Supporting Information

Early-Stage Oral Cancer Diagnosis by artificial intelligence-Based SERS using Ag NWs@ZIF core-shell nanochains

Xin Xie[1], Wenrou Yu[1], Zhaoxian Chen[1], Li Wang[2], Junjun Yang[1,*], Shihong Liu[3], Linze Li[1], Yanxi Li[1], Yingzhou Huang[1,*]

1. State Key Laboratory of Coal Mine Disaster Dynamics and Control, Chongqing Key Laboratory of Soft Condensed Matter Physics and Smart Materials, College of Physics, Chongqing University, Chongqing 400044, China

2. School of Optoelectronics Engineering, Chongqing University, Chongqing 401331, China

3. Department of Geriatric Oncology and Department of Palliative care, Chongqing University Cancer Hospital, Chongqing, 400030, China

Email: yzhuang@cqu.edu.cn (Y.H.); junjun@cqu.edu.cn

Contents

1. Experiments and methods

1.1 Materials.

Silver nitrate (AgNO3, ≥99.9%), methanethiol, sodium borohydride ($NaHB_4$),PVP (Mw = 58,000, K29-32), ethylene glycol (EG)，hexadecyl trimethylammonium bromide (CTAB), 2-Methylimidazole (98%) and methanol (≥99.9%) were purchased from Aladdin (Shanghai) Biochemical Technology Inc. zinc nitrate hexahydrate

($Zn(NO3)_2·6H_2O$), acetone, n-hexane, toluene and ethanol absolute (≥99.5%) are purchased from Sinopharm Group Co., Ltd. Distilled water (Millipore Milli-Q grade) with a resistivity of 18.2 MΩ cm^{-1} was used in all experiments. All chemicals were at least analytical reagent grade and applied without further purification.

1.2 The Synthesis of Ag nanowires (Ag NWs).

The Ag NWs were prepared according to a previous synthetic method.[1] In brief,, 5 mL of an EG solution was added into a flask and then the flask was moved into an oil bath at a temperature of 160 °C under stirring for 5 min. Next, 12 mL of a PVP solution (135 mg) and 0.6 mL of an $AgNO_3$ solution (1M) were respectively added to the previous flask in turn by stirring for 1 h. Afterward, the grey-white silky suspension was obtained. In the end, the resultant Ag NWs were purified by centrifugation at 2000g several times and were dispersed in methanol solution for ZIF coating.

1.3 Fabrication of the Ag NWs@ZIF core–shell nanochains.

The Ag NWs@ZIF core–shell nanochains were prepared by in-situ growth of ZIF on the as-synthesized Ag NWs.[2] In the beginning, 25 mL of $Zn(NO3)_2$ solution (25 mM) and 0.5 mL or 1 mL of synthetic Ag NWs (10 mg/mL) was sequentially added to 25 mL of 2-methylimidazole solution (25 mM) while stirring. Then, the mixture reacted at 30 °C for 2 h without stirring. After that, the residual reagents were removed by centrifugation and then washed twice with ethanol to obtain the Ag NWs@ZIF core–shell nanochains. Finally, the Ag NWs@ZIF core–shell nanochains was deposited on Si film to dry for further use.

1.4 Adsorption of gaseous methanethiol molecules

In the beginning, the dried SERS substrate and the petri dish with 2 mL of alcohol solution of the different concentration of methanethiol placed on the heating platform at 60°C. Then, large petri dish was covered on the heating plate in order for the SERS substrate to fully absorb gaseous methanethiol molecules. After waiting 5 h, the SERS substrate was taken out collect SERS spectrum. The simulated health breath (ethanol, acetone, n-hexane, toluene) and the simulated oral cancer (OC) breath (ethanol, acetone, n-hexane, toluene, methanethiol) was a mixture according to the literature. The dried SERS substrate and the petri dish with 3 mL of alcohol solution of the simulated health breath or the simulated oral cancerplaced on the heating platform at 60°C. Then,

