Application of EBL fabricated nanostructured substrates for SERS detection of protein A in aqueous solution

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Surface enhanced Raman spectroscopy (SERS) allows detection of unique signatures of molecular vibrations, which identify molecules (analytes) adsorbed on nanostructured substrates. The excitation of localized surface plasmons by light interacting with metallic nanostructures on dielectric substrates increases dramatically the intensity of Raman scattering by the analyte making SERS a very promising technique for detection of various molecules including biological polymers[1]. However, for SERS detection techniques to be reliable, highly regular metallic nanostructures must be fabricated. While electron beam lithography (EBL) is a powerful tool for direct-write nanostructuring[2], the fabrication of metallic patterns on dielectric substrates required for an enhanced SERS signal[3] results in accumulation of charge during EBL exposure, thereby degrading the process[4].

In this work, we have optimized high resolution EBL to fabricate gold nanostructures on fused silica (FS) substrates for efficient SERS bio-detection. To avoid charging issues, we employed aquaSAVE (Mitsubishi Rayon) anti-charging film on top of PMMA resist as described earlier[4] (Fig. 1a). 30 keV EBL exposures using a Raith 150^{TWO} instrument followed by development have been used to fabricate arrays of nano-pits in PMMA on a FS substrate, with subsequent evaporation of Au and lift-off to obtain arrays of Au dots (Fig. 1b-1c). The EBL exposure dose and development time have been tuned to obtain arrays of dots with various pitches (50 nm, 100 nm, and 200 nm) and different inter-dot gaps (10-25 nm). The SERS substrates have been bio-functionalized by self-assembled monolayers (SAMs) of 11-mercaptoundecanoic acid (MUA)[5] and a biological analyte (protein A) immobilized on the SAM. In order to detect the protein in its natural environment, the samples were kept in deionized water. SERS spectra were acquired employing a Thermo Nicolet Almega XR instrument (532 nm).

Figs. 2 and 3 show the results for dot arrays with a 50 nm pitch. It can be seen that the SERS signal increases when the inter-dot gap is decreased, which can be attributed to stronger plasmon coupling between closely spaced structures[1]. In particular, inter-dot gaps narrower than 20 nm have resulted in the highest Raman intensities, allowing detection of many vibrational modes of protein A (Figs. 2, 3). By controlling the EBL exposure and development conditions, arrays of Au dots with optimized geometry have been fabricated on FS substrates for more sensitive SERS detection of immobilized protein A. Further work using nano-imprint lithography (NIL) is also being developed in order to increase device fabrication throughput as well as consistency in features. Testing of devices with alternative metal designs is also in progress.

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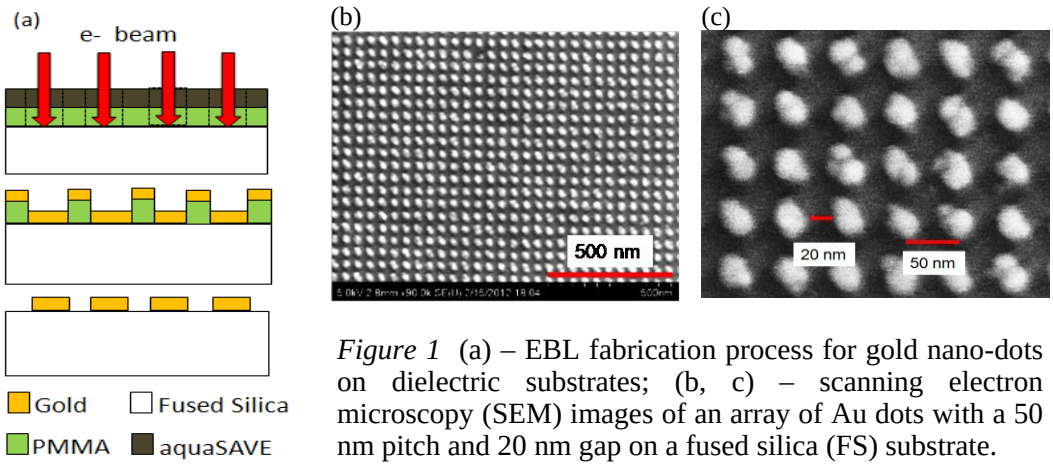

Figure 1 (a) – EBL fabrication process for gold nano-dots on dielectric substrates; (b, c) – scanning electron microscopy (SEM) images of an array of Au dots with a 50 nm pitch and 20 nm gap on a fused silica (FS) substrate.

