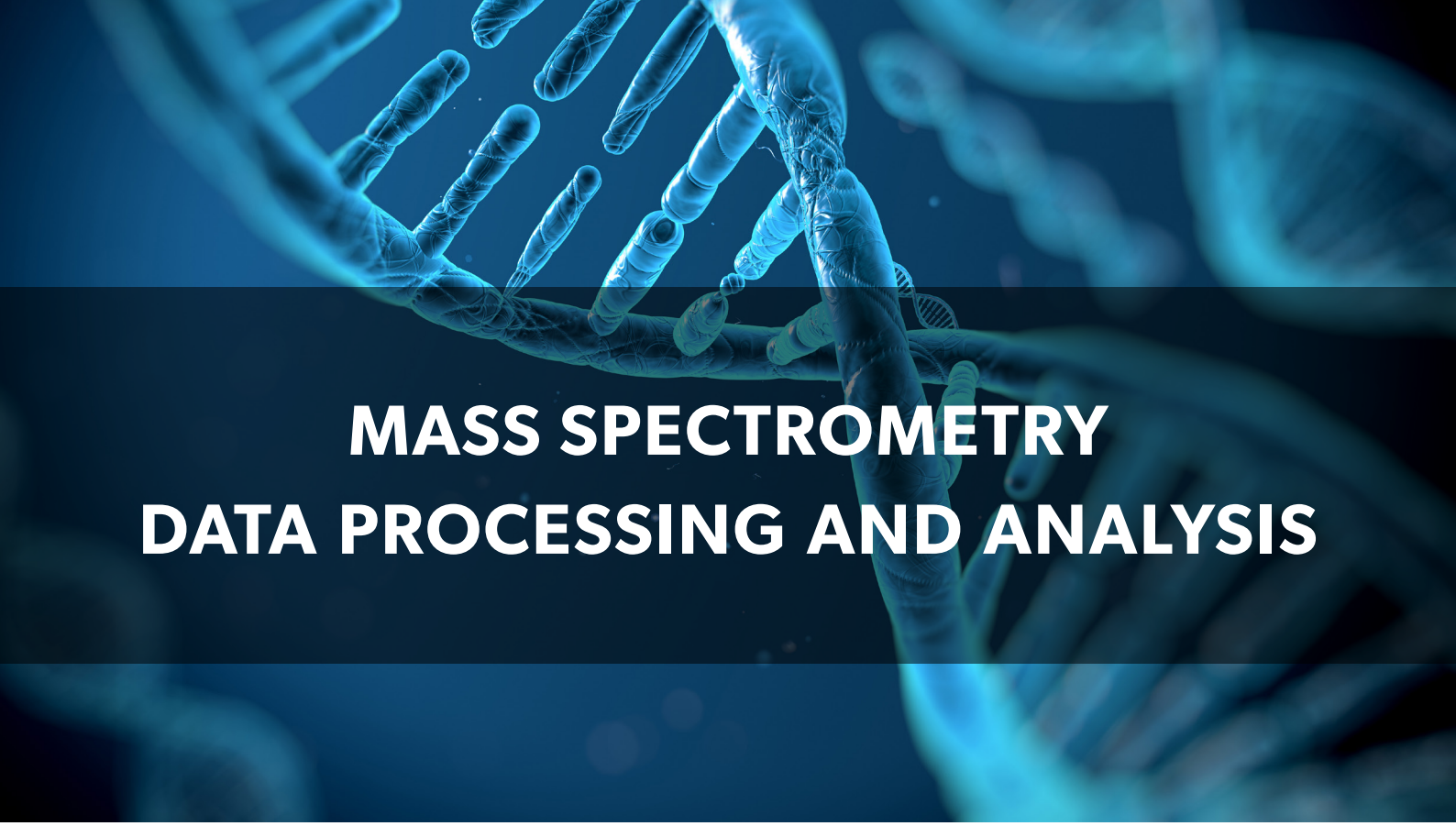
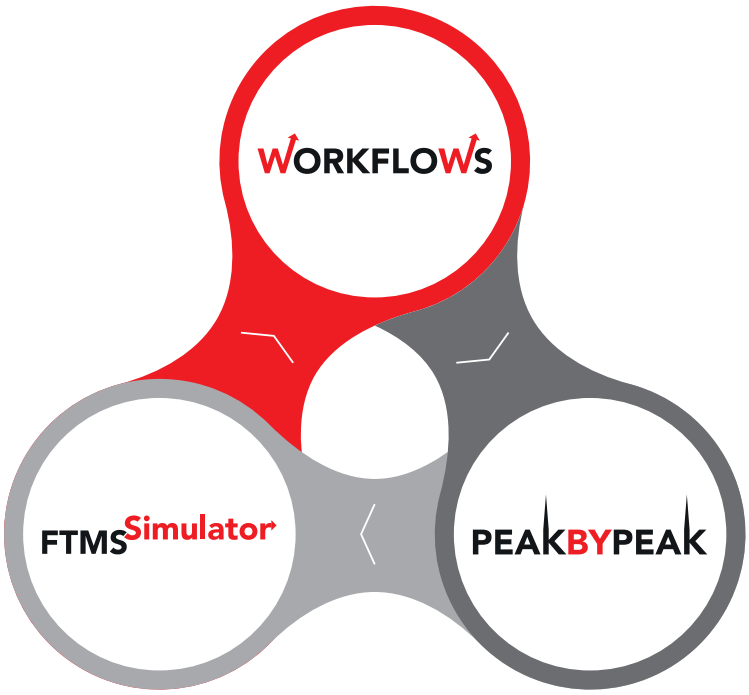


MASS SPECTROMETRY DATA PROCESSING AND ANALYSIS



NEW

Peak-by-Peak Multiomics: All-in-One Workflow

Spectroswiss

Software Tools for Data Processing and Analysis

Spectroswiss software solutions provide a step-change in mass spectrometry data quality and processing sophistication. Our computationally-efficient tools are capable of processing any size datasets, whether these are **unreduced data** (time-domain transients and absorption mode FT, or aFT, mass spectra) or **reduced data** (e.g., full and reduced profile enhanced FT, or eFT, and magnitude mode FT, or mFT, mass spectra). These tools empower the application-specific workflows offering step-by-step data processing and analysis routines.

Advanced Modular Software

PEAKBYPEAK

Peak-by-Peak suite: a powerful set of computational modules to process time-domain transients or mass spectra. Available in Base and Expert Editions.

Software	Base edition	Expert edition
Simulation of FTMS data (The FTMS Isotopic Simulator)	⊕	⊕
RAW file statistics (data distributions: scan time, IT, charge state) and metadata overview	⊕	⊕
Mass spectra processing: baseline correction, noise thresholding and peak-picking	⊕	⊕
Plot multiple mass spectra: experimental and/or simulated data comparison	⊕	⊕
Mass spectra re-calibration: single mass spectra	⊕	⊕
Mass spectra re-calibration: LC-MS/GC-MS (1D datasets)	–	⊕
Mass spectra re-calibration: imaging (2D datasets)	–	⊕
Process multiple FTMS experiments:		
plot TICs and SICs for each input file	⊕	⊕
plot single and/or average mass spectra	⊕	⊕
align LC-MS or LC-MS/MS chromatograms	–	⊕
average data between any files (including between technical replicates)	–	⊕
SIM-window stitching from spectral or time-domain data	–	⊕
SIM-window averaging and stitching	–	⊕
Deconvolution of low-resolution data	–	⊕
Deconvolution of high-resolution data	⊕	⊕
Data visualization and output: high quality images and/or mass spectra in open file formats	⊕	⊕

FTMS Simulator

FTMS Simulator allows to in-silico accurately represent isotopic envelopes and full range mass spectra of any complexity for OrbitrapTM & ICR FTMS platforms.

