Electronic Supplementary Material (ESI) for Analyst

Depth of penetration as a function of angle of incidence and wavenumber

Fig. S1 and Fig. S2 compare the d_e and d_p from samples of different refractive indices. For water, these values converge at ≈ 40°; whilst for tissue specimen, this occurs at ≈ 35°, thus in our study where the angle falls between 35°- 40°, both Eq. 3 and Eq. 4 are similarly applicable. The angle where d_e and d_p is equal decreases when n_2 increases, depicted in Fig. S 3. All calculations are carried out with Ge crystal with a refractive index of 4.0.

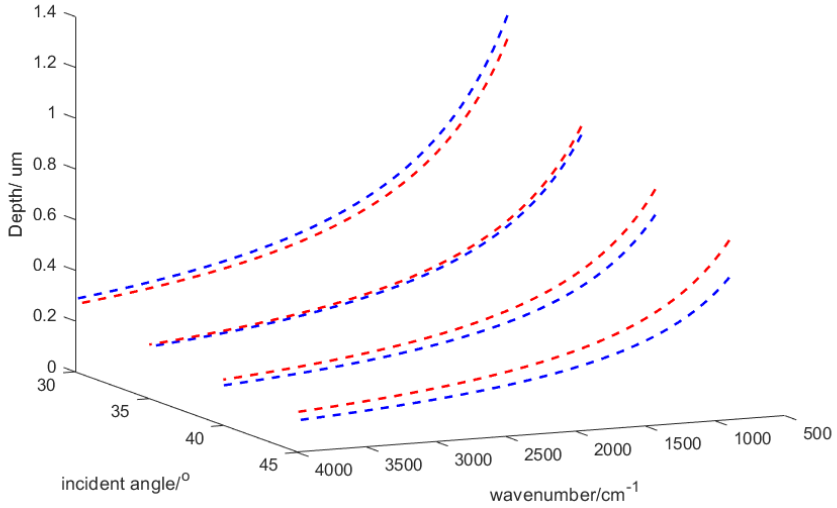

Fig. S 1 Plot of depth as a function of angle of incidence and wavenumber of a tissue sample (n = 1.45), blue line is for d_p while red line is for d_e. With the same IRE (Ge), both values differ from each other and the difference increases with wavenumber, but the values converge at an angle depending on the refractive index of the sample investigated.

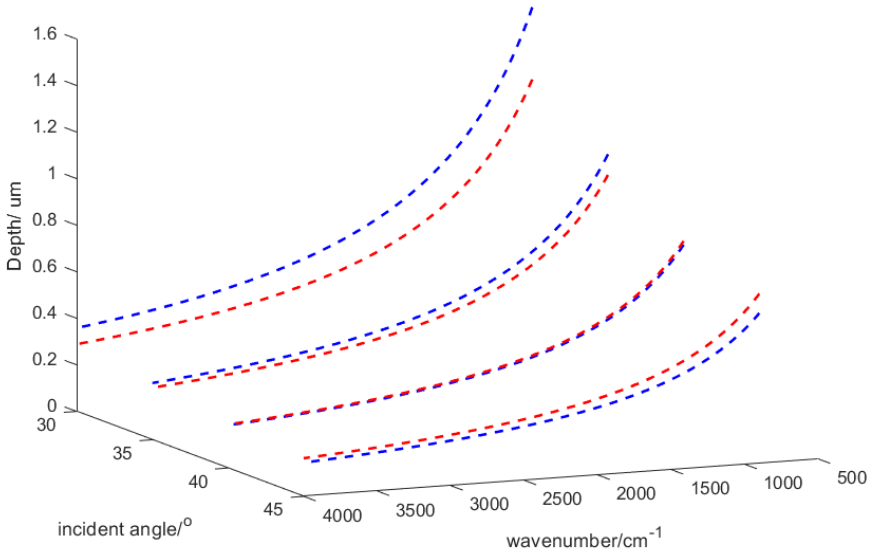

Fig. S 2 Plot of depth as a function of angle of incidence and wavenumber of liquid water (n = 1.45), blue line is for d_p while red line is for d_e.

