

## Supportive information

### Nondestructive molecular imaging by Raman spectroscopy vs. marker detection by MALDI IMS for early diagnostics of HCC

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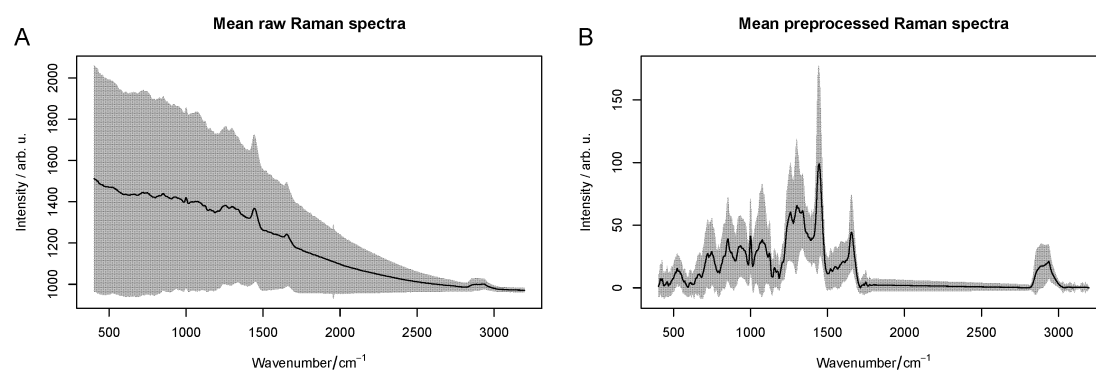


Figure S1 – Mean raw (A) and preprocessed (B) Raman spectrum of the entire data set. Shaded areas represent the standard deviations within the data set.

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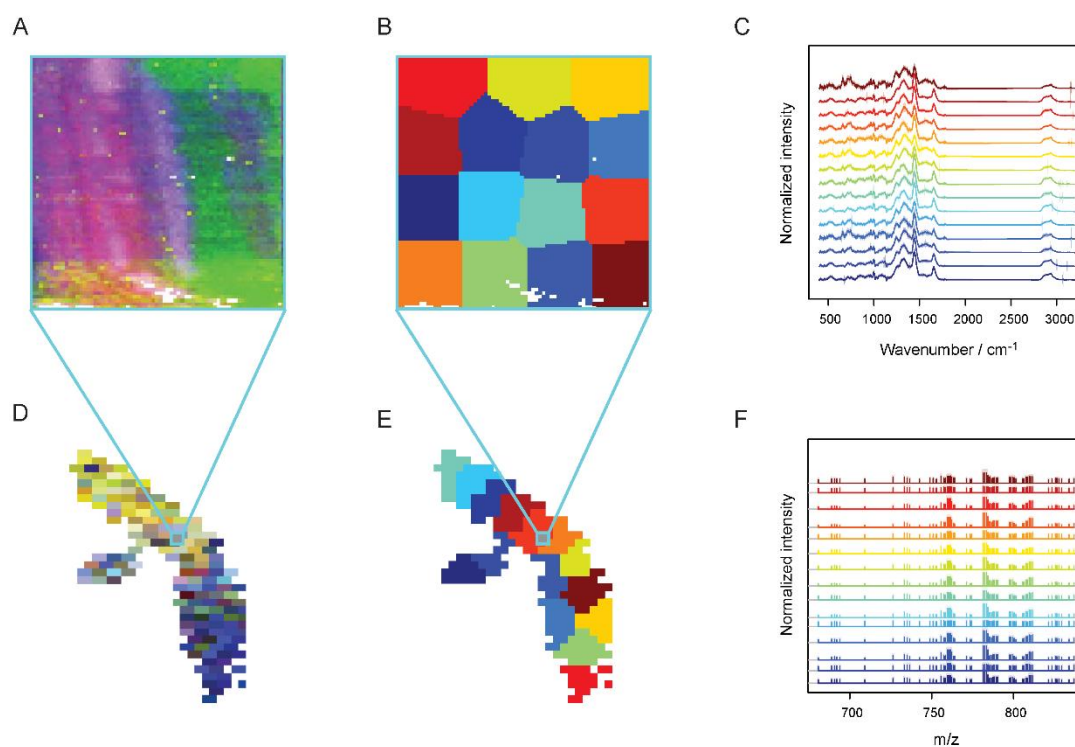


Figure S2 – An example of the data from a single sample. False-colour images of the first, second, and third PCA components for Raman imaging (A) and MALDI IMS data (D) are visualised in red, green, and blue, respectively. Subsampling of the scans into 15 subareas, using k-means clustering on the coordinates, is visualized for Raman imaging (B) and MALDI IMS (E) using 15 colours. A mean spectrum for each subarea is calculated and displayed in C and F for Raman imaging and MALDI IMS with respective colours per subarea. The spectra are shifted vertically for clarity.

