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SUPPORTING INFORMATION

SERS characterization of aggregated and isolated bacteria

deposited on silver-based substrates

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Section 1. Preparation of silver-based substrates and their optimization for dye probes detection

Silver nanostructures (Ag NSs) were formed on glass surfaces by thermal evaporation of a thin silver film. Four different thicknesses of 4, 5, 7 and 9 nm were deposited. Their UV-vis transmission spectra are displayed in **Figure S1-1A**. They show a clear red shift of the absorption peak characteristic of the localized surface plasmon resonance (LSPR) band together with a width enlargement upon increasing the nominal thickness of the film. In order to generate a SERS effect, it is well-admitted that the plasmon bands of the SERS substrates have to overlap with the laser excitation wavelength (at 633 nm), which is the case only for the 5- and 7 nm-thick films with maxima at ~500 and 550 nm, respectively [1]. These two films were then further analysed by SEM imaging to investigate the size, shape and density of the silver nanostructures (**Figure S1-1B**). The 5 nm-thick Ag film is characterized by a high density of particles with a large size distribution, extending from 3 to 45 nm (determined from the statistical evaluation of the Ag NSs size, **Figure S1-2**). For the 7 nm-thick Ag film, the structure is heterogeneous with partially coalesced metal islands and large domains up to 100 nm, leading to extreme broadening of the LSPR band.

