

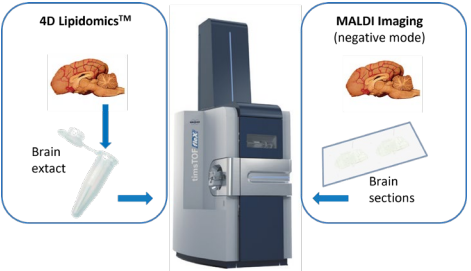
CCS-enabled SpatialOMx[®] for automatic annotation of lipids in MALDI Images based on 4D-Lipidomics™ data

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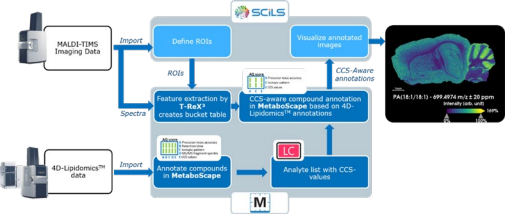
Introduction

The CCS-enabled SpatialOMx workflow opens new dimensions by combining the molecular and spatial information measured by MALDI-TIMS Imaging with highly confident 4D-Omics annotations. MetaboScape[®] 2021b and SciLS™ Lab 2021b provide the interface to match data from both ionization techniques and enable automatic and CCS-enabled annotations of MALDI Imaging data. The CCS-value is a key component of this workflow.

Methods

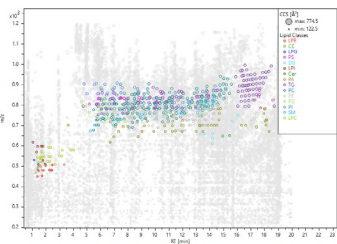


Mouse brain lipids were annotated after LC-ESI PASEF using a brain homogenate. Annotations were based on exact mass, retention time, MS/MS spectra and CCS-value. The resulting list was used to annotate lipids after MALDI Imaging of sections from the same brain sample. In addition to the exact mass, the mobility information (CCS-value) adds an additional confirmation criterion for reliable annotations.

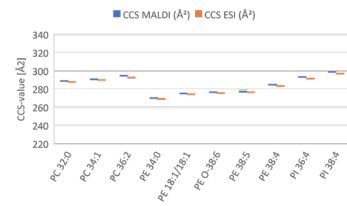


Computational pipeline using SciLS™ Lab 2021b and MetaboScape™ 2021b for CCS-enabled annotation of MALDI Imaging data.

Results



Annotated lipids from the 4D-Lipidomics™ (LC-ESI PASEF) experiment. 292 unique lipids were annotated in negative mode and 295 in positive mode using the rule-based lipid annotation tool of MetaboScape.

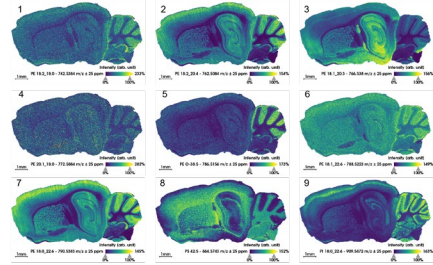


Reproducibility of CCS-values across ESI- and MALDI-ionization for different lipids.

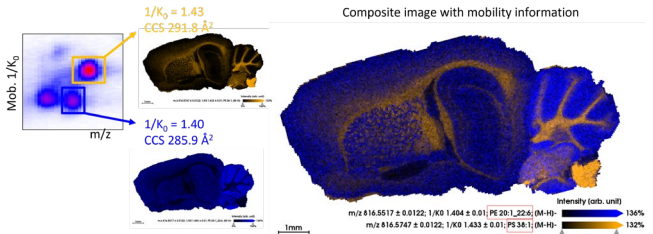
Extract of feature table listing the selected lipids shown below.

Rank	m/z ⁺ mass	MS mass	Isom	Name	Δm/z [ppm]	ΔCCS [Å²]	Molecular For.	Annotations	ΔQ
1	742.53843	743.54866	+	PE 18:2, 18:0	-1.079	0.0	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
2	762.50055	763.51033	+	PE 38:4	0.162	0.0	C ₄₈ H ₈₄ N ₂ O ₆ P	CCS, ESI	1.1
3	766.53053	767.54080	+	PE 18:1, 20:3	-1.559	0.2	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
4	772.56837	773.56955	+	PE 20:1, 18:0	2.642	0.2	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
5	786.52753	787.53297	+	PS 18:1/18:1	-2.175	0.0	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
6	788.52289	789.53057	+	PE 18:1, 22:6	-1.633	0.0	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
7	790.53831	791.54539	+	PE 18:2, 22:4	-1.199	0.1	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1
8	864.57427	865.58135	+	PS 42:5	-2.010	0.0	C ₆₄ H ₁₀₄ N ₂ O ₆ P	CCS, ESI	1.1
9	909.54725	910.55432	+	PI 18:0, 22:4	-2.865	0.4	C ₃₄ H ₆₄ N ₂ O ₆ P	CCS, ESI	1.1

Visualization of annotated lipids in SciLS™ Lab 2021b.



*Missing ΔCCS indicates that a different adduct was detected with ESI.



Visualization of MALDI-TIMS Imaging data in SciLS™ Lab 2021b.

Conclusions

- Mobility enhanced MALDI-TIMS Imaging enables the separation of isobaric or even isomeric compounds and thereby delivers unprecedented imaging results, especially for spatial lipidomics.
- The novel CCS-enabled SpatialOMx[®] workflow increases the confidence in lipid annotations for MALDI images through the acquisition of CCS-tagged data.
- The CCS-enabled SpatialOMx[®] workflow is facilitated by a seamless communication between MetaboScape[®] 2021b and SciLS™ Lab 2021b.