Supplementary Information for

Intelligent convolution neural network assisted SERS to realize highly accurate identification of six pathogenic Vibrio

Hui Yu[a], Zhi-Lan Yang[a], Shi-Ying Fu[a], Yue-Jiao Zhang[a], Rajapandiyan Panneerselvamc[b], Bao-Qiang Li[c], Lin Zhang[c,*], Ze-Hui Chen[d,*], Xin Wang[a,*], Jian-Feng Li[a,*]

[a] College of Materials, State Key Laboratory for Physical Chemistry of Solid Surfaces, College of Chemistry and Chemical Engineering, College of Energy, School of Aerospace Engineering, Xiamen University, Xiamen 361005, China

[b] Department of Chemistry, SRM University AP, Amaravati, Andhra Pradesh 522502, India

[c] State Key Laboratory of NBC Protection for Civilian, Beijing 102205, China

[d] Xiamen City Center for Disease Control and Prevention, Xiamen 361005, China

Table of contents

1. Experimental Section

Bacterial strains and chemicals

The bacteria used in the experiments were Vibrio Ordaii strain, Vibrio Xuii, and Vibrio Tasmanian strain. They were from the Marine Microbial Species Conservation Management Center, and the strain numbers were MCCC1A00004, MCCC1B00093, and MCCC1B00674. All of them had been verified by sequencing before activation and export. The remaining six kinds of bacteria were Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus, all provided by Xiamen City Center for Disease Control and Prevention. The water used in the experiments was ultrapure Milli-Q water (18.2 MΩ·cm), and the consumables used were sterilized by a high temperature sterilization pot to prevent contamination in subsequent tests. LB solid broth medium, Marine bacteria broth 2216 were purchased from PERFEMIKER company.

Preparation of Au@Ag NPs

Au@Ag nanoparticles were prepared by a two-step growth method. In the first step, 45 nm spherical Au nanoparticles were synthesized as gold seeds by the classical method. 200 mL HAuCl4 aqueous solution (wt 0.01%) was added into a 250 mL round bottom flask and then heated to boiling under stirring. After that, 2 mL sodium citrate (wt 1%) was quickly injected into the round-bottom flask. In the second step, 10 mL of 45 nm Au sol and 55 mL ultrapure water were added into a 150 mL round-bottom flask, and then 2.4 mL citric acid solution (mass fraction 1%) and ascorbic acid solution were added respectively. Finally, 9.6 mL silver nitrate solution with a mass fraction of 20 mM was slowly added into the round bottom flask, and the whole process was under stirring. Au@Ag nanoparticles with the required particle size could be obtained, and the morphology of the prepared Au@Ag NPs is shown in the Fig. S2.

Preparation of SERS sample

A single colony from each pure culture on an Agar plate was inoculated into a 10 mL liquid medium and then cultured in a shaking incubator for 18-24 hours. In addition, each strain is cultured independently in at least three different batches for biological replication. Then 1 mL solution was absorbed from the liquid medium by an aseptic pipette tip and centrifuged in the 1.5 mL centrifuge tube. The bacteria were centrifuged at the speed of 8000 rpm for 3 minutes and washed two times with PBS buffer with pH 7.4. Finally, the concentrated 150 nm Au@Ag nanoparticles of the same volume were mixed, and the 3.6 mL droplets were added to the gold wafer with the gold film as a substrate, and add 3.6 mL droplets onto the gold substrate, then wait for approximately 7-8 minutes to air dry on a workbench at 25 ℃.

SERS measurements

The above prepared biological samples were used to collect SERS spectral data. The instrument used for SERS spectra acquisition was a SHINs-P784V, Xiamen SHINs Technology Co., Ltd., using

the 785 nm laser beam as the excitation source. The measurement condition was 40 mW, the integration time was 2 s, accumulation 2 times, each prepared biological sample was randomly collected 5 times, and a total of 400 Vibrio data were collected.

