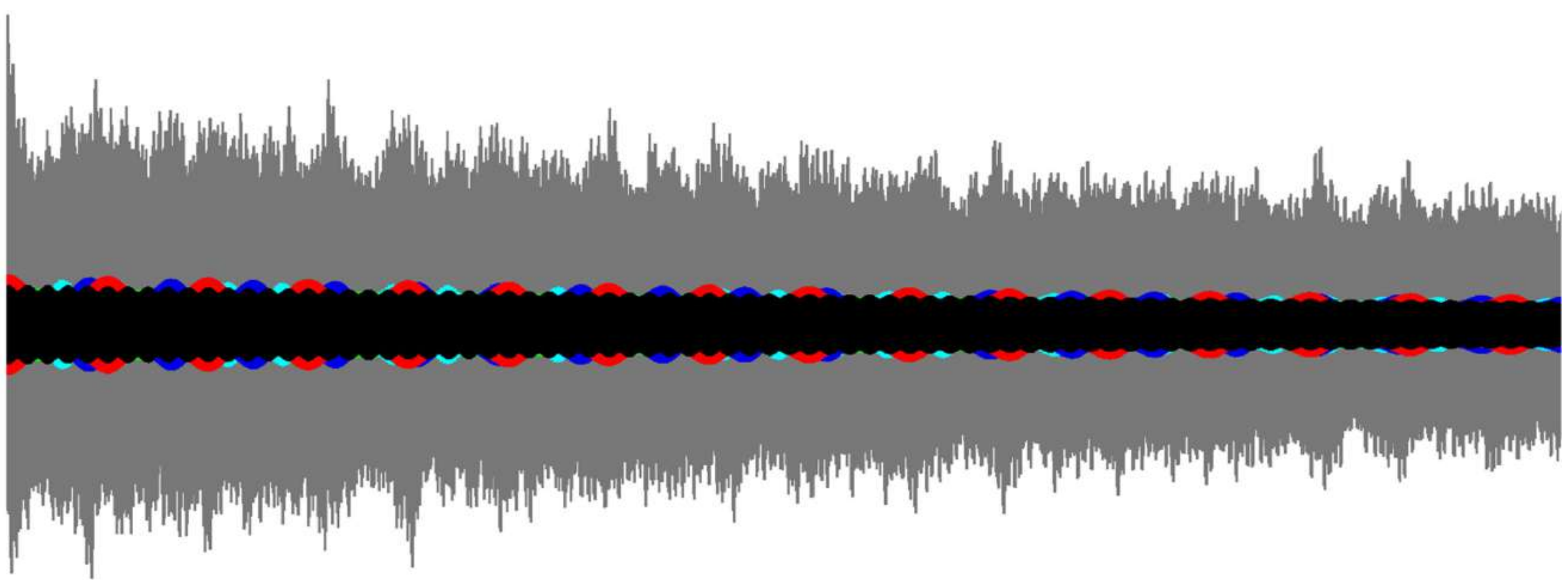
Spectroswiss
FTMS Science and Technology

IMSF Workgroup Seminar Series - Instrumentation

Harnessing the power of the unreduced data in FTMS

… or how to squeeze out maximum information from ion signals



16 March 2021 @ 17:00 CET

Yury Tsybin
tsybin@spectroswiss.ch

Spectroswiss
Lausanne, Switzerland

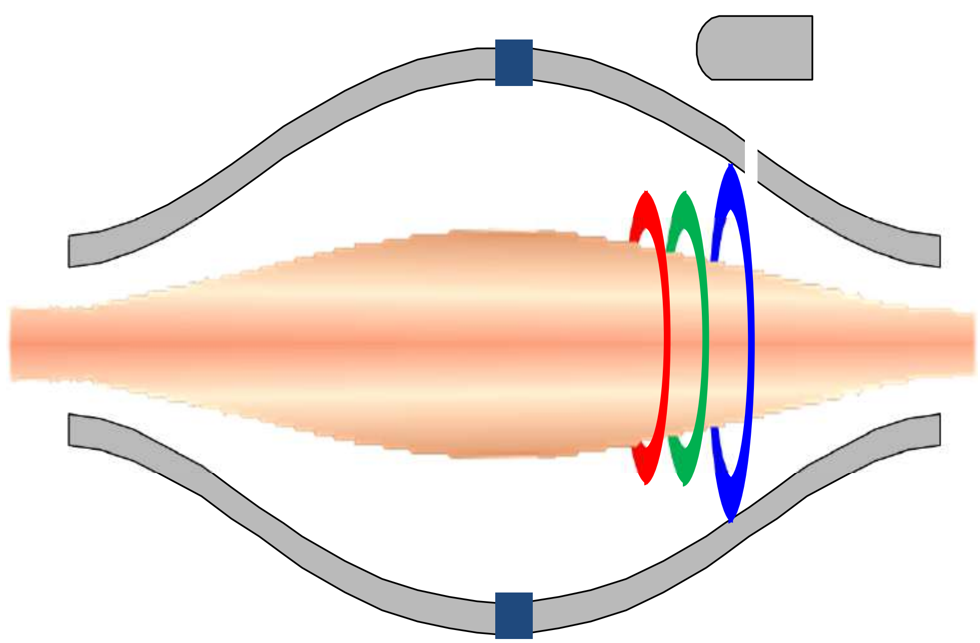
Spectroswiss

FTMS: Fourier Transform Mass Spectrometry

- Ion identity (*m/z*) is encoded as a frequency of ion oscillations in an ion trap
- Frequencies of ion oscillations are measured as time-domain signals (**transients**)
- Fourier transform (FT) decodes transients to reveal frequencies (*m/z*) values

Electrostatic field-based

Orbitrap



$$\omega \approx \sqrt{\frac{k}{m/z}}$$

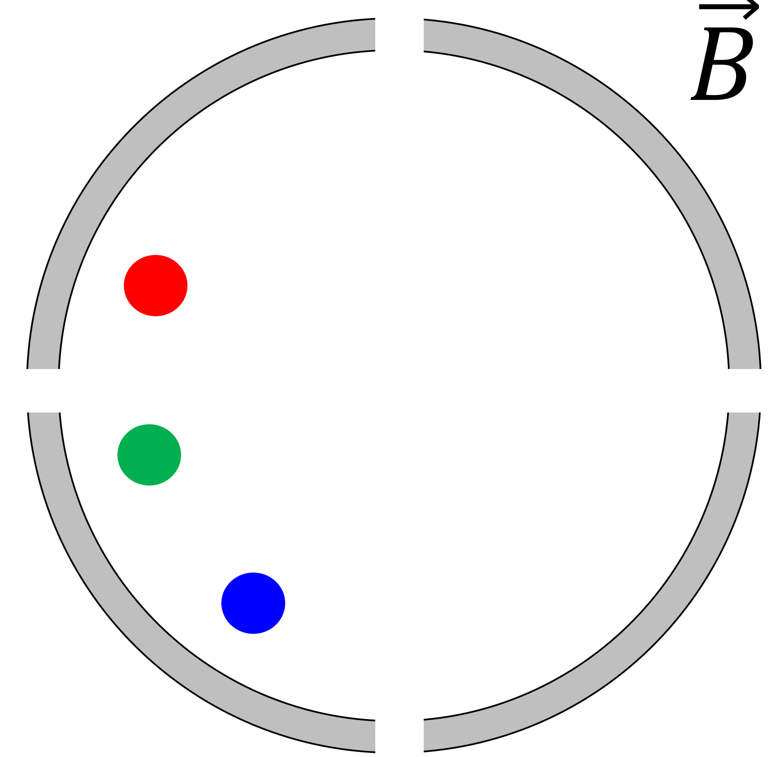
Orbitrap™ families: LTQ Orbitrap; Exactive; Q Exactive; Exploris; Fusion; Lumos; Eclipse

Magnetic field-based

Ion Cyclotron Resonance (ICR)

Magnetic Resonance MS (MRMS)