WORKFLOWS

Together, Peak-by-Peak and FTMS Simulator power the application-specific **Workflows** that enable step-by-step advanced data processing and data analysis for FTMS and other MS platforms.

Key Features

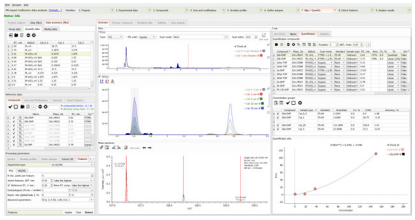
- step-by-step workflows for advanced processing and analysis of mass spectrometry data
- a **multi-vendor** approach, featuring a direct data import of major vendor and open-source file types
- support of reduced and unreduced data, including full profile mass spectra and time-domain transients
- targeted deconvolution and untargeted low-resolution (UniDec) and high-resolution (Hardklor) deconvolution
- targeted experimental data search and annotation with simulated centroid and profile isotopic envelopes
- parallel computing on multiple CPU-cores and GPUs for fast data processing
- powerful and memory-efficient handling of any size datasets

Workflows

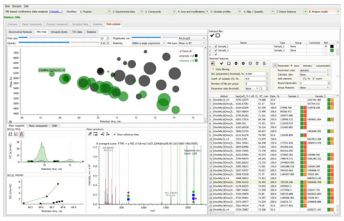
Customer- and application-centered, computationally-efficient, ergonomic, and flexible workflows for your routine and most challenging mass spectrometry data processing and analysis needs – capable to handle **any size datasets** from any type of a mass spectrometer. Our computational algorithms and flexible graphical user interface (GUI) templates can be custom-configured to match your ideas and vision!

NEW

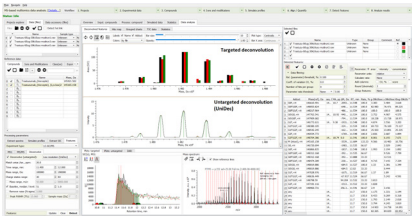
Peak-by-Peak Multiomics: All-in-One Workflow



Small molecules: metabolomics, lipidomics, protein-ligand interactions

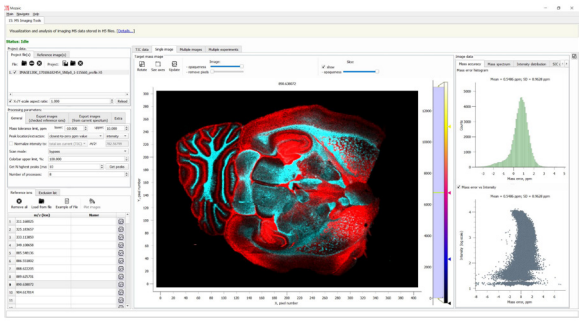


Peptides & peptidoforms: profiling & quantitation of glycopeptides & peptides with other modifications



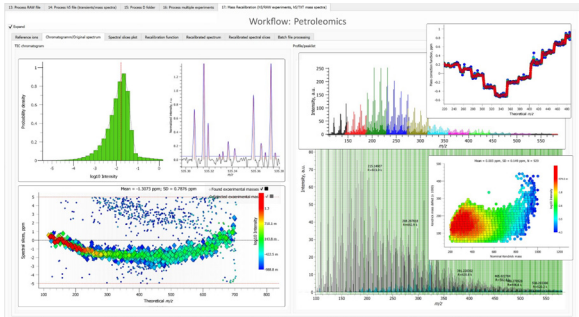
Proteins & proteoforms: intact mass & quantitation protein-ligand interactions

Application-Specific Workflows

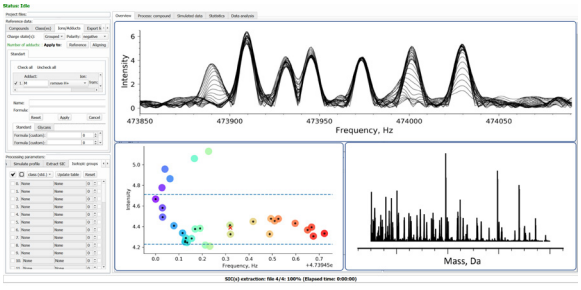


MS Imaging: MALDI & DESI

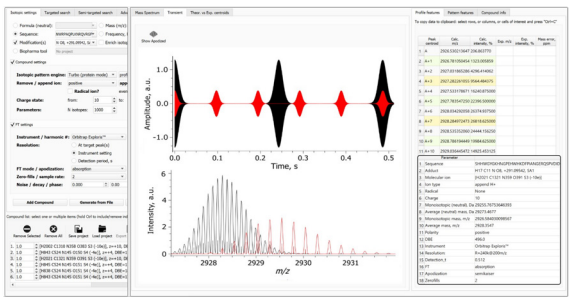
Proteoforms sequencing: top-down & middle-down analysis



Complex mixture analysis (direct infusion & LC-MS)



Charge detection mass spectrometry (CDMS)



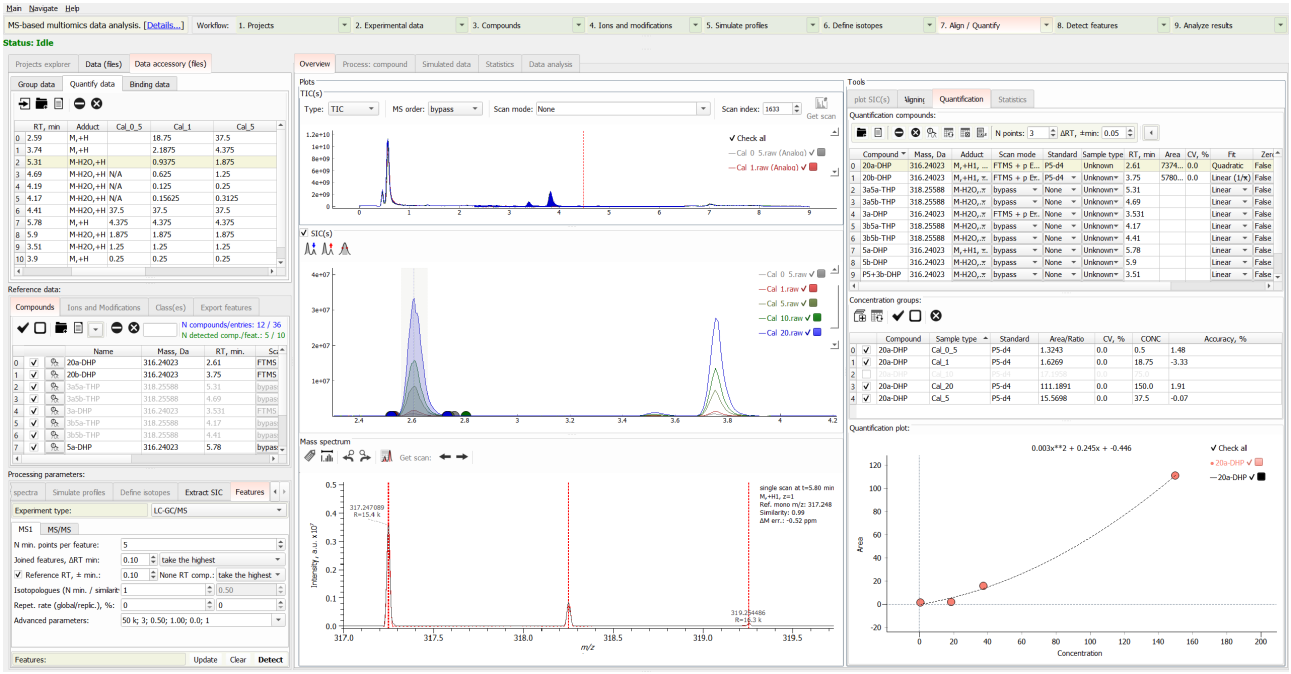
Accurate simulations of FTMS transients, isotopic envelopes, & mass spectra

Custom workflows to meet your specific requirements & needs!

