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Visible wavelength surface-enhanced Raman spectroscopy from In-InP nanopillars for biomolecule detection

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Abstract – Visible wavelength surface-enhanced Raman spectroscopy (SERS) has been observed from bovine serum albumin (BSA) using In-InP nanopillars synthesised by Ar gas cluster ion beam sputtering of InP wafers. InP provides a high local refractive index for plasmonic In structures, which increases the wavelength of the In surface plasmon resonance. The Raman scattering signal was determined to be up to 285 times higher for BSA deposited onto In-InP nanopillars when compared with Si wafer substrates. These substrates demonstrate the label-free detection of biomolecules by visible wavelength SERS, without the use of noble metal particles.

Surface-enhanced Raman spectroscopy (SERS) utilises the resonant excitation of localised surface plasmons to boost the scattering efficiency from samples containing few or even single molecules¹⁻³. The technique's usefulness for non-destructive and label-free characterisation has resulted in a large body of research since its discovery in 1974^{3,4}.

Gold and silver nanoparticles have historically been the most often employed SERS substrates due to their strong visible wavelength localised surface plasmon resonance (LSPR)^{5,6}. However, the price and scarcity of noble metals remains one of the major drawbacks of SERS and, as such, it has been recognised that more cost effective alternatives must be developed⁷. Hence, the plasmonic properties of other metals^{8,9}, semiconductors¹⁰ and transparent conducting oxides (TCOs)¹¹ have been recently investigated. In and Al have been identified as the most promising 'poor metal' plasmonic materials, especially for applications in the UV⁷. While SERS has been demonstrated experimentally using both materials, the majority of the attention has been paid to Al in spite of the reported challenges in synthesising stable Al nanoparticles⁷. Although the price of In has approached that of Ag in recent years, its relative Earth abundance is three times that of Ag and its extraction rates remain significantly lower. The first experimental SERS studies on indium island films were conducted in the 1980's^{12,13} but since then there has been little development of SERS applications for In. Attempts have recently

been made to address this issue theoretically⁷ and experimentally¹⁴, however, these studies have focused on UV-SERS.

While UV-SERS can offer benefits including reduced interaction volumes, fluorescence suppression and amplified SERS (which scales according to λ^{-4}), UV Raman is an inherently more sophisticated technique and considerably more expensive. Additionally, as the energy per photon is increased so too is the risk of sample degradation, especially for biological samples¹⁵. Therefore, it would be advantageous to be able to employ plasmonic In substrates for use in SERS at visible wavelengths.

Additionally, in order for quantitative SERS to be a possibility, methods which rapidly produce uniform nanostructures over wafer-scale areas must be developed. Tailored growth of InP nanostructures has been achieved using broad beam monatomic Ar⁺ ion sputtering^{16,17}. A recent report has also demonstrated the wafer-scale production of InP nanopillars for photovoltaic applications using maskless nitrogen ion beam etching¹⁸. There is potential for similar techniques to be employed for the production of SERS substrates.

Here, we report on visible wavelength SERS observed from bovine serum albumin (BSA) using In-InP nanopillars synthesised by Ar gas cluster ion beam sputtering (GCIB) of InP wafers. InP provides a high local refractive index for plasmonic In structures, which has been predicted to increase the wavelength of the In LSPR⁷.

GCIB sputtering utilises the high mass-to-charge ratio of singly-charged Ar atomic clusters to enable high current density, low energy-per-atom irradiation of surfaces even when the total beam energy is high¹⁹. GCIB is routinely used for secondary ion mass spectrometry (SIMS) and X-ray photoelectron spectroscopy (XPS) depth profiling of soft and organic materials²⁰. It has also been reported to reduce preferential sputtering in some inorganic compound semiconductors²¹. However, GCIB sputtering of In-containing semiconductors, such as InAs and InP, has been shown to produce compositional changes and nanostructured surfaces²². These two phenomena are expected to be linked and are likely a result of the sputter rate differential between In and As/P. This can be exploited to achieve the mask-less fabrication of InP nanopillars.