Spectral data preprocessing

Since an appropriate pretreatment method can effectively improve the applicability of the model, it is necessary to conduct standard pretreatment steps for SERS. The first step involved cosmic ray reduction, followed by using the adaptive iterative reweighted penalty least squares method (AirPLS), which was used for baseline fitting to remove the fluorescent background. And then the data was normalized to between [0,1] to eliminate the difference in acquisition at different times. Finally, the Savitzky-Golay filter was used for smoothing processing. The whole process was completed in MATLAB R2022a.

Model architecture and training details

In this study, we designed a convolutional neural network (CNN) model for Vibrio Raman detection and analysis in seafood. The CNN model is mainly composed of three convolution layers, three pooling layers, and one fully connected layer. The output of the six dimensions is achieved through the maximum pooling layer and the full connection layer. See Table. S1 for parameter details. In addition, during the training of the CNN model, to maintain the conservative characteristics of Raman data, signal data every 20 wavelengths were selected as the feature region, that is, the original 1×1300 dimensions data is converted to 20×65 dimensions data. Read each segment of valid feature data gradually.

2. Deep learning convolutional neural network model architecture developed

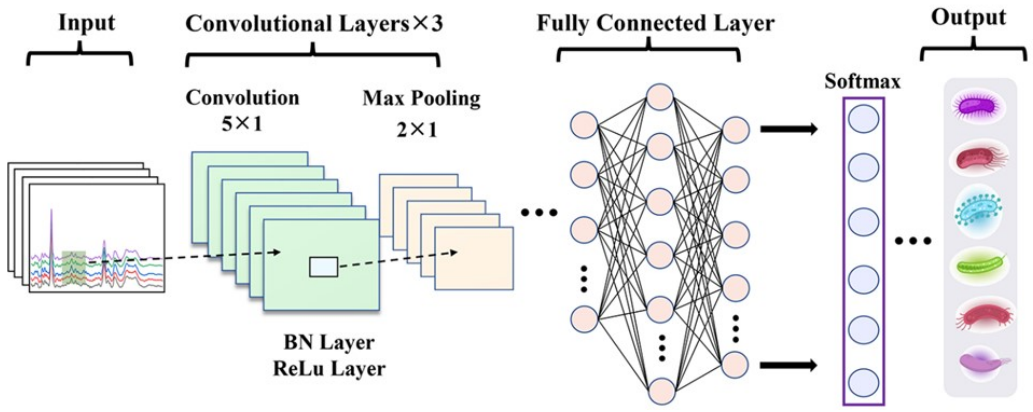

Fig. S1 CNN model architecture, designed with a deep learning classification model consisting of three convolutional layers, a fully connected layer, and Softmax classifier, in which the convolutional layer contains 32, 32, and 64 neurons respectively, and the convolutional filter of each layer is 5×1. After being activated by the Relu function, the network enters the maximum pooling layer and carries out 1×2 pooling. The last six-dimensional data are output through the fully connected layer. Abbreviations: BN Layer: Batch Normalization Layer, and ReLU: Rectified Linear Unit.

