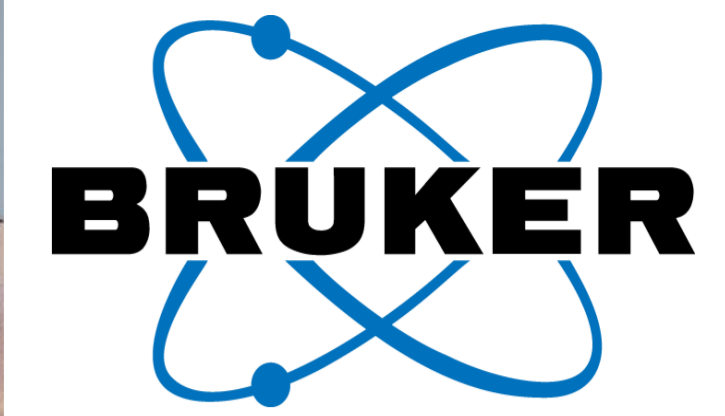


4D-Lipidomics™ based automated annotation of MALDI Imaging data using a dedicated bioinformatics pipeline

Janina Oetjen¹, Christian Marsching², Sven Meyer¹, Corinna Henkel¹, Annika Koch¹, Nikolas Kessler¹, Wiebke Timm¹, Aiko Barsch¹, Jan Hendrik Kobarg¹, Dennis Trede¹, Heiko Neuweger¹, Carsten Hopf²



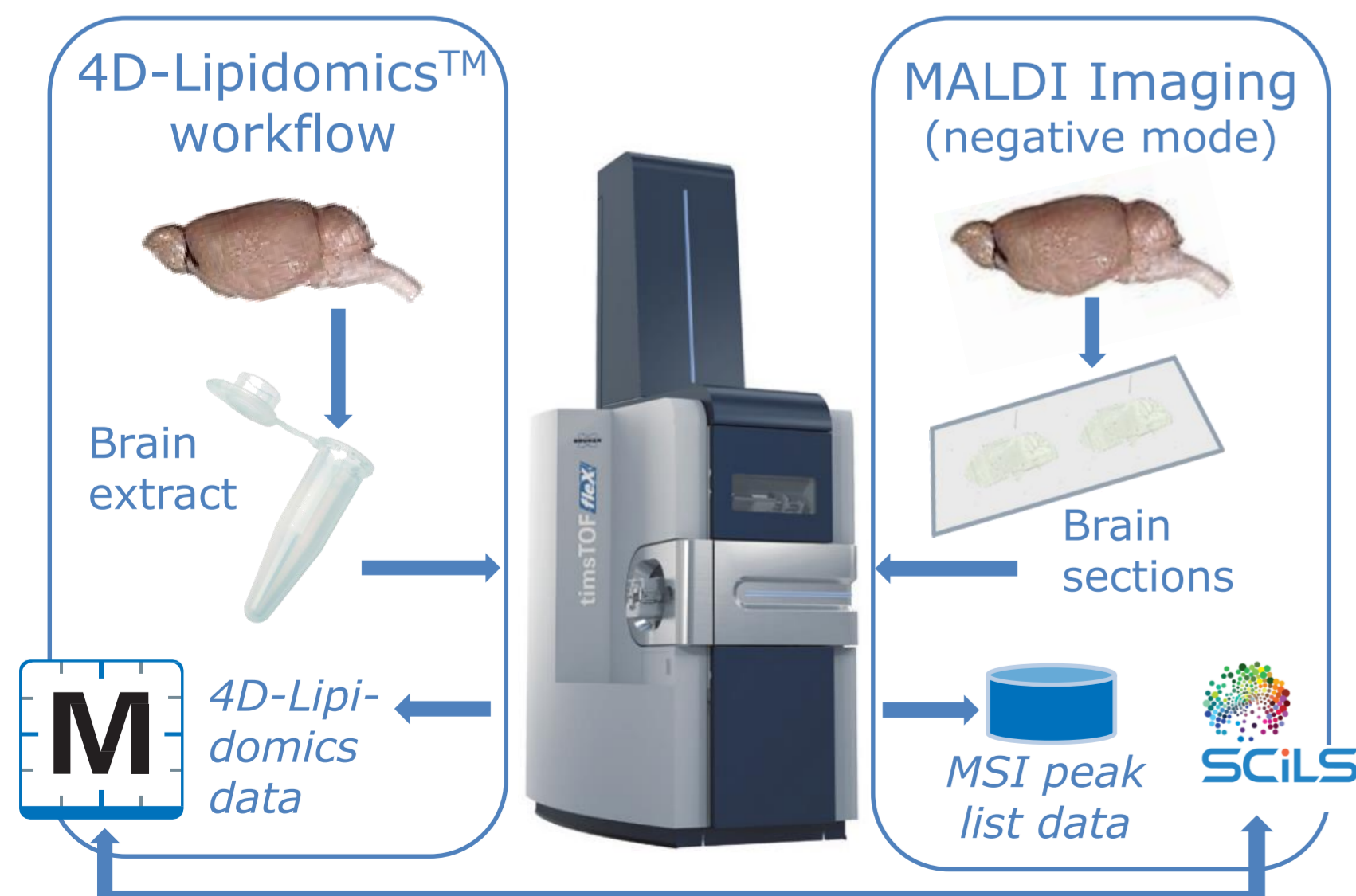
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1. Bruker Daltonics Inc., Fahrenheitsstr. 4, 28359 Bremen, Germany
2. Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim Technical University, Mannheim, Germany

