SUPPLEMENTARY INFORMATION

SUPPLEMENTARY NOTE 1

Acquisition parameters and experimental conditions per time period:

2016: A fiber-optic probe produced by Emvision, LLC that allowed intrinsic fluorescent spectroscopy (IFS), diffuse reflectance spectroscopy (DRS) and Raman spectroscopy (RS) was used. The probe is connected to an illumination and detection assembly using 3 m long fiber-optic cable with 2.1 mm diameter. The cable and probe consist in nine low hydroxyl content 300 μm core silica fibers (of which seven are used to collect Raman scattered photon) and notch filter for Raman collection, and a central 272 μm core silica fiber with an inline short-pass filter for Raman excitation. A two-component lens at the tip of the probe ensures spatial correlation between RS and other modalities. The seven RS collection fibers combine into one optical connector and are connected to a spectrograph composed of a diffraction grating and high-resolution charge-coupled device (CCD) camera (ANDOR Technology). Excitation is provided by a 785 nm spectrum stabilized laser (Innovative Photonic Solutions)[1].

During this period, sequential acquisition of DRS, IFS and RS was performed on all samples. Patient population included 7 patients with stage 2–4 glioma, for a total of 70 brain samples. The acquisition protocol is described in the main text. For RS, one 50 ms background measurement (laser turned off) and three 50 ms RS measurements were performed on each sample. The output power at the end of the probe varied from 27 to 75 mW to maximize gain.

2017 (SR): A fiber-optic probe produced by Emvision LLC for RS was used. The probe consists in seven low-hydroxyl, 300 μm core silica fibers and a notch filter, surrounding a central 272 μm laser-delivery fiber with a small band-pass filter. The front lens consists in a Plano convex 2 mm diameter curvature sapphire black portion a flat front and a of 1mm thick Plano magnesium fluoride to limit interference from the sapphire while allowing overlap of the focus from the illuminated and collected lights. The probe's

collection system and its connection are identical as the one described above. The exception is the use of a ….[2]

During this period, the probe was used solely for RS. The configuration allowed to interrogate Raman signal for both the fingerprint and the high wavenumber spectral range. The population includes 4 patients undergoing neurosurgery for tumor resection of a glioma grade 2–4, for a total of 41 brain samples. Again, the acquisition protocol and parameter were identical as the previous study period.

2018 (ODS): The same probe and collection systems were used.

The patient population included 22 patients undergoing neurosurgical resection of a glioma grade 2–4, for a total of 276 brain samples. The acquisition protocol and parameter were identical as the previous study period, with the exception that every Raman measurement consisted in one 50 ms background measurement (laser turned off) and ten 50 ms RS measurements

2019 (TRIAL): The same probe and collection systems were used.

The patient population included 32 patients undergoing neurosurgical resection of a glioma grade 2–4, for a total of 160 brain samples. The acquisition protocol and parameter were identical as the previous study period, with the exception that every Raman measurement consisted in one 50 ms background measurement (laser turned off) and five 50 ms RS measurements.

The resulting spectra for each period are illustrated in **Supplementary Fig. 1**.

SUPPLEMENTARY NOTE 2

The fitting of the Raman peaks estimates three parameters: the peak location, width (more precisely, full-width-at-half-maximum), and height. The parameter estimation can fail at three points: 1) peak maximum search, 2) optimization of the fitting procedure and 3) estimation of the parameters for the gaussian distribution. First, if no local maximum is found in a range of +/− 5 cm^{-1}, the band with the maximum intensity in this range is used as the peak location and the gaussian function is fitted accordingly. Second, the gaussian fitting optimization could failed if it did not find an optimal solution after 1,000 iterations. Third, if the fitting was successful, the estimated parameters were compared to a range of "plausible" values according to the location of the target band and height and standard deviation of the signal at that location. If the optimization failed or any of the estimated parameters was flagged as erroneous, the values of all parameters were imputed: the maximum value of the spectra around the band was used as height, and deviation of the intensities around the maximum as width. Gaussian and Lorentzian densities were tested: fitting with Lorentzian function resulted in a normalized Root-mean-squared-error (nRMSE) at target band location of 0.128, while the nRMSE reduced to 0.119 with gaussian shapes. Gaussian shapes were used throughout the study.