$\vec{B} \odot$



$$\omega \approx \frac{1}{m/z}$$

Apex
Solarix
Scimax

FTMS is the highest resolution & mass accuracy MS technique

FTMS Information Output

Unreadable data

full information content

Reduced data

10-fold smaller file size

metabolites
identified & quantified

proteins
identified & quantified

% sequence coverage in
protein structural analysis

confidence for precursor
and product ion detection

The use of the unreduced data aims to provide performance improvement

The Unreduced Data in FTMS

I. The Unreduced Data in FTMS

II. Data Reduction Approaches

III. How to Get and Process the Unreduced Data

IV. Examples of Applications

The Unreduced Data in FTMS

Spectroswiss

I. The Unreduced Data in FTMS

II. Data Reduction Approaches

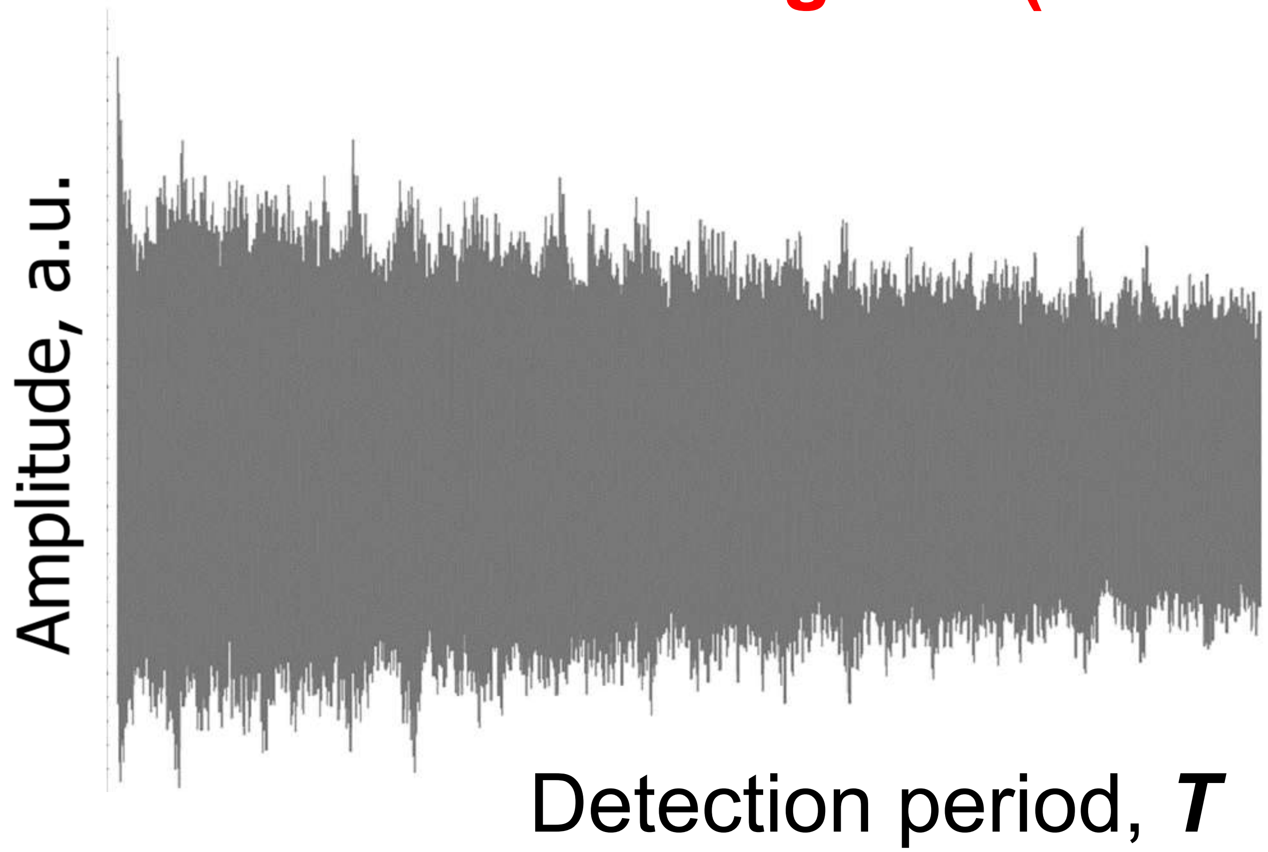
III. How to Get and Process the Unreduced Data

IV. Examples of Applications

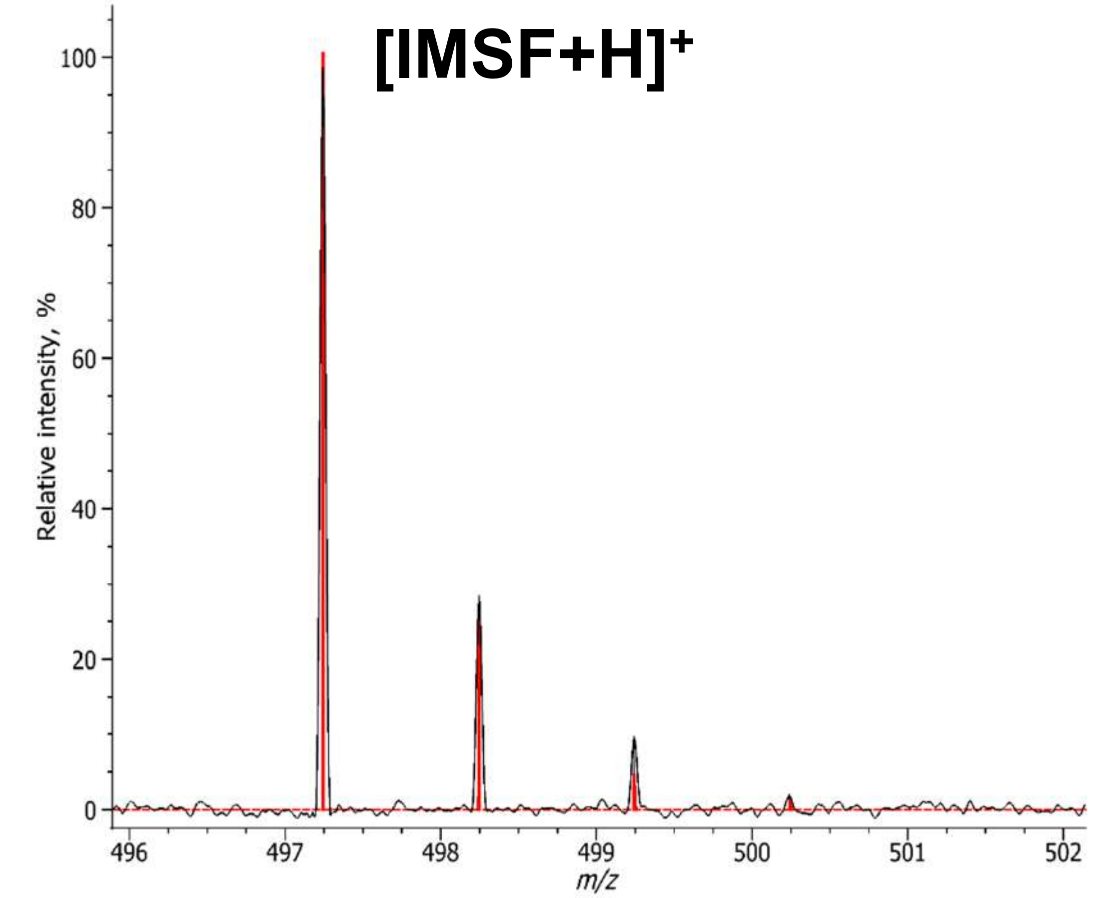
The Unreduced Data Types in FTMS

Spectroswiss

Time-domain ion signals (transients) | **Absorption mode mass spectra**



FT



$R \sim T$ • Resolution increases linearly with transient length (detection period)

$S/N \sim \sqrt{T}$ • Sensitivity increases as a sqrt of transient length (detection period)

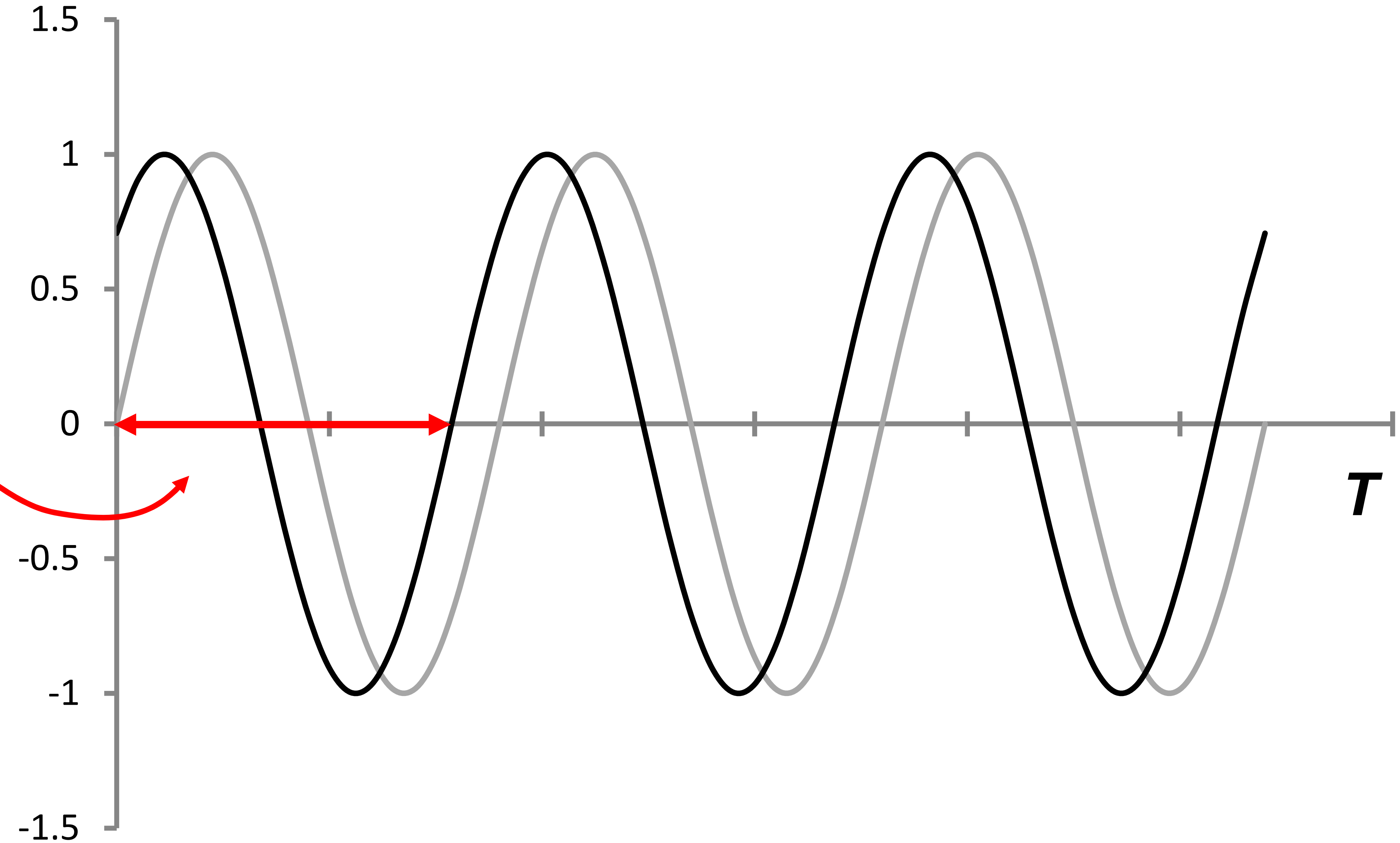
Transients & aFT mass spectra provide equal information output

Time-Domain Ion Signal: **Transient**

The Unreduced Data

Three components to define a wave:

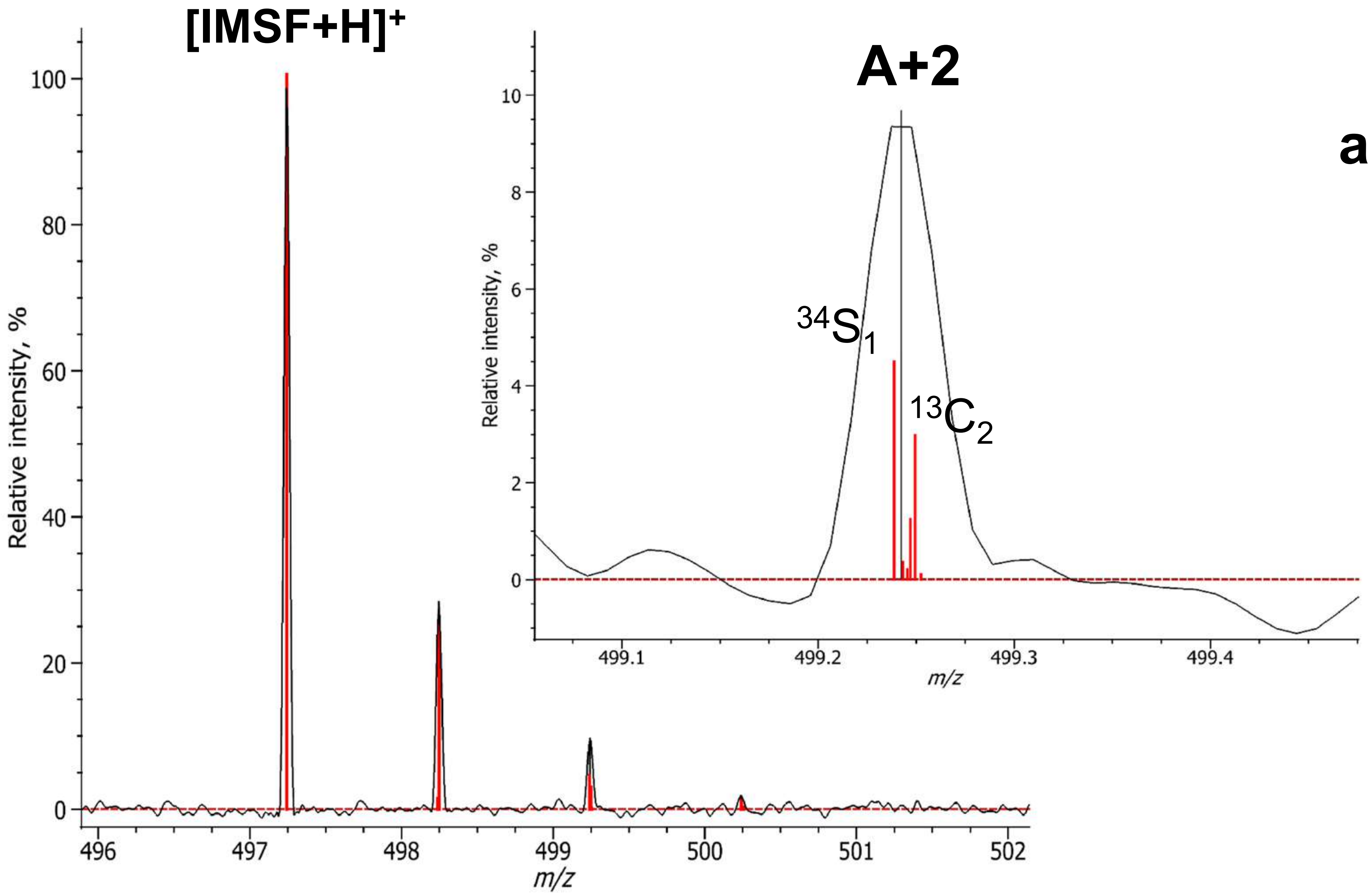
- Frequency
- Magnitude (measure of amplitude)
- Phase (angle)



- example: two waves of the same frequency and amplitude, but different phase

Absorption Mode FT (aFT) Mass Spectrum

The Unreduced Data



aFT preserves all information:

- Frequency
- Amplitude
- Phase

noise (+ and -)

Simulation of FTMS data:
DOI: 10.1021/jasms.0c00190

Simulations: IMSF, $[M+H]^+$, 497.2 *m/z*, Orbitrap Q Exactive, 15k @ 200 *m/z*

Why Reduce FTMS Data?

- **The unreduced data file size:**
  - a single transient: 1-20 MB;
  - a single unreduced mass spectrum: 2-40 MB
  - a single imaging/LC-MS run: **1-50 GB**
- **Technological challenges/cost**
  - High-performance electronics for transient acquisition
  - Real-time digital signal processing algorithms (on a chip)
  - Efficient software tools for post-processing of “big data”
- **Ease of use**
  - Extended data processing time for the unreduced data processing
  - A larger storage space is needed for the unreduced data

Spectroswiss

Cost of Reducing FTMS Data?

- **It is a one way street**
  - Once data is reduced, the full data cannot be recovered
  - (Unless the original data is also saved – defeating the purpose)

- **Data reduction always loses information/capability**
  - Spectral quality
  - Unresolved components
  - Noise distribution
  - Unexpected analytes
  - Sub-threshold peaks
  - *Etc*.

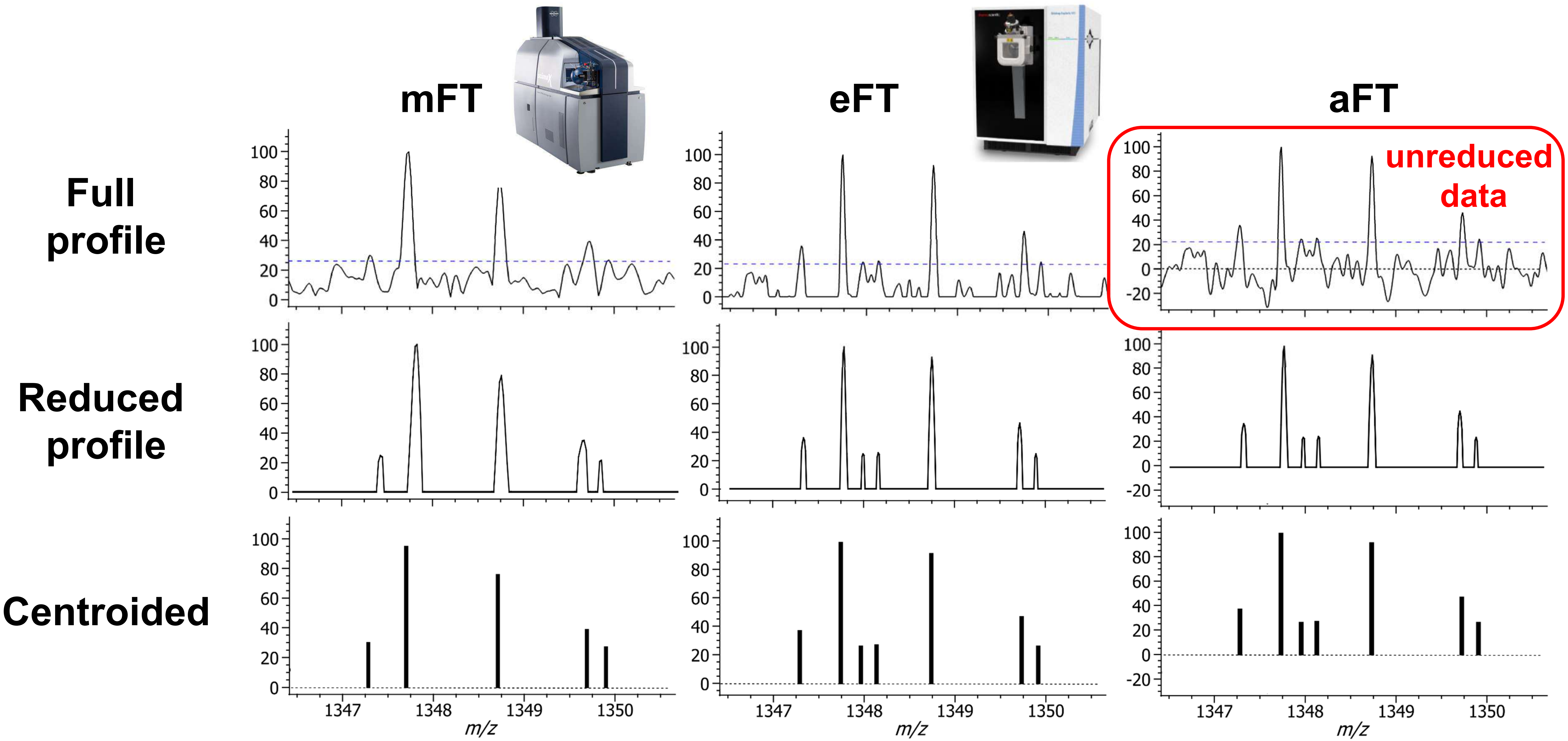
Note: the reduced data use is sufficient for many FTMS applications

The Unreduced Data in FTMS

Data Reduction Approaches in FTMS

- Transient length: ion detection during a reduced period of time (*not* all time ions induce signals)
- Calibration of initial phases: post-processing instead of in real time
- Performing other modes of FT: magnitude (mFT) or enhanced (eFT)
- Noise reduction/thresholding: full vs. reduced profile
- Visible mass (*m/z*) range: low and high mass cut-off
- Centroiding mass spectra
- Deisotoping, deconvolution
- Transients are not stored, typically

