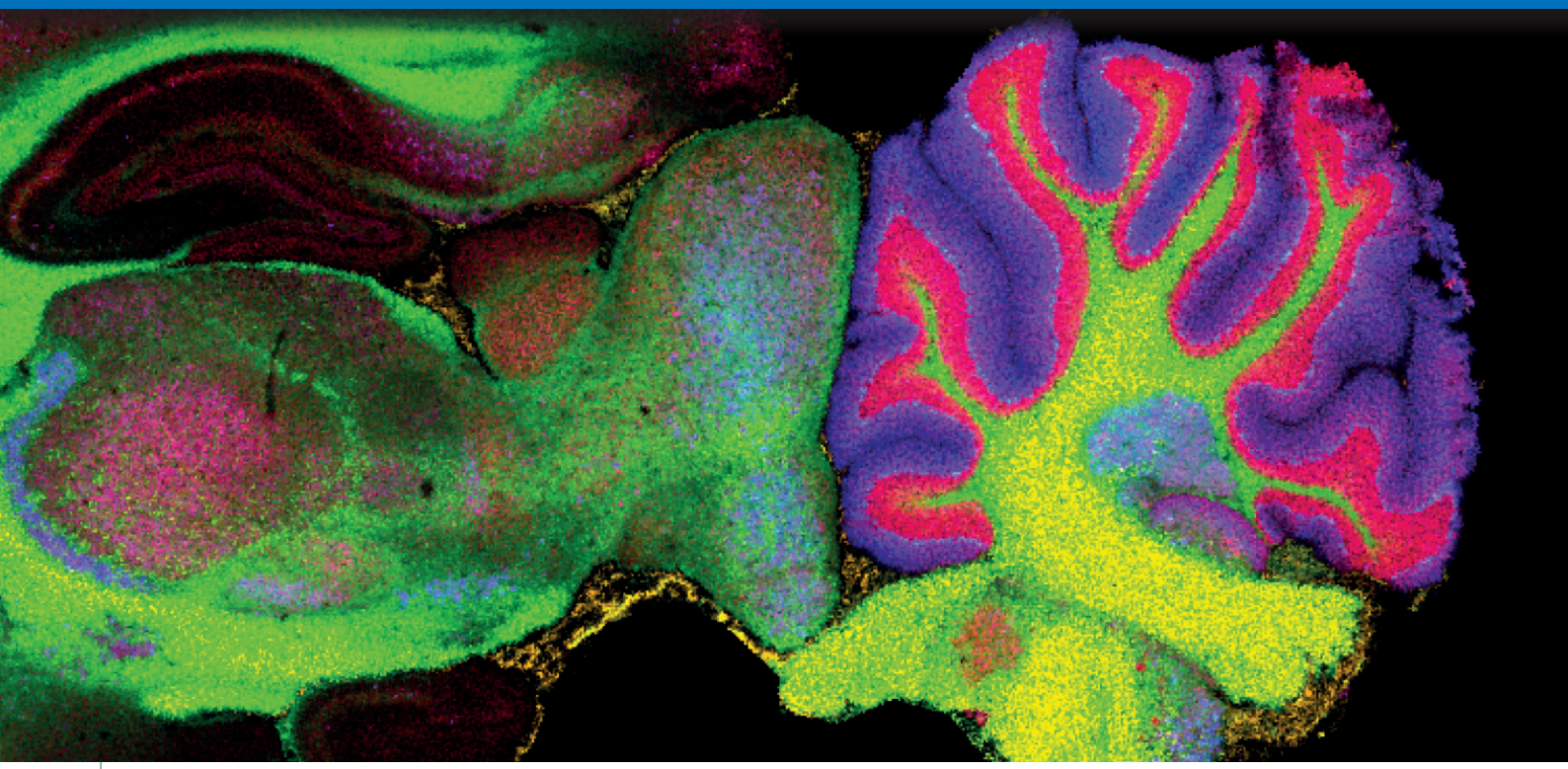

Novel MALDI Imaging solution empowered by a timsTOF fleX and dedicated bioinformatics pipeline for identification of lipids from tissue

MALDI Imaging spectra can be used to investigate how compounds are localized across tissue samples. In this application note, we present a novel software workflow for the identification of signals using data from the high-speed, high spatial resolution timsTOF fleX instrument.

