Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Fourier transform infrared spectroscopy imaging as a screening tool to predict the grade and invasiveness of urothelial carcinoma of the bladder

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Table S1. Positions of FTIR bands with their assignment to vibrational modes and biomolecules.[10,15,21,37]

Band [cm^{-1}]	Assignment
967	DNA; ν(CNC)
995	RNA, glycogen; carbohydrate rings stretch and deformation
1026	Glycogen; δ(COH)
1052	Sugar moieties in DNA, polysaccharides, glycoproteins, collagen; ν(C–O)
1081	Collagen, nucleic acids, phosphate-containing molecules, glycogen; $\nu_s(PO_2^-)$, ν(CC)
1119-1111	Polysaccharides, RNA, lactate; ν(C-O), ν(CC-OC)
1154	Glycogen, ν_{as}(CO-O-C)
1200	Collagen, ν(COH, COC)
1233	Nucleic acids and other phosphate-containing molecules, $\nu_{as}(PO_2^-)$; collagens, amide III
1280	Collagens, amide III,
1335	Collagens amide III:
1370-1393	free fatty acids and amino acids; $\nu_s(COO^-)$
1450	Proteins, lipids, carbohydrates; $\delta(CH_2, CH_3)$
1516	Tyrosine residue, elastin; $\nu(CC)_{ring}$
1544	Proteins; amide II; δ(NH) + ν(C-N)
1573	Lipids, proteins, nucleic acids; ν(C=N, C=C)
1591	free amino acids; $\nu_{as}(COO^-)$
1637	Proteins (β-sheets); amide I; collagen and elastin: ν(C=O) + δ(N-H)
1652	Proteins (α-helix); amide I; ν(C=O) + δ(N-H)
1673	Amide I; ν(C=O) + δ(N-H)
1687	Proteins (β-turns); amide I, DNA; ν(C=O)+δ(N-H), ν(C=C, C=O)
1743	Triglycerides, phospholipids; ν(C=O) ester group

ν – stretching mode, as – antisymmetric, s - symmetric; δ – in-plane deformation.

Table S2. The position of regression vectors for the constructed models for BC classification (in cm^{-1}). Bold numbers indicate the specific IR features of the epithelium and subepithelium.

N (ve–) vs BC (ve+)		**Tni (ve–) vs Tinv (ve+)**		**HG (ve–) vs LG (ve+) BC**		**N (ve–) vs BC (ve+)**		**Tni (ve–) vs Tinv (ve+)**	
Epithelium						**Subepithelium**			
ve–	**ve+**	**ve–**	**ve+**	**ve–**	**ve+**	**ve–**	**ve+**	**ve–**	**ve+**
1697	1683	**1590**	**1660**	1660	1682	1697	1683	**1682**	**1652**
1670	**1608**	**1552**	**1565**	1633	1609	1672	**1657**	**1640**	**1623**
1586	**1547**	**1524**	1477	1576	1547	1585	**1635**	**1544**	1562
1397	**1485**	1460	**1431**	1487	1457	**1547**	**1558**	1464	1447
1310	**1447**	**1337**	**1165**	1435	1215	1398	1414	**1364**	**1397**
1140	1413	**1226**	**1158**	1164	1043	1311	**1247**	**1214**	**1136**
1043	**1060**	**1188**	**1108**	980	992	1049	996	**1082**	**1068**
	996	**1043**				**1010**		**1023**	**980**

