

Integrated annotation pipeline using *in-silico* prediction for on-target chemical derivatization MALDI Imaging

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Introduction

On-tissue/on-target chemical derivatization (OTCD) is a widely applied method to improve the ionization efficiency and detection of low molecular weight and/or nonpolar analytes for MALDI Imaging.

The portfolio of applied derivatization agents is highly diverse, depending on the targeted compound class and the respective attributes desired for the analysis. Ideally, the derivatization agents should have high reactivity and specificity towards the targeted compound class. Additionally, they should contribute to the absorption of laser energy and introduce a specific ionization site. Furthermore, derivatization agents are often designed to improve the identification of derivatized compounds from a complex imaging dataset.

To enhance the annotation of derivatized compounds, MetaboScape[®] introduced an *in-silico* derivatization prediction, which is directly available via the MetaboScape-powered Molecular Annotation pipeline in SCiLS[™] Lab.

Methods

MALDI Imaging data was generated on timsTOF flex MALDI-2 instruments. The schematic workflow is depicted in **Figure 1**. To annotate the derivatized compounds, the MALDI Imaging data was imported into SCiLS Lab 2025b, and T-ReX[®] Feature Finding was performed during import. The respective *in-silico* derivatizations were defined in MetaboScape 2025b by providing the structural information of the derivatization agents as InChi or SMILES and the reaction sites of both the agent and target analytes as SMARTS strings (**Figure 2**, **Figure 4**). This *in-silico* derivatization was automatically applied to all target list entries that included structural information. Molecular annotation was triggered from SCiLS Lab 2025b, and the T-ReX mass and/or mass-mobility features were annotated for both underivatized and derivatized states of the target list compounds (**Figure 3**, **Figure 5**).