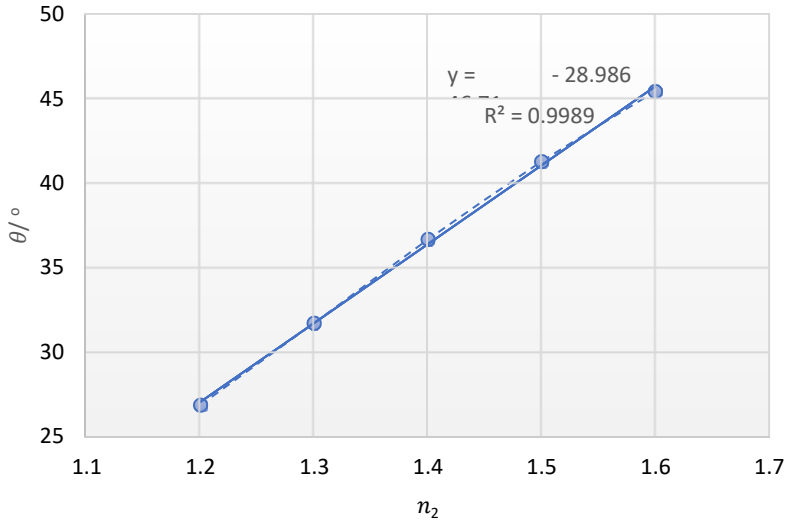

Fig. S 3 Plot of the angle of incidence where d_p is equal to d_e at various refractive indices (n_2) of sample. A linear correlation can be inferred from the figure; as n_2 increases, the angle increases as well.

Water spectra

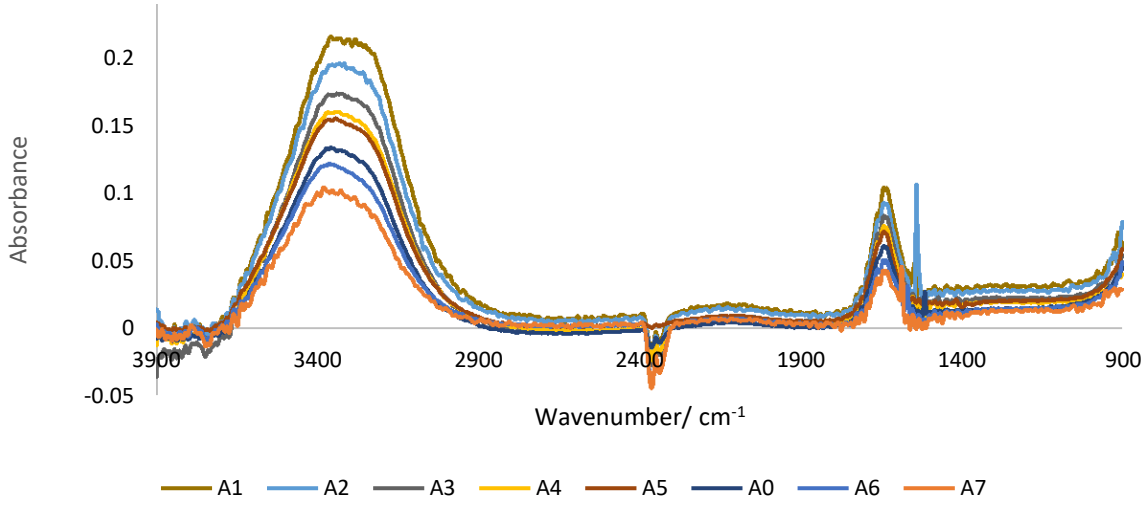

Fig. S 4 Mean IR absorption spectra of liquid water from all pixels within field of view measured with apertures at various angles of incidence.

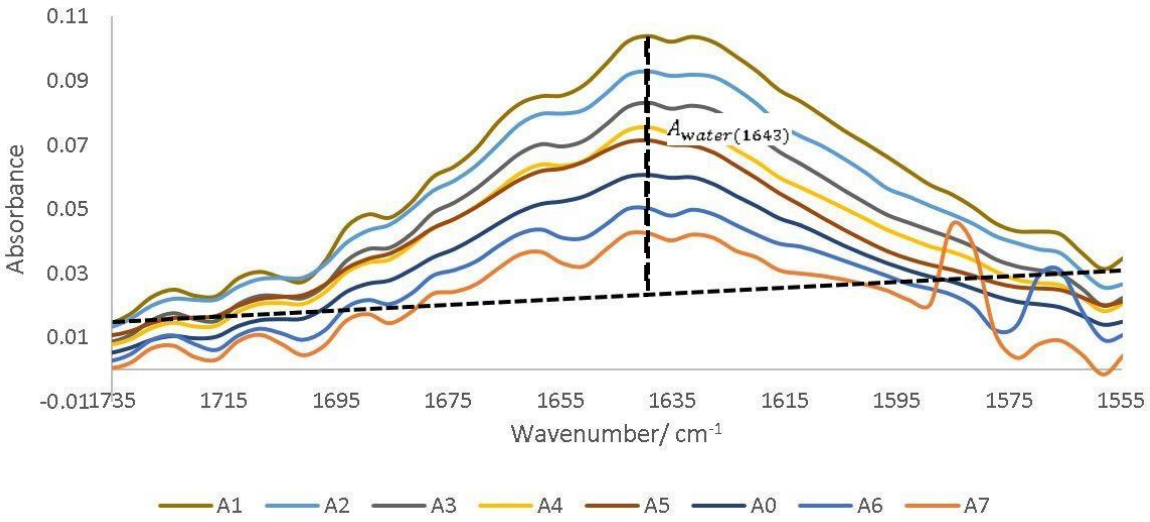

Fig. S 5 Zoom in absorbance of the spectral band corresponding to the bending mode of water in the region of 1735 – 1555 cm^{-1} (peak absorbance at 1643 cm^{-1}) measured with different apertures. This wavenumber range coincides with the spectral absorption for Amide I and Amide II of biological specimens. Absorbance at 1662 cm^{-1}, 1677 cm^{-1}, 1689 cm^{-1}, 1708 cm^{-1}, and 1724 cm^{-1} are attributed to the presence of spectral bands of water vapour (J.D. Ingle Jr. and S.R. Crouch, Spectrochemical Analysis, p. 409. Eaglewood Cliffs, Prentice-Hall, 1988).

