Electronic Supplementary Information

Rapid single-cell detection and identification of pathogens by using surface-enhanced Raman spectroscopy

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HRTEM/STEM/EDS. A drop of suspension of the sample was deposited and dried on a copper grid coated by a thin carbon film prior to the electron microscopy analysis. Both analysis (TEM and SEM/EDS) was carried out using a Hitachi HD-2700 scanning transmission electron microscope (STEM), equipped with a cold field emission gun, working at an acceleration voltage of 80 kV and designed for high-resolution (HRTEM) imaging with a resolution of 0.144 nm. EDS spectra and chemical maps for the elements were acquired using a Dual EDX System (X-Max N100TLE Silicon Drift Detector (SDD)) from Oxford Instruments.

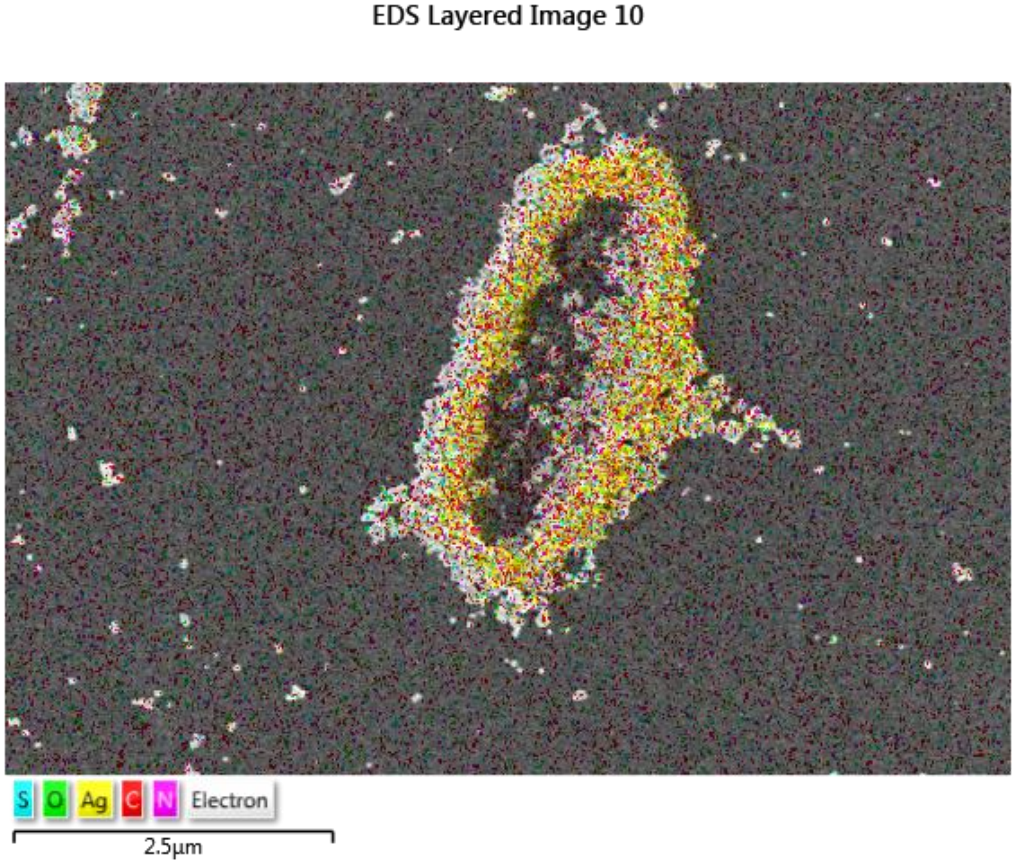

Figure S1. *SEM/EDS image showing the most relevant elements present in and around a Gram-negative bacterium (M. morganii) a few minutes after generating AgNPs by in situ synthesis.*