3. TEM and SEM image of 150 nm Au@Ag NPs

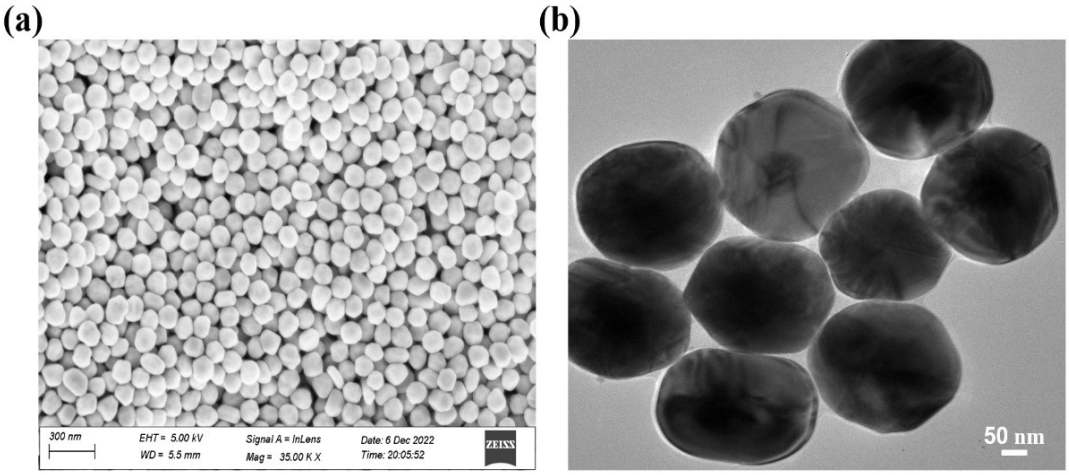

Fig. S2 Image of 150 nm Au@Ag NPs.

4. Testing the Raman enhancement effect of Au@Ag using E. coli as an example

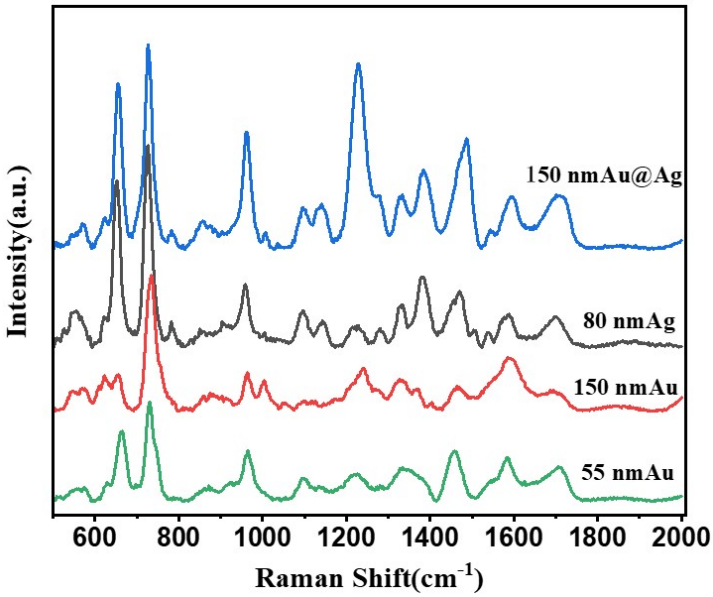

Fig. S3 Comparison of the effects of different enhancement particles on E. coli testing

4. Baseline correction of SERS data

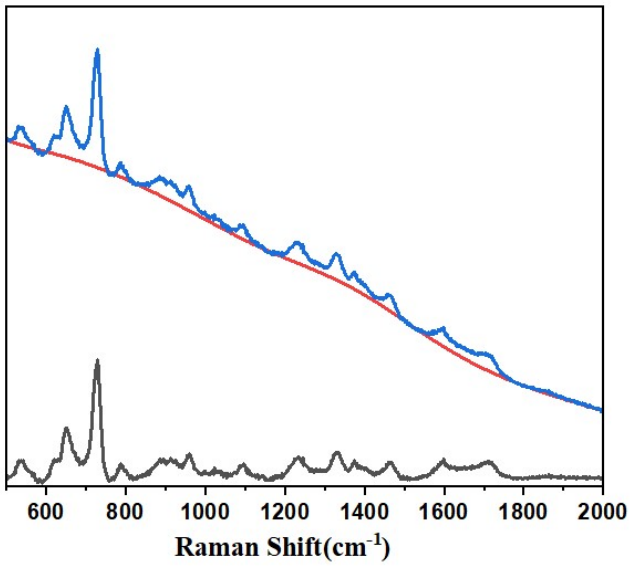

Fig. S4 The adaptive iterative re-weighted penalized least squares (airpls) algorithm was used to remove background noise from the Vibrio Raman spectrum data for baseline correction.