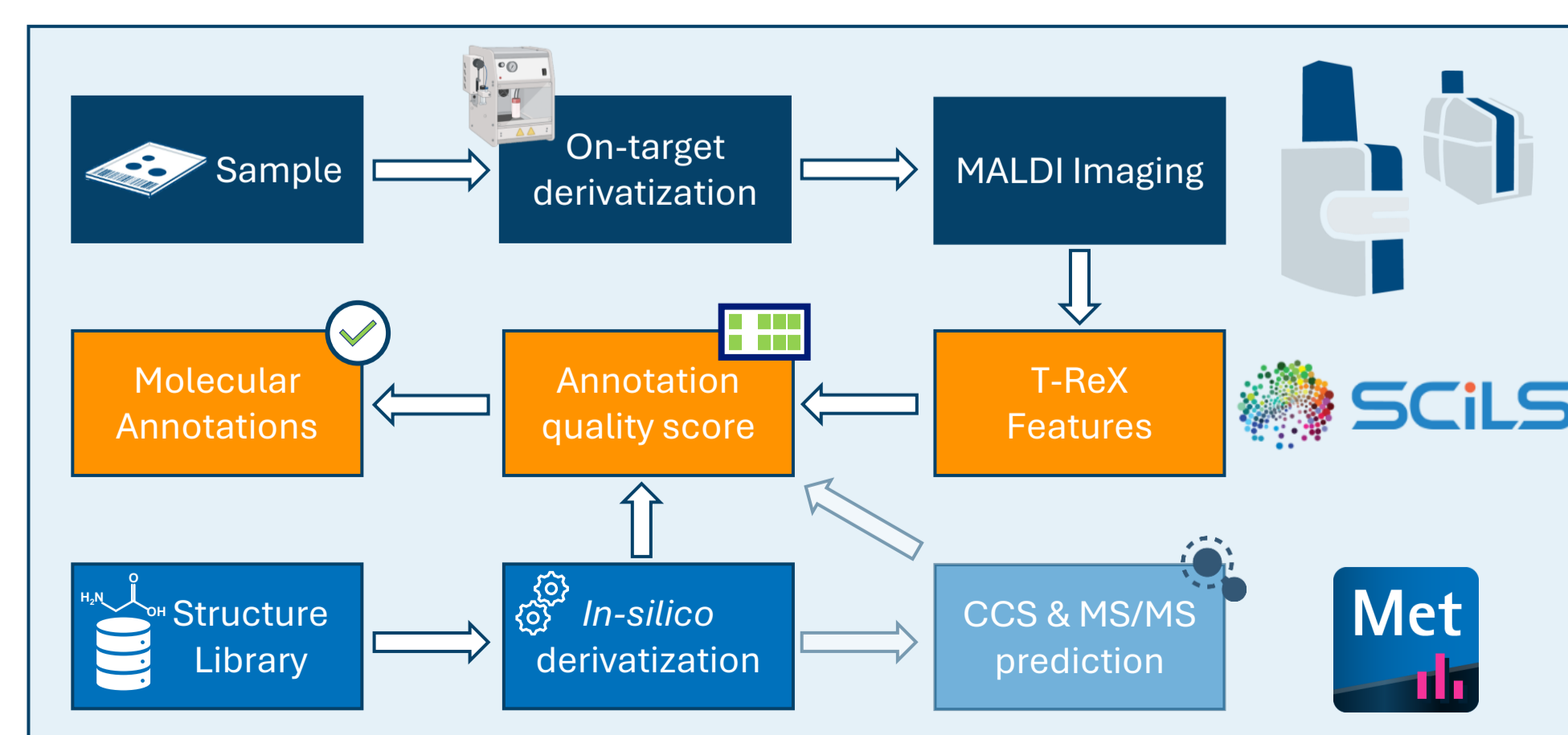


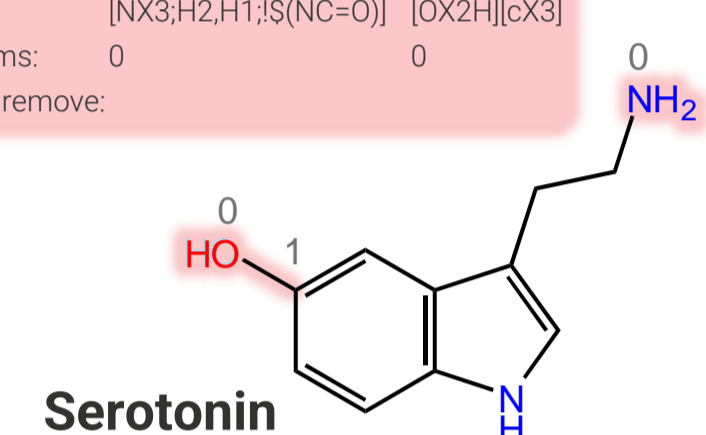
Figure 1: Schematic workflow for OTCD measurement and data analysis.

FMP-10 Derivatization for Neurotransmitter Imaging

- Reactive matrix: 4-(anthracene-9-yl)-2-fluoro-1-methyl pyridinium (FMP-10)
- Reactivity towards primary and secondary amines, as well as phenolic hydroxyls
- Established protocols for advanced mapping of neurotransmitters (Shariatgorji, 2019)
- Exemplary data: Rat brain, coronal section
- MALDI-MS Imaging @ 50 μm raster width
- Accurate mass and isotopic pattern scoring for high annotation confidence

Target Molecule Test Structure

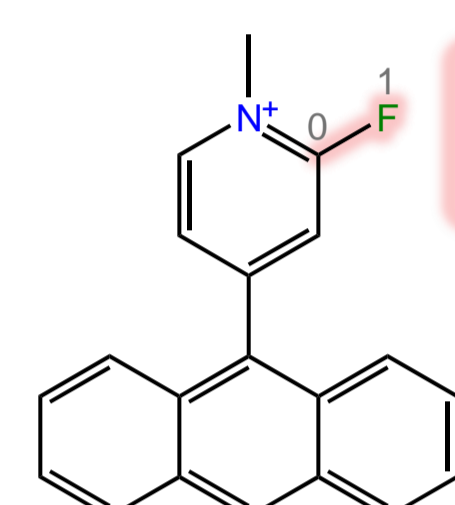
Reaction Site: Primary or sec. amine Phenol
 SMARTS: [N](C)C1=CC=C(C=C1)O
 Index of Target Atoms: 0
 Indices of Atoms to remove: 0