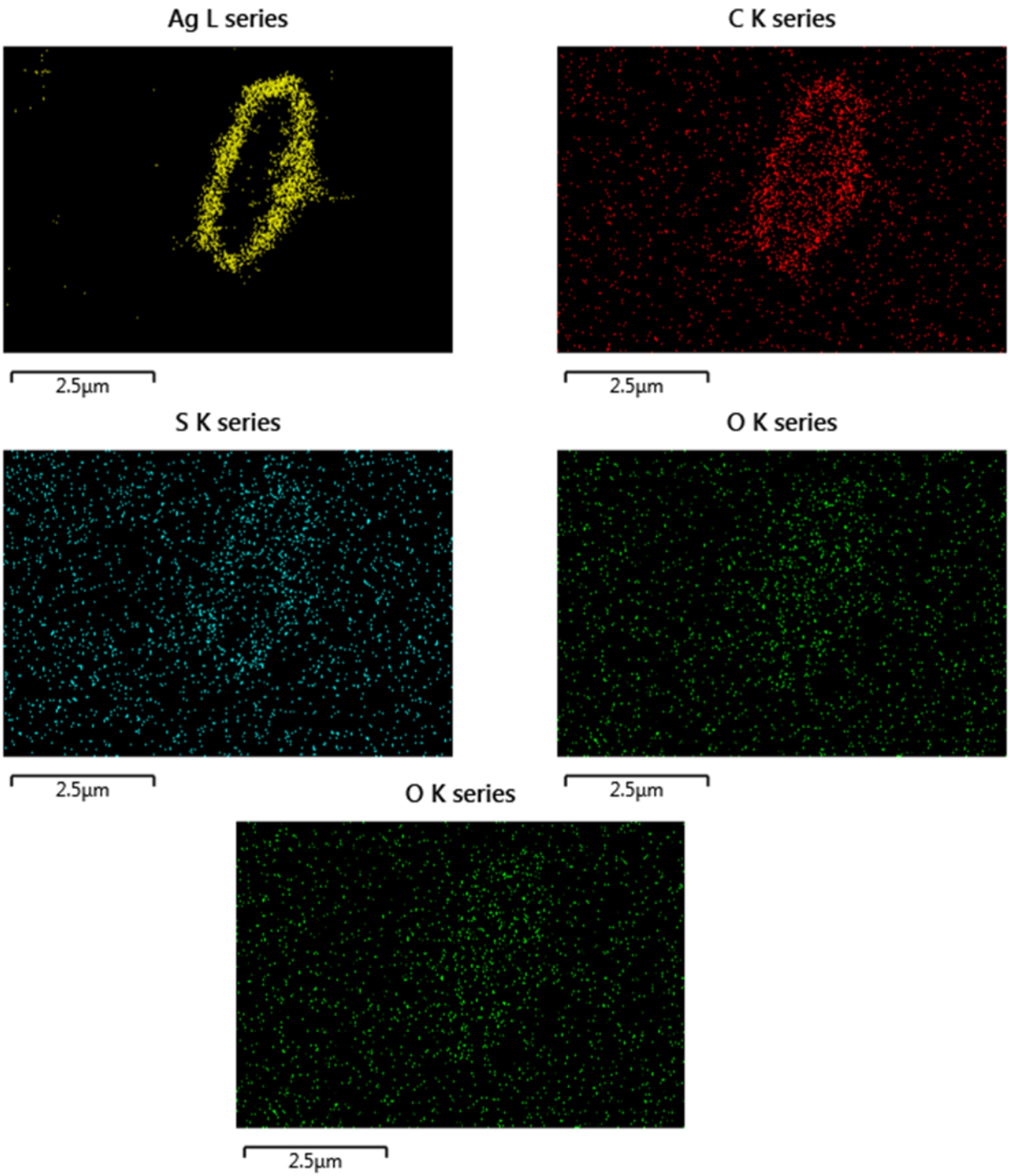

Figure S2. *SEM/EDS image as shown in Fig. S5, displaying each element separately, present in and around a Gram-negative bacterium (M. morganii) a few minutes after generating AgNPs by in situ synthesis. It can be appreciated that Ag is only covering the bacterial cell wall and not entering into the cell.*