## Supportive information

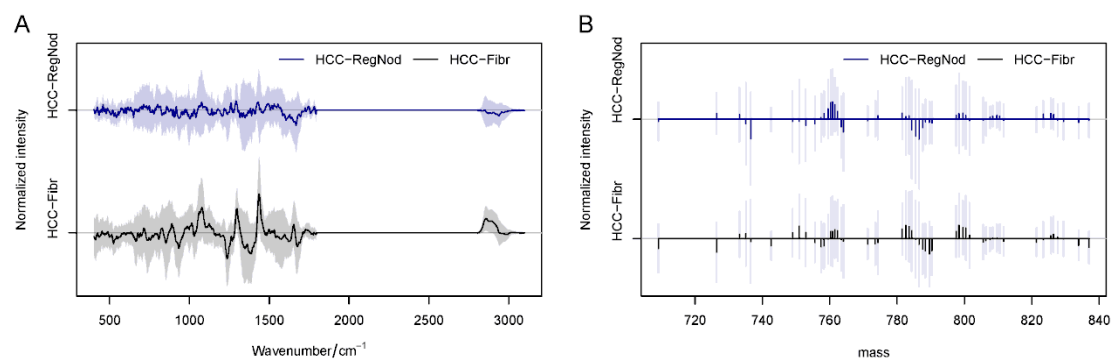


Figure S3 - Difference spectra calculated for HCC and regenerative nodules (Reg Nod) and HCC and fibrotic tissue (Fibr) for (A) Raman and (B) MALDI datasets. Shaded areas represent the standard deviations for each data subset.

Table S1 - Annotations of identified Raman bands ( $\text{cm}^{-1}$ ) for detected differences between HCC and fibrotic liver tissue based on Movasaghi et al.<sup>1</sup>; Tolstik et al.<sup>2</sup>; Tolstik et al.<sup>3</sup>; and Bonifacio et al.<sup>4</sup>

Experimental data		References				Identification
$\text{cm}^{-1}$	Tissue type	Ref <sup>1</sup> : Raman bands	Ref <sup>2</sup> : HCC tissue	Ref <sup>3</sup> : HCC cells	Ref <sup>4</sup> : Collagen	Substance class
525	Fibrosis	524 Phosphatidylserine				Lipids
717	HCC	717-719 Choline group	719 Phosphatidylcholine	717 Choline group		Lipids
756	Fibrosis	755 Symmetric breathing of tryptophan			760 Trp ring deformation	Proteins
774	Fibrosis	776 Phosphatidylinositol				Lipids
810	Fibrosis	810 phosphodiester			816 collagen–other proteins	Collagen
852	Fibrosis	852 Glycogen	856 Collagen			Collagen, Glycogen
918	Fibrosis	918 Glycogen				Glycogen
933	Fibrosis	933 v(C-C) skeletal of collagen backbone	937 Collagen			Collagen
999	HCC	1000 Phenylalanine	1001 band in HCC	1002 Phenylalanine		Proteins
1032	Fibrosis	1032 CH <sub>2</sub> CH <sub>3</sub> bending modes of collagen			1031 Collagen	Collagen
1059	HCC	1060 C-C stretching	1063 Unsaturated fatty acids	1059 C-C stretching		Lipids
1077	HCC	1078 Phospholipids	1076 band in HCC	1078 Lipids		Lipids
1125	HCC		1123 band in HCC			
1158	Fibrosis	1158 C-C/C-N stretching of proteins				Proteins
1182	Fibrosis	1180-84 Cytosine, guanine, adenine				DNA
1239	Fibrosis	1240 Collagen	1242 Collagen		1245 Collagen, amide III	Collagen
1275	Fibrosis	1275 Amide III (Collagen)			1271 Collagen	Collagen
1299	HCC	1299 CH <sub>2</sub> deformation	1299 band in HCC			Lipids
1341	Fibrosis	1343 CH <sub>3</sub> , CH <sub>2</sub> wagging collagen; Glucose	1343 L-Valine		1342 glycosaminoglycans, d C-H (CH <sub>2</sub> )	Collagen, Glucose
1380	Fibrosis	1379 CH <sub>3</sub> symmetric lipids			1380 glycosaminoglycans, unassigned	Lipids, polysaccharides
1389	Fibrosis		1387 band in Fibrosis			
1401	Fibrosis	1401 bending mode of CH <sub>3</sub> in collagen				Collagen
1440	HCC	1440 CH <sub>2</sub> deformation	1439 Palmitic acid	1440 Lipids		Lipids
1569	HCC			1573 Guanine, adenine, tryptofan		Proteins
1587	HCC	1586-8 Phenylalanine				Proteins
1653	HCC	1652-3 C=C stretch	1655 Amide I, C=C stretching	1655 Lipids		Lipids
1677	Fibrosis	1676 Amide I	1677 band in Fibrosis		1670 collagen, amide I	Collagen
1743	HCC	1744 Carbonyl feature				Lipids
2856	HCC	2856 CH <sub>2</sub> stretch	2856 band in HCC	2854 Lipids		Lipids
2880	HCC	2853-81 CH <sub>2</sub> stretch				Lipids and Proteins
2889	HCC	2889 CH <sub>2</sub> asymmetric stretch		2888 Lipids		Lipids and Proteins
2895	HCC	2889-908 CH <sub>2</sub> asymmetric stretch	2895 band in HCC	2898 Lipids		Lipids and Proteins

