Electronic Supplementary Material (ESI) for Analytical Methods.

Innovative sandwich assay with dual optical and SERS sensing mechanisms for bacterial detection
Supplementary Information

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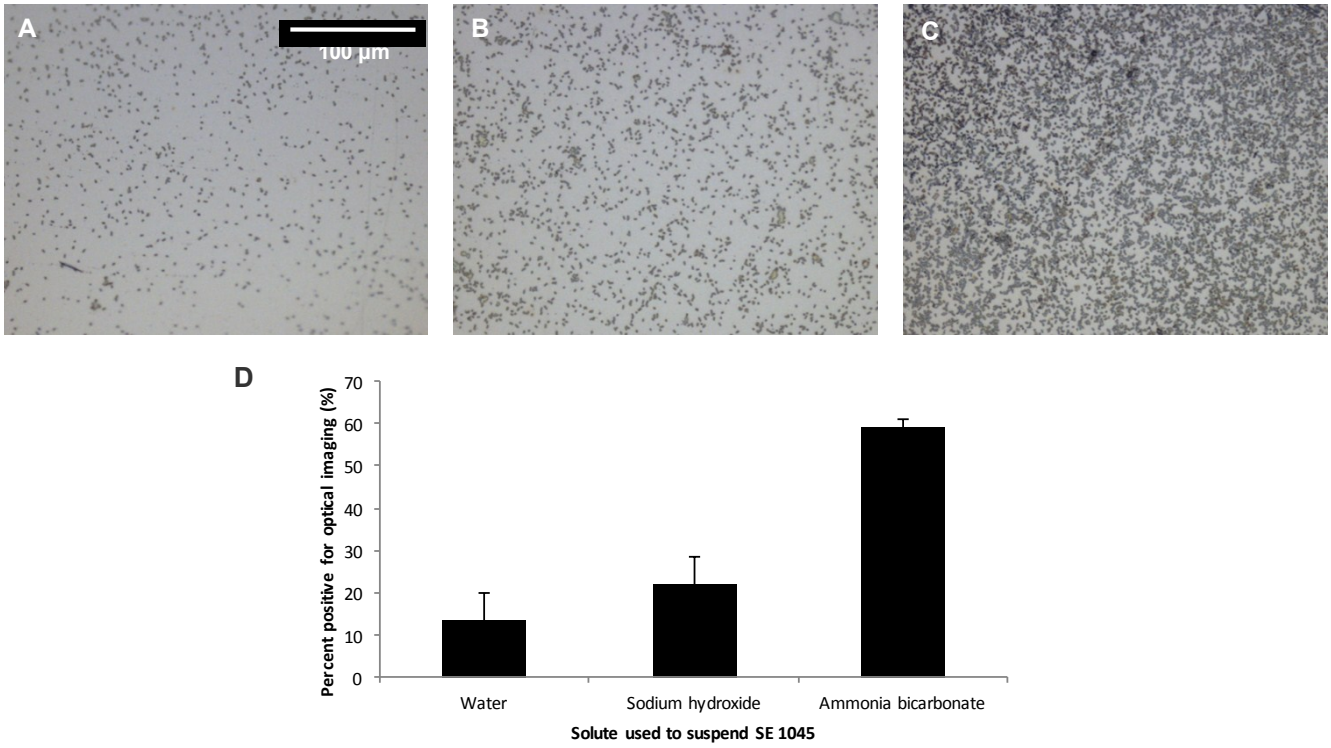


Fig. S1. Effect of solute on Salmonella enterica's ability to bind to 3-MPBA coated gold chip. The same culture of Salmonella enterica was washed using the method described in Section 2.1. Then bacteria were suspended in (A) autoclaved double distilled water (B) 0.1 mM sodium hydroxide (pH 8.4) (C) 50 mM ammonia bicarbonate (pH 8.4) and diluted to 10^7 CFU mL^{-1} concentration. Images were taken using a 20x objective Raman microscope (D) Comparison of optical imaging using the three different solutes in triplicate using pixel intensity analysis.

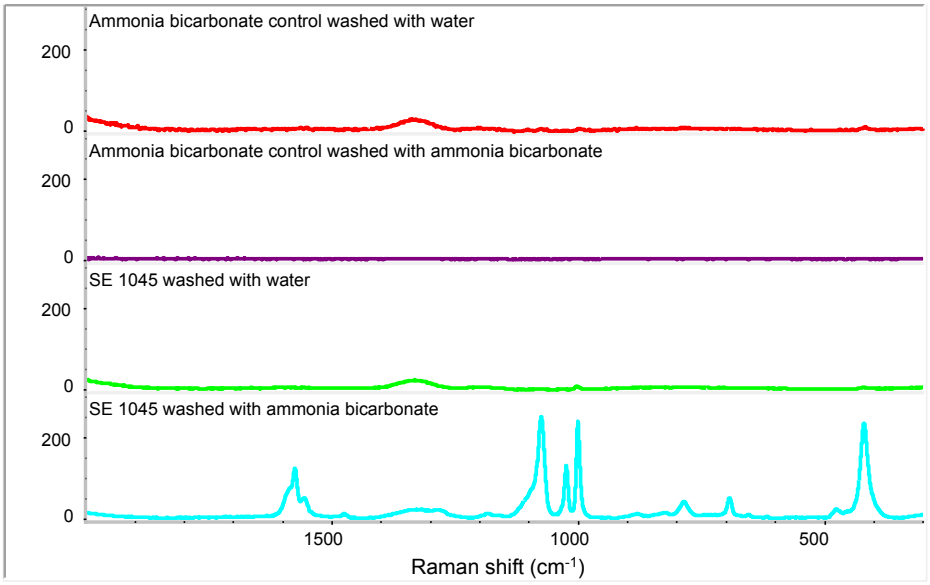


Fig. S2. Effect of washing solution on intensity of Raman spectra in the 3-MPBA sandwich assay. The washing solutions were autoclaved double distilled water or 50 mM ammonia bicarbonate. The concentration of *Salmonella enterica* was 10^7 CFU mL^{-1}.