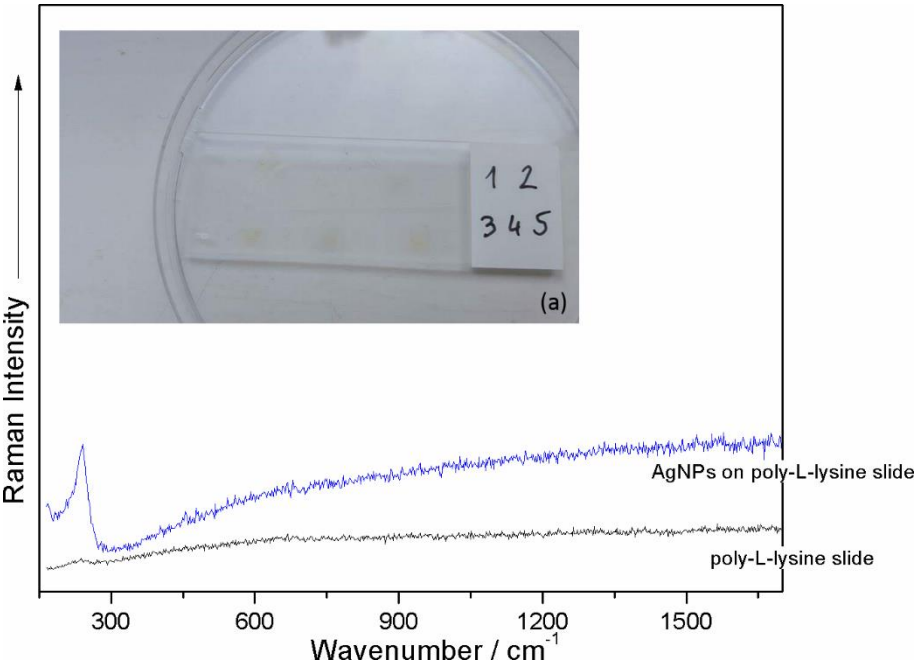

Figure S3. *Raman signal of the polyslides and the slide covered with in situ synthesized AgNPs when irradiated with 532 nm laser.*

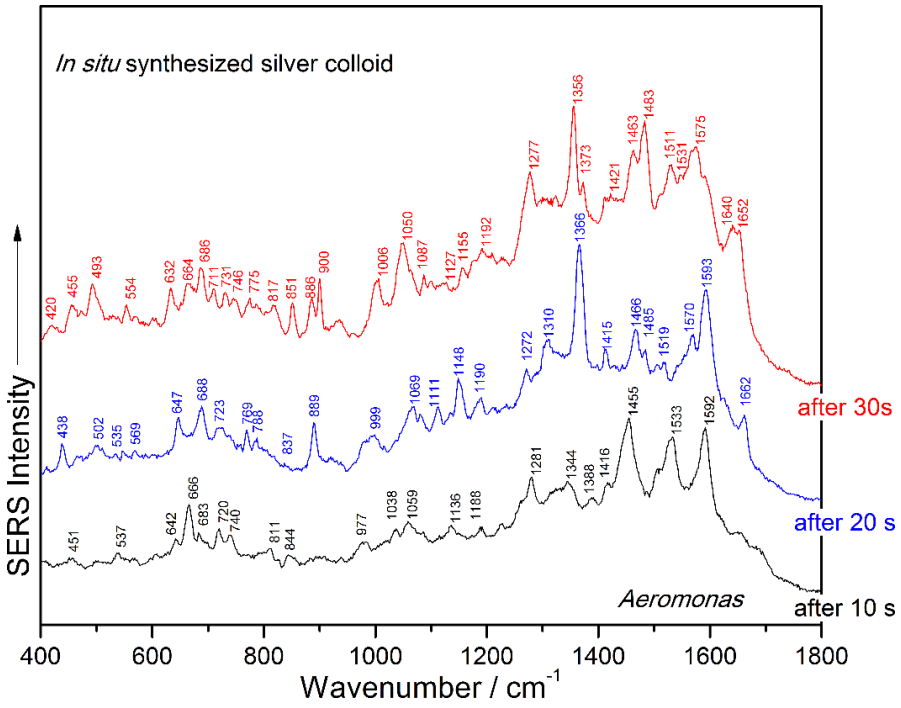

Figure S4. *SERS spectra of Aeromonas at single cell level showing the effects of long exposure times when irradiated with 633 nm laser.*

By dividing the SERS profiles of the microorganisms investigated in two sections, below and above 1100 cm^{-1}, we identified several SERS marker bands that contribute to the SERS-based identification at strain level, which are highlighted in **Figure S3**, (see below for the 633 nm laser line). Therefore, independent of the laser line used for SERS detection, reliable results are obtained, ensuring the pathogens identification.

