Supplementary information

Label-free determining of liver cancer stages using surface-enhanced Raman scattering coupled with preferential adsorption of hydroxyapatite microsphere

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1. Collection of human serum samples

In this study, we had obtained the ethical approval for studying human blood samples. 25 serum samples of liver cancer patients with T1 stage, while 23 serum samples of liver cancer patients with T2-T4 stage as well as 35 serum samples of healthy volunteers were provided by the MengChao Hepatobiliary Hospital of Fujian Medical University. Table S1 lists the clinical details information about liver cancer patients. After 12 hours of overnight fasting, 5 mL of peripheral blood samples were collected from the subjects between 7:00 and 8:00 A.M., and then the serum was obtained by extracting the supernatant from the collected blood centrifuged at 3000 rpm for 10 minutes. The obtained serum samples were frozen at -80 °C before use.

Table 1. Clinical information of T1 stage and T2-T4 stage cancer patients

	T1 stage cancer	T2-T4 stage cancer
Age		
Mean	58.3	56.6
Median	59	55
Gender		
Male	19	17
Female	6	6
Cancer stage		
TI	25	NA
T2-T4	NA	23

2. Preparation of Ag nanoparticles

The preparation method of Ag nanoparticles (NPs) were reported by Leopold and Lendl.[1] Briefly, 4.5 mL of 0.1 M sodium hydroxide solution was added with 5 ml of 0.06 mM hydroxylamine hydrochloride solution accompanied by uniformly mixed. Then, the mixture was quickly added to 90 mL of 0.0011 M silver nitrate solution, the resulting mixture was kept stirring until obtain a homogeneous mixture. Finally, the Ag colloidal was centrifuged at 10000 rpm for 10 minutes, and the supernatant was discarded to obtain high-concentration Ag NPs for protein-SERS measurement. Figure S1 shows the UV-Vis-NIR absorption spectra of Ag NP with a maximum absorption of 416 nm, it was also shown in the figure that the particle sizes were determined by transmission electron microscope with a mean diameter of 35 nm and a standard deviation of 6 nm.

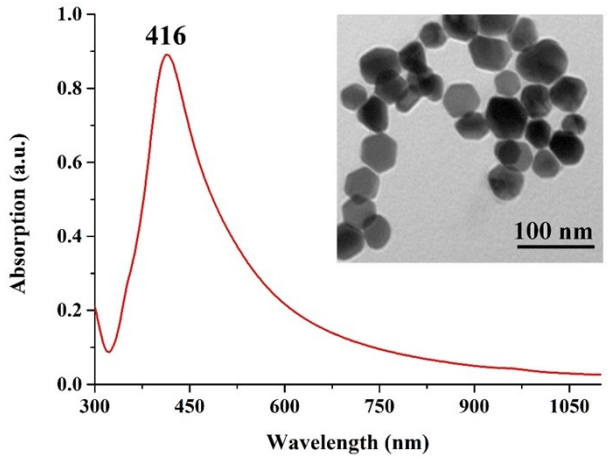

Fig. S1. The UV-Vis-NIR absorption spectrum of Ag NP with average size of 35 ± 6 nm. The inserted image is the transmission electron micrograph of Ag NPs.

3. Data preprocessing and analysis

The SERS data set was inputted into SPSS 15.0 software package (SPSS Inc., Chicago) for PCA and LDA analysis. PCA was performed to reduce the high dimensions of complex data set and generated the appropriate number of principal components (PCs) that involve most of the entire variance in the original spectra while retaining the most diagnostically information for serum protein differentiation.[2] And then, an independent sample T test was used to determine the diagnostically significant PC scores calculated by PCA in each case using an alpha of 5%. Subsequently, the most statistically significant PC score ($p < 0.05$) were retained and fed into LDA model to correctly predict the samples obtained from three sample groups with the leave-one-out, cross-validation method (i.e. T1 stage cancer+T2-T4 stage cancer vs. normal, T1 stage cancer vs. normal, T2-T4 stage cancer vs. normal and T1 stage cancer vs. T2-T4 stage cancer). To further test the performance of PCA-LDA diagnostic algorithm, the receiver operating characteristic (ROC) curve were generated by continuously varying the different threshold to determine discrimination sensitivity and specificity of all samples.

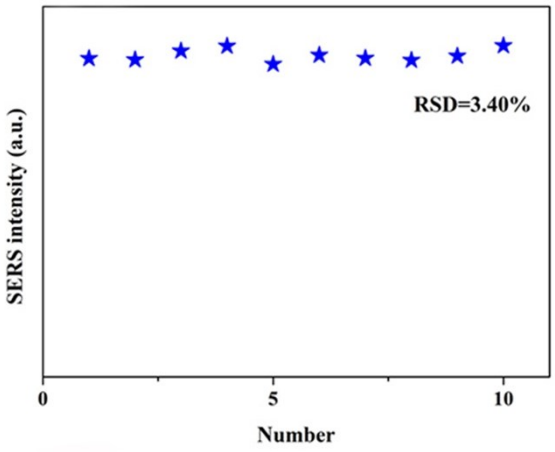

Figure S2. The corresponding SERS signal intensity of peak at 1004 cm^{-1}.

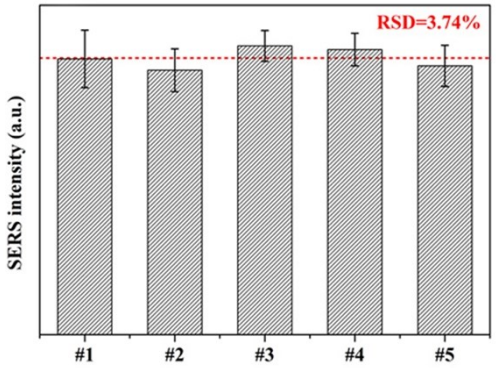

Figure S3. SERS peak intensity distribution of albumin at 1004 cm^{-1} obtained from five batches of independent SERS measurements.

References

1 N. Leopold, B. Lendl, *J. Phys. Chem. B*., 2003, **107**, 5723-5727.

2 Q. Wu, S. Qiu, Y. Yu, W. Chen, H. Lin, D. Lin, S. Feng, R. Chen, *Biomed. Opt. Express*, 2018, **9**, 3413-3423.