Lipid Annotation for MALDI Imaging using Isotope Pattern, Spectral Pattern and Co-Localization Information

Jeff Dahl and Md Amir Hossen
Shimadzu Scientific Instruments, Columbia, Maryland

Introduction

MALDI imaging of animal tissue slices provides an incredibly detailed, spatially resolved data set for omics analysis. MALDI imaging using high resolution, accurate mass measurement acquired in an untargeted mode results in the benefit of a large comprehensive data set. Without chromatographic separation however, the annotation of lipid species is challenging. Using commercially available imaging MS software combined with isotope pattern, spectral pattern, and co-localization information, lipid annotation can be substantially improved.

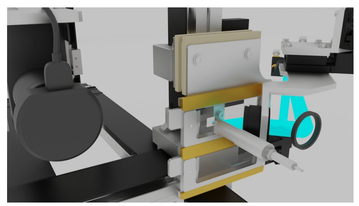

Figure 1. The IMScopeQT, a high-speed API-MALDI source connected to a QTOF mass spectrometer, was used to acquire data from mouse brains. A typical full mouse brain data set could be acquired in under 2 hours.

Methods

Mouse brains were sliced to a thickness of about 10 µm and attached to ITO slides. Slides were coated with 9AA matrix (0.9 µm thickness) using sublimation coating (using the Shimadzu iMLayer). Samples were analzyed on the Shimadzu iMScopeQT at 25 µm spatial resolution in positive ion mode. Datasets were processed using Shimadzu ImageReveal software in combination with separately developed isotope pattern matching functions and image similarity functions.

Setting	Value
Pitch X	25.0 µm
Pitch Y	25.0 µm
Polarity	Positive
Scan Range (*m/z*)	650 to 900
Number of Pixels	67,288
Laser Irradiation Number	100 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 brain tissue, 9AA coated, in positive MS mode.

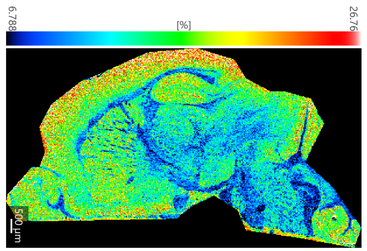

Figure 2. TIC image of typical mouse brain slice.

Results

A typical MS image of a mouse brain is shown in Figure 3. A summary table of annotations is shown in Table 2. For each possible annotation, the mass accuracy, isotope pattern, and co-localization score (where applicable) was calculated. The A-number, showing whether the peak was the monoisotopic peak, was calculated. The monoisotopic peak has an A-number of zero and isotopes have A-numbers greater than zero.

