FTMS Teaching Pack: an interactive resource to teach FTMS fundamentals

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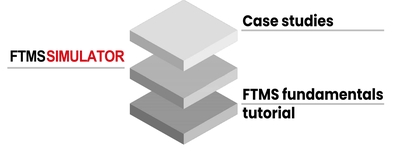
Introduction

FTMS instruments are inherently complex and require FTMS fundamentals understanding to unlock their full capability and avoid results misinterpretation. Here we present the FTMS Teaching Pack - the package which includes the FTMS fundamental tutorials, FTMS Simulator software, and customized training case studies.



FTMS SIMULATOR

FTMS Simulator is 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's ranking
- to design FTMS experiments by selecting optimum settings and FT data processing parameters

Key features

- 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

Workflow

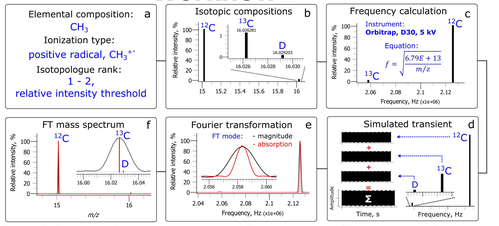


Figure 1. Schematic diagram of the workflow of Fourier transform (FT) mass spectra simulation for user-defined chemical compounds via *in silico* time-domain signal (transient) generation for ICR or Orbitrap FTMS instruments. (a) Definition of molecular formulas of interest, (b) calculation of a complete isotopic distribution on a *m/z* scale, (c) conversion of the calculated isotopic distribution from a *m/z* scale into a frequency scale, (d) simulation of transients composed of sinusoidal components that correspond to individual isotopologues (*m/z* values and abundances), (e) FT processing of transients, and (f) visual representation of transients, isotopic distributions, and mass (or frequency) spectra. (*JASMS*, 2020, 1927; DOI: 10.2021/jasms.0c00190)

Settings

Compound definition:
- Elemental composition
- Amino acid sequence
- Isotopes relative abundances
- Modifications
- Mass (*m/z*) or frequency value

Ion (charged compound) definition:
- Charge carrier: electron, H^+, K^+, Na^+, Cs^+, I^-, $HCOO^-$
- Ionization: positive, negative, or neutral
- Charge state: selected or range
- Isotopologues: how many and which ones

FT processing settings:
- FTMS instrument type and model: ICR/MRMS, Orbitraps
- Harmonics order: which harmonic to calculate (1, 2, 3, ...)
- Resolution: at target peak, instrument setting, transient length
- FT mode: absorption or magnitude
- Apodization window: none, full (Kaiser), half (semi Kaiser)
- Number of zero fills: 0, 1, 2, or 3
- Sampling rate (digitization frequency): 1, 2, 4, 6 MHz, or any
- Noise (added to the transient): noise amplitude
- Decay rate: ion signal decay rate in a transient, $e^{-(decay\ rate)t}$
- Phase: initial phase (angle) of ion detection in a transient

FTMS SIMULATOR

Overview

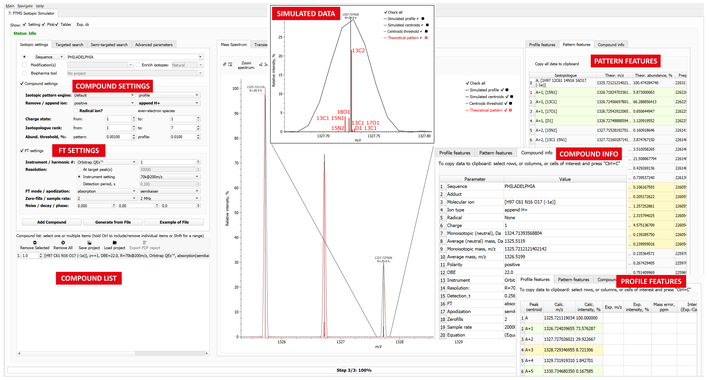


Figure 2. FTMS Simulator graphical user interface. The adjustable parameters include compound, charged species (ion), instrument, and FT settings. FTMS Simulator allows to visualize simulated isotopic envelopes, mass spectra, and time-domain transients. The set of adjustable parameters and available functions enables accurate representation of FTMS data of practically any complexity.

Frequency and resolution

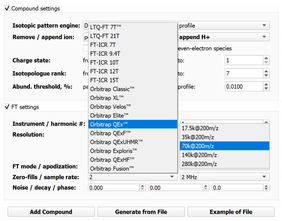


Figure 3. FTMS simulator allows the user to specify a particular model of an Orbitrap or an ICR FTMS instrument. Both commercially available instruments and those custom developed, e.g., 21 T FT-ICR MS instruments, are included. Once a specific instrument model is selected, simulations will take into account the corresponding calibration equations and frequency-*m/z* relationships. For those FTMS instruments that support transient period (ion detection time) setting via selection of a resolution target - the typically employed settings are provided to the user. Moreover, the ion detection frequency can be specified at the fundamental harmonic as well as on the higher order harmonics specified by the user. The frequency spectrum can also be visualized.