## Supportive information

Table S2 - Annotations of identified m/z ratio for differences between HCC and fibrotic tissue detected by MALDI IMS based on LIPID MAPS® Lipidomics Gateway<sup>5</sup> and Passos-Castilho et al.<sup>6</sup>

Experimental data			Identification by LIPID MAPS <sup>5</sup>			Ref <sup>6</sup>
Found m/z	Tissue Type	Mass	Formula	Main Class	Sub Class	
709,12	Fibrosis	709,47	C <sub>39</sub> H <sub>68</sub> NO <sub>8</sub> P	Glycerophosphoethanolamines [GP02]	Diacylglycerophospho-ethanolamines, [GP0201]	
750,94	HCC	750,58	C <sub>41</sub> H <sub>83</sub> O <sub>9</sub> P	Glycerophosphoglycerols [GP04]	1-alkyl,2-acylglycerophosphoglycerols, [GP0402]	
782,59	HCC	782,58	C <sub>45</sub> H <sub>83</sub> O <sub>8</sub> P	Glycerophosphates [GP10]	Diacylglycerophosphates [GP1001]	+
783,56	HCC	783,58	C <sub>44</sub> H <sub>82</sub> NO <sub>8</sub> P	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]	+
784,35	HCC	784,53	C <sub>43</sub> H <sub>77</sub> O <sub>10</sub> P	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]	
788,39	Fibrosis	788,45	C <sub>40</sub> H <sub>69</sub> O <sub>13</sub> P	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]	+
789,61	Fibrosis	789,62	C <sub>44</sub> H <sub>88</sub> NO <sub>8</sub> P	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]	+
790,38	Fibrosis	790,46	C <sub>40</sub> H <sub>71</sub> O <sub>13</sub> P	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]	+
798,36	HCC	798,54	C <sub>44</sub> H <sub>79</sub> O <sub>10</sub> P	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]	
799,56	HCC	799,57	C <sub>44</sub> H <sub>82</sub> NO <sub>9</sub> P	Glycerophosphoserines [GP03]	1-alkyl,2-acylglycerophosphoserines [GP0302]	+
800,38	HCC	800,45	C <sub>41</sub> H <sub>69</sub> O <sub>13</sub> P	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]	+

Table S3 - Annotations of identified m/z ratio for differences between well- and moderate-differentiated HCC detected by MALDI IMS based on LIPID MAPS® Lipidomics Gateway.<sup>5</sup>

