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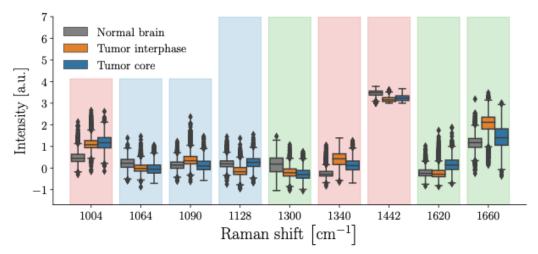
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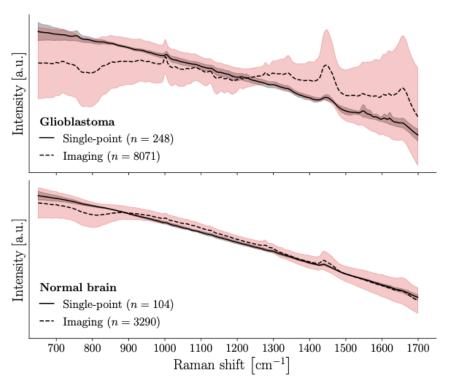


**Supplemental Table 1**. Prominent Raman peaks observed in glioblastoma and normal brain with corresponding molecular assignment and associated literature references.

Raman peak (cm <sup>-1</sup> )	Molecular assignment
1004	Phenylalanine tissue content <sup>38</sup>
1064	Carbon skeletal stretching of lipids <sup>38</sup>
1090	Carbon skeletal stretching of lipids <sup>38</sup>
1128	CH <sub>2</sub> wagging mode of lipids <sup>38</sup>
1300	CH <sub>2</sub> twist and wag, Amide III band of proteins <sup>38</sup>
1340	Tryptophan, CH <sub>2</sub> /CH <sub>3</sub> deformations in lipids and proteins
1430-1460	Multiple vibrational modes of carbon and hydrogen in lipids and proteins 41,42
1620	Aromatic amino acids (tyrosine, tryptophan, phenylalanine) 39-41
1660	Amide I band of proteins (C=O bond in peptide bonds) 38



**Supplemental Figure 1.** Differences between the intensity of inelastic scattering bands in spectra acquired with the Raman imaging system that were predicted as *Normal brain* or *Tumor*. The tumor spectra either belonged to specimens labeled *Tumor core* (cancer cells mixed with necrotic tissue) or *Tumor interphase* (no necrotic tissue). Boxplots are shown that were associated with the Raman band intensity highlighted (red, green, or blue) in Figure 3: 1004, 1064, 1090, 1128, 1300, 1340, 1442, 1620 and 1660 cm<sup>-1</sup>. The boxplots displayed the dataset minimum, first quartile (Q1), median (Q2), third quartile (Q3), and maximum values. The box spans Q1 to Q3, showing the interquartile range (IQR), while the line inside marks the median (Q2). The whiskers extend to the minimum and maximum within 1.5 times the IQR, and any points beyond are plotted individually as outliers. One-way ANOVA conducted between each tissue type, for each band, all resulted in p < 0.0001 except for *Normal brain* vs. *Tumor Interphase* at 1620 cm<sup>-1</sup> which resulted in p < 0.01.



**Supplemental Figure 2.** Average SNV-normalized Raman spectra prior to background removal for the single-point probe system (solid line) and the Raman imaging instrument (dashed line) datasets. Shadowed areas represent the standard deviation computed across all measurements. The first graph shows data from spectra associated with glioblastoma while the second graph shows data associated with normal brain.