**SUPPLEMENTARY MATERIAL**

**Supplementary Table**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Band (cm-1)** | **Dominant** | **Molecular Species** | **Details** | **p-value** |
| 614 | Cancer | cholesterol |  | 0.0007 |
| 647 | Normal | proteins | phenylalanine (C-C twist) | < 0.0001 |
| 727 | Cancer | nucleic acid, proteins, lipids |  | 0.0125 |
| 742 | Cancer | nucleic acid, proteins | tryptophan | < 0.0001 |
| 767 | Cancer | nucleic acid |  | 0.5458 |
| 845 | Normal | cholesterol |  | 0.1658 |
| 863 | Normal | lipids |  | 0.0040 |
| 883 | Normal | proteins | proline, valine, tryptophan, CH2 rock | < 0.0001 |
| 926 | Normal | cholesterol, proteins, lipids | proline, valine | < 0.0001 |
| 940 | Normal | proteins | C-C stretching, alpha helix | < 0.0001 |
| 957 | Normal | cholesterol |  | 0.0034 |
| 1005 | N/A | proteins | breathing mode of phenylalanine | 0.8452 |
| 1036 | Normal | proteins | collagen | < 0.0001 |
| 1053 | Normal | proteins | C-O stretching, C-N stretching | < 0.0001 |
| 1096 | Cancer | nucleic acid |  | 0.0734 |
| 1130 | N/A | cholesterol, lipids, proteins | C-C stretching | 0.9842 |
| 1171 | Normal | nucleic acid, proteins | tyrosine (C-H bending) | < 0.0001 |
| 1220 | Normal | proteins | C=N=C stretching, amide III | < 0.0001 |
| 1270 | Cancer | lipids, proteins | amide III | < 0.0001 |
| 1296 | Cancer | lipids | CH2 deformation | < 0.0001 |
| 1309 | Cancer | lipids | CH2 twisting and bending | 0.0008 |
| 1324 | Cancer | proteins, nucleic acid | collagen, purine (CH3CH2 wagging) | < 0.0001 |
| 1335 | Cancer | nucleic acid |  | < 0.0001 |
| 1441 | Cancer | cholesterol, lipids, proteins | CH2 deformation, CH2 bending collagen | < 0.0001 |
| 1456 | Cancer | lipids | phospholipids | < 0.0001 |
| 1488 | Normal | nucleic acid, proteins | amide II | < 0.0001 |
| 1554 | Cancer | proteins | amide II | 0.4101 |
| 1580 | Cancer | nucleic acid |  | 0.0275 |
| 1600 | Cancer | proteins | amide I | < 0.0001 |
| 1611 | Cancer | nucleic acid |  | < 0.0001 |
| 1628 | Cancer | proteins | amide I | 0.0006 |
| 1634 | Cancer | proteins | amide I | < 0.0001 |
| 1656 | Cancer | proteins | amide I | < 0.0001 |
| 1667 | Cancer | proteins | amide I, alpha helix, collagen, elastin | 0.0024 |
| 1700 | Normal | proteins | amide I | < 0.0001 |

**Supplementary Table S1**. Summary of prominent Raman peaks and tissue bands, with details of the associated molecular species. For each peak, a bootstrapping student t-test was applied for cancer tissue vs. normal, and the corresponding p-value is provided. See Table 1 for sample sizes. The RS spectra show bands associated with proteins for amide I (for which an important component is C=O stretches) at 1600 cm-1, 1628 cm-1, 1634 cm-1, 1656 cm-1, 1667 cm-1 and 1700 cm-1; amide II (for which the main contributions are N-H bending and C-N stretches around 1550) at 1242 cm-1, 1488 cm-1, 1554 cm-1; amide III (for which the main contributions are also N-H bending and C-N stretches around 1300) at 1220 cm-1, 1270 cm-1; C-C twisting of phenylalanine at 647 cm-1; the breathing mode of phenylalanine at 1005 cm-1; tryptophan at 742 cm-1, 883 cm-1; proline and valine at 883 cm-1, 926 cm-1; collagen at 1036 cm-1, 1324 cm-1, 1441 cm-1. Cancer tissue displays increases in many of the bands associated with nucleic acids, identified at 727 cm-1, 742 cm-1, 767 cm-1, 1096 cm-1, 1171 cm-1, 1324 cm-1, 1334 cm-1, 1488 cm-1, 1580 cm-1, 1611 cm-1. Bands associated with lipids and phospholipids were observed at 727 cm-1, 863 cm-1, 1130 cm-1, 1270 cm-1, 1296 cm-1, 1380 cm-1, 1441 cm-1, 1456 cm-1. Bands associated with cholesterol/cholesterol esters were identified at 614 cm-1, 845 cm-1, 926 cm-1, 957 cm-1, 1130 cm-1, 1441 cm-1.

