Molecular Specific Biosensing Based on Engineered Quasi-3D Plasmonic Nanostructures

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Abstract and Introduction:

Raman scattering is inelastic light scattering, wherein the incoming photon collides with a molecule and transfers part of its energy to the vibrational energy of that molecule. The emitted photon leaves the molecule with less energy than when it entered the molecule. Due to the fact that Raman scattering is related to the vibrational energy of a molecule, a spectrum of this data is capable of demonstrating unique spectral fingerprints for the molecular vibrations of anything from a single molecule to unicellular organisms [1]. Raman scattering typically demonstrates a very low probability, in which only 1 in 10^{12} photons exhibits this phenomenon [2].

Surface-Enhanced Raman Scattering (SERS) will be employed to greatly enhance the number of photons that will be inelastically scattered by placing the molecule under investigation at the surface of the nanostructure of noble metals (e.g., gold (Au), silver (Ag), or copper (Cu)).

In this study, two substrates, indium tin oxide (ITO)-coated glass and silicon (Si), were compared in order to determine which substrate yielded the greatest Raman signal enhancement. The goal was to aid in the optimization of nanostructure parameters in order to achieve an enhanced Raman signal. Also, we were looking to detect a single bacterium on our quasi-3D nanostructures in order to characterize and identify species of bacteria.

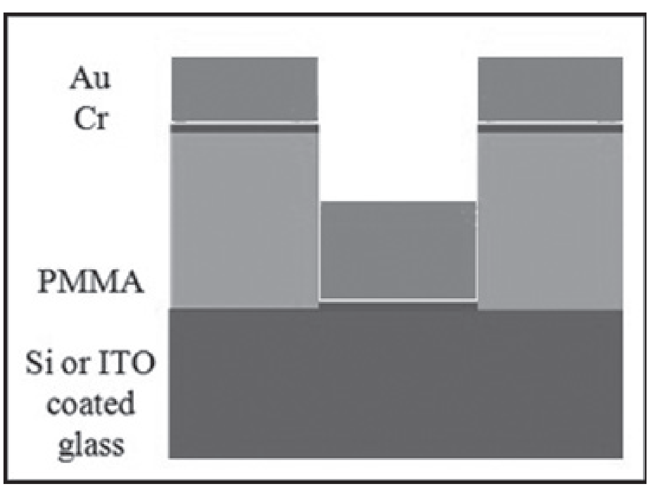

Figure 1: Schematic for Quasi 3-D nanostructures [3].

Experiment Methodology:

These quasi-3D nanostructures were created by employing electron beam lithography (EBL) on two different substrates, ITO-coated glass and Si, using two different nanopattern arrays, circular nanoholes and rectangular nanoholes.

First, a poly(methyl methacrylate) (PMMA) photoresist was spin-coated onto the two substrates until it reached a thickness of 300 nm. Next, the patterns were exposed to an electron beam with a current of 95.6 pA and a dose of 0.36 nC/cm^2, developed in 1:3 methyl isobutyl ketone/isopropanol (MIBK/IPA) PMMA developer for 70 seconds, rinsed with IPA, blown dry with N_2, then hard-baked at 95°C for 30 minutes.

Finally, a 2 nm layer of chromium adhesion was evaporated to the surface of the nanostructures, followed by a 50 nm layer of Au film. Figure 1 illustrates a schematic for the quasi-3D gold nanostructures. 4-mercaptopyridine (4-MP) was used as a probe molecule in order to determine the enhancement factor for quasi-3D nanostructures. A self-assembled monolayer of 4-MP was formed on the surface of the nanostructures by first cleaning the substrate in UV ozone for 20 minutes and then immersing the substrate into a 3 mM 4-MP solution for three hours. The resultant substrate was then washed with deionized water and dried using N_2 [3].

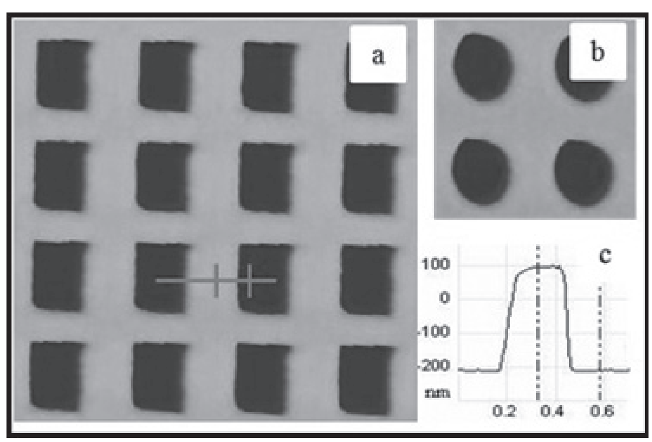

Figure 2: AFM images for rectangular (left) and circular (right) nanopatterns.