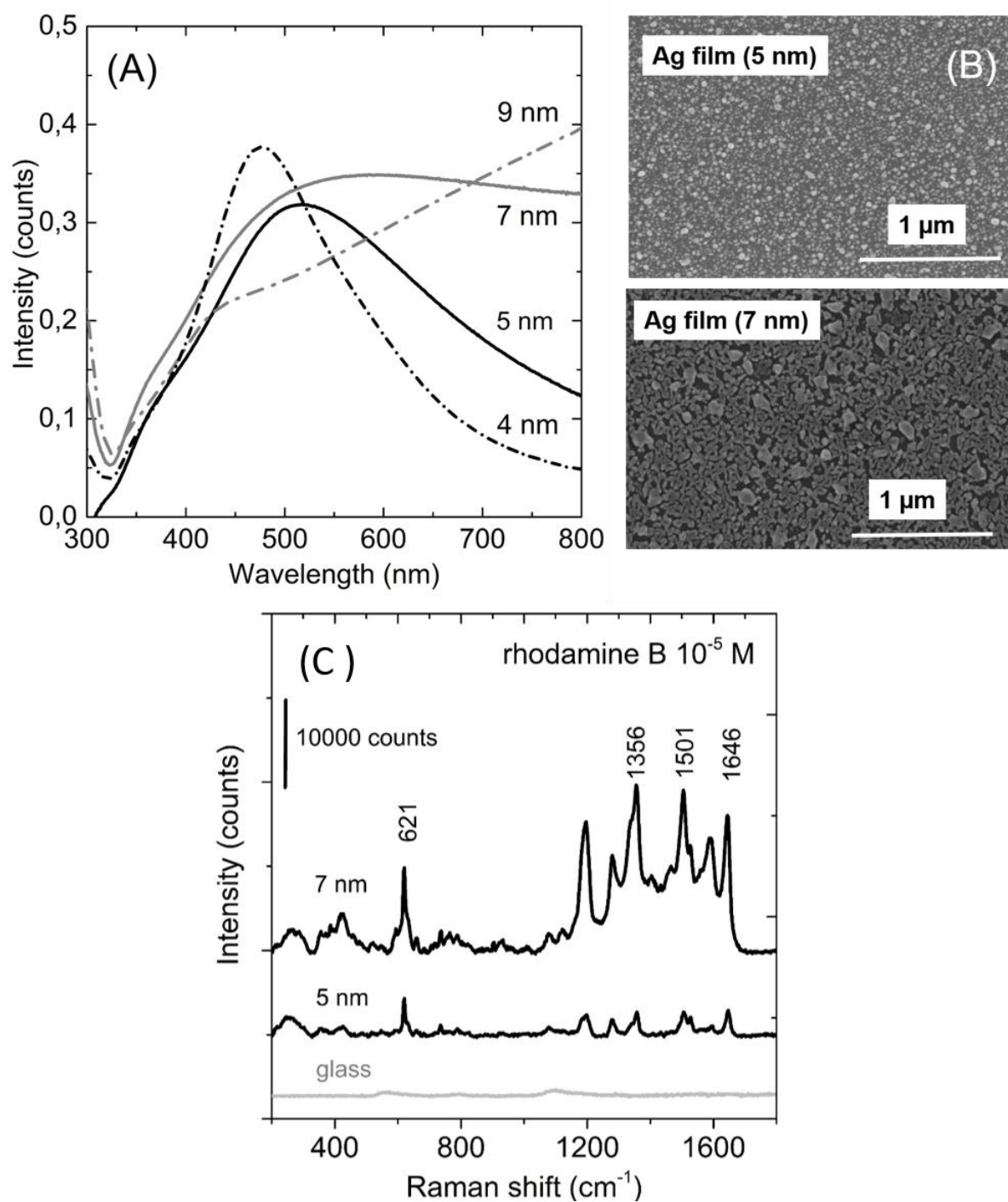


Figure S1-1. UV-vis transmission spectra of 4, 5, 7 and 9 nm-thick silver films recorded in air (A); SEM images of 5 nm- (top image) and 7 nm- (bottom image) thick silver films deposited on glass substrates by thermal evaporation (B); SERS spectra of rhodamine B (deposited from a 10 μM solution) on bare glass and glass slides coated with 5 nm- and 7 nm-thick Ag films (C).

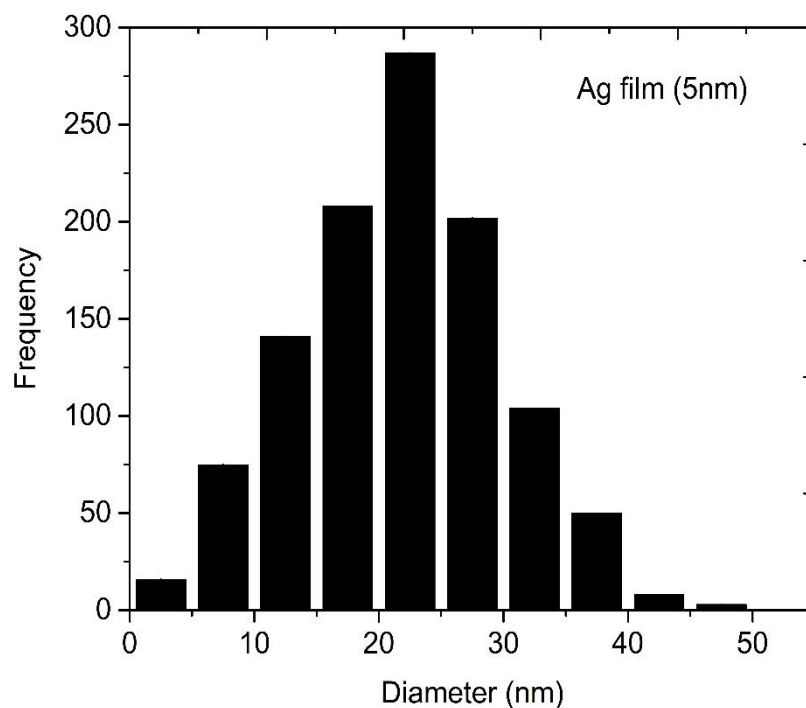


Figure S1-2. Particle size distribution of a 5 nm-thick Ag film, determined from the analysis of the image of Figure S1-1B.

These Ag films were investigated for their SERS activity using rhodamine B (10 μM in water) as a probe. A droplet of 3 μL was deposited on the substrates and once dried, Raman spectra were recorded. As shown in **Figure S1-1C**, SERS enhancement is visible on both substrates, but the signal intensity is much pronounced on the 7 nm for which the λ_{max} is closer to the laser excitation ($\lambda_{\text{ex}}=633$ nm). The Raman signature of rhodamine B exhibits characteristic bands related to the aromatic C-C stretching modes at 1646, 1501 and 1356 cm^{-1} and to C-C-C ring in-plane bending at 621 cm^{-1} [2],[3]. The intense vibration band located at 231 cm^{-1} is due to silver-oxide mode or ionic species adsorbed on the metal surface [4].

In order to test the homogeneity of the substrate response, SERS mapping was realized on different areas of the 7 nm-thick Ag film wet by a rhodamine B aqueous droplet. The signature of rhodamine B reveals to be highly reproducible since the SERS spectra extracted from the mapping are identical (**Figure S1-3A**). Subsequently, a second dye, crystal violet

(CV), was tested. It was deposited from a 10^{-5} M solution on the 7 nm-thick Ag film. Raman spectra were recorded with the same acquisition parameters as those used for rhodamine B. In such a case, the SERS response was fully saturated. By using a laser power 25 times lower (0.1 mW), the SERS spectra, shown in **Figure S1-3B**, have similar intensities as for rhodamine B. This higher SERS exaltation may be explained by a resonant Raman effect of CV, which exhibits an absorption band at 590 nm closer to the laser excitation wavelength, as compared to 553 nm for rhodamine B. The SERS peaks are associated to the main vibrations of CV, notably the two C-C stretching modes of the phenyl ring at 1622 and 1590 cm^{-1} , the C-H bending modes at 1178 and 806 cm^{-1} , the radical ring skeletal vibration at 914 cm^{-1} and the C-N bending vibration at 423 cm^{-1} [5].

