NANO LETTERS

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Letter

Bacterial Wastewater-Based Epidemiology Using Surface-Enhanced Raman Spectroscopy and Machine Learning

Liam K. Herndon,* Yirui Zhang, Fareeha Safir, Babatunde Ogunlade, Halleh B. Balch, Alexandria B. Boehm, and Jennifer A. Dionne*

Cite This: Nano Lett. 2025, 25, 1250–1259

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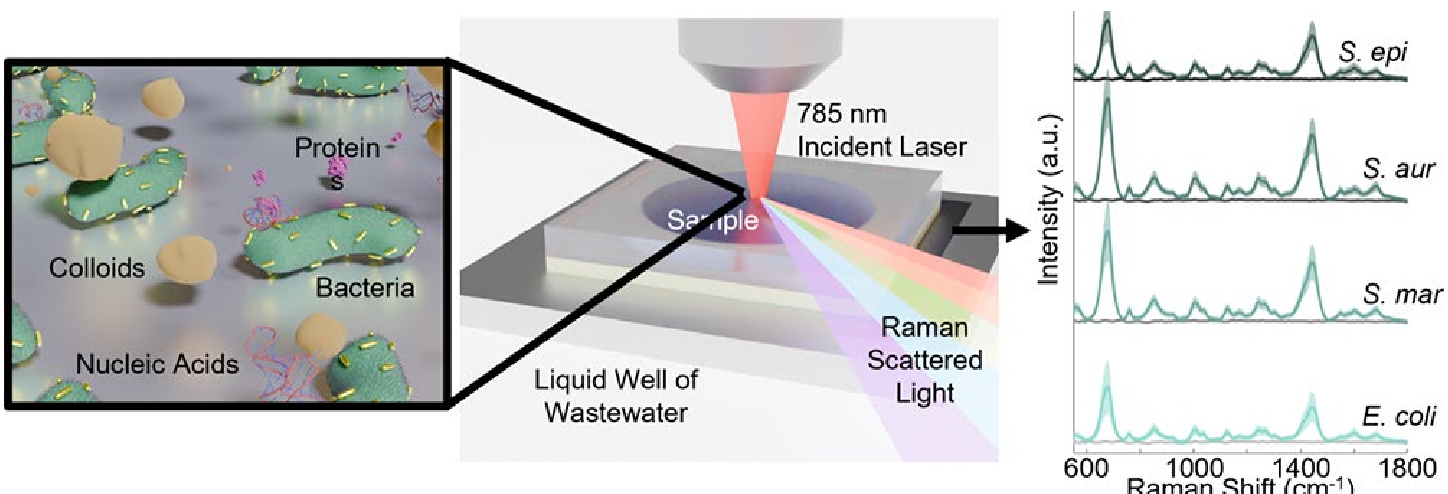

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12/04/2025

Sujan Manna

The dirty picture

ARTS, HUMANITIES, & SOCIAL SCIENCES | JULY 8, 2024

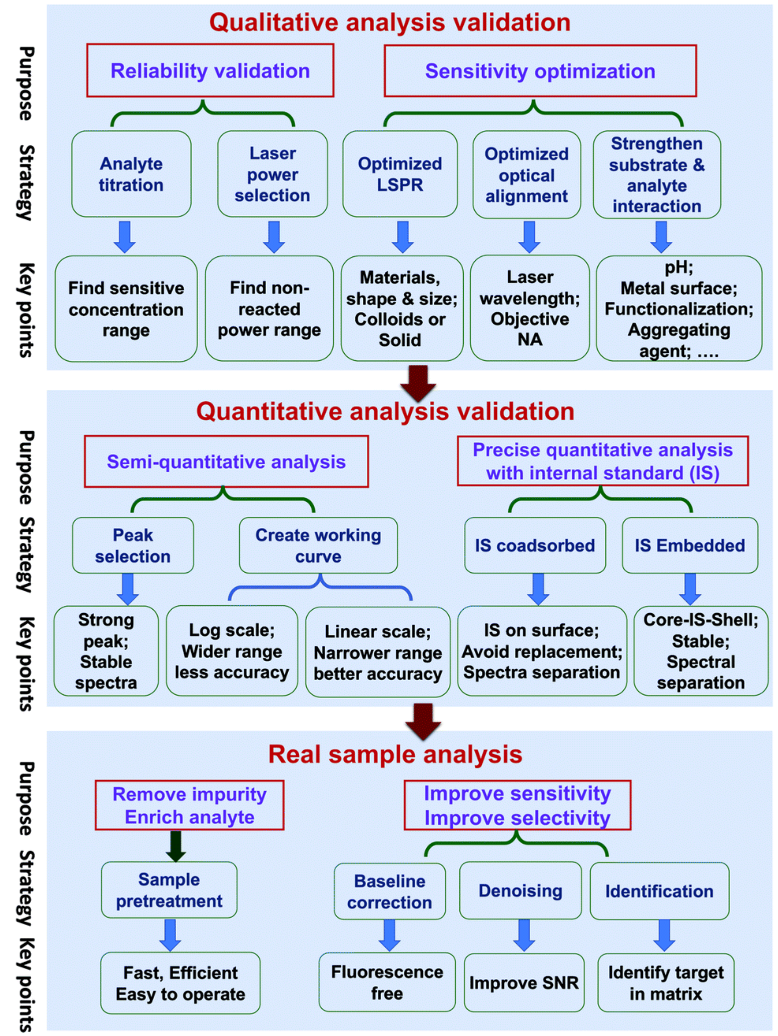
The anthropology of plastics in India

Doctoral candidate Adwaita Banerjee uses ethnographic research to document the ecological transition of the Deonar dumping ground, where thousands of Dalits and Muslim migrants mine the area for plastic that can be resold and recycled.

