Characteristics of MALDI-imaging on a new dual ion source QTOF with TIMS separation

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Introduction

MALDI Imaging has emerged as a technique with a broad range of applications. We present here the timsTOF fleX, a system consisting of a timsTOF Pro QTOF mounted with a fully integrated high throughput, high spatial resolution MALDI source and stage, enabling measurement of analytes at high mass resolving power with both high mass accuracy and high lateral resolution. The instrument has both an ESI and MALDI source (figure 1), making it ideal for SpatialOMx studies. Additionally, we present a novel software workflow for the identification of signals using data from the high-speed, high spatial resolution timsTOF fleX instrument (figure 2).

Methods

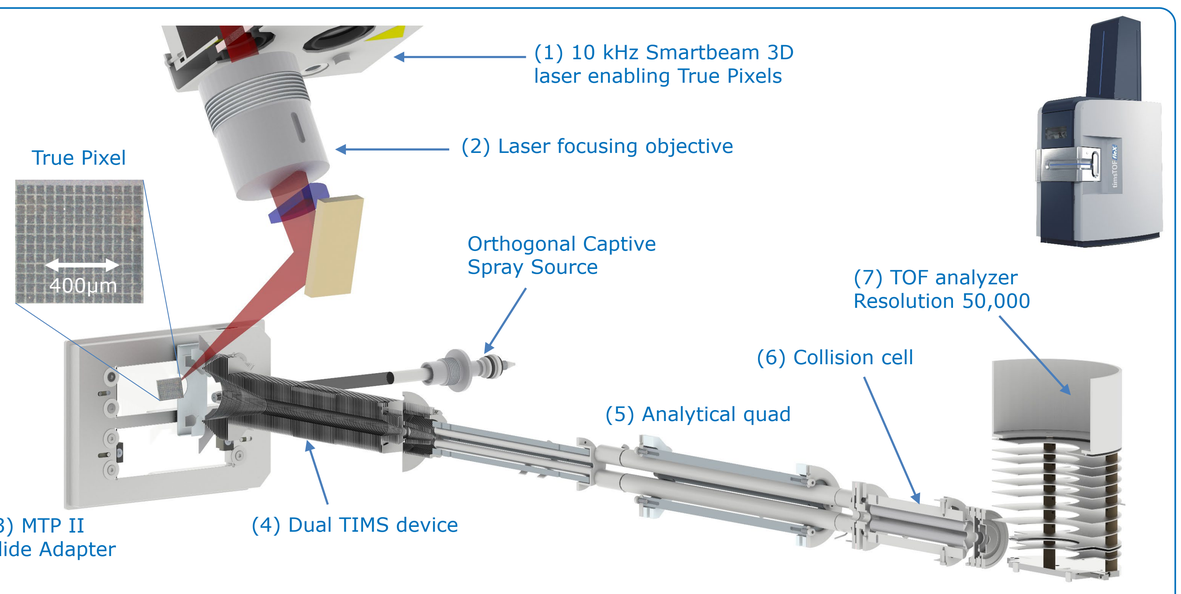
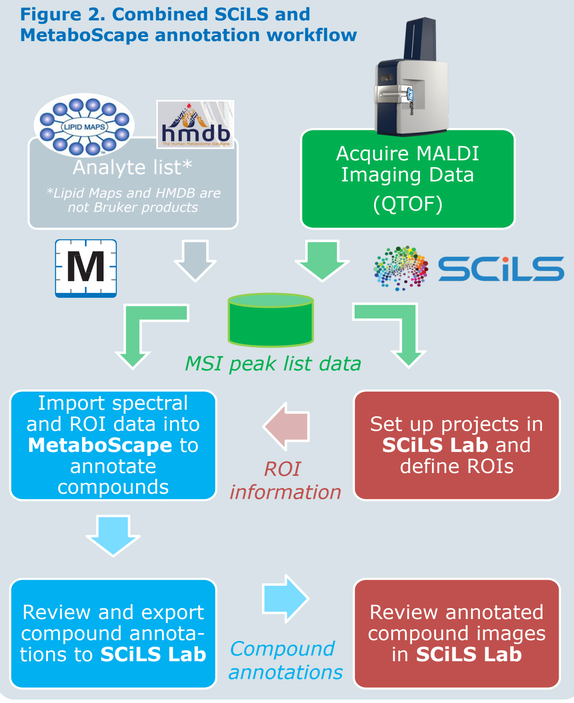
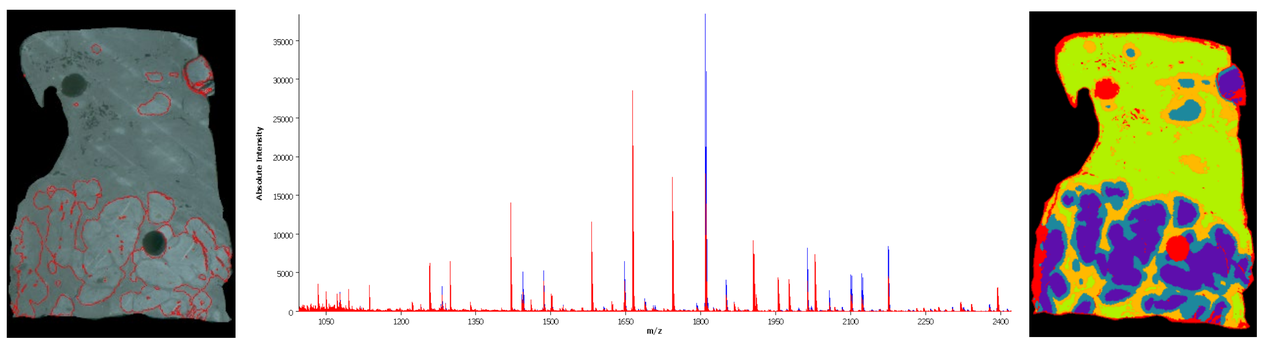