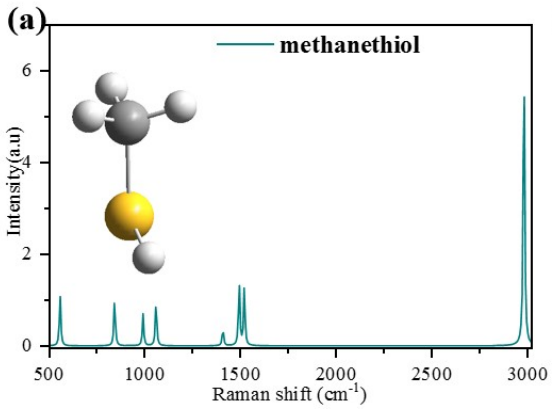
large petri dish was covered on the heating plate in order for the SERS substrate to fully absorb the simulated gaseous molecules. After waiting 5 h, the SERS substrate was taken out collect SERS spectrum.

1.5 Characterization and SERS measurements.

Scanning electron microscopy (SEM) images were acquired with a TESCAN MIRA 3FE device operating at 25 kV. Transmission electron microscopy (TEM) images were obtained using a FEI Taloes-F200S instrument at an operating voltage of 200 kV. SERS measurements were performed using a Raman spectrometer (iHR550, Horiba) equipped with a 632.8 nm He−Ne laser. The SERS spectra were collected under 100× objective lens at the excitation of 632.8 nm. The laser power was around 0.7 mW at the sample position. All SERS spectrum was collected in high-resolution mode with an accumulation time of 20 s.

1.6 Artificial intelligence Analysis. AI algorithms are used to analyze SERS data from simulated health and cancer exhalations. SERS data were preceded by preprocessing done using Labspec 5 software (Horiba; standard values: 4 degrees, 9 dimensions), including smoothing; baseline subtraction (function: polynomial type, 8 degrees). The simulated SERS spectrum of health breath and OC breath were analyzed in the 400 cm^{-1} to 3000 cm^{-1} range (4111 1). In SERS spectra data, the simulated health samples are considered positive and the simulated OC samples are considered negative. The partial least-squares discriminant analysis (PLS) model, principal component analysis (PCA) model, artificial neural networks (ANN) model are constructed. The ANN model consists of three parts: input layer, hidden layer, and output layer. The input data width of SNN model is composed of 4111 one-dimensional floating data. The ANN model parameters are set to randomly combine the hidden layers from 1 to 4, 1 to 50 neurons, and RMSprop optimizer ($lr=3e^{-4}$-$3e^{-3}$). At the same time, EarlyStopping (5 steps) and ModelCheckpoint methods are used to assist in obtaining the optimal model.

1.7 Computational Details. In this work, the ground-state geometry structure of methanethiol is calculated using density functional theory (DFT), B3LYP functional, 6-311+G basis set for all atoms. In order to compare well with the experimental data, the Raman spectra have been corrected using a frequency correction factor (0.9580 for single molecules).



(b)

Number	Raman shift (cm^{-1})	Peak Assignment
1	684	υ_{CS}
2	1069	β_{CH}
3	1424	β_{CH}
4	1495	β_{CH}
5	2981	υ_{SH}

Fig. S1. (a) The simulated SERS spectra of methanethiol and the corresponding vibration modes at these characteristic peak (b).

