SHIMADZU

ThP 426

MS Imaging Spectrum Annotation Tool using Accurate Mass and Isotope Pattern Analysis

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Introduction

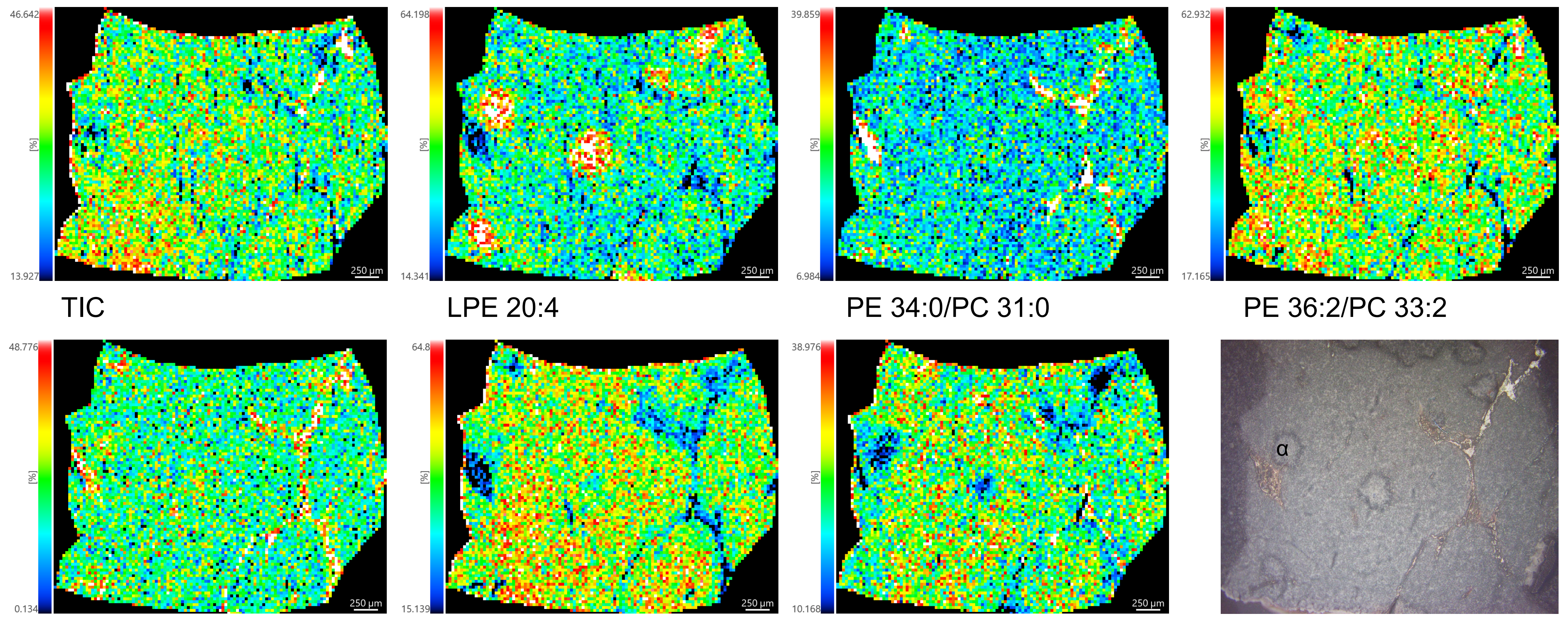
MS Imaging reveals the spatial distribution of important biomolecules to discover the details of disease processes, find drug distribution patterns, or characterize new materials. Real-world samples can result in complex mass spectra which are time-consuming to process manually. Searching for targets may turn up false matches, even having less than one part-per-million mass error. Using isotope pattern can exclude false matches and speed up processing. We developed a new MS spectrum annotation tool using accurate mass and isotope pattern analysis to quickly match targets to data.

Methods

Tissue samples were prepared by vapor-depositing 1,5-Diamino-naphthalene onto mouse pancreas affixed to conductive glass slides using a Shimadzu iMLayer^{TM} sublimation unit. Then tissues were analyzed by MALDI MS imaging using a Shimadzu IMScope QT^{TM}, composed of an ionization unit connected to a QTof mass spectrometer. Spectra were exported from the acquisition software and analyzed by a newly developed data processing tool.