Introduction

The complexity of lipid samples makes their analysis directly from tissue sections extremely challenging. Ion mobility helps in this respect by introducing an additional analytical dimension that can separate isomeric and isobaric molecules. Moreover, annotation of lipids from MALDI Imaging data is often hampered by the limited ability to perform large-scale MS/MS experiments directly from tissue. Here we demonstrate a workflow combining 4D-Lipidomics™ and MALDI Imaging data obtained from just one instrument, timsTOF fleX, in conjunction with a bioinformatics pipeline for automatic lipid annotation.

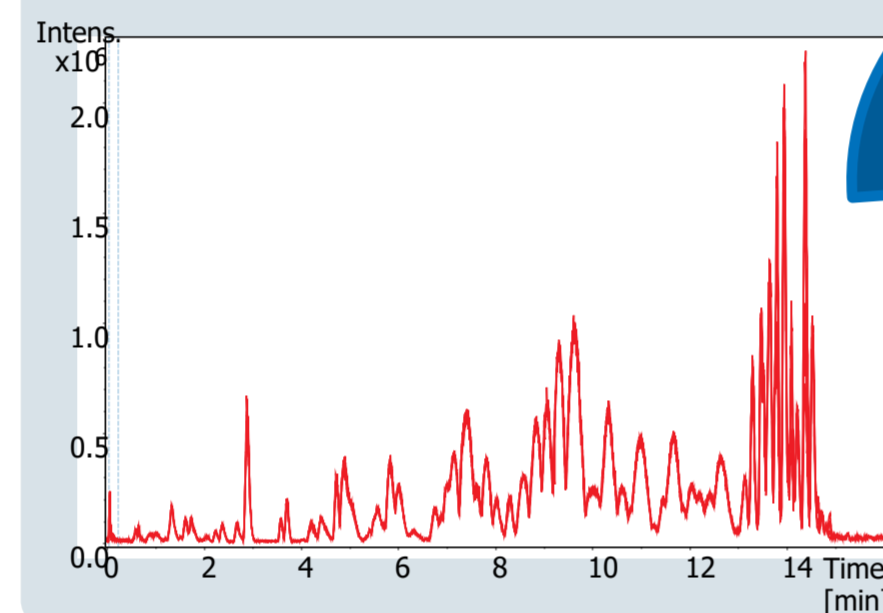
Methods



Results

4D-Lipidomics results

Apply five quality criteria on PASEF empowered 4D-Lipidomics data for confident annotations. Merge positive and negative mode data to increase the number of hits.