Broadband mass spectrum

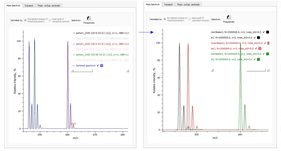


Figure 4. Broadband (wide *m/z* range) mass spectra of practically any complexity can be simulated for the user-selected parameters. The summed mass spectra can be extracted as high quality figures or as data points. The latter option shows utility in quality control applications and experimental data search. The relative intensity of peaks in broadband mass spectra are user-defined. Top-down mass spectra for protein analysis is a particularly interesting application of this capability. The thus generated data can be used to benchmark and validate software tools, and help in optimizing the processing settings of these tools.

Apodization

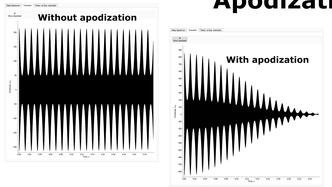


Figure 5. The apodization windows "shape" transients prior to Fourier transform processing. (Left panel) shows the unapodized transient of a peptide $[PHILADELPHIA+H]^+$ analyzed in Figure 2. (Right panel) shows this time-domain transient after half-window (semi Kaiser) apodization.

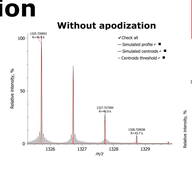


Figure 6. Comparison of mass spectra processing without (left panel) and with (right panel) apodization (number of zero-fills in both cases is 2). Expectedly, apodization significantly reduces the FT artefacts and characteristics sidelobes, such as Gibbs oscillations. The associated reduction in resolution can be also noted.

Zero-fills

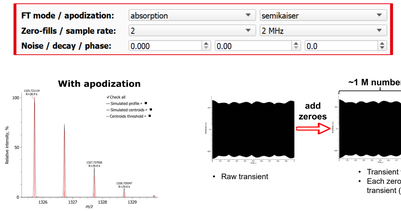


Figure 7. Adding zeroes allows to improve peak digitalization (peak shape) for enhanced resolution and mass accuracy. Typically, 2 zero-fills are applied to process modern FTMS data.

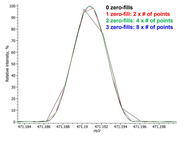


Figure 8. Peak shape changes depending on the number of zero-fills. Higher numbers of zero-fills increase the number of points that define mass spectral peaks.

Noise

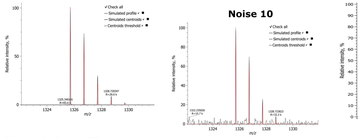


Figure 9. Before FT processing, the simulated transients can be mixed with a noise signal of Gaussian distribution with a user-defined standard deviation. The latter can help to account for the noise in experimental transients and provide a realistic mass spectra representation.

Decay factor

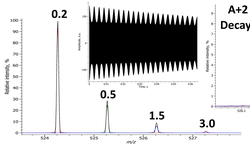


Figure 10. Mass spectrum of MRFA $[M+H]^+$ simulated for Orbitrap Q Exactive (black color, no transient decay) with FTMS Simulator. Decay factors for the isotopologues (starting from the monoisotopic peak) are: 0.2; 0.5; 1.5; and 3.0. The corresponding individual transients (64 ms) are shown in the inset. Another inset shows an expanded view on the A+2 isotopologue.

Phase

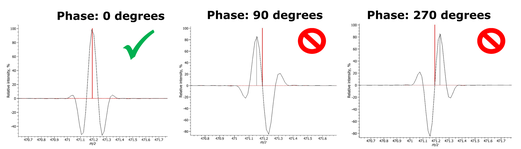


Figure 11. The *m/z*-dependent initial phase value directs the outcome of absorption mode FT processing. In case this value is not taken into account upon data acquisition or upon post-processing of transients, its shift can result in mass spectral peak artefacts: peak position (*m/z*) and abundance. Accurate phasing is crucial for artefact-free aFT mass spectra.

BioPharma tool

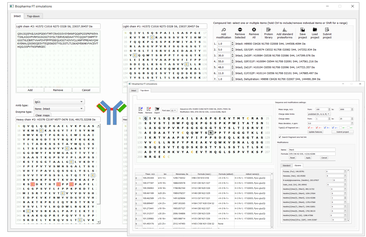


Figure 12. The BioPharma tool facilitates definition of structural information (elemental composition, sequence, and modifications). The mass spectra of the thus defined species can be visualized with the user-defined relative abundances in narrowband and broadband mass spectra views.