Figure 1. Ion optical components of the timsTOF fleX, a dual ion source trapped ion mobility separation quadrupole orthogonal time of flight instrument (MALDI/ESI-TIMS-Qq-TOF).

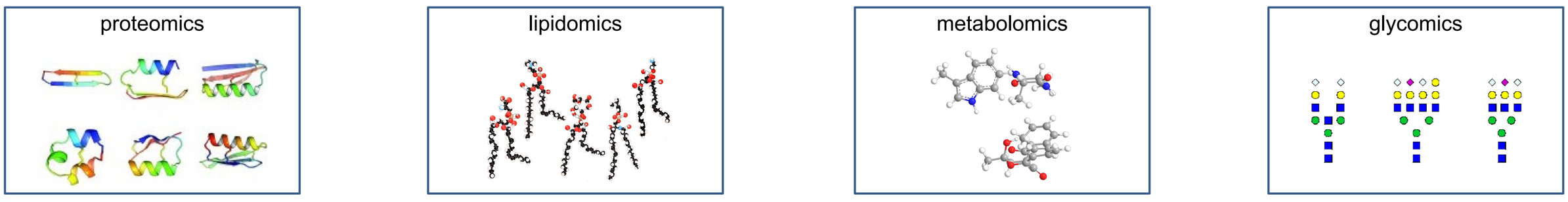
Figure 2. Combined SCiLS and MetaboScape annotation workflow