Setting	Value
Pitch X	30.0 µm
Pitch Y	30.0 µm
Polarity	Negative
Scan Range (*m/z*)	500 to 1000
Number of Pixels	8752
Laser Irradiation Number	80 shots
Laser Repetition Frequency	2000 Hz
Laser Diameter	Approximately 35 µm
Laser Intensity	65%
DL Temperature	250 deg C

Table 1. Imaging MS settings optimized for mouse pancreas tissue, DAN coated, in negative MS mode.



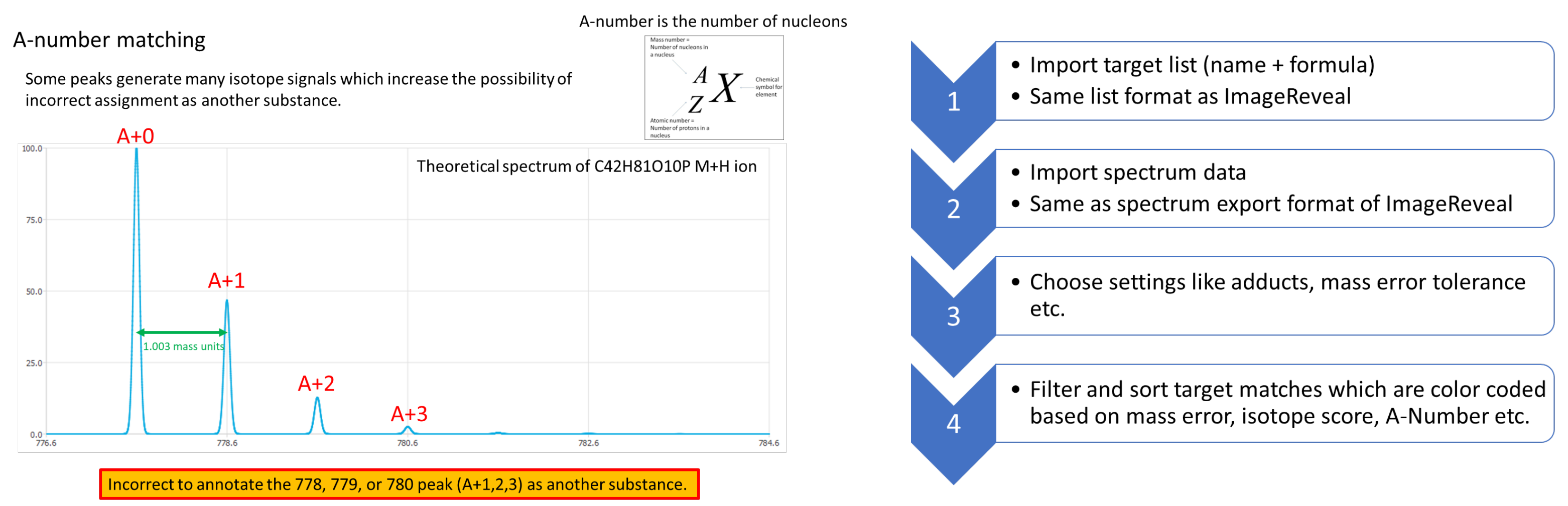
TIC | LPE 20:4 | PE 34:0/PC 31:0 | PE 36:2/PC 33:2

SM 41:2;O2b/SM 41:2;O2 | PI 36:4 | PS 38:4 | Optical image

Figure 1. MS images of mouse pancreas: TIC, representative lipids with acceptable matching criteria, and optical image. Alpha cells (round clusters, one example marked α) are visible in the optical and selected MS images.

Results

Mass spectra were imported into the newly developed tool along with a list of targets of interest which might be expected to be detected in the sample. The settings in the tool allow to select ionization mode, adducts, mass error and intensity tolerances, and other match settings. For each target with a matching signal in the MS spectrum, the isotope pattern match, monoisotopic ion (A-number) and other characteristics were calculated and shown in a summary table.



