Supplementary information

Raman spectroscopic Stratification of Multiple Myeloma patients based on Exosomes profiling

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Figure S1 – Raman spectrum of Exosome Resuspension buffer

Raman spectrum of Exosome Resuspension Buffer has been measured in order to investigate potential interferences with Raman spectra of Exosome samples from patients.

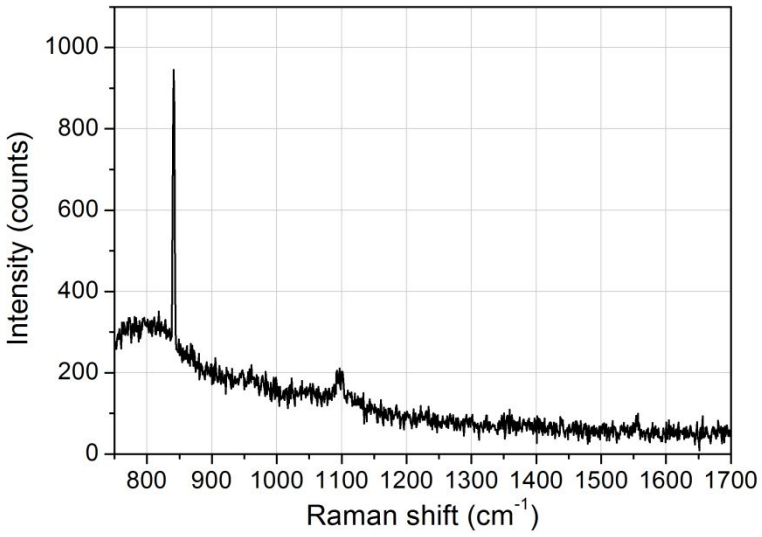

Figure S1: The buffer present in the miRCURY™ Exosome Isolation Kit and used for the exosome resuspension has been measured and its spectrum is here reported. Negligible Raman signal has been detected, except for a sharp peak at 860cm^{-1} which is however easily detectable in Raman measurements of exosomes.

Table S1 – Patients list

List of all patients from which exosomes have been extracted. Patient sex, neoplastic stage and age are reported too. The color code is in agreement with the colors reported in the main text.

ID Sample	Stage	Sex	Age (years)
CP	MGUS	M	81
LGP1	MGUS	M	45
1 CA	MGUS	M	80
2 CB	MGUS	M	54
22 CN	MGUS	F	76
24 GC	MGUS	M	61
38 SF	MGUS	F	51
48 CR	MGUS	F	53
59 SF	MGUS	F	51
8 VV	MGUS	M	58
12 BT	MGUS	F	63
36 DD	aMM	M	67
35 CL	aMM	F	73
11 VS	aMM	F	55
10 CD	aMM	F	54
IG	sMM	M	73
MF	sMM	F	48
FI	sMM	F	81
AR	sMM	F	70
SF	sMM	F	75
MC	sMM	F	81
DLM	sMM	F	61
PR	sMM	M	71
ZF	sMM	M	69
EM	sMM	F	67
BD	sMM	M	83
FF	sMM	M	69
DA	sMM	M	90

Figure S2 – Loading curve of rotated PC2

Rotated-PC2 loads, calculated after the rotation of the Principal Components in the PC1-PC2 plane.

