FT Mass Spectra Simulation: Fundamentals and Applications

Simulated transient  \[ \sum \]

FT mass spectrum  \[ m/z \]

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How & Why to Simulate the FTMS Data?

- Approaches to simulate the FTMS isotopic envelopes and mass spectra
- Teaching & training: understanding the FTMS concepts and definitions
- Reviewing & writing: manuscripts, project applications, ideas verification
- Support FTMS data interpretation and experiment design (settings selection)
- Advancing the FTMS fundamentals and generating novel insights
- Support of FTMS data processing workflows (simulated vs experimental data)
FTMS Data Simulation Approaches

- Numerical simulations of ion motion and induced current detection
  - SIMION, Particle-in-Cell (PIC): Amster (IJMS, 2020), Hendrickson (IJMS, 2009), etc.
- Analytical models for estimation of ion oscillation frequencies and resolution
  - For each FTMS instrument: parameters of ion oscillations (e.g., frequency); dependency of resolution on frequency, relationship between frequency and \( m/z \)
- Empirical estimation of resolution: peak shape addition to isotopic distributions
  - Gaussian peak shapes are added using the resolution relationship with \( m/z \)
    https://www.envipat.eawag.ch/ - as employed in LIPIC (Cataldi, JASMS 2021)
  - The resolution values are estimated from the experimental peaks (FasmaTech, etc.)
- Accurate simulation of the FTMS data processing workflow
  - Simulation and processing of time-domain transients, for each instrument (this work)
FTMS Data Simulation Approaches

Isotopic distribution calculation

Resolution (FWHM) calculation

Gaussian simulation

Gaussians summation

D20, D30, 5 kV, etc.

Resolution (FWHM) estimation in experimental data

Gaussian simulation

Gaussians summation

Frequency calculation

Transient simulation

Fourier transform

D20, D30, 5 kV, etc.

Protein analysis? (isotopic beats)

Peak interference?

Computational speed?

ChemCalc
IsoSpec2
EnviPat
...

13C_1

15N_1

196.08 196.10

195 196 197

m/z

m/z
FTMS Data Simulation via Time-Domain Transients

Elemental composition: \( \text{CH}_3 \)
Ionization type: positive radical, \( \text{CH}_3^+ \)
Isotopologue rank: 1 - 2, relative intensity threshold

Isotopic compositions

<table>
<thead>
<tr>
<th>Isotopic Mass</th>
<th>Relative Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12C</td>
<td>1</td>
</tr>
<tr>
<td>13C</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Frequency calculation

Instrument: Orbitrap, D30, 5 kV
Equation:
\[
f = \sqrt{\frac{6.79E + 13}{m/z}}
\]

FT mass spectrum

<table>
<thead>
<tr>
<th>Mass/Charge (m/z)</th>
<th>Relative Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12C</td>
<td>100</td>
</tr>
<tr>
<td>13C</td>
<td>90</td>
</tr>
</tbody>
</table>

Fourier transformation

FT mode: magnitude, absorption

Simulated transient

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**Compound definition:**
- elemental composition, isotopic labelling / enrichment
- amino acid sequence
- mass ($m/z$) or frequency value
- proteoforms – sequence and modifications (mAbs, viruses, …)

**Ion (charged compound) definition:**
- Charge carrier: electron, $H^+$, $K^+$, $Na^+$, $Cs^+$, $I^-$, $HCOO^-$
- Ionization mode: positive, negative, or a neutral species
- Charge state: from the lowest to the highest
- Isotopologues: how many and which ones

**FT processing settings:**
- FTMS instrument and model: ICR/MRMS, Orbitraps
- Harmonics order: fundamental and higher order harmonics
- 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, $e^{-(\text{decay rate})}$
- Phase: initial phase (angle) of ion detection in a transient
THALIA + H$^+$
unapodized magnitude mode
7 T FT-ICR MS
192 ms transient
Full window, Kaiser apodization
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. A+1
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. (Details...)