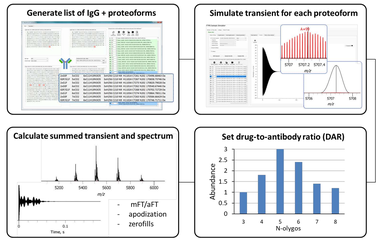


Figure 13. The BioPharma tool can support structural analysis of monoclonal antibodies (mAbs)-derived species, including antibody-drug conjugates (ADCs). For example, it can aid in the validation and development of novel methods for the analysis of drug-to-antibody ratios (DARs) of ADCs (Nagornov *et al.*, *Anal. Chem.* 2021, 12930)

Validation

Appropriate use of the simulated FTMS data in the quality control and method development applications requires careful validation of the employed tool, FTMS Simulator. Therefore the validation procedures have been performed on diverse molecular sizes and complexity levels.

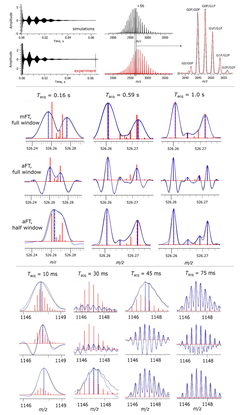


Figure 14. Benchmarking FTMS Simulator: comparison of the simulated (top panel) and experimental (bottom panel) data. Results are shown for the analysis of trastuzumab (including its proteoforms) performed with a Q Exactive HF Orbitrap FTMS in the denaturing mode (electrospray ionization). The expanded view of the 56 charge state shows the resolved proteoforms, but unresolved isotopic envelopes (64 ms transients). Intensities of the simulated peaks are assigned by the user.

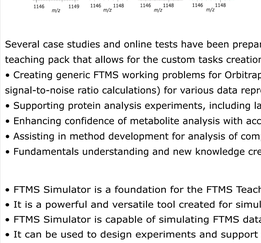


Figure 15. Analysis of peptide isotopic fine structure. Expanded views into (black color, solid line) simulated and (blue color, dashed line) experimental profile mass spectra showing the A+2 isotopologues of a singly charged MRFA peptide. Peak centroids are shown (blue color, dashed line) for experimental and (black color, solid lines) for simulated peaks. Calculated IFS distributions are shown with red color solid lines. Experimental data were acquired using a Q Exactive HF FTMS with 10 *m/z* isolation (SIM mode); simulated data were obtained for corresponding settings. Both simulated and experimental transients of different length, Tacq = 160 ms (left column), Tacq = 590 ms (middle column), and Tacq = 1000 ms (right column), were apodized using the Kaiser function and FT processed via (top panel) mFT and full window, (middle panel) aFT and full window, and (bottom panel) aFT and half window. (*JASMS* 2021, 1927; DOI: 10.2021/jasms.0c00190)

Figure 16. Analysis of a 5 kDa protein isotopic distribution. Expanded views into (black color, solid line) simulated and (blue color, dashed line) experimental profile mass spectra showing isotopic distribution of 5+ insulin protein. Peak centroids are shown for experimental peaks (blue color, dashed line) and for simulated peaks (black color, solid lines). Theoretical isotopic pattern is shown with red color solid lines. Experimental data acquired using a Q Exactive HF FTMS, simulated data obtained using the corresponding settings. Both simulated and experimental transients of different length from left: Tacq=10 ms, Tacq=30 ms, Tacq=45 ms and Tacq=75 ms were apodized using Kaiser function and FT processed via (top panel) mFT, full window, (middle panel) aFT, full window, and (bottom panel) aFT, half window. (*JASMS* 2021, 1927; DOI: 10.2021/jasms.0c00190)

Case studies

Several case studies and online tests have been prepared by Spectroswiss team. Further developments can be easily made due to the flexibility of the FTMS teaching pack that allows for the custom tasks creation including but not limited to:

- Creating generic FTMS working problems for Orbitraps and ICR/MRMSs instruments (resolution change, transient parameters, frequencies, signal amplitudes, signal-to-noise ratio calculations) for various data representation types (mFT, aFT)
- Supporting protein analysis experiments, including large proteins, such as monoclonal antibodies and viruses
- Enhancing confidence of metabolite analysis with accurate representation of the isotopic fine structure
- Assisting in method development for analysis of complex ADCs, including verification of their critical quality attributes, such as DARs
- Fundamentals understanding and new knowledge creation, for example via analysis of the time-domain isotopic beat patterns

Conclusions

- FTMS Simulator is a foundation for the FTMS Teaching Pack
- It is a powerful and versatile tool created for simulation of realistic FTMS data via transient generation
- FTMS Simulator is capable of simulating FTMS data for any current FTMS application, for example for DAR analysis of complex ADC species
- It can be used to design experiments and support data analysis in FTMS, including for benchmarking data analysis software tools
- Simulated isotopic profiles and centroids can be used to search the experimental data and rank the results using the similarity scores
- The accurately simulated results can reveal FT processing-related artefacts or characteristic features, which are lost in typical calculators
- The didactic nature of the FTMS Simulator appears extremely useful in educating and training the rigorous FTMS scientists
- Didactic materials include the FTMS fundamentals tutorial and case studies, which can be customized or adapted by the user

Poster for the 69th ASMS Conference, Philadelphia, Pennsylvania, USA, Fall 2021