# Deep Metabolome Profiling Using mzmine and Self-Supervised Deep Learning Molecular Networking on the timsMETABO MS Instrument

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**Introduction**

Metabolomic experiments generate vast amounts of data, in which only a small proportion of compounds can be annotated. Trapped ion mobility spectrometry (TIMS) enhances peak capacity and enables mobility-resolved MS2 spectra collection via parallel accumulation-serial fragmentation (PASEF), leading to deeper insights per injection. Transforming these complex datasets into knowledge requires advanced data mining strategies, including molecular networking for exploring unknown chemical space.

**Methods**

We present a workflow for deep metabolome profiling using a novel TIMS-MS instrument, mzmine data processing, and interactive molecular networking. Two data-dependent acquisition (DDA) LC-MS2 methods were developed: one without TIMS (autoMSMS) and another with TIMS (PASEF). mzmine processing covered raw data import, spectral processing, 3D feature detection (LC-MS2), 4D feature detection (LC-TIMS-MS2), ion identity and feature-based molecular networking (FBMN), and spectral library matching using MSnLib and MassBank of North America (MoNA). For FBMN, we applied both modified cosine similarity and the recently introduced DreaMS (Deep Representations Empowering the Annotation of Mass Spectra) transformer-based neural network for spectral similarity.

**Results**

mzmine enabled rapid comparison of both acquisition methods. The LC-TIMS-MS2 approach using PASEF outperformed conventional DDA LC-MS2 in total detected features, acquired MS2 spectra, and annotated compounds. Mobility-resolved MS2 acquisition via PASEF reduced chimeric spectra, while the LC-TIMS-MS2 method generated more connected molecular networks with fewer singleton nodes compared to the conventional LC-MS2 method. The DreaMS neural network further minimized singleton nodes compared to modified cosine similarity, which is tuned for single modifications. In contrast, DreaMS connects structural analogs with multiple or complex modifications, forming complementary networks.

**Conclusion**

The combination of a novel TIMS-MS instrument and mzmine enhances feature detection, spectral acquisition, and annotation. TIMS-PASEF reduces chimeric spectra, while DreaMS improves molecular networking by linking structural analogs, advancing metabolomics research.