Rapid bacteria detection using a novel SERS substrate produced by oblique angle vapour deposition on nano-imprinted polymer template

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Introduction

Rapid bacteria detection can lead to better medical treatment strategies, current methods such as PCR, cell cultures and immunoassays generally require specialist lab equipment or can take long processing times depending on the species. Surface Enhanced Raman Scattering (SERS) can provide faster and more sensitive measurements capable of detecting single molecules.[1] By using polymer templates and oblique angle deposition (OAD), noble metal nanorod arrays can be produced cheaply and used as sensitive SERS substrates.[2] Depositing material at an oblique angle to the substrate normal can produce nanostructures, such as nanorods, through atomic self-shadowing. Fig. 1 illustrates this effect and how by using templates we can further develop these thin films. This project explores from deposition simulations, SERS substrate fabrication to rapid bacteria detection.

Templates

Oblique angle deposition enables arrays of nanorods to be produced using templates. The templates, see Fig. 2, contain elevated surface structures evenly with ABA packing distribution. These surface structure define the nucleation sites for where nanorods will form and therefore array and packing density is determined by the template design.

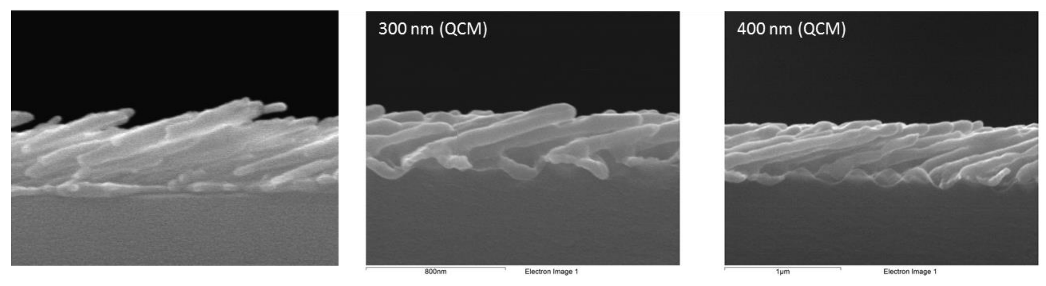

Figure 1 – (left) oblique angle deposition of copper on silicon (100) substrate, no template features mean that nucleation for nanostructures rely on random sites. (middle and right) oblique angle deposition of silver onto polymer templates at varying thicknesses measured *in-situ* by a quartz crystal microbalance.

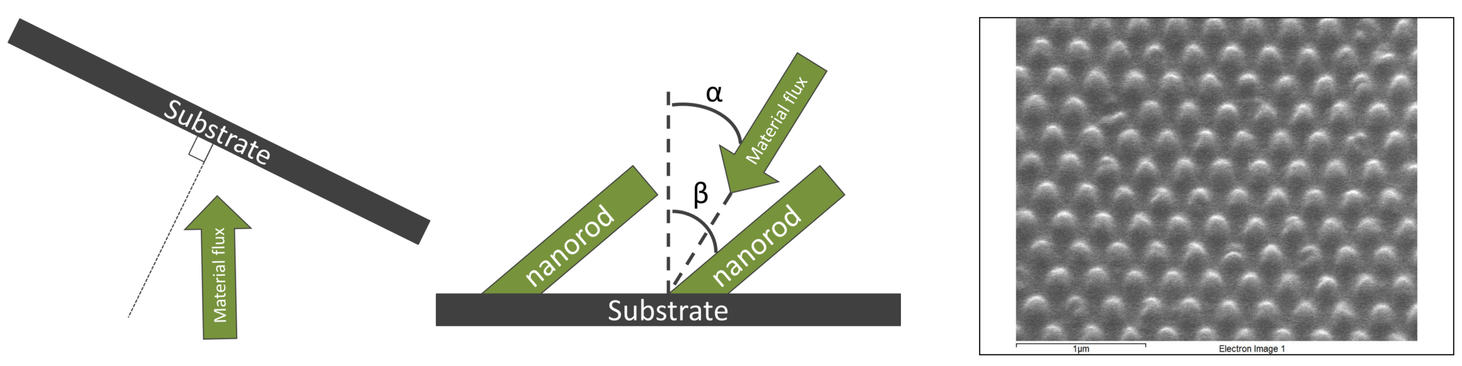

Figure 2 – (left) Brief diagram illustrating the operation of oblique angle deposition and the angles involved when carrying out such depositions. (right) The hexagonal packing structure allows for nanorod arrays to be form due to the atomic self-shadowing effect at high deposition angles.