Peak-by-Peak Multiomics: All-in-One Workflow

Peak-by-Peak Multiomics unifies several omics approaches to molecular structural analysis under one roof:

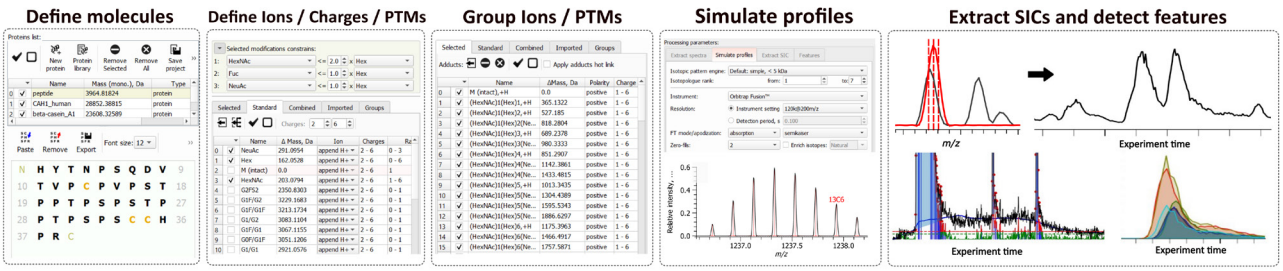
- metabolomics and lipidomics (feature extraction and quantitation)
- ligand analysis in protein-ligand interactions (screening and binding)
- quantitative compound classes analysis of (polymers, biofuels, complex mixtures, etc.)
- PTMs profiling (glycosylation, phosphorylation, etc.) and site-specific analysis in proteomics
- quantitative protein analysis in biopharmaceutical applications, including monoclonal antibodies analysis
- ligand-protein complex (large molecule) analysis in protein-ligand interactions
- analysis of proteoforms in complex mixtures (feature extraction and quantitation)



Targeted Deconvolution and Feature Extraction Workflow

Data analysis in FTMS typically begins with mass spectra deconvolution and deisotoping (an **untargeted approach**). The obtained monoisotopic or average mass lists are then searched against the suspect database by mass accuracy, retention time, fragments, and isotopic ratios.

An alternative approach is to do the inverse (a **targeted approach**), namely to start with the suspect database, targeted or large-scale, simulate the isotopic envelopes in diverse charge states, and identify compounds directly from the experimental data by their signals correlation with the simulated data.

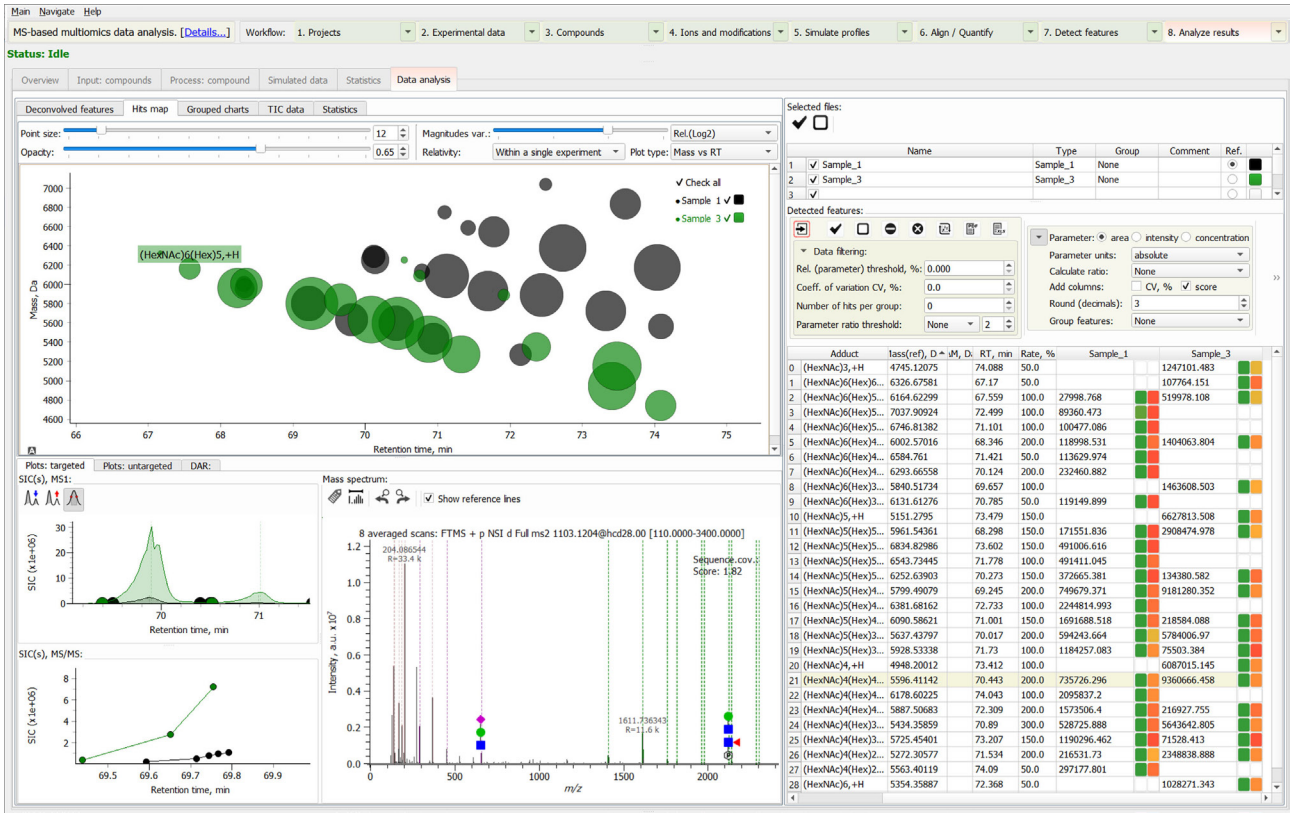


Targeted deconvolution and feature extraction step-by-step workflow (shown above) starts with transient-mediated data simulation (using FTMS Simulator) based on the database of suspects, followed by similarity scoring between experimental & simulated data (*m/z* space, without untargeted deconvolution).

The targeted workflow is implemented in the Peak-by-Peak Multiomics offering an all-in-one workflow for small (metabolites, peptides) and large (mAbs) molecules. It delivers improved proteoform & peptidoform characterization in intact, top-down, and bottom-up MS.

