Extending Dynamic Range and Enhancing Compound Identification for Untargeted Ion Mobility-MS Workflows

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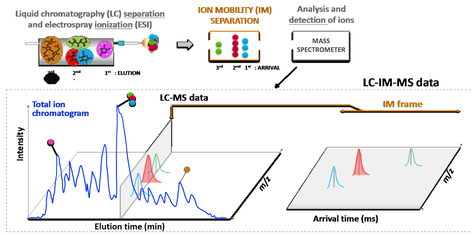
Overview

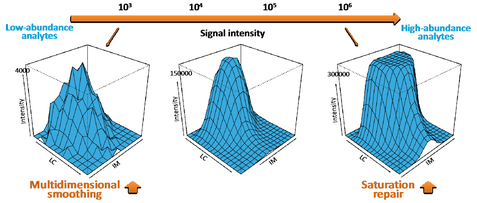
- PNNL PreProcessor is free software for enhancing signal quality of raw spectra at the limits of the MS detection system.
- Multidimensional moving average smoothing improves ion peaks with poor definition at low abundances.
- A new saturation repair algorithm reconstructs the intensities of ion peaks at high abundances.

Introduction

LC-IM-MS data acquisition

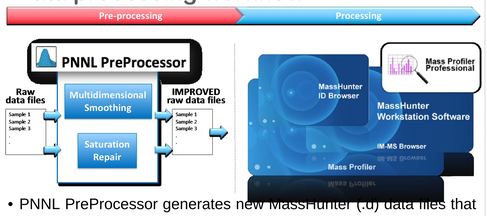


Algorithms for each end of the dynamic range



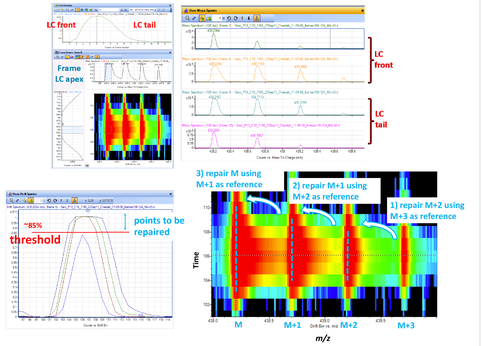
Methods

Data processing workflow



- PNNL PreProcessor generates new MassHunter (.d) data files that can be processed with all other existing software.
- Size of data files is reduced and downstream processing uses less time and memory.
- Implemented in C# and provides batch processing with GUI and command-line access.

Saturation repair algorithm

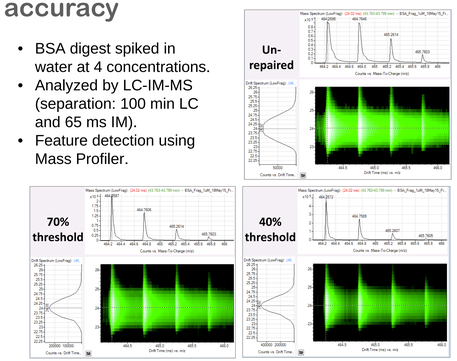


- Complete raw data files from untargeted analyses are automatically preprocessed to find ions exceeding a defined threshold (percentage of the raw intensity limit given the MS instrumentation).
- The undistorted isotopic distribution is recovered from spectra at unsaturated time from the peak tail (intensity below the saturation threshold). In contrast to our previous algorithm [1], this new method does not require theoretical isotopic distributions and works with raw spectra (non-centroid).
- The most intense unsaturated isotopic peak is utilized as reference to successively reconstruct the time profile of the saturated ones.

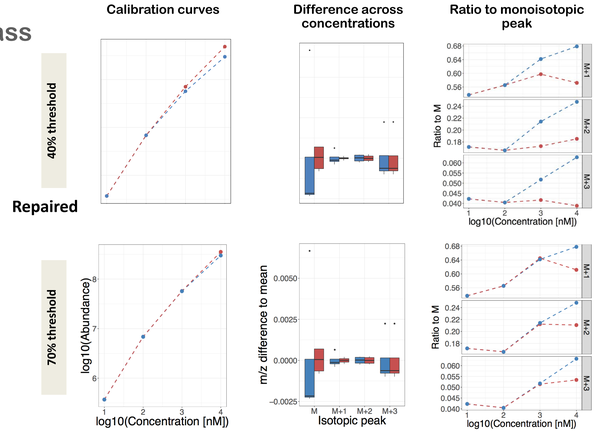
Results

Enhanced quantitation linearity and mass accuracy

- BSA digest spiked in water at 4 concentrations.
- Analyzed by LC-IM-MS (separation: 100 min LC and 65 ms IM).
- Feature detection using Mass Profiler.



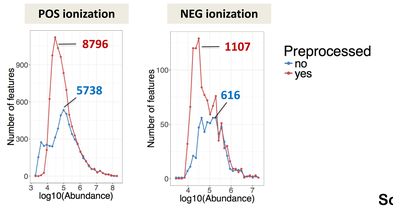
Screenshots of raw data (frame at LC apex)
Using IM-MS Browser. Thresholds are a percentage of the raw intensity limit (92160 counts)



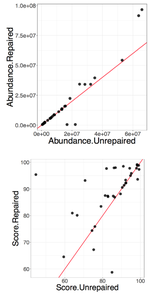
Example of saturation repair using different intensity thresholds
Peptide feature 464.25682 *m/z* (+2), 46.3 min and 23.4 ms

Improved feature detection and isotopic score

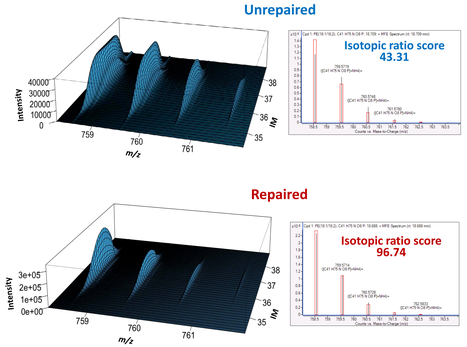
- NIST Standard Reference Material for Human Plasma.
- Analyzed by LC-IM-MS (separation: 30 min LC and 70 ms IM) in 5 replicates.
- Preprocessing: smoothing (3 points chromatography, 5 points drift) and saturation repair (threshold 40%).



Distribution of abundances
Lipid features detected across all replicates using Mass Profiler



Scatter plot of abundances and isotopic scores
Using ID Browser and libraries using LIQUID [2] and PNNL CCS DB [3]



Example raw data (frame at LC apex) and isotopic score
Score using ID Browser and max ion volume as abundance

Conclusions

- Software is as important as hardware.
- Multidimensional smoothing improves detection reproducibility of low-abundance analytes.
- Our new saturation repair algorithm recovers the intensity, isotopic ratios and mass accuracy of high-abundance analytes prior to identification and is applicable to different molecular ions (i.e. peptides, metabolites, lipids).
- PNNL PreProcessor provides consistent characterization of more analytes in an extended dynamic range.
- Free download: https://omics.pnl.gov/software/pnnl-preprocessor

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References

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3. Zheng X, Aly NA, Zhou Y, Dupuis KT, Bilbao A, Paurus VL, Orton DJ, Wilson R, Payne SH, Smith RD, Baker ES. ***A structural examination and collision cross section database for over 500 metabolites and xenobiotics using drift tube ion mobility spectrometry.*** Chemical Science. 2017; 8(11):7724-36.

Career Opportunities: For potential openings in Integrative Omics Group at PNNL please visit http://omics.pnl.gov/careers

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