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

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Introduction

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) has emerged as a technique with a broad range of applications, particularly for clinical and pharmaceutical research. There is currently a gap between desired analysis properties, such as high speed and mass resolution capabilities in current instrumentation used in these fields.

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. The instrument has both an ESI and MALDI source. The MALDI source operates at medium vacuum (2-3 mbar), while the laser operates at 10 kHz laser frequency allowing for 15 pixels/second acquisition and 20 µm pitch.

In this work, we present the technical challenges, their solutions and results.

Methods

In a prototype setup we equipped a trapped ion mobility separation quadrupole orthogonal time of flight mass spectrometer (timsTOF Pro, Bruker) with a LASER (Smartbeam 3D, Bruker) and a MALDI stage (MTP-II, Bruker) (see Fig. 1).

MALDI operation:
Images had been measured from fresh-frozen rat brain tissue sections spray coated with 15 mg/ml DHB matrix in 90% ACN/H_2O using a TM Sprayer (HTX Technologies). MALDI imaging experiments were acquired with a new acquisition control SW. The measured range was *m/z* 300-1000. Spatial resolution was set to 20 µm pitch. Each pixel is averaged from 400 shots of the 10 kHz LASER. LASER shots of each pixel are geometrically arranged to ionize a square shape of 20 x 20 µm (True Pixel, Bruker [1]). The acquisition of each pixel takes 40 ms and the imaging speed is 15 pixels/second. Mass spectra were imported into and visualized using SCiLS Lab MVS software (Bruker Daltonik). For some samples, the tissues were stained with H&E following measurement, digitised and co-registered to the mass spectrometric data using SCiLS Lab. Brain regions were identified using the Allen Brain Atlas (http://mouse.brain-map.org/static/atlas).

ESI operation:
A nanoElute HPLC was coupled to the timsTOF fleX mass spectrometer (both Bruker Daltonik). Peptides of a Pierce HeLa digest (200 ng) were separated on a C18 column (25 cmx75 µm, 1.6 µm, IonOpticks, Australia) using a linear 90-minute gradient of 6 – 35% B (0.1% FA in ACN) at a constant flow rate of 400 nL/min. Data were acquired using Parallel Accumulation and Sequential Fragmentation (PASEF) acquisition mode with an MS/MS acquisition rate of 109 Hz [2] using the standard timTOF Pro control software. Database search has been performed with Mascot against human Uniprot database at a false discovery rate of below 1%.

Results

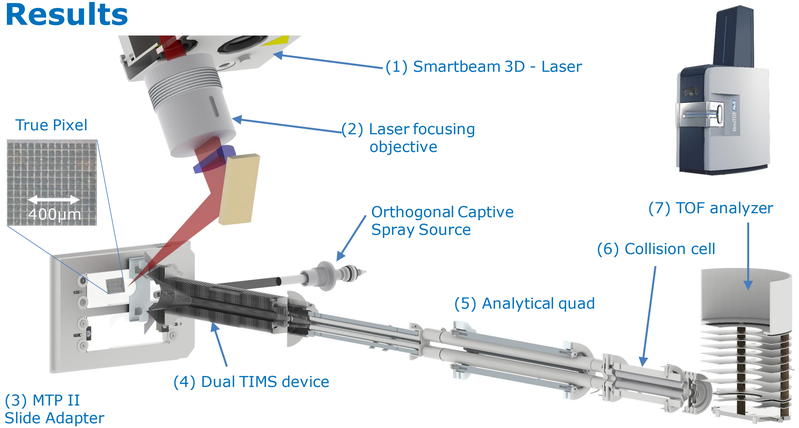


Fig. 1: Ion optical components of the timsTOF fleX, a dual ion source trapped ion mobility separation quadrupole orthogonal time of flight instrument (MALDI/ESI-TIMS-Qq-oaTOF). (1) Smartbeam 3D Laser enables ns laser pulses with maximum 10kHz repetition rate. (2) Laser objective design allows focusing of the laser beam over a long distance. This allows for no optical elements inside of the vacuum system. (3) 50 x 50 µm True Pixel MALDI stage with imaging speed of up to 15 pixel/sec. MALDI or ESI ions are accumulated and parallel analyzed in the dual TIMS device (4). The quadrupole (5) allows precursor mass selection up to m/z=3000. The collision cell (6) with axial field gradient and gating towards the orthogonal accelerator is designed for fast, high duty cycle transfer to the TOF analyzer (7) with full sensitivity resolution of 50,000.

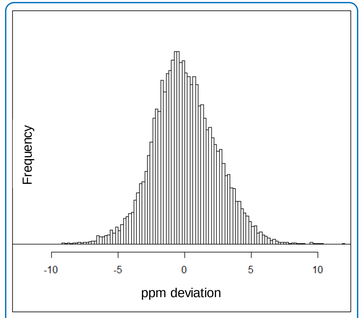
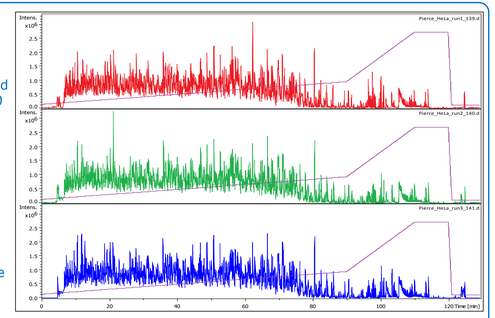


Fig. 2: Mass precision during imaging performance without lock mass. Histogram plotting the mass drift for the peak *m/z* 556.23 over six consecutive imaging measurements of rat brain sections. In total, ~2.2 million pixels with a lateral resolution of 20 µm pitch size were acquired; the acquisition of all six sections was approximately 30 hours.

