Spatial systems lipidomics reveals nonalcoholic fatty liver disease heterogeneity

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Contents

1. Materials

1.1. Chemicals

Name	Supplier	Purity	Cas number
Mayers Hematoxylin solution	Merck KGaA, Darmstadt, Germany	99%	517-28-2
Eosin	Merck KGaA, Darmstadt, Germany	99%	15086-94-9
Ethanol	Biosolve B.V., Dieuze, France	99.9%	7732-18-5
Entellan	Merck KGaA, Darmstadt, Germany	-------	107961
Norharmane	Merck KGaA, Darmstadt, Germany	99%	244-63-3
Water ULC/MS	Biosolve B.V. Dieuze, France	99.98%	67-17-5
Red Phosphorus	Merck KGaA, Darmstadt, Germany	≥99.9%	7723-14-0

1.2. Consumables

Name	Supplier	Type
Microscope Glass slides	Thermo Fisher Scientific, Bremen, Germany	ISO 8037/1
Indium tin oxide -coated glass slides	Delta Technologies, Loveland, CO, USA	CG-40IN-1115

1.3. Instrumentation

Name	Supplier	Type
MALDI-TOF-MS	Bruker Daltonik GmbH, Bremen, Germany	Rapiflex TissueTyper
MALDI-Orbitrap	Thermo Fisher Scientific, Bremen, Germany	HF Elite
Microscope	Leica, Buffalo Grove, IL, USA	DM6000 B
Balance	Mettler, Columbus, OH, USA	AT261

Cryostat	Microm, Walldorf, Germany	HM525
MIRAX Scanner	Zeiss, Breda, The Netherlands	----------
Sublimation device	IDEE, Maastricht, The Netherlands	----------

2. Lipid Nomenclature

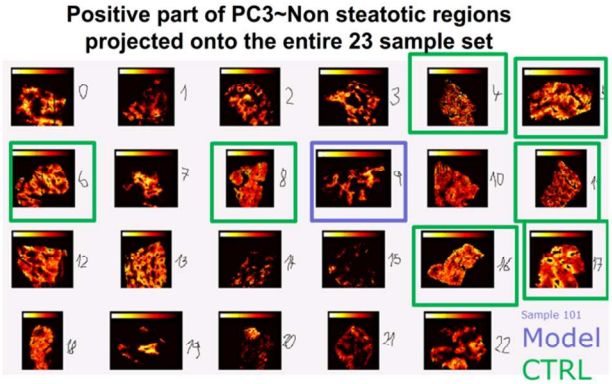
Lipid nomenclature is based on the recommendations reported by Lipid MAPS consortium.
A two letter abbreviation is commonly used, for example: PI corresponds to a phosphatidylinositol, whereas PG stands for Phosphatidylglycerol. Individual fatty acyls are indicated in the format of "total number of carbons:number of double bonds". For instance 22:6 indicates a fatty acyl containing 22 carbons and six double bonds. In case of lipids containing two fatty acyls, two notations can be used. Notation with underscore indicates the sn-position of the fatty acyls is unknown. For example PE(18:0_20:4) indicates a phosphatidylethanolamine containing an 18:0 and a 20:4 fatty acid with unknown sn-positions. If the sn-position of the fatty acyls is known, a slash can be used in the notation. Following the same example PE(18:0/20:4) indicates a phosphatidylethanolamine containing an 18:0 fatty acid at sn1-position and a 20:4 fatty acid at sn2- position.

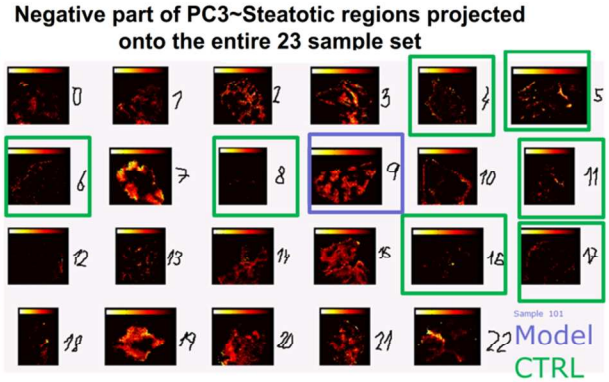
If the assignment of individual fatty acyls is impossible, given the type of collected data, the sum composition of both fatty acids is indicated. For instance, PE(38:4) indicates a phosphatidylethanolamine containing 38 carbons and 4 double bonds distributed over both fatty acyls.