FTMS Data Hierarchy: Information vs. File Size



Tsybin *et al.*, DOI: 10.1016/B978-0-12-814013-0.00005-3

The Unreduced Data in FTMS

I. The Unreduced Data in FTMS

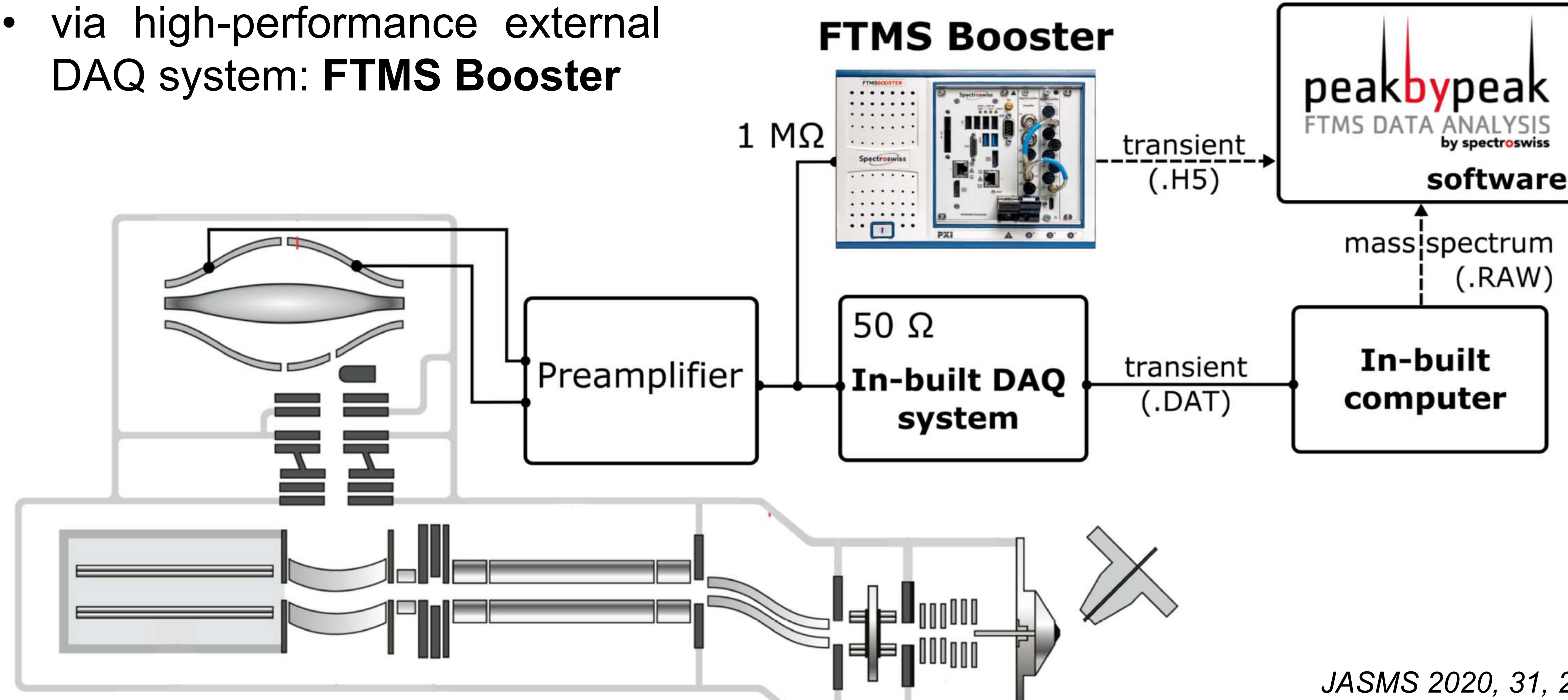
II. Data Reduction Approaches

III. How to Get and Process the Unreduced Data

IV. Examples of Applications

How to Get & Process the Unreduced FTMS Data

- via high-performance external DAQ system: **FTMS Booster**

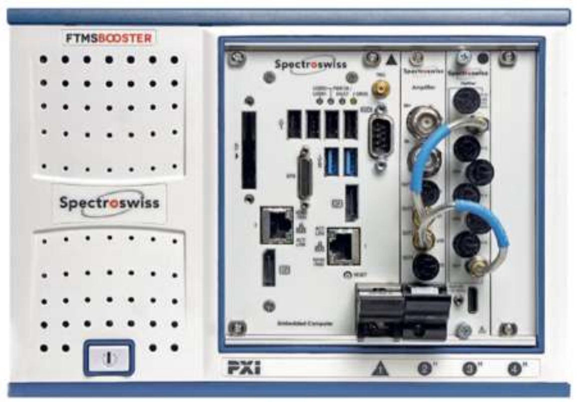


Exploris[TM] schematics: https://planetorbitrap.com/

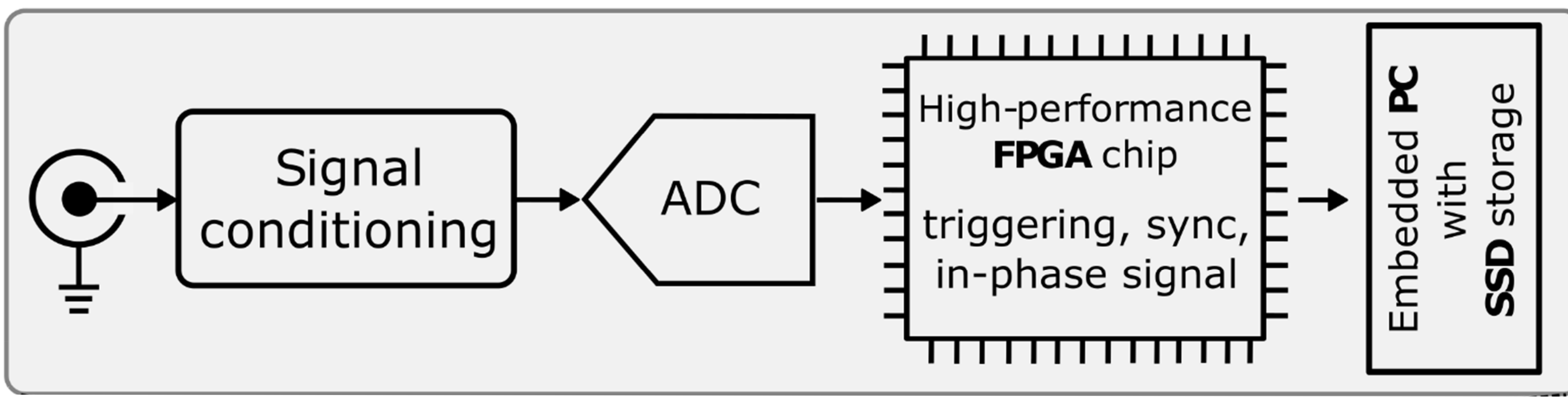
JASMS 2020, 31, 257-266
***DOI:** 10.1021/jasms.9b000*

Overcoming the technological limitations in acquiring phased & full transients

FTMS Booster: a high-performance DAQ system



- Analogue transient capture after the original built-in pre-amplifier
- Phase artefact-free digitization (sampling) of transients
- Ability to maximize the duty cycle of ion detection event (throughput)
- Ability to record (much) longer transients (due to enhanced flexibility)
- Transient storage (.H5 files) for post-processing, reduced file size



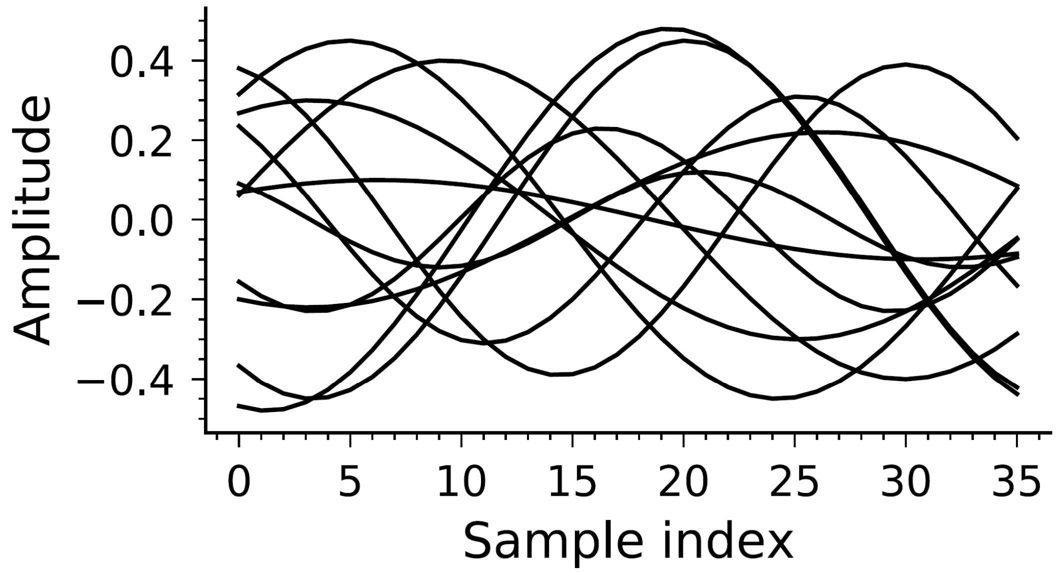
FPGA: field programmable gate array (chip)

JASMS 2020, 31, 257-266

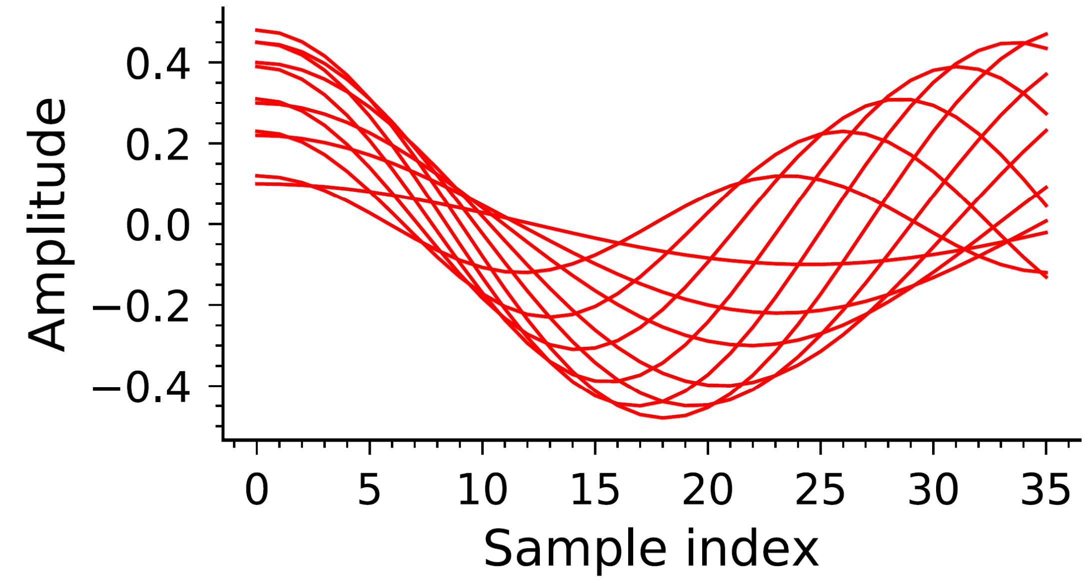
Phased Transients: Initial Phases All Close to **Zero**