Peak-by-Peak Multiomics: All-in-One Workflow

Supports comparative analysis and reporting of extracted and annotated features via a dynamic global result matrix with a **quick access** to the experimental data: multiparameter filtering (CV, intensity, repetition rate, etc.), selectable experiments, compounds, groups and parameter table, original and deconvolved mass spectra in MS and MS/MS modes, bubble plot, hits map, bar-chart (grouped and stacked), statistical analysis output.

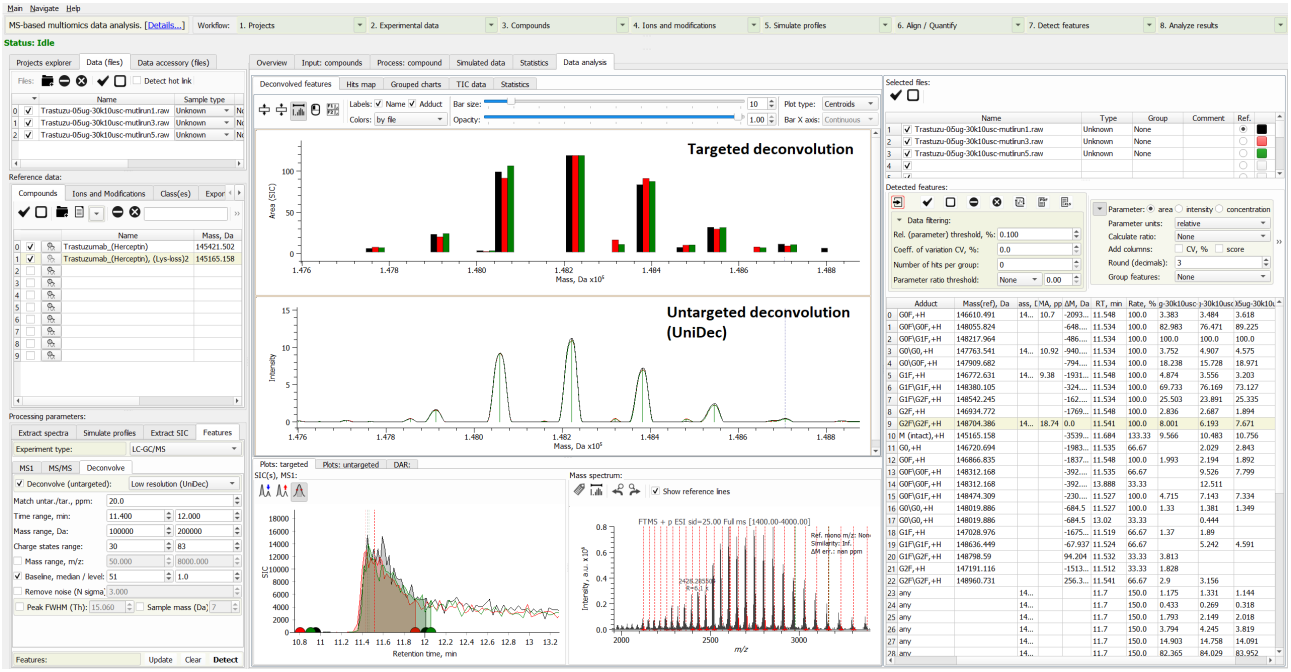


Key Features

- rapid switching between projects from diverse omics applications, & automated batch file processing
- **multi-vendor** support, featuring a direct data import of major vendor and open-source file types
- automatic recognition of meta-information (e.g., instrument model and experimental settings), and
- support of multiple acquisition modes (MS, MS/MS, SIM, etc.)
- processes reduced and unreduced **data of any size**, including full profile mass spectra and transients
- empowered by **FTMS Simulator** enabling accurate simulation of isotopic envelopes & full mass spectra
- targeted MS and MS/MS experimental data search and annotation with simulated isotopic envelopes
- targeted & untargeted low-resolution & high-resolution deconvolution, own & third-party approaches
- multiple parameters for feature extraction and filtering: number of data points in the elution peak,
- experimental vs. simulated profile similarity, repetition rate, number of adducts, etc.
- user-defined isotopic grouping: averaged isotopologues or isotopic fine structure (IFS) level
- elemental formula assignment by accurate mass, with a similarity score ranking vs the simulated data
- data validation through mass accuracy evaluation and mass spectra re-calibration via linear (on-the-fly,
- lock mass) and non-linear approaches; mass spectral peak alignment
- quantification of compounds and classes of compounds (via area under the curve in LC/GC-MS): absolute quantification, semi-quantification, and standard addition (external calibration)
- advanced & routine data processing and data analysis capabilities, user-defined results **reporting** formats
- user-defined selection of parameters output for further data analysis with third party tools

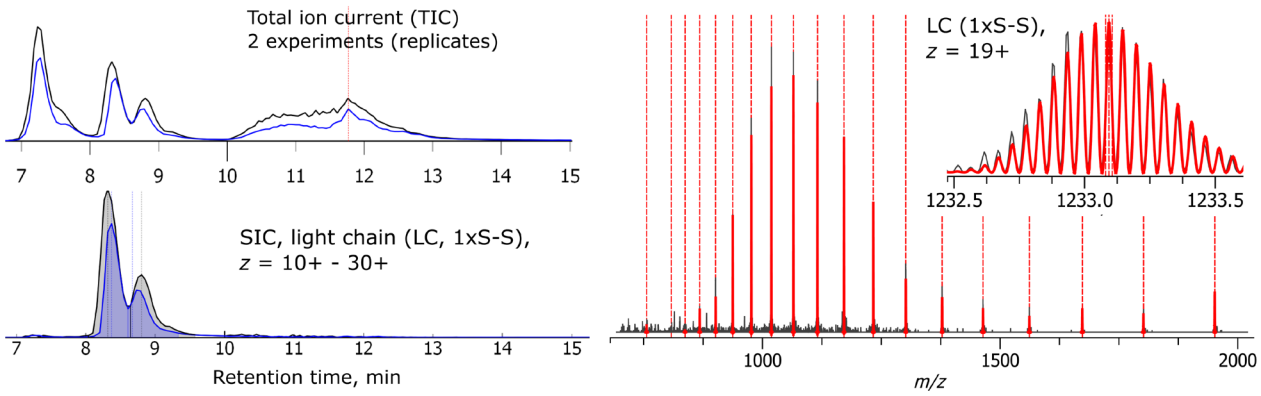
Peak-by-Peak Multiomics: Protein Analysis (BioPharma)

In-depth processing and quantitative analysis of mass spectrometry protein analysis data.