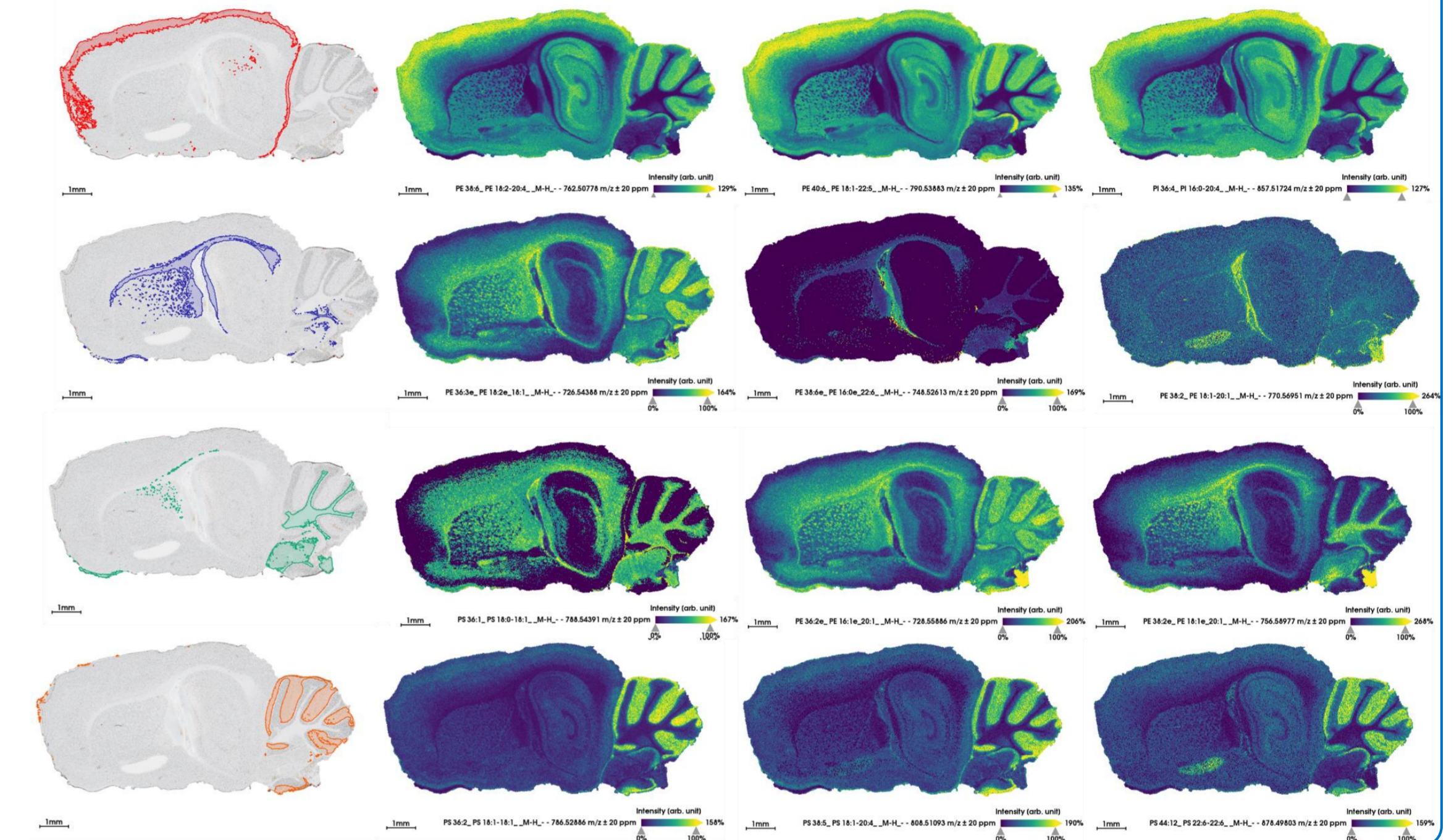
AQ score



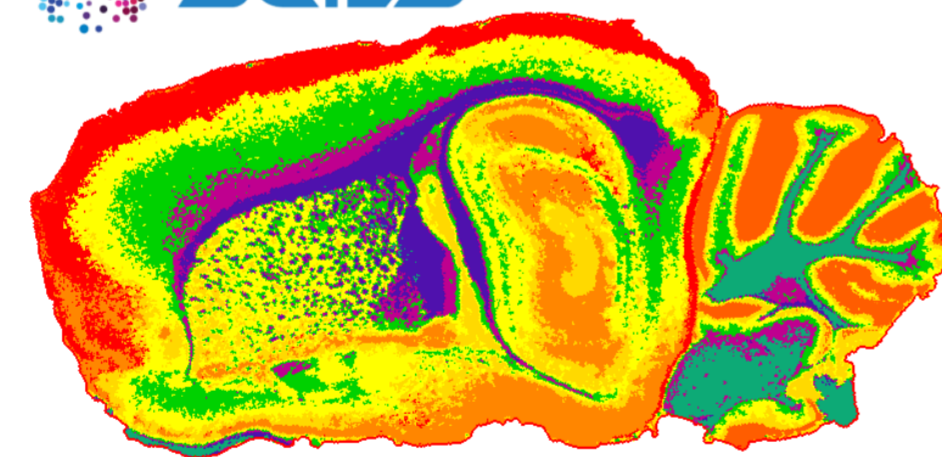
- A Precursor mass accuracy
- B Retention time
- C Isotopic pattern
- D MS/MS fragment spectra
- E CCS values