Dotting the fields of discarded plastic are about 2,000 people, mostly Dalits and Muslim migrants, who mine the area for plastic that can be resold and recycled. Waste pickers measure their longevity and status by the access they have to more lucrative forms of plastic and by how far they live from the dumping ground itself. Here, life expectancy is around 40 years of age.

Spectra-culous

- Reproducibility and uniformity
- Substrate fabrication
- Sensitivity and detection limits
- Interference and background signals
- Complex sample matrices
- Stability and durability of SERS substrates
- Standardization
- Theoretical understanding
- Applications in Biological and Medical fields



A. I. Pérez-Jiménez *et al*., *Chem. Sci*. **2020**, *11*, 4563-4577

Background work

Rapid identification of pathogenic bacteria using Raman spectroscopy and deep learning

Chi-Sing Ho, Neal Jean, Catherine A. Hogan, Lena Blackmon, Stefanie S. Jeffrey, Mark Holodniy, Niaz Banaei, Amr A. E. Saleh, Stefano Ermon & Jennifer Dionne

Nature Communications **10**, Article number: 4927 (2019) | Cite this article

Nano Letters > Vol 20/Issue 10 > Article

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Open Access Editors' Choice

LETTER | September 11, 2020

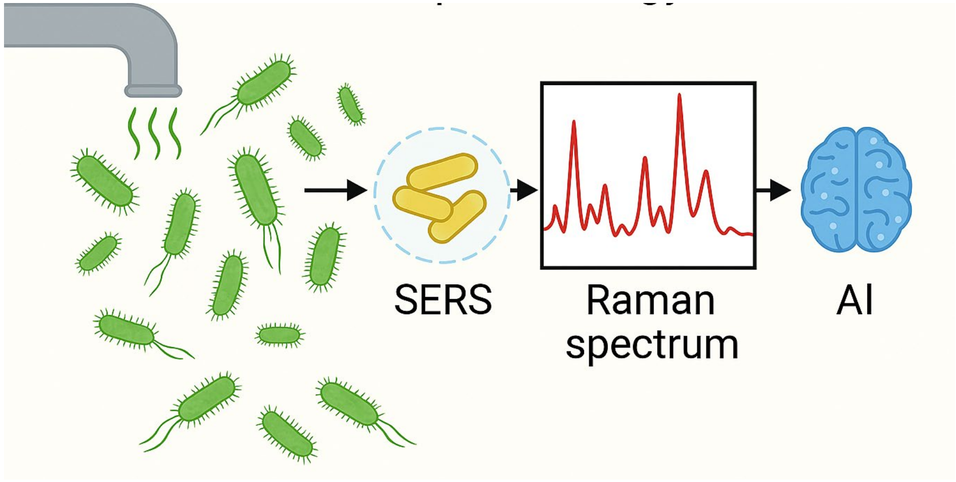
Plasmonic and Electrostatic Interactions Enable Uniformly Enhanced Liquid Bacterial Surface-Enhanced Raman Scattering (SERS)

Loza F. Tadesse*, Chi-Sing Ho, Dong-Hua Chen, Hamed Arami, Niaz Banaei, Sanjiv S. Gambhir, Stefanie S. Jeffrey, Amr A. E. Saleh*, and Jennifer Dionne*

Motivation

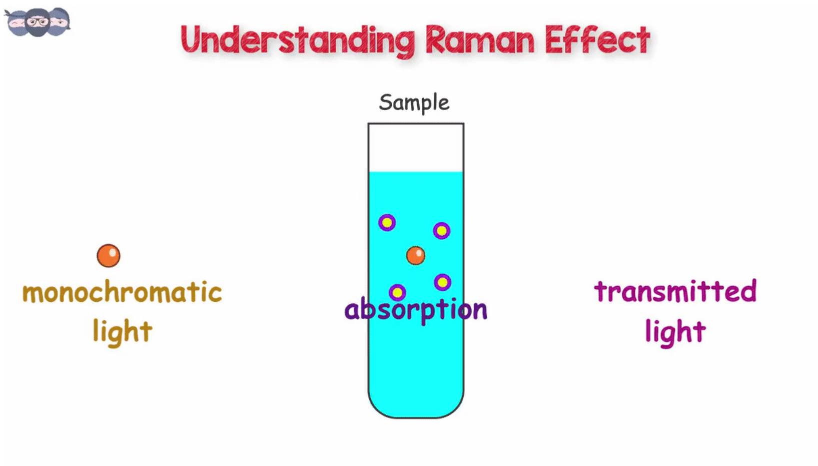
From 'say ah' to 'say SERS'

- Addresses Public Health Priority
- Fills a Research Gap
- Label-Free Detection Strategy
- Integration of Nanochemistry and AI
- Real-World Applicability
- Mechanistic Innovation
- Multidisciplinary Impact