The main peaks observed in the SERS spectra of the two dyes (highlighted in grey in **Figure S1-3 A and B**) were used to evaluate the reproducibility of the SERS intensity by calculating the Relative Standard Deviation (RSD) values. As it can be observed in **Figure S1-3C**, in the case of rhodamine B, the values obtained for RSD range from 6.3% for the peak at 620 cm^{-1} to 11.3% for the peak at 1356 cm^{-1} . Concerning crystal violet, the lowest value was 6.1% obtained for the peak at 1368 cm^{-1} and 9.1% for the peak at 442 cm^{-1} (**Figure S1-3C**). Such values demonstrate the homogeneity as well as good reproducibility in the SERS detection of molecules simply deposited on top of the 7nm-thick Ag substrate.

In addition to its SERS performance, the 7 nm-thick Ag substrate displays a remarkable stability over time since the plasmon band does not significantly change in time (after 4 months storage under vacuum and after one year in air), as shown in **Figure S1-4**; it was therefore chosen for the study of bacteria.

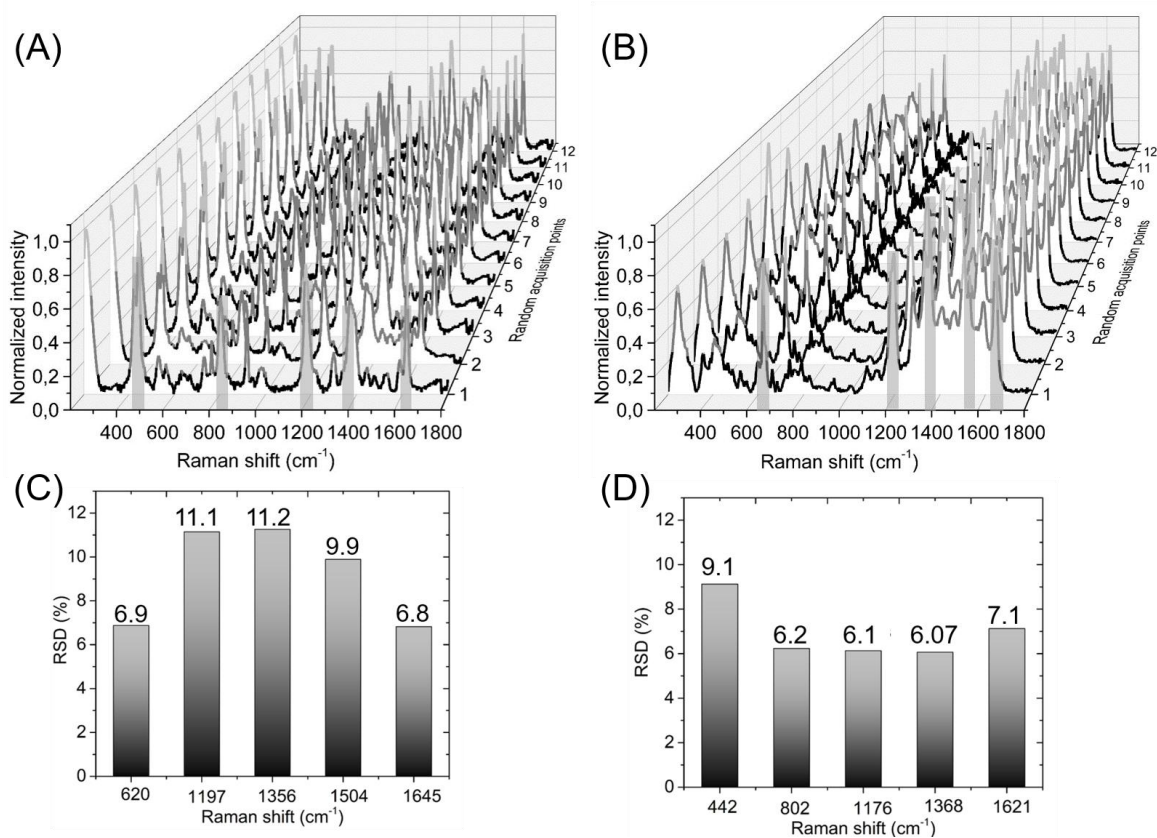


Figure S1-3. SERS spectra at different acquisition points corresponding to (A) rhodamine B and (B) crystal violet deposited on 7 nm-thick Ag film from a 10^{-5} M solution (633 nm laser excitation, 10 \times objective, 30 s acquisition time, 1 accumulation, 2.5 mW laser power in case of rhodamine B and 0.1 mW power for crystal violet) and RSD (relative standard deviation) of the major bands highlighted in grey for rhodamine B (C) and crystal violet (D) for 12 acquisition points.

