

FAST AND ACCURATE IDENTIFICATION OF PATHOGENIC BACTERIA USING EXCITATION-EMISSION FLUORESCENCE SPECTROSCOPY AND MACHINE LEARNING

Jacob Henry,¹ Jennifer Endres,² Marat R. Sadykov,² Kenneth W. Bayles,² Denis Svechkarev^{1}*

¹ Department of Chemistry, University of Nebraska at Omaha, Omaha NE, United States

² Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha
NE, United States

SUPPLEMENTARY INFORMATION

Figure S1. Neural network architecture	S2
Figure S2. Excitation-emission spectra of bacteria	S2
Figure S3. Pattern differences between Gram-positive and Gram-negative bacteria	S3
Figure S4. Pattern differences in Gram-positive and Gram-negative bacteria	S4
Figure S5. Confocal fluorescence microscopy of representative bacteria	S5
Figure S6. ROC curve for the Gram status classification model	S6

* Corresponding author: Department of Chemistry – Durham Science Center, University of
Nebraska at Omaha, 6601 University Drive N, Omaha, NE 68182-0109;
dsvechkarev@unomaha.edu

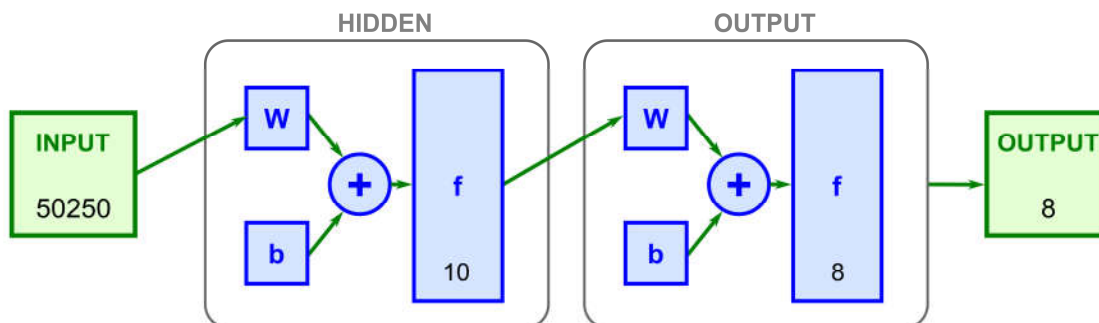


Figure S1. Architecture of the convolutional neural network used for bacterial pathogens classification.

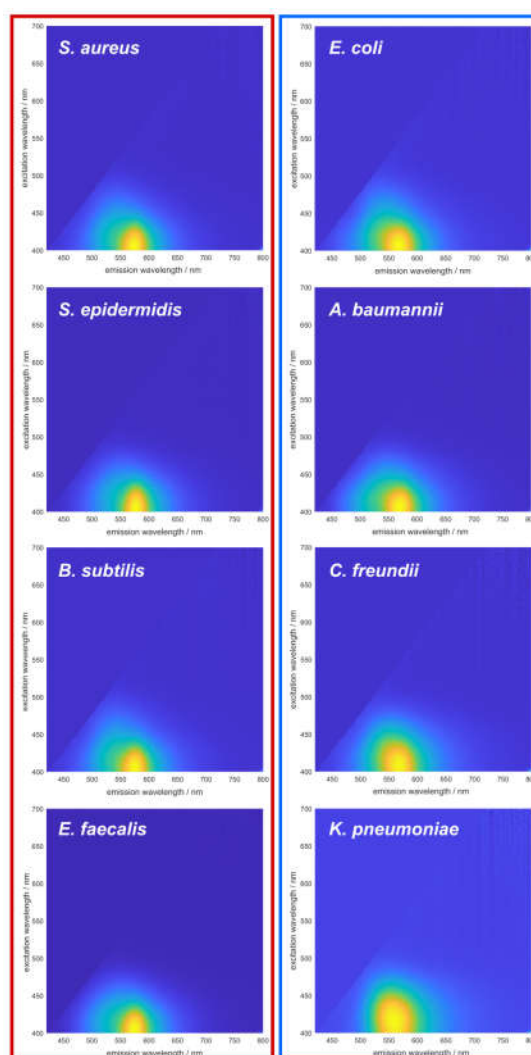


Figure S2. Excitation-emission spectra of Gram-positive (left) and Gram-negative (right) bacteria exhibit distinct Gram-status-specific shape patterns: for the Gram-negative microorganisms, fluorescence is shifted to the area of hydrogen-bonded complex emission (around 540-550 nm) and farther into longer-wavelength excitation (past 440-450 nm).