To prepare the SERS substrates undoped InP (100) were irradiated by Ar₃₀₀ clusters (i.e. 300 atoms per cluster) at 8 keV using a monomer and gas-cluster ion source (MAGCIS™) (mounted inside a Thermo Theta Probe XPS equipped with monochromated Al K α X-ray source) at an angle of 45°. A total beam current of 5 nA was measured for this particular mode of the ion gun, and the beam was rastered across an area of 1×2 mm² for a

total etch time of 4800s. A Helium Ion Microscope (HIM) (Zeiss Orion NanoFab) image is shown in Figure 1(a). After etching nanopillars can be observed oriented towards the incident angle of the ion beam. A large contrast difference is observed between spherical regions localised at the tip of the nanopillars, thought to be clusters of metallic indium, and the remainder of the substrate. The mechanism for the cluster formation is under debate, but it has been proposed that they form via the Kirkendall effect²³. Itinerant In atoms from within a characteristic diffusion length coalesce, resulting in a narrow distribution of nanoparticle cluster sizes. The average cluster size was determined to be 58nm using the image processing software, ImageJ.

The substrates were submerged in 2×10^{-5} M solutions of BSA in phosphate-buffered saline (PBS) for 12 hours and air dried. Identical solutions were prepared and deposited onto bare Si wafers and Si wafers with 20nm thick Au films for comparison. Elaborate ‘feather-like’ patterns are formed during the drying process (Fig 1(b)). These patterns have been observed by numerous authors previously^{24,25}. Ensuring a conformal coating of BSA on the nanopillar surface is important to enable an accurate determination of the enhancement factor. Fig 1(c) and (d) show HIM images of the nanopillar surface in close proximity to a crystal, revealed below to be comprised of NaCl, which has precipitated during drying of the BSA in PBS solution. The NaCl crystal displays no signs of inhomogeneity and looks to be resting on the nanopillars rather than conformally coating them. A carbonaceous layer is observed coating the nanopillar surface, which is revealed more clearly after sputtering by He^+ (Fig 1(d)). A square area of the nanopillar surface is shown to be mostly cleaned by the ion beam, while some remnants of the carbonaceous layer remain. The conformal carbonaceous layer is evidence of a uniformly distributed BSA coating. The BSA coating conformity and distribution is revisited below.

FIG 1: (a) HIM image of GCIB-irradiated InP, showing the large scale fabrication of In-InP nanopillars. (b) Patterns arising from dried BSA in PBS deposited onto SERS substrates. (c) HIM image of a NaCl crystal which precipitates from the BSA in PBS solution. A carbonaceous layer can be seen coating the nanopillars. (d) A region of the carbonaceous layer after etching by the helium ion beam. Patterns also emerge on the NaCl crystal due to ion beam damage (Scale bars are 500nm unless labelled).

Figure 2(a) shows the In3*d* XPS from the uncoated InP substrate both prior to GCIB etching (0s) and after 4800s irradiation. High energy shoulders are observed at 453.3eV and 445.7eV prior to etching, which correspond to a surface oxide on the InP surface. This is reduced after GCIB sputtering and the primary In-P peaks at 452.3eV and 444.8eV increase in intensity. This coincides with the emergence of low binding energy peaks at 451.6eV and 444.0eV attributed to metallic In. A loss feature at 455.6eV also appears which is generally ascribed to the presence of metallic In. The P2*p* XPS (Fig 2(b)), however, remains relatively unaffected by the sputtering, though does not display the same increase in intensity. The In:P ratio was determined to be 3.3 after 4800s GCIB irradiation. This indicates the preferential removal of P atoms, while those that remain are in an unchanged bonding state. The valence band XPS is shown in Fig 2(c), which shows a

shift in the valence band maximum (E_{VB}) from 1.13eV to the Fermi level, indicating a metallic surface. The XPS measurements along with the material contrast observed in the HIM images (Fig 1(a)) suggest that the spherical clusters observed at the nanopillar tips are indeed metallic In clusters, while the remainder of the structure and substrate is InP.

FIG 2: (a) In3d, (b) P2p and (c) valence band XPS from the InP wafer before and after etching (prior to BSA coating). The appearance of the In⁰ component and the shift of E_{VB} to 0eV confirm the presence of metallic In at the surface. The P2p appears unaffected.