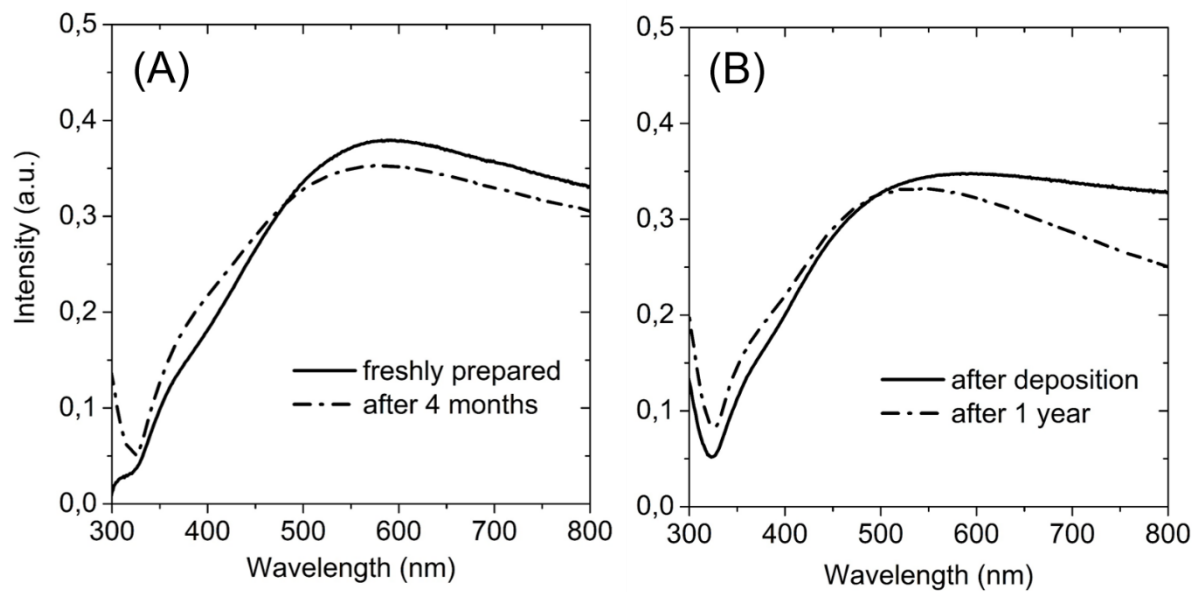


Figure S1-4. UV-vis absorption spectra of (A) as-deposited 7 nm-thick Ag film and after 4 months of storage under vacuum, (B) another 7 nm-thick Ag film after 1 year in air.

