Supplementary Materials

Detection and identification of human fungal pathogens using surface-enhanced Raman spectroscopy and principal component analysis

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1. SERS substrate characteristic.

In order to examine the SERS activity of the (AgNPs/FTO) substrate, the enhancement factor (EF) was calculated for *p*-MBA as a standard analyte. The AgNPs/FTO substrate was incubated in 1.0 × 10^{-6} M p-MBA solution in ethanol for 60 min and then washed with deionized water. The surface enhancement factor (EF) for p-MBA was calculated according to the following equation:

$$EF = \frac{I_{SERS} N_{NR}}{I_{NR} N_{SERS}}$$

where N_{SERS} and N_{NR} refer to the number of molecules adsorbed on the SERS probe within the laser spot area and the number of molecules probed by regular Raman spectroscopy, respectively. I_{SERS} and I_{NR} correspond to the SERS intensity of *p*-MBA on the modified surface and to the normal Raman scattering intensity of p-MBA in the bulk. I_{NR} and I_{SERS} were measured at 1079 cm^{-1}. From the relative intensity of both normal Raman and SERS band at 1079 cm^{-1} and the number of molecules sampled from the regular Raman and SERS measurements, the enhancement factor was calculated to be about 9.0×10^6.

The reproducibility of the recorded *p*-MBa signals has been also examined. To verify the signal reproducibility of our substrate, SERS spectra of molecules (10^{-6} M solution in ethanol) from randomly 30 selected places on a AgNPs/FTO substrate were collected under the same experimental conditions. As can be seen in Fig. 1S, the AgNPs/FTO substrate exhibits very good SERS sensitivity and reproducibility. The Raman spectra of *p*-MBa are enhanced strongly at each acquisition point. To get statistically meaningful results, the strong band at 1079 cm^{-1} was chosen to calculate the relative standard deviation (RSD). The RSD of the intensity of these Raman vibrations in the collected spectra on the same platform is around 4 %.

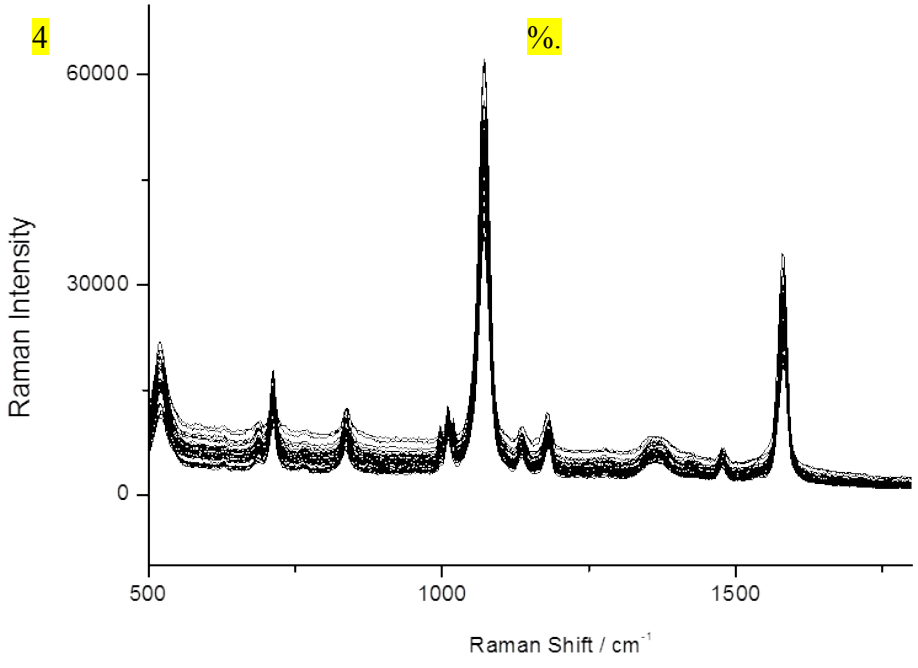

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Fig. 1S. The representative SERS spectra of *p*-MBA recorded from 30 different spots on the SERS surface. Experimental conditions: 10mW of 785 nm excitation, 1 x 2 seconds acquisition time.