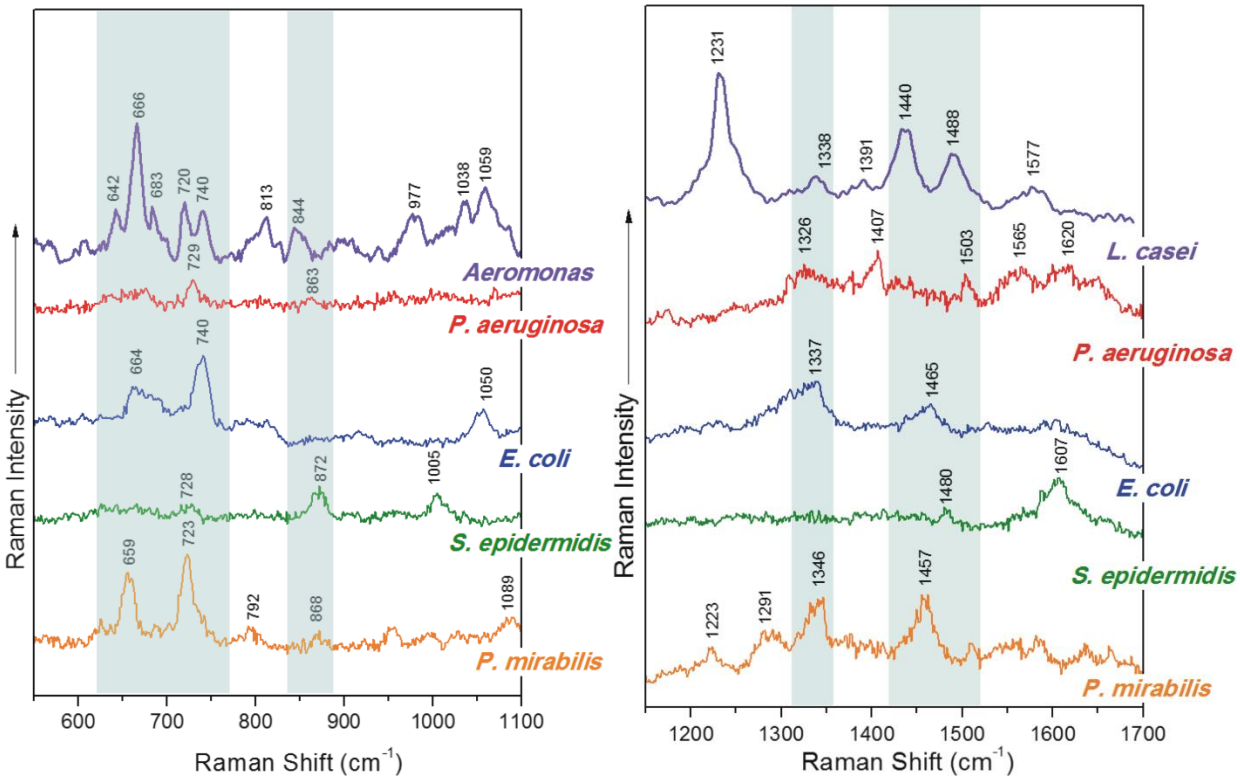

Figure S5. *SERS spectra of different microorganisms at single cell level irradiated with 633 nm laser line (right – wavenumbers below 1100 cm^{-1}; left – wavenumbers above 1100 cm^{-1} of the SERS spectra of five pathogens are displayed).*