The Cosine Rule

Empirical relations such as the cosine rule (see equation (1), this applies deposition angles >60°) and tangent rule (relatively low deposition angles, i.e. <60°) are applied in predicting resulting column angles.

$$\beta = \alpha - \operatorname{asin}\left(\frac{1-\cos\alpha}{2}\right) \tag{1}$$

The cosine rule is based on geometry and does not take into account thermally activated effects, such as adatom diffusion. Our experiments and literature show that equation (1) under estimates the results and our observations indicate that a multiplier to the asin term (found to be 0.588) corrects for this.[3] Fig. 3 relates the tangent rule ($\tan\alpha = 2\tan\beta$), cosine rule, modified cosine rule [($\beta = \alpha - \gamma\operatorname{asin}(\frac{1-\cos\alpha}{2})$)] and observations with copper nanorod arrays on template substrates. It was found that the templates themselves did not significantly affect the resulting nanostructure angle.

NASCAM

Kinetic Monte Carlo can simulate deposition onto substrates, by taking into account relevant energies such as surface diffusion activation energy, the deposition of metals on various templates can modelled at larger timescales than molecular dynamics. The NASCAM kinetic Monte Carlo package was used in this study,[4] different deposition angles and surface diffusion activation energies were considered and this affected the resulting nanorod structures. Fig. 4 shows the difference the deposition angle, surface diffusion activation energy can make to the resulting nanostructures.

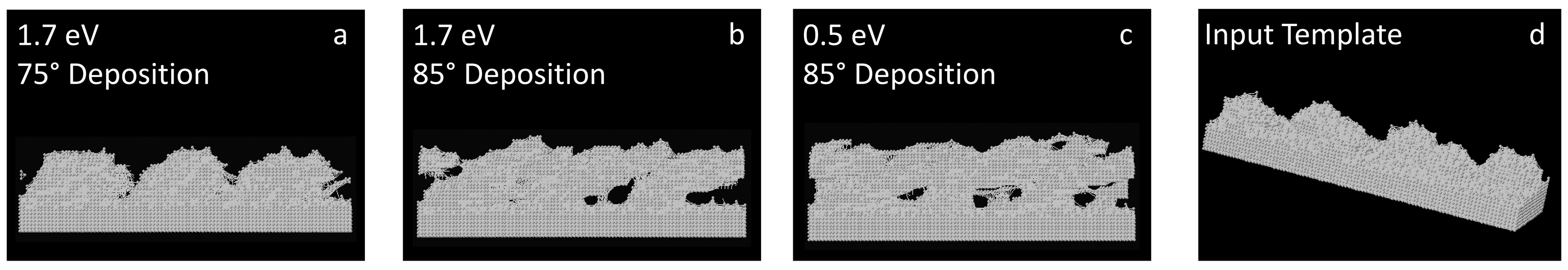

Figure 4 – NASCAM simulations of Ag deposition onto a template surface (d) at lower deposition angles (a) and surface diffusion energy closest to those found in literature (c).