Key Features

- Advanced and proprietary mass spectrometry solutions for mAb analysis, including complex ADCs/AOCs
- Automatic recognition of meta-information (e.g., instrument model and experimental settings)
- Input of target and semi-target databases of individual proteoforms of interest; charge state grouping;
- **Deconvolution** of low-resolution and high-resolution mass spectra with **targeted** (see Figure below) and untargeted approaches. Perhaps a unique commercial implementation of **UniDec** for deconvolution

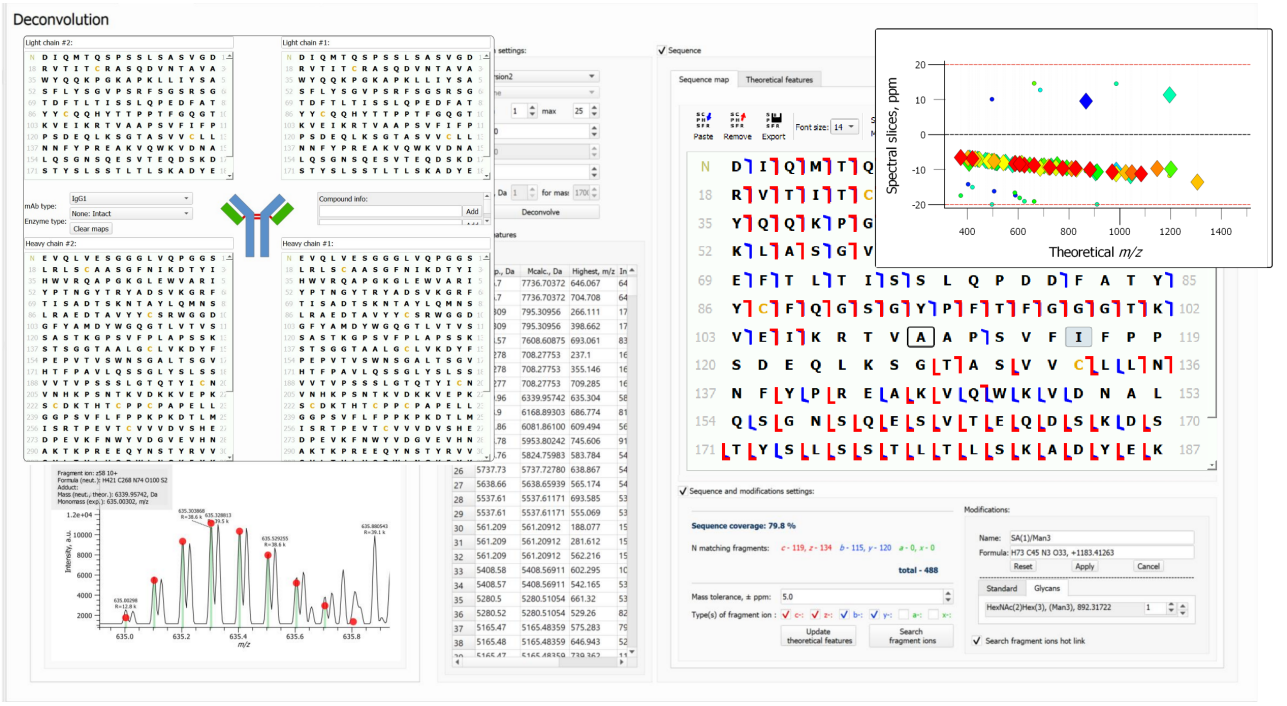


- Data validation through mass accuracy evaluation and mass spectra re-calibration via linear (on-the-fly, lock mass) and non-linear approaches; mass spectral peak alignment
- Experimental data matching with the simulated isotopic envelopes. User-defined analyte parameters for simulations: amino acid sequence, charge carrier, adduct, modifications, isotopic enrichment/depletion, number of charge states and isotopologues
- Support for batch file processing of large data sets: practically any number of LC-MS data sets
- Advanced time-domain transient processing: matching the mass spectral resolution to the sample characterization needs – from high-resolution to ultra-low resolution for enabling proteoform peak integration approach

Drug-to-Antibody Ratio Estimation via Proteoform Peak Integration in the Analysis of Antibody–Oligonucleotide Conjugates with Orbitrap Fourier Transform Mass Spectrometry. Konstantin O. Nagornov, Natalia Gasilova, Anton N. Kozhinov, Pasi Virta, Patrik Holm, Laure Menin, Victor J. Nesatyy, and Yury O. Tsybin. Anal. Chem. 2021, 93, 38, 12930–12937

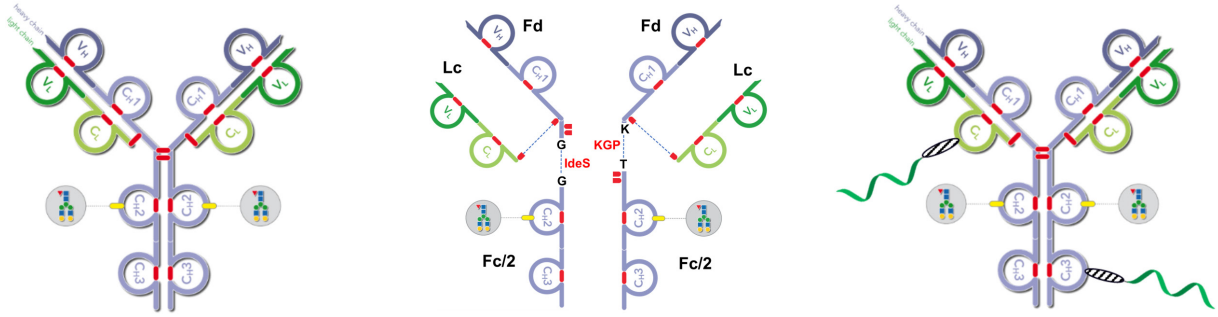
Protein Analysis via Top-Down and Middle-Down Approaches

Advanced processing and analysis of intact, middle-down, and top-down data.



Key Features

- Advanced and proprietary mass spectrometry solutions for mAb analysis, including complex ADCs/AOCs
- Superior quality data owing to proprietary methods and techniques for Orbitrap FTMS
- Integration of intact mass, middle-up, middle-down, top-down, and bottom-up mass spectrometry data
- Data averaging of LC-MS/MS and MS/MS data from multiple experiments (technical replicates)
- Recalibration of intact (MS data) and middle/top-down (MS/MS data) mass spectra
- Embedded database of common monoclonal antibodies with sequences and modifications
- Accurate simulation of mass spectra based on proteoform sequence and modifications
- Comparison of experimental and simulated isotopic envelopes and mass spectra
- High-resolution and low-resolution deconvolution, charge state grouping, quantitation
- Intact mass and product ion assignment to the deconvolved data
- Interactive graphical representation of sequence maps (add proteoforms, remove assignments, etc.)
- Graphical output (images), mass spectral data and peak lists