Introduction

Understanding the content and distribution of molecules in tissue and the changes that occur to them in disease is integral to enhancing our understanding of the tissue-based diseases, such as cancers and neurological-based conditions. MALDI Imaging is a powerful label-free technique that allows mapping of a wide range of molecules from tissue sections and is capable of detecting hundreds to thousands of unique compounds in a single sample. This allows the understanding of molecular distributions over entire samples or even to classify distinct regions of interest. We present a novel workflow solution for automatically annotating MALDI Imaging data from a timsTOF fleX, allowing

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Authors: Janina Oetjen[1], Lucy Woods[1], Shannon Cornett[2], Jens Fuchser[1], Nikolas Kessler[1], Heiko Neuweger[1], Aiko Barsch[1], Alice Ly[1];
[1] Bruker Daltonik GmbH, Bremen, Germany; [2] Bruker Scientific LLC, Billerica, MA, USA.

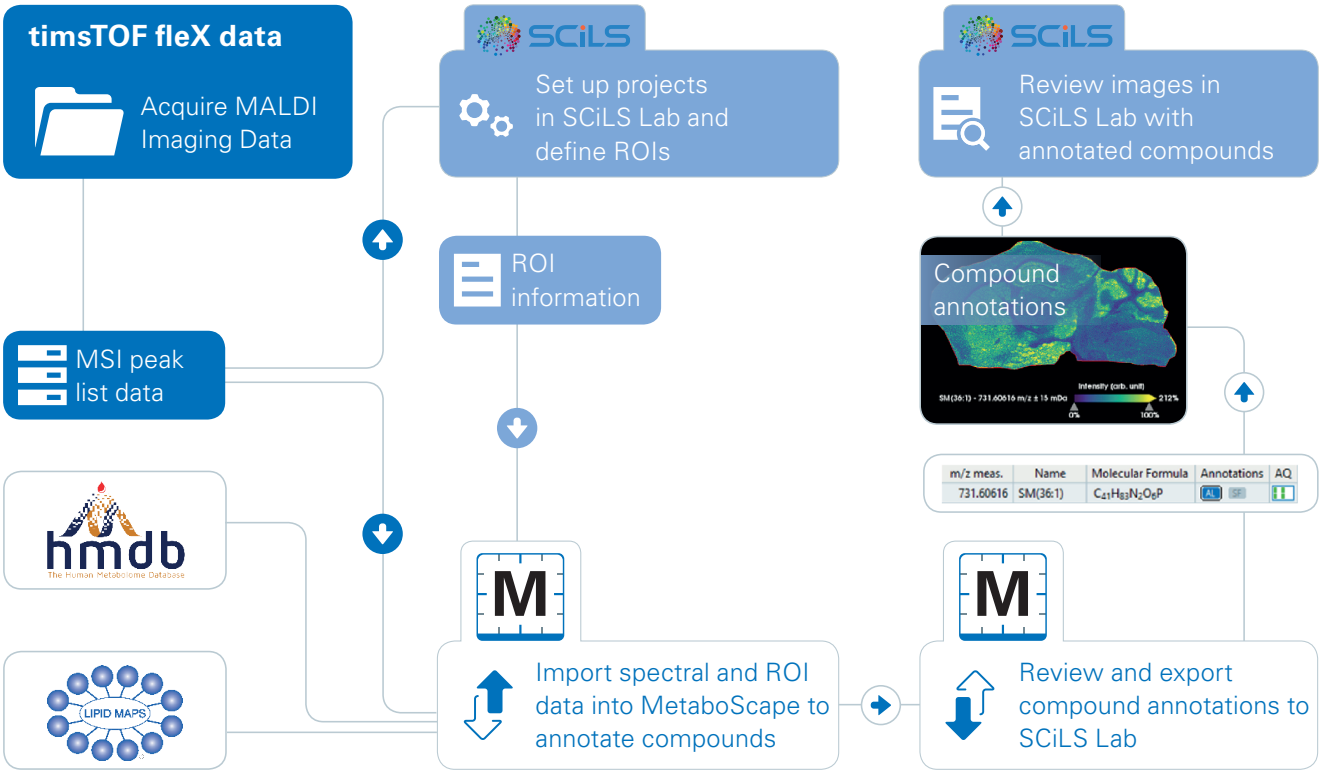

*Figure 1: Automated annotation of metabolites and lipids from MALDI Imaging data workflow. *Lipid Maps and HMDB are not Bruker products.*

subsequent generation of annotated images. In this application note, the workflow above is demonstrated on measurements of lipids from rat brain sections.

Methods

Fresh-frozen tissue sections on conductive glass slides were dried then matrix coated using a TM Sprayer (HTX Technologies, Chapel HIll, NC, USA). MS data were acquired on a timsTOF fleX. The measurement parameters were: positive mode, m/z range 300-1000, 20 µm pixel size, 10 kHz laser repetition rate. Data were