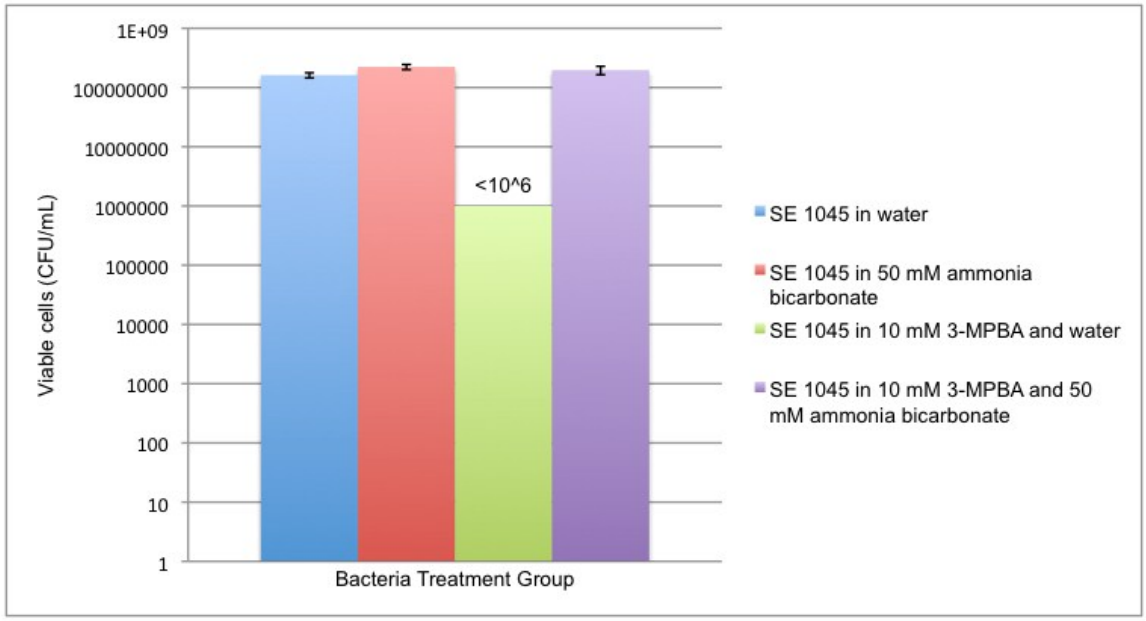


Fig. S3. Effect of immersion of *Salmonella enterica* in different solutions for 1 hour on the bacteria cell viability.