**Supplementary Figures**



**Supplementary Figure S1.** **(A)** Histopathology images (H&E) are shown for 3 samples from patients with colon brain metastasis (left), lung metastasis (middle), and melanoma that metastasized to the brain (right). **(B)** The corresponding spectra obtained for each sample using RS, DRS, and IFS.



**Supplementary Figure S2.** Boxplots of the Raman spectra at prominent peaks, showing increases in cancer tissue. See Table 1 for sample sizes.



**Supplementary Figure S3.** Boxplots of the Raman spectra at prominent peaks, showing increases in normal brain tissue. See Table 1 for sample sizes.



**Supplementary Figure S4.** **(A-B)** Boxplots of the IFS spectra integrated under the curve, as a measure of bulk fluorescence. A bootstrapping student t-test was applied for cancer tissue vs. normal, and the corresponding p-value were p = 0.8 for tissue excitation at 365 nm and p = 1 for excitation at 455 nm. **(C-D)** The effect of the number of spectral bands used for the IFS spectra for tissue classification on the classification accuracy. The fitted polynomial curves of order 3 are displayed. See Table 1 for sample sizes. This illustrates the importance of using spectroscopic intrinsic fluorescence for cancer detection, based on spectral deformations from different tissues, rather than single band or bulk fluorescence analysis. In fact when using only the total fluorescence intensity (sum over all spectral bands), univariate statistical testing for cancer vs. normal tissue leads to p values > 0.7 for both fluorescence wavelengths (**Figure S3**).

**Supplementary Methods**

**Data processing for IFS and DRS**

Firstly, the diffuse reflectance signals needs to be calibrated in order to be able to extract useful and quantitative information. The trajectory of light in the current system can be described as:

[eq. 1]

where:

= signal measured by the spectrometer when the sample is excited by the white broadband light (intensity in a.u.)

= white source spectrum

= real reflectance spectrum of the sample

= probe’s transmission (inner fibers and filters included)

= optical switch’s transmission

= optical fibers’ transmission

= response due to the detection system (spectrometer and connexion)

When a reference material with a known reflectance spectrum is used as the excited sample, it is possible to remove unknown but constant variables in this equation by a calibration procedure. A chemically inert optical standard called spectralon (Labsphere, Inc., New Hampshire, USA) is used here, with a flat spectrum from 250 to 2 500 nm and 99 % of reflection. The next formula then allows to extract the real reflectance spectrum of any imaged samples:

[eq. 2]

where:

= signal measured by the spectrometer when the spectralon is excited by the white broadband light (see eq. 1).

The IFS spectra are calibrated using a SRM 2940 relative intensity correction standard for fluorescence spectroscopy (National Institute of Standards and Technology, Maryland, USA) in order to determine instrument response. Wavelet decomposition is used to find spectral components in the raw IFS spectra. Then a least-squares spectral unmixing approach is used on every raw IFS spectrum, using the calculated instrument response and wavelet spectral components as base spectra, in order to eliminate instrument response and wavelet components that are similar to the instrument response function from the spectra. This calibration procedure is used only for the purpose of visually displaying the IFS spectra in the paper figures, not for spectral analysis. Calibration is performed for each acquisition of DRS and IFS. The region of interest is then selected for each wavelength according to their long-pass filter threshold.