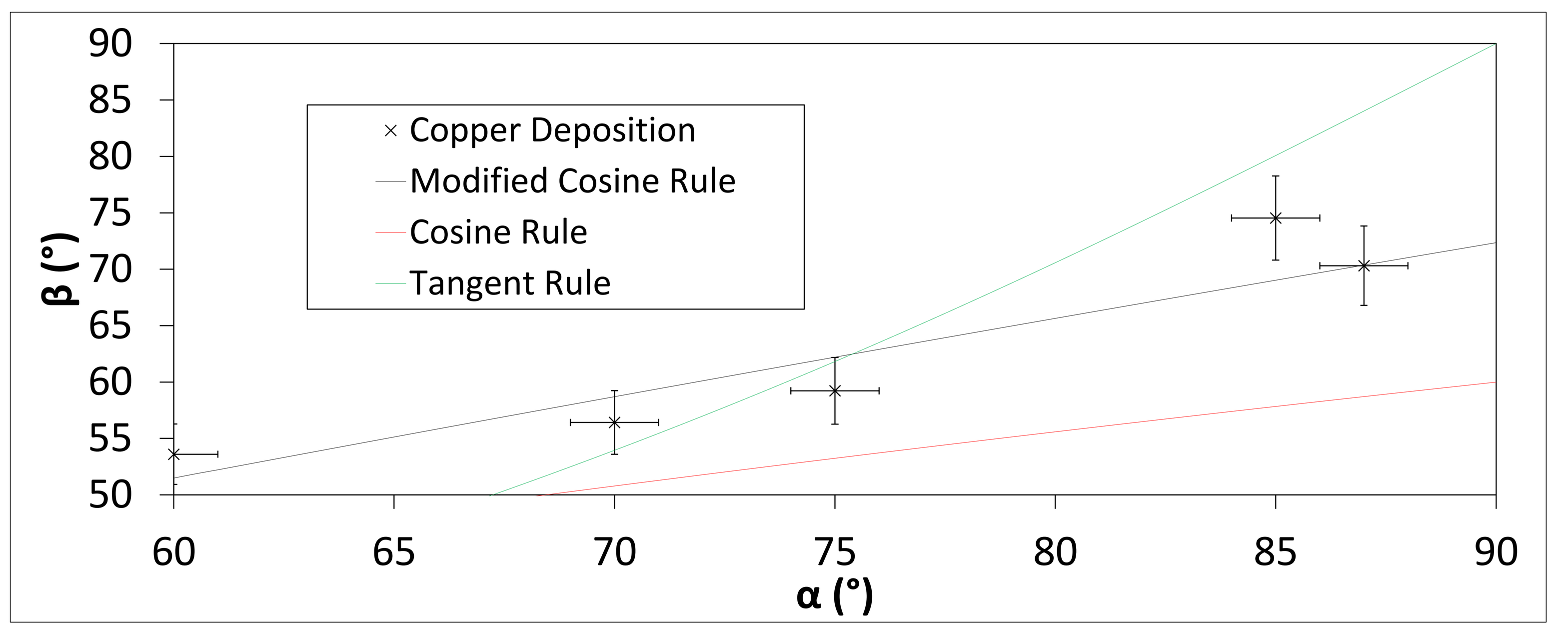

Figure 3 – β angles related by deposition angle, empirical relations such as the tangent, cosine and modified cosine rule are compared against experimental observations.