Spectroswiss

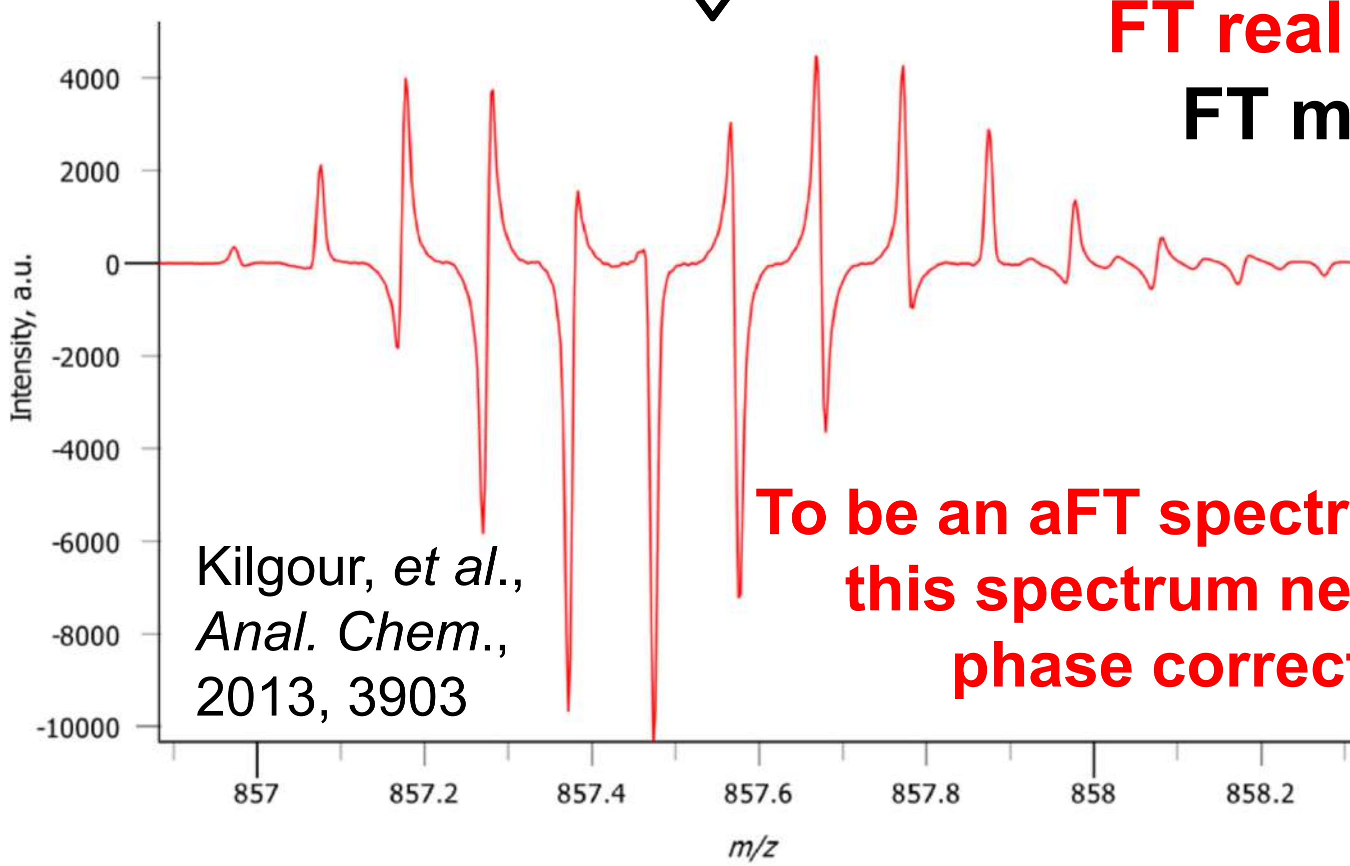
Un-phased transients (state of the art)



Phased transients

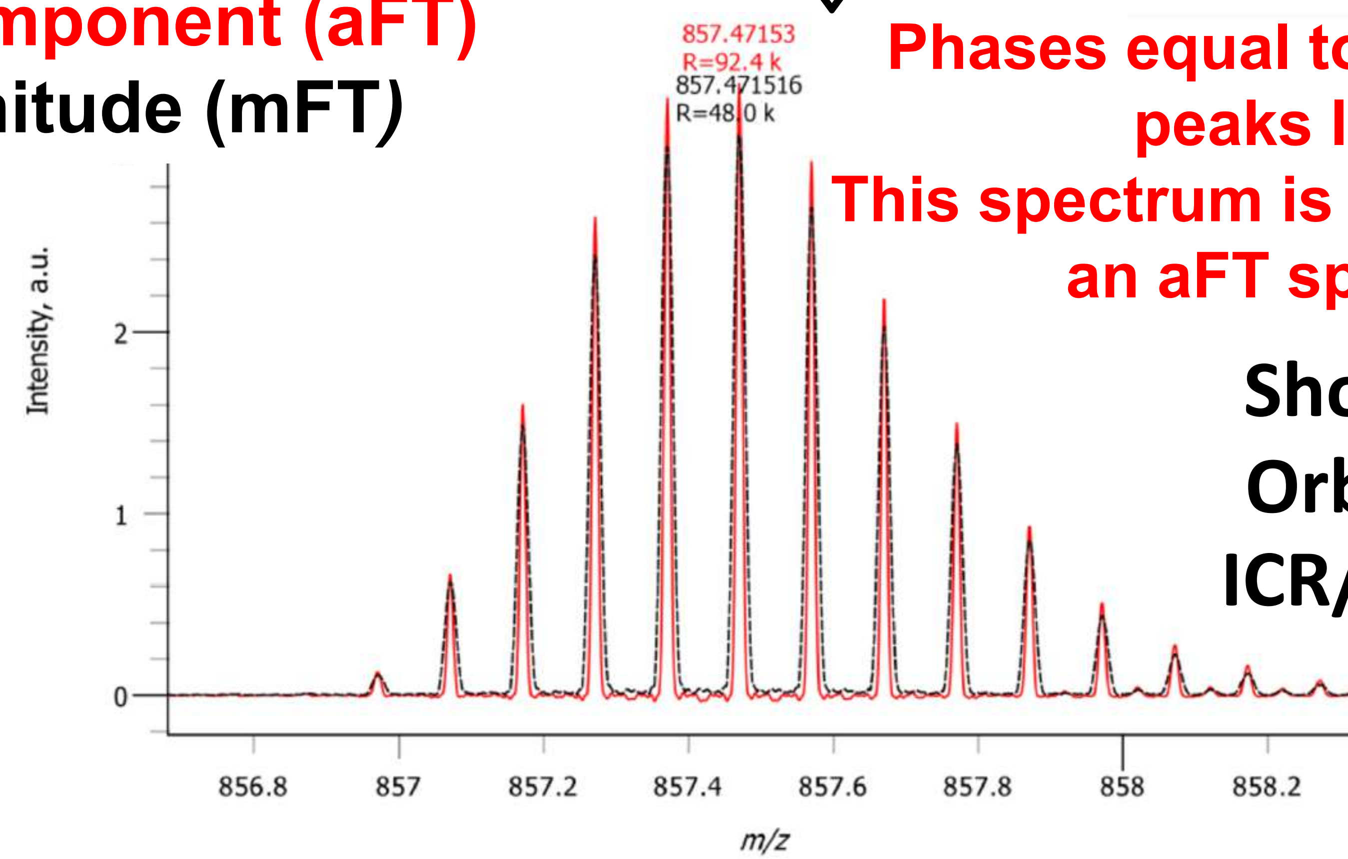


Mass spectra computed as:
FT real component (aFT)
FT magnitude (mFT)