5. Normalization and smoothing denoising

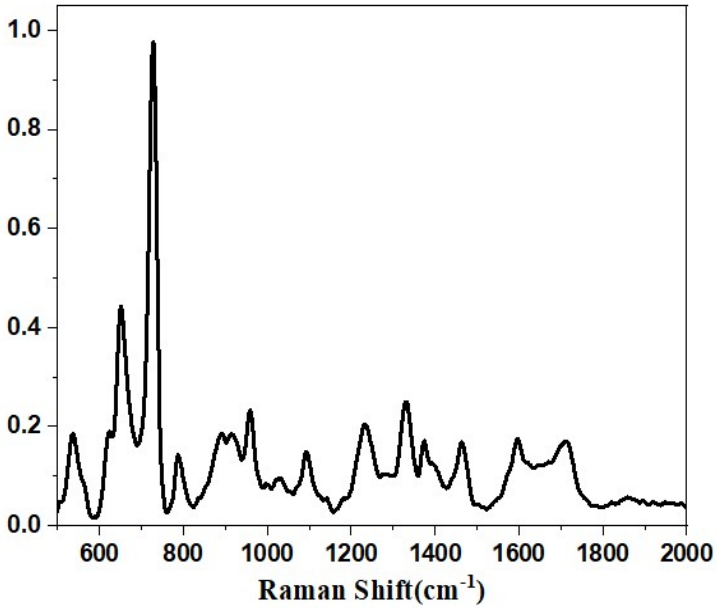

Fig. S5 The Raman data after baseline correction was normalized [0,1], and smoothed by Savizkg-Golag algorithm.

6. Principal component analysis is viewable in three dimensions

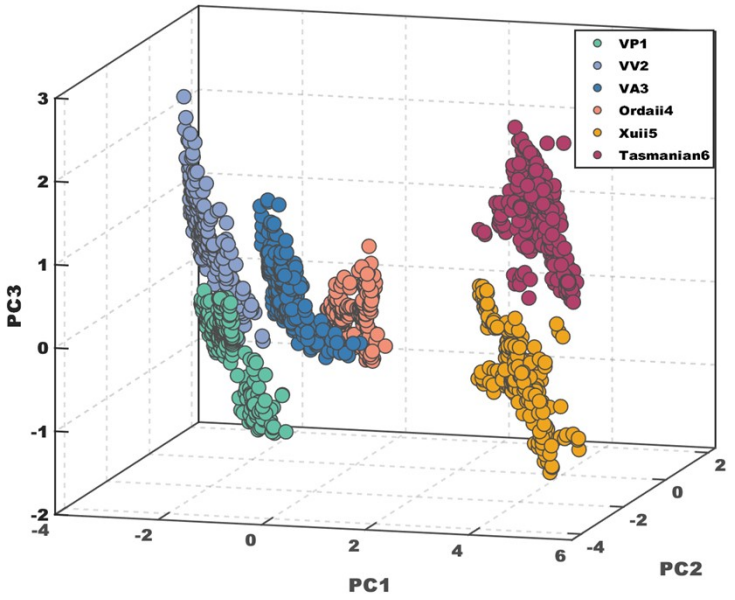

Fig. S6 Three-dimensional data distribution of six pathogenic Vibrio species in seafood after dimensionality reduction.