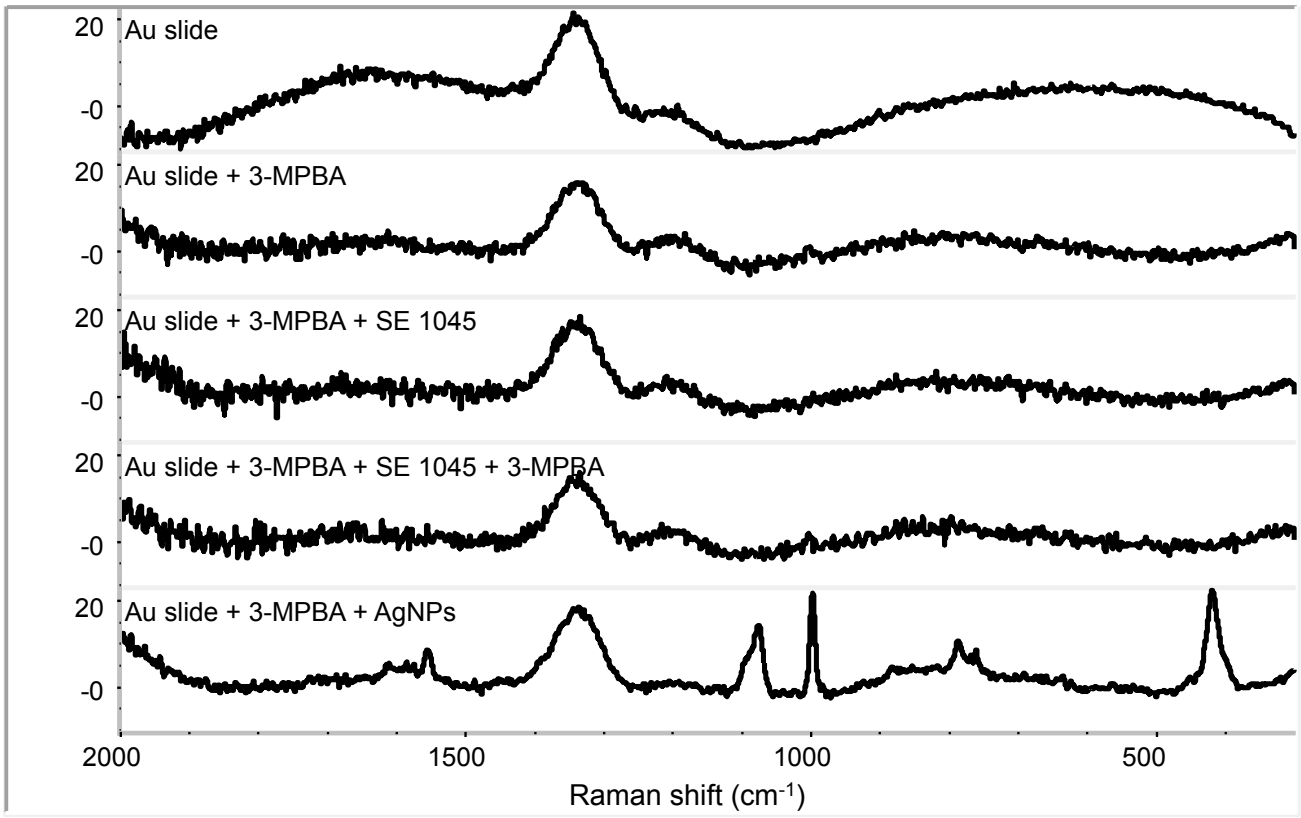


Fig. S4. Controls for 3-MPBA sandwich assay showing Raman spectra at each stage of the sandwich assay (refer to Fig. 3). Concentration of 3-MPB was 1 mM before bacteria was added and 0.1 mM for indicator solution. Concentration of *Salmonella enteria* was 10^7 CFU mL^{-1}. Silver nanoparticles were also dropped on the gold coated chip to demonstrate that the 1023 cm^{-1} peak does not appear before bacteria are added.

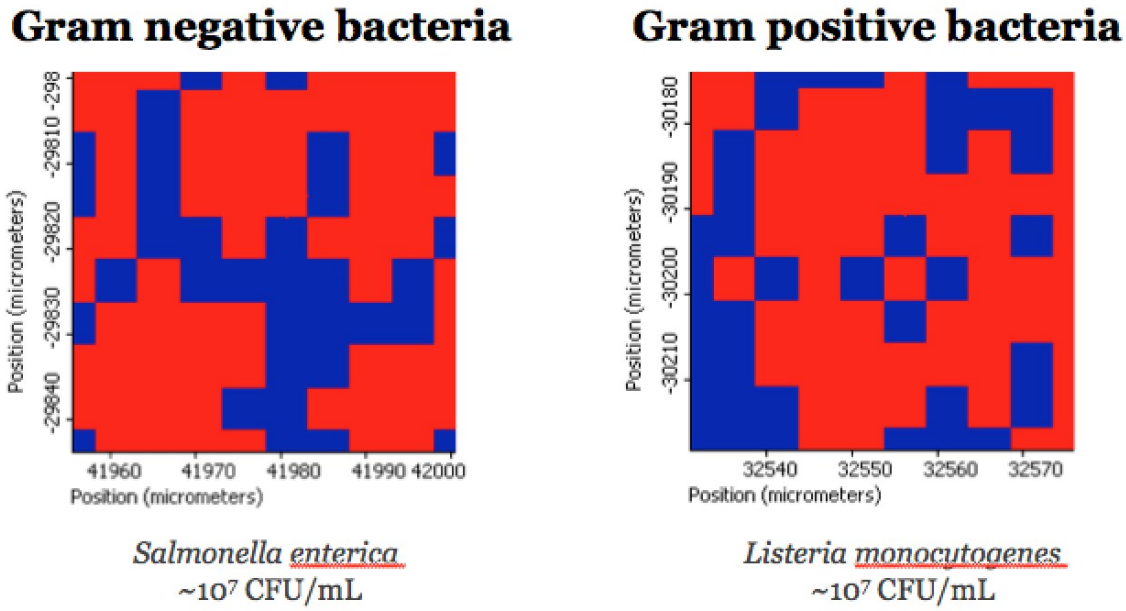
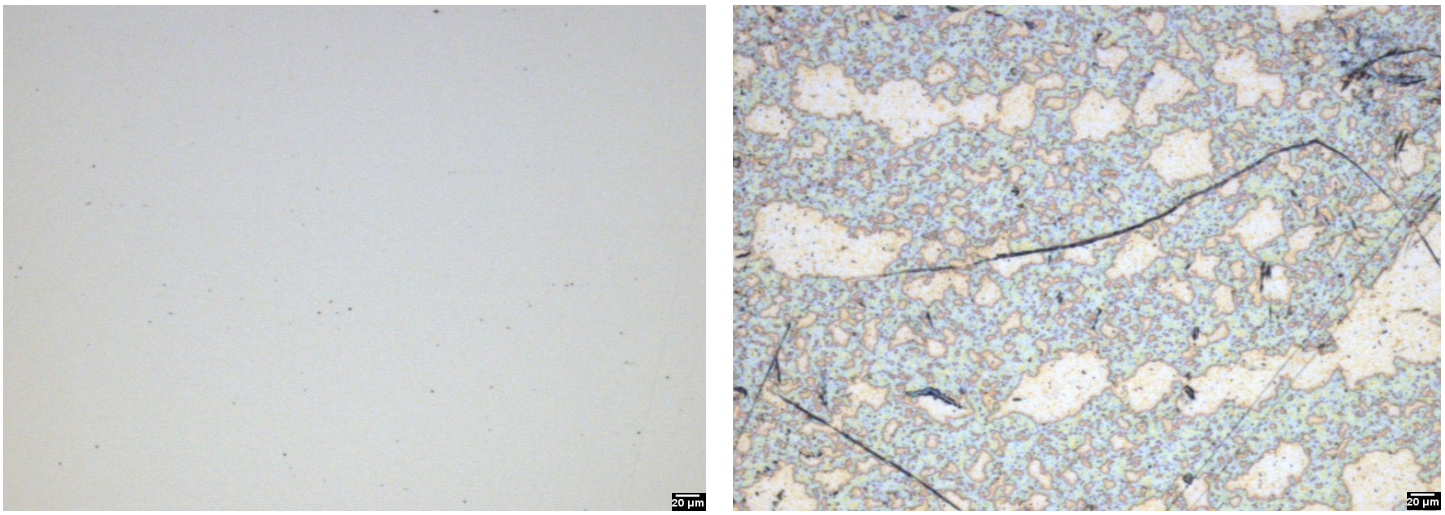