The stability of a SERS substrate determines the range of its practical applications in chemical and biological analysis. The crucial parameters are the stability against oxidation for an extended period of time. Considering the 1079 cm^{-1} band as a reference, the SERS platform exhibits the stability of the recorded *p*-MBA signal intensity during 1.5 month. Such high stability and reproducibility enables the quantitative SERS studies of numerous biomolecules and improves the SERS potential in real applications.

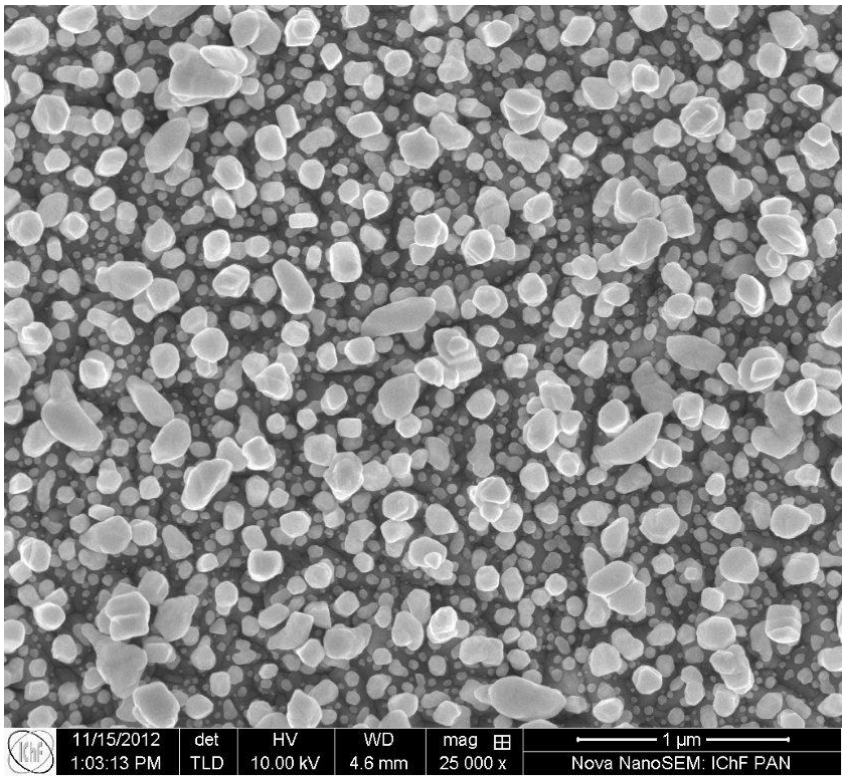


Fig. 2S. The SEM image presents the morphology of the AgNPs/FTO substrate.

2. **Fungal detection**.

Reproducibility of fungal SERS signals.

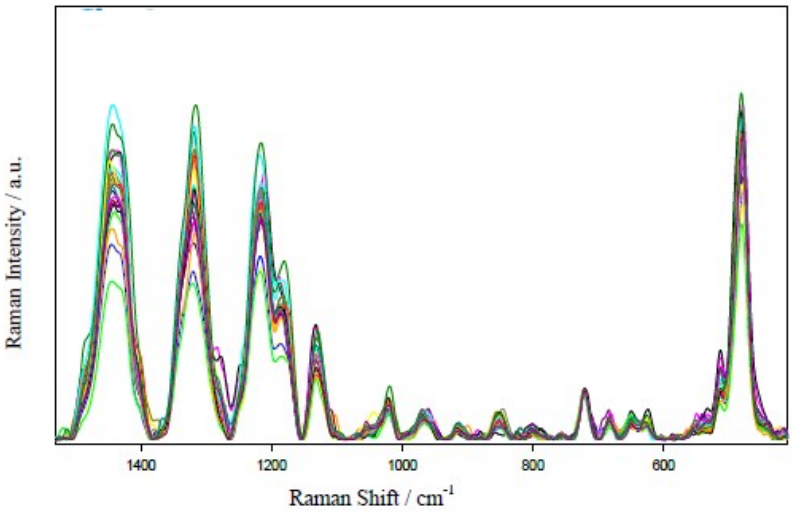
The reproducibility of the recorded SERS signals plays a crucial role in the analytical and biomedical applications of SERS technique. The average standard deviations (Av.STD) of the SERS signals of *S. brumptii, A. flavus, T. rubrum, and C. krusei* were calculated and are presented in the Table 1S. The SERS spectra of these fungal species recorded from different spots within the same samples are also presented in Figure 3S.