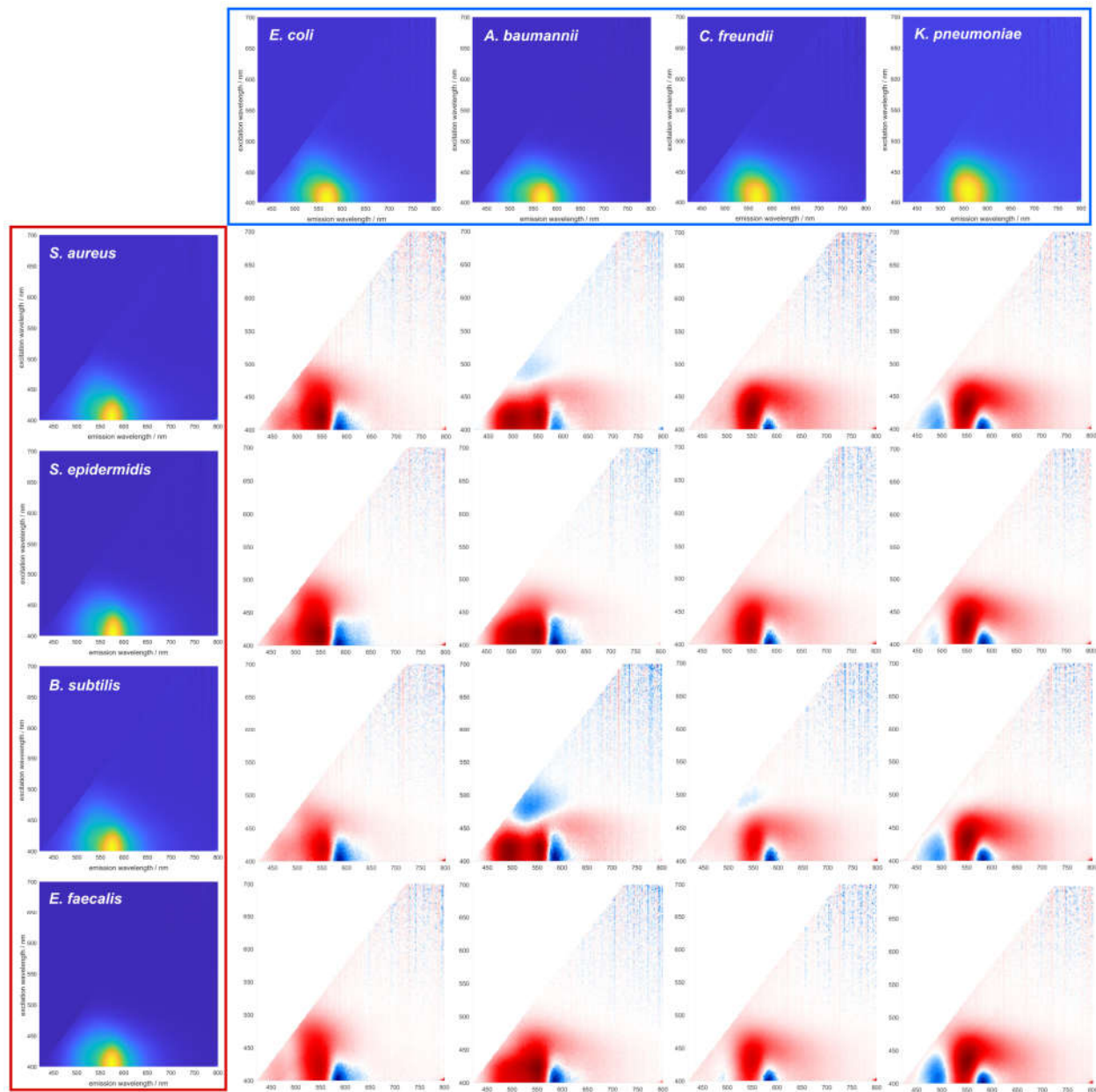


Figure S3. Excitation-emission spectra of Gram-positive and Gram-negative bacteria and the difference matrices illustrating the signals of Gram(+) bacteria subtracted from those of Gram(-) bacteria. A common pattern is observed where the emission intensity redistributes to the shorter wavelengths (red color = increase of intensity) and towards longer excitation wavelengths. At the same time, a decrease of intensity is observed in the area of the tautomeric fluorescence of DMAF (blue color).