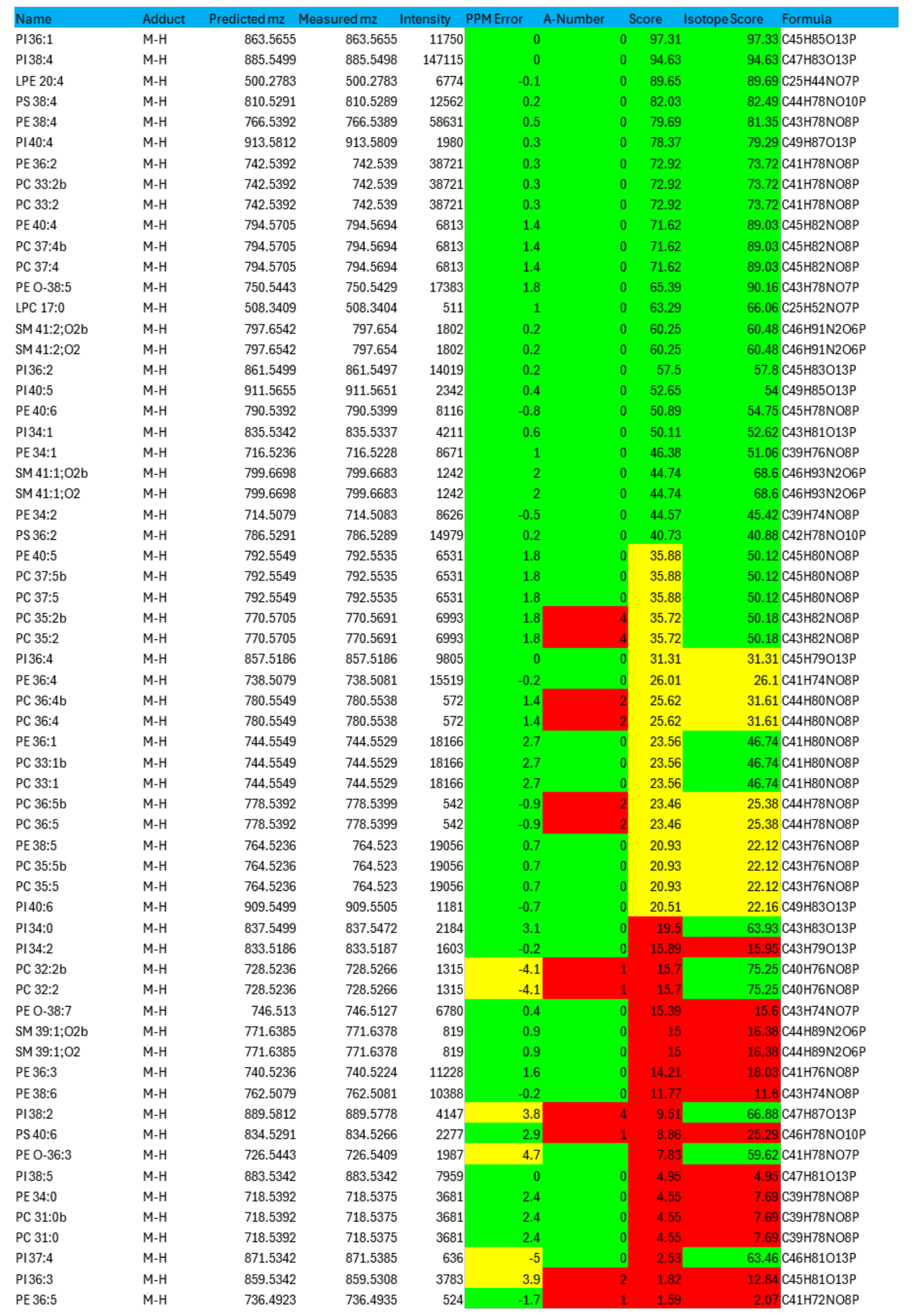
Some peaks generate many isotope signals which increase the possibility of incorrect assignment as another substance.

Theoretical spectrum of $C_{42}H_{81}O_{10}P$ M+H ion

Incorrect to annotate the 778, 779, or 780 peak (A+1,2,3) as another substance.