Section 2. Characterization of bacteria using 7 nm-thick silver films

NEW FIGURE

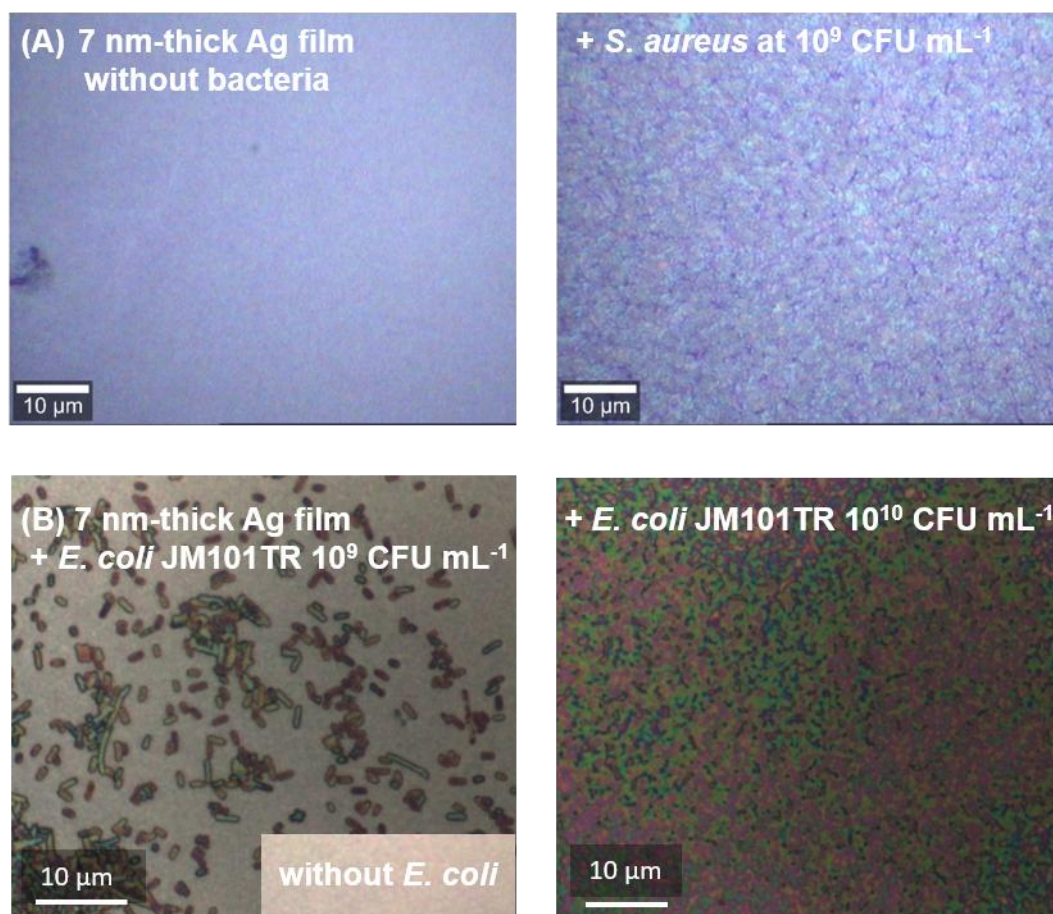


Figure S2-1. Optical images of 7 nm-thick Ag film without and with *S. aureus* deposited from a 10^9 CFU mL⁻¹ solution (A) and with *E. coli* JM101TR deposited from 10^9 and 10^{10} CFU mL⁻¹ solutions (B).