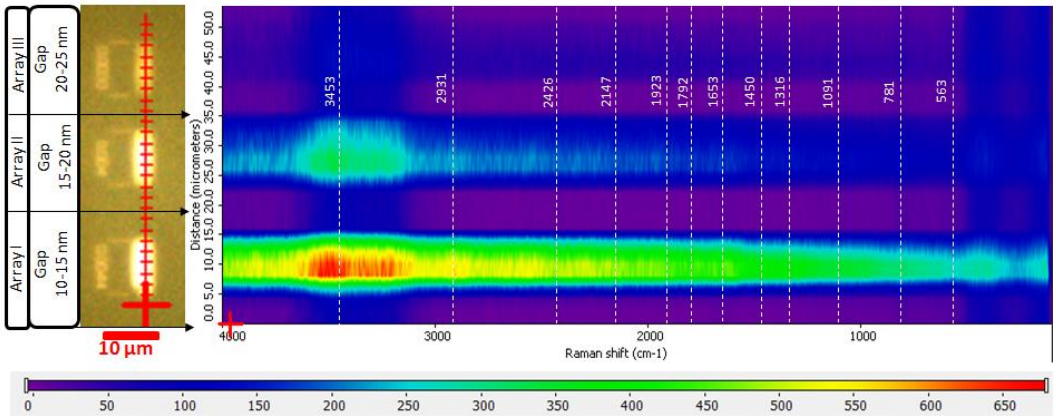

Figure 2 (Left) – optical microscope images of a bio-functionalized SERS substrate in water. The sample comprised three arrays of Au dots with a 50 nm pitch and different inter-dot gaps on a FS substrate, covered by a MUA SAM with immobilized protein A. (Right) – Raman mapping for the three different arrays (532 nm laser excitation). Vertical dashed lines represent the Raman bands of free protein A in solution.

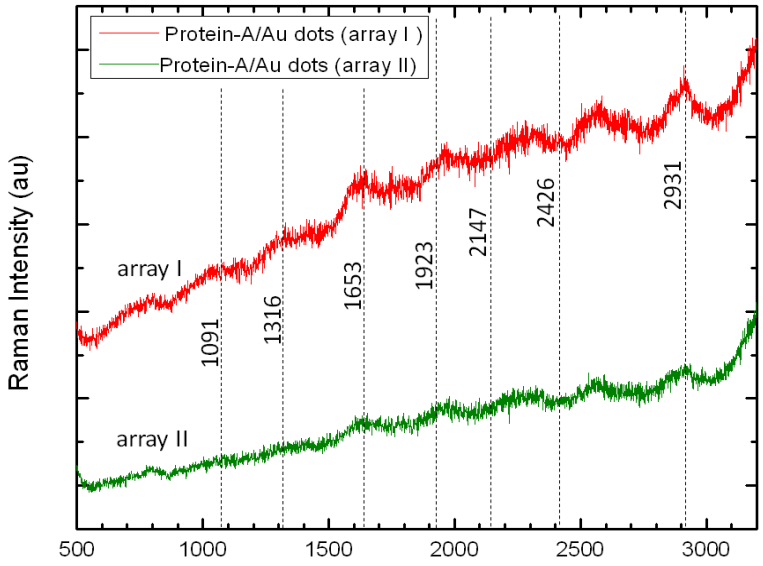

Figure 3: SERS spectrum for the immobilized protein A on the functionalized Au nanodots with a 50 nm pitch and dot arrays with different inter-dot gap,10-15 nm (array I) and 15-20 nm (array II). Dot arrays correspond to the figure 2.Dashed lines represent the Raman bands found for free protein A in solution (2mg/ml).