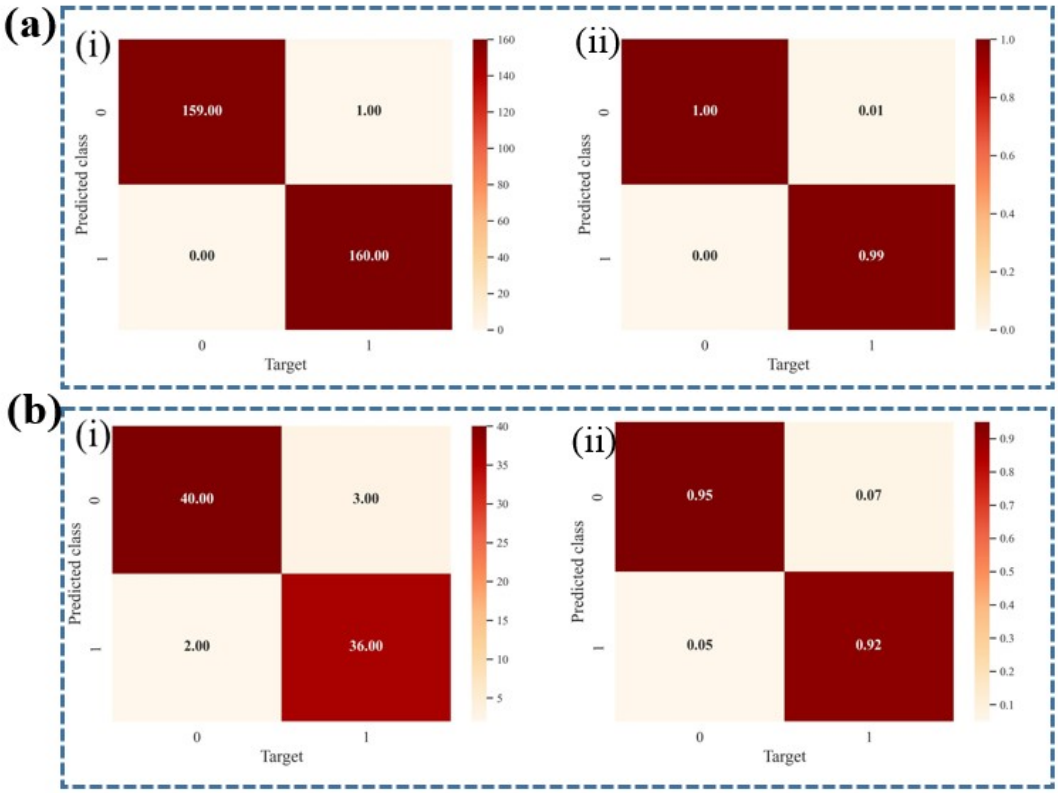


Fig. S2. Confusion matrices of the results (a) training data and test data (b) predicted by ANN algorithm.

