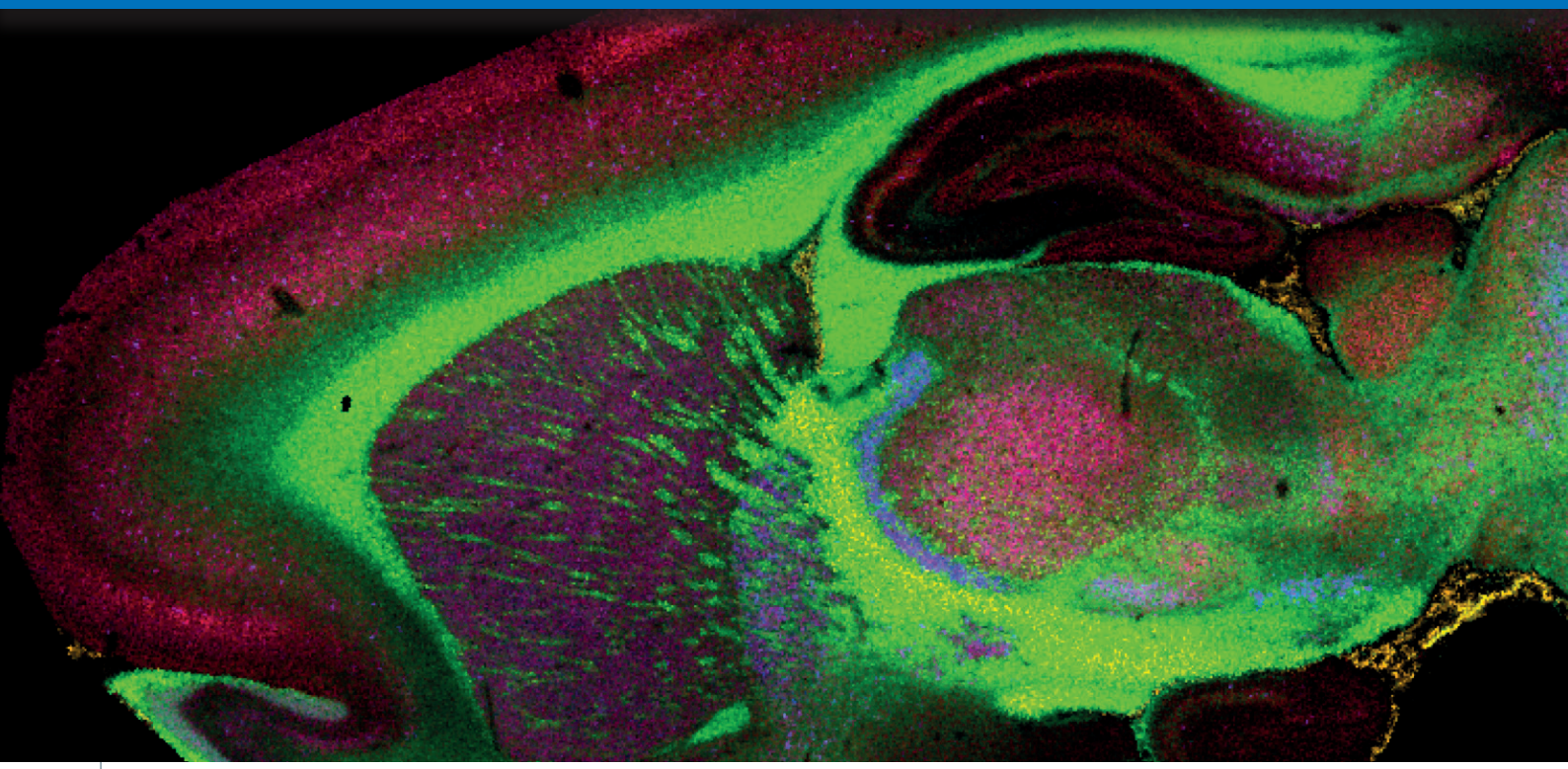

High Speed, High Lateral Resolution Lipid Imaging using a timsTOF fleX

Changes in lipid profiles are known to occur in response to stress and disease. This structurally and functionally diverse class of compounds play important roles in various biological processes and therefore lipids represent an interesting class of biomolecules to study.

Introduction

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 instrument for MALDI-MSI measurements of lipids. This configuation consisting of the game changing timsTOF Pro platform with Smartbeam 3D laser technology and a fast and precisely moving stage allows for high speed and high spatially resolved imaging of lipids.