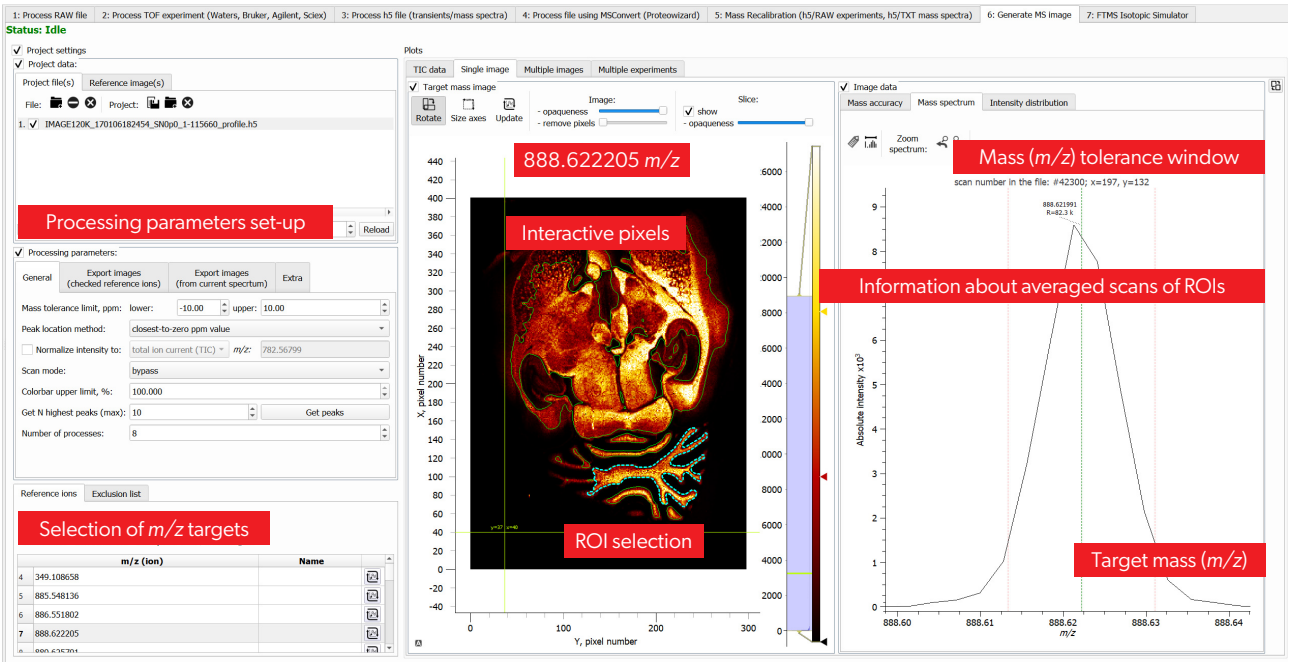


Structural Analysis of Monoclonal Antibodies with Top-down and Middle-down Electron Transfer Dissociation Mass Spectrometry: The First Decade. Luca Fornelli, Daniel Ayoub, Kristina Srzentić, Konstantin Nagornov, Anton Kozhinov, Natalia Gasilova, Laure Menin, Alain Beck, and Yury Tsybin. CHIMIA, 2022, 76, 114-126

Imaging: Mozaic

Process, analyze, and visualize MSI data - no matter the file size or the MS platform.

Individual *m/z* Image



The presented dataset (mouse brain tissue imaging , 50 x 50 µm pixel size) was acquired on a LTQ Orbitrap Elite™ FTMS instrument (Thermo Fisher Scientific) equipped with a MALDI injector from Spectroglyph.

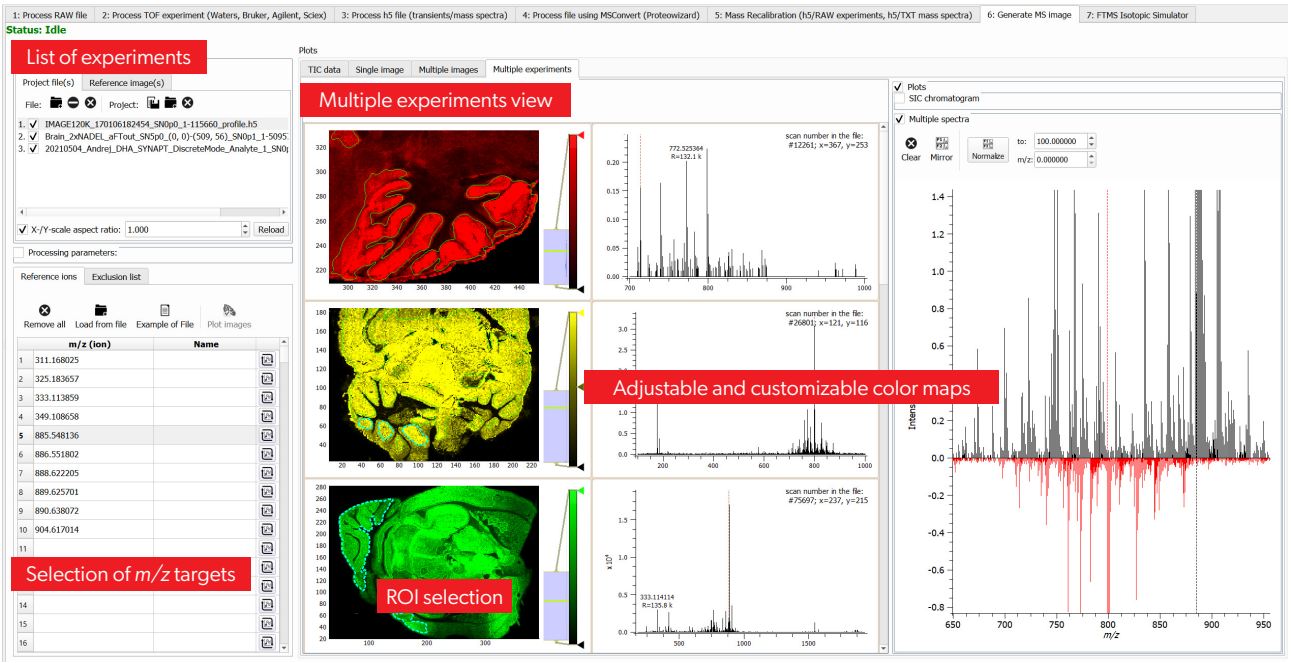
Data Processing

Comprehensive processing of MSI datasets of any size from diverse MS instruments, including Orbitrap™ FTMS, FT-ICR MS, TOF MS, and QQQ MS (vendor-specific file formats and imzML datasets)

- Support for MALDI, DESI, and MALDESI ion sources, including from MassTech, Spectroglyph, & TransMIT
- Automatic recognition of meta information (e.g., instrument model and experimental settings), support of multiple acquisition modes (MS, MS/MS, SIM, etc.), and MS/MS scans grouping via precursor mass rounding
- Import functionality for all types of reduced and unreduced data, including reduced and full profile enhanced FT (eFT) mass spectra, and time-domain transients from FTMS instruments
- A full cycle of FTMS data processing: unreduced (time-domain transients and absorption mode (aFT) mass spectra) and reduced (centroided, reduced, and full profile eFT and magnitude mode (mFT) mass spectra)
- Data validation via mass accuracy evaluation and mass spectra re-calibration using either linear (on-the-fly, single and multiple lock mass (*m/z*) values) or non-linear approaches
- Mass spectra processing with user-defined parameters, e.g., noise thresholding and data averaging
- Averaging mass spectra from adjacent pixels to increase sensitivity and dynamic range
- Flexible image viewers for interactive viewing and investigation from single and multiple *m/z* values
- Image processing: normalization, noise reduction, hotspot removal, etc.
- Super-resolution signal processing (e.g., least squares fitting) of FTMS transients

MOZAIC

Multiple Experiments



Shown are results of MALDI MSI analysis of different mouse brain tissue sections performed with: (top panel) an FT-ICR MS instrument (a 9.4 T SolariX™ XR from Bruker Daltonics); (middle panel) a TOF MS instrument (Synapt™ from Waters); and (bottom panel) an Orbitrap FTMS instrument (LTQ Orbitrap Elite from Thermo Fisher Scientific) equipped with a MALDI injector from Spectroglyph.