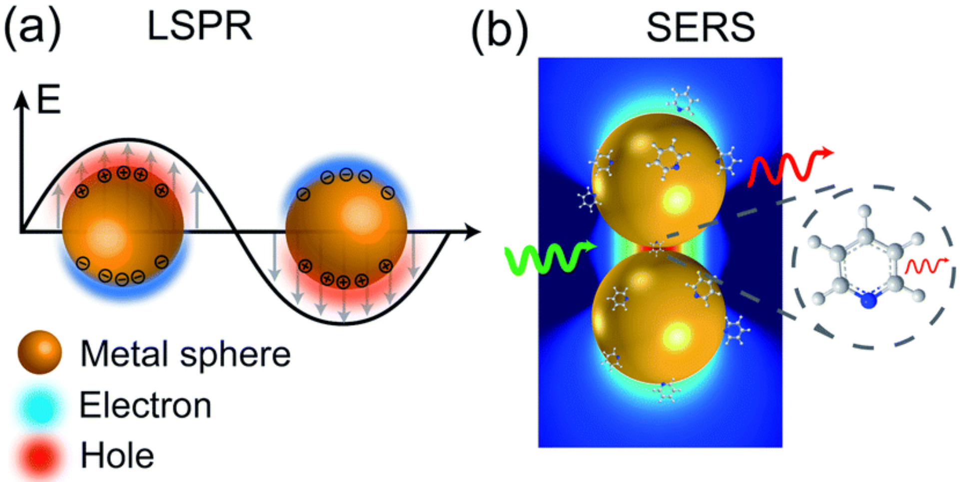


Jargon box

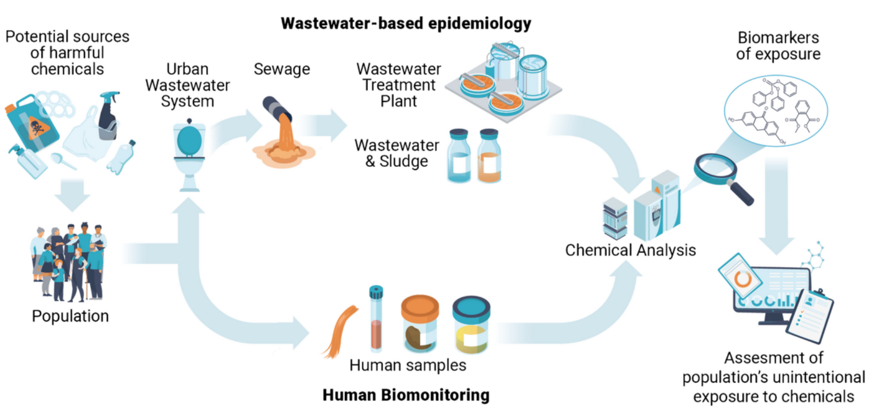
i) Raman spectroscopy



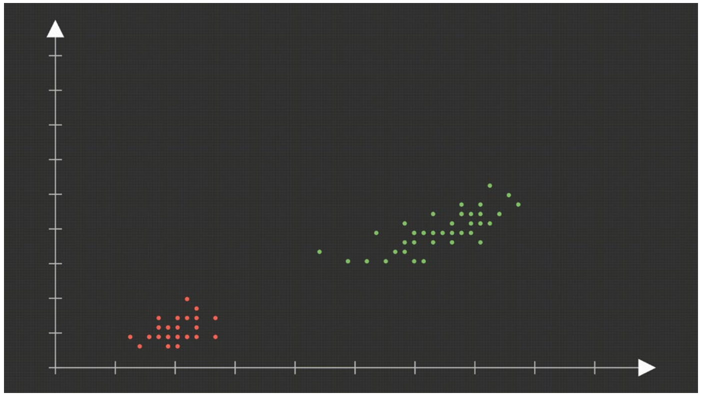
ii) Surface enhanced Raman spectroscopy (SERS)