Serotonin

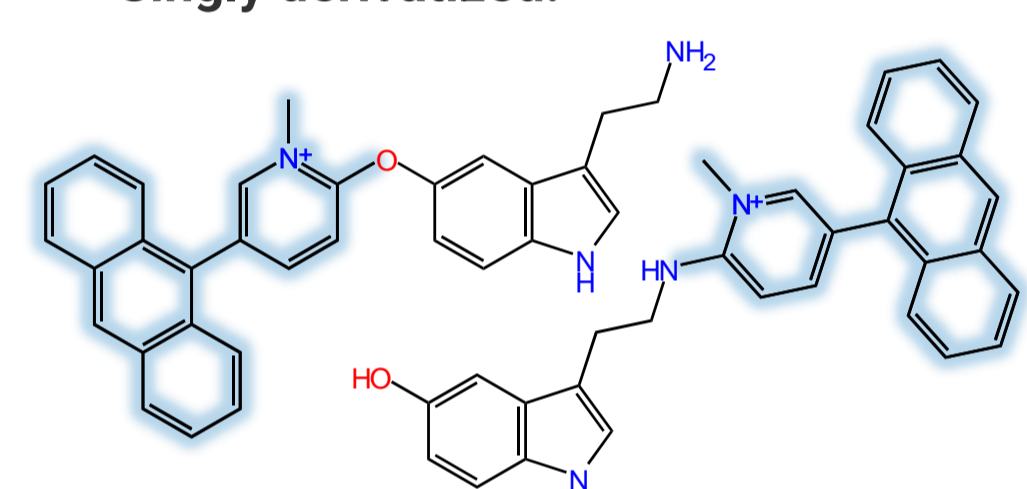
Reagent Structure

Reaction Site: C-F
 SMARTS: [F]C1=CC=C(C=C1)
 Index of Target Atoms: 0
 Indices of Atoms to remove: 1

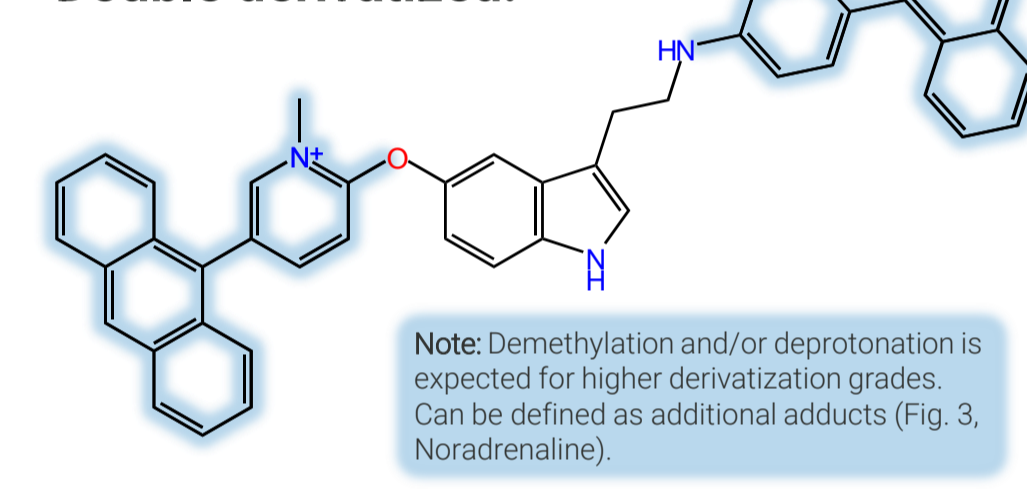


Derivatization Product Structures:

Singly derivatized:



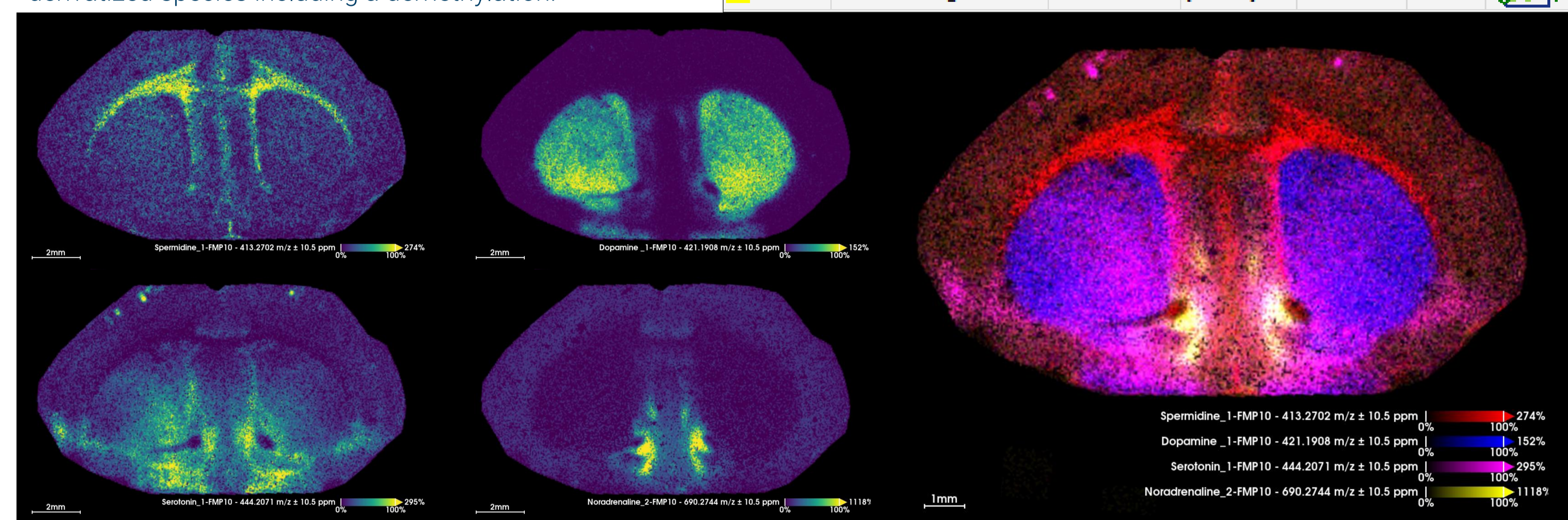
Double derivatized:



Note: Demethylation and/or deprotonation is expected for higher derivatization grades. Can be defined as additional adducts (Fig. 3, Noradrenaline).

Figure 2: Definition of *in-silico* derivatization parameters for FMP-10 using Serotonin as Target Molecule Test Structure.

Figure 3: Annotation details and ion images of 4 exemplarily selected compounds from rat brain derivatized with FMP-10. Spermidine, Dopamine and Serotonin are shown as singly derivatized species, Noradrenaline is shown as double derivatized species including a demethylation.



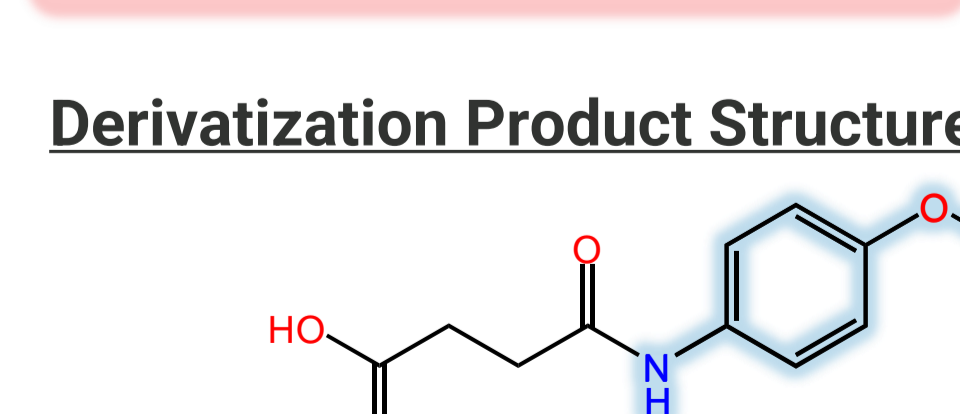
Data from rat brain after FMP-10 derivatization was kindly provided by Michael Becker, Boehringer Ingelheim.