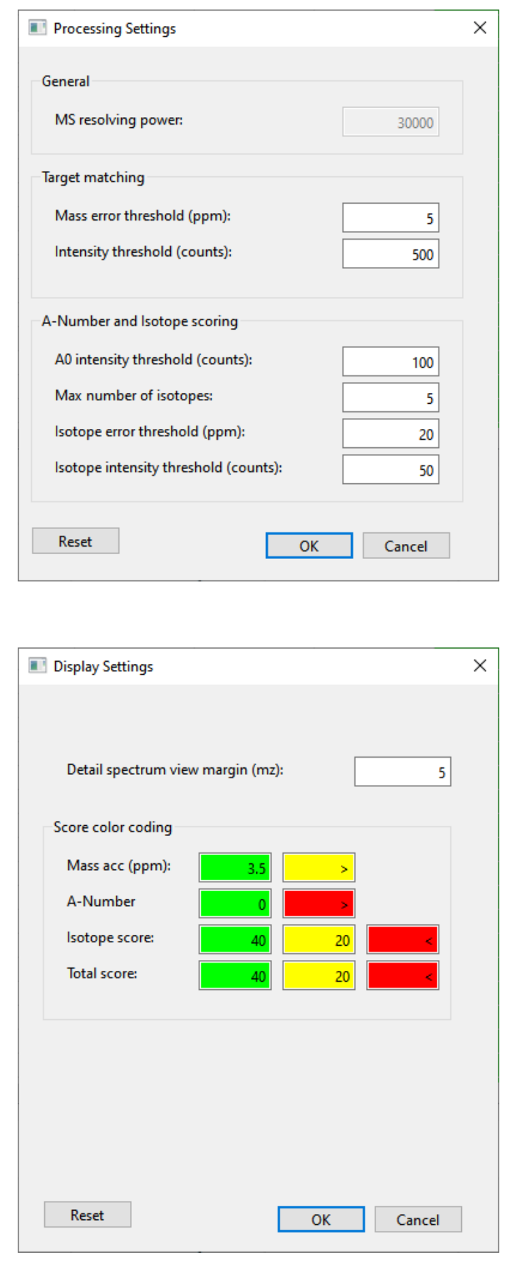
1.
   - Import target list (name + formula)
   - Same list format as ImageReveal
2.
   - Import spectrum data
   - Same as spectrum export format of ImageReveal
3.
   - Choose settings like adducts, mass error tolerance etc.
4.
   - Filter and sort target matches which are color coded based on mass error, isotope score, A-Number etc.

Figure 2. (Left) Concept of A-number assignment. **(Right)** Workflow of data processing using the tool.