Our attention now returns to the coating of the substrates with BSA in PBS solution. Figure 3(a) and (b) show mass-filtered negative ion SIMS images of NaCl and CNO⁻, respectively. Time-of-flight SIMS (ToF-SIMS) images were acquired on an ION-TOF ‘TOF-SIMS IV – 200’ instrument (ION-TOF GmbH, Münster, Germany) of single-stage reflectron design²⁶. Negative ion spectra and images were obtained using a Bi₃²⁺ focused liquid metal ion gun at 25 keV energy, incident at 45° to the surface normal and operated in ‘bunched’

mode for high mass resolution. The CNO^+ signal has been used previously in ToF-SIMS as a marker for BSA proteins²⁵. Here, it can be observed that the ‘feather-like’ pattern, also observed in Fig 1(b), is NaCl. The CNO^+ signal is predominantly negatively correlated with the NaCl signal and shows little structure besides that which is NaCl, indicating a uniform distribution over the SERS substrate surface. These observations are supported by scanning electron microscopy energy dispersive X-ray spectroscopy (SEM-EDS) images (acquired using a Hitachi TM3030 equipped with Bruker XFlash 430H EDS detector). The EDS images of Na (Fig 3(c)) and Cl (Fig 3(e)) are correlated and localised to the ‘feather-like’ patterns. The images of C and N (Fig 3 (d) and (f), respectively) are negatively correlated with the NaCl signal and show no other features. Note that different sample regions were used to acquire ToF-SIMS and SEM-EDS images to avoid recording beam damage introduced by either technique. These images, together with the HIM images (Fig 1) provide conclusive evidence that the precipitated crystals are NaCl and, also, that the BSA is evenly distributed in a conformal coating on the SERS substrate surface. Additionally, the C1s and N1s XPS from the BSA on the SERS substrate are shown in Fig 3 (g) and (h) respectively (plots for BSA deposited onto bare Si wafer and 20nm Au/Si wafer for comparison are shown inset). The peak corresponding to C=O bonds is clearly observable at $\sim 288\text{eV}$, while the larger broad peak centred at $\sim 286\text{eV}$ is representative of the C-C (285 eV) and C-N (286eV) bonds which cannot be resolved from each other. However, evidence of C-N bonds is clear from the N1s spectrum, confirming the presence of BSA on each of the substrates²⁷.

FIG 3: (a) ToF-SIMS images of NaCl ($\text{Cl}^- + \text{NaCl}_2^+$) and (b) BSA ($\text{CN}^- + \text{CNO}^-$) show predominantly negative correlation. SEM-EDS images of (c) Na, (d) C, (e) Cl and (f) N confirm the precipitation of NaCl from the BSA in PBS solution. (g) C1s and (h) N1s XPS spectra also confirm the presence of BSA on the substrate (C1s and N1s for BSA on bare Si and 20nm Au/Si wafer are shown inset). (Scale bars are 80 μm).

SERS measurements, acquired using a Horiba Jobin Yvon HR800 UV at 2.4mW, from the 2×10^{-5} M solutions of BSA on In-InP were obtained at 514nm and 457nm and are shown in Figure 4(a). Equivalent Raman spectra were acquired from 2×10^{-5} M solutions of BSA deposited onto bare Si wafers (Fig 4(b)) and Si wafers with 20nm sputtered Au for comparison (Fig 4(c)). A HIM image showing the surface roughness of the Au film is shown inset in Fig 4(c). In each of the spectra we observe both the longitudinal optical (LO) phonon and forbidden transverse optical (TO) phonons for InP due to the breaking of crystal symmetry after sputtering²⁸. Second order InP peaks can also be observed at 615.4 cm^{-1} , 650.6 cm^{-1} and 681.7 cm^{-1} attributed to the 2TO, (TO+LO) and 2LO peaks, respectively. The second most prominent features are bands at 1300 - 1400 cm^{-1} and 1550 -1650 cm^{-1} , which correspond to the tryptophan/amide III and amide I regions of BSA^{29,30}. These peaks are

also observed using the Au on Si substrate, albeit at a slightly reduced intensity. This is likely due to a combination of the reduced surface roughness compared to the In-InP nanopillars and the mismatch in energy between the 514nm laser and the LSPR of the Au film. The tryptophan/amide peaks are greatly reduced in the Raman spectrum obtained from BSA solution deposited onto Si wafer (Figure 1. (c)). However, a peak at 1458cm^{-1} is observable and corresponds to CH_2 ³⁰. Evidence of CH_2 is present in Fig 4(a) but it is dominated by the amide bands. The spectra acquired at 514nm exhibits stronger enhancement of the BSA signal for each of the nanostructured InP samples, while this is not true of the InP TO and LO modes, indicating that the LSPR of the In nanoparticles is red-shifted above 457nm.