4-APEBA Derivatization for TCA Cycle Imaging

- Reagent: 4-(2-((4-bromophenethyl)-dimethylammonio)-ethoxy)benzenaminium bromide (4-APEBA), Activator: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Matrix: DHB
- Reactivity towards carbonyl and carboxy functionalities
- Established protocols for advanced mapping of TCA metabolites from plant materials (Zemaitis, 2023)
- Exemplary data: Poplar root cross section
- MALDI-TIMS-MS Imaging @ 5 μm raster width
- In-silico* CCS scoring supported by MetaboScape's CCS Predict Pro algorithm

Target Molecule Test Structure

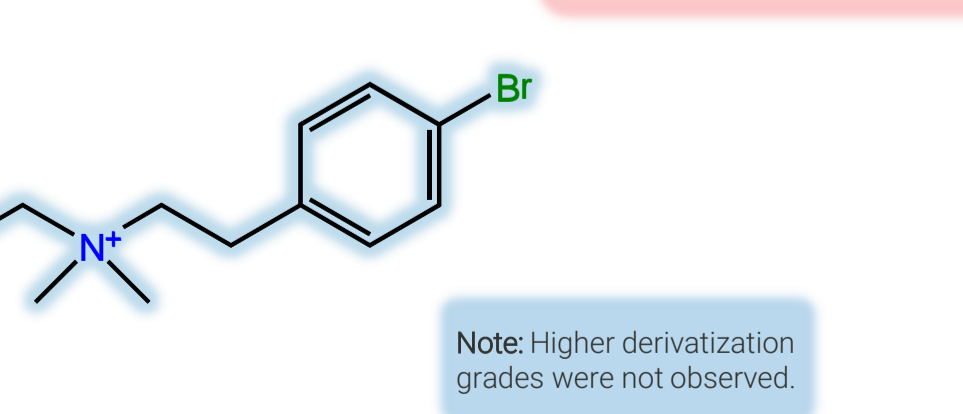
Reaction Site: Carboxy Carbonyl
 SMARTS: [C](=O)O [C](=O)O
 Index of Target Atoms: 0
 Indices of Atoms to remove: 2



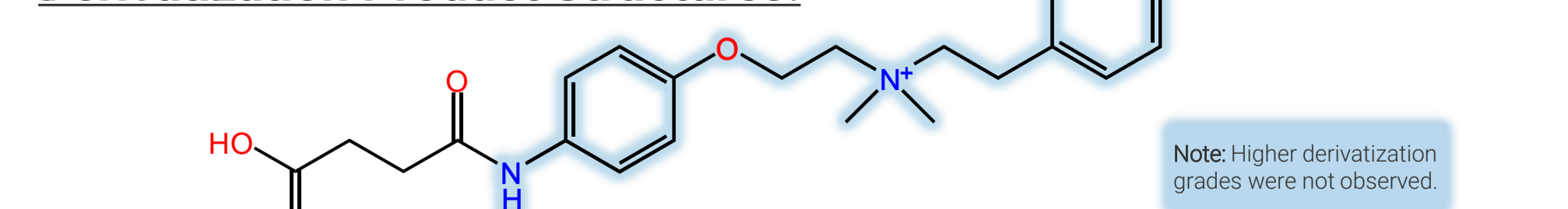
Malic Acid

Reagent Structure

Reaction Site: C-F
 SMARTS: [F]C1=CC=C(C=C1)
 Index of Target Atoms: 0
 Indices of Atoms to remove: 1



Derivatization Product Structures:



Note: Higher derivatization grades were not observed.

Figure 4: Definition of *in-silico* derivatization parameters for 4-APEBA using Malic Acid as Target Molecule Test Structure.

Data from poplar root thin sections after 4-APEBA derivation was kindly provided by PNNL.

m/z	CCS [Å ²]	Name	Ion Notation	$\Delta\text{m/z}$ [ppm]	ΔCCS [%]	AQ score
479.1182	215.3455	Malic acid_1-4-APEBA	[M] ⁺	1.301	1.9	100%
463.1206	211.7334	Succinic acid_1-4-APEBA	[M] ⁺	-4.574	1.2	100%
519.1150	220.7359	cis-Aconitic acid_1-4-APEBA	[M] ⁺	4.823	-0.4	100%
537.1264	223.0449	Isoctiric acid_1-4-APEBA	[M] ⁺	6.233	0.2	100%