Over all observations (n = 547), the variance across all spectra for each parameter was highly correlated with the quality assessment of the spectra ($F_{4, 24610} = 47.19$, $p < 0.0001$). Post-hoc analyses revealed that the estimated height and location of the peaks showed higher variance in low quality samples. This motivated the removal of poor-quality observations from the dataset; only higher-quality observations are considered (n = 223).

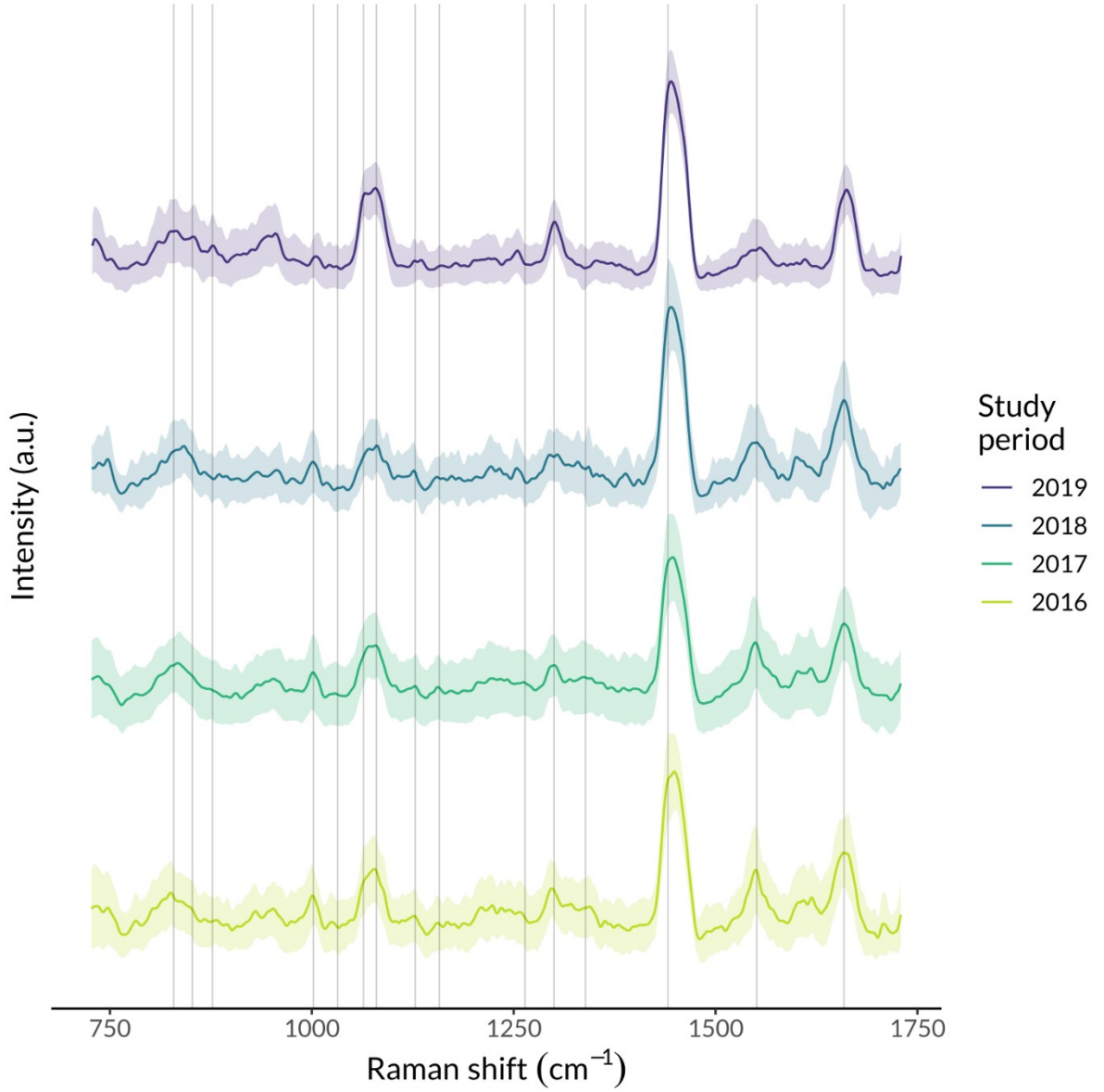
To assess the quality of the band fitting, we calculated the proportions of successful parameter estimation in the high-quality observations (15 target peaks over 223 spectra, for a total of 3,345 band fitting procedures). For 81.2% of the 3,345 peaks, all three parameters were successfully estimated. In all, 91.3% of total parameter estimations were successful. There is a slight variation of the fit quality between peaks: the bands at 1339, 1268 and 1032 cm^{-1} had the lowest rate of correct parameter estimation (81.6%, 84.3% and 85.7% respectively).