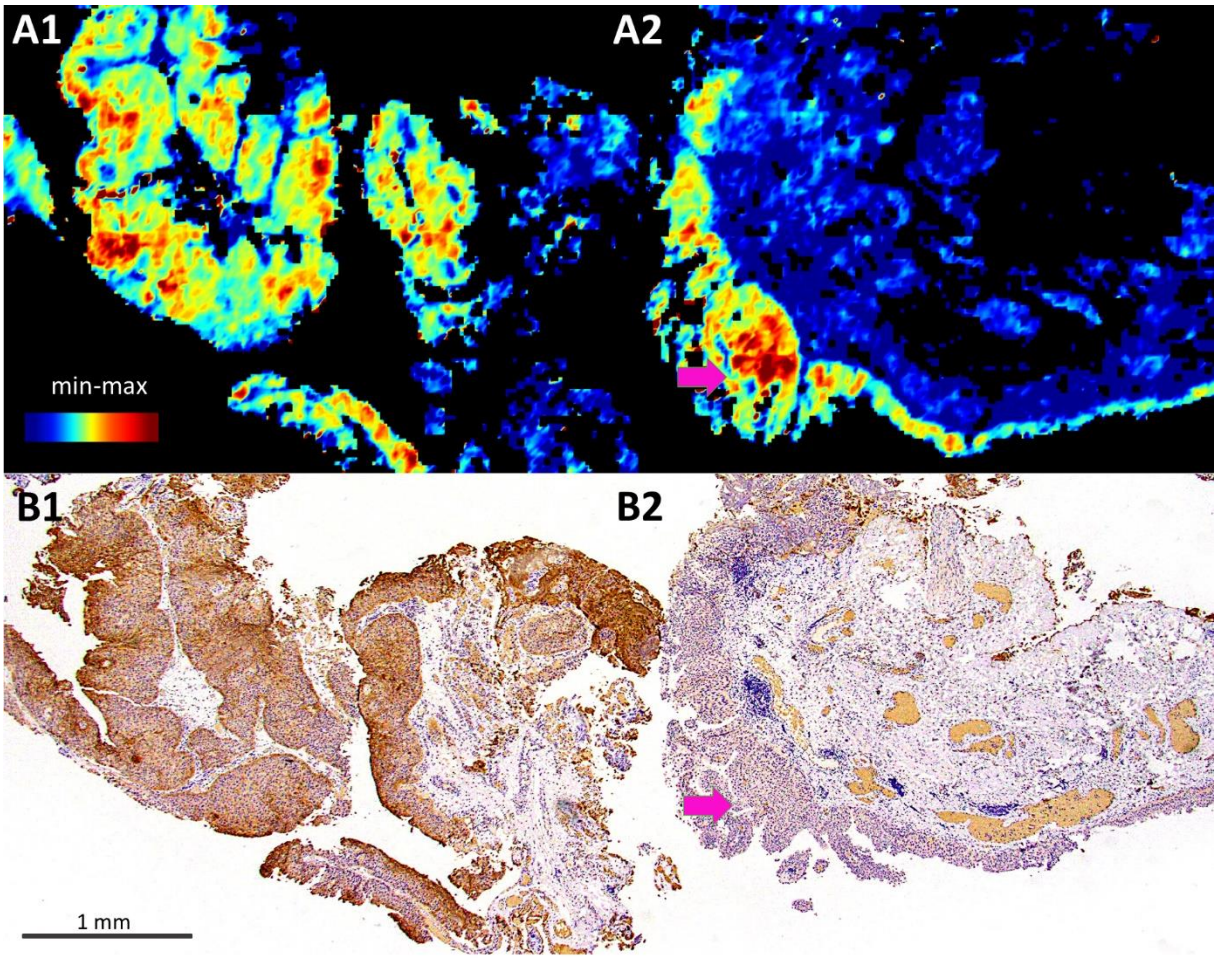

Figure S1. The comparison of IR distribution images for carbohydrates (**A1**, **A2**) and IHC Glut-1 expression (**B1**, **B2**, magnification 2×) for two excision samples from the T0 patient. Pink arrows in **A2** and **B2** indicate the early neoplastic changes which were not recognised in IHC.