Fungal species	Selected bands [cm^{-1}]	Av.STD (%)

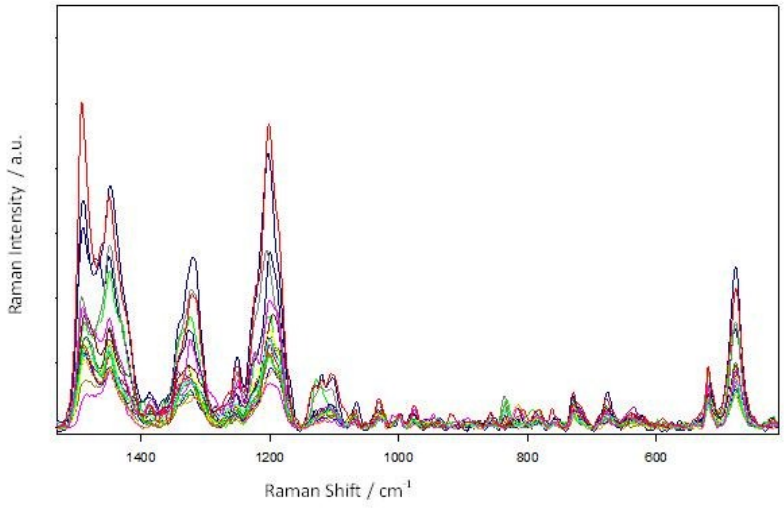
S. brumptii	1200	8
A. flavus	485	12
T. rubrum	1190	7.2
C. krusei	650	10

Table 1S. The Av.STD of the selected intensities of SERS signals of *S. brumptii, A. flavus, T. rubrum, and C. krusei* recorded from 30 different spots within the same sample.

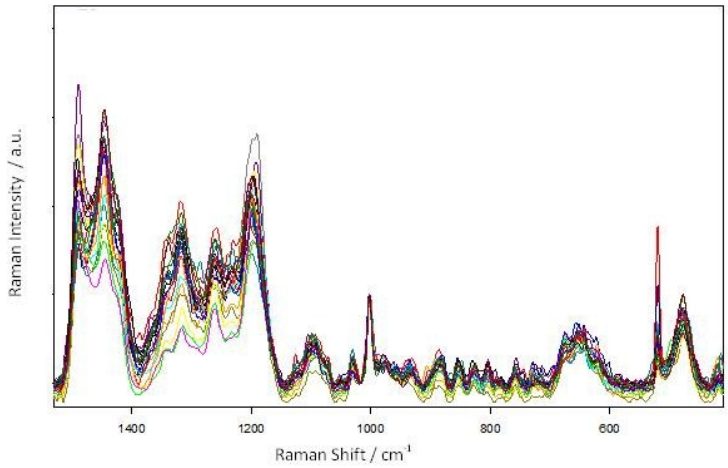
(a)



(b)



(c)



(d)