Name	Adduct	mz (theor.)	mz (obs)	Intensity	A-number	ppm err	isotope score	co-loc. score	Comment
Phosphatidylcholine(32:1)	M+H	732.5538	732.5545	158.9	0	-0.9	36.9	NA	inconclusive
Phosphatidylcholine(32:0)	M+H	734.5694	734.5702	6606.4	0	-1.1	85.9	NA	accept
Phosphatidylcholine(32:0)	M+Na	756.5514	756.552	2079.8	0	-0.7	60.8	2220	accept
Phosphatidylcholine(34:3)	M+H	756.5538	756.552	2079.8	0	2.4	79.0	NA	accept
Phosphatidylcholine(34:1)	M+H	760.5851	760.5859	10822.8	0	-1.0	77.0	NA	accept
Phosphatidylcholine(34:0)	M+H	762.6007	762.5976	2901.1	0	4.1	45.2	NA	accept
Phosphatidylethanolamine(36:3)	M+Na	764.5201	764.522	27.9	0	-2.5	31.5	NA	inconclusive
Phosphatidylethanolamine(38:6)	M+H	764.5225	764.522	27.9	0	0.6	6.9	NA	reject (isotope pattern)
Phosphatidylethanolamine(38:3)	M+H	770.5694	770.5656	340.2	1	4.9	NA	NA	reject (A+1 peak)
Phosphatidylethanolamine(38:1)	M+H	774.6007	774.6012	879.3	0	-0.7	69.7	NA	accept
Phosphatidylglycerol(36:1)	M+H	777.564	777.5631	16.3	1	1.2	NA	NA	reject (A+1 peak)
Phosphatidylcholine(34:1)	M+Na	782.567	782.5683	4889.3	0	-1.6	70.7	2028	accept
Phosphatidylcholine(36:4)	M+H	782.5694	782.5683	4889.3	0	1.5	76.5	NA	accept
Phosphatidylethanolamine(36:1)	M+K	784.5259	784.5236	49.4	0	3.0	3.6	NA	reject (isotope pattern)
Phosphatidylcholine(34:0)	M+Na	784.5827	784.5789	1116.3	0	4.9	27.9	NA	reject (mass error and pattern)
Phosphatidylcholine(36:2)	M+H	786.6007	786.6006	469.8	0	0.2	38.2	NA	inconclusive
Phosphatidylcholine(36:1)	M+H	788.6164	788.6171	2787.0	0	-0.9	71.9	NA	accept
Phosphatidylethanolamine(38:3)	M+Na	792.5514	792.5546	302.3	0	-4.1	51.3	4324	reject (not co-localized)
Phosphatidylethanolamine(40:6)	M+H	792.5538	792.5546	302.3	0	-1.1	48.6	NA	accept
Phosphatidylcholine(34:2)	M+K	796.5259	796.526	56.3	0	-0.1	0.7	NA	reject (isotope pattern)
Phosphatidylglycerol(36:2)	M+Na	797.5303	797.5275	16.8	1	3.5	NA	NA	reject (A+1 peak)
Phosphatidylcholine(34:1)	M+K	798.5415	798.5417	7704.3	0	-0.3	61.8	NA	accept
Phosphatidylglycerol(36:1)	M+Na	799.546	799.5452	3526.3	1	1.0	NA	NA	reject (A+1 peak)
Phosphatidylglycerol(38:4)	M+H	799.5484	799.5452	3526.3	1	4.0	NA	NA	reject (A+1 peak)
Phosphatidylethanolamine(40:1)	M+H	802.632	802.6316	75.4	0	0.5	64.6	NA	accept
Phosphatidylcholine(36:4)	M+Na	804.5514	804.5522	201.5	0	-1.0	36.1	4929	reject (not co-localized)
Phosphatidylcholine(38:7)	M+H	804.5538	804.5522	201.5	0	2.0	34.3	NA	inconclusive
Phosphatidylethanolamine(38:4)	M+K	806.5102	806.5097	375.8	0	0.6	32.0	NA	inconclusive
Phosphatidylcholine(36:3)	M+Na	806.567	806.5694	880.2	0	-3.0	51.2	NA	accept
Phosphatidylcholine(36:3)	M+H	806.5694	806.5694	880.2	0	0.0	48.5	NA	accept
Phosphatidylcholine(36:2)	M+Na	808.5827	808.5827	355.9	0	-0.1	31.2	2450	inconclusive
Phosphatidylcholine(38:5)	M+H	808.5851	808.5827	355.9	0	2.9	29.6	NA	reject (isotope pattern)
Phosphatidylcholine(36:1)	M+Na	810.5983	810.5998	1905.8	0	-1.9	67.1	1615	accept
Phosphatidylcholine(38:4)	M+H	810.6007	810.5998	1905.8	0	1.1	64.2	NA	accept
Phosphatidylcholine(36:4)	M+K	820.5259	820.5259	597.7	0	0.0	56.2	3776	reject (not co-localized)
Phosphatidylglycerol(38:4)	M+Na	821.5303	821.5293	193.2	1	1.2	NA	NA	reject (A+1 peak)
Phosphatidylglycerol(40:7)	M+H	821.5327	821.5293	193.2	1	4.2	NA	NA	reject (A+1 peak)
Phosphatidylcholine(36:2)	M+K	824.5572	824.5569	230.4	0	0.4	30.7	NA	inconclusive
Phosphatidylglycerol(40:5)	M+H	825.564	825.56	55.0	1	4.8	NA	NA	reject (A+1 peak)
Phosphatidylcholine(36:1)	M+K	826.5728	826.5728	1884.1	0	0.0	48.3	NA	accept
Phosphatidylethanolamine(40:7)	M+K	828.4946	828.4929	60.2	0	2.1	4.9	NA	reject (isotope pattern)
Phosphatidylethanolamine(40:6)	M+K	830.5102	830.5106	840.0	0	-0.5	51.2	7477	reject (not co-localized)
Phosphatidylcholine(38:5)	M+Na	830.567	830.5644	23.7	1	3.1	NA	NA	reject (A+1 peak)
Phosphatidylcholine(38:4)	M+Na	832.5827	832.5839	242.0	0	-1.4	44.5	2477	accept
Phosphatidylcholine(40:7)	M+H	832.5851	832.5839	242.0	0	1.5	42.3	NA	accept
Phosphatidylcholine(38:3)	M+Na	834.5983	834.6003	442.1	0	-2.4	50.0	NA	accept
Phosphatidylcholine(40:6)	M+H	834.6007	834.6003	442.1	0	0.5	60.8	NA	accept
Phosphatidylcholine(38:6)	M+K	844.5259	844.5259	541.1	0	0.0	62.5	5042	reject (not co-localized)
Phosphatidylglycerol(40:6)	M+Na	845.5303	845.5293	164.5	1	1.1	NA	NA	reject (A+1 peak)
Phosphatidylcholine(38:4)	M+K	848.5572	848.5567	504.2	0	0.6	49.0	2374	accept
Phosphatidylcholine(40:6)	M+Na	856.5827	856.5831	61.7	0	-0.5	45.3	2366	accept
Phosphatidylcholine(40:6)	M+K	872.5572	872.5572	242.9	0	0.0	44.6	2121	accept
Phosphatidylinositol(34:1)	M+K	875.5052	875.503	18.7	1	2.6	NA	NA	reject (A+1 peak)