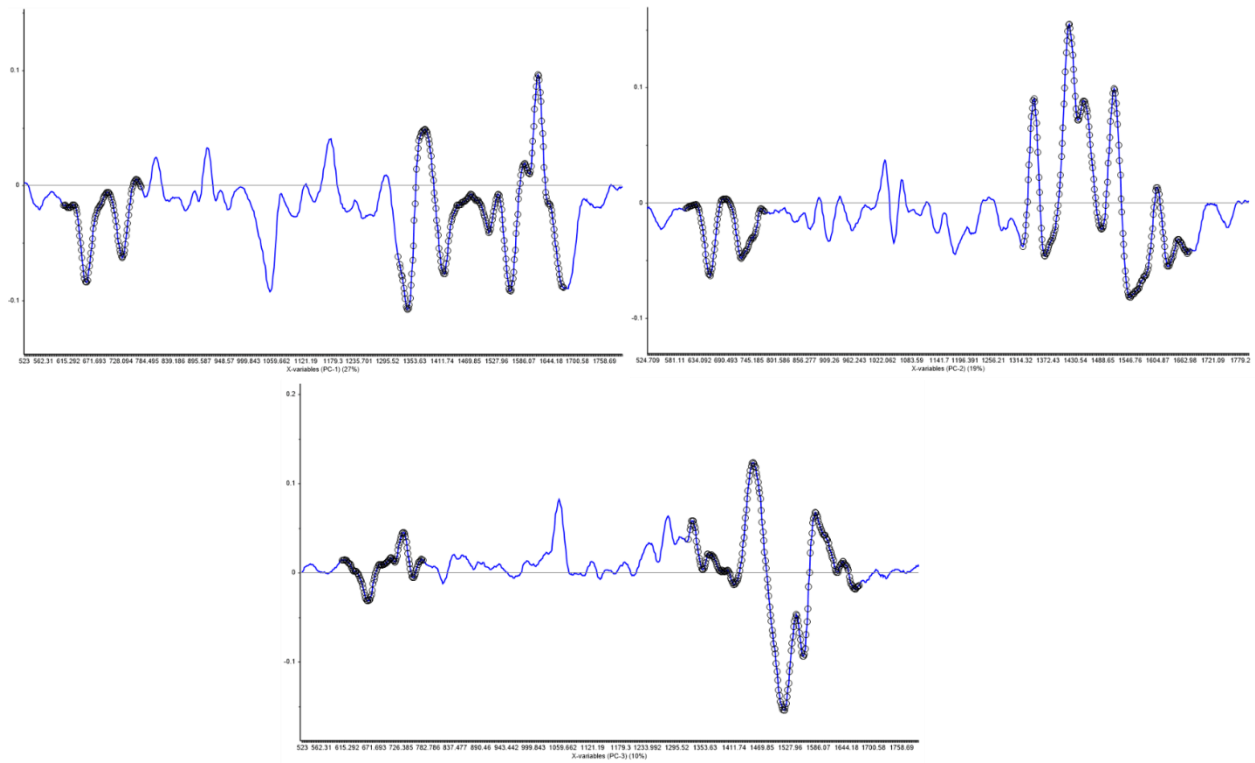

Figure S6. *PCA loadings for the first three principal components, considered also in the PCA analysis with 3D scores plot shown in Figure 5. Highlighted are the variables which are corresponding to the main SERS marker bands.*

Table S1. *Tentative assignments for the SERS marker bands of microorganisms*

Wavenumbers (cm^{-1})	*Assignments*	*References*
642	*δ(COO−) guanine*	*1, 2*
659-666	*δ(COO−) guanine*	*1, 2*
683	*δ(COO−) guanine*	*1, 2*
720-740	*ν(Adenine, glycosidic ring)*	*3-5 6*
792	*ν(CN) Tyr*	*2, 3*
813	*ν(CN) Tyr*	*2, 3*
844	*ν(C-C) in glycosidic link*	*7*
863-872	*ν(C–C) skeletal proteins*	*1, 8*
923-929	*"Breathing" in aromatic rings*	*9*
977	*"Breathing" in aromatic rings*	*9*
1005	*"Breathing" in aromatic rings*	*9*
1038	*In-plane ν(CH)*	*10*
1050-1059	*ν(C–C)*	*1*
1089	*ν(C–C), ν(C–O) in carbohydrates*	*10*
1151-1166	*(=C–C=) lipids*	*4, 10*
1223-1231	*Amide III*	*1*
1291	*δ(CH) proteins*	*1*
1324-1328	*adenine*	
1337-1346	*δ(CH) and ν_s(COO−) proteins*	*4, 12, 13*
1391-1407	*ν(C–O), symmetric (COO^-)Phe*	*7*
1440	*δ(CH_2) saturated lipids*	*3*
1453-1467	*δ(CH_2) saturated lipids*	*3*
1480-1488	*δ(CH_2) saturated lipids*	*3*
1503	*Phe*	*7*
1565	*δ(NH, CH), ν(CC)*	*1*
1577	*ν(ADN)*	*3*
1607	*ν(ADN)*	*3*
1620	*ν(ADN)*	*3*

δ – deformation, ν_s – symmetric stretching, Phe - phenylalanine, Tyr – tyrosine,

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