Biosensing Based on Surface-Enhanced Raman Scattering

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Abstract and Introduction:

Raman scattering involves the inelastic scattering of a photon from a sample in which the incident photon transfers energy to a molecule and is emitted at a different frequency. This change in energy allows for a spectroscopic technique that provides a molecular fingerprint of a sample. However, Raman scattering is only a small fraction of the light that is scattered from a molecule. Noble metal nanoparticles or nanostructures can greatly enhance Raman scattering to the orders of 10^6-10^{14}-fold due to the localized surface plasmon resonance, i.e., the so-called surface-enhanced Raman scattering (SERS). SERS is an effective technique for detection and distinction of biological samples.

Using specially tailored substrates with quasi-three-dimensional (3D) gold nanostructures, developed by Dr. Yu's group at the University of Washington, the Raman spectra of numerous biological species were enhanced greatly. SERS was first used to confirm that two different strains of the bacteria *Vibrio parahaemolyticus* could be identified and distinguished in a mixed sample by comparison with the SERS barcoding of each strain. Additionally, the optimal concentration of bacteria to obtain high reproducibility and intensity was determined. Furthermore, SERS was employed to detect chemicals spiked in whole milk to explore its capability of being used for direct detection of chemical contaminates in complex media.

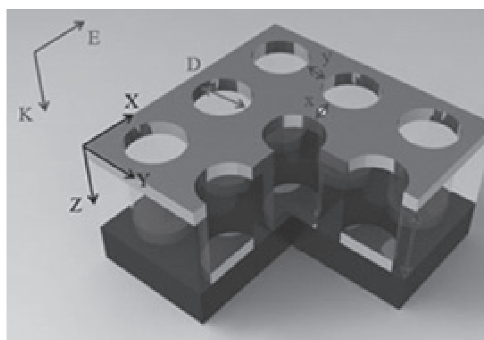

Figure 1: 3D illustration of the quasi-3D gold nanostructure composed of a separated gold thin film.

Methods:

Specially tailored substrates with quasi-3D gold nanostructures, developed by Dr. Yu's group at the University of Washington, were used for biosensing [1]. Four 50 μm × 50 μm nanohole arrays with a 400 nm hole diameter and 100 nm edge-to-edge distance were generated on each chip. For bacterial samples, the arrays were fabricated on indium tin oxide (ITO) coated glass with a 300 nm hole depth. For milk samples, 390 nm hole depths were produced on a silicon chip. Each chip was coated with 50 nm gold as illustrated in Figure 1.

Bacterial Samples. A 1:1 ratio of *V. parahaemolyticus* strains 551 and 3256 was prepared *in situ* by placing a SERS-active chip, cleaned in UV ozone for 20 minutes and rinsed with deionized water, in a custom-made Teflon® holder. After vortexing, 500 μl of the *V. parahaemolyticus* solution was deposited onto the quasi-3D nanohole patterns, and a microscope cover slide was carefully positioned on top. To determine the optimal concentration of bacteria for SERS detection, five samples of *V. parahaemolyticus* 551 of different concentration, ranging from 10^4 to 10^8 cfu/ml, were prepared *in situ* in the same fashion.

Milk Samples. Three samples were prepared: whole milk, whole milk spiked with 0.1% 4-mercaptopyridine (4-MP), and whole milk spiked with 0.0001% rhodamine 6G (R6G). These samples were prepared using a dip-and-dry method in which a clean SERS-active chip was placed in a vial containing 2 ml of the sample. The chips were carefully removed and dried with air after the vials were refrigerated for three hours.

Using a Renishaw inVia Raman microscope, SERS spectra were gathered from 400 to 2000 cm^{-1} with a 785 nm laser and 5 mW power.

Results and Conclusions:

As seen in Figure 2, the barcode of the mixed sample of bacteria clearly displays the characteristic peaks of each pure strain, especially the most intense peak of *V. parahaemolyticus* 551 at 1532 cm^{-1} and the most intense peak of *V. parahaemolyticus* 3256 at 525 cm^{-1}.

Ergo, it was shown that SERS can be used to detect separate strains of the same bacteria in a mixed sample.

Figure 3 illustrates the peak intensities and error bars of different bacterial concentrations. The optimal concentration of bacteria to obtain high reproducibility and intensity to prepare bacterial samples was found to be 10^8 cfu/ml. At this concentration, the greatest peak intensity was found, which is necessary for efficient detection of bacteria. Additionally, at concentrations below 10^8 cfu/ml, the normalized intensity of peak 1539 cm^{-1} drops significantly, and considerable error is introduced.