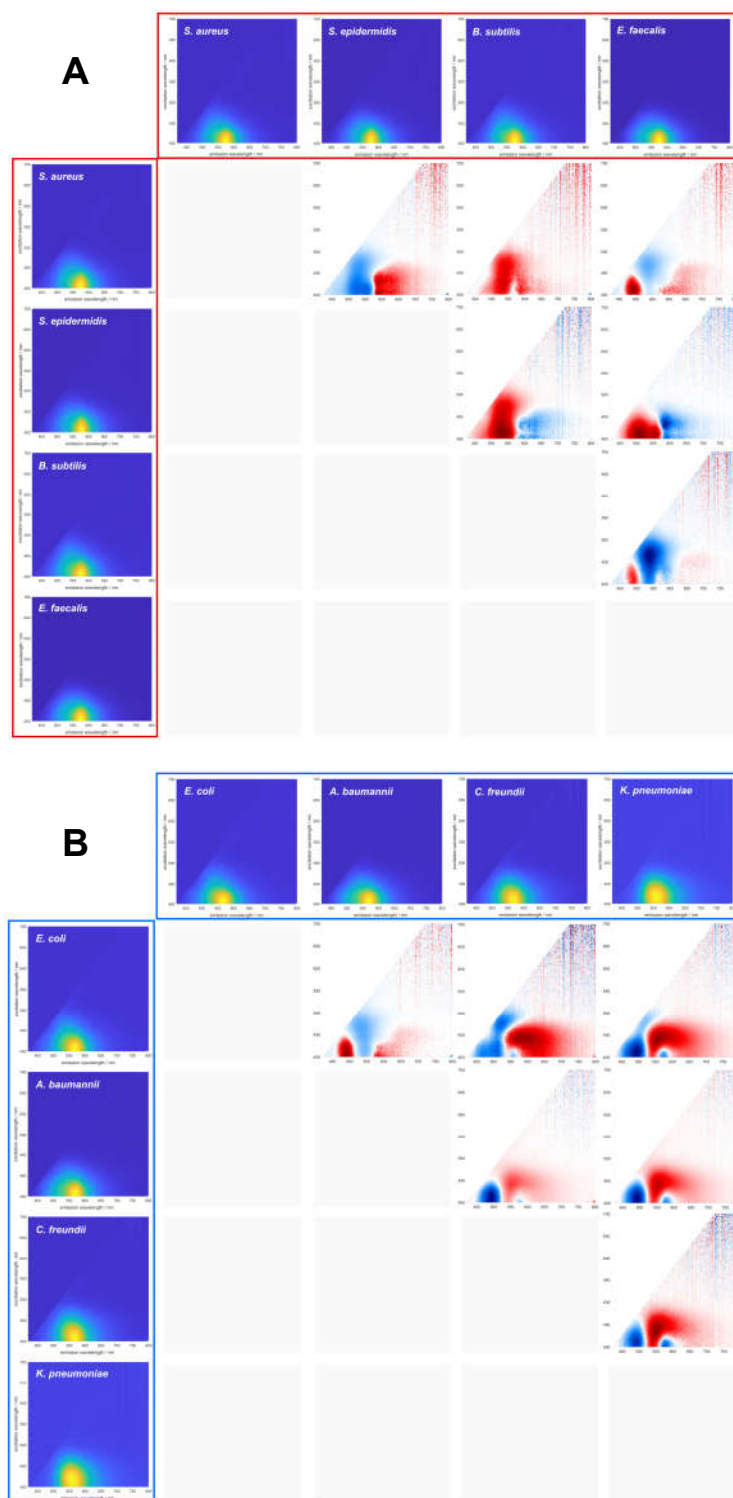


Figure S4. Difference spectra between Gram positive (A) and Gram-negative (B) bacteria illustrate more intricate variations in the excitation-emission signal within a group of bacteria representing the same Gram status, lacking a common trend that dominates the transition from Gram(+) to Gram(-) samples shown in Figure S2.