Spatial resolution of the images

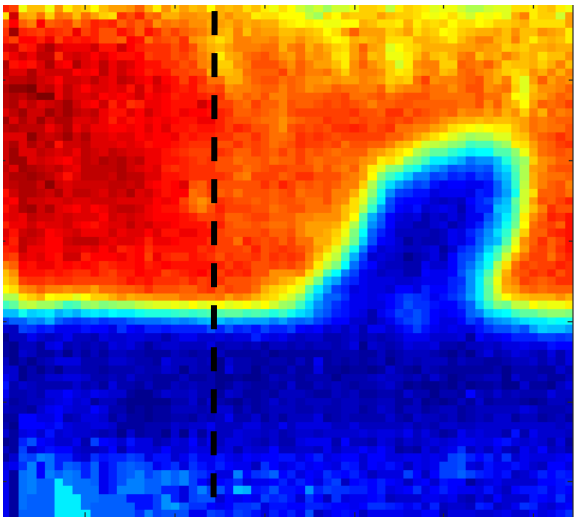

Fig. S 6 Chemical image of a polyurethane/PMMA interface, horizontally aligned for vertical resolution to be measured, constructed at 1600 cm^{-1}.

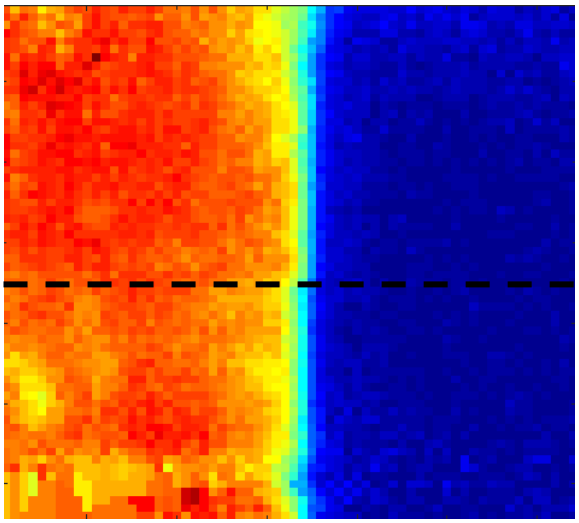

Fig. S 7 Chemical image of a polyurethane/PMMA interface, vertically aligned for horizontal resolution to be measured, constructed at 1600 cm^{-1}.

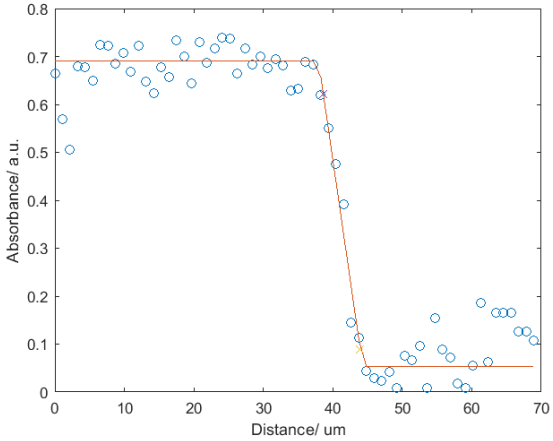

Fig. S 8 Plot of absorbance vs distance (top to bottom) along the dotted line in Fig. S6 at 1600 cm^{-1}. Drawn in red is the line of best fit.

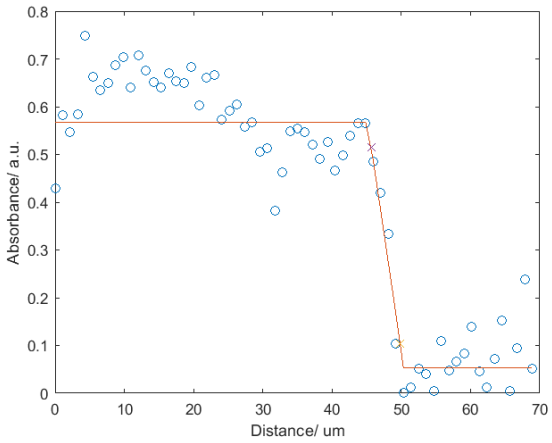

Fig. S 9 Plot of absorbance vs distance (left to right) along the dotted line in Fig. S6 at 1600 cm^{-1}. Drawn in red is the line of best fit.