Keywords: SpatialOMx, lipidomics, MALDI Imaging, timsTOF fleX, SCiLS Lab

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Methods

For lipid imaging, fresh frozen rat or mouse brain was sectioned at 10 µm and mounted onto conductive glass slides (Bruker Daltonik GmbH, Bremen, Germany). After drying, sections were sprayed with 15 mg/mL DHB in 90% ACN/H_2O or 2.5 mg/ml ZSA matrix in 70% ACN/H_2O using a TM sprayer (HTX Technologies, Chapel Hill, NC, USA) (1). Tissues were measured using the following parameters if not indicated otherwise: m/z range: 300-1000, shots: 400, laser frequency: 10 kHz, pitch: 20 µm. Mass spectra were imported into and visualized using SCiLS Lab MVS software (Bruker Daltonik GmbH). Brain regions were identified using the Allen Brain Atlas (http://mouse.brain-map.org/static/atlas).

Results and Discussion

MALDI-MSI allows the detection of many different molecular species in their native histological context and has emerged as a powerful technique for fields where knowledge of molecular spatial distribution is essential, such as pharmaceutical and biomedical research. However, until now there has been a gap between desired analysis properties, such as high speed and high mass resolution in current instrumentation in these fields. In this application note, we present a timsTOF fleX, a timsTOF Pro Q-TOF equipped with a fully integrated high speed, high spatial resolution MALDI source and stage and Smartbeam 3D.

Table 1 shows the performance of the timsTOF fleX compared to a dedicated high-throughput true pixel axial MALDI-TOF MSI instrument, and an extreme mass resolving magnetic resonance mass spectrometer. The timsTOF fleX ranks between these instruments in terms of resolution and signal to noise (S/N). Imaging results from the three

Matrix	Instrument	Sum formula	m/z calculated	m/z measured	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

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 comparative 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).

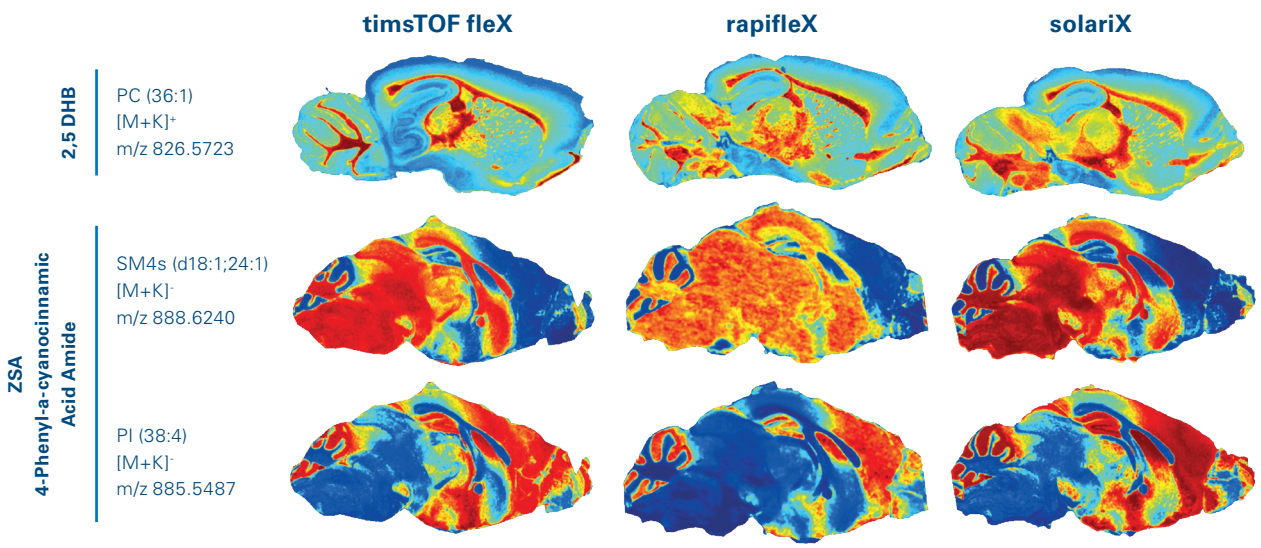

Figure 1: Comparison of different MALDI-MSI lipid imaging results from three instruments in positive and negative mode. Distributions of the potassium adduct of PC(36:1) with similar patterns for the timsTOF fleX, rapifleX and solariX (upper panel). Sections were prepared with DHB matrix. The middle and lower panels show ion density maps with similar patterns for the deprotonated species of PI(38:4) and SM4s(d18:1;24:1) after ZSA 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.

different instruments in positive and negative mode are shown in Figure 1. Although there are some differences due to the serial sections not being identical, the same structures are present for each of the m/z values across the different instruments.

Different aspects of instrument stability are shown in Figure 2. The distribution of m/z 556.23 is shown across four different brain sections without normalization. The image consists of approximately 1.5 million pixels for a total acquisition time of nearly 20 hours measured without lockmass calibration. The ion is present in white matter regions of the brain, and despite the long duration, signal fading is not present despite the high number of pixels indicating no matrix sublimation or sensitivity to source fouling. In the mass drift plot for the four measurements, it can be seen that the majority of the drift is within ± 5 ppm.