Fig. S5. Raman mapping of representative Gram positive and negative bacteria. Presence/absence criteria was determined by examining all the 1023 cm^{-1} peak, and selecting a cutoff of 3 times the standard deviation plus mean of the negative control (ammonia bicarbonate).



Au slide

SE 1045 10^8 CFU/mL in water air dried on a Au slide

Fig. S6. Optical video images (20x bright field objective) of a plain gold slide (left) and *Salmonella enterica* 1045 10^8 CFU mL^{-1} suspended in water and air dried on a plain gold slide.

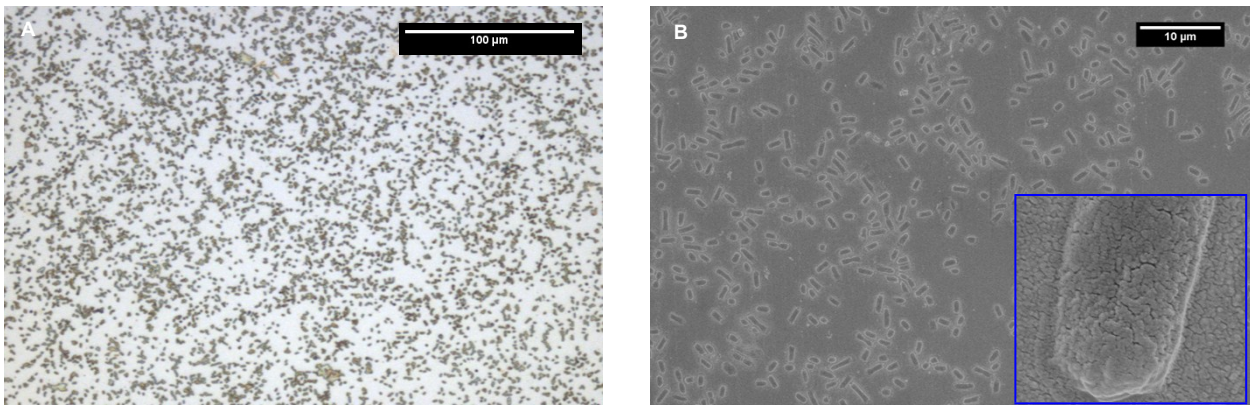


Fig. S7 *Salmonella enterica* 10^8 CFU/mL on 3-MPBA sandwich assay under (A) Raman microscope 20x bright field objective and (B) SEM (inset is the enlarged image).