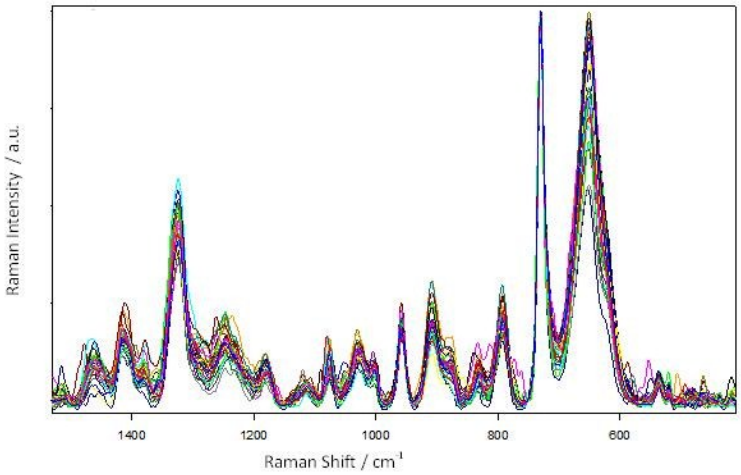
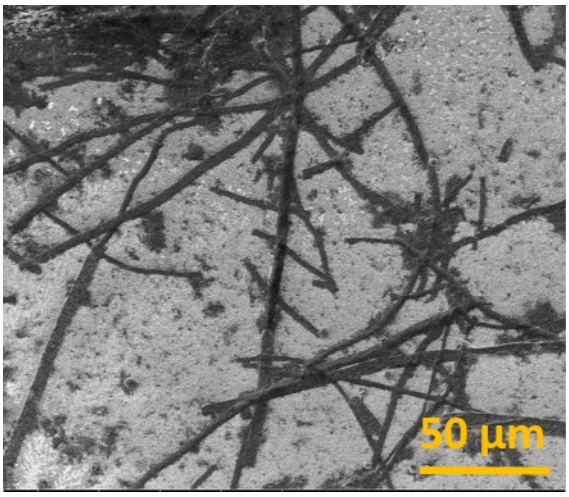


Figure 3S. SERS spectra of (a) *A. flavus,* (b) *S. brumptii,* (c)*T. rubrum, and* (d) *C. krusei* recorded from different spots within the same sample. The excitation wavelength was at 785 nm, laser power was 5 mW, and the acquisition time was 60 s.

a) 

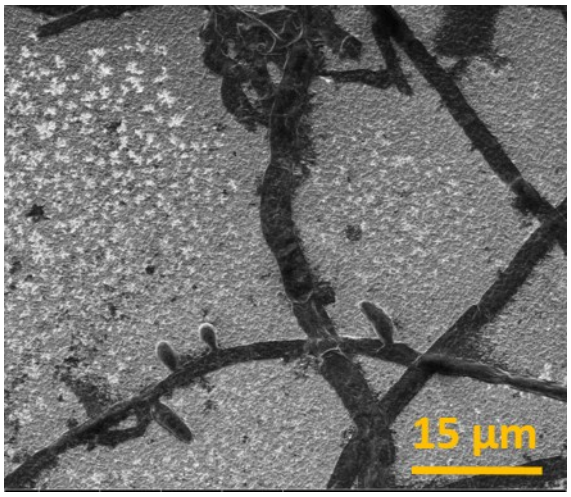
b) 

Figure 4S. SEM images of homogenized hyphae of a) *A. flavus* and b) *T. rubrum* deposited onto SERS platform.

3. Raman and SERS spectra of fungi.

For fungal samples deposited onto a glass slide we were not able to obtain any reasonable Raman spectra of examined fungi. Fig.5S below presents the comparison of the Raman and SERS spectra for *S. brumptii* sample.