visualized using SCiLS Lab (Bruker Daltonik GmbH). MetaboScape (Bruker Daltonik GmbH) was used for lipid annotation using the customizable Analyte List tool to match against the LIPID MAPS structure database and lipids reported in literature. MS data were processed in MetaboScape using the T-ReX^2 algorithm for feature extraction, de-isotoping and ion deconvolution. 2500 total pixels were averaged in 5x5 blocks yielding 100 spectra with averaged signal intensities. Mass tolerances were set to 5 mDa, and 250 mSigma for isotopic pattern fits.

	m/z meas.	M meas.	Ions	Name	mSigma	Δm/z [ppm]	Molecular For...	An...	AQ
1	788.61566	787.60839	±⁰	PC(18:0/18:1(11Z))	14.2	-0.978	$C_{44}H_{86}NO_8P$	AL	
2	369.35237	368.34509	±⁰	3-Deoxyvitamin D3	20.5	2.060	$C_{27}H_{44}$	AL	
3	744.49387	743.48659	±⁰	LMGP02080001	20.4	-3.129	$C_{43}H_{70}NO_7P$	AL	
4	772.52494	771.51766	±⁰	LMGP02080002	36.8	-3.426	$C_{45}H_{74}NO_7P$	AL	
5	734.56932	733.56204	±⁰	PC(10:0/22:0)	15.2	-0.172	$C_{40}H_{80}NO_8P$	AL	
6	706.53835	705.53108	±⁰	PC(10:0/20:0)	8.1	-0.279	$C_{38}H_{76}NO_8P$	AL	
7	651.53501	650.52773	±⁰	LMGL02070009	16.8	0.450	$C_{43}H_{70}O_4$	AL	
8	496.34015	495.33288	±⁰	PC(O-14:0/2:0)	15.7	0.781	$C_{24}H_{50}NO_7P$	AL	
9	746.60565	745.59838	±⁰	PC(O-16:0/18:1(9Z))	20.5	-0.680	$C_{42}H_{84}NO_7P$	AL	
10	792.55377	791.54649	±⁰	PC(15:0/22:6(4Z,7Z,10...	17.3	-0.669	$C_{45}H_{78}NO_8P$	AL	

Table 1: 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-ReX^2 algorithm were automatically annotated. The entries of the Analyte List, containing 42,755 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 1 shows 10 hits out of 47 automatically annotated features.