7. Comparison of clustering effect and original data distribution

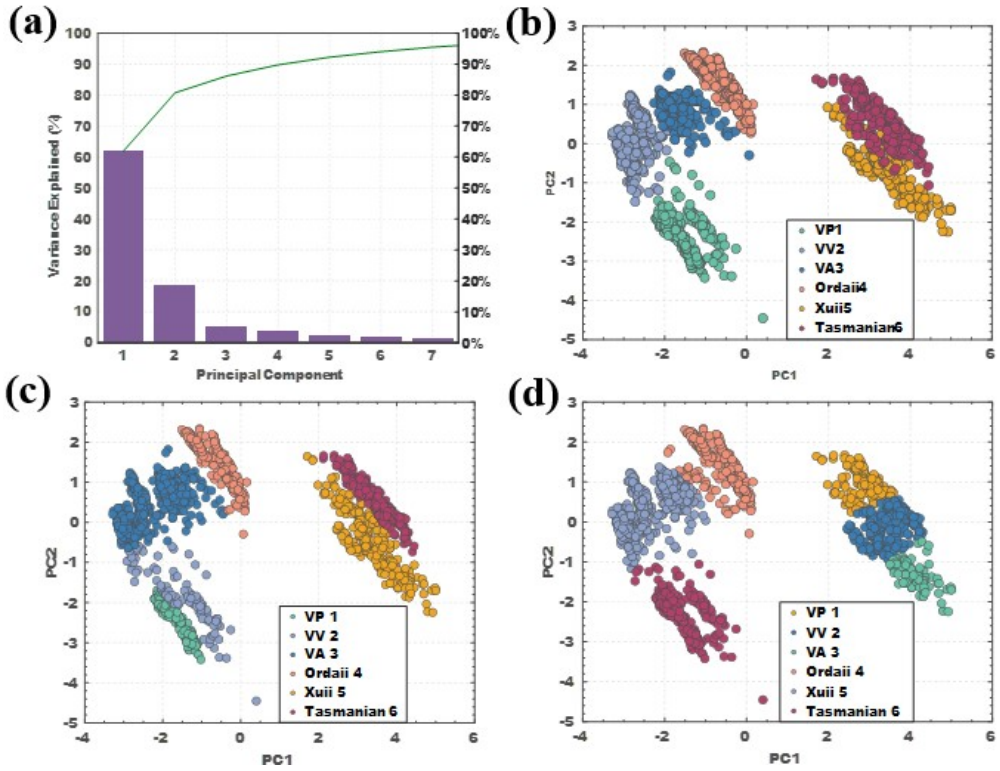

Fig. S7 A principal component analysis is used to reduce the dimension of all SERS data sets and extract effective features. (a) the principal component score with a cumulative variance contribution rate of 95%. (b) two-dimensional visualization of the first two principal components with large variance contribution. (c) K-means clustering effect. (d) K-means clustering effect of particle swarm optimization (PSO).

8. Convolutional neural network model parameter setting

Table S1. The construction and parameter design of the CNN algorithm model.

Name	Type	Detail
data input	Input Layer	20×65×1 data with 'zerocenter' normalization
conv_1	Convolution	32 5×1 convolution with stride [1, 1] and padding [0, 0, 0, 0]
batchnorm_1	Batch Normalization	---
relu_1	ReLu	---
maxpool_1	Max Pooling	2×1 max pooling with stride [1, 1] and padding [0, 0, 0, 0]s
conv_2	Convolution	32 5×1 convolution with stride [1, 1] and padding [0, 0, 0, 0]
batchnorm_2	Batch Normalization	---
relu_2	ReLu	---
maxpool_2	Max Pooling	2×1 max pooling with stride [1, 1] and padding [0, 0, 0, 0]
conv_3	Convolution	64 5×1 convolution with stride [1, 1] and padding [0, 0, 0, 0]
batchnorm_3	Batch Normalization	---
relu_3	ReLu	---
maxpool_3	Max Pooling	2×1 max pooling with stride [1, 1] and padding [0, 0, 0, 0]
fc	Fully Connected	6 fully connected layer
softmax	Softmax	----
class output	Classification Output	crossentropyex

9. Band distribution of SERS characteristic peaks of Vibrio pathogens

Table. S2 Band assignments of characteristic peaks to potential metabolites in the average Raman spectra of six Vibrio pathogens (υ-Stretching, δ-Denatured, Tyr-Tyrosine).

Raman Shift (cm^{-1})	Assignment
650-683	δ(COO-) guanine
720-735	glycosidic ring, adenine
792-797	υ(CN) Tyr
840-852	υ(C-C)
875	υ(C-C) skeleton protein
952-958	υ(CN), protein
1034-1042	CH group, C-C, protein
1085-1093	Lipid, nucleic acid
1128	υ(COC), nucleic acid
1319-1333	Adenine, polyadenine, DNA
1453-1467	υ(CH2), COO-, Lipid
1565-1568	δ(NH), υ(CC)
1581-1591	Adenine, guanine
1662-1689	amide