Experimental data		Identification by LIPID MAPS <sup>5</sup>			
Found m/z	HCC Type	Formula	Mass	Main Class	Sub Class
206,12	Moderate	C <sub>13</sub> H <sub>18</sub> O <sub>2</sub>	206,13	Fatty esters [FA07]	Wax monoesters [FA0701]
212,08	Moderate	C <sub>12</sub> H <sub>20</sub> O <sub>3</sub>	212,14	Fatty Acids and Conjugates [FA01]	Hydroxy fatty acids [FA0105]
213,09	Moderate	C <sub>10</sub> H <sub>15</sub> NO <sub>4</sub>	213,10	Fatty amides [FA08]	Fatty acyl homoserine lactones [FA0803]
222,10	Moderate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222,09	Fatty Acids and Conjugates [FA01]	Dicarboxylic acids [FA0117]
223,09	Well	C <sub>12</sub> H <sub>17</sub> NO <sub>3</sub>	223,12	Fatty amides [FA08]	Primary amides [FA0801]
228,06	Moderate	C <sub>11</sub> H <sub>16</sub> O <sub>5</sub>	228,10	Fatty alcohols [FA05]	-
229,10	Well	C <sub>11</sub> H <sub>19</sub> NO <sub>4</sub>	229,13	Fatty esters [FA07]	Fatty acyl carnitines [FA0707]
229,36	Well	C <sub>13</sub> H <sub>27</sub> NO <sub>2</sub>	229,20	Fatty Acids and Conjugates [FA01]	Amino fatty acids [FA0110]
234,06	Moderate	C <sub>9</sub> H <sub>15</sub> BrO <sub>2</sub>	234,03	Fatty Acids and Conjugates [FA01]	Halogenated fatty acids [FA0109]
235,11	Moderate	-			
250,04	Moderate	C <sub>10</sub> H <sub>19</sub> BrO <sub>2</sub>	250,06	Fatty Acids and Conjugates [FA01]	Halogenated fatty acids [FA0109]
251,10	Well	C <sub>16</sub> H <sub>29</sub> NO	251,22	Fatty amides [FA08]	N-acyl amines [FA0802]
265,97	Moderate	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> P	266,09	Fatty Acids and Conjugates [FA01]	Hydroxy fatty acids [FA0105]
299,17	Moderate	C <sub>18</sub> H <sub>37</sub> NO <sub>2</sub>	299,28	Fatty Acids and Conjugates [FA01]	Amino fatty acids [FA0110]
750,94	Well	C <sub>41</sub> H <sub>83</sub> O <sub>9</sub> P	750,58	Glycerophosphoglycerols [GP04]	1-alkyl,2-acylglycerophosphoglycerols [GP0402]
758,26	Well	C <sub>33</sub> H <sub>59</sub> O <sub>17</sub> P	758,35	Glycerophosphoinositolglycans [GP15]	Monoacylglycerophosphoinositolglycans [GP1504]
759,49	Well	C <sub>40</sub> H <sub>74</sub> NO <sub>10</sub> P	759,51	Glycerophosphoserines [GP03]	Diacylglycerophosphoserines [GP0301]
760,32	Well	C <sub>33</sub> H <sub>61</sub> O <sub>17</sub> P	760,36	Glycerophosphoinositolglycans [GP15]	Monoacylglycerophosphoinositolglycans [GP1504]
760,83	Well	C <sub>44</sub> H <sub>89</sub> O <sub>7</sub> P	760,63	Glycerophosphates [GP10]	1-alkyl,2-acylglycerophosphates [GP1002]
761,52	Well	C <sub>40</sub> H <sub>76</sub> NO <sub>10</sub> P	761,52	Glycerophosphoserines [GP03]	Diacylglycerophosphoserines [GP0301]
762,32	Well	C <sub>33</sub> H <sub>63</sub> O <sub>17</sub> P	762,38	Glycerophosphoinositolglycans [GP15]	Monoacylglycerophosphoinositolglycans [GP1504]
763,44	Well	C <sub>43</sub> H <sub>74</sub> NO <sub>8</sub> P	763,52	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]
764,05	Well	C <sub>42</sub> H <sub>69</sub> O <sub>10</sub> P	764,46	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]
774,31	Well	C <sub>34</sub> H <sub>63</sub> O <sub>17</sub> P	774,38	Glycerophosphoinositolglycans [GP15]	Monoacylglycerophosphoinositolglycans [GP1504]
782,59	Well	C <sub>45</sub> H <sub>83</sub> O <sub>8</sub> P	782,58	Glycerophosphates [GP10]	Diacylglycerophosphates [GP1001]
783,56	Well	C <sub>44</sub> H <sub>82</sub> NO <sub>8</sub> P	783,58	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]
784,35	Well	C <sub>43</sub> H <sub>77</sub> O <sub>10</sub> P	784,53	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]
785,49	Well	C <sub>45</sub> H <sub>72</sub> NO <sub>8</sub> P	785,50	Glycerophosphoethanolamines [GP02]	Diacylglycerophospho-ethanolamines [GP0201]
786,52	Well	C <sub>43</sub> H <sub>79</sub> O <sub>10</sub> P	786,54	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]
787,60	Well	C <sub>44</sub> H <sub>86</sub> NO <sub>8</sub> P	787,61	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]
788,39	Well	C <sub>40</sub> H <sub>69</sub> O <sub>13</sub> P	788,45	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]
798,36	Well	C <sub>44</sub> H <sub>79</sub> O <sub>10</sub> P	798,54	Glycerophosphoglycerols [GP04]	Diacylglycerophosphoglycerols [GP0401]
799,56	Well	C <sub>43</sub> H <sub>78</sub> NO <sub>10</sub> P	799,54	Glycerophosphoserines [GP03]	Diacylglycerophosphoserines [GP0301]
800,38	Well	C <sub>41</sub> H <sub>69</sub> O <sub>13</sub> P	800,45	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]
801,52	Well	C <sub>43</sub> H <sub>80</sub> NO <sub>10</sub> P	801,55	Glycerophosphoserines [GP03]	Diacylglycerophosphoserines [GP0301]
811,64	Well	C <sub>46</sub> H <sub>86</sub> NO <sub>8</sub> P	811,61	Glycerophosphocholines [GP01]	Diacylglycerophosphocholines [GP0101]
826,45	Well	C <sub>43</sub> H <sub>71</sub> O <sub>13</sub> P	826,46	Glycerophosphoinositols [GP06]	Diacylglycerophosphoinositols [GP0601]
827,67	Well	C <sub>48</sub> H <sub>94</sub> NO <sub>7</sub> P	827,68	Glycerophosphocholines [GP01]	1-alkyl,2-acylglycerophosphocholines [GP0102]
829,42	Well	C <sub>46</sub> H <sub>72</sub> NO <sub>10</sub> P	829,49	Glycerophosphoserines [GP03]	Diacylglycerophosphoserines [GP0301]