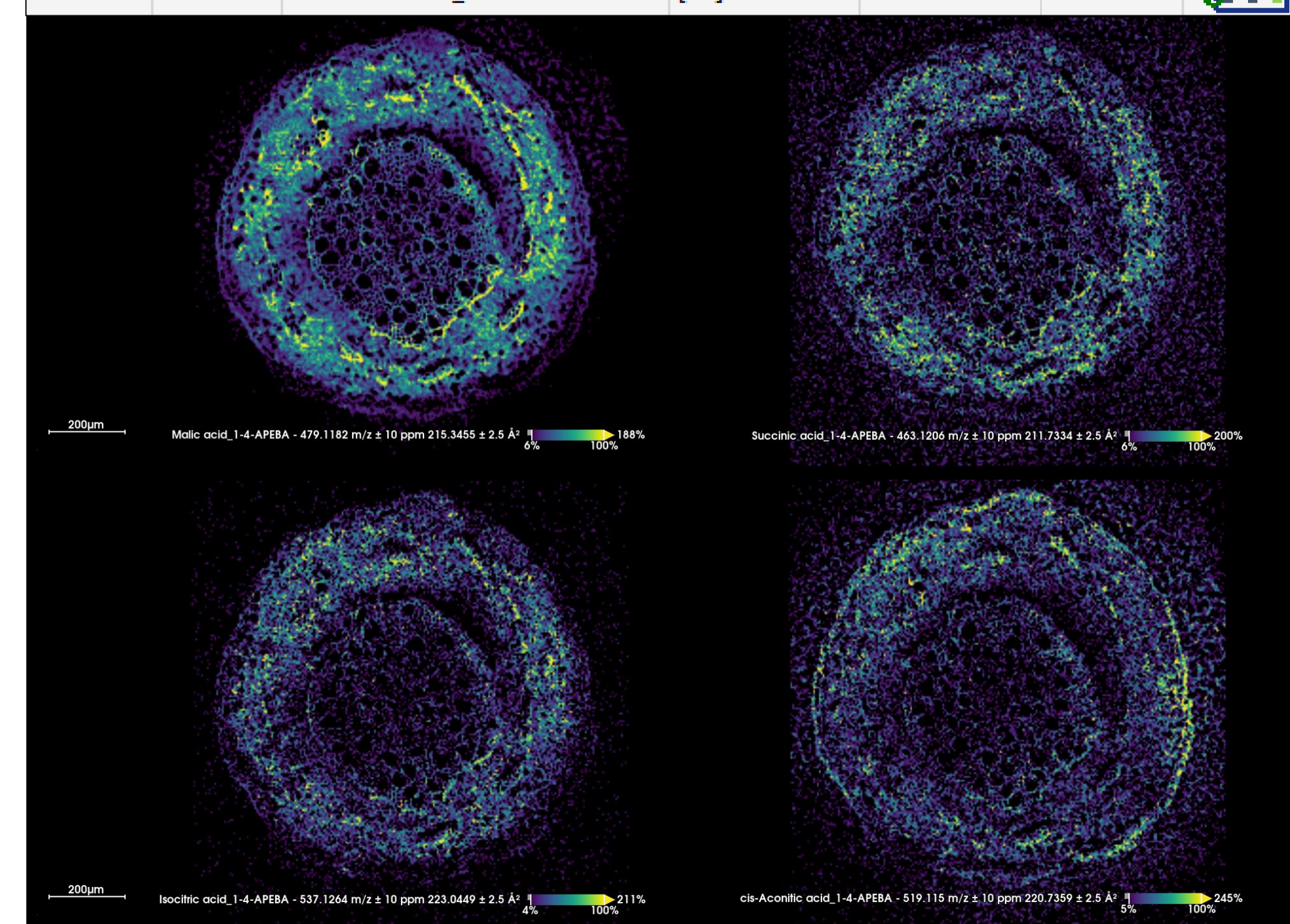


Figure 5: Annotation details and ion images of 4 exemplarily selected compounds from poplar root derivatized with 4-APEBA. In addition to *m/z*-based scoring, CCS-Predict Pro allows to match towards structure candidates from the *in-silico* derivatization.

Improved experience for on-target derivatization analysis:

- Seamless integration of MetaboScape's *in-silico* derivatization into SCiLS[™] Lab annotation workflow
- Fast and direct annotation feedback
- High annotation confidence by *m/z*, mSigma and CCS scoring
- CCS-Predict Pro for candidate structure prediction

Imaging MS: Computational Methods, Software & Analysis

COI Disclosure: ABE, NK, SW, BH, TB, NT, AE, AD and ME are employees of Bruker Corporation. Bruker manufactures and sells analytical instruments including mass spectrometers and software used in this study.