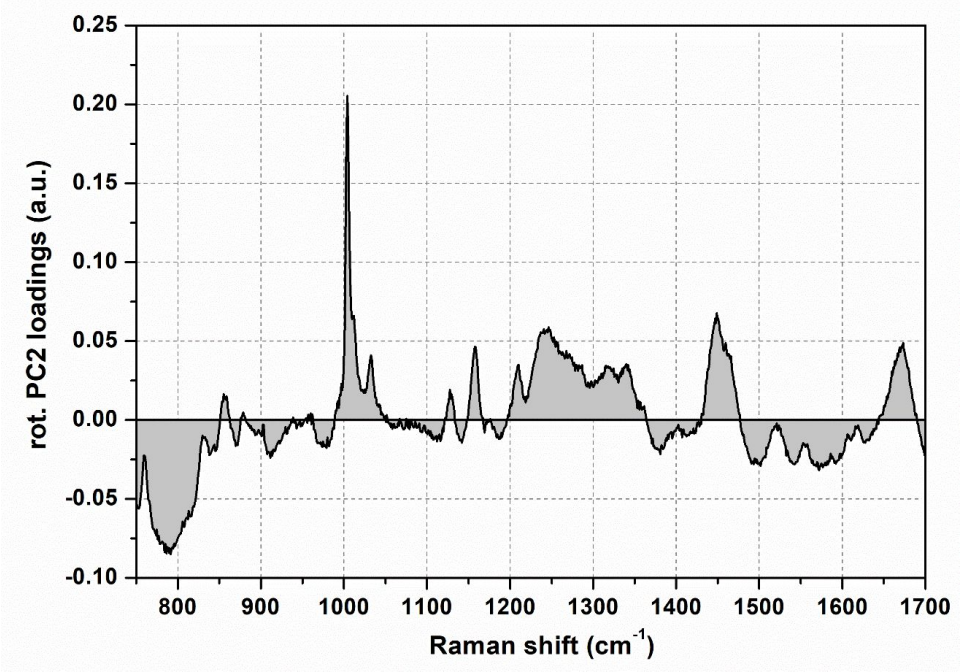

Figure S2: Rot.PC2 loading spectrum. It is a 32° counterclockwise rotated PC2 that is able, solely, to discriminate among all the exosome groups. The assignment of most important peaks can be found in the Table S2.

Table S2 – Assignment of the main peaks of the rot.PC2 loadings

Biochemical assignment of the main important peaks present in the rot.PC2 loading spectrum (Fig.S2 above). For the References of SI please see at the end of this document.

Raman Shift (cm^{-1})	Tentative Peak Assignment	Reference in SI
787	Nucleic Acids	[1]
850-860	Polysaccharide Structure	[2]
1003	Phenylalanine	[2]
1032	Phospholipid and/or Polysaccharide	[3]
1128	Proteins and/or Ceramides	[2]
1157	β-Carotene Accumulation	[1]
1209	Tryptophan and/or Phenylalanine	[1]
1235-1285	Proteins and/or Nucleic Acids and/or Lipids	[3]
1316	Nucleic Acids and/or Collagen and/or Guanine	[4]
1335-1345	Nucleic Acids (Purine Bases) and/or Tryptophan and/or Glycine Backbone and/or Proline Side Chain	[2]
1378	Carbohydrate and/or Nucleic Acids	[4]
1440-1450	Proteins and/or Lipids	[5]
1490-1500	Nucleic Acids	[1]
1539	Nucleic Acids	[6]
1560-1580	Tryptophan and/or Nucleic Acids and/or Proteins and/or Carbohydrates	[7]
1672	Ceramide	[1]

Table S2: Tentative Raman assignment, from literature, of the main peaks in rot.PC2 loadings. In red are reported the peaks downregulated in the sMM EX population, while in black the upregulated ones.