## Supportive information

Table S4 - Annotations of identified Raman bands ( $\text{cm}^{-1}$ ) for detected differences between well- and moderate- differentiated HCC based on Movasaghi et al.<sup>1</sup>

Experimental data		Raman reference database <sup>1</sup>	Identification
Raman band	Tissue type		Substance class
489	Moderate	484-90 Glycogen	Polysaccharide
576	Moderate	576 Phosphatidylinositol	Lipids
753	Moderate	753 Symmetric breathing of tryptophan	Proteins
849	Moderate	850 Tyrosine	Proteins
858	Moderate	859 Tyrosin, collagen	Collagen
873	Moderate	873 Hydroxyproline, tryptophan	Proteins
939	Moderate	938 C-C stretch backbone	
1002	Moderate	1002 Phenylalanine	Proteins
1009	Moderate	1008 Phenylalanine	Proteins
1035	Moderate	1035 Collagen	Collagen
1044	Moderate	1044 symmetric stretching v3PO43-	
1065	Moderate	1065 Palmic acid, Fatty acids	Lipids
1089	Moderate	1070-90 Symmetric PO2- stretching of DNA	DNA
1104	Moderate	1104 Phenylalanine	Proteins
1116	Moderate	1115/6 CH2 bend and C1-Ca-Ha bend	
1155	Moderate	1155 Glycogen, C-C stretching of proteins	Proteins
1209	Moderate	1209 Tryptophan, Phenylalanine	Proteins
1230	Moderate	1230 Antisymmetric phosphate stretching vibration	Proteins
1245	Moderate	1246 Amide III (collagen)	Collagen
1263	Moderate	1263 T, A of DNA	DNA
1281	Moderate	1280 Amide III, Collagen	Collagen
1317	Moderate	1317 Guanine	DNA
1335	Moderate	1335 CH3CH2 wagging, collagen	Collagen
1356	Moderate	1355-7 G	DNA
1374	Moderate	1373 T, A, G of DNA	DNA
1551	Moderate	1552 Tryptophan	Proteins
1587	Moderate	1586-8 Phenylalanine	Proteins
1635	Moderate	1635 Collagen	Collagen
1662	Moderate	1662 Nucleic acid modes	DNA
1677	Moderate	1676 Amide I	Proteins
2916	Moderate	2915 CH band of lipids and proteins	Lipids, Proteins
2928	Moderate	2928 Symmetric CH3 stretch	Proteins
600	Well	600 Nucleotide conformation	DNA
777	Well	776 Phosphatidylinositol	Lipids
822	Well	820 Protein	Proteins
1023	Well	1023 Glycogen	Polysaccharide
1179	Well	1180 C, G	DNA
1290	Well	1290 C	DNA
1305	Well	1304 CH2 deformation lipids, A, C	Lipids, DNA
1434	Well	1436 CH2 deformation lipids	Lipids
1446	Well	1446 CH2 deformation proteins and lipids	Proteins and Lipids
1509	Well	1510 A	DNA
1647	Well	1647 Random coils in proteins	Proteins
1719	Well	1716-41 C=O in Lipids	Lipids
2838	Well	2817-49 CH2 symmetric stretch of lipids	Lipids
2850	Well	2850 CH2, fatty acids	Lipids

## References

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