	Vertical resolution	Horizontal resolution
Angle of incidence	38.2 °	
Maximum value of line of best fit @ 1600 cm^{-1}	0.6882	0.5668
Maximum value of line of best fit @ 1600 cm^{-1}	0.2226	0.0530
Distance at 90% of maximum value	38.6243 (0.6216)	45.6614 (0.5154)
Distance at 10% of maximum value	43.9383 (0.0892)	49.8386 (0.1044)
Difference in distance (µm)	5.3140	4.1772

Table S 1 Calculation showing the distance or resolution in µm. The values are different for vertical and horizontal resolution and is dependent on angle of incidence, which can be approximated by taking the inverse cosine of the ratio of resolution in both directions. The resolution is calculated by taking the distance between 90% and 10% of the maximum absorbance.

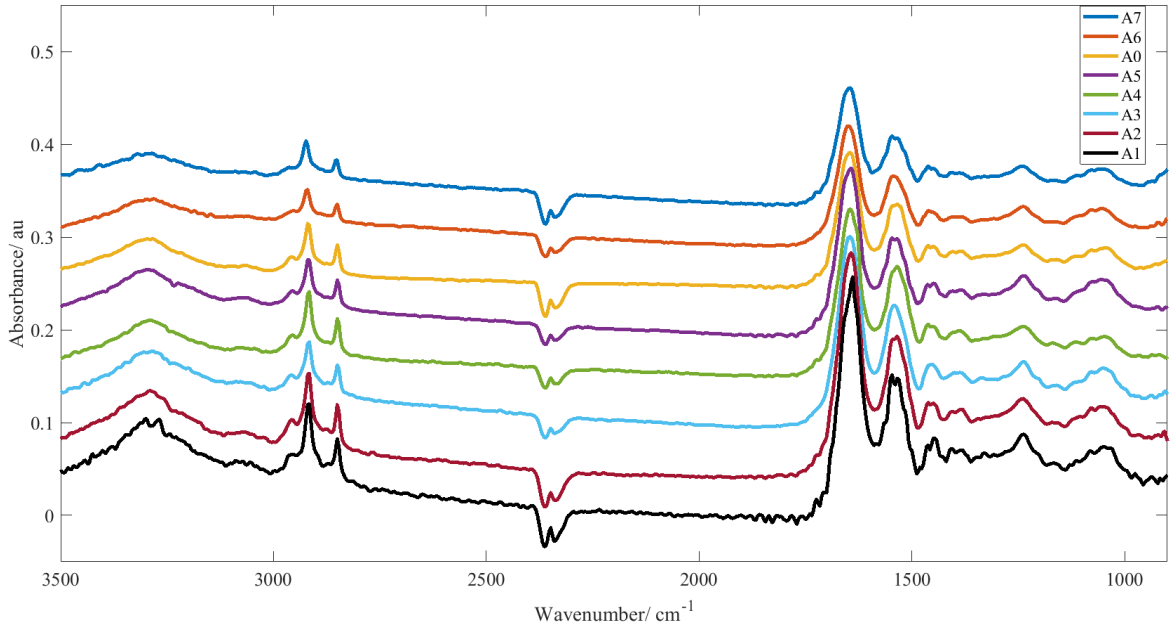

Fig. S 10 Averaged raw spectra obtained from a 3 × 3 binned tissue area (centre coordinates: x = 50; y = 40). For clarity and easier comparison, all spectra were plotted on the same scale with an offset of 0.05 from one another.