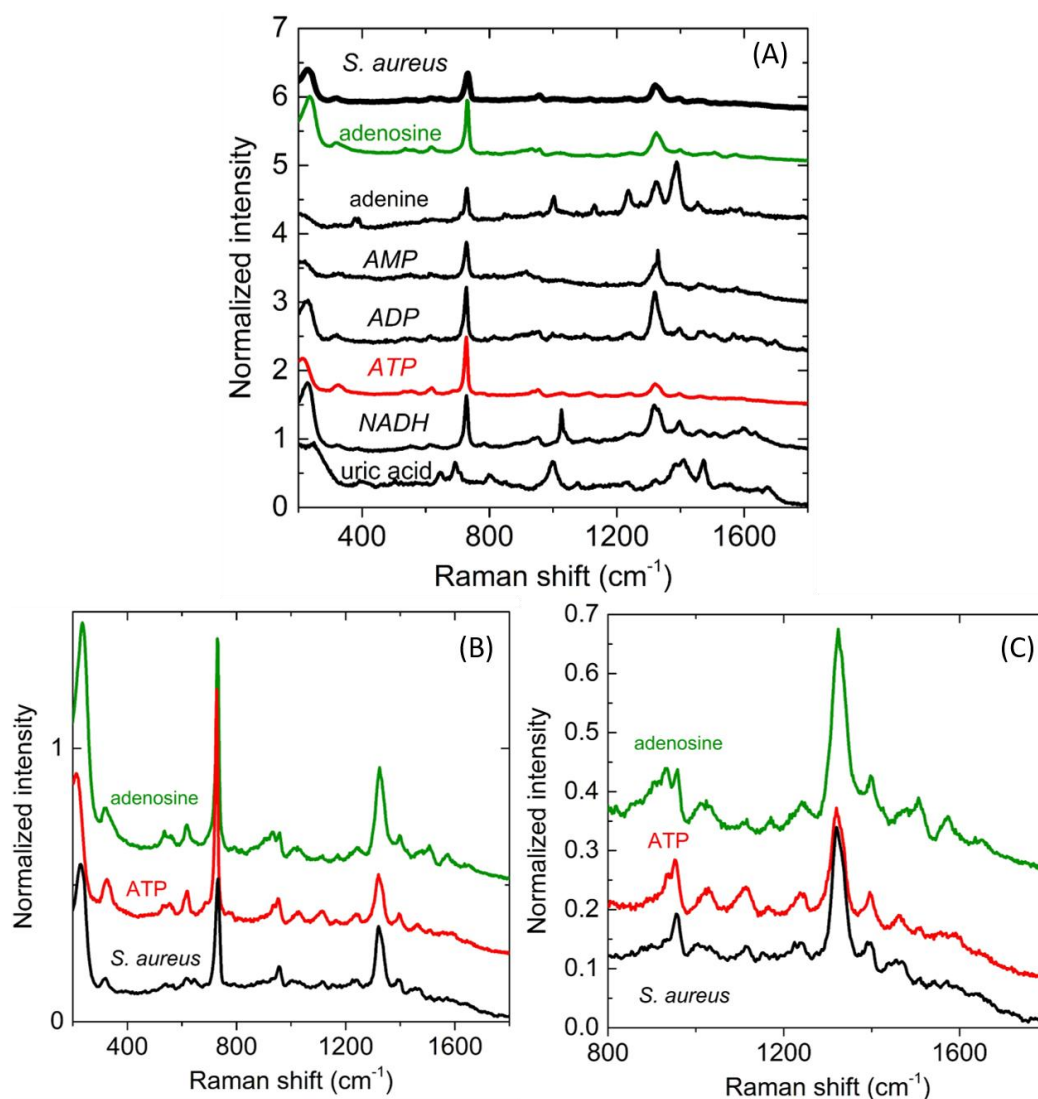
NEW FIGURE

Figure-S2-2. SERS spectra of *S. aureus* (10^9 CFU mL⁻¹) using 633 nm laser excitation at 2.5 mW, $\times 100$ objective, 10 s acquisition time, 1 accumulation ($I \times 5$), compared with SERS spectra of some biomolecules (adenosine, adenine) and some possible metabolites (AMP, ADP, ATP (red spectrum), NADH, uric acid) deposited from solutions at 18.5 mM ($\lambda_{\text{ex}}=633$ nm at 0.1 mW, $100\times$ objective, 10 s acquisition time, 1 accumulation), all deposited on top of 7 nm-thick Ag films (A); Zoom of SERS spectra of *S. aureus*, ATP (in red) and adenosine (in green) for a better comparison (B) and in the 800-1800 cm⁻¹ spectral range (C).