SUPPLEMENTARY TABLES

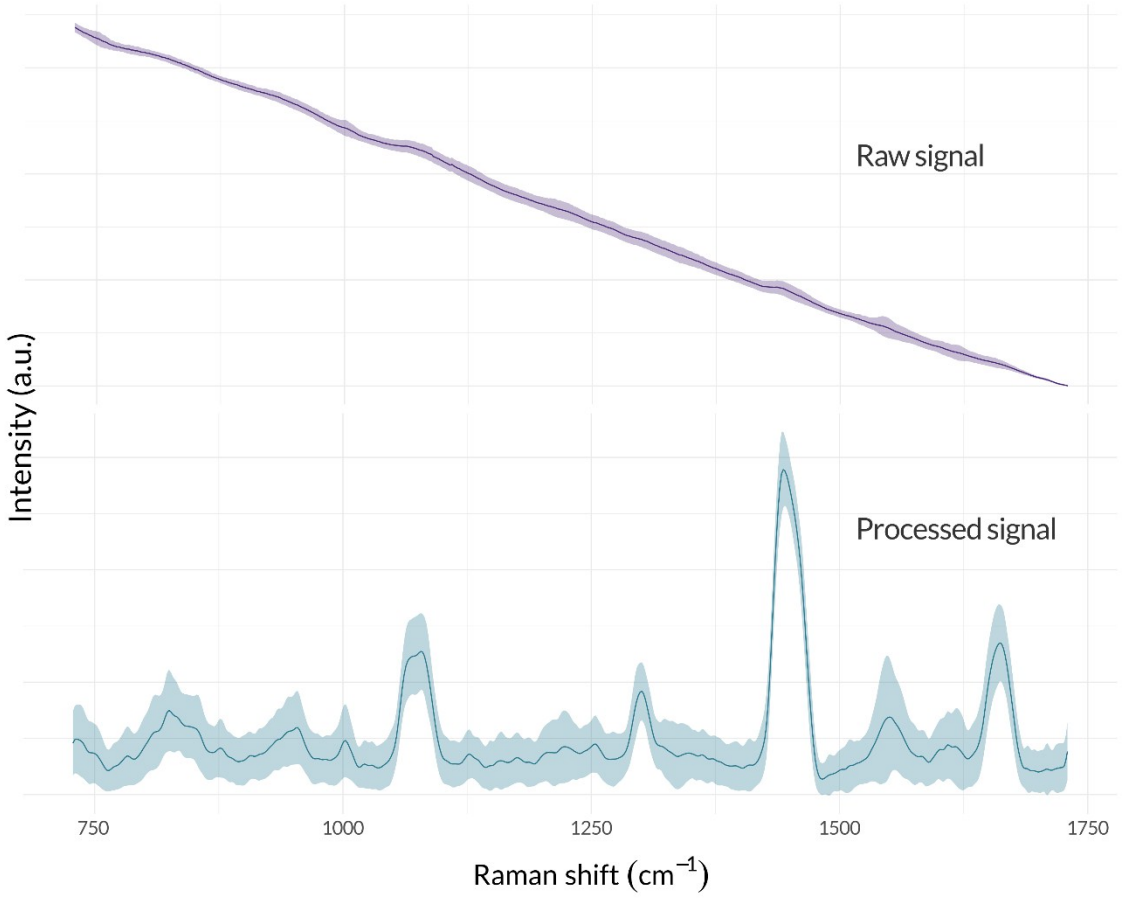
Table 1: Raman peaks reported in literature on brain tissue from 1999 to 2019.