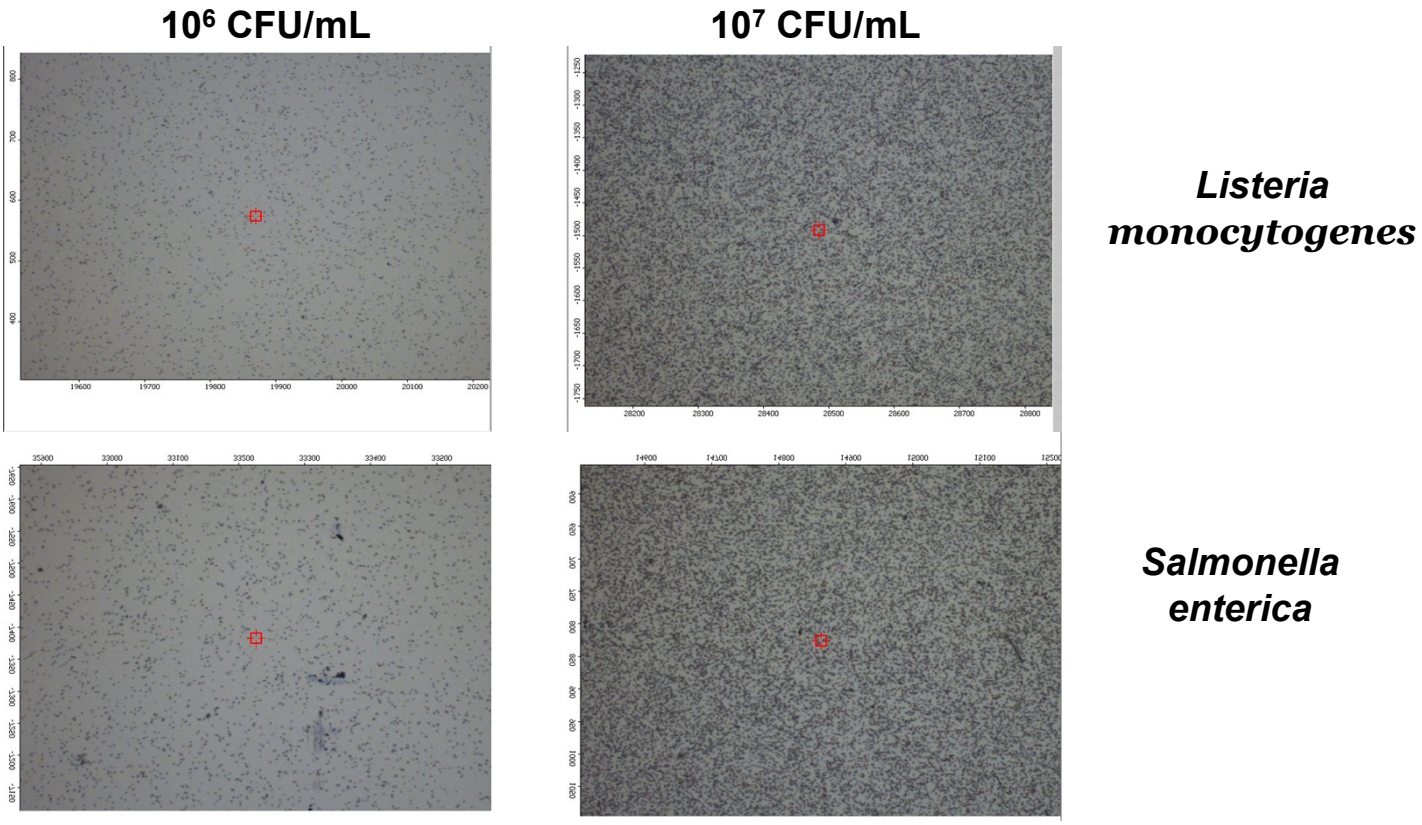


Fig. S8. Optical video images of *Listeria monocytogenes* and *Salmonella enterica* (10^6 CFU mL^{-1}, left, and 10^7 CFU mL^{-1}, right) under bright field 10x microscope objective.

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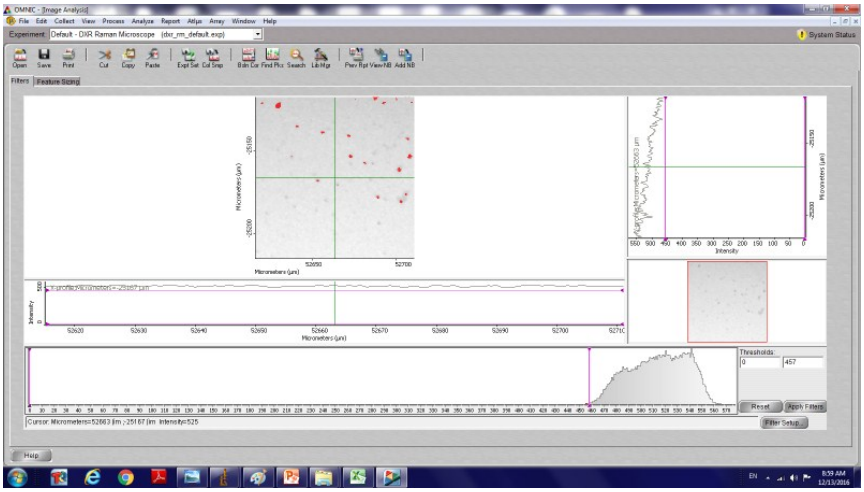

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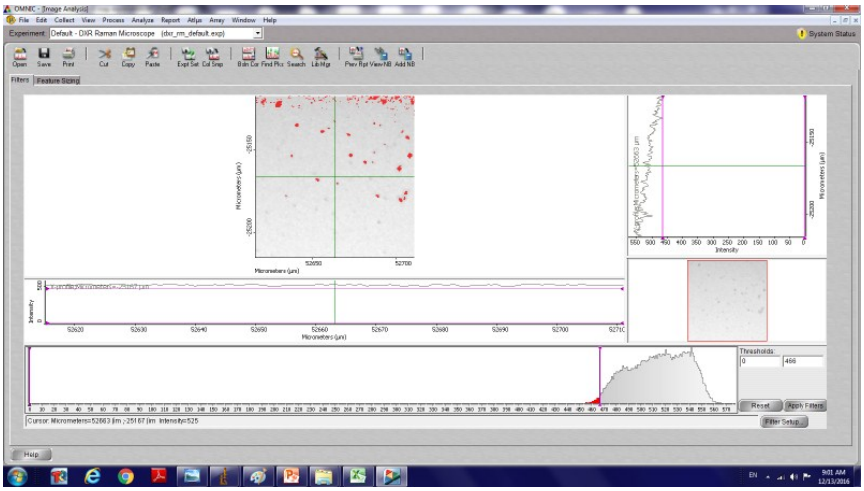