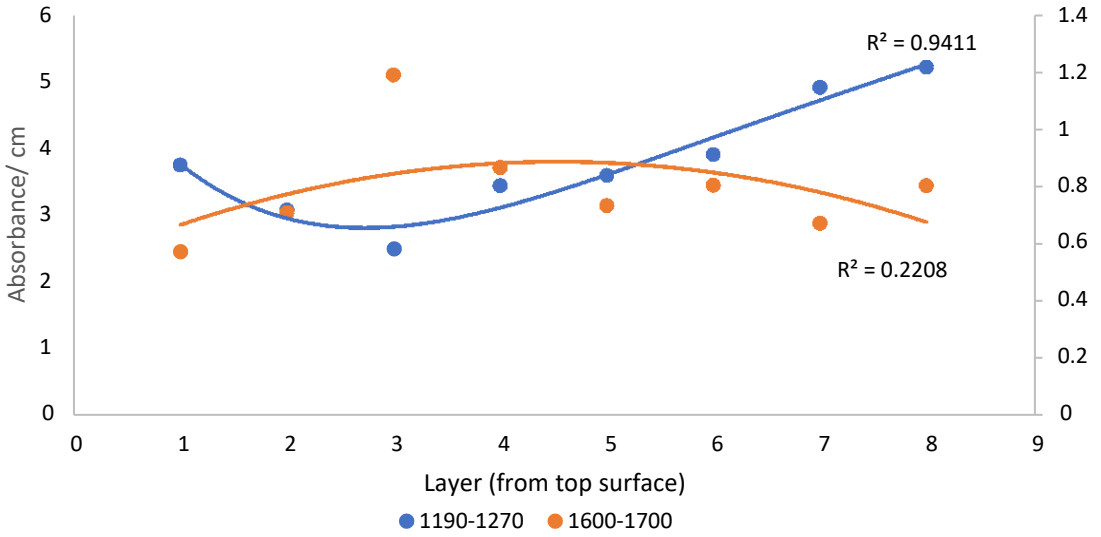

Fig. S 11 Mean integrated absorbance of a single cell in the square area shown in fig 9 in the main article at 1270 – 1190 cm^{-1} (orange) and 1700 – 1600 cm^{-1}(blue) measured for different layers of the sample.

Spectra of tissue samples

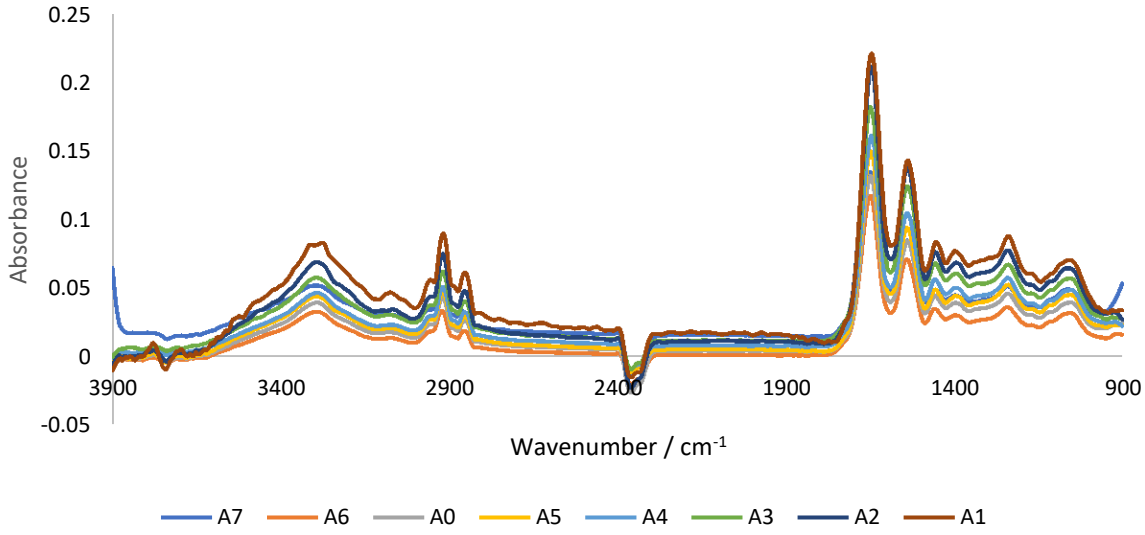

Fig. S 12 Mean ATR-FTIR spectra in the 3900 – 900 cm^{-1} region of prostate cancer tissue.

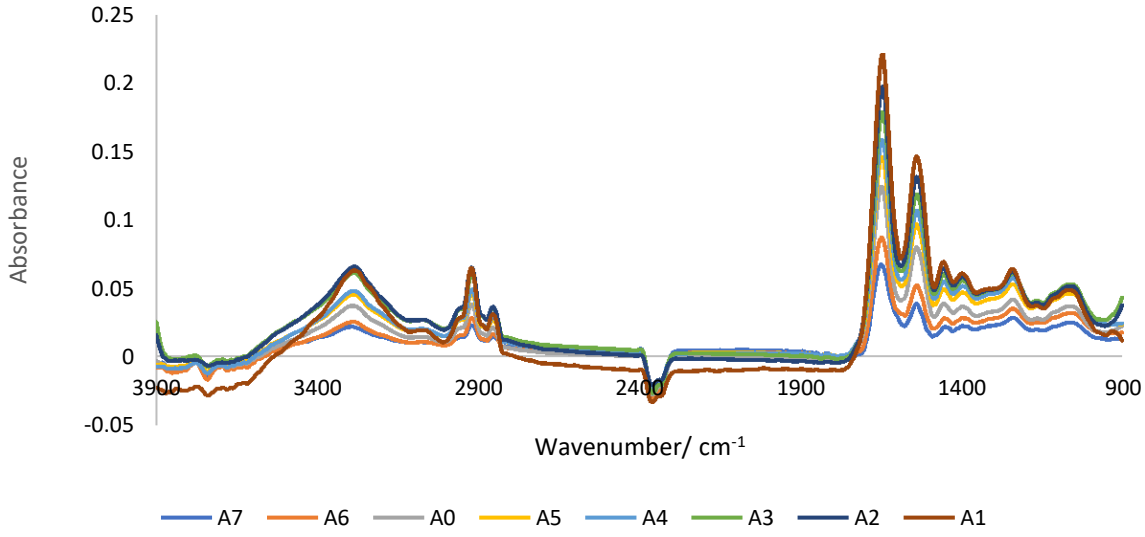

Fig. S 13 Mean ATR-FTIR spectra in the 3900 – 900 cm^{-1} region of healthy prostate tissue.