Results and Discussion

Label-free MALDI Imaging is capable of demonstrating localization of more molecules and molecular classes in a single tissue section than immunohistochemistry or other tag-based imaging techniques. A single MALDI Imaging dataset may contain hundreds to thousands of unique, label-free ion images, which can be used for molecular marker discovery or investigating the molecular content of specific regions of interest. Particularly for SpatialOMx studies, MALDI Imaging provides morphological topography to a given sample. For timsTOF fleX MALDI Imaging datasets, we present a pipeline for compound identification and visualization (Figure 1). Using this, timsTOF fleX peak list data is loaded into SCiLS Lab (version 2020a) for statistical analysis and defining regions of interest (ROIs). The spectral and ROI data is loaded into MetaboScape (version 5.0) for annotation e.g. via databases. The annotated data can be exported back to SCiLS Lab for visualization.

Table 1 is an example of a MetaboScape 5.0 data output from a timsTOF fleX MALDI Imaging dataset. The first two column headings indicate features, i.e. the de-isotoped m/z signals belonging to the same molecule extracted by the T-ReX^2 algorithm. The Ions column shows which ion adducts are combined into a feature (e.g. M+H). mSigma indicates goodness of fit of the isotopic pattern (measured vs simulated), where a low value denotes better fit. Δm/z [ppm] specifies mass error of measured versus predicted mass in parts per million. Annotation specifies how the annotation was completed (e.g. database or analyte list), and AQ (Annotation Quality) provides a fast overview of the scoring based on accurate mass and isotopic fidelity matching based on user-defined settings.

Figure 2 shows the distribution of m/z 788.623 in rat brain, identified as PC(36:1) in MetaboScape with the annotation in SCiLS Lab. As a comparison of the quality of the annotation and distribution, the right panel shows the distribution of PC(36:1) previously identified from published literature. In both the timsTOF fleX and published data, the signal is located in the white matter tracts of the brain.

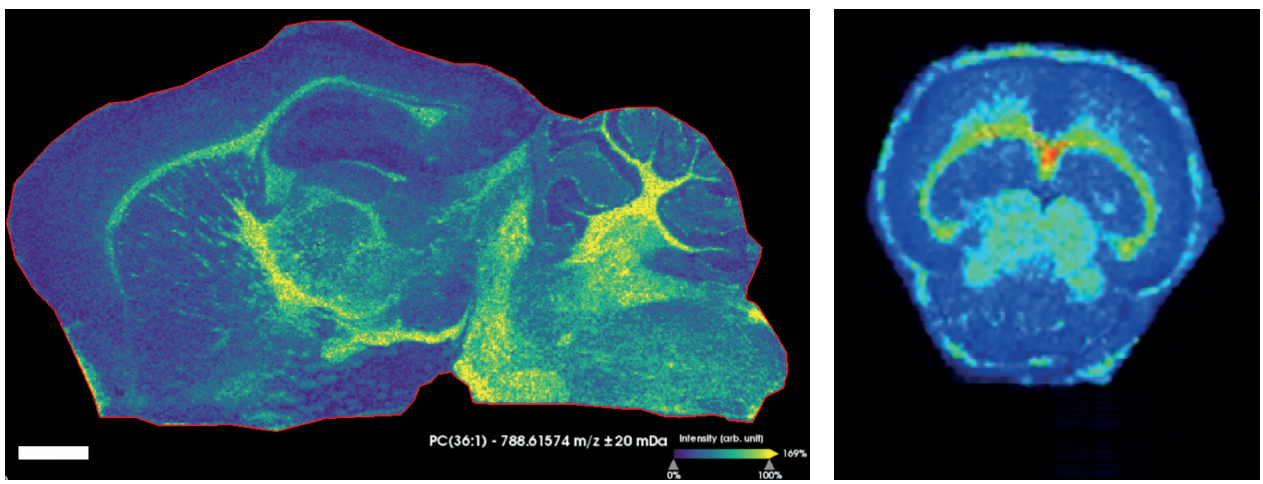

Figure 2: Lipid distribution. Visualizaiton of PC(36:1) in the dataset used for compound annotation with MetaboScape. The lipid distribution could be confirmed with a reference from literature. Scale bar represents 1 mm.

Conclusions

- 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.
- Lipid distributions map to the expected localizations.
- This workflow enables SpatialOMx by providing annotations to the morphological topography.

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com