iii) Wastewater-based epidemiology



iv) Support Vector Machine



Results and discussion

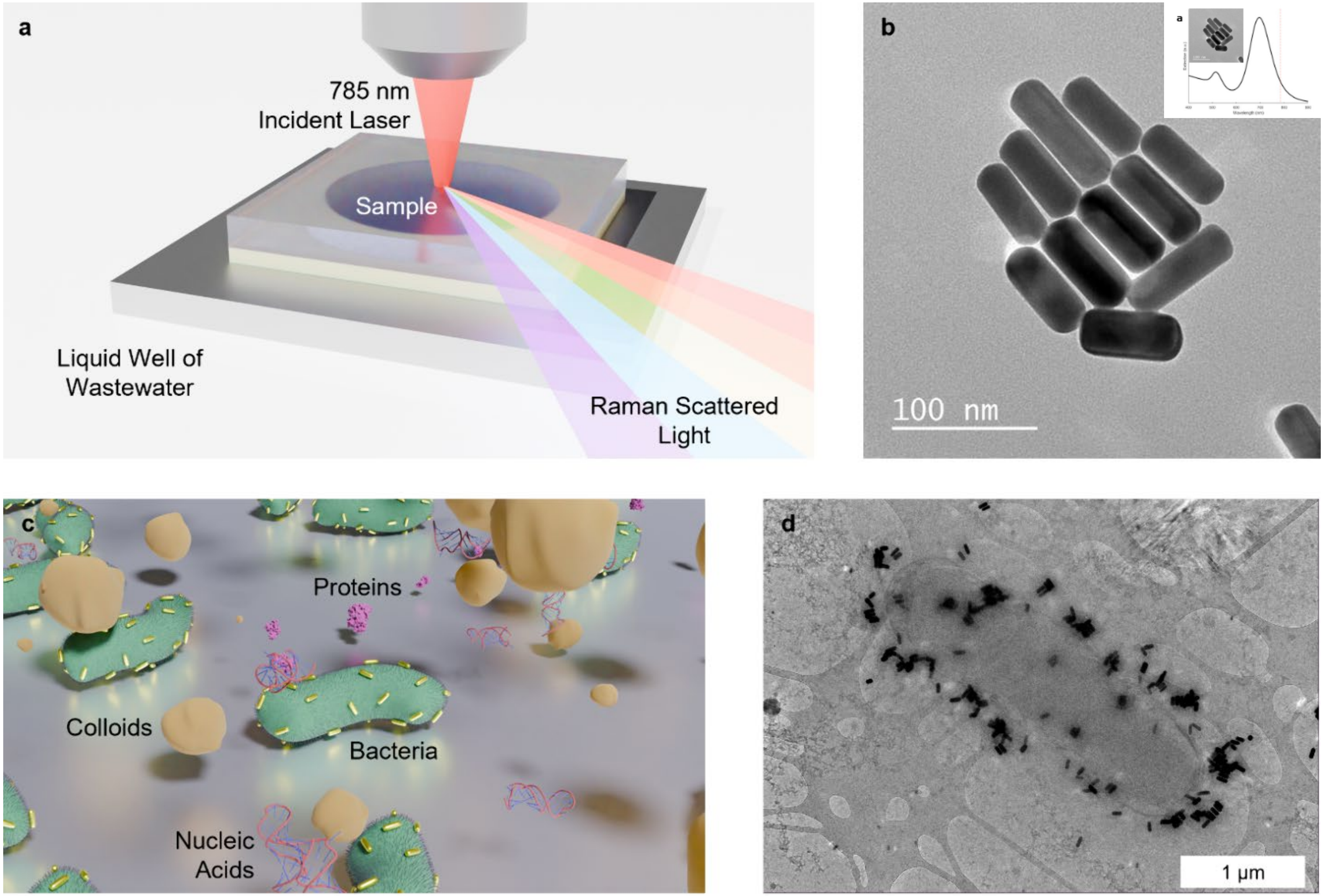
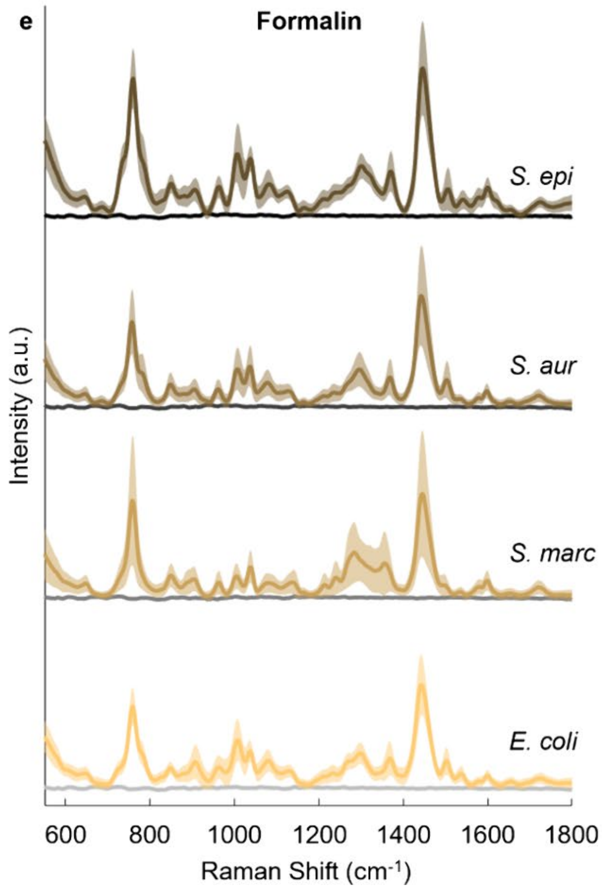


Figure 1. (a) Raman spectra are collected from liquid samples in microfluidic wells. (b) Electron micrographs of AuNRs used for SERS. (c) Schematic illustrating that in the liquid wells AuNRs selectively enhance scattered signals from bacteria over a complex mixture of background components, such as colloids, proteins, and nucleic acids. (d) Cryoelectron micrograph of electrostatic binding of AuNRs to the E. coli surface.

Results and discussion



760 cm^{-1}: adenine ring breathing
851 cm^{-1}: thymine
1125 cm^{-1}: phosphate groups
1599 cm^{-1}: carboxyl group vibrations

