# Maximizing Metabolomic Discovery on timsMetabo: MoRE Mode Combined with Met4DX and MetDNA3 Workflow

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## Introduction

4D-metabolomics—integrating retention time, m/z, ion mobility, and intensity—has revolutionized metabolite characterization. However, balancing sensitivity, coverage, and collision cross-section (CCS) accuracy remains challenging for complex biological samples. The Bruker timsMetabo platform addresses this through its MoRE (Mobility Range Enhancement) scan mode, which optimizes small molecule transmission, while the Met4DX workflow streamlines data acquisition, processing, and annotation. Together, this integrated solution enhances the depth and reliability of metabolomic profiling, facilitating biomarker and pathway discovery.

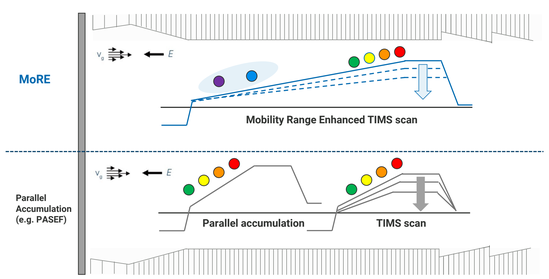


Fig. 2 MoRE increased the m/z range observable in a single TIMS cycle

## Methods

Samples were analyzed on a Bruker timsMetabo system coupled with a UHPLC system, which ensured efficient separation of complex metabolites (Fig. 2). Data acquisition was performed in the advanced MoRE scan mode to maximize metabolite coverage. For CCS accuracy evaluation, 16 metabolite standards were analyzed to guarantee data reproducibility. Low m/z detection capability was assessed under MoRE and PASEF scan mode by using liver samples (Fig. 1).

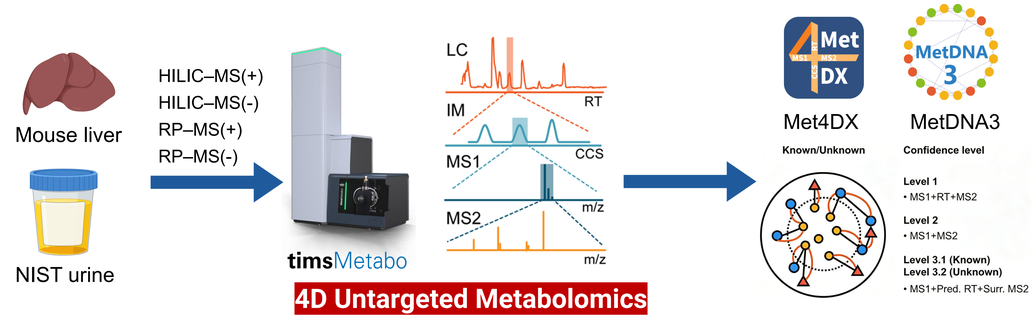


Fig. 1 Schematic of 4D-metabolomics bio-samples analysis workflow.

Metabolite separation was achieved with a 12-min gradient optimized for polar and non-polar metabolites. All raw data were processed *via* the Met4DX workflow to ensure peak picking, alignment, CCS calibration, and high-confidence annotation.

## Results

CCS and m/z accuracy were evaluated using 16 metabolite standards under MoRE mode, yielding median CCS errors of 0.5% (Pos) / 0.6% (Neg) and mass errors of 0.3 ppm (Pos) / 0.6 ppm (Neg), enabling reliable 4D annotation (Fig. 3).

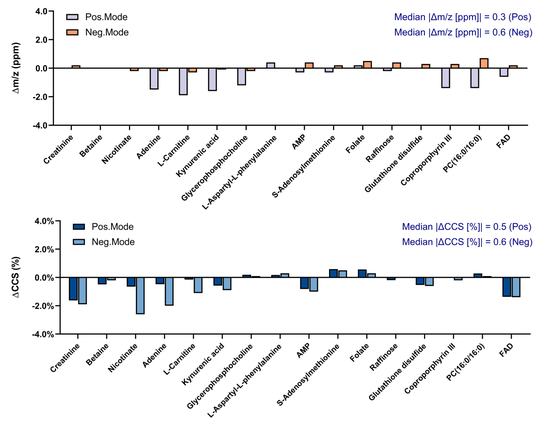


Fig. 3 Mass Accuracy and CCS Precision of 16 STDs (MoRE mode).