Data Analysis

- Instant visualization of interactive images and mass spectra (click and see)
- Manual and automatic definition of regions of interest (ROI), analysis and geometry handling
- Comparative analysis of (averaged) mass spectra from multiple ROIs for the same or different data sets
- Multiple pixel interpolation approaches, image sharpening
- Co-localization and overlay of (multi-mode) images
- Image transparency, add color function, customizable and adjustable color maps
- Results are readily exportable in common (imzML) and advanced (H5) data formats
- High quality images are exportable in different formats: PDF, png, jpeg, tiff, svg, etc.
- Peak annotation and further data analysis: via imzML upload to third-party software tools

Enhanced MSI via Unreduced Data Acquisition and Processing

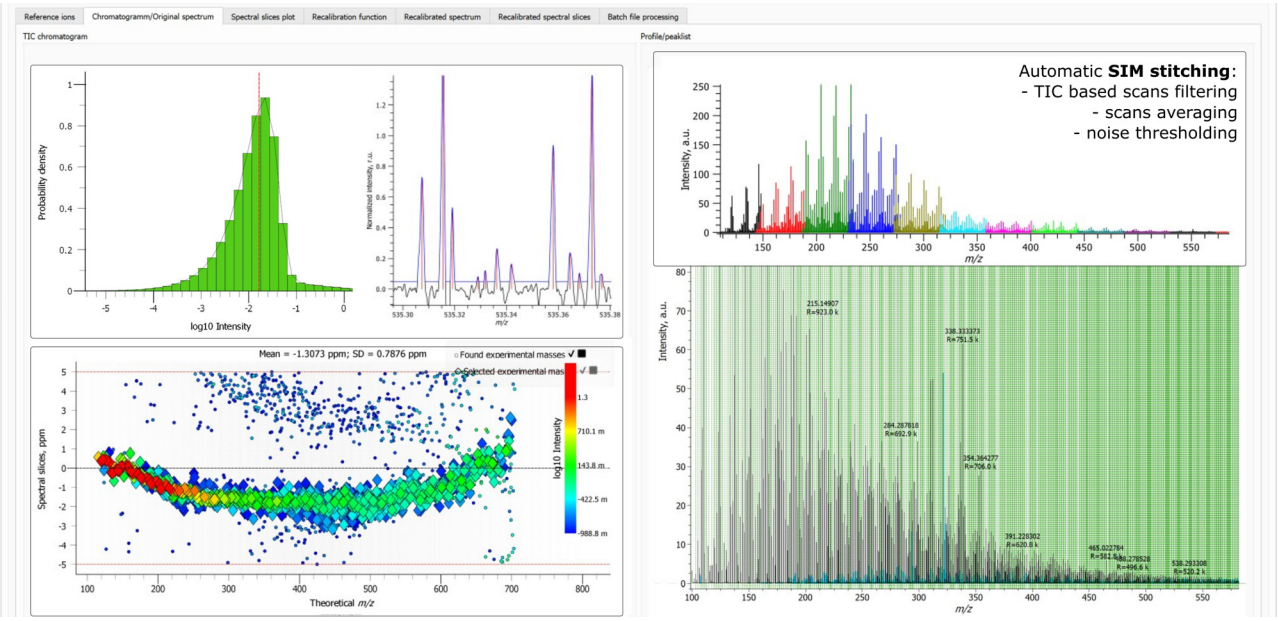
Integrated hardware-software solution to access and process time-domain transients

Mozaic is a stand-alone software tool. In addition, it can form an integrated solution with the Spectroswiss **FTMS Booster** - a high-performance data acquisition system providing access to the time-domain transients from Orbitrap and Ion Cyclotron Resonance (ICR) FTMS instruments, see https://spectroswiss.ch/hardware/

Imaging and Direct Sampling Capabilities of Nanospray Desorption Electrospray Ionization with Absorption-Mode 21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Gregory W. Vandergrift, et al.,. Anal. Chem. 2022, 94, 8, 3629-3636

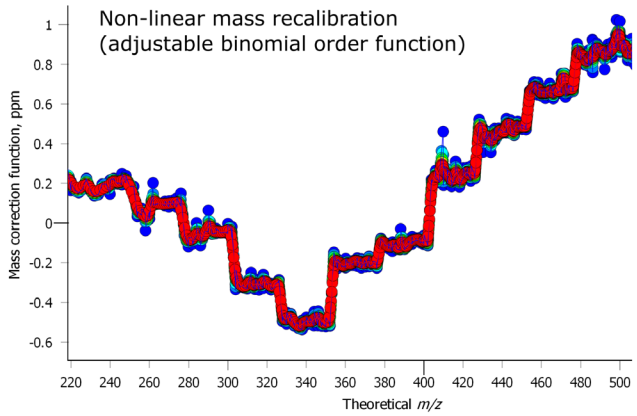
Complex Mixture Analysis, Petroleomics & Bio-Fuels

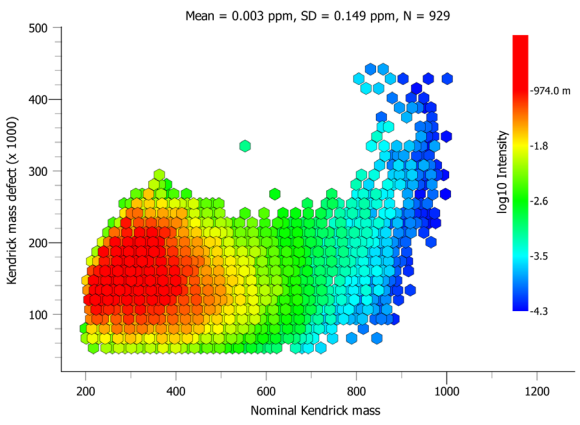
Advanced processing and analysis of (very) complex mixtures mass spectrometry data



Key Features

- Integration and processing of practically any size .RAW, .d folders, and .H5 files
- Open file formats for transients & mass spectra: H5, DAT, MGF, mzXML, and mzML
- Time-domain transient processing: advanced FT with pre- and post-processing, including apodization, zero-filling, magnitude and absorption mode FT
- Visualization of time-domain transients and mass spectra
- Visual comparison of mass spectra from separate data sets
- Spectral and time-domain transient averaging for high sensitivity and dynamic range
- Processing of LC-MS data for petroleomics and biofuels applications
- Data averaging across MS and LC-MS multiple technical replicates
- Narrow *m/z* window (SIM) stitching of the unreduced data for enhanced performance
- Advanced peak picking and efficient baseline correction
- Noise thresholding, reduced-profile mode mass spectra representation
- Mass scale calibration equations, including iterative re-calibration for complex mixtures