Kilgour, *et al.*, *Anal. Chem.*, 2013, 3903

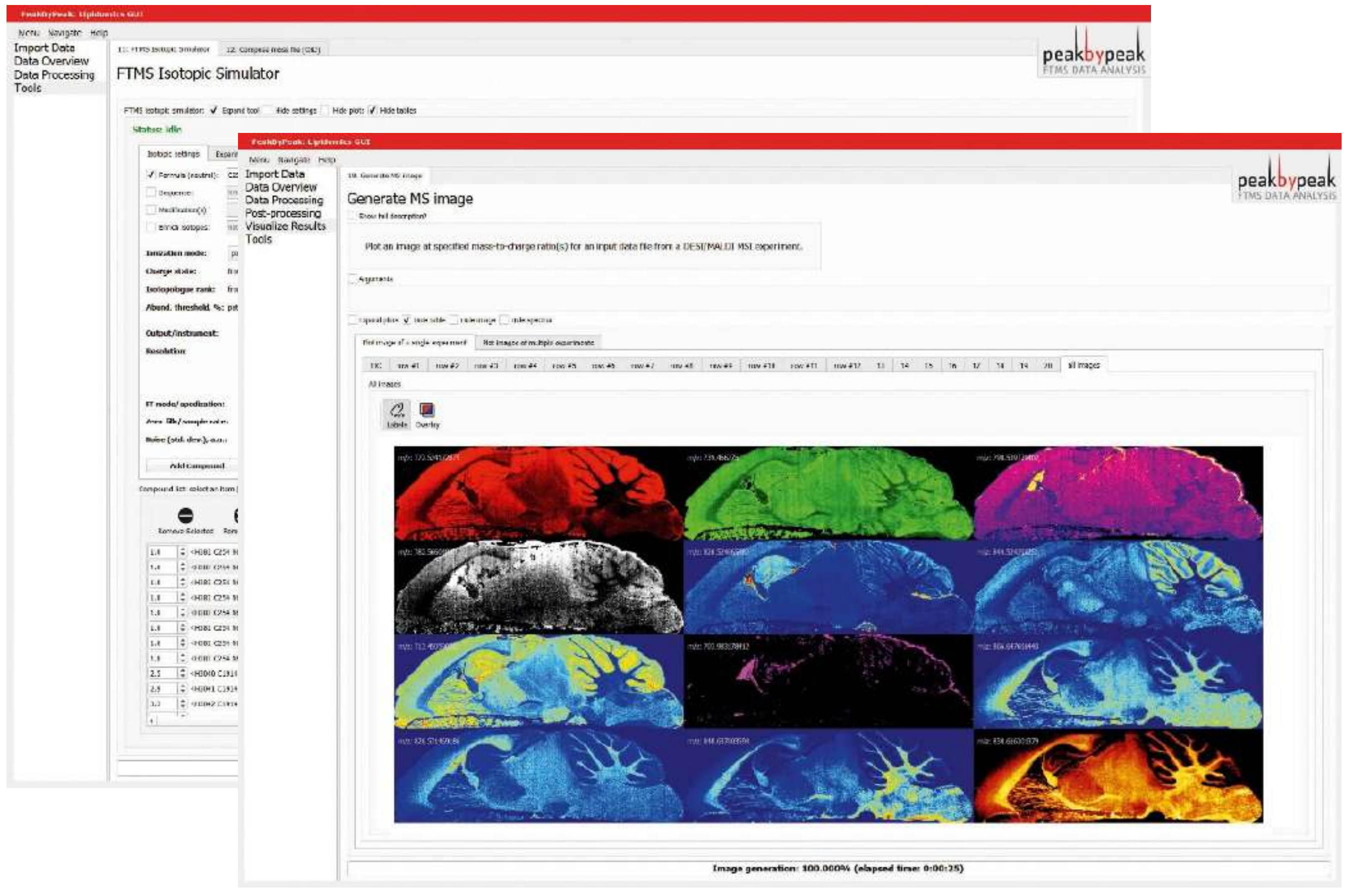
To be an aFT spectrum, this spectrum needs phase correction

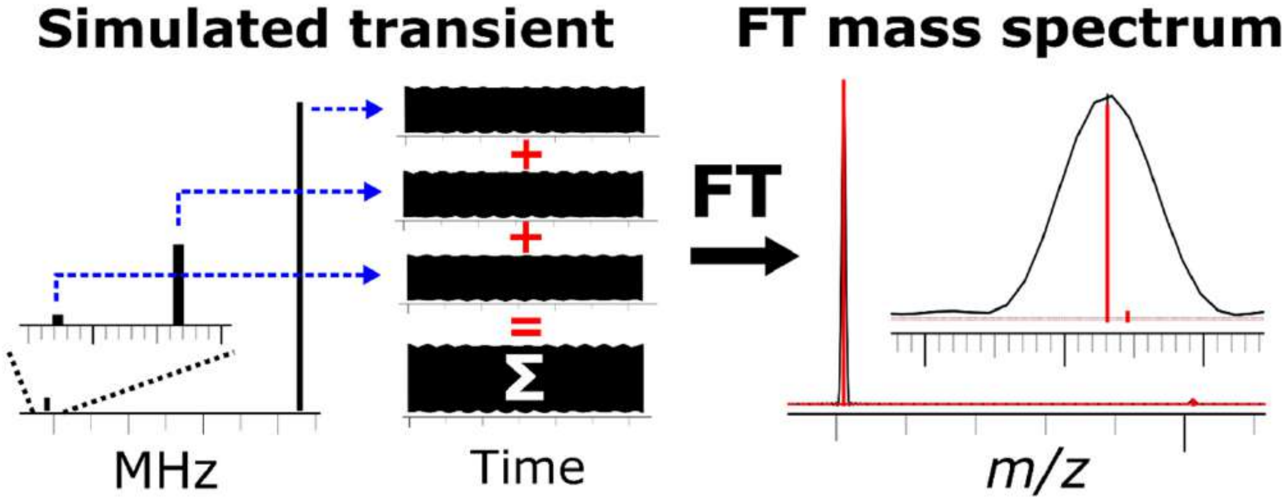


Phases equal to zeros: peaks look up. This spectrum is already an aFT spectrum

Shown for Orbitraps ICR/MRMS

Software Requirements for Processing of the «Big Data»





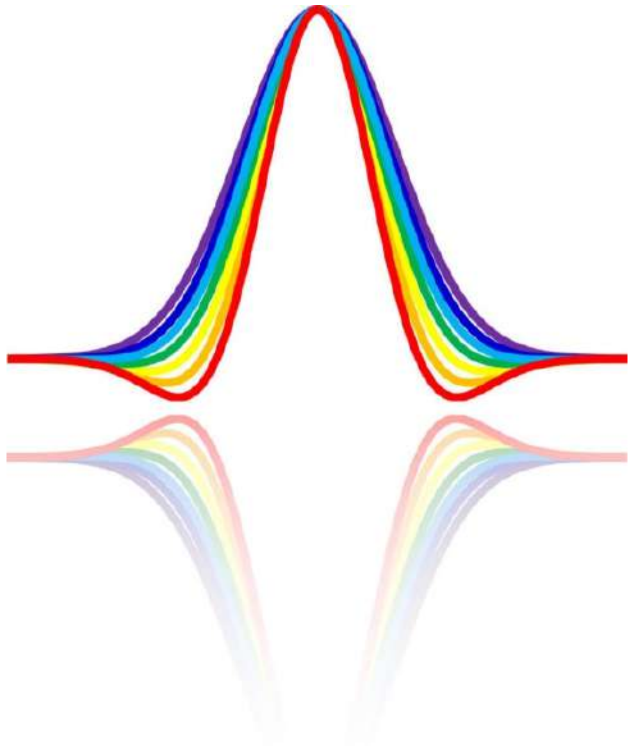
- Processing of any size and number of transients: user-defined FT parameters
- Mass spectra generation in absorption mode FT (aFT): *vs.* the eFT & mFT (*e.g.*, RAW)
- Processing of any size & number of mass spectra (*e.g.*, RAW in reduced & full profile)
- Processing of mass spectra (*e.g.*, RAW) from different experiments (*e.g.*, tech. replicates)
- Transient-mediated accurate simulation of FTMS data (*DOI: 10.1021/jasms.0c00190*)
- Data analysis of mass spectra (*e.g.*, RAW) using the accurately simulated FTMS data

Peak-by-Peak software package (Spectroswiss) fulfills these requirements

Spectroswiss

Another Example of the «Big Data» FTMS Software

AutoVectis



- Suite of MS data (post) processing modules
- Uses transient as starting point – unreduced data!
- Can be combined into many different workflows
  - AutoPhaser – aFT mode processing
  - AutoPiquer – peak picking
  - AutoSeequer – top-down assignment
  - Discharger – deconvolution & decomposition
  - AutoLogis – DOM and petroleomics:

David Kilgour

10.1016/j.marchem.2021.103955

Streamlined recalibration | aFT image reprocessing | TOF import | aFT fine structure | Deconvolution Decomposition | aFT peak detection | Top down sequencing | DOM/Oil Assignment

https://www.kilgourlab.com/

The Unreduced Data in FTMS

Spectroswiss

I. The Unreduced Data in FTMS

II. Data Reduction Approaches

III. How to Get and Process the Unreduced Data

IV. Examples of Applications

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

Transient post-processing: super-resolution algorithms

Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

Transient post-processing: enhanced spectral quality

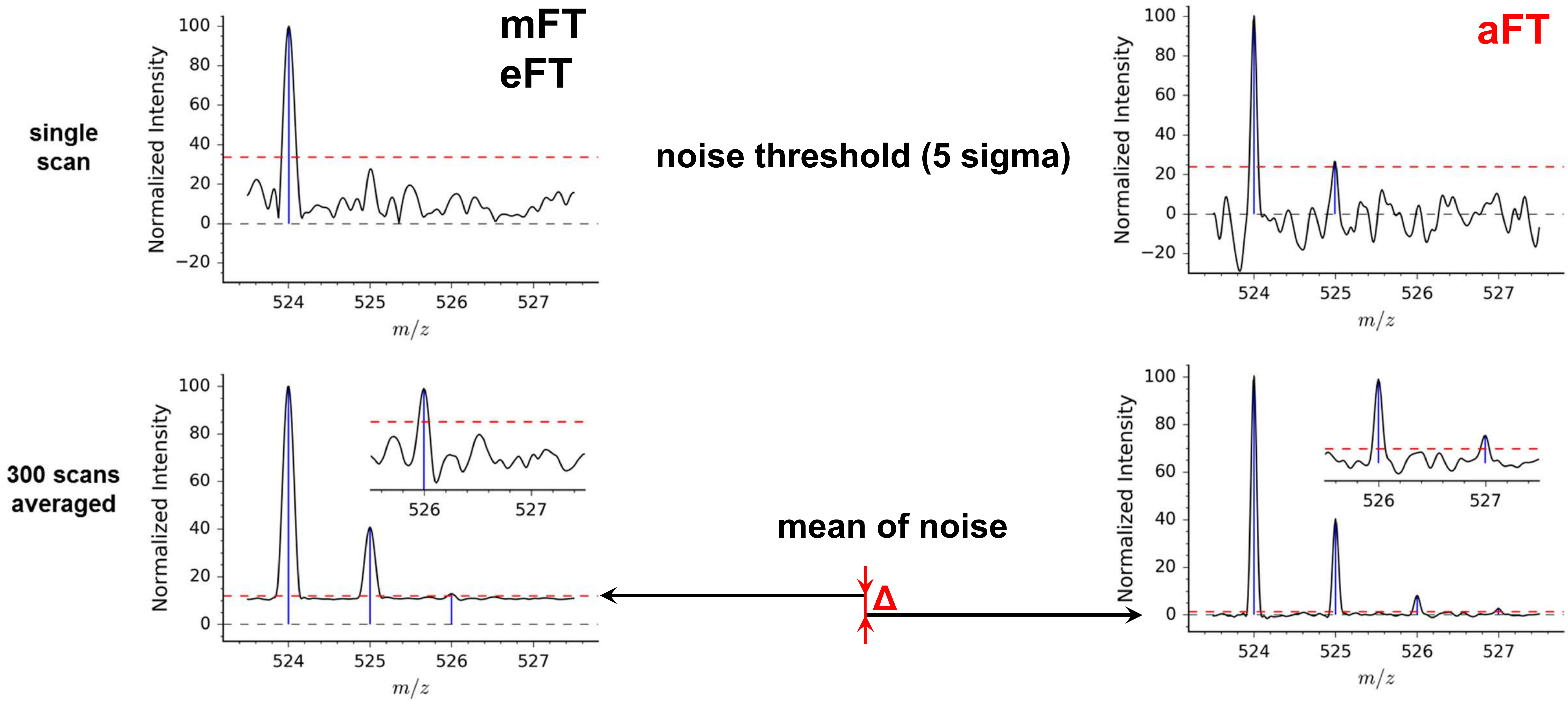
Transient post-processing: single ion counting