Fig. 3: Proteomics performance. Around 5,780 protein families and on average 45,000 unique peptides sequences were identified in the shotgun "QC" samples at a false discovery rate of below 1 %. This confirmed uncompromised proteomics performance of the nano HPLC-TIMS-Qq-oTOF system.



Run	Protein Families	Unique Peptide Sequences	Peptide Spectrum Matches (PSMs)
Pierce_HeLa_run1_139.d	5784	44665	103554
Pierce_HeLa_run2_140.d	5783	45140	104117
Pierce_HeLa_run3_141.d	5793	45122	104718

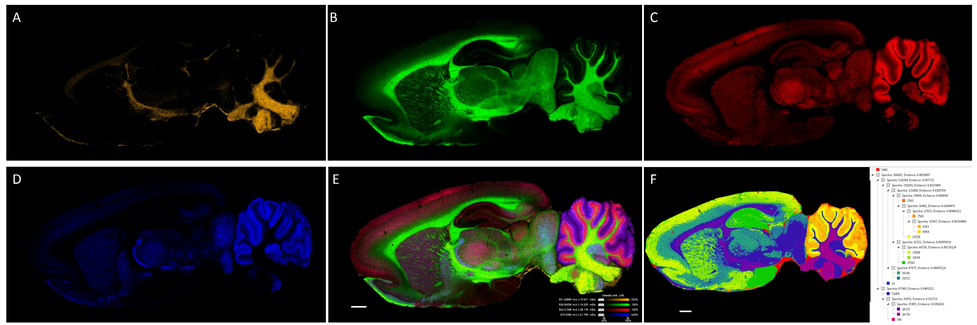
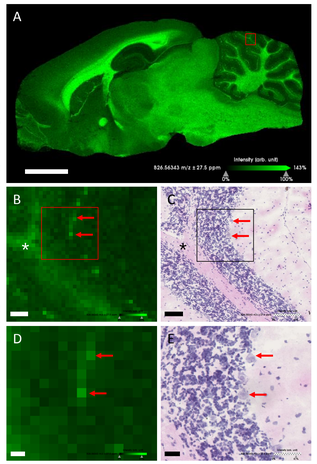


Fig. 4: Visualization of MALDI-MSI data acquired on the timsTOF fleX.
Sagittal rat brain section spray coated with 15 mg/ml DHB matrix and measured for lipids on the timsTOF fleX. Measurement parameters were: range m/z 300-1000; 10kHz laser repetition rate; 400 laser shots per pixel; 20µm pitch. In total, 312,095 spectra or approximately 2.2 million pixels were acquired; the acquisition time of the section was approximately 5 hours. (A) *m/z* 851.629 and (B) *m/z* 826.566 are in fibre tracts. (C) *m/z* 844.513 is in grey matter, but most intense in the granule cell layer of the cerebellum. (D) *m/z* 872.539 is most intense in the cerebellar molecular layer. (E) Merge of the preceding ion images. (F) Segmentation map and cluster tree calculated using the bisecting k-means algorithm with correlation distance with weak denoising clearly divides the spectra into regions corresponding to different brain regions. Dark & light green – neurons in the cortex. Yellow & orange – neurons in the cerebellum. Blues & purples – nerve fibre tracts. Scale bars represent 1 mm.

Fig. 5: MALDI-MSI at 20 µm pitch distance reveals signal belonging to discrete structures. (A). Overview of the distribution of *m/z* 826.572, putative K^+ adduct of PC (36:1), after RMS normalization in SCiLS Lab MVS version 2019c in a sagittal rat brain section indicates it is highly present in white matter tracts (corpus callosum in the cortex and arbor vitae of the cerebellum), and as a fine punctate layer in the cerebellum (red box). Scale bar indicates 3 mm. (B-C) Zoom of the area indicated by red box as the *m/z* image (B) and post-measurement H&E stained sample (C). This confirms the distribution of *m/z* 826.572 in the white matter (asterisks), and localizes the punctate signal to the border of the cerebellar granular layer. Scale bars indicate 90 µm. (D – E) Further zoom of the areas indicated by the boxes in B & C. The punctate signals in (D) correspond with Purkinje cells (arrows) demonstrating true 20 µm lateral resolution. Scale bars indicate 30 µm.



Conclusions

- The timsTOF fleX delivers uncompromised shotgun proteomics performance making use of PASEF technology while providing MALDI imaging capabilities with 20 µm pitch and 15 pixel/sec speed.
- Seamless change of operation modes from MALDI to ESI and back is possible.
- Addition of the MALDI source to the timsTOF Pro does not compromise proteomics performance
- Stable mass precision during MALDI imaging performance without lock mass calibration
- High quality MALDI data can be used for SpatialOMx workflows.

Acknowledgements
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References
[1] J. Hoehndorf ASMS 2015 Oral
[2] F. Meier et al. MCProt. December 1, 2018, 17 (12) 2534-2545;

timsTOF fleX