Table 2. Lipid annotation for a typical mouse brain sample, including mass accuracy, isotope pattern match, A-number (A = 0 for monoisotopic peak), and co-localization. Each annotation was accepted, rejected, or left inconclusive based on a combination of each factor.

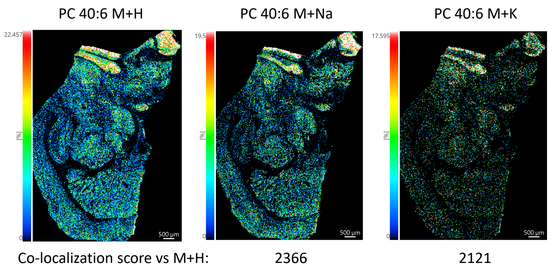

Figure 3. Example of closely co-localized analytes (adducts of PC 40:6). The co-localization score (vs the M+H adduct) is shown. Lower scores indicate closer co-localization.

Co-localization scores of signals corresponding to M+Na or M+K adducts of detected M+H signals were calculated. Examples are shown in Figures 3 and 4. In some cases, such as shown in Figure 4, it is not possible to distinguish between two species with nearly the same *m/z*, as the mass accuracy, isotope pattern, and co-localization are nearly the same for more than one annotation. However as shown in Table 2, many incorrect annotations can be rejected.

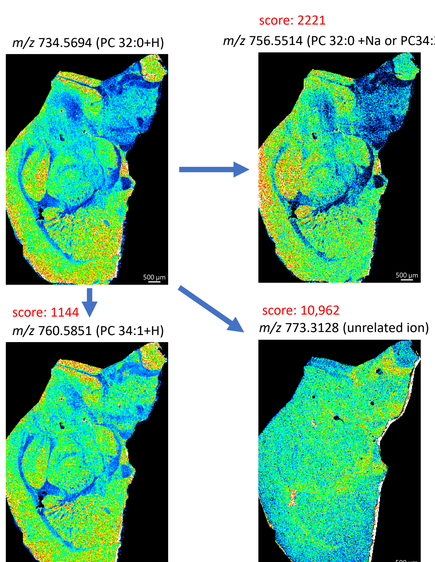

Figure 4. MS image for four ions and a co-localization score. In the case of m/z 756.5514, the annotation is consistent with either PC 32:0 (M+Na) or PC 34:3 (M+H) based on mass accuracy, isotope pattern, and co-localization.

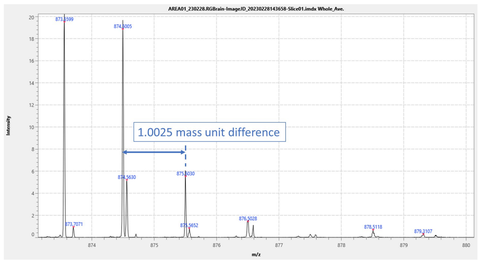

Figure 5 Magnified area-average spectrum around *m/z* 875. Although the peak at *m/z* 875.5030 is consistent with assignment as the $[M+K]^+$ ion of phosphatidylinositol (34:1), applying an isotope searching function revealed it matched the A+1 isotope peak of *m/z* 874.5005. Therefore the assignment as the $[M+K]^+$ ion of phosphatidylinositol (34:1) must be rejected.

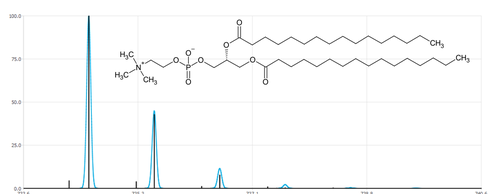

Figure 6. Observed mass spectrum (black) and theoretical mass spectrum (blue) of PC 32:0 M+H adduct (structure inset). The isotope score was 85.9 and the mass accuracy of the A+0 peak was -1.1 ppm. This indicates an excellent match between observed data and expected result.

Conclusions

- Fast and high-sensitivity atmospheric pressure MALDI could be carried out on mouse brain slices.
- Using mass accuracy, isotope pattern, and co-localization scoring, lipid annotations could be supported or rejected.
- An isotope searching function could detect whether a peak matched an isotope of a lower *m/z* peak by means of A-number matching.

Acknowledgement

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