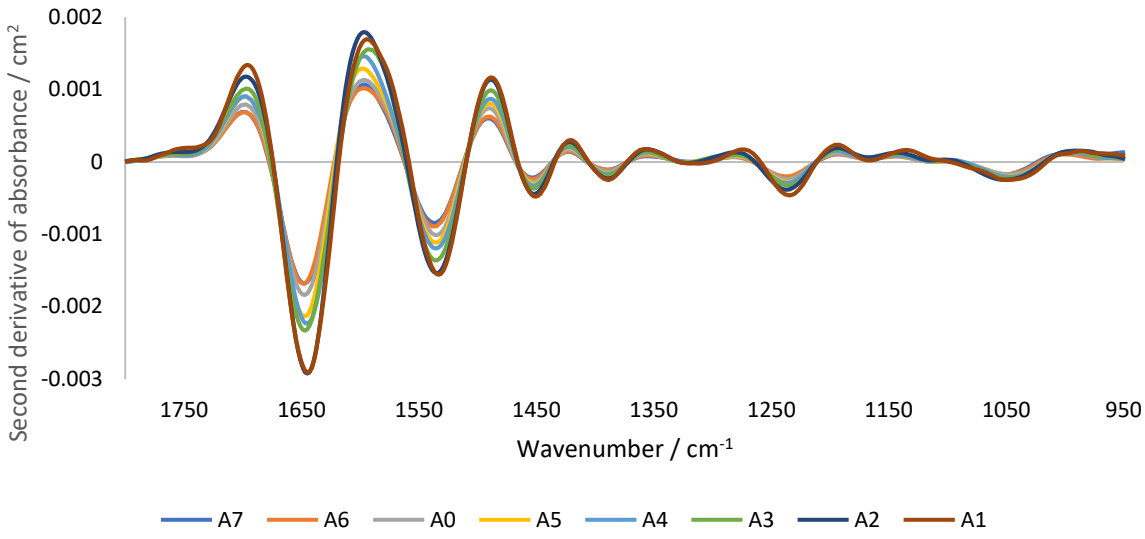

Fig. S 14 Mean second derivative spectra in the 1800 – 950 cm^{-1} region of prostate cancer tissue.

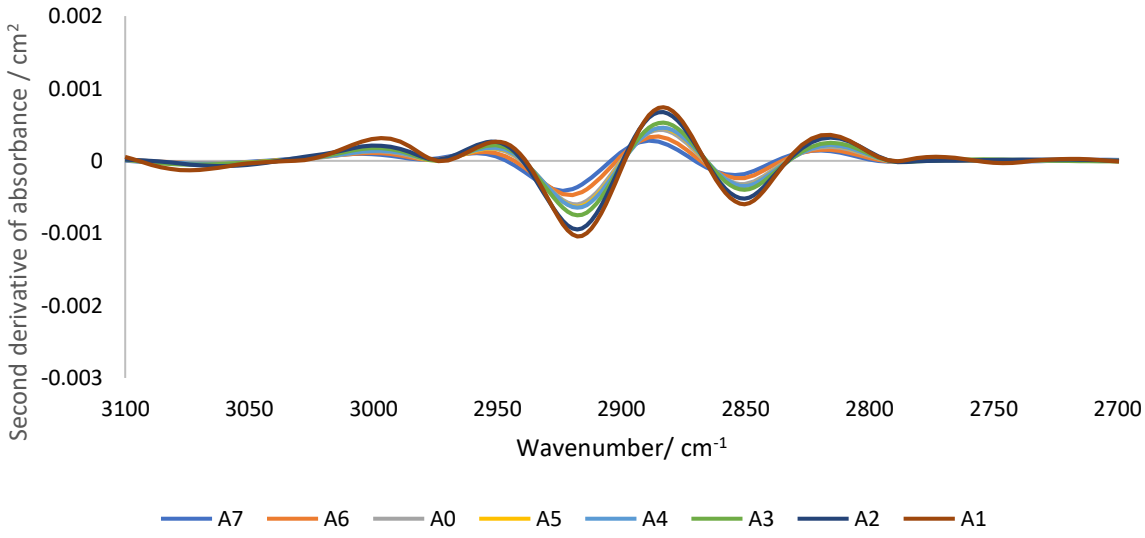

Fig. S 15 Mean second derivative spectra in the 3100 – 2700 cm^{-1} region of prostate cancer tissue.