When 4-mercaptopyridine (4-MP) was added to whole milk, it was found that the spectra of 4-MP predominated over the spectra of the milk. This is most likely due to the aromatic thiol adsorbing quickly and easily to the gold, forming a self-assembled monolayer before fats, proteins, carbohydrates, minerals, and other larger components of milk could reach the gold surface. However, as illustrated in Figure 4, R6G was successfully detected, proving as a basis for SERS detection of chemical contaminates in milk.

Future Applications:

Future work includes testing strains of *V. parahaemolyticus* from different sources, along with collecting Raman spectra of live toxic marine phytoplankton using an optical tweezer method. Additionally, future work involves spiking milk with pesticides to imitate a more natural contamination, and testing unpasteurized milk for contaminants using SERS.

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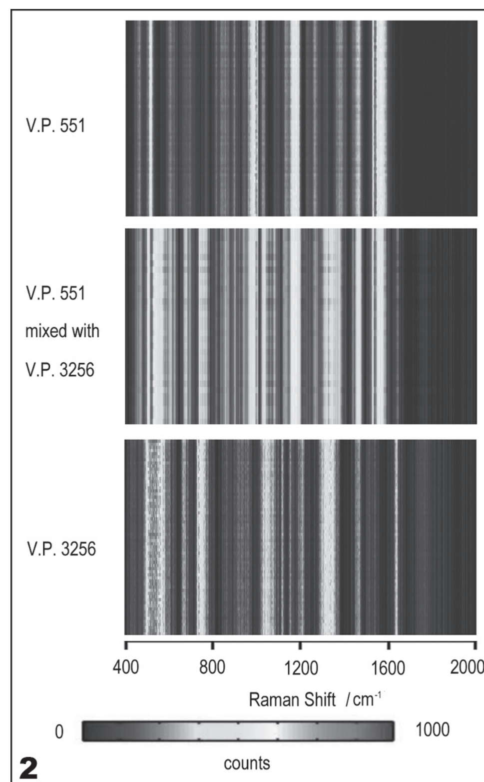
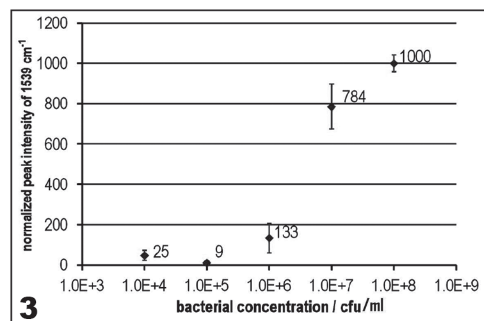
References:

[1] Xu, J., Zhang, L., Gong, H., Homola, J. and Yu, Q. (2011), Tailoring Plasmonic Nanostructures for Optimal SERS Sensing of Small Molecules and Large Microorganisms. Small, 7: 371–376. doi: 10.1002/smll.201001673.





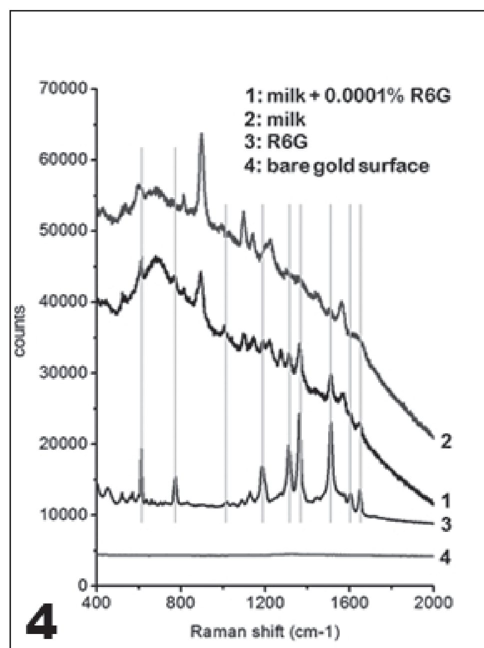


Figure 2, top: Comparison of the normalized SERS barcoding of V. parahaemolyticus *551, a 1:1 ratio of* V. parahaemolyticus *551 to* V. parahaemolyticus *3256, and* V. parahaemolyticus *3256.*

Figure 3, middle: The normalized intensities of peak 1539 cm^{-1} for bacterial concentrations of V. parahaemolyticus *551 ranging from 10^4 to 10^8 cfu/ml.*

Figure 4, bottom: Comparison of SERS spectra of milk, R6G, and milk spiked with R6G.