

## **SUPPLEMENTARY INFORMATION**

### **Repeated double cross-validation applied to the PCA-LDA classification of SERS spectra: a case study with serum samples from hepatocellular carcinoma patients**

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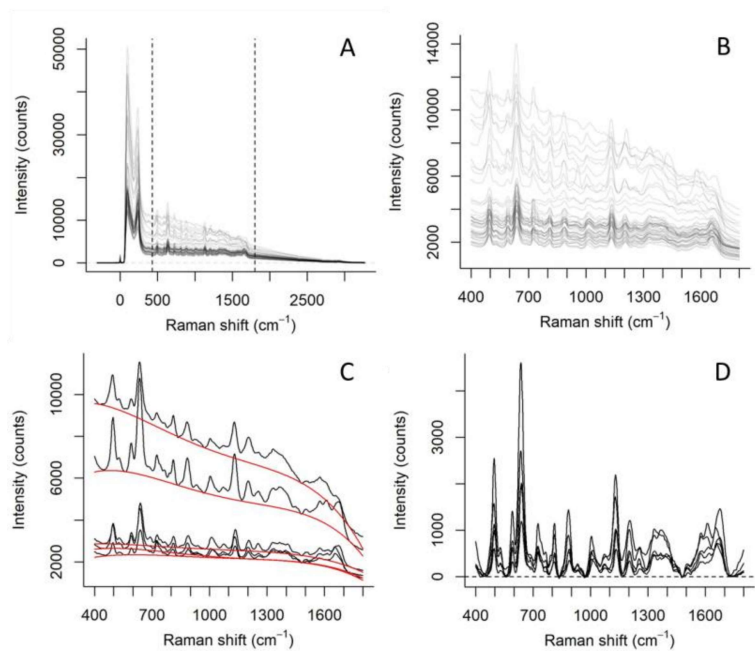
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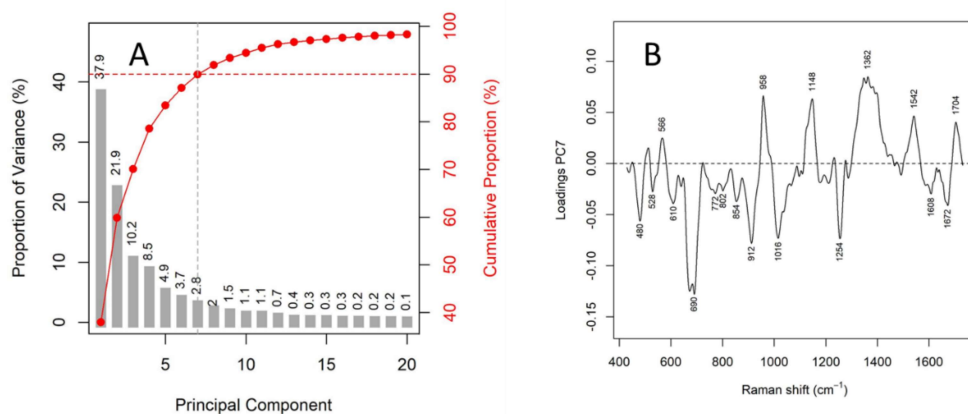
(4) Clinica Patologie Fegato, Azienda Sanitaria Universitaria Giuliano Isontina (ASUGI), Via Costantino Costantinides 2, Trieste, Italy.