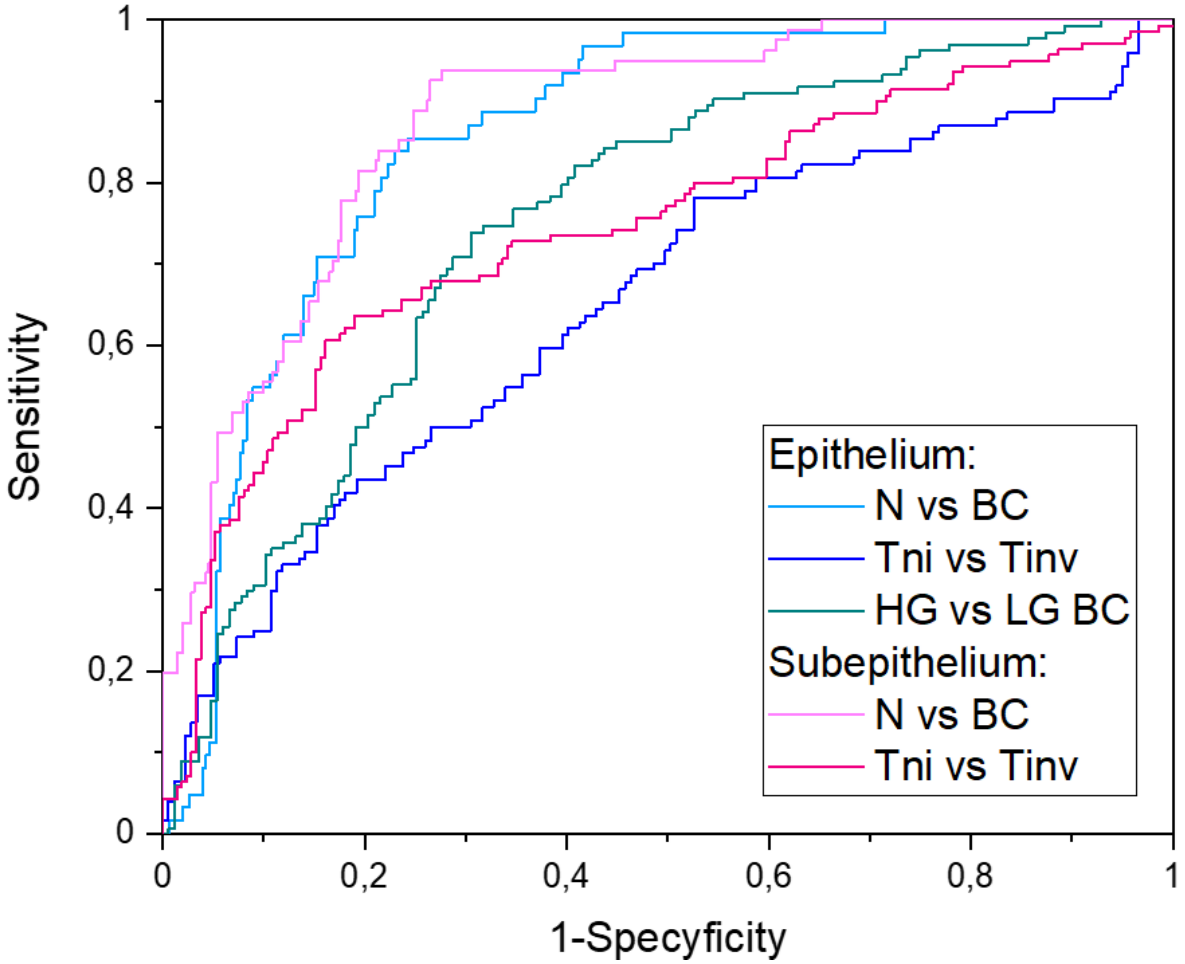

Figure S2. Area Under the Receiver Operation Curves for the cross validation of the patient groups.

Legend: Epithelium (n=417): n(N) = 62, n(Tni) = 124, n(Tinv) = 177, n(LG) = 167, n(HG) = 134; Subepithelium (n=494): n(N) = 81, n(Tni) = 140, n(Tinv) = 206.