Data	Buckets	Buckets with MS/MS	Annotations based on LipidBlast Search
Pos. Ionization	7107	2984	811
Neg. Ionization	2915	1535	546
Pos / Neg merged	9797	4353	1232

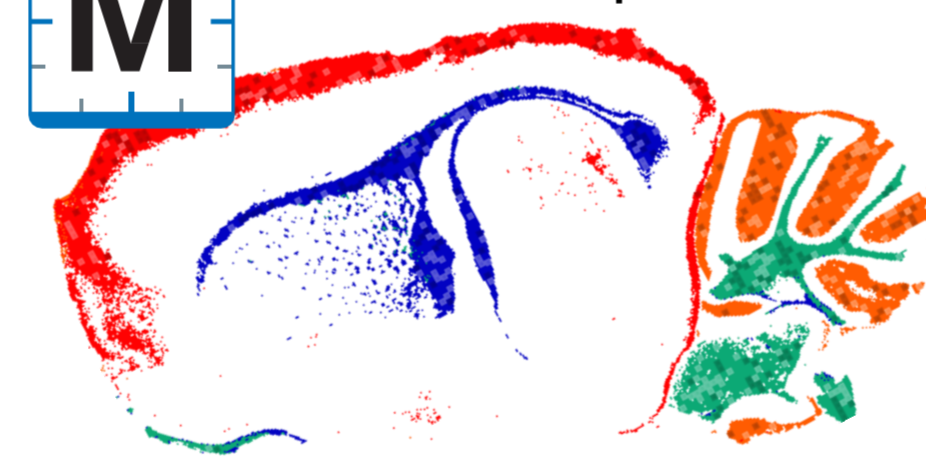
Correlate molecular information with spatial information.



MALDI Imaging results.



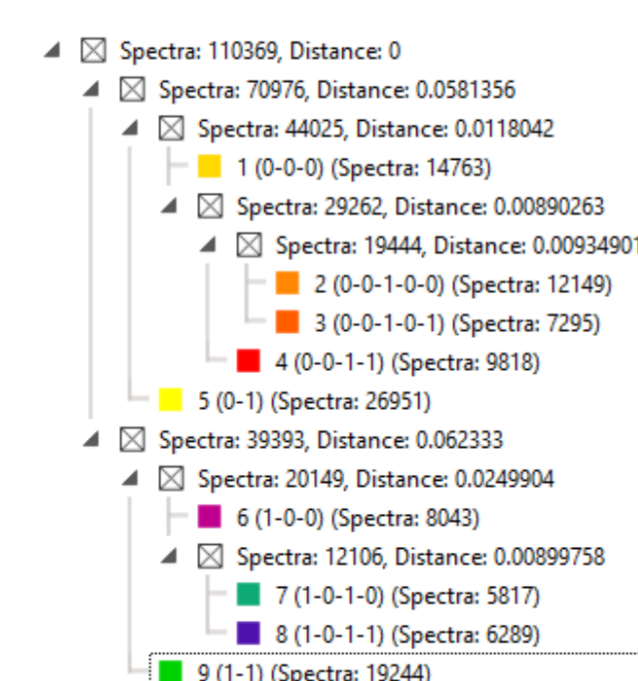
Region of interest (ROI) map of automatically generated molecular features.



Extract data from ROIs and import to MetaboScape 5.0

- Annotation
- Red: Cortex
 - Blue: Corpus callosum
 - Orange: Molecular layer
 - Green: White matter of cerebellum

Retrieve automatic annotations using an Analyte List composed of the 4D-Lipidomics results.