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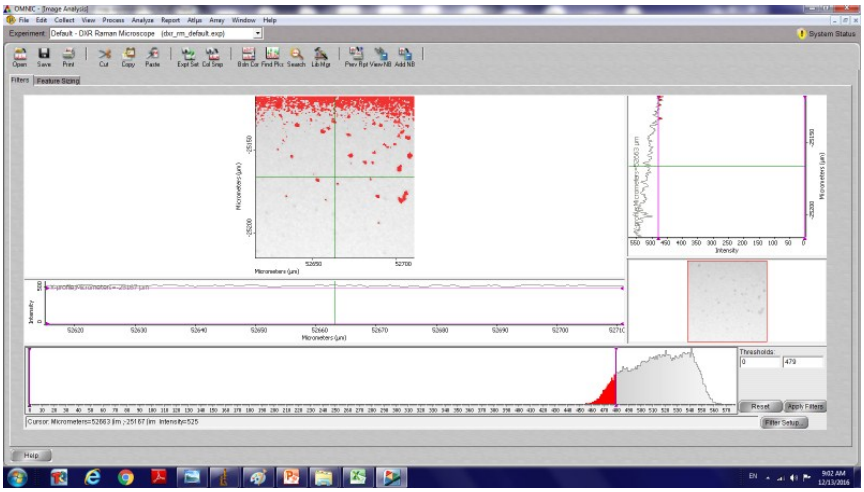

Fig. S9. Demonstration of how the threshold for the optical imaging was determined for the 400 point images in Fig. 4. The first picture demonstrates the threshold that was actually used. Picture 2 shoes the optical image just past the threshold, where tiny red dots begin to appear in the upper right corner of the image. Picture 3 shows the optical image well past the threshold, with red dots covering the entire top of the optical image.