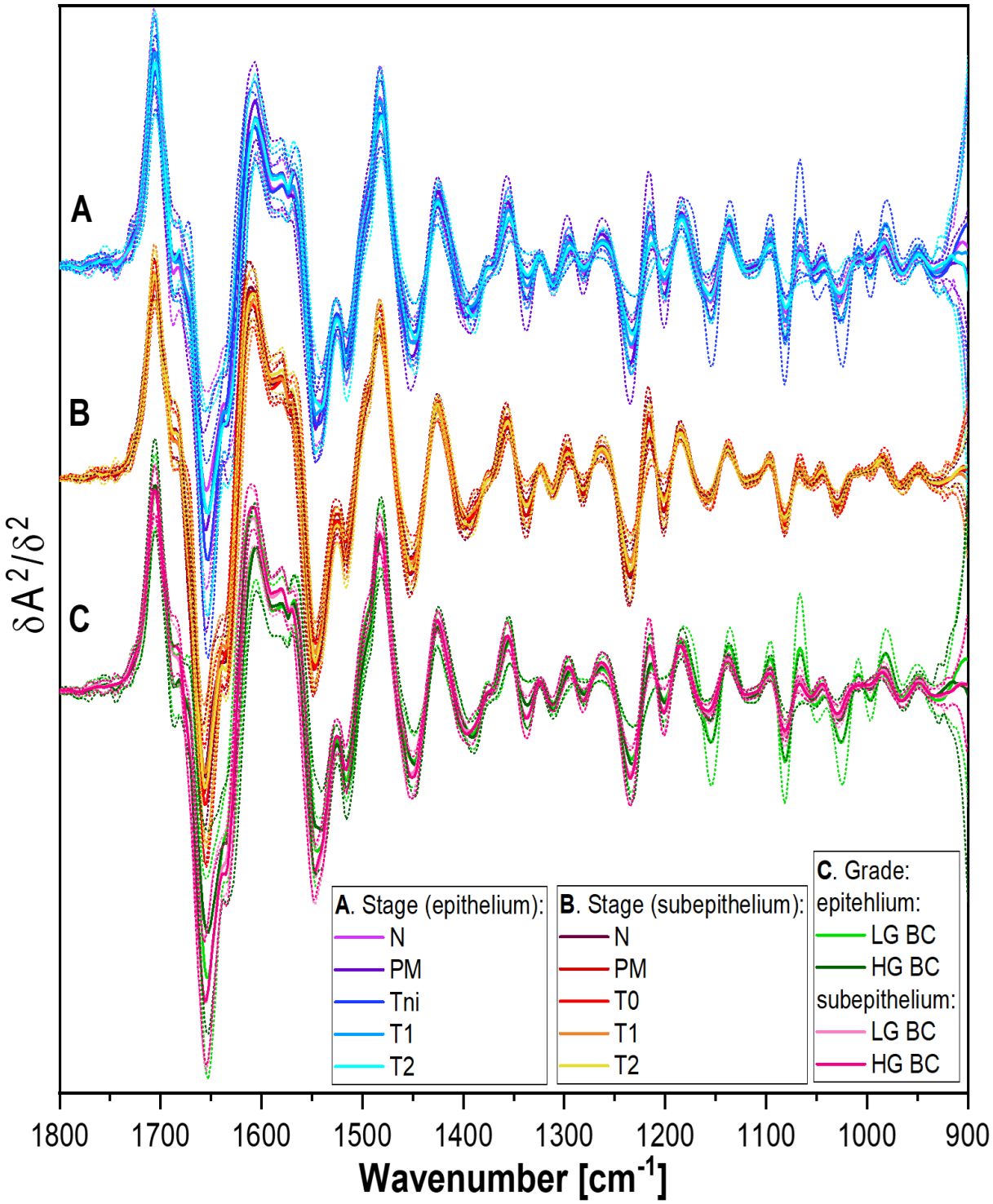

Figure S3. Averaged second derivatives IR spectra (±SD, marked with dotted lines) subjected to the epithelial and subepithelial bladder tissue (extracted from classes of UHCA).

