High Speed, High Lateral Resolution Lipid Imaging in a MALDI-Q-TOF

Janina Oetjen[1], Arne Fuetterer[1], Juergen Suetering[1], Niels Goedecke[1], Sören-Oliver Deininger[1], Christian Marsching[3], Carsten Hopf[3], Lucy Woods[1], Oliver Raether[1], Jens Höhndorf[1], Shannon Cornett[2], Jens Fuchser[1], Aiko Barsch[1], Nikolas Kessler[1], Heiko Neuweger[1], Alice Ly[1]

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[1]Bruker Daltonik GmbH, Bremen, Germany
[2]Bruker Daltonics Inc., Billerica, MA, USA
[3]Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim Technical University, Mannheim, Germany

Introduction

Changes in lipid compositions are known to occur in response to stress and disease. This structural and functional diverse class of compounds plays important roles in various biological processes and therefore lipids represent an interesting group of biomolecules to study. MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a powerful tool for the *in-situ* examination of lipids. Here we present the performance of the timsTOF fleX, a MALDI-Q-TOF instrument, for MALDI-MSI measurements of lipids. This configuration together with the Smartbeam 3D laser technology and a fast and precisely moving stage allows for high speed and high spatially resolved imaging of lipids. The intelligence from MALDI can guide SpatialOMx analyses of select structures or cell populations.

Methods

The workflow for MALDI-MSI sample preparation and data analysis is outlined in Figure 1. For lipid imaging, fresh frozen rat or mouse brain was sectioned at 10 µm and mounted onto conductive slides (Bruker Daltonik GmbH, Bremen, Germany). After drying, sections were sprayed with 15mg/ml DHB in 90% ACN/H_2O or 2.5 mg/ml ZSA in 70% ACN/H_2O using a TM sprayer (HTX Technologies, Chapel Hill, NC, USA). Tissues were measured using the following parameters if not indicated otherwise: m/z range: 300-1000, 400 shots, 10 kHz laser frequency, pitch: 20 µm. Mass spectra were imported into and visualized using SCiLS Lab MVS software (Bruker Daltonik). Brain regions were identified using the Allen Brain Atlas (http://mouse.brain-map.org/static/atlas). Imaging data was imported to MetaboScape 5.0 and lipids annotated by a database search against a custom Analyte list in MetaboScape.

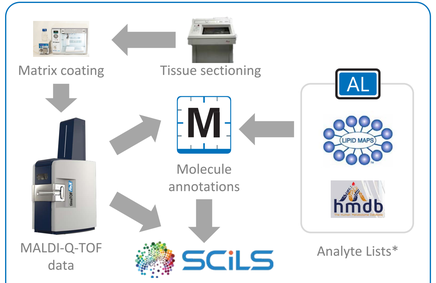


Fig. 1: MALDI-MSI workflow

*Lipid Maps and HMDB are not Bruker products.

Results

Table 1: Lipid imaging performance for PC(36:1) $[M+K]^+$ after MALDI-MSI measured on three instruments. A rat brain section was prepared with DHB and about 1500 pixel measured by MALDI-MSI using comparable settings (100 µm pitch, m/z range 150-3000). Data was imported to Data Analysis 5.0 and quality metrics were calculated for averaged spectra. The performance of the timsTOF fleX ranked between the axial MALDI-TOF instrument (rapifleX) and the MRMS system (scimaX).

Matrix	Instrument	Mol. formula	m/z measured	m/z calculated	Delta mass [ppm]	Res.	FWHM	S/N
DHB	rapifleX	$C_{44}H_{86}NO_8P$	826.571	826.572264	1.53	19563	0.042	83
	timsTOF fleX	$C_{44}H_{86}NO_8P$	826.5722	826.572264	0.08	40058	0.0206	568
	scimaX	$C_{44}H_{86}NO_8P$	826.57233	826.572264	-0.08	115066	0.00718	10970

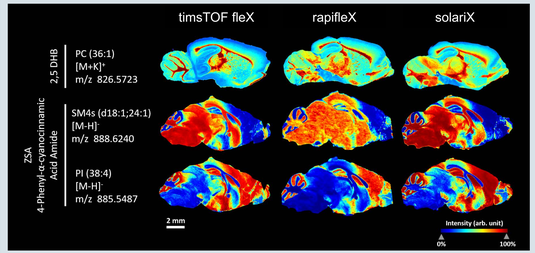


Fig. 2: Comparison of different MALDI-MSI lipid imaging results from three instruments in positive and negative mode. Lipid ID's were made by comparison with literature[1]. Distributions of the potassium adduct of PC(36:1) with similar patterns for the timsTOF fleX, rapifleX and solariX (upper panel). Sections were prepared with DHB matrix. The middle and lower panels show ion density maps with similar patterns for the deprotonated species of SM4s(d18:1;24:1) and PI(38:4) after ZSA[2] matrix preparation using a HTX TM sprayer. Data was acquired in negative mode using comparative settings with 20 µm pitch resolution. RMS-normalization and weak denoising was applied in SCiLS Lab MVS version 2019c for all datasets. Scale bars represent 2 mm; Relative intensities are indicated by false color coding using a jet color bar.