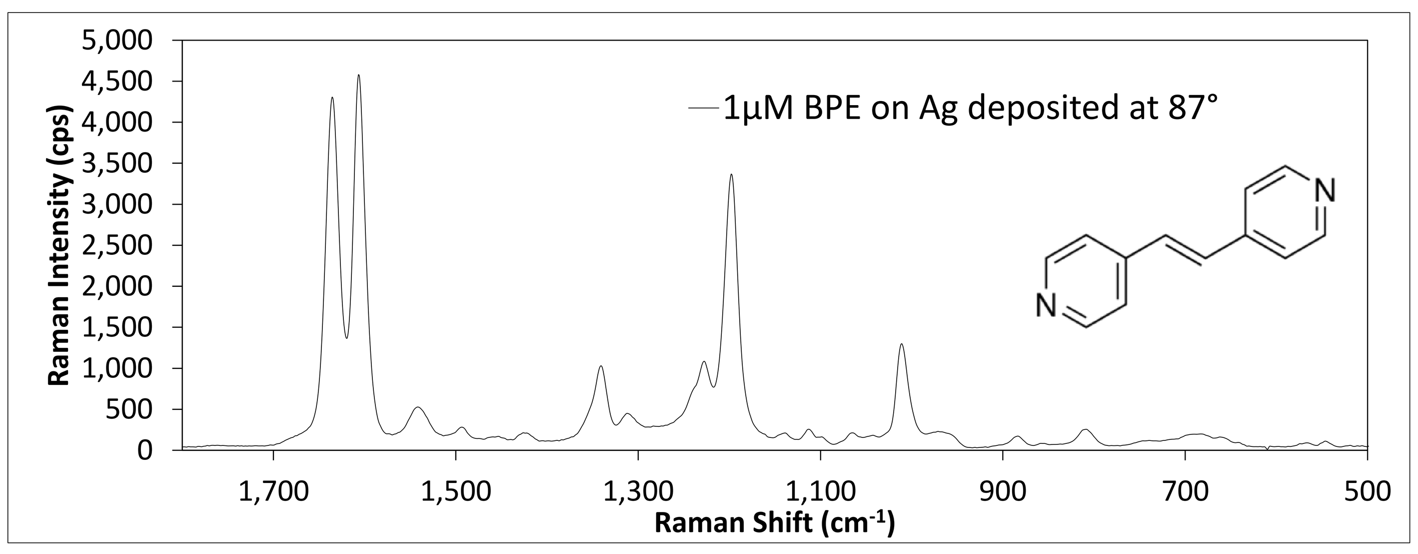

Figure 5 – SERS spectrum of BPE on Ag nanorod array produced by 87° OAD. These arrays produced the best enhancement signal, giving approximately 100,000 enhancement factor.

Surface Enhanced Raman Spectroscopy

SERS substrates were evaluated using the 1,2-Bis(4-Pyridyl)-ethylene (BPE) Raman probe. The copper based nanorods gave, relative to silver, poor Raman signal enhancement. Indicating that copper only contributed a chemical enhancement rather than generating an intense region of enhancement. Silver nanorods, as seen in the graph above, can give signals for low concentrations of BPE solutions. The asymmetry of the doublet peak near 1600-1700 cm^{-1} gives evidence of BPE-Ag adsorption as the higher peak is contributed from the pyridyl ring adsorbed onto the metal surface. These enhancement factors are comparable with previous work done and result in a relation similar to the modified cosine rule, which is more applicable for metal deposition. Fig. 5 shows the Raman spectrum for BPE on Ag nanorod array produced at 87° deposition, compared to the control which was at a much higher concentration on Si substrate, this gave an enhancement factor of ca. 10^5.

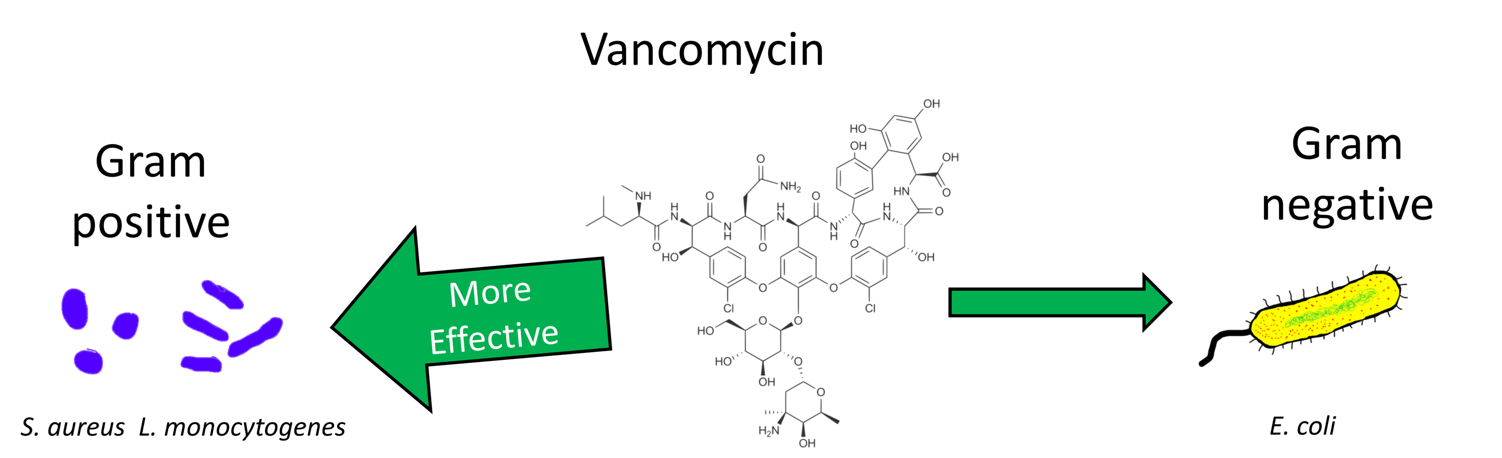

Figure 6 – Gram negative bacterium have additional layers in the cell wall structure, where the peptidoglycan should be inaccessible by the vancomycin. Therefore the activity of vancomycin differs significantly for gram negative and gram positive bacteria.