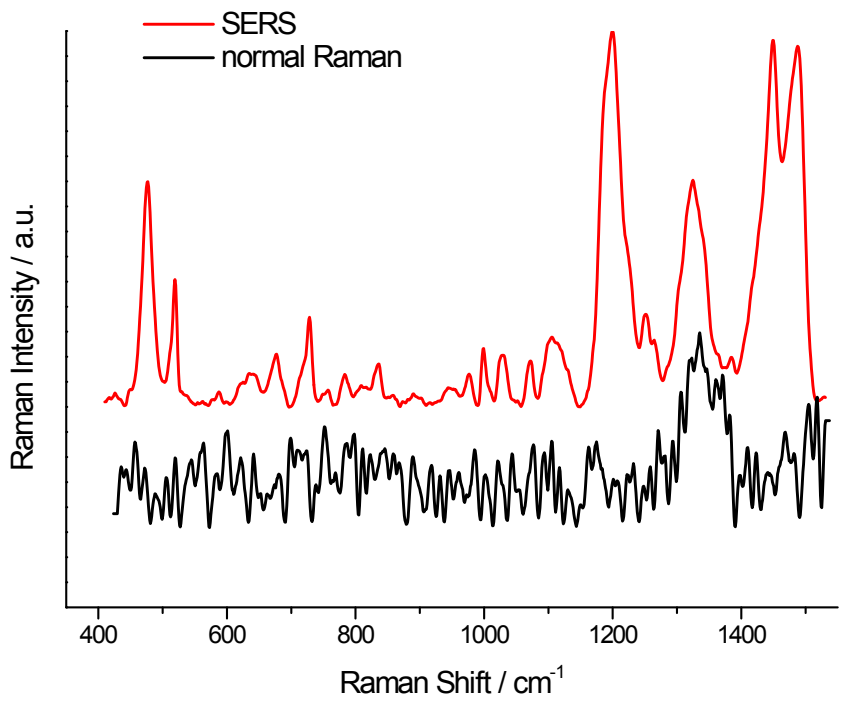


Figure 5S. The comparison of normal Raman (black) and SERS (red) spectra of *S. brumptii* fungal cells. Experimental conditions: Raman system - laser 785 nm, 20× microscope objective, NA = 0.25, 20 s time of accumulation.

4. Chemometric analysis

The PCA is the data reduction technique where the new variables, called principal components (PC), are calculated from original variables. The first principal component (PC-1) accounts for the greatest variability in the data as possible. The method of PCA is based on a model assuming $X = TP^T + E$, where the X matrix is decomposed by PCA into two smaller matrices, one of scores (T) and other of loadings (P) [i], and E is the error matrix. PC's scores are related to a linear combination of the original variables, and describe the differences or similarities in the sample (plots of PC-1 vs PC-2). PCA provides insight into the percentage of variance explained by each PC and shows how many PCs should be kept to maintain the maximum information from the original data without adding noise to the current information. Loadings describe the data structure in terms of variable correlation and reflect how well PC takes into account the variation of that variable. By analysing the plot of PC-loadings as a function of the variables (i.e. Raman shifts) one can indicate the most important diagnostic variables or regions related to the differences found in the data set.

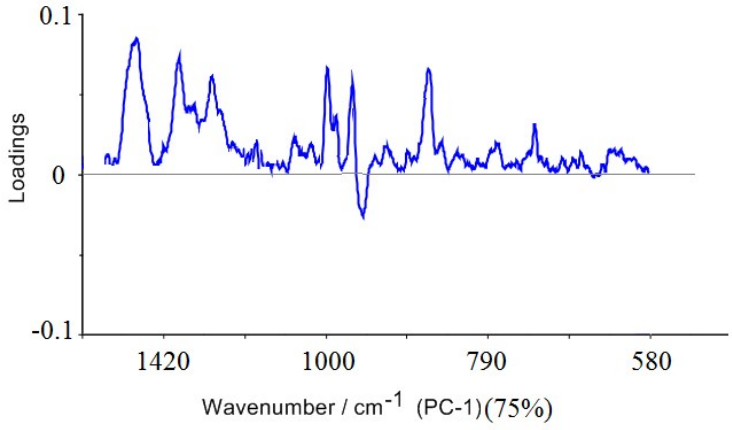


Figure 6S. Loadings plots of the first principal components PC-1 calculated for the all data recorded for healthy and *T. rubrum* infected human skin samples in the whole wavenumber region 500 - 1600 cm^{-1}.

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[i] Mobili P, Londero A, De Antoni G, Gomez-Zavaglia A, Araujo-Andrade C, Avila Donoso H, Ivanov-Tzonchev R, Moreno I, Frausto-Reyes C, Multivariate analysis of Raman spectra applied to microbiology: Discrimination of microorganisms at the species level. Rev Mex Fis. 2010;56:378-85.