Legend: Epithelium (n=417): n(N) = 62, n(PM) = 44, n(Tni) = 124, n(T1) = 94, n(≥T2) = 83, n(LG) = 167, n(HG) = 134; Subepithelium (n=494): n(N) = 81, n(PM) = 61, n(Tni) = 140, n(T1) = 122, n(≥T2) = 84; and Tinv is T1 + ≥T2.

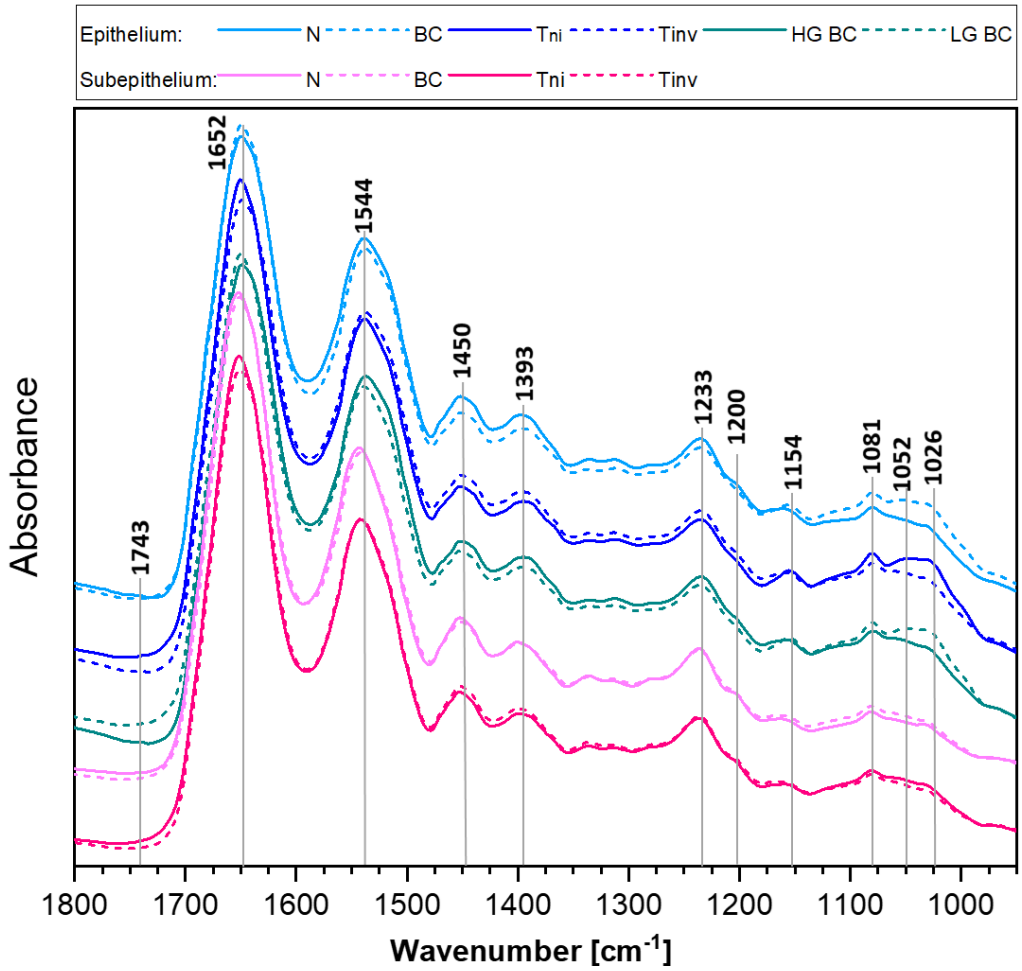

Figure S4. Averaged absorbance FTIR spectra of the epithelial and subepithelial tissues of the bladder (extracted from classes of UHCA).

Legend: Epithelium (n=417): n(N) = 62, n(Tni) = 124, n(Tinv) = 177, n(LG) = 167, n(HG) = 134; Subepithelium (n=494): n(N) = 81, n(Tni) = 140, n(Tinv) = 206.