FIG 4: Raman spectra from 2×10^{-5} M BSA in PBS solution deposited onto (a) In-InP nanopillars, (b) Si wafer and (c) Au on Si substrates. The In-InP nanopillar and Au on Si substrates exhibit signal enhancements of $\sim 97\times$ and $9\times$ compared to Si substrates, respectively. A HIM image of the Au surface is shown inset in (c) (scale bar 100nm).

The SERS substrate enhancement factor EF is given by,

$$EF = \frac{I_{SERS} / N_{surf}}{I_R / N_{Vol}} \quad (1)$$

where I_{SERS} and I_R are the SERS and non-SERS Raman intensities, respectively. N_{surf} is the average number of molecules attached to the SERS surface and N_{Vol} is the average number of molecules in the non-SERS Raman scattering volume³¹. An estimation of the surface area calculated from the average nanopillar dimensions as determined from the HIM images (Fig 1) yields an increase of ~2.5x compared with planar Si wafer. I_{SERS}/I_R was found to vary depending on the wavenumber, with 97 and 285 recorded at 1350 cm⁻¹ and 1594 cm⁻¹, respectively. Assuming a monolayer of BSA coats both the SERS substrate and the Si wafer, this yields EF s of between 39 and 114. This result suggests that the SERS effect recorded for the In-InP nanopillars is a minimum 3 times higher than has been recorded for UV-SERS using In nanoparticles¹⁴ in spite of the wavelength dependent Raman scattering enhancement law, $I \propto \lambda^{-4}$.

Finite-difference time-domain (FDTD) calculations (performed using the OptiFDTD software package) support the observation of strong electric field enhancement due to plasmonic excitation at 514nm. A model of a single In-InP nanopillar was constructed from the average dimensions determined from the HIM images using ImageJ. The spherical In cluster diameter was 58nm, while the base was set to twice this diameter to mimic the tapered conical shape of the pillars and the height was fixed at 2.5 times the In cluster diameter. The refractive index of the InP pillars was $n = 3.75 + 0.46i$. For the metallic In nanoparticles a Drude dispersion model was used with plasma frequency ω_p and damping constant γ of 12.8 eV and 0.46eV, respectively. Figure 5(a) and (b) shows the electric field intensity distribution $|E|$ and calculated SERS enhancement factor $|E|^4$ calculated for a single nanopillar³². $|E|$ was also calculated for nanopillars coated with a 4nm BSA layer ($n = 1.55 + 0i$) and was found to be relatively unperturbed (not shown). The average enhancement at the surface is 10^3 which is within an order of magnitude of the I_{SERS}/I_R value measured for the Amide I band of BSA on the In-InP nanopillar substrate.

FIG 5: (a) Local electric field $|E|$ and (b) SERS enhancement factor $|E|^4$ calculated for In-InP nanopillars with 58nm spherical In nanoparticle tips at 514nm.

In summary, we have shown that large scale In-InP nanopillar SERS substrates can be manufactured rapidly using GCIB sputtering of InP wafers. The conformity and uniform distribution of the BSA analyte was proven using SEM-EDS and ToF-SIMS, allowing the determination of SERS enhancement factors. The Raman scattering signal was determined to be up to 285 times higher for BSA deposited onto In-InP nanopillars when compared with BSA deposited on Si wafer substrates, in reasonable agreement with FDTD calculations. These substrates enable the detection of low concentrations of BSA at with visible wavelength SERS, without the use of noble metal particles. Our results show the ability of In-based SERS substrates to be used effectively outside the UV for label-free biomolecule detection.

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