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

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On-tissue/on-target chemical derivatization (OTCD) is a widely applied method to improve the ionization 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 support 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 annotation pipeline in SCiLS™ Lab.

Introduction

On-tissue/on-target chemical derivatization (OTCD) is a widely applied method to improve the ionization 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 support 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 annotation pipeline in SCiLS™ Lab.

Methods

MALDI Imaging data was provided from timsTOF fleX MALDI-2 systems for different derivatization agents and sample types. Derivatization agents like FMP10 or 4-APEBA introduce an intrinsic charge through their quaternary ammonium subunit. FMP10 is commonly used for the detection of neurotransmitters, including primary or secondary amines as well as phenolic hydroxy functionalities. 4-APEBA reacts with carbonyl functionalities and is therefore applied in the enhanced visualization of TCA cycle metabolites from tissue.

To annotate the derivatized compounds, the MALDI Imaging data was imported into SCiLS Lab 2025b, and T-ReX® Feature Finding was applied during import. The respective in-silico derivatizations were defined in MetaboScape 2025b by providing the structural information about the derivatization agents as InChi or SMILES and the reaction sites of both the agent and target analytes as SMARTS strings. This in-silico derivatization was automatically applied to all target list entries that include 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.

Results

This workflow significantly improved molecular annotation and data evaluation for OTCD MALDI Imaging. The MetaboScape-powered molecular annotation pipeline in SCiLS Lab enhances annotation confidence and speed by providing annotations within short processing times and includes quality scoring for m/z, isotope pattern, and, if available, CCS and MS/MS.

Conclusion

Seamless integration of an in-silico derivatization-based annotation workflow for OTCD MALDI Imaging analysis within SCiLS Lab.