Figure 3 illustrates the visualization of the potassium adduct of PC(36:4) (m/z 820.532) primarily localized in the granule cell layer of the cerebellum 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 Figure 3C co-localizes with heme signal at m/z 616.177 (Figure 3D) and a blood vessel in the H&E stained sample.

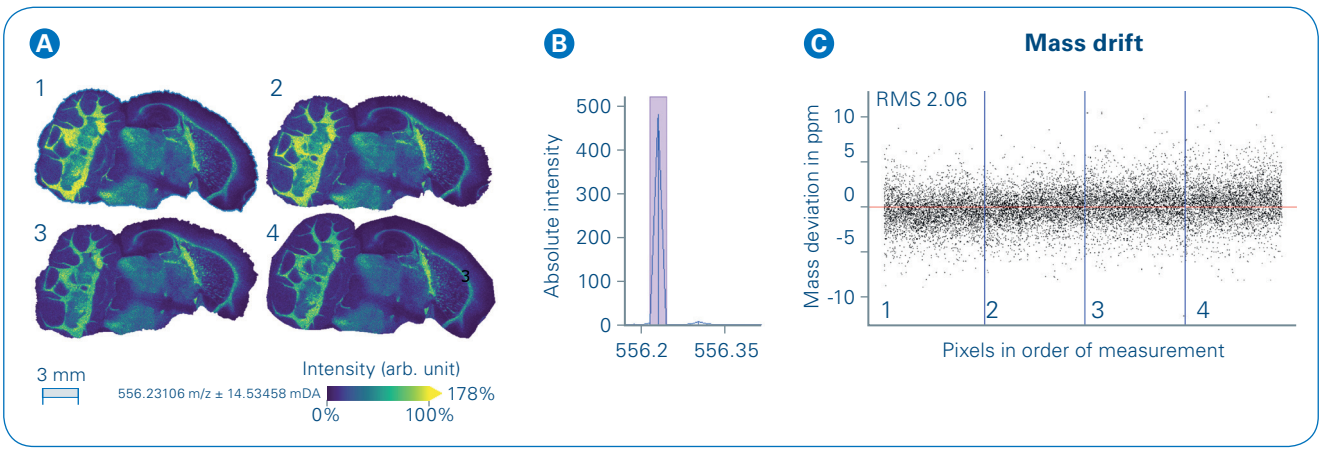

Figure 2: Robust imaging performance and stable signal intensity. A Distribution of a low abundant ion (m/z 556.23) without normalization for four consecutive imaging experiments visualized with SCiLS Lab MVS version 2019c. In total, ~1.5 million pixel with a lateral resolution of 20 µm pitch size were 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

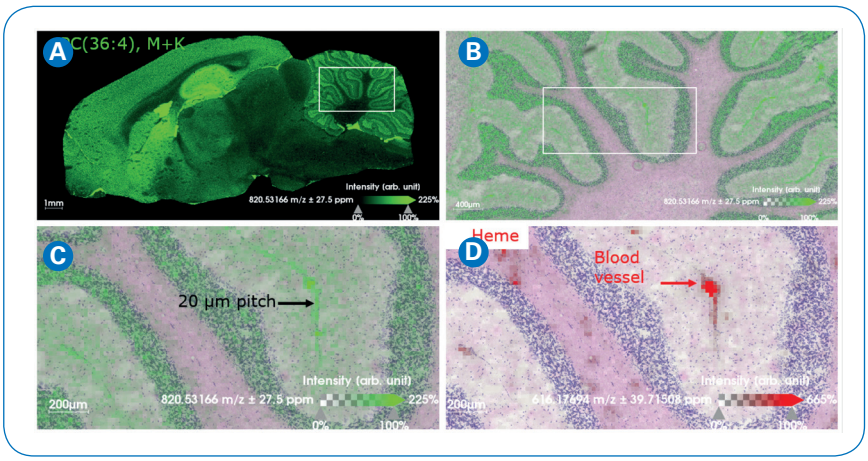

Figure 3: Spatial resolution. Visualization of the potassium adduct of PC(36:4) after RMS normalization in SCiLS Lab MVS version 2019c (A, B, C). DHB was sprayed with a HTX TM-sprayer™ on a 10 µm thick fresh-frozen rat brain section that has been cut sagittally. Data was acquired with 20 µm pitch size 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 co-localizes with a blood vessel, in which the heme signal at m/z 616.177 is high abundant (D).

Conclusions

- Lipid imaging with high speed and high lateral resolution robustly conducted on a 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.
- The timsTOF flex can be used to map the distribution of molecules across a section for SpatialOMx workflows.

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