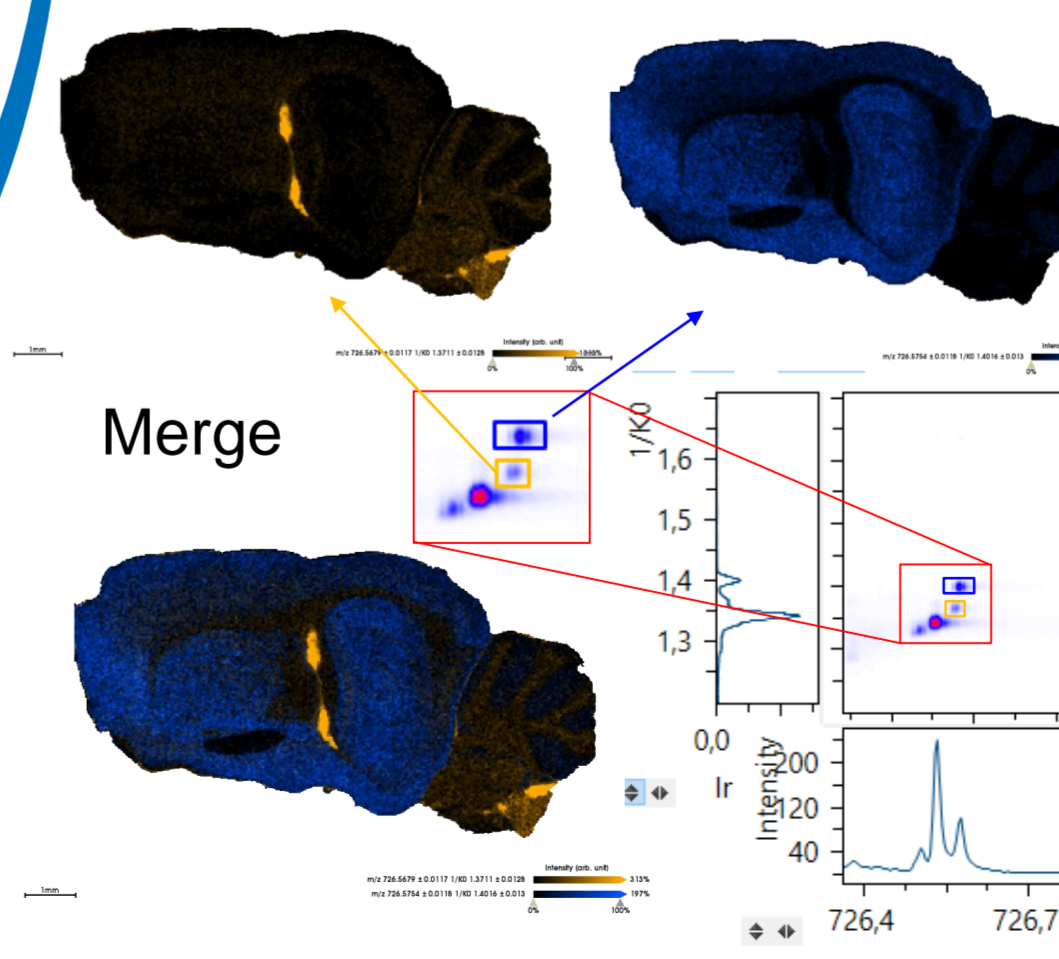
Bucket table (feature list) with 55 annotated compounds = images.

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 ₄₀ H ₇₈ NO ₂ P	[A][B]	[E]
2	788.52374	789.53101	PE 40:7...PE 18...	89.6	0.200	C ₄₀ H ₇₇ NO ₂ P	[A][B]	[E]
3	647.46436	648.47163	DA 35:1...DA 16...	75.0	0.671	C ₃₅ H ₆₂ O ₂	[A][B]	[E]
4							[A][B]	[E]
5							[A][B]	[E]
6							[A][B]	[E]
7							[A][B]	[E]
8							[A][B]	[E]
9	719.46551	720.47279	PA 38:6...PA 16...	38.6	1.064	C ₃₈ H ₆₂ O ₂ P	[A][B]	[E]
10	885.54916	886.55644	PI 38:4...PI 18:0...	26.2	-1.086	C ₃₈ H ₆₃ O ₂ P	[A][B]	[E]

4D-Molecular Imaging

Separate isobaric or isomeric compounds using trapped ion mobility spectrometry (TIMS).

m/z 726.571
 $1/K_0 = 1.37$ $1/K_0 = 1.40$



Conclusions

- Highly confident annotations of PASEF empowered 4D-Lipidomics™ data were retrieved using five quality scoring criteria.
- SpatialOMx® workflow for automatic annotation of MALDI Imaging data using SCiLS Lab and MetaboScape presented.
- 4D-Molecular Imaging applying TIMS reveals distributions of isobaric compounds without having hundreds of thousands of resolving power.

timsTOF fleX