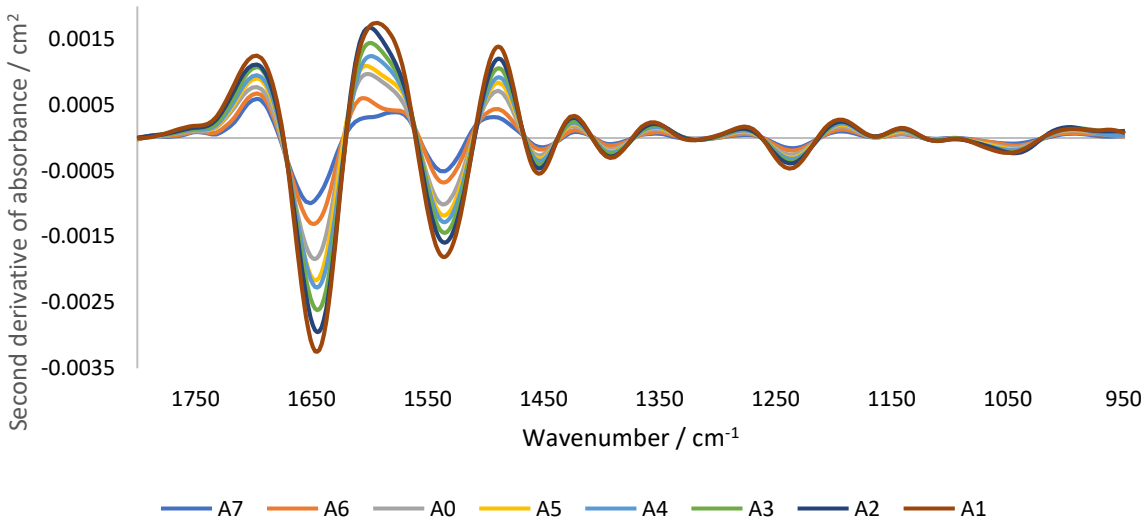

Fig. S 16 Mean second derivative spectra in the 1800 – 950 cm^{-1} region of healthy prostate tissue.

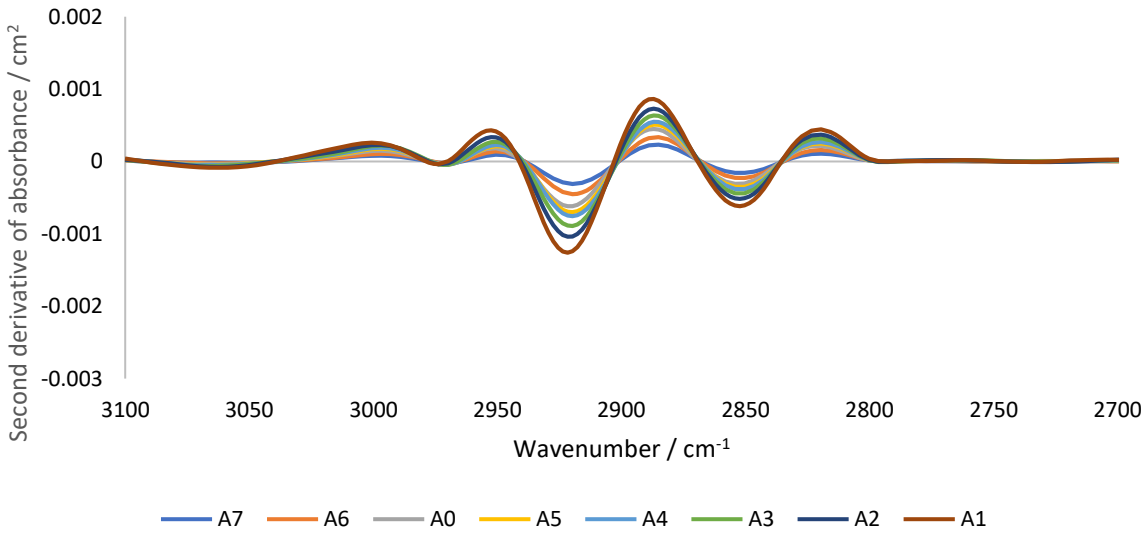

Fig. S 17 Mean second derivative spectra in the 3100 – 2700 cm^{-1} region of healthy prostate tissue.

***t*-test analysis**

Wavenumber/cm^{-1}	995 - 1087	1087 - 1137	1137 - 1191	1191 - 1272	1272 - 1353	1353 - 1423	1423 - 1486
	0	1	1	1	1	0	0
A7	0.1030	0.0007	0.0000	0.0000	0.0000	0.7250	0.2341
	0	1	0	1	1	1	0
A6	0.1734	0.0000	0.0514	0.0005	0.0009	0.0005	0.4879
	0	1	0	0	0	0	1
A0	0.0300	0.0000	0.8487	0.0731	0.9403	0.0288	0.0001
	0	1	0	0	0	0	1
A5	0.0529	0.0001	0.8772	0.0766	0.9466	0.0839	0.0007
	0	1	0	0	0	0	1
A4	0.4197	0.0001	0.2297	0.3998	0.9088	0.0636	0.0018
	1	1	0	0	0	0	0
A3	0.0000	0.0001	0.5705	0.1767	0.9755	0.0879	0.1641
	1	1	0	1	0	0	0
A2	0.0000	0.0000	0.7902	0.0003	0.5125	0.1022	0.0447
	1	1	0	0	0	0	0
A1	0.0001	0.0009	0.0395	0.5488	0.0278	0.1989	0.1554