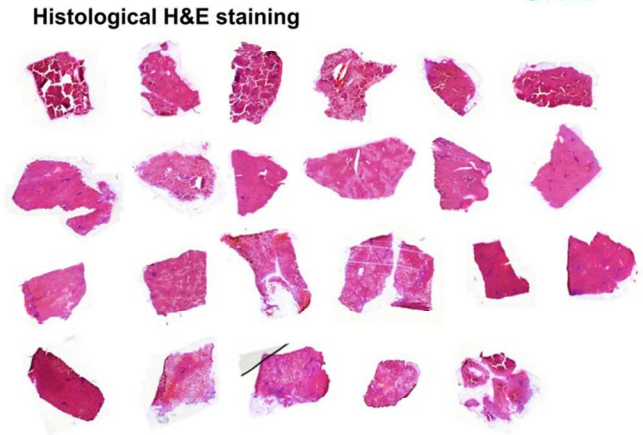
We note given the limitations of CID and HCD it is not possible to assign sn- or double bond positions.

Finally, the 'O-' prefix is used to indicate the presence of an alkyl ether substituent e.g. PC(O-16:0/18:1(9Z)), whereas the 'P-' prefix is used for the 1Z-alkenyl ether (Plasmalogen) substituent e.g. PC(P-16:0/18:1(9Z)).