Frequency spectrum
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. The diagram shows an unapodized transient.
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra.

**Apodized Transient**

![Graph showing an apodized transient](image-url)
half window apodization absorption mode FT (aFT)
apodized transient
full window apodization
# zero fills = 0
# zero fills = 1
# zero fills = 2
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. # zero fills = 3
The FTMS Isotropic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

noise
The FTMS Isotopic Simulator is a software tool to accurately simulate FTMS isotopic envelopes and mass spectra. [Details...]

The image displays a graph with peaks labeled 'noise'.
Transient decay
transient decay
phase = 0°
phase = 25°
different FTMS instruments
large molecules: mAbs

$[M+30H]^{30+}$
isotopic beats: mAbs
Isotopes: mAbs
Isotopes: mAbs

A+115
Isotopic fine structure: mAbs
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Question 12. A typical monoclonal antibody (approx. 150 kDa) has an isotopic distribution that is 25 Da wide (measured as full-width at half-maximum) and appears at m/z 5000, when sprayed intact, under native conditions. With an Orbitrap FTMS (eFT mass spectra) having available resolution settings 15,000; 30,000; 60,000; and 120,000 at m/z 200, what setting should be chosen to provide the highest resolution for the antibody peak, before the signal-to-noise ratio starts to drop?

- 15,000
- 30,000
- 60,000
- 120,000
Monoclonal Antibody (mAb) Analysis

Isotopic Envelope

$15k @ m/z 200: 3'100$

$30k @ m/z 200: 4'900$

$60k @ m/z 200: 6'000$

$120k @ m/z 200: 6'300$

$240k @ m/z 200: 6'400$

Q Exactive HF of trastuzumab

$[M+30H]^{30+}$

$[M+30H]^{30+}$

$[M+30H]^{30+}$

$[M+30H]^{30+}$

~25 Da
Isotopic Beats in FTMS Transients

The constructive and destructive interferences between close frequency ion signals - beats

Hofstadler, S. A.; Bruce, J. E.; Rockwood, A. L.; Anderson, G. A.; Winger, B. E.; Smith, R. D. Isotopic beat patterns in Fourier transform ion cyclotron resonance mass spectrometry: implications for high resolution mass measurements of large biopolymers. IJMS and Ion Processes 1994, 132, 109-127

Easterling, M. L.; Amster, I. J.; van Rooij, G. J.; Heeren, R. M. A. Isotope beating effects in the analysis of polymer distributions by Fourier transform mass spectrometry. JASMS 1999, 10, 1074-1082

Makarov, A.; Denisov, E. Dynamics of ions of intact proteins in the Orbitrap mass analyzer. JASMS 2009, 20, 1486-1495
Isotopic Beats in FTMS Transients: mAbs

- **Sample:** a monoclonal antibody, trastuzumab
- **Instrument:** a Q Exactive HF BioPharma, native mode, [M+30H]^{30+}
- **Transient simulation with FTMS Simulator**

![Graphs showing transient behavior](attachment:image.png)

*Nagornov et al., JASMS 2022, 33, 1113–1125*
Resolution and SNR Dependencies: mAbs

- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, [M+30H]\(^{30+}\)
- Transient simulation and data processing with FTMS Simulator

- Colors: unapodized aFT; half window apodized aFT; full window apodized aFT
Consequences for mAb Analysis

• Only proteoforms with > 25 Da mass difference can be resolved (regular settings)
Isotopic Beats in **High-Resolution FTMS**: mAbs

- **Sample**: a monoclonal antibody, trastuzumab
- **Instrument**: a Q Exactive HF BioPharma, native mode, [M+30H]$^{30+}$
- **Transient simulation with FTMS Simulator**

Nagornov et al., JASMS 2022, 33, 1113–1125
Resolution & SNR Dependencies in HR FTMS: mAbs

- Sample: a monoclonal antibody, trastuzumab
- Instrument: a Q Exactive HF BioPharma, native mode, [M+30H]^{30+}
- Transient simulation and data processing with FTMS Simulator

Colors: unapodized aFT; half window apodized aFT; full window apodized aFT
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Resolution «Resonance» in the mFT ICR

- Sample: a biopolymer with an average mass of 150 kDa, H$_{10282}$C$_{6646}$N$_{1724}$O$_{2141}$S$_{44}$
- Instrument: a 12 T FT-ICR MS, [M+10H]$^{10+}$, full window (Kaiser-type) apodization
- Simulations: FTMS Simulator

To be verified experimentally!