MoRE scan mode demonstrates superior sensitivity for low-mass metabolites, detecting 41% more features than PASEF in the sub-200 m/z range (6,440 vs 4,577 features) in liver samples (Fig. 4), This enhanced coverage is particularly beneficial for investigating central carbon metabolism, including the tricarboxylic acid (TCA) cycle and amino acid metabolism, which rely heavily on low-molecular-weight intermediates.

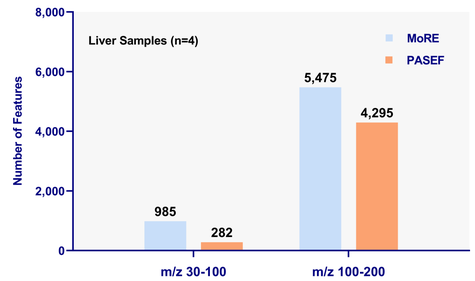


Fig. 4 MoRE vs. PASEF: Enhanced Features (m/z<200) in liver samples

The integrated 4D-metabolomics workflow combines timsMetabo with Met4DX for streamlined data acquisition and processing, and MetDNA3 for comprehensive metabolite annotation. This pipeline achieved high-confidence Level 1 annotations (MS1 + RT+ MS2) [1], identifying 717 metabolites in mouse liver and 516 metabolites in NIST Urine (Fig.5).

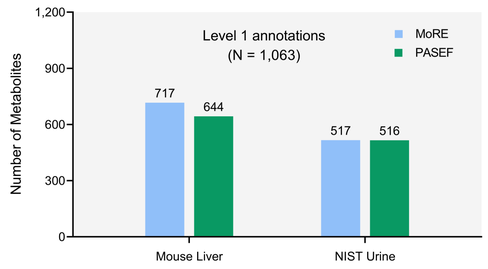


Fig. 5 MoRE vs. PASEF: Level 1 annotation in mouse liver and NIST urine

For Level 3 annotations, MetDNA3 substantially expanded known metabolite coverage (2,806 in mouse liver and 3,249 in NIST Urine), demonstrating superior depth and reliability in complex biological matrices (Fig.6).

Collectively, the MoRE-Met4DX-MetDNA3 integrated solution maximizes feature discovery while enhancing sensitivity, accuracy, and annotation confidence in 4D-metabolomics workflows.

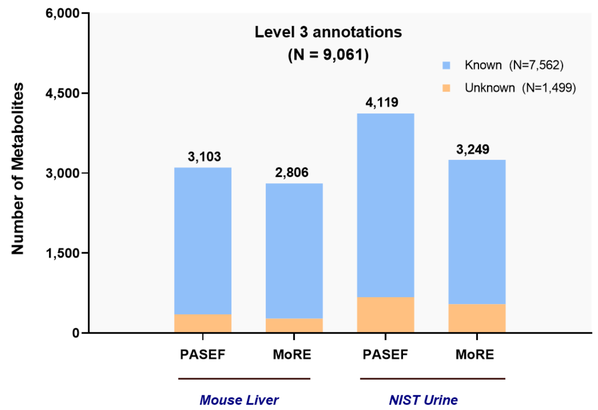


Fig. 6 MoRE vs. PASEF: Level 3 annotation in mouse liver and NIST urine

Reference: [1] Zhou Z, Luo M, Zhang H, Yin Y, Cai Y, Zhu ZJ. Metabolite annotation from knowns to unknowns through knowledge-guided multi-layer metabolic networking. Nat Commun. 2022 Nov 4;13(1): 6656.

## Conclusion

- Enhanced Ion Transmission — MoRE detects 41% more low-mass features (m/z < 200) vs. PASEF.
- Exceptional Precision — Median CCS error < 0.6% and mass accuracy < 0.6 ppm ensure reliable 4D annotation.
- Streamlined Processing — Met4DX workflow automates peak picking, alignment, and CCS calibration for robust data handling.
- Deep Annotation — MetDNA3 achieves 717/516 Level 1 annotations (mouse liver/NIST urine) and expands known coverage to 2,806/3,249 features.
- Integrated Solution — Combined MoRE-Met4DX-MetDNA3 maximizes sensitivity, accuracy, and confidence for comprehensive 4D-metabolomics.

**Discovery MoRE on timsMetabo**

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