Name	Adduct	Predicted mz	Measured mz	Intensity	PPM Error	A-Number	Score	Isotope Score	Formula
PI 36:1	M-H	863.5655	863.5655	11750	0	0	97.31	97.33	$C_{45}H_{85}O_{13}P$
PI 38:4	M-H	885.5499	885.5498	147115	0	0	94.63	94.63	$C_{47}H_{83}O_{13}P$
LPE 20:4	M-H	500.2783	500.2783	6774	-0.1	0	89.65	89.69	$C_{25}H_{44}NO_{7}P$
PS 38:4	M-H	810.5291	810.5289	12562	0.2	0	82.03	82.49	$C_{44}H_{78}NO_{10}P$
PE 38:4	M-H	766.5392	766.5389	58631	0.5	0	79.69	81.35	$C_{43}H_{78}NO_{8}P$
PI 40:4	M-H	913.5812	913.5809	1980	0.3	0	78.37	79.29	$C_{49}H_{87}O_{13}P$
PE 36:2	M-H	742.5392	742.539	38721	0.3	0	72.92	73.72	$C_{41}H_{78}NO_{8}P$
PC 33:2b	M-H	742.5392	742.539	38721	0.3	0	72.92	73.72	$C_{41}H_{78}NO_{8}P$
PC 33:2	M-H	742.5392	742.539	38721	0.3	0	72.92	73.72	$C_{41}H_{78}NO_{8}P$
PE 40:4	M-H	794.5705	794.5694	6813	1.4	0	71.62	89.03	$C_{45}H_{82}NO_{8}P$
PC 37:4b	M-H	794.5705	794.5694	6813	1.4	0	71.62	89.03	$C_{45}H_{82}NO_{8}P$
PC 37:4	M-H	794.5705	794.5694	6813	1.4	0	71.62	89.03	$C_{45}H_{82}NO_{8}P$
PE O-38:5	M-H	750.5443	750.5429	17383	1.8	0	65.39	90.16	$C_{43}H_{78}NO_{7}P$
LPC 17:0	M-H	508.3409	508.3404	511	1	0	63.29	66.06	$C_{25}H_{52}NO_{7}P$
SM 41:2;O2b	M-H	797.6542	797.654	1802	0.2	0	60.25	60.48	$C_{46}H_{91}N_{2}O_{6}P$
SM 41:2;O2	M-H	797.6542	797.654	1802	0.2	0	60.25	60.48	$C_{46}H_{91}N_{2}O_{6}P$
PI 36:2	M-H	861.5499	861.5497	14019	0.2	0	57.5	57.8	$C_{45}H_{83}O_{13}P$
PI 40:5	M-H	911.5655	911.5651	2342	0.4	0	52.65	54	$C_{49}H_{85}O_{13}P$
PE 40:6	M-H	790.5392	790.5399	8116	-0.8	0	50.89	54.75	$C_{45}H_{78}NO_{8}P$
PI 34:1	M-H	835.5342	835.5337	4211	0.6	0	50.11	52.62	$C_{43}H_{81}O_{13}P$
PE 34:1	M-H	716.5236	716.5228	8671	1	0	46.38	51.06	$C_{39}H_{76}NO_{8}P$
SM 41:1;O2b	M-H	799.6698	799.6683	1242	2	0	44.74	68.6	$C_{46}H_{93}N_{2}O_{6}P$
SM 41:1;O2	M-H	799.6698	799.6683	1242	2	0	44.74	68.6	$C_{46}H_{93}N_{2}O_{6}P$
PE 34:2	M-H	714.5079	714.5083	8626	-0.5	0	44.57	45.42	$C_{39}H_{74}NO_{8}P$
PS 36:2	M-H	786.5291	786.5289	14979	0.2	0	40.73	40.88	$C_{42}H_{78}NO_{10}P$
PE 40:5	M-H	792.5549	792.5535	6531	1.8	0	35.88	50.12	$C_{45}H_{80}NO_{8}P$
PC 37:5b	M-H	792.5549	792.5535	6531	1.8	0	35.88	50.12	$C_{45}H_{80}NO_{8}P$
PC 37:5	M-H	792.5549	792.5535	6531	1.8	0	35.88	50.12	$C_{45}H_{80}NO_{8}P$
PC 35:2b	M-H	770.5705	770.5691	6993	1.8	4	35.72	50.18	$C_{43}H_{82}NO_{8}P$
PC 35:2	M-H	770.5705	770.5691	6993	1.8	4	35.72	50.18	$C_{43}H_{82}NO_{8}P$