Peak	N reported	Percentage reported	Raman shift range (cm^{-1})
1659	20	90.9	[1657 - 1661]
1441	19	86.4	[1436 - 1447]
1004	18	81.8	[1002 - 1008]
1300	17	77.3	[1296 - 1305]
1064	15	68.2	[1061 - 1068]
1339	14	63.6	[1333 - 1345]
1128	13	59.1	[1122 - 1132]
1268	13	59.1	[1262 - 1270]
829	9	40.9	[826 - 835]
852	9	40.9	[850 - 854]
877	9	40.9	[873 - 881]
1032	9	40.9	[1030 - 1035]
1087	9	40.9	[1081 - 1090]
1158	9	40.9	[1154 - 1161]
1553	9	40.9	[1550 - 1559]
785	8	36.4	[780 - 788]
936	8	36.4	[934 - 940]
1251	8	36.4	[1247 - 1255]
1175	7	31.8	[1172 - 1179]
1208	7	31.8	[1206 - 1212]
1671	7	31.8	[1669 - 1673]
757	6	27.3	[754 - 760]
1449	6	27.3	[1446 - 1453]
1523	6	27.3	[1518 - 1527]
925	5	22.7	[921 - 927]
1096	5	22.7	[1093 - 1100]
1583	5	22.7	[1581 - 1585]
1731	5	22.7	[1725 - 1735]
960	4	18.2	[958 - 962]
1315	4	18.2	[1313 - 1318]
1633	4	18.2	[1629 - 1635]
1742	4	18.2	[1739 - 1746]
1240	3	13.6	[1239 - 1240]
1375	3	13.6	[1374 - 1376]
1420	3	13.6	[1419 - 1422]
1563	3	13.6	[1560 - 1566]
1613	3	13.6	[1610 - 1614]
1656	3	13.6	[1655 - 1657]
748	2	9.1	[746 - 751]
898	2	9.1	[896 - 900]
1106	2	9.1	[1104 - 1107]
1226	2	9.1	[1225 - 1226]
1260	2	9.1	[1259 - 1260]
1275	2	9.1	[1272 - 1278]
1487	2	9.1	[1486 - 1488]
1577	2	9.1	[1576 - 1578]
1604	2	9.1	[1604 - 1604]
1668	2	9.1	[1668 - 1668]
739	1	4.5	[739 - 739]
845	1	4.5	[845 - 845]
865	1	4.5	[865 - 865]
954	1	4.5	[954 - 954]
977	1	4.5	[977 - 977]
1133	1	4.5	[1133 - 1133]
1199	1	4.5	[1199 - 1199]
1255	1	4.5	[1255 - 1255]
1295	1	4.5	[1295 - 1295]
1397	1	4.5	[1397 - 1397]
1461	1	4.5	[1461 - 1461]
1616	1	4.5	[1616 - 1616]
1623	1	4.5	[1623 - 1623]