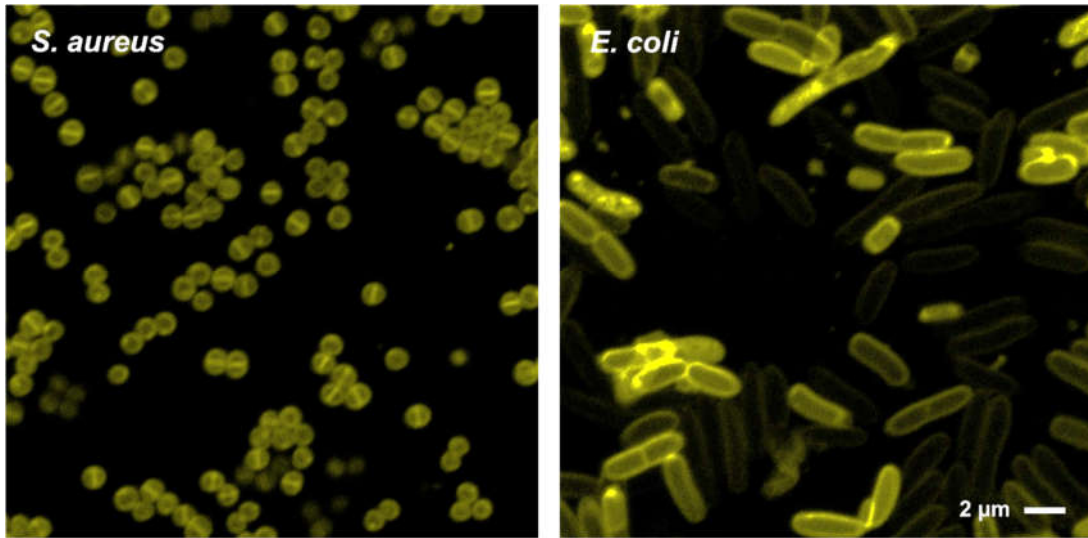


Figure S5. Confocal fluorescence microscopy of representative bacteria (*S. aureus* and *E. coli*) stained with DMAF confirms its localization in the cell envelope.

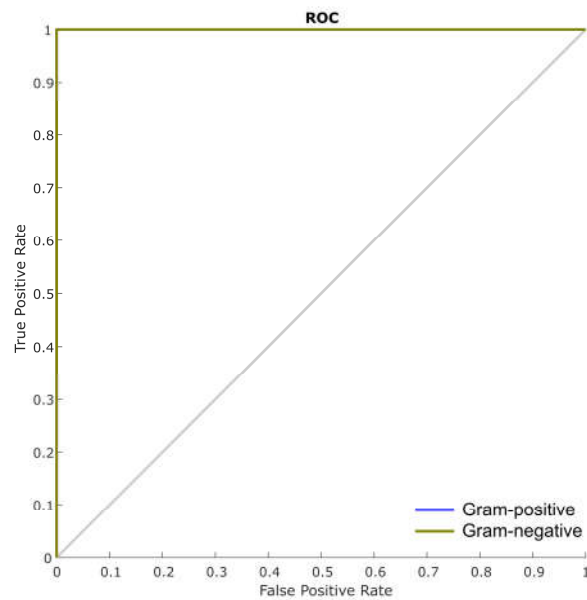


Figure S6. The receiver operating characteristic curve for the Gram status classification model.