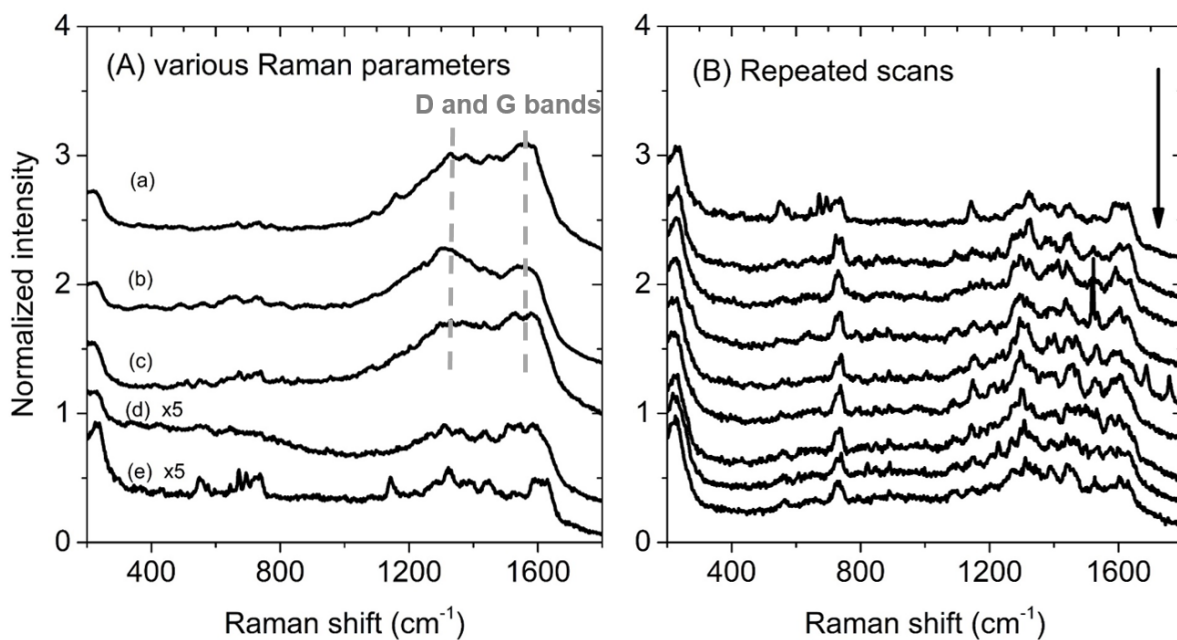


Figure S2-3. SERS spectra of *E. coli* Katushka (deposited from a 10^{10} CFU mL⁻¹ solution) recorded using 633 nm laser excitation and 100× objective (A) at different acquisition parameters: (a) 1 mW power, 10 s acquisition time, 1 accumulation; (b) and (c) 0.5 mW power, 10 s acquisition time, 1 accumulation; (d) 0.5 mW power, 1 s acquisition time, 5 accumulations; (e) 0.1 mW power, 10 s acquisition time, 1 accumulation. In the last spectrum (e), the contribution of the D and G bands of amorphous carbon are negligible; SERS spectra of *E. coli* Katushka (deposited from a 10^{10} CFU mL⁻¹ solution) recorded using reduced laser power (0.1 mW), 100× objective, 10 s acquisition time, 1 accumulation, repeated scan on the same location.

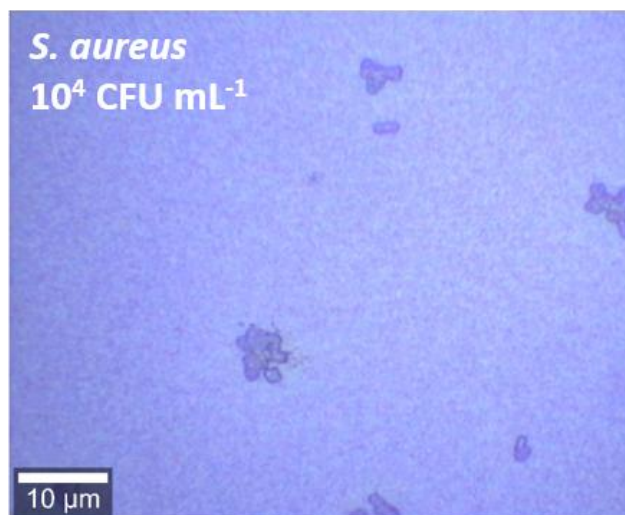


Figure S2-4. Optical image of *S. aureus* deposited from a 10^4 CFU mL⁻¹ suspension on 7 nm-thick Ag film.