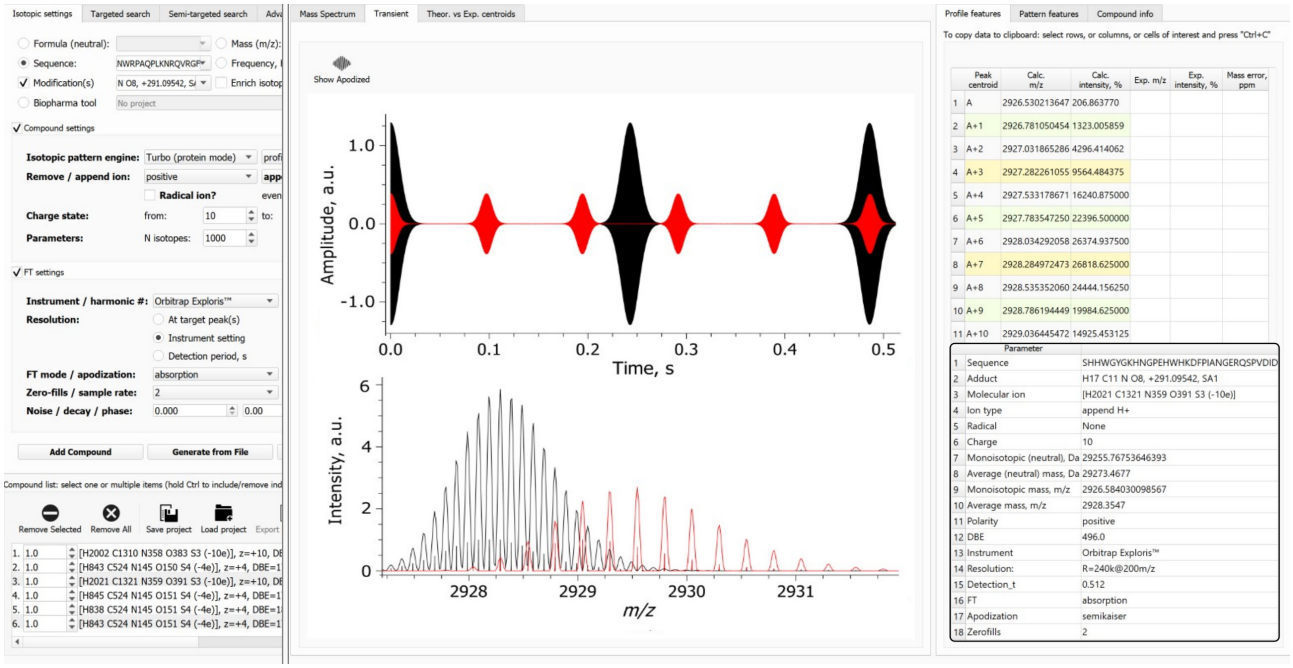


Iterative Method for Mass Spectra Recalibration via Empirical Estimation of the Mass Calibration Function for Fourier Transform Mass Spectrometry-Based Petroleomics. Anton N. Kozhinov, Konstantin O. Zhurov, and Yury O. Tsybin. ***Anal. Chem.*** 2013, 85, 13, 6437–6445

FTMS Simulator

A software tool to accurately simulate FTMS isotopic envelopes and mass spectra:

- to visually validate and verify the experimental results and hypotheses, perform data quality control
- to teach the FTMS subject by demonstrating realistic transients and corresponding mass spectra
- to search the experimental data with a like-for-like simulated data using similarity score ranking
- to design FTMS experiments by selecting optimum settings and FT data processing parameters



Key Features

- Applicable to (almost) any size molecules – from metabolites to monoclonal antibodies and viruses
- Applicable to (almost) any complexity mass spectra – from isotopic envelopes to full scale top-down data
- Analyte parameters selection: elemental composition, amino acid sequence, charge carrier, modifications, isotopic enrichment/depletion, number of charge states, and number of isotopologues
- FTMS experiment parameters: FTMS instrument selection (models of Orbitrap™ & ICR), resolution setting (@ *m/z*), add noise, set signal intensity thresholds, set initial phase, sampling rate, and decay rate
- Data processing parameters: absorption or magnitude FT, unapodized or apodization function, number of zero-fills, full or reduced profile mass spectra, centroids
- Visualize transients with and without apodization, visualize frequency spectra
- Use mass spectra: manually or automatically set peak intensities, visualize isotopic envelopes, plot multiple isotopic envelopes for a broadband view, extract high quality figures or data points for analysis
- Results output: use simulated data to search experimental data, rank search results by the similarity score (*m/z*, intensity), save project for future use, create report as a PDF file

FTMS Simulator

Transient-Mediated Simulations of FTMS Isotopic Distributions and Mass Spectra to Guide Experiment Design and Data Analysis. Konstantin O. Nagornov, Anton N. Kozhinov, Natalia Gasilova, Laure Menin, and Yury O. Tsybin. *JASMS,* 2020, 31, 9, 1927–1942

Characterization of the Time-domain Isotopic Beat Patterns of Monoclonal Antibodies in Fourier Transform Mass Spectrometry. Konstantin O. Nagornov, Anton N. Kozhinov, Natalia Gasilova, Laure Menin, and Yury O. Tsybin. *JASMS,* 2022, 33, 7, 1113–1125

FTMS Didactics: Unreduced and Reduced Data

Unreduced Data

Reduced Data

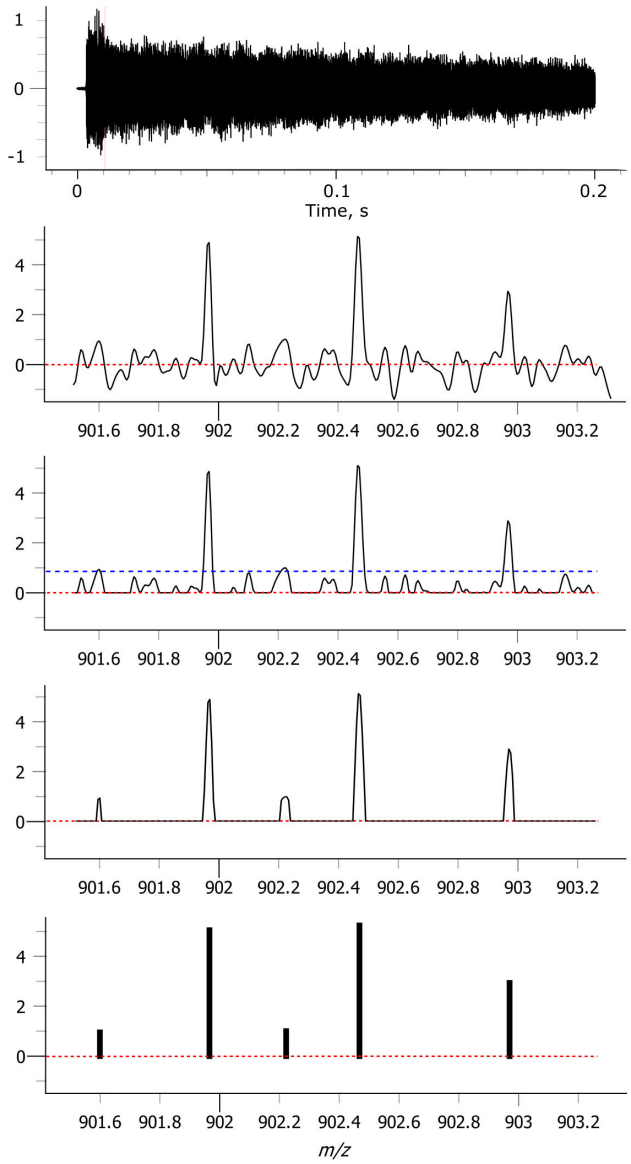
FTMS transient
time domain data

aFT (absorption)
true full profile

eFT, RAW
full profile

eFT, RAW
reduced profile

eFT, RAW
centroided



Information

Software Information and Distribution

To learn more about our software tools or to get your free evaluation license, you are most welcome to visit https://spectroswiss.ch/software/ and to contact us at info@spectroswiss.ch

PEAKBYPEAK FTMS Simulator WORKFLOWS

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