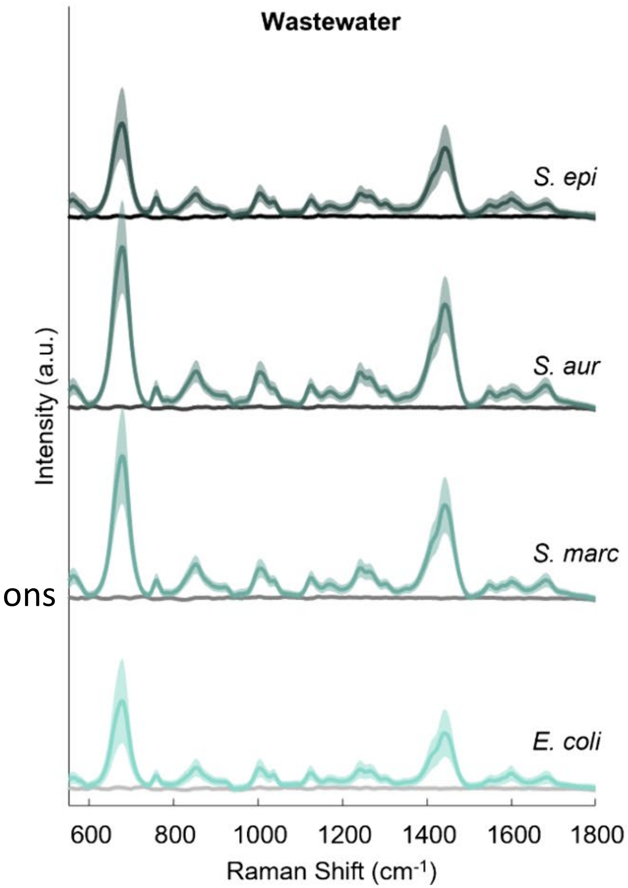
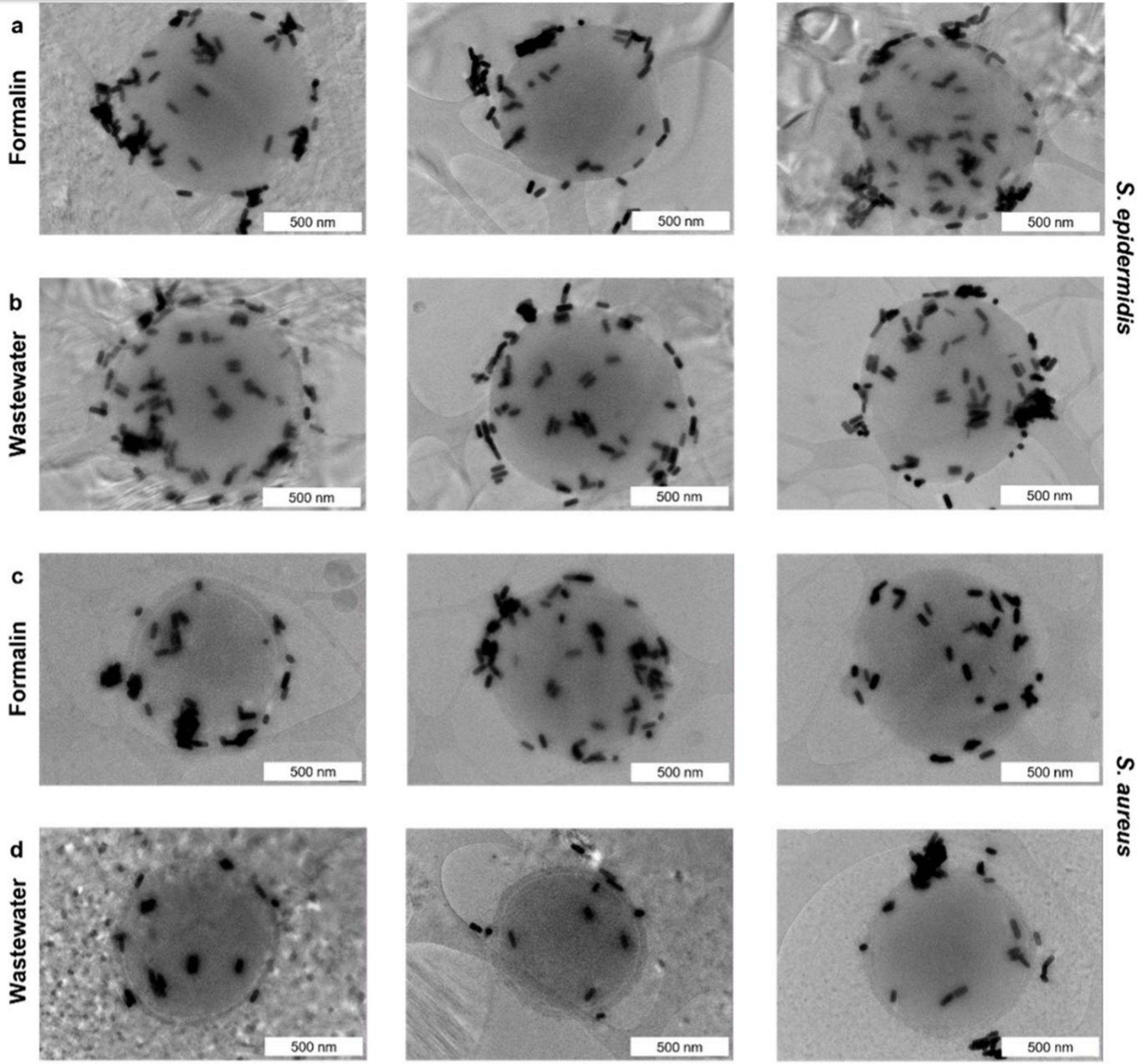