Wavenumber/cm^{-1}	1488 - 1600	1600 - 1697	1697 - 1754		2819 - 2884	2884 - 2950	2950 - 3000
	0	0	1		0	0	0
A7	0.7950	0.1277	0.0000		0.7794	0.1864	0.0416
	0	0	1		0	0	0
A6	0.7319	0.0641	0.0005		0.9022	0.0456	0.1488
	0	0	0		0	0	0
A0	0.2242	0.0750	0.3300		0.8177	0.1754	0.1538
	0	0	0		0	0	0
A5	0.3919	0.2648	0.1606		0.7547	0.5063	0.6934
	0	0	1		0	0	0
A4	0.6729	0.5526	0.0000		0.6680	0.7663	0.8295
	0	0	1		0	0	0
A3	0.4900	0.9306	0.0000		0.8045	0.9097	0.5444
	0	0	0		0	0	0
A2	0.0239	0.2007	0.0501		0.8364	0.6166	0.9647
	0	0	0		0	0	0
A1	0.2680	0.6604	0.0243		0.5676	0.9654	0.6022

Table S 2 Results of t-test analysis at each range of wavenumber specified. For each aperture, the top row gives the value 0 or 1 (1 is returned if the null hypothesis is rejected, otherwise 0 is returned) and bottom row gives the p value.

PCA analysis

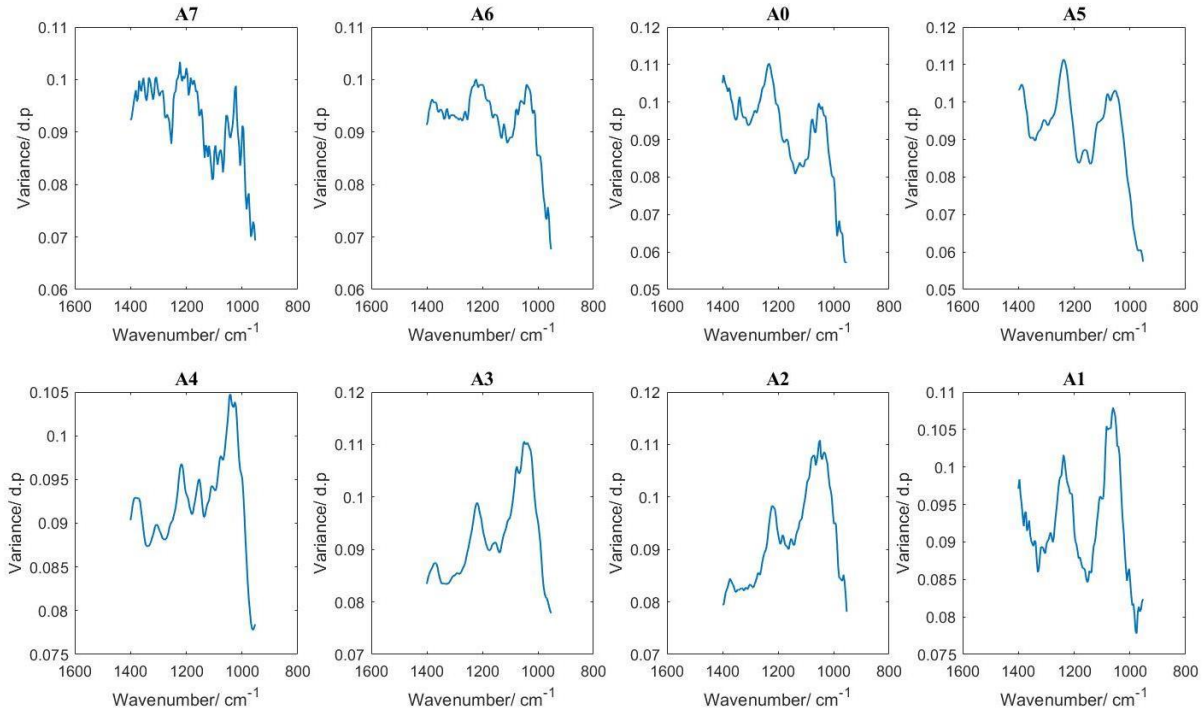

Fig. S 18 Percentage variance at each wavenumber from 1500 – 850 cm^{-1} along which the first principal component (PC) aligned. Higher variance indicates a dominating wavenumber for the PC. In the top four plots, 1235 cm^{-1} has the highest variance, whereas for the bottom four, ~1062 cm^{-1} is the dominating band.