Examples of Applications

Spectroswiss

Small molecules (metabolites/peptides)	**Large molecules (proteins)**
Data averaging: sensitivity & quantitation accuracy	Data averaging: top-down & middle-down sequencing
Full transients: ultra-high resolution, throughput	Transient post-processing: enhanced spectral quality
Transient post-processing: super-resolution algorithms	Transient post-processing: single ion counting

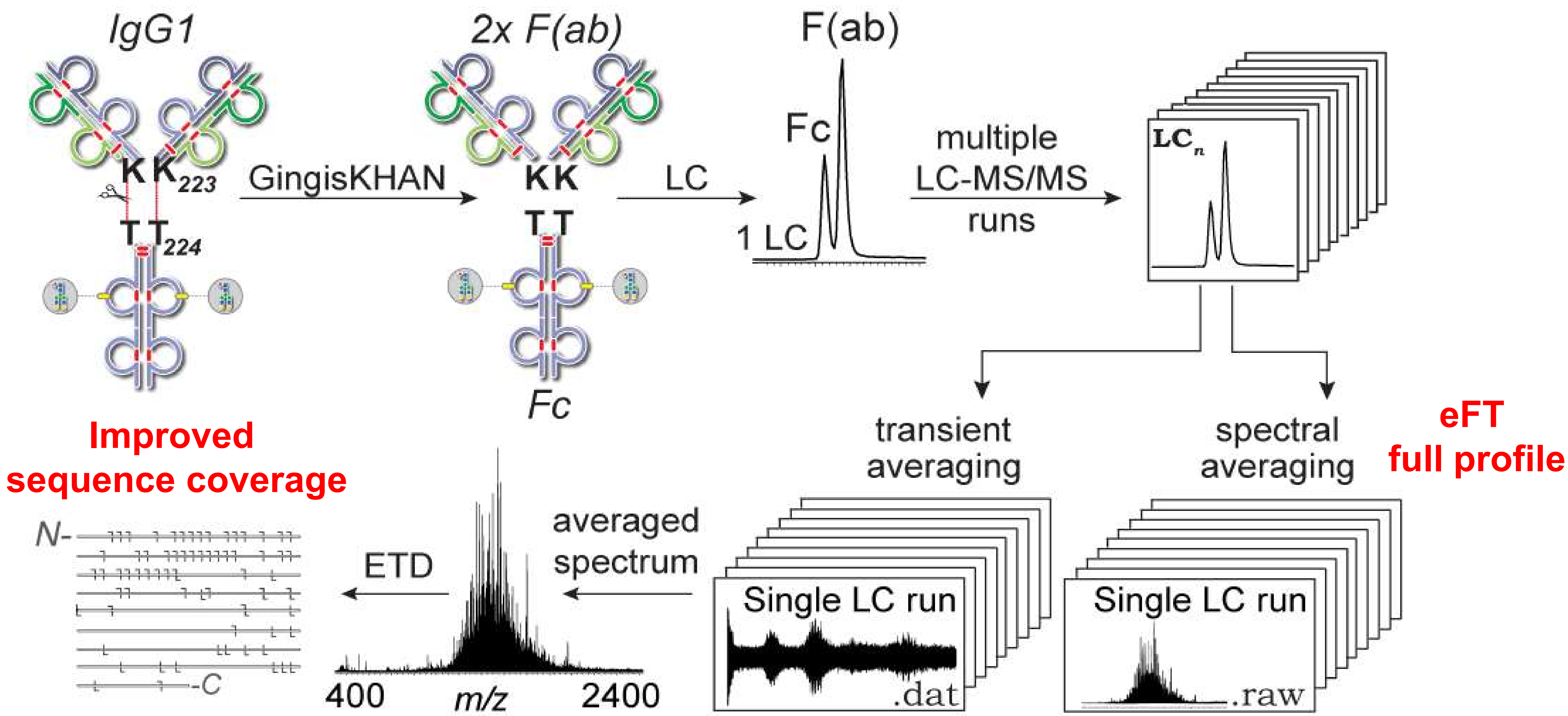
Benefits of the Unreduced Data: Full Profile Averaging



Srzentic et al., Anal. Chem. (2018) 12527

Enhanced Protein Sequencing with Top/Middle-Down

2D Data Averaging: Use of Multiple LC-MS/MS Technical Replicates



Srzentic *et al.*, *Anal. Chem.* (2018) 12527; Fornelli *et al.*, *J. Proteomics* (2017) 67

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

Transient post-processing: super-resolution algorithms

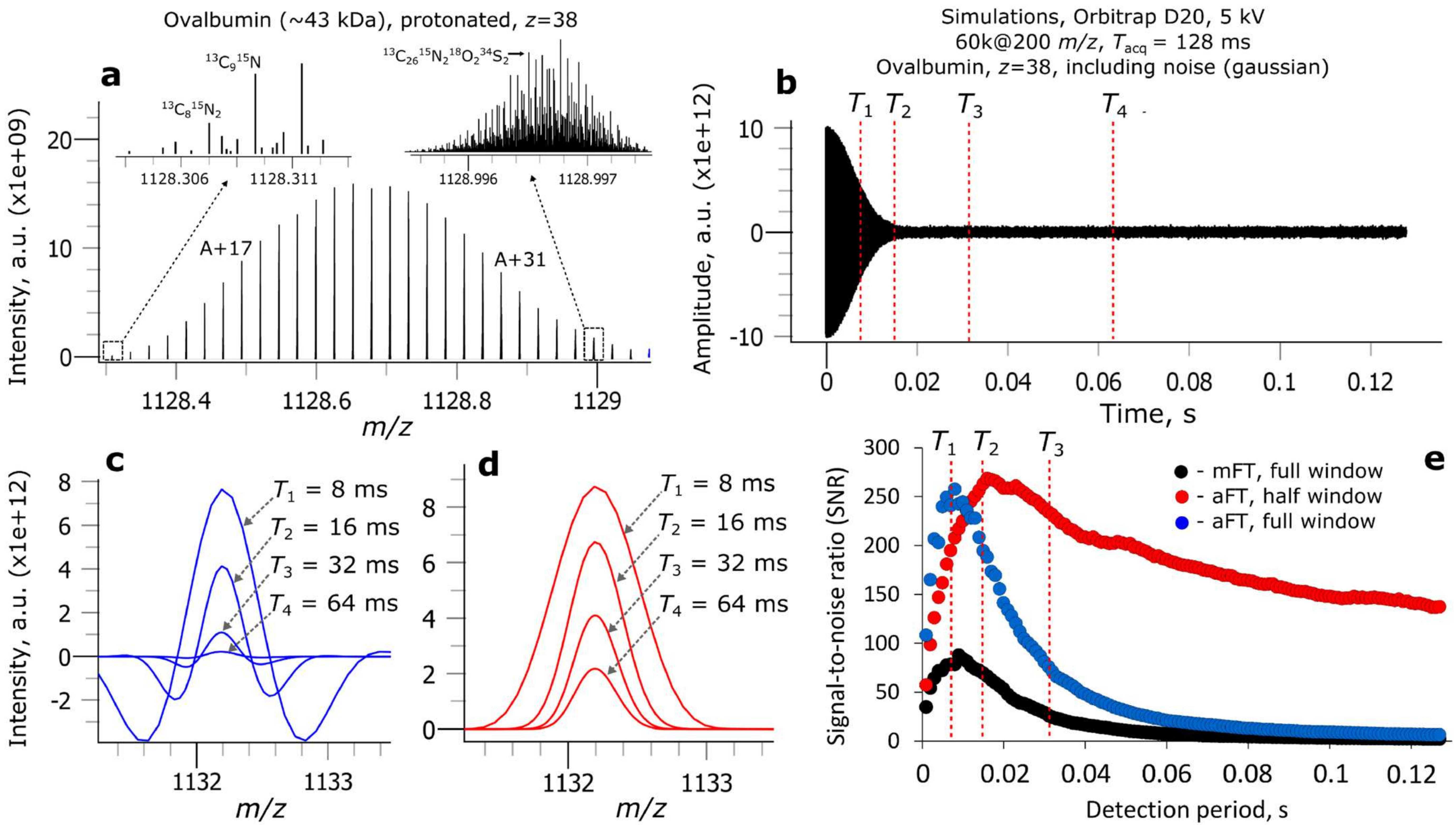
Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