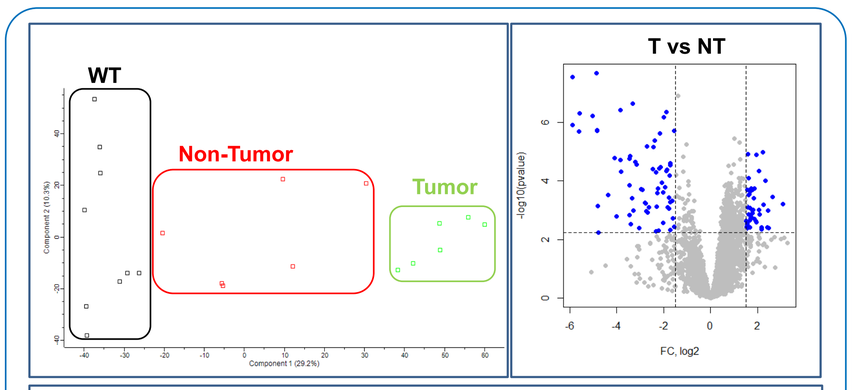


Results



LC-PASEF





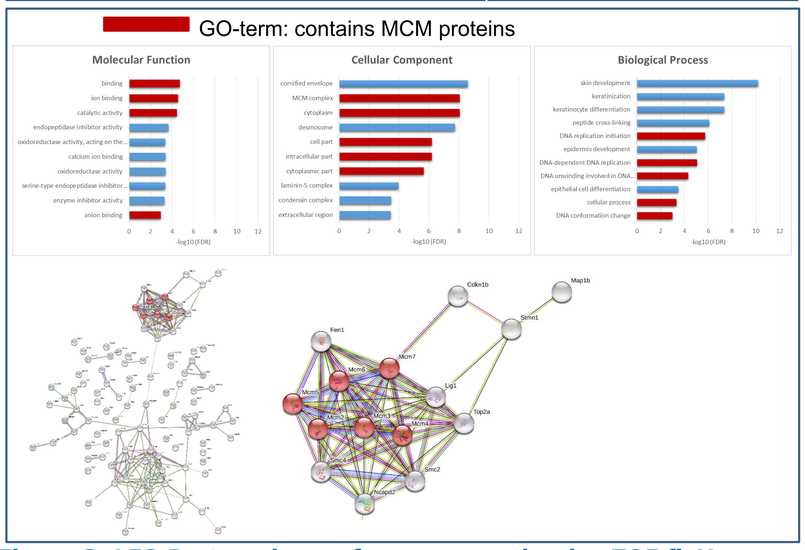
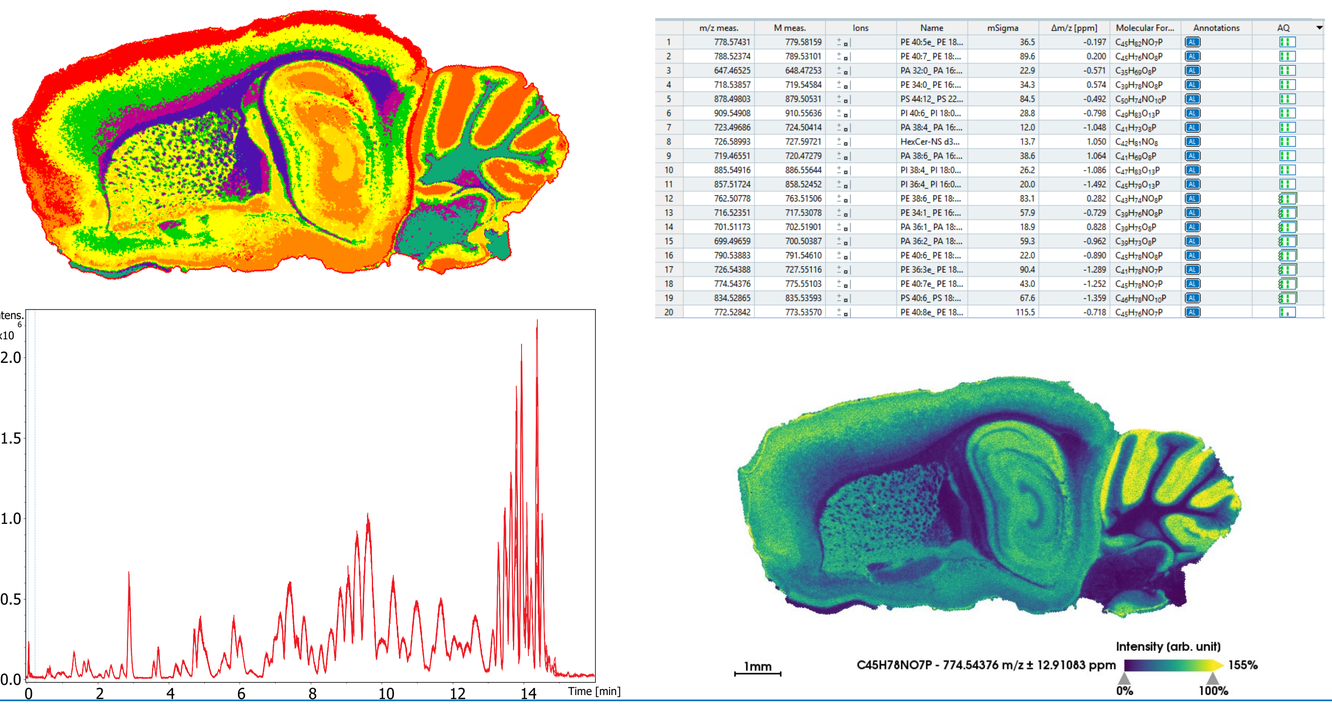


Figure 3: LFQ Proteomics performance on the timsTOF fleX. Tissue from frozen TCEA-positive and WT mouse stomach were prepared as described elsewhere [1], and analysed using PASEF acquisition mode with MS/MS acquisition rate of 109 Hz [2]. PCA of 5001 identified protein groups with their respective LFQ intensities separates the three groups (WT, NT, and T). Volcano plot of all identified protein groups comparing tumor and non-tumor tissues finds 110 significantly regulated proteins are highlighted. The analysis considers technical and biological replicates. Threshold for significance is an absolute fold-change value > 1.5 and an adjusted p-value < 0.05 after Limma statistics. STRING: functional protein association networks database GO annotation analysis of the 110 significantly regulated protein groups comparing tumor and non-tumor tissues based on imma statistics. The top ten hits within molecular function, cellular component, and biological process are shown. The minichromosome maintenance protein complex (MCM-complex) is shown to be enriched in tumor over non-tumor tissues. GO-terms including MCM-complex proteins are highlighted in red.