3. Supplementary figures

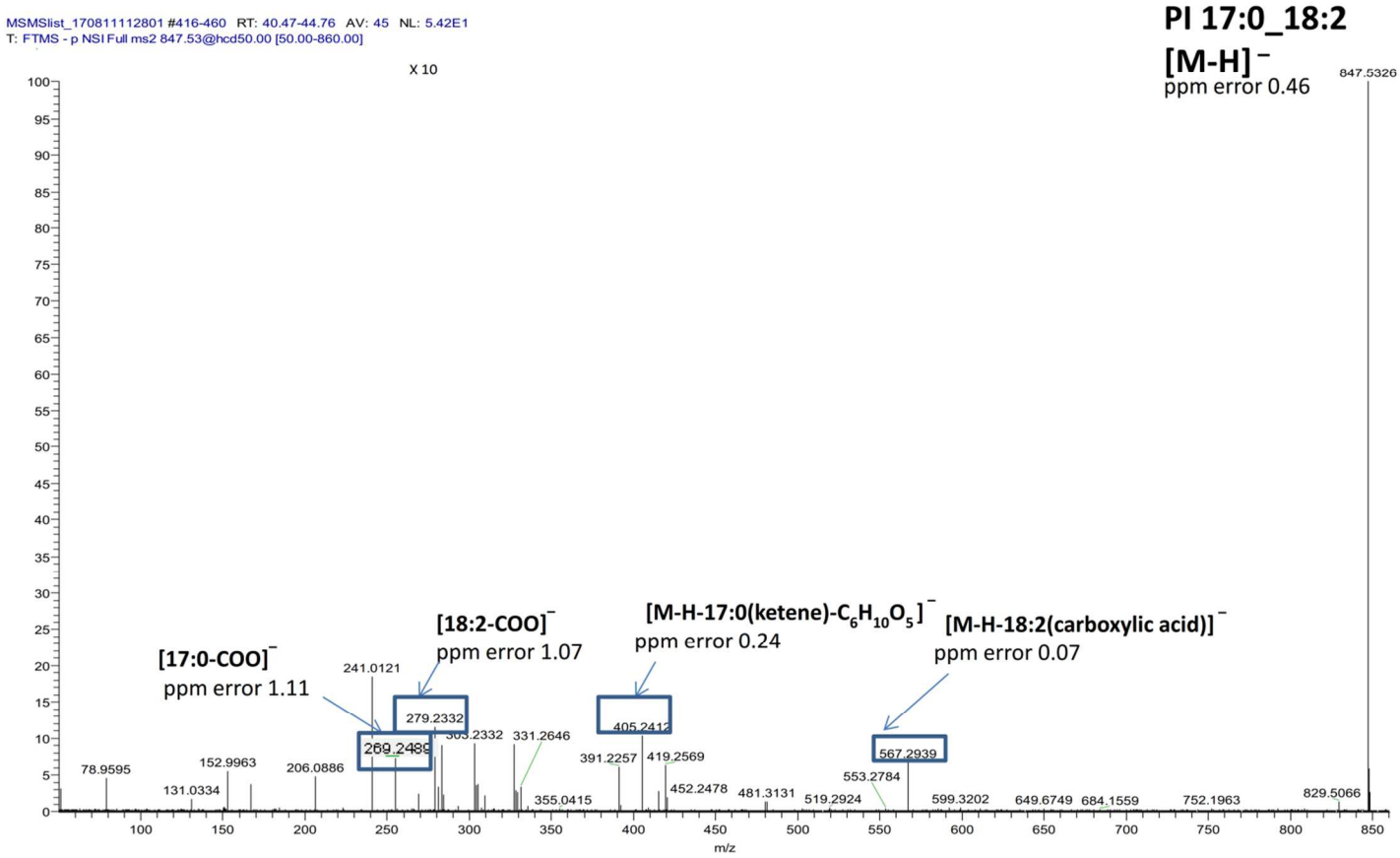




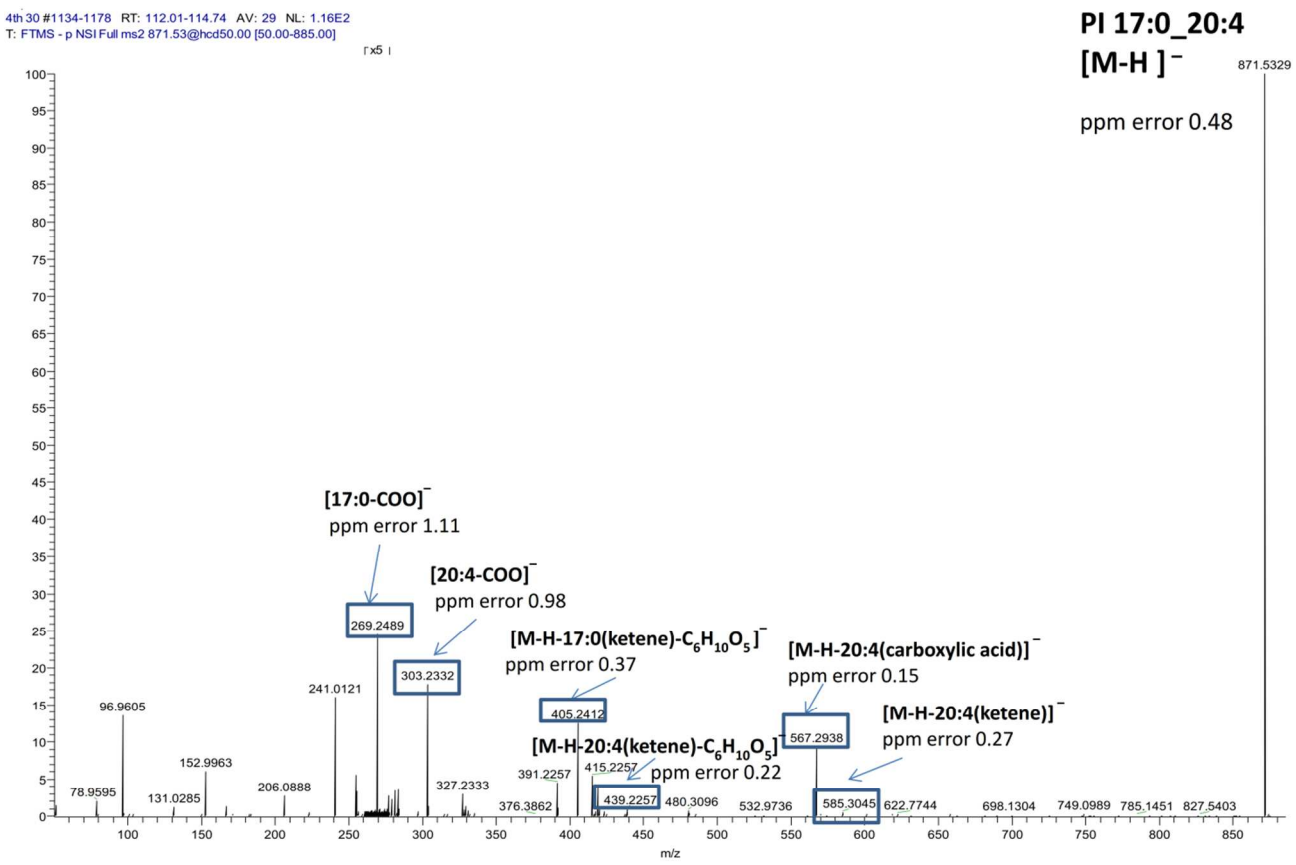


Sample number	Code number	Steatosis grade
68	0	5-33%
80	1	5-33%
100	2	>33-66%
105	3	>66+%
122	4	<5%
134	5	<5%
66	6	<5%
83	7	>33-66%
86	8	<5%
101	9	>33-66%
108	10	>33-66%
112	11	<5%
118	12	>33-66%
103	13	>33-66%
49	14	>33-66%
55	15	>66+%
89	16	<5%
117	17	<5%
128	18	>33-66%
130	19	>33-66%
126	20	>66+%
131	21	5-33%
48	22	5-33%

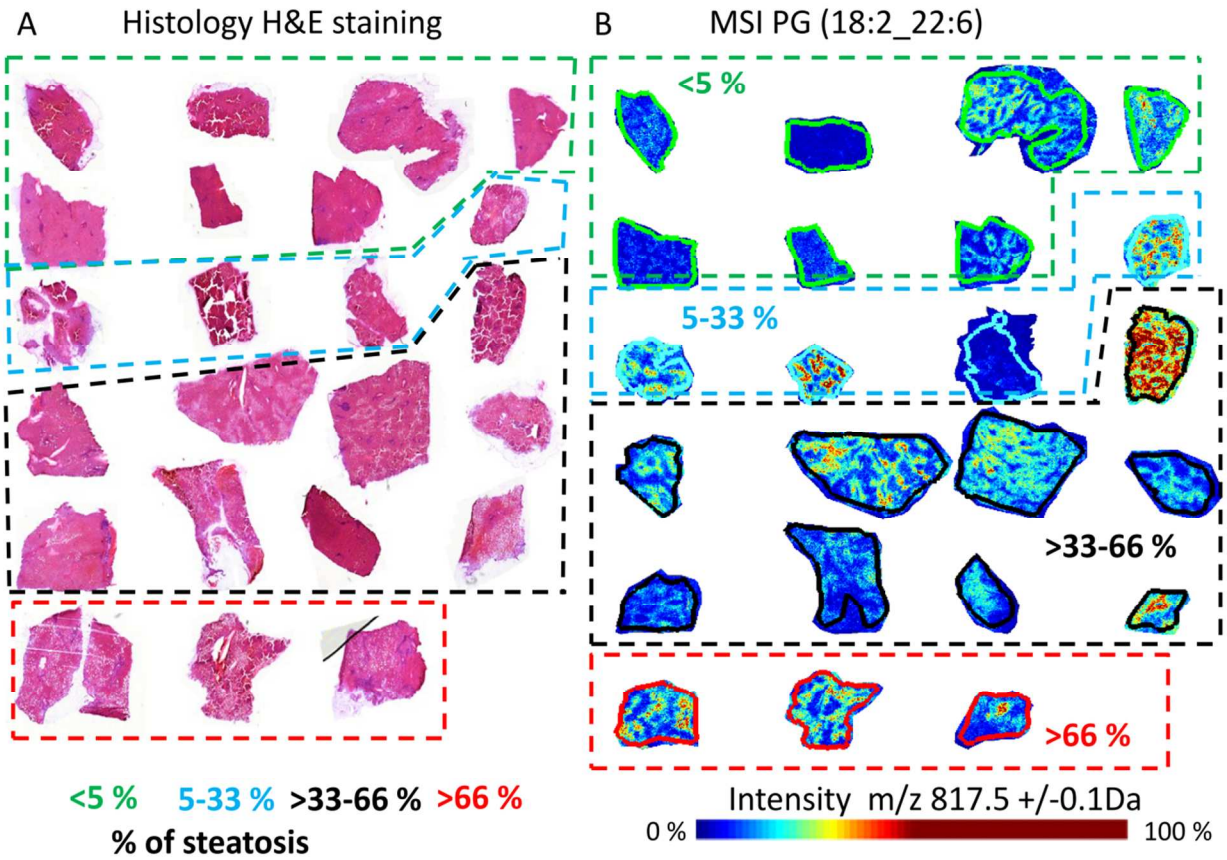
Supplementary Figure 1: Projection of one sample built PCA onto the remaining 22 samples showing the robustness and sensitivity of the steatotic signature captured by the PC3. Top left: PC+3 projected delineating the non-steatotic regions in the 23 samples dataset. Top right: PC-3 projected displaying the steatotic regions in the 23 samples dataset. In both, the purple box indicates the sample that was used to build the PCA model. Green boxes denote the control (steatosis grade 0, <5%) samples. Bottom left: The corresponding H&E histology images of the same sample set. Bottom right: Table with sample numbers and their pathological classification according to the Kleiner scoring system.