Figure S3 – SERS substrates

Scanning electron microscopy (SEM) picture of SERS substrates

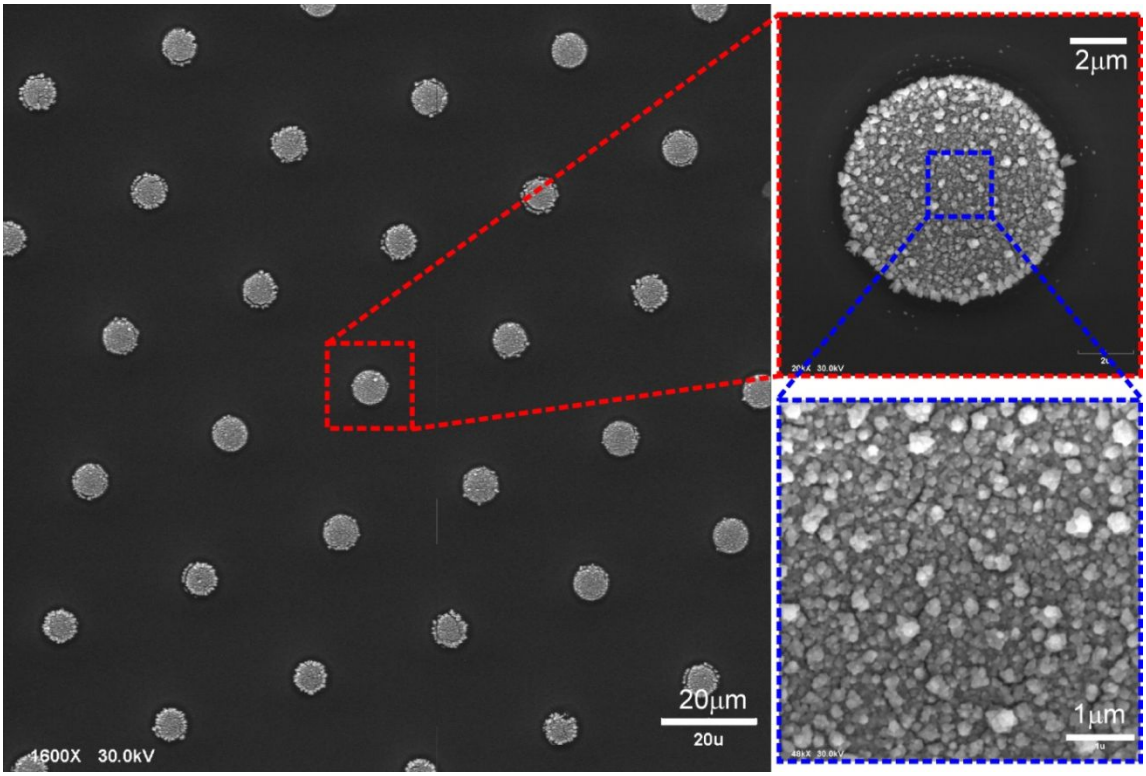

Figure S3: Scanning electron microscopy of SERS substrates. Microdisks containing Au nanoparticles are fabricated by means of optical lithography and Au electroless deposition.

Figure S4 – Raman spectra recorded with 633nm and 830nm excitation wavelengths

Comparison between standard Raman spectra recorded with 633nm and 830nm excitation wavelengths, in order to investigate potential wavelength-dependent effects.

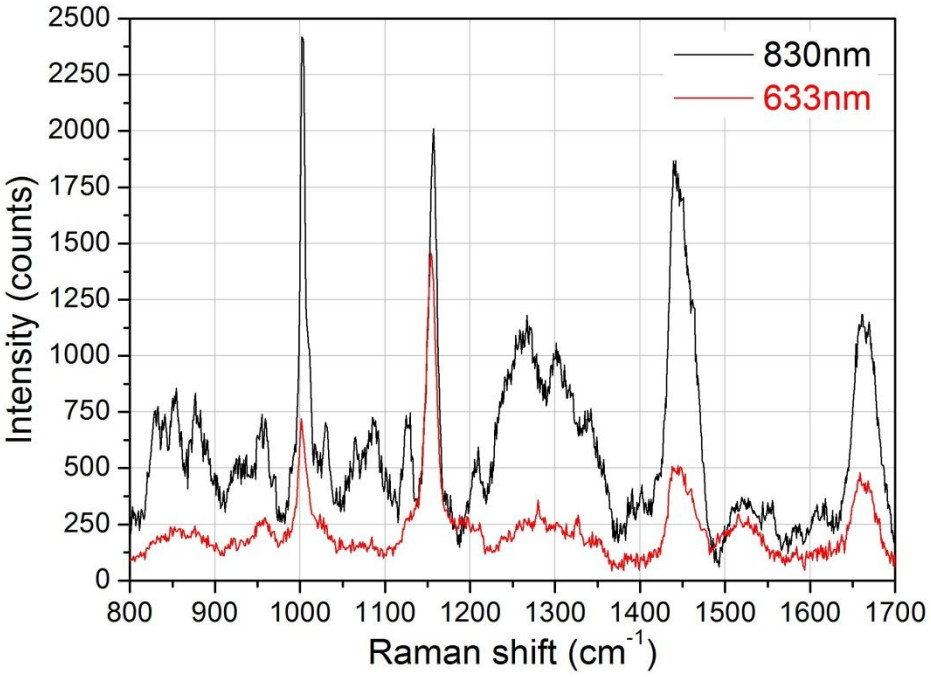

Figure S4: Raman spectra recorded on one of the Multiple Myeloma derived exosomes samples using two different excitation sources at 633nm and 830nm. It can be observed that all the major Raman peaks/bands are the same for the two sources, thus proving that no wavelegnth-dependent effects are present. Moreover some minor spectral features can be better appreciated with the 830nm laser, mainly due to the smaller fluorescence effects arising with IR sources.

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