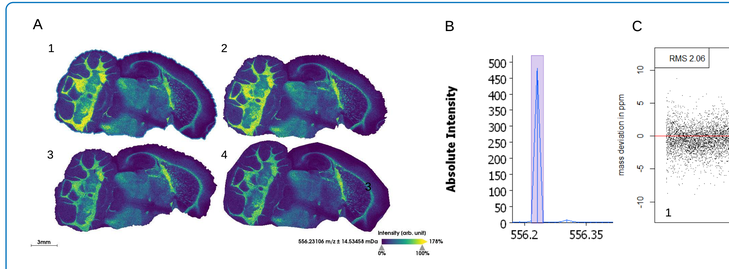


Fig. 3: Robust imaging performance and stable signal intensity.
(A) Distribution of a low abundant ion (m/z 556.23) without normalization for four consecutive imaging experiments visualized with SCiLS Lab MVS version 2019c. In total, ~1.5 million pixel with a lateral resolution of 20 µm pitch size were acquired in the order indicated. Each image is composed of ~370,000 pixel and the acquisition took about 5 hours per section. The relative intensity is indicated by the color bar. (B) Overall mean spectrum for the four measurements shown in (A) zooming on peak of interest. (C) Mass drift plots for the individual measurements shown in (A) for the peak at m/z 556.23.

Fig. 4: Spatial resolution
Visualization of the potassium adduct of PC(36:4) after RMS normalization in SCiLS Lab MVS version 2019c (A, B, C). DHB was sprayed with a HTX TM-sprayer™ on a 10 µm thick fresh-frozen rat brain section that has been cut sagittally. Data was acquired with 20 µm pitch size in positive mode. In the rat cerebellum (B), M+K of PC(36:4) is mainly localized in the granule cell layer and in a single cell layer in the middle of the white matter demonstrating true 20 µm lateral resolution. The anatomical fine structure enlarged in (C) colocalizes with a blood vessel, in which the heme signal at m/z 616.18 is high abundant (D).

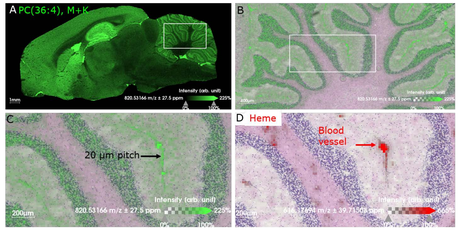
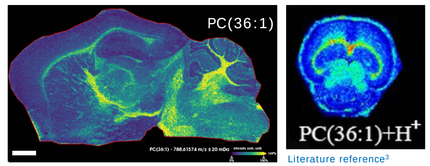


Table 2: Automatically annotated compounds using MetaboScape 5.0.
Using a custom Analyte List of known compounds the features (de-isotoped m/z signals belonging to the same molecular formula) extracted by the T-ReX² algorithm were automatically annotated. The entries of the Analyte List, containing 42755 compounds were downloaded from Lipid Maps (http://www.lipidmaps.org). Annotation Quality Scoring provided a fast overview of the confidence of each annotation based on accurate mass and isotopic fidelity (AQ column). Table 2 shows 10 hits out of 47 automatically annotated features.

	m/z meas.	M meas.	Ions	Name	mSigma	Δm/z [ppm]	Molecular For...	An...	AQ
1	788.61566	787.60839	[M]+	PC(18:0/18:1(11Z))	14.2	-0.978	$C_{44}H_{86}NO_8P$		
2	369.35237	368.34509	[M]+	3-Deoxyvitamin D3	20.5	2.060	$C_{27}H_{44}$		
3	744.49387	743.48659	[M]+	LMGP02080001	20.4	-3.129	$C_{43}H_{70}NO_7P$		
4	772.52494	771.51766	[M]+	LMGP02080002	36.8	-3.426	$C_{45}H_{74}NO_7P$		
5	734.56932	733.56204	[M]+	PC(10:0/22:0)	15.2	-0.172	$C_{40}H_{80}NO_8P$		
6	706.53835	705.53108	[M]+	PC(10:0/20:0)	8.1	-0.279	$C_{38}H_{78}NO_8P$		
7	651.53501	650.52773	[M]+	LMGL02070009	16.8	0.450	$C_{43}H_{70}O_4$		
8	496.34015	495.33288	[M]+	PC(O-14:0/2:0)	15.7	0.781	$C_{24}H_{50}NO_6P$		
9	746.60565	745.59838	[M]+	PC(O-16:0/18:1(9Z))	20.5	-0.680	$C_{42}H_{84}NO_7P$		
10	792.55377	791.54649	[M]+	PC(15:0/22:6(4Z,7Z,10...	17.3	-0.669	$C_{45}H_{78}NO_8P$		

Fig. 5. Lipid distribution. Visualization of PC(36:1) in the dataset used for compound annotation with MetaboScape. The lipid ID and distribution could be confirmed with a reference from literature[3]. Scale bar represents 1 mm



Literature reference[3]

Conclusions

- Lipid imaging with high speed and high lateral resolution robustly conducted on a Qq-oaTOF system, the timsTOF fleX
- Lipid distributions map to the expected localizations.
- Quality metrics for MALDI-MSI of a representative lipid demonstrate ranking between a MRMS system and an axial MALDI-TOF instrument.
- True 20 µm lateral resolution demonstrated by correlating positive signals with a histological feature.
- Lipid annotation automated with MetaboScape 5.0 featuring T-ReX² feature extraction technology and AQ scoring. Visualization of annotated signals with SCiLS Lab completing the intuitive workflow.

Acknowledgements

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References

1. Murphy, RC et al., J Lipid Res. 2009 Apr;50 Suppl:S317-22.
2. Fülöp A et al., Anal Chem. 2013 Oct 1;85(19):9156-63
3. Li et al., SciRep. 2016, 6:37903

MS-Imaging