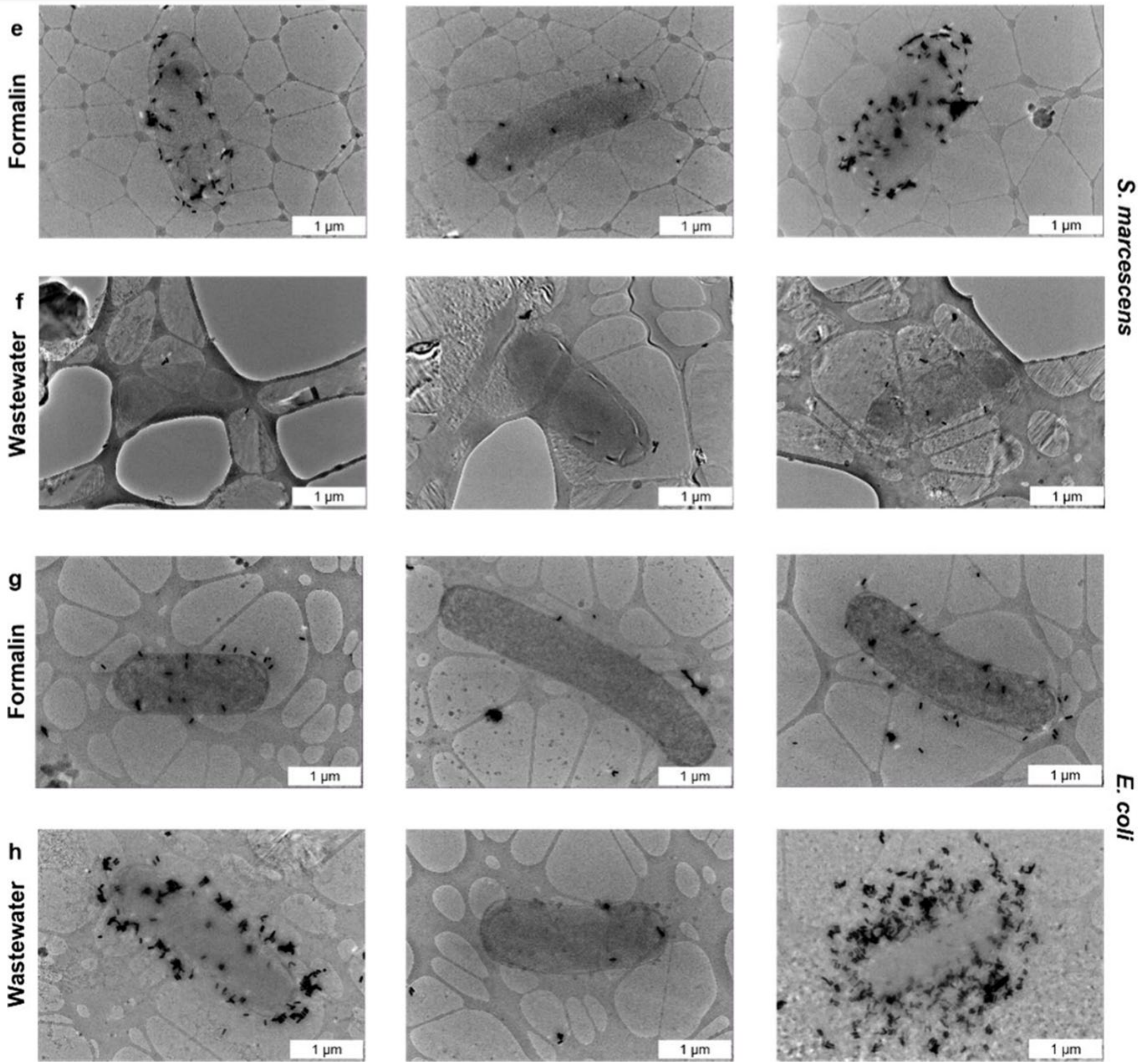


