

Supplementary information:

Optical photothermal infrared spectroscopy and discrete wavenumber imaging for high content screening of single cells

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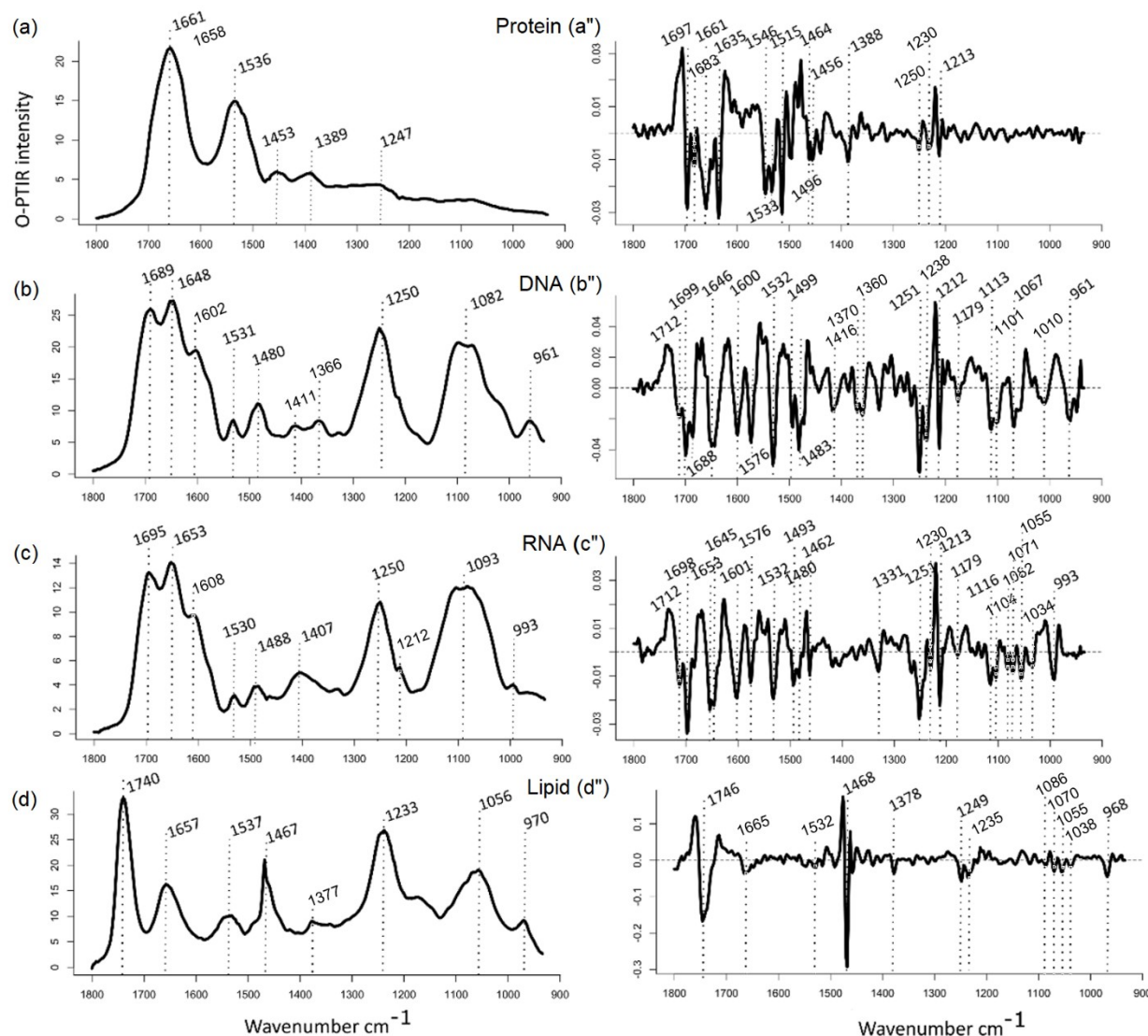
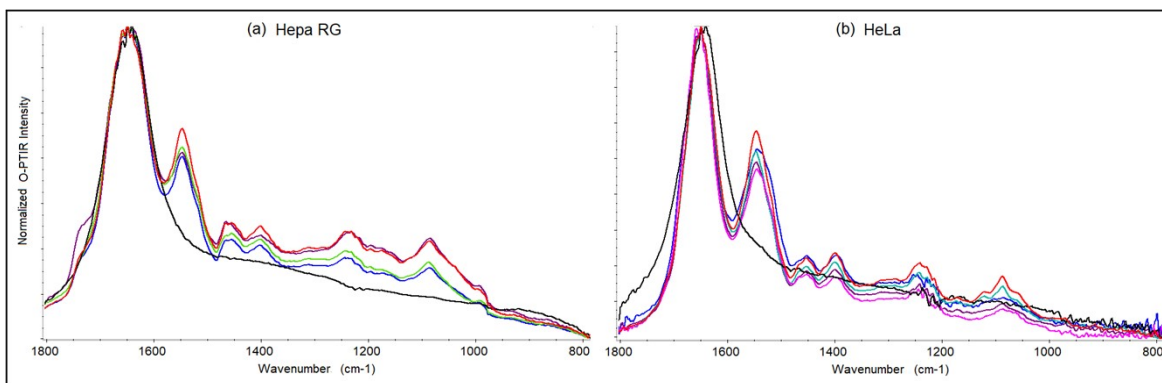


Figure S1: O-PTIR spectra and second derivative of pure compounds protein (a, a''), DNA (b, b''), RNA (c, c''), and lipid (d, d'').