	HCC (n= 72)	Healthy (n=72)
<b>Patients characteristics</b>		
<b>Age (mean, 95%CI)</b>	69 (67-71)	56 (55-57)
<b>Etiology</b>		
Alcohol metabolic	44	
Alcohol metabolic viral	13	
Viral	10	
Other*	5	
<b>Disease scores</b>		
CTP A/B/C	50/17/3	
BCLC 0/A/B/C-D	7/38/21/6	
<b>Tumor parameters</b>		
<b>Number of lesions</b>		
Single $\leq$ 2cm	9	
Single or 3 $\leq$ 3cm	21	
Large-single or multi	41	
<b>Alpha-fetoprotein</b>		
<20 ng/mL	37	
20 - 400 ng/mL	8	
>400 ng/mL	9	

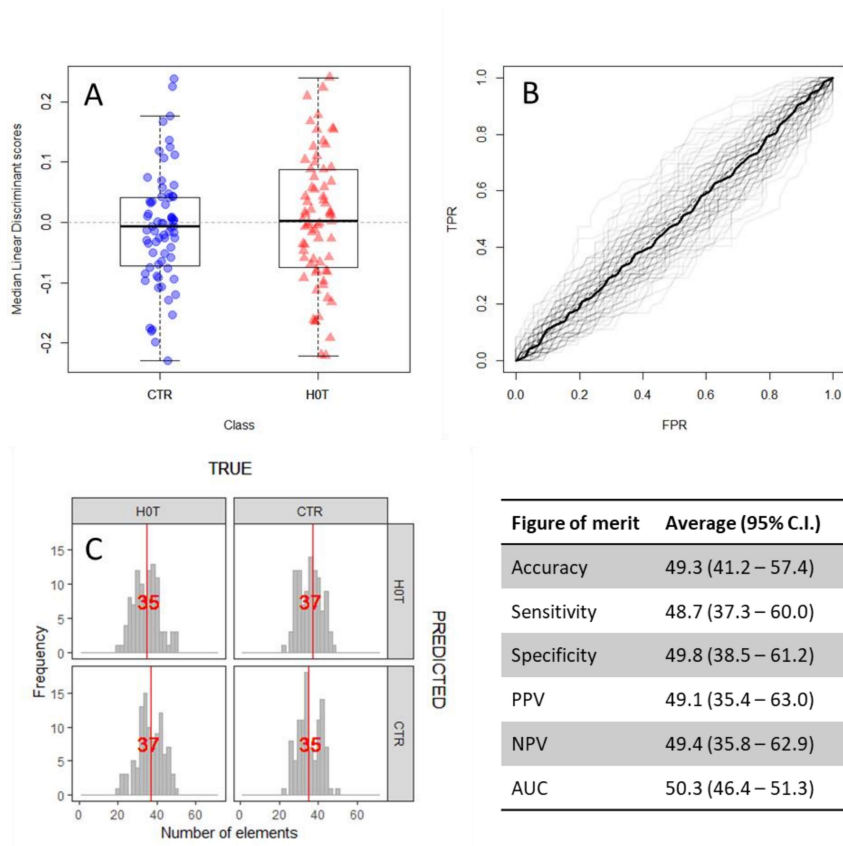
**Table S1** Detailed characteristics of the study population.



**Fig. S1** Spectral preprocessing: (A) un-processed data and spectral region used for cropping; (B) data after cropping; (C) examples of spectra before baseline subtraction, with calculated baseline reported as red lines; (D) the same spectra shown in (C) after baseline subtraction.



**Fig. S2** Cumulative proportion of explained Variance (A) for the first 20 principal components of the dataset, together with the loadings for the PC7, since the first 7 PC explain up to 90% of the spectral variance.



**Fig. S3** Results (corresponding to figures 3 and 4, and to table 2 or the manuscript) of a permutation test (with 100 permutations) for the optimized PCA-LDA model (i.e.  $n^{\circ}\text{PC} = 4$ ), in which the class labels were randomly shuffled (i.e. each sample has a class label randomly chosen). Results are those expected from pure chance (i.e. “zero hypothesis”  $H_0$  classification), with all Figures of Merit close to 50%, markedly different from the results obtained from the dataset with the correct class labels. In particular: Figures of Merit (bottom right) calculated from all the permutations; medians of the LD scores (A) for each sample, calculated over the different permutations; ROC curves (B) of all permutations (the average ROC is shown as non-transparent, black trace); (C) statistics for the Confusion Matrices resulting from the permutations (median values are shown in red).