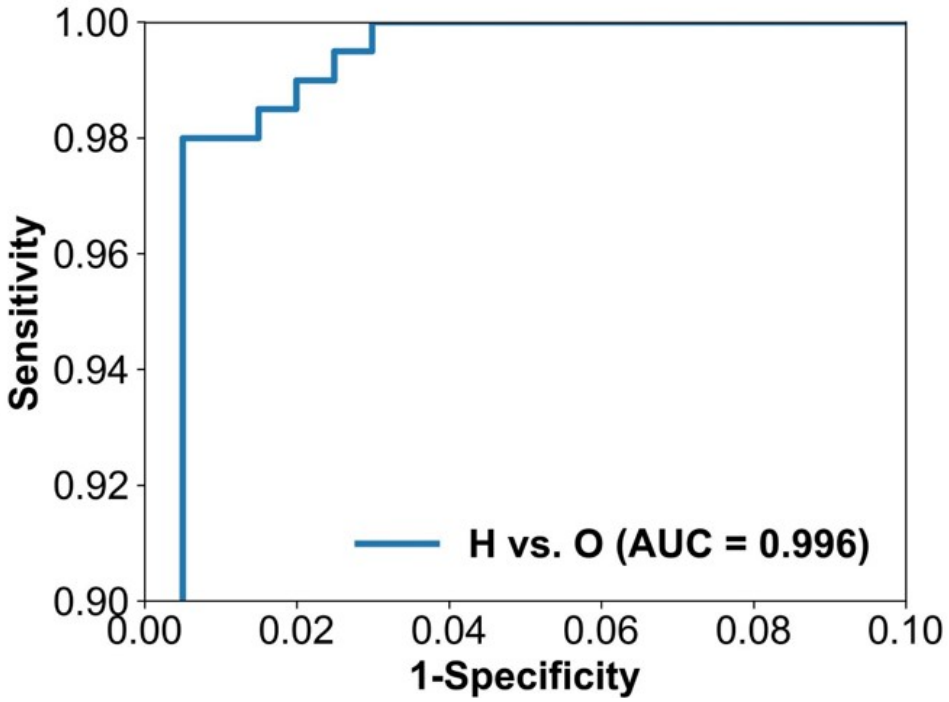


Fig. S3. An area under the curve (AUC) of receiver operating characteristic (ROC) curves of 0.996 for simulated breath samples.

Fig. S4. The selectivity, reproducibility and stability of the proposed SERS method.

As illustrated in Fig. S4a, the selectivity of the Ag NWs@ZIF core–shell nanochains (0.5 mL) was investigated by exposing it to 10^{-4} M methanethiol gas molecules and 10^{-4} M of other interfering gases, including ethanol, acetone, n-hexane, toluene. It indicates that the proposed sensor exhibits excellent selectivity toward methanethiol compared to the other interfering gases and gas mixtures. The 10 SERS spectrum of methanethiol at the concentration of 10^{-7} M was measured to explore the reproducibility of the Ag NWs@ZIF core–shell nanochains (0.5 mL)-based SERS platform. As illustrated in Fig. S4b-c, the Raman intensity of the 10^{-7} M methanethiol does not show any noticeable change from 10 consecutive measurements. The relative standard deviation (RSD) of the intensity at 2931 cm^{-1} is as low as 21%. This observation indicates that the SERS substrate possesses excellent uniformity, guaranteeing the effective signal reproducibility and stability for portable detection.

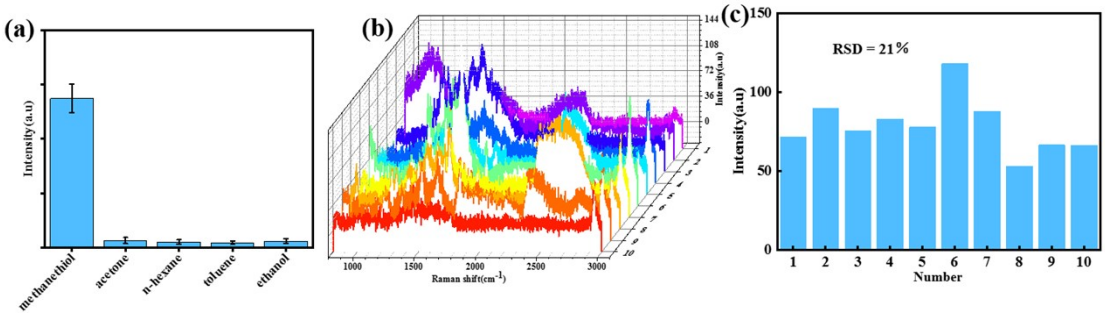


Fig. S4. (a)The stability of the Ag NWs@ZIF core–shell nanochains (0.5 mL) toward 10-4 M ppb methanethiol gas molecules. (b)The 10 SERS spectrum of methanethiol at the concentration of 10^{-7} M and The relative standard deviation (RSD) of the intensity at 2931 cm^{-1}(c).

References

[1] Q. Li, S. Gong, H. Zhang, F. Huang, L. Zhang, S. Li, Tailored necklace-like Ag@ZIF-8 core/shell heterostructure nanowires for high-performance plasmonic SERS detection, Chem. Eng. J. 371 (2019) 26-33. https://doi.org/10.1016/j.cej.2019.03.236.

[2] J. Yang, M. Pan, X. Yang, K. Liu, Y. Song, S. Wang, Effective adsorption and in-situ SERS detection of multi-target pesticides on fruits and vegetables using bead-string like Ag NWs@ZIF-8 core-shell nanochains, Food Chem. 395 (2022) 133623. https://doi.org/10.1016/j.foodchem.2022.133623.