	m/z meas.	M meas.	Ions	Name	mSigma	Δm/z [ppm]	Molecular For...	Annotations	AQ
1	778.57431	779.58159		PE 40:5e_ PE 18:...	36.5	-0.197	$C_{45}H_{82}NO_7P$		
2	788.52374	789.53101		PE 40:7_ PE 18:...	89.6	0.200	$C_{45}H_{76}NO_8P$		
3	647.46525	648.47253		PA 32:0_ PA 16:...	22.9	-0.571	$C_{35}H_{69}O_8P$		
4	718.53857	719.54584		PE 34:0_ PE 16:...	34.3	0.574	$C_{39}H_{78}NO_8P$		
5	878.49803	879.50531		PS 44:12_ PS 22...	84.5	-0.492	$C_{50}H_{74}NO_{10}P$		
6	909.54908	910.55636		PI 40:6_ PI 18:0...	28.8	-0.798	$C_{49}H_{83}O_{13}P$		
7	723.49686	724.50414		PA 38:4_ PA 16:...	12.0	-1.048	$C_{41}H_{73}O_8P$		
8	726.58993	727.59721		HexCer-NS d3:...	13.7	1.050	$C_{42}H_{81}NO_8$		
9	719.46551	720.47279		PA 38:6_ PA 16:...	38.6	1.064	$C_{41}H_{69}O_8P$		
10	885.54916	886.55644		PI 38:4_ PI 18:0...	26.2	-1.086	$C_{47}H_{83}O_{13}P$		
11	857.51724	858.52452		PI 36:4_ PI 16:0...	20.0	-1.492	$C_{45}H_{79}O_{13}P$		
12	762.50778	763.51506		PE 38:6_ PE 18:...	83.1	0.282	$C_{43}H_{74}NO_8P$		
13	716.52351	717.53078		PE 34:1_ PE 16:...	57.9	-0.729	$C_{39}H_{76}NO_8P$		
14	701.51173	702.51901		PA 36:1_ PA 18:...	18.9	0.828	$C_{39}H_{75}O_8P$		
15	699.49659	700.50387		PA 36:2_ PA 18:...	59.3	-0.962	$C_{39}H_{73}O_8P$		
16	790.53883	791.54610		PE 40:6_ PE 18:...	22.0	-0.890	$C_{45}H_{78}NO_8P$		
17	726.54388	727.55116		PE 36:3e_ PE 18...	90.4	-1.289	$C_{41}H_{78}NO_7P$		
18	774.54376	775.55103		PE 40:7e_ PE 18...	43.0	-1.252	$C_{45}H_{78}NO_7P$		
19	834.52865	835.53593		PS 40:6_ PS 18:...	67.6	-1.359	$C_{46}H_{78}NO_{10}P$		
20	772.52842	773.53570		PE 40:8e_ PE 18...	115.5	-0.718	$C_{45}H_{76}NO_7P$		

Figure 4: SpatialOMx workflow for mouse brain lipids. Mouse brains were dissected, with sections from one hemisphere prepared for MALDI Imaging; lipids were extracted from the other hemisphere (25µg/µl) for LC-MS/MS analysis. Top left panel shows spatial segmentation from SCiLS Lab 2020 of a mouse brain section coated in ZSA matrix [3], and measured in negative mode at 20µm spatial resolution. Key brain regions of interest were exported: cortex (red), corpus callosum (purple), cerebellar white matter and hindbrain (teal) and grey matter (orange). Lower left panel shows 5 replicate LC-PASEF measurements in negative mode of brain lipid extracts from the timsTOF fleX from a 10 µl injection [4]. LC-MS/MS measurement of total brain extract contained 200 compounds. The LC-MS/MS data and MALDI Imaging ROIs were combined in MetaboScape 5.0. Top right panel shows 20 out of 49 annotated features from the combined datasets. Lower right panel shows an example of an annotated lipid in SCiLS Lab 2020.

Conclusions

- The timsTOF fleX delivers uncompromised shotgun proteomics performance making use of PASEF technology while providing MALDI imaging capabilities with 20 µm pitch spatial resolution and 15 pixel/sec speed.
- Addition of the MALDI source to the timsTOF Pro does not compromise proteomics performance
- Lipid annotation automated with MetaboScape 5.0 featuring T-ReX^2 feature extraction technology and AQ scoring. Visualization of annotated signals with SCiLS Lab completing the intuitive workflow.
- This workflow enables SpatialOMx by providing annotations to the morphological topography

timsTOF fleX

Acknowledgements

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References

[1] Erich K et al. (2018). Molecular & Cellular Proteomics. doi: mcp.RA118.000980;
[2] F. Meier et al. MCProt. December 1, 2018, 17 (12) 2534-2545
[3] Fülöp A et al., Anal Chem. 2013 Oct 1;85(19):9156-63
[4] ???