Pure compounds	Bands	Second derivative
Protein	1658, 1536, 1453, 1389, 1247	1697, 1683, 1661, 1635, 1546, 1533, 1515, 1496, 1464, 1456, 1388, 1250, 1230, 1213
DNA	1689, 1648, 1602, 1531, 1480, 1411, 1366, 1250, 1082, 961	1712, 1699, 1688, 1646, 1600, 1576, 1532, 1499, 1483, 1416, 1370, 1360, 1251, 1238, 1212, 1179, 1113, 1101, 1067, 1010, 961
RNA	1695, 1653, 1608, 1530, 1488, 1407, 1250, 1212, 1093, 993	1712, 1698, 1653, 1645, 1601, 1576, 1532, 1493, 1480, 1462, 1331, 1251, 1230, 1213, 1179, 1116, 1104, 1082, 1071, 1055, 1034, 993
Lipid	1740, 1657, 1537, 1467, 1233, 1056, 970	1746, 1665, 1532, 1468, 1378, 1249, 1235, 1086, 1070, 1055, 1038, 968

Table S1: Summary of band positions in the pure compounds shown in figure S1.

Pure Cells (figure S2)	Assignment	2 nd derivative (figure S3)
Wavenumber cm ⁻¹		Wavenumber cm ⁻¹
1740	C=O stretching of lipids	1743, 1718
1648	Amide I of proteins, water	1679/1665 (turn), 1658 (α helix), 1690/1635 (β sheet), 1643 (water)
	RNA/DNA	1575
1547	Amide II of proteins	1552, 1519
	RNA/DNA	1496, 1472
1447	CH ₂ deformation of proteins	1452
1400	COO ⁻	1402
	RNA, DNA	1371
	CH _{2/3} deformation of proteins	1344, 1317
1240	Asymmetric stretching PO ₂ ⁻ in RNA/DNA, amide III of proteins	1248, 1226
	-	1190, 1175, 1126
1088	Symmetric stretching PO ₂ ⁻ in RNA/DNA	1088
	RNA	1054

Linear discriminant of model 1 (figure 3b)		2 nd derivative (figures S1 and S3)
1738	Lipid (C=O)	1743
1709	DNA/RNA (C=O)	1712/1699
1646/1631	Protein (amide I)	1643/1635
1555/1536	Protein (amide II)	1552/1533
1496	DNA/RNA	1499/1496
1478	DNA/RNA	1472/1480
1400	Protein (COO ⁻)	1402/1389
1248	DNA/RNA (asym. PO ₂ ⁻) or amide III of protein	1251
1156	-	
1090	DNA/RNA (asym. PO ₂ ⁻)	1088
1030	RNA	1034

Linear discriminants of model 2 (figure 3d)		2 nd derivative (figures S1 and S3)
1694/1665/1651	Protein (amide I)	1690/1665/1658/1643
1552	Protein (amide II)	1546
1488	Protein or DNA/RNA	1496 or 1493/1483
1400	Protein (COO ⁻)	1402
1243	Asymmetric stretching PO ₂ ⁻ RNA/DNA	1251
1125	RNA	1126
1027	RNA	1034
994	RNA	993

Linear discriminants of model 3 (figure 3f)		2 nd derivative (figures S1 and S3)
1734	Lipid (C=O)	1743
1716	DNA/RNA (C=O)	1712
1695, 1666	Protein (amide I)	1697, 1665
1620	-	
1603, 1575	DNA/RNA	1601, 1576
1552	Amide II	1546
1498	Protein	1496
1420	DNA	1416
1400	COO ⁻	1402
1371	DNA	1371
1231	Asymmetric stretching PO ₂ ⁻ of RNA/DNA or phospholipid, amide III of protein	1226, 1233
1180	DNA/RNA	1179, 1175
1048	Lipid	1055

Table S2: Band assignments of cells in O-PTIR spectra and second derivatives according to Diem et al. and references cited therein¹, linear discriminants (three PLS-LDA models) and second derivative spectra of the cell lines. (-) no assignment

Reference:

- (1) Diem, M.; Mazur, A.; Lenau, K.; Schubert, J.; Bird, B.; Miljković, M.; Krafft, C.; Popp, J. Molecular Pathology *via* IR and Raman Spectral Imaging. *J Biophotonics* **2013**, 6 (11–12), 855–886. <https://doi.org/10.1002/jbio.201300131>