Figure 1. (e) SERS spectra of four model species collected in formalin (left) and wastewater (right). Gray lines indicate negative control without AuNRs. SERS spectra are averaged over a minimum of 216 samples and unenhanced spectra are averaged over a minimum of 36 samples. Shaded regions indicate 1 standard deviation error.

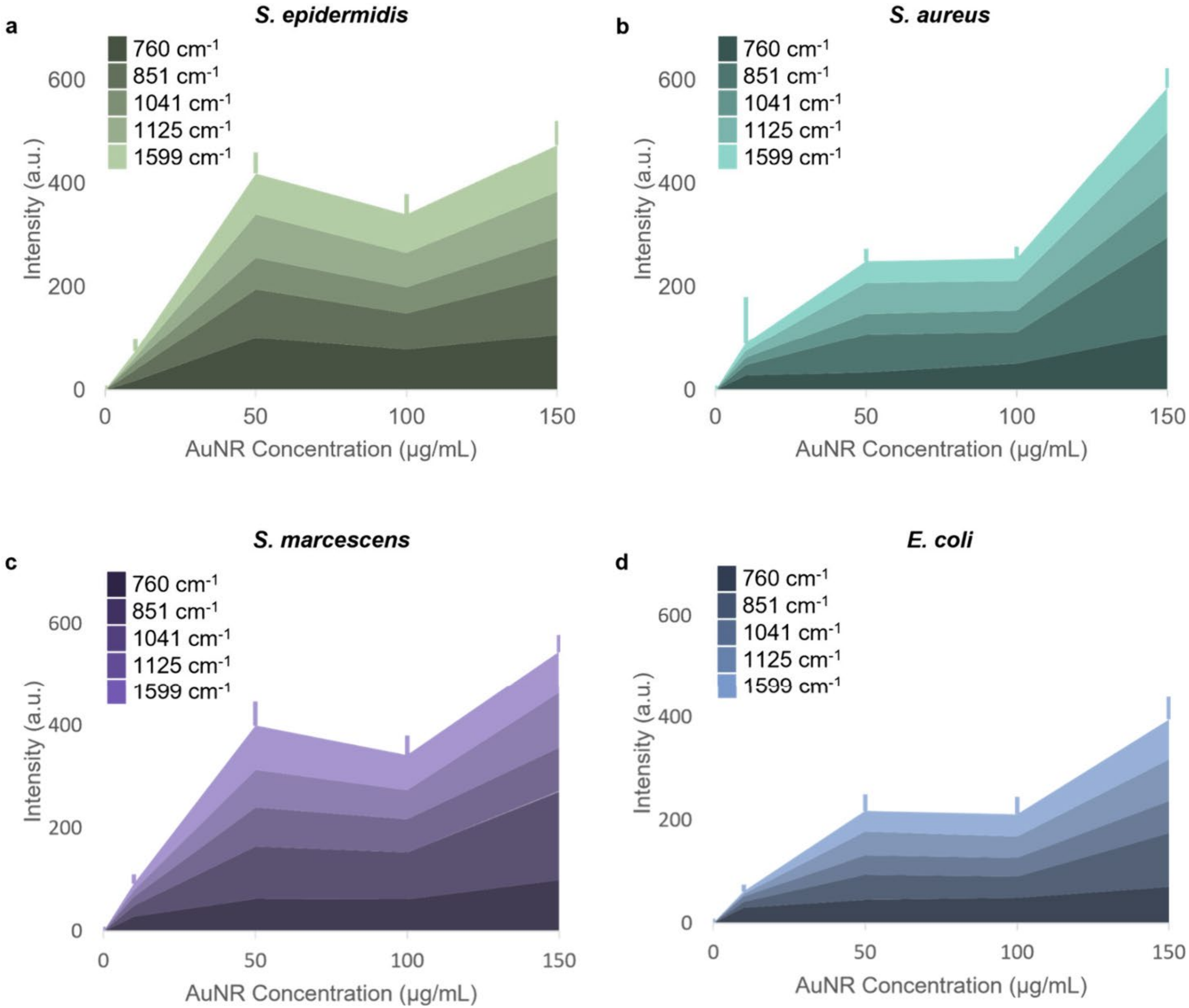
Results and discussion



Results and discussion

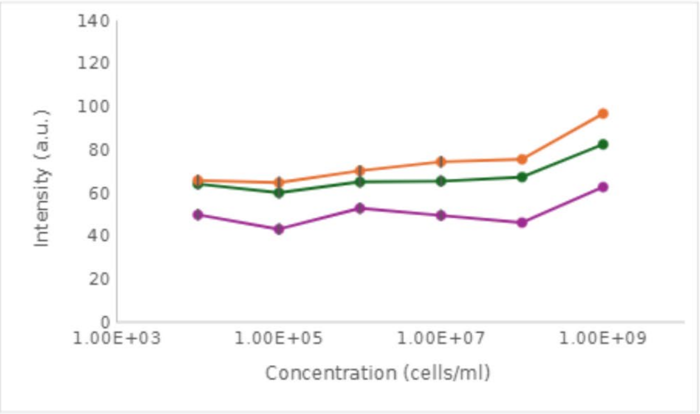


Results and discussion

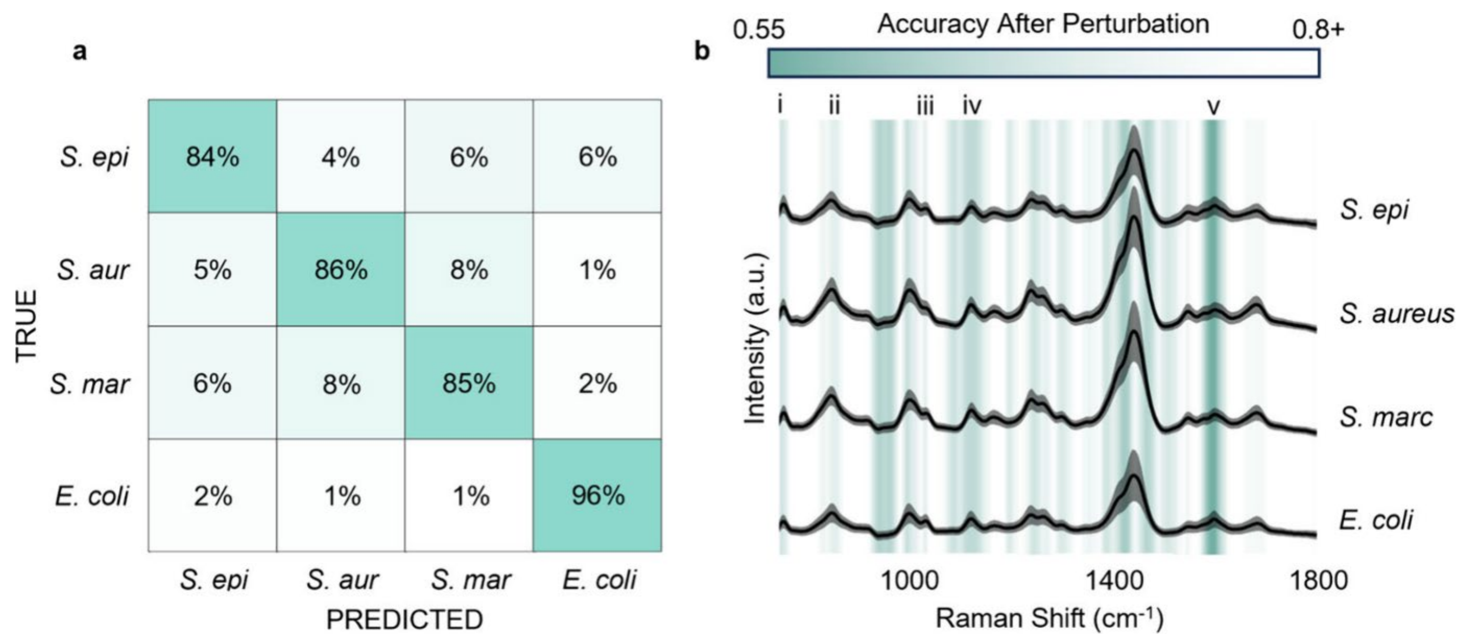


Results and discussion

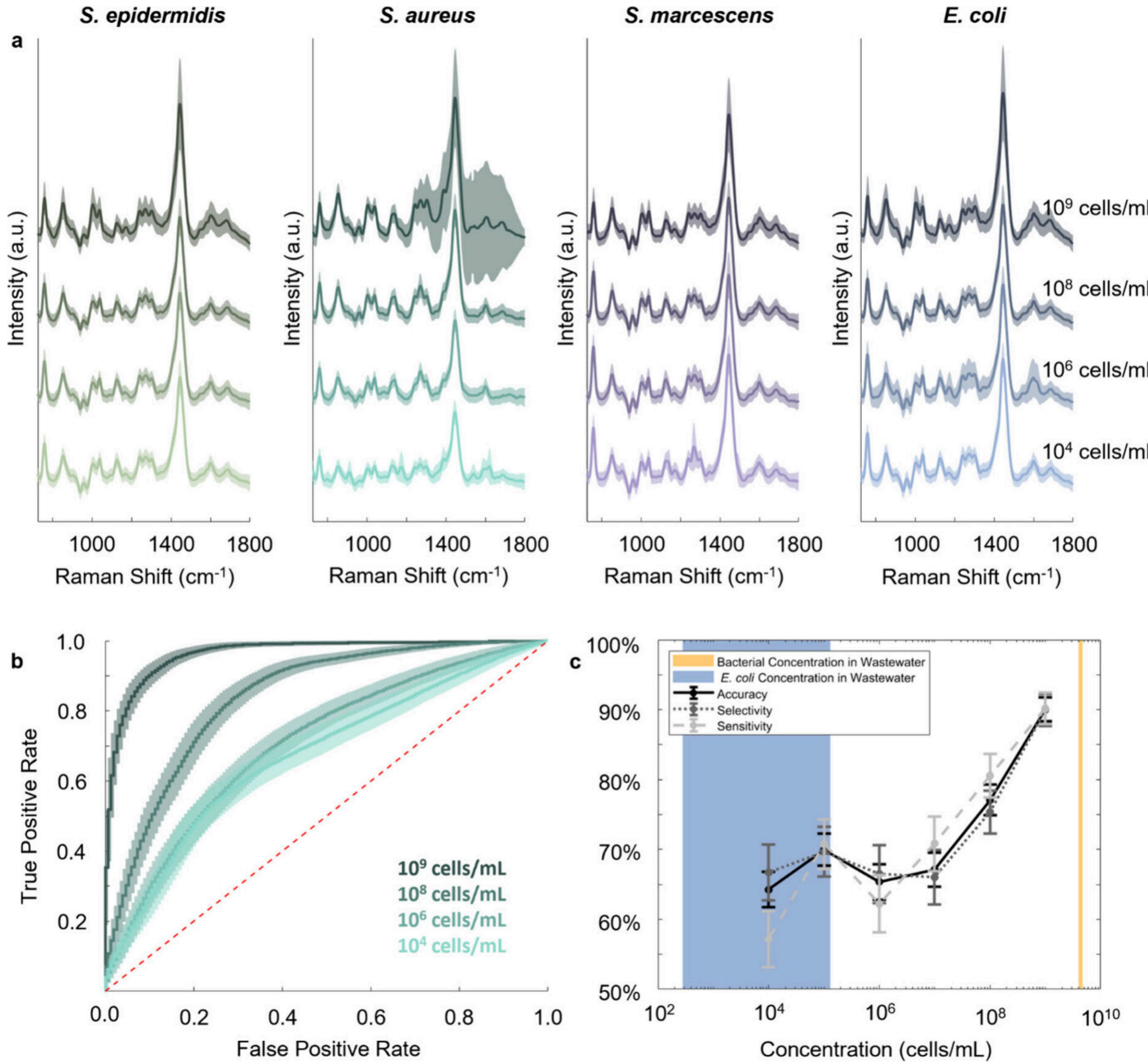
Figure S6



Average peak intensities for spectra with bacteria at each concentration at 851 cm^{-1} (orange), 1040 cm^{-1} (green), and 1,599 cm^{-1} (purple). These values display a positive correlation between bacterial concentration and peak intensity.



Results and discussion



Conclusion

- **Enabled SERS in Wastewater:** Demonstrated reliable Raman signal enhancement even in complex, real-life wastewater environments.
- **Electrostatic AuNR Binding:** Positively charged gold nanorods selectively bind to negatively charged bacterial surfaces, ensuring focused SERS signal amplification.
- **Species-Specific Spectral Fingerprints:** Each bacterium shows reproducible and distinguishable Raman peaks, including adenine, phosphate, and carboxyl groups.
- **Accurate ML Classification:** Achieved 87% overall classification accuracy using SVM, with *E. coli* reaching 96% — highlighting model robustness.