Color coding: magnitude mode FT; absorption mode FT

Nagornov et al., JASMS 2022, 33, 1113–1125
The 2nd Beat Challenge: Full Window Apodization

• Transient period: 11.5 s
The 2\textsuperscript{nd} Beat Challenge: Full Window Apodization

- Transient period: 12.25 s
The 2nd Beat Challenge: Full Window Apodization

- Transient period: 13 s
The 2nd Beat Challenge: Full Window Apodization

- Isotopic envelopes are correlated for each charge state (no deconvolution)
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FTMS Workflows Embedding Data Simulation

1. Calculate mass spectral features

2. Simulate reference profile library

3. Align experiments (targeted/untargeted)

4. Extract SIC(s)

5. Process SIC(s) and detect features

6. Data analysis (mass recalibrate, quantify)

Nagornov et al., ASMS 2022, poster
Profile Library Simulations: Low Resolution

- Automatically determine FTMS instrument model and settings from metadata
- Specify a database of target compounds (from small molecules to proteins)
- Suggest adducts, modifications, charge carriers, and charge states
Correlate Experimental and Simulated Data

- Isotopic envelopes are compared for each charge state (no deconvolution)
- Charge state distributions are used to filter out the false positives
- Selected Ion Current (SIC) chromatograms show proteoform-specific elution periods
Targeted & Untargeted Deconvolution: Low Resolution

Herceptin, G0F/G1F, z = 49+

G1F/G2F, z = 49+

G0F/G1F (148217.96)

G0/G0F (147909.68)

G1F/G2F (148542.245)

UniDec low-res deconvolution

148220.28

147912.59

148546.25
Profile Library Simulations: High-Resolution

- Isotopically resolved envelopes
Targeted Deconvolution: High Resolution

• Analysis of monoclonal antibodies subunits (LC – light chains, 25 kDa)

QExact HF, R = 120k, 2 replicates
Humira, light chain

SIC: sum of signals of found isotopologues for multiple charge states of protein
Targeted & Untargeted Deconvolution: High Resolution

- Deconvolution approaches for isotopically-resolved data: FLEXDeconv, Hardklor, ...
Targeted Deconvolution: Small Molecule Analysis

- Analysis of steroids in human seminal fluid with a Q Exactive Focus
- Data annotation using 789 steroids database and 5 ppm mass tolerance
- Efficient feature extraction with reduced artifacts introduction and false positives
Targeted Deconvolution: Complex Mixture Analysis

- LC-MS analysis of a complex mixture on a 7 T LTQ FT Ultra
- Feature extraction of compound classes: LC/GC-MS complex mixture analysis

S class
dbe: 12

Area = 2.46 M

S class
dbe: 14

S class
dbe: 16

Area = 3.61 M
Conclusions:

Why to Simulate FTMS Data via Time-Domain Transients?

- Resolution dependence on mass, charge state, instrument model, etc.
- Peak interference and unresolved peaks – artifacts in resolution estimation
- Peak shape dependence on the FT processing – apodization, zero fills, etc.
- Computationally, data processing speeds are comparable with peak overlay methods
- Step-by-step visualization of the FT processing workflow – teaching & training
- Understanding the FTMS data and hypothesis verification (w/out experimental data)
- Revealing novel insights into FTMS, with subsequent experimental verification

Disclaimer: FTMS Simulator is only an example tool – it validates the approach