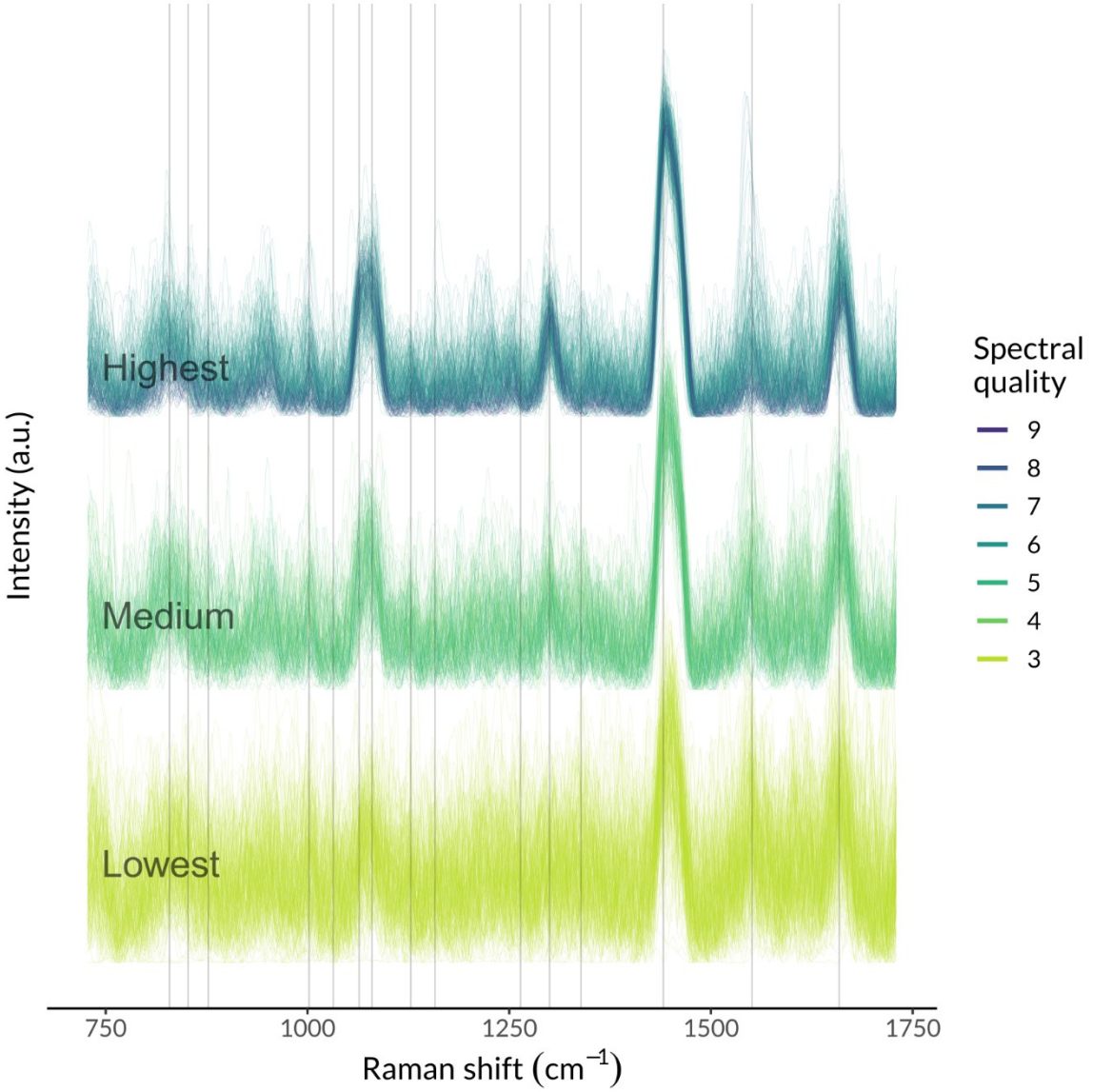
SUPPLEMENTARY FIGURES



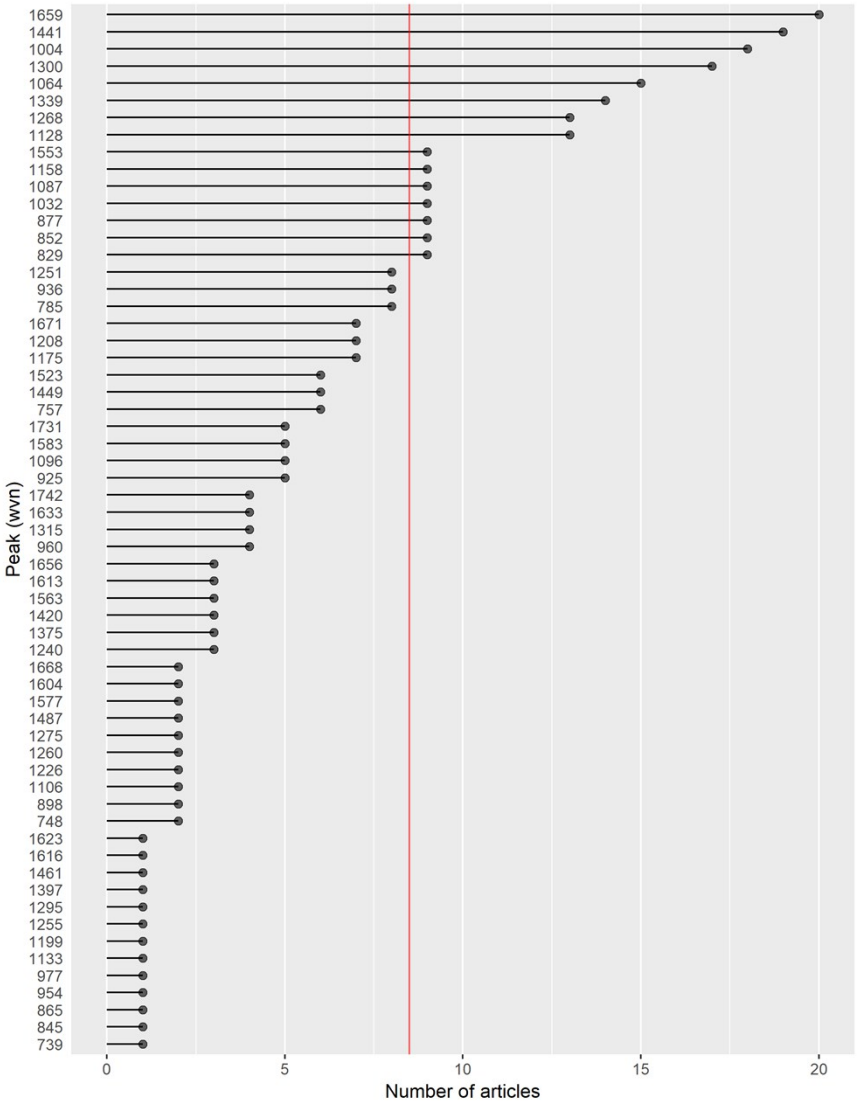
Supplementary Figure 1: Averaged Raman spectra with standard deviation acquired in human brain samples during neurosurgical glioma resection, stratified by acquisition time periods. The vertical lines represent the 15 target bands used in the statistical analysis.



Supplementary Figure 2 : Averaged Raman acquisitions before ("Raw spectra") and after data processing.



Supplementary Figure 3: All individual Raman acquisitions (*n* = 547) stratified by data quality. Data quality was assessed by three independent reviewers with a rating of 1 (lower quality) to 3 (higher quality). The final score is the sum of all three ratings. A score of 5 or higher ("Highest" in the figure) was set as cut-off before statistical analysis. The vertical lines represent the 15 target bands used in the statistical analysis.



Supplementary Figure 4 : All Raman bands mentioned in the literature on Raman spectroscopy of brain tissue from 1999–2019. The red line indicates the cut-off for bands that were selected as target bands in the study.