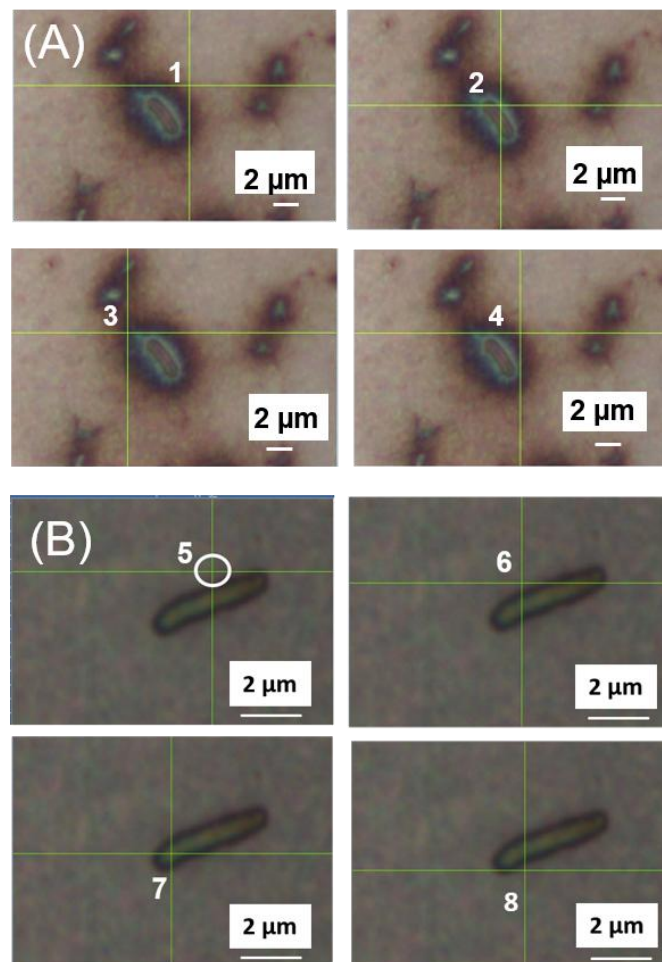
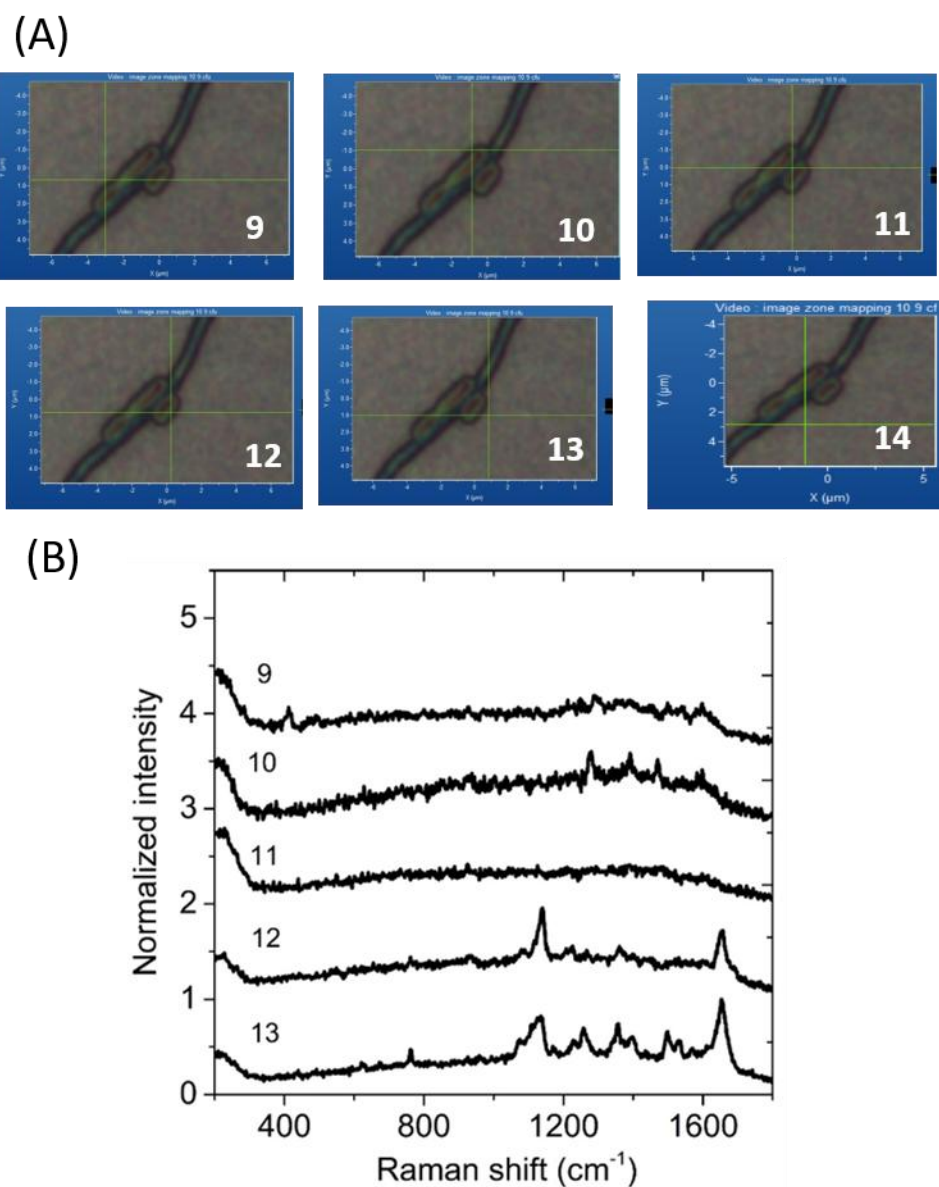


Figure S2-5. Locations corresponding to the spectra displayed in Figure 6B, extracted from SERS mapping around *E. coli* JM101TR single bacterium, droplet deposited from a 10^4 CFU mL^{-1} suspension, recorded at 633 nm with 0.1 mW, 100 \times objective, 10 s acquisition time, 1 accumulation: mapping 1 (A) and mapping 2 (B). The number displayed on each image refers to that indicated on each trace of spectra displayed in Figure 8B and the white circle in image 5 displays the approximate size of the spot when using 633 nm laser excitation and 100 \times objective.

Figure S2-6. Locations (A) corresponding to the spectra displayed in (B), extracted from SERS mapping (mapping 3) around *E. coli* JM101TR group of bacteria, droplet deposited from a 10^9 CFU mL⁻¹ suspension) recorded at 633 nm with 0.1 mW, 100× objective, 10 s acquisition time, 1 accumulation. The number displayed on each image refers to that indicated on each trace of spectra.

NEW FIGURE



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