Vancomycin Surface Functionalisation & Bacteria Detection

Vancomycin is an anti-biotic that targets the peptidoglycan of bacteria cell walls. (see Fig. 6) As a result, the activity on gram negative bacteria differs from gram positive. This project aims to use the hydrogen bonding abilities of this antibiotic for surface-cell adhesion at specific sites. While gram negative bacteria have multiple layers between their surface and their peptidoglycan, these intermediates are typically relatively much thinner than most gram positive bacteria. Another point to mention is that vancomycin is most effective with metabolically active bacteria, by the time bacteria is deposited onto the surface these would be significantly lower, how much lower has yet to be studied for this case. Fig. 7 illustrates the Raman spectra for *E. coli*, *L. monocytogenes* and *S. aureus*, the detection time was on the scale of seconds to minutes and provide us with spectral "fingerprints" for differentiating bacteria species.

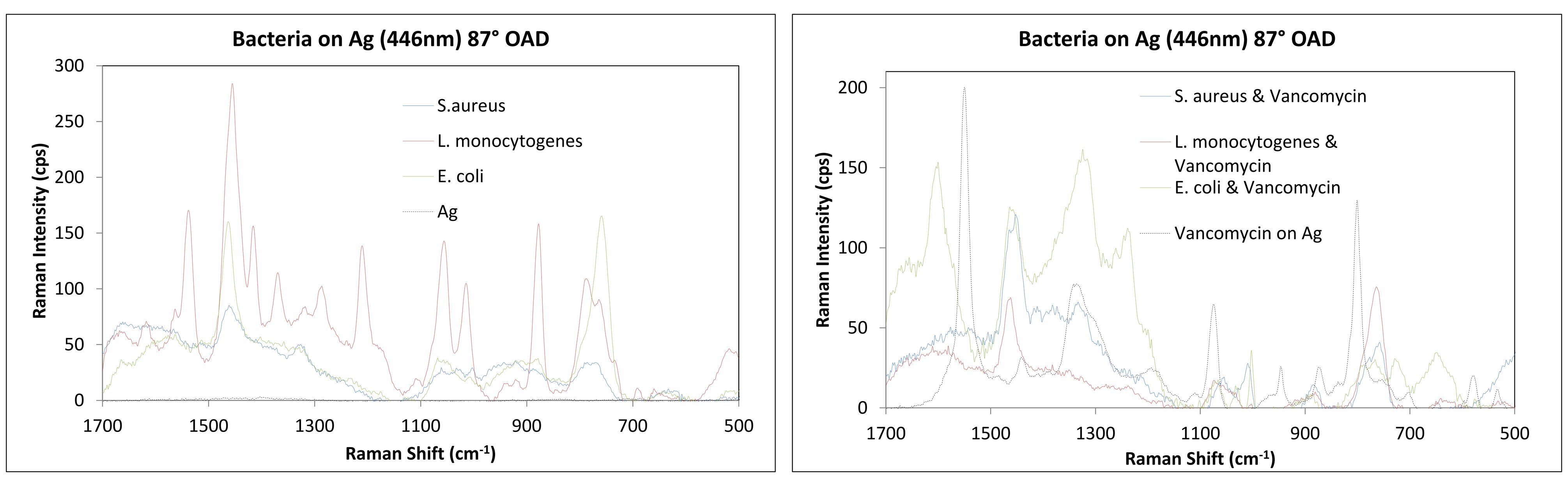

Figure 7 – (left) SERS spectrum of various bacteria on the SERS substrate. (right) The Raman spectrum is changed for all analytes, these can provide spectral "fingerprints" for determining species.

Summary

It was found that empirical relations typically used for oblique angle deposition studies do not apply well for all materials. Some attribute this to thermally activated effects such as adatom migration, as such the surface diffusion activation energy should be taken into account for metals such as copper and silver. This study found that applying a γ coefficient to the asin term, where γ is 0.588 for Cu and Ag, gives angles that are closer to what is experimentally observed. Rapid bacteria detection had been achieved with these SERS substrates, capable of detection in under 5 minutes. Coupled with vancomycin surface functionalisation, these substrates have potential for screening antibiotic activity on different bacteria.

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