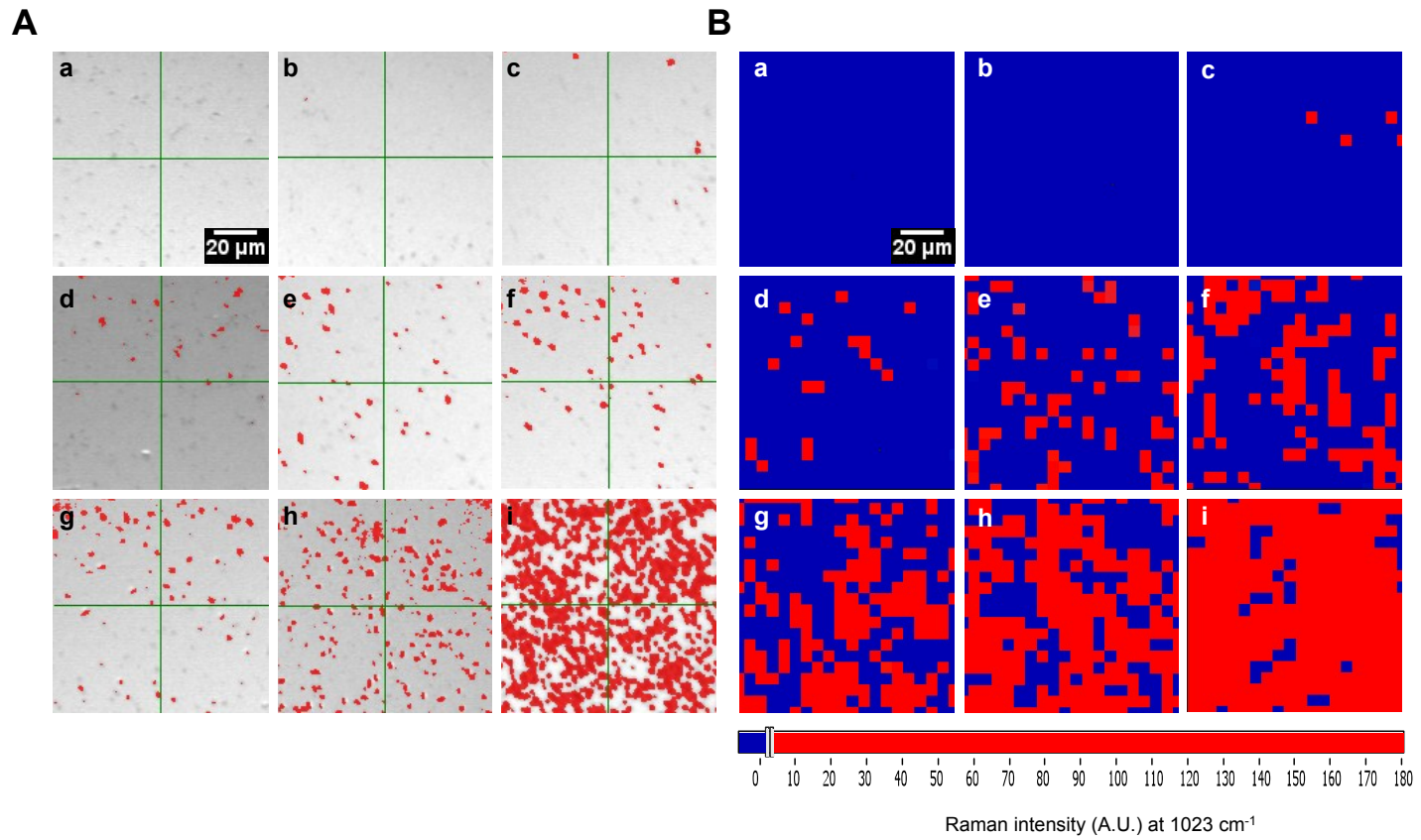

Fig. S10 Demonstration of reproducibility of the dual sensing mechanism (refer to Fig. 4). (**A**) Optical sensing mechanism of a 100 µm × 100 µm area of *Salmonella enterica* on 3-MPBA sandwich chip at log concentrations (a) Ammonia bicarbonate negative control (b) 2.0×10^{0} CFU mL^{-1} (c) 2.0×10^{1} CFU mL^{-1} (d) 2.0×10^{2} CFU mL^{-1} (e) 2.0×10^{3} CFU mL^{-1} (f) 2.0×10^{4} CFU mL^{-1} (g) 2.0×10^{5} CFU mL^{-1} (h) 2.0×10^{6} CFU mL^{-1} (i) 2.0×10^{7} CFU mL^{-1}. (**B**) Chemical imaging of adjacent video images. Refer to labeling stated previously for concentration.

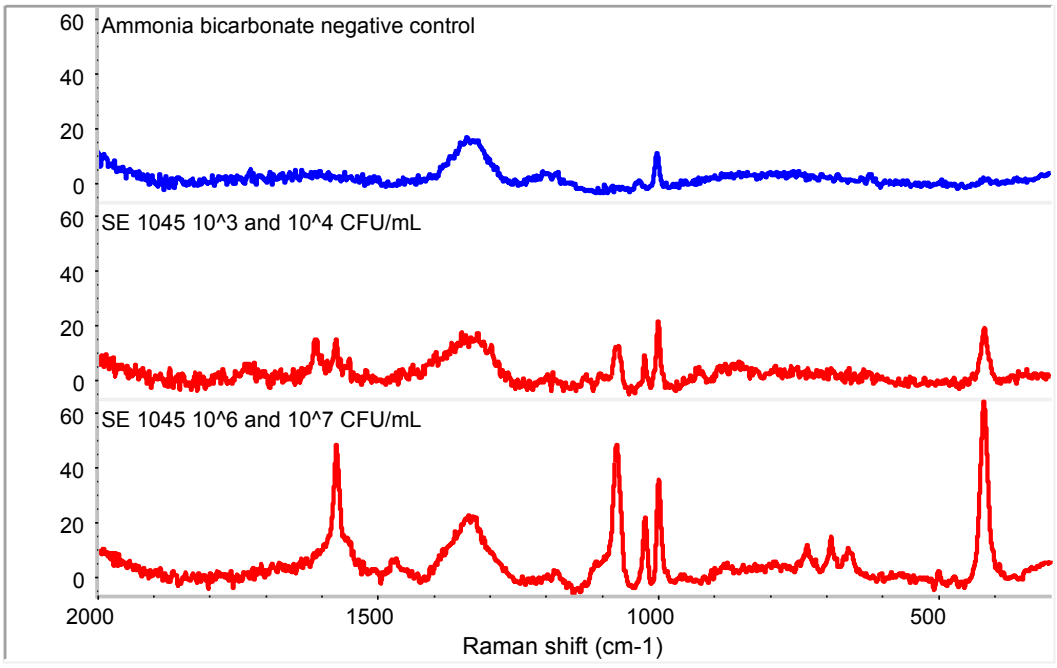

Fig. S11. Representative spectra of *Salmonella enterica* at various concentrations (refer to Fig. 4).