PI 36:4	M-H	857.5186	857.5186	9805	0	0	31.31	31.31	$C_{45}H_{79}O_{13}P$
PE 36:4	M-H	738.5079	738.5081	15519	-0.2	0	26.01	26.1	$C_{41}H_{74}NO_{8}P$
PC 36:4b	M-H	780.5549	780.5538	572	1.4	2	25.62	31.61	$C_{44}H_{80}NO_{8}P$
PC 36:4	M-H	780.5549	780.5538	572	1.4	2	25.62	31.61	$C_{44}H_{80}NO_{8}P$
PE 36:1	M-H	744.5549	744.5529	18166	2.7	0	23.56	46.74	$C_{41}H_{80}NO_{8}P$
PC 33:1b	M-H	744.5549	744.5529	18166	2.7	0	23.56	46.74	$C_{41}H_{80}NO_{8}P$
PC 33:1	M-H	744.5549	744.5529	18166	2.7	0	23.56	46.74	$C_{41}H_{80}NO_{8}P$
PC 36:5b	M-H	778.5392	778.5399	542	-0.9	2	23.46	25.38	$C_{44}H_{78}NO_{8}P$
PC 36:5	M-H	778.5392	778.5399	542	-0.9	2	23.46	25.38	$C_{44}H_{78}NO_{8}P$
PE 38:5	M-H	764.5236	764.523	19056	0.7	0	20.93	22.12	$C_{43}H_{76}NO_{8}P$
PC 35:5b	M-H	764.5236	764.523	19056	0.7	0	20.93	22.12	$C_{43}H_{76}NO_{8}P$
PC 35:5	M-H	764.5236	764.523	19056	0.7	0	20.93	22.12	$C_{43}H_{76}NO_{8}P$
PI 40:6	M-H	909.5499	909.5505	1181	-0.7	0	20.51	22.16	$C_{49}H_{83}O_{13}P$
PI 34:0	M-H	837.5499	837.5472	2184	3.1	0	19.5	63.93	$C_{43}H_{83}O_{13}P$
PI 34:2	M-H	833.5186	833.5187	1603	-0.2	0	15.89	15.95	$C_{43}H_{79}O_{13}P$
PC 32:2b	M-H	728.5236	728.5266	1315	-4.1	1	15.7	75.25	$C_{40}H_{76}NO_{8}P$
PC 32:2	M-H	728.5236	728.5266	1315	-4.1	1	15.7	75.25	$C_{40}H_{76}NO_{8}P$
PE O-38:7	M-H	746.513	746.5127	6780	0.4	0	15.39	15.6	$C_{43}H_{74}NO_{7}P$
SM 39:1;O2b	M-H	771.6385	771.6378	819	0.9	0	15	16.38	$C_{44}H_{89}N_{2}O_{6}P$
SM 39:1;O2	M-H	771.6385	771.6378	819	0.9	0	15	16.38	$C_{44}H_{89}N_{2}O_{6}P$
PE 36:3	M-H	740.5236	740.5224	11228	1.6	0	14.21	18.03	$C_{41}H_{76}NO_{8}P$
PE 38:6	M-H	762.5079	762.5081	10388	-0.2	0	11.77	11.8	$C_{43}H_{74}NO_{8}P$
PI 38:2	M-H	889.5812	889.5778	4147	3.8	4	9.51	66.88	$C_{47}H_{87}O_{13}P$
PS 40:6	M-H	834.5291	834.5266	2277	2.9	1	8.86	25.29	$C_{46}H_{78}NO_{10}P$
PE O-36:3	M-H	726.5443	726.5409	1987	4.7		7.83	59.62	$C_{41}H_{78}NO_{7}P$
PI 38:5	M-H	883.5342	883.5342	7959	0	0	4.95	4.95	$C_{47}H_{81}O_{13}P$
PE 34:0	M-H	718.5392	718.5375	3681	2.4	0	4.55	7.69	$C_{39}H_{78}NO_{8}P$
PC 31:0b	M-H	718.5392	718.5375	3681	2.4	0	4.55	7.69	$C_{39}H_{78}NO_{8}P$
PC 31:0	M-H	718.5392	718.5375	3681	2.4	0	4.55	7.69	$C_{39}H_{78}NO_{8}P$
PI 37:4	M-H	871.5342	871.5385	636	-5	0	2.53	63.46	$C_{46}H_{81}O_{13}P$
PI 36:3	M-H	859.5342	859.5308	3783	3.9	2	1.82	12.84	$C_{45}H_{81}O_{13}P$
PE 36:5	M-H	736.4923	736.4935	524	-1.7	1	1.59	2.07	$C_{41}H_{72}NO_{8}P$