In the bacteria study, bacteria were placed on the surface of the nanostructures by preparing a solution of bacteria of a species and forming a solution of 10^6 CFU/mL and dropping this solution onto the substrate. The bacterial solution was allowed to dry on the surface then the Raman spectrum was taken.

In both cases, the 4-MP and bacteria, a Raman spectrometer and a 50x objective were used to illuminate the sample with a 785 nm excitation laser and to collect the scattered light. Once the Raman spectra for the bacteria and 4-MP were attained, scanning electron microscope (SEM) and atomic force microscope (AFM) images were taken in order to ensure that the correct patterns with the correct dimensions had been attained via EBL. Figure 2 shows an AFM image of the circular and rectangular quasi-3D nanostructures and a depth profile for the nanostructures.

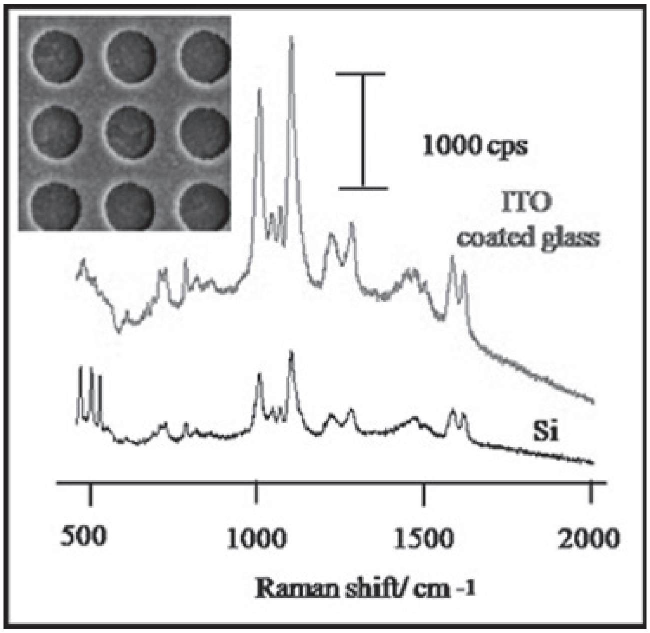

Figure 3: Raman spectra comparison for ITO-coated glass and Si.

Results and Conclusion:

Figure 3 illustrates a raw Raman spectral comparison of 4-MP on ITO-coated glass and Si. Based on this, it is clearly shown that the ITO-coated glass demonstrates about a two to three times higher Raman enhancement than the silicon substrate. It has been determined that this is due to the differences in optical properties between the two substrates. ITO coated glass demonstrates backside reflection, in which the light is reflected at different stages within the glass, thus "trapping" the light for a longer amount of time and increasing the level of localized surface plasmon resonances (LSPR) and increasing the amount of Raman signal. This is compared to the Si substrate, which was optically reflective and did not exhibit this extra electric field enhancement.

A Raman spectrum focused on a single *Escherichia coli* (*E. coli*) bacterium on both the Au circular nanopattern and flat Au surface is shown in Figure 4. The top spectrum is the Raman spectra focused on a single bacterium on the nanopattern surface.

It is clearly shown that the nanopatterns induce the SERS effect and make capable molecular characterization of the bacteria's surface and the detection of a single bacterium. The bottom, flat Raman spectra is taken on a flat Au surface focused on a single bacterium and does not exhibit a significant enough Raman intensity in order to detect, nor identify the *E. coli* bacterium.

Future Work:

In the future, we plan on employing this molecular specific Raman technology in order to identify between different cancer cells and make comparisons of cancer cells versus normal tissue cells in order to aid in cancer diagnostics.

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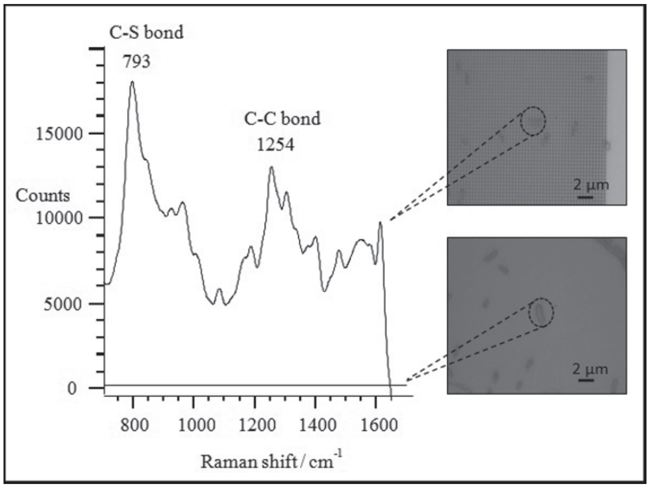

Figure 4: Raman spectra comparison for bacterium on nanopatterns (top) and on a flat, gold surface (bottom).