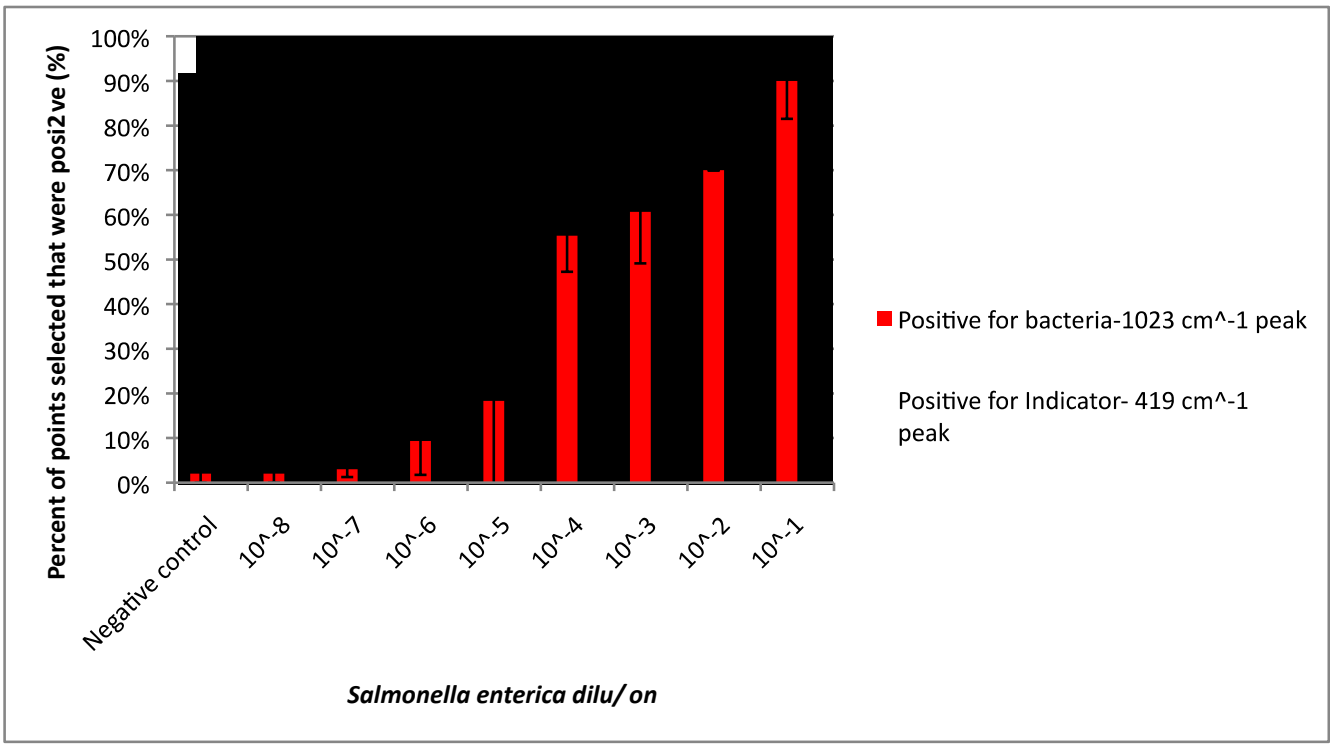

Fig. S12. Percent of postive signals for bacteria (1023 cm^{-1}) and indicator (419 cm^{-1}) at different bacteria concentrations.

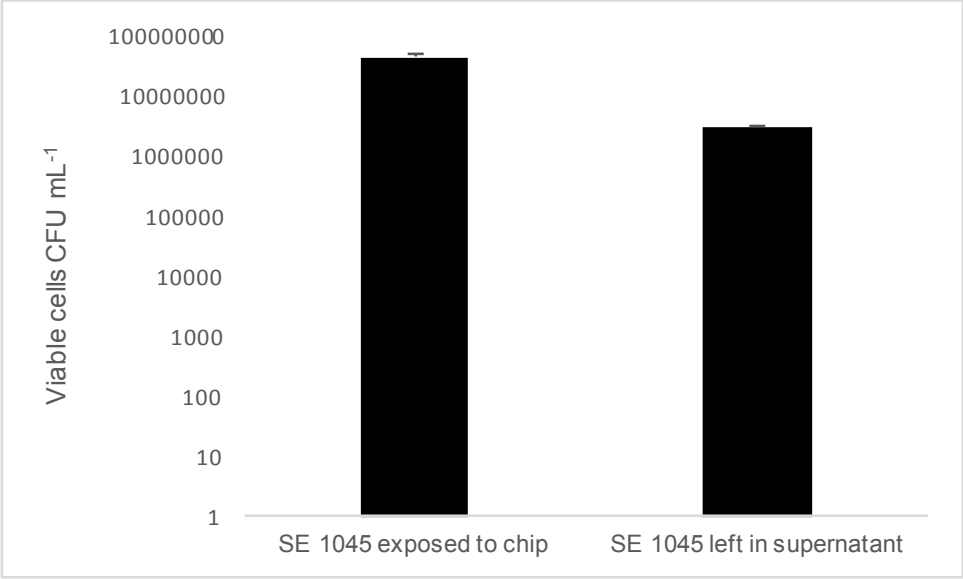

Fig. S13. Capture efficiency of the 3-MPBA coated gold chip exposed to *Salmonella enterica* 10^7 CFU mL^{-1} using the plate count method. The gold coated 3-MPBA chip captured 93.1% of bacteria cells.

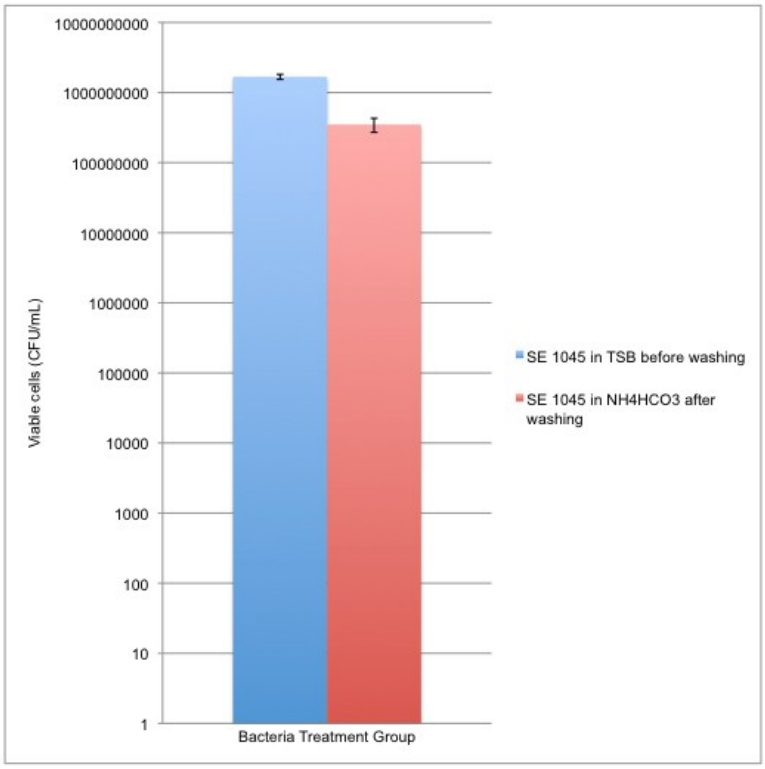

Fig. S14. Comparison of viable *Salmonella enterica* cells before and after washing.