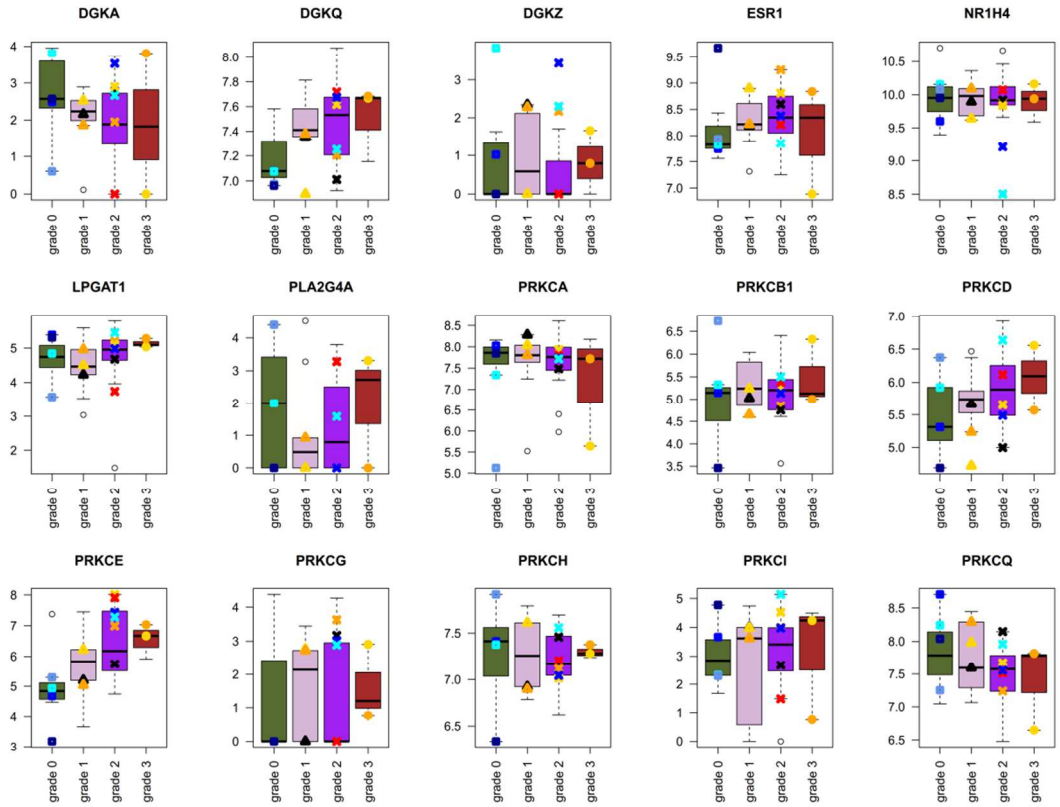
Supplementary Figure 2: HCD MS/MS spectrum of the ion at *m/z* 847.5326 detected from human liver tissue in negative ion mode using the Orbitrap Elite. Ions supporting the assignment of [PI(17:0_18:2)-H]-are annotated with their corresponding mass accuracy.