- **Low Detection Threshold:** Successfully detected bacteria at concentrations as low as 10^4 cells/mL, aligning with real-world wastewater bacterial loads.
- **Molecular Spillover Detection:** Captured Raman signals even without direct bacterial contact, suggesting detection of shed biomolecules.
- **Fully Label-Free Strategy:** Bypasses PCR and antibodies, enabling rapid, untargeted bacterial surveillance in unknown or mixed samples.
- **Field-Ready Workflow:** Combines simple preprocessing with portable Raman and ML tools for on-site public health diagnostics.
- **Scalable Surveillance Platform:** Expandable to additional pathogens, antimicrobial resistance profiling, and broader environmental monitoring.

The paper ends, but the questions continue

Drawbacks	Possible Solutions
Limited bacterial diversity (only 4 species tested)	Include more pathogens, e.g., AMR strains like Pseudomonas, Klebsiella
Only one ML model used (SVM)	Compare performance with RF, CNN, ensemble models
No profiling of antimicrobial resistance (AMR)	Incorporate Raman features of resistant strains or gene probes
Detection limit (~10^4 cells/mL) could be improved	Optimize nanostructures for better enhancement (e.g., hotspots)
Spiked wastewater lacks real-world variability	Validate using diverse, field-collected wastewater samples
No time-series or real-time monitoring	Introduce temporal data collection and monitoring campaigns
No single-cell resolution	Integrate with microfluidics or optical tweezers
Lack of bacterial quantification	Apply regression models (e.g., PLSR, SVR) for concentration estimation