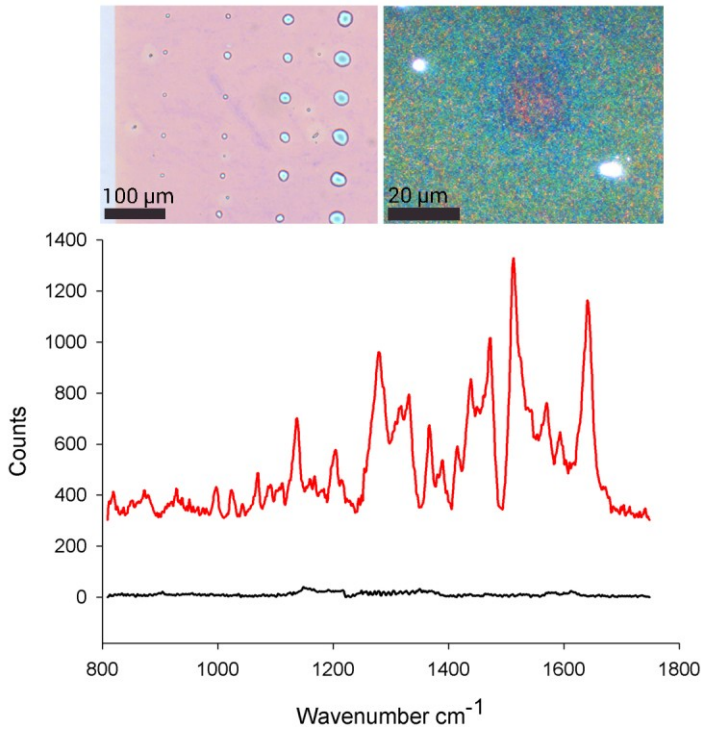

Figure 4. Raman detection of DPN produced DNA binding assay. Top left –DPN patterned spot of different sizes, ranging from 7 µm to 40 µm**. Top right –** Dark-field image of DPN patterned area after DNA-nanoparticle hybridization. A red spot is clearly visible in the center of the green background, indicating that nanoparticles are located within this area and are coupling to the plasmon of the Ag surface features. **Bottom –** SERS spectra from the center of the DPN patterned spot (red) and from an area of the Ag surface not functionalized with the initial probe DNA strand (black). The presence of the malachite green spectra in the center of the DPN sot confirms that DNA-hybridization took place in this area.

The focused laser beam, when used in conjunction with the 100x 0.75NA objective, has an area which corresponds to approximately 13 surface nanostructures. In turn this corresponds to approximately 34 DNA-nanoparticle binding events (given the average number is 2.6 nanoparticles per Ag diamond), a sensitivity only achieved due to the engineered nature of the sensor surface and the knowledge of the exact areas of DNA patterning that DPN provides.

Conclusion

We have demonstrated a plasmonic biosensing platform and patterning technique which allows for colorimetric and SERS detection of DNA hybridization. By engineering the sensor surface we are able to observe DNA hybridization using low-magnification dark-field colorimetry *and* Raman spectroscopy at sensitivities that would not be possible in a traditional solution based system. By carefully controlling both the plasmonic Ag diamond structures and the areas of molecular patterning, we also demonstrate a further step towards engineered metasurfaces with geometries and plasmonic properties which can be altered through biomolecular binding events.

Acknowledgements

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