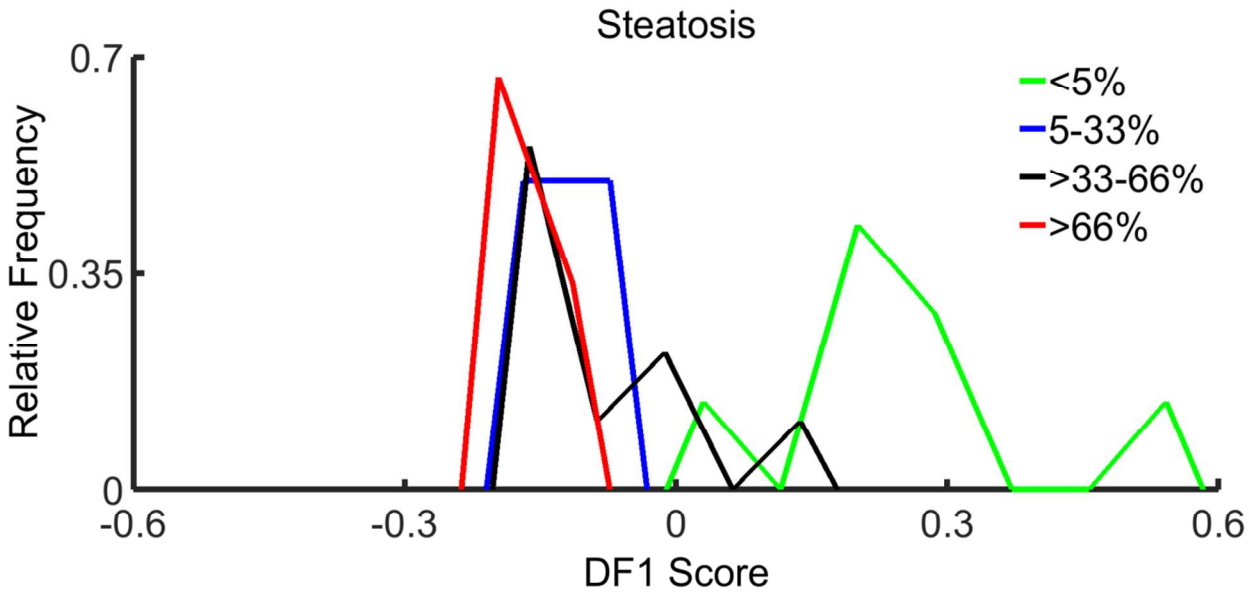
Supplementary Figure 3: HCD MS/MS spectrum of the ion at *m/z* 871.5329 detected from human liver tissue in negative ion mode using the Orbitrap Elite. Ions supporting the assignment of [PI(17:0_20:4)-H]-are annotated with their corresponding mass accuracy.



Supplementary Figure 4: Localized lipid accumulation and potential discriminatory power of a single lipid biomarker of steatosis. (A) Histological H&E images of all biopsies categorized to steatosis groups (colored accordingly). (B) MS-images of PG (18:2_22:6)(*m/z* 817.5) in the entire cohort.



Supplementary Figure 5: Boxplots showing hepatic expression of the genes from the network analysis. Gene expression from 35 patients in the same cohort was available, of which 15 samples were from the same patients that were MS-imaged. The exact expression levels of the 15 MSI analyzed samples are indicated by colored markers.



Supplementary Figure 6: PCA-LDA classifier based on averaged 23 spectra showing the homogenization effect. Histogram showing the distribution of the 4 steatosis classes along the discriminant function 1.