Table 2. Matched hits with scores using the newly developed tool on mouse pancreas data.



Processing Settings

General
MS resolving power: 30000

Target matching
Mass error threshold (ppm): 5
Intensity threshold (counts): 500

A-Number and Isotope scoring
A0 intensity threshold (counts): 100
Max number of isotopes: 5
Isotope error threshold (ppm): 20
Isotope intensity threshold (counts): 50

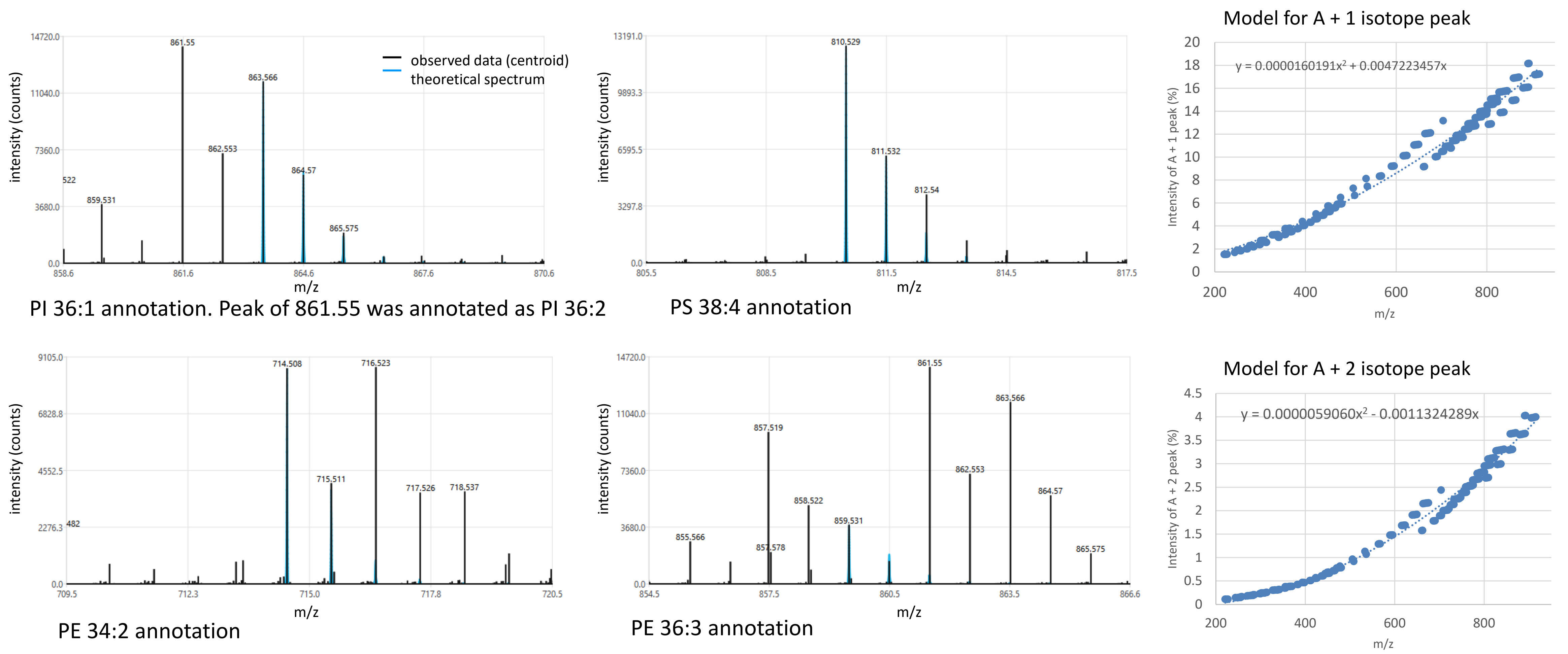
Display Settings

Detail spectrum view margin (mz): 5

Score color coding

Score color coding	🟩	🟨	🟥
Mass acc (ppm):	3.5	>	
A-Number	0		>
Isotope score:	40	20	<
Total score:	40	20	<

Figure 4. Data processing settings (top) and color codes (bottom).



PI 36:1 annotation. Peak of 861.55 was annotated as PI 36:2

PS 38:4 annotation

PE 34:2 annotation

PE 36:3 annotation

Figure 3. (Top) Selected annotations showing observed and theoretical isotope patterns. **(Upper right)** Plot of intensity for A+1 and A+2 ions for group of typical lipids used to create the model for monoisotopic peak identification.

The isotope model, based on typical lipid composition and shown in figure 3, is especially important for annotation of the monoisotopic peaks in complicated spectra.

Conclusions

- Using mass accuracy and isotope pattern matching based on expected average patterns, lipid annotations could be supported or rejected.
- Combination of scores from mass accuracy, isotope pattern, and A-number matching used to remove incorrect hits.

Acknowledgement

Tissue samples and raw data was kindly provided by Dr. Ruth Gordillo, University of Texas Southwestern Medical Center.