Transient post-processing: enhanced spectral quality

Transient post-processing: single ion counting

Data Post-Processing for Improved Protein Analysis



Experiment: Fornelli et al., J. Proteome Res. 2017, 16, 609−618

Simulations: DOI: 10.1021/jasms.0c00190

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

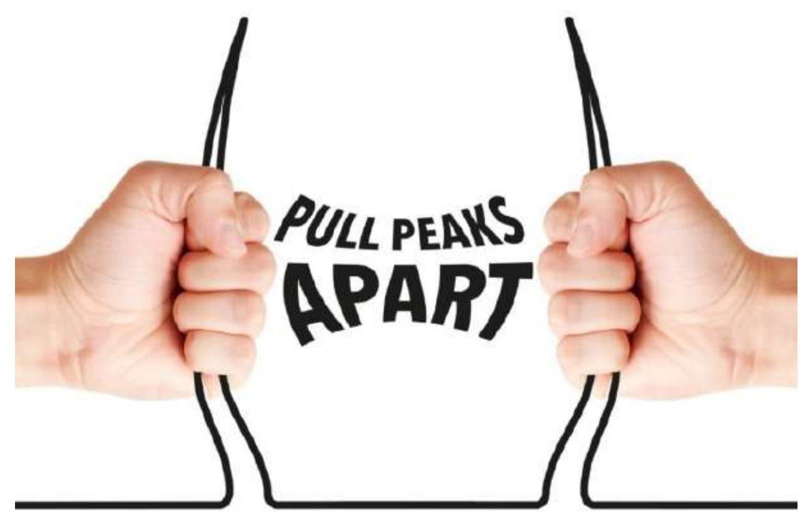
Transient post-processing: super-resolution algorithms

Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

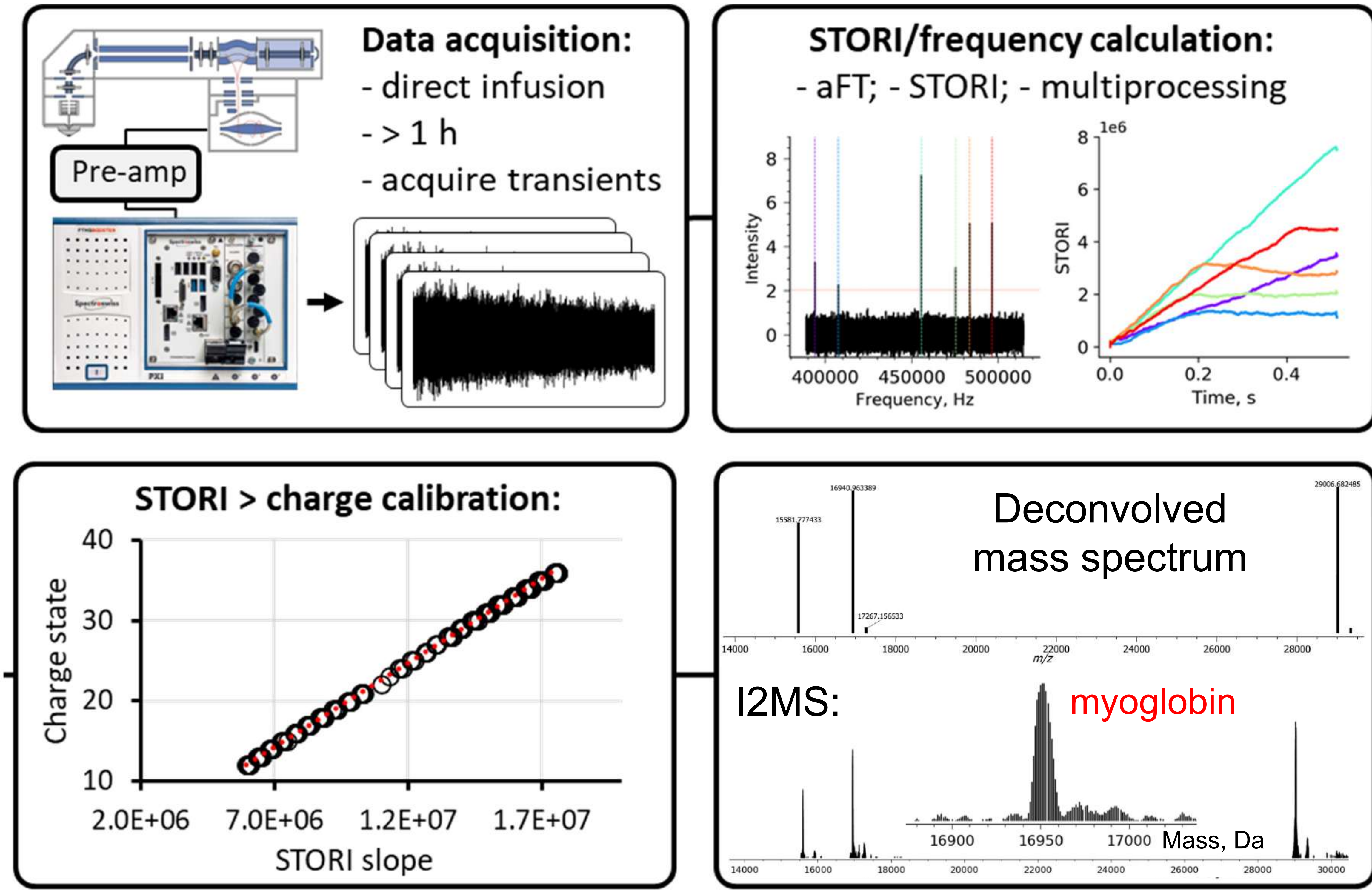
Transient post-processing: enhanced spectral quality

Transient post-processing: single ion counting



Single Ion Counting: Resolving Protein Interference

Spectroswiss



- Detection of single ions reduces space charge artefacts (peak interference), but hides peak's charge state
- Charge state of a single ion peak can be deduced from its transient, *e.g.*, via STORI (I2MS)

Williams, Kelleher, Heck

DOI: 10.1038/s41592-020-0764-5
DOI: 10.1021/acs.analchem.9b01669

STORI: Selective Temporal Overview of Resonant Ions, DOI: 10.1021/jasms.8b06253

Spectroswiss

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

Transient post-processing: super-resolution algorithms

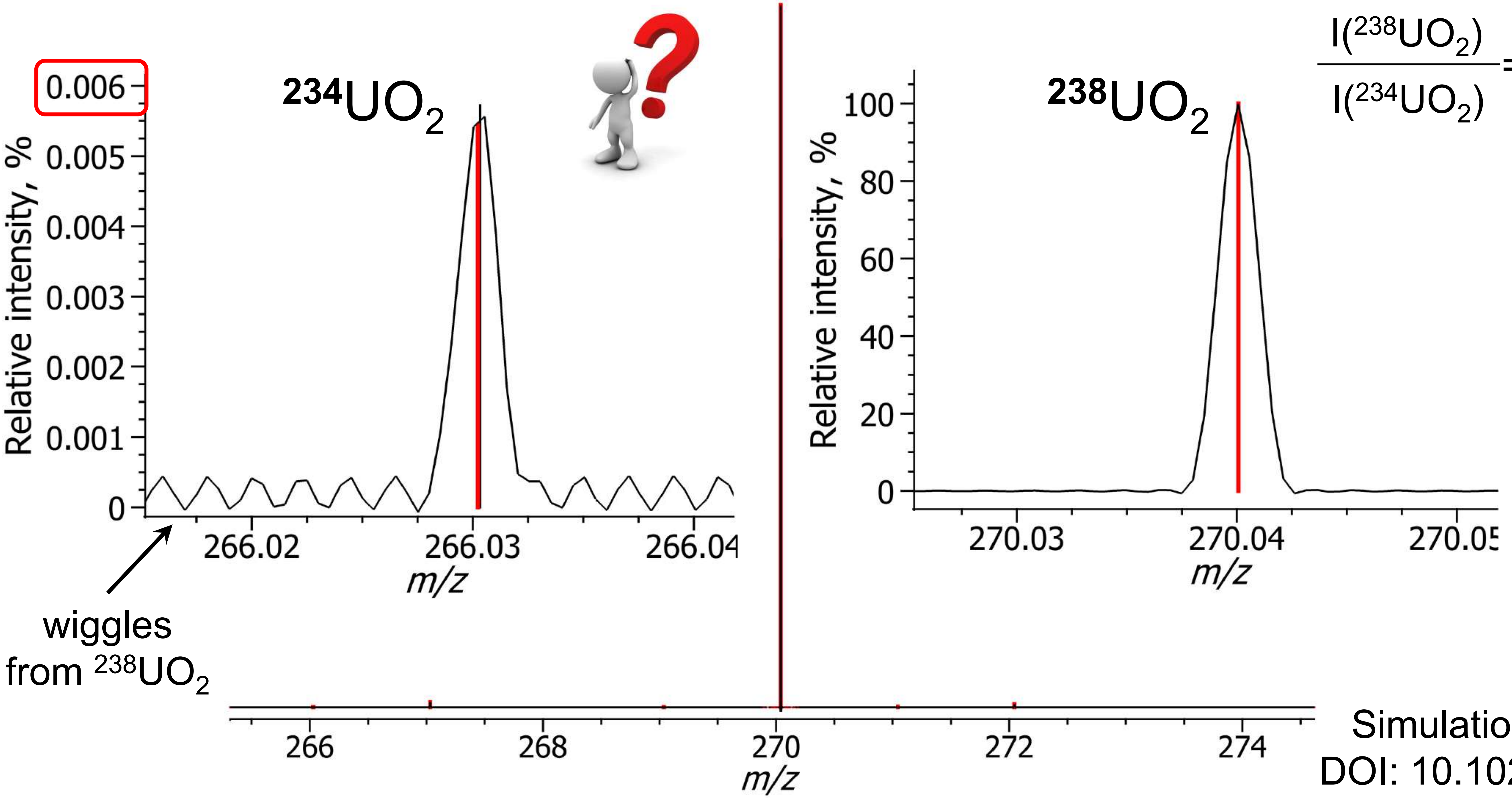
Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

Transient post-processing: enhanced spectral quality

Transient post-processing: single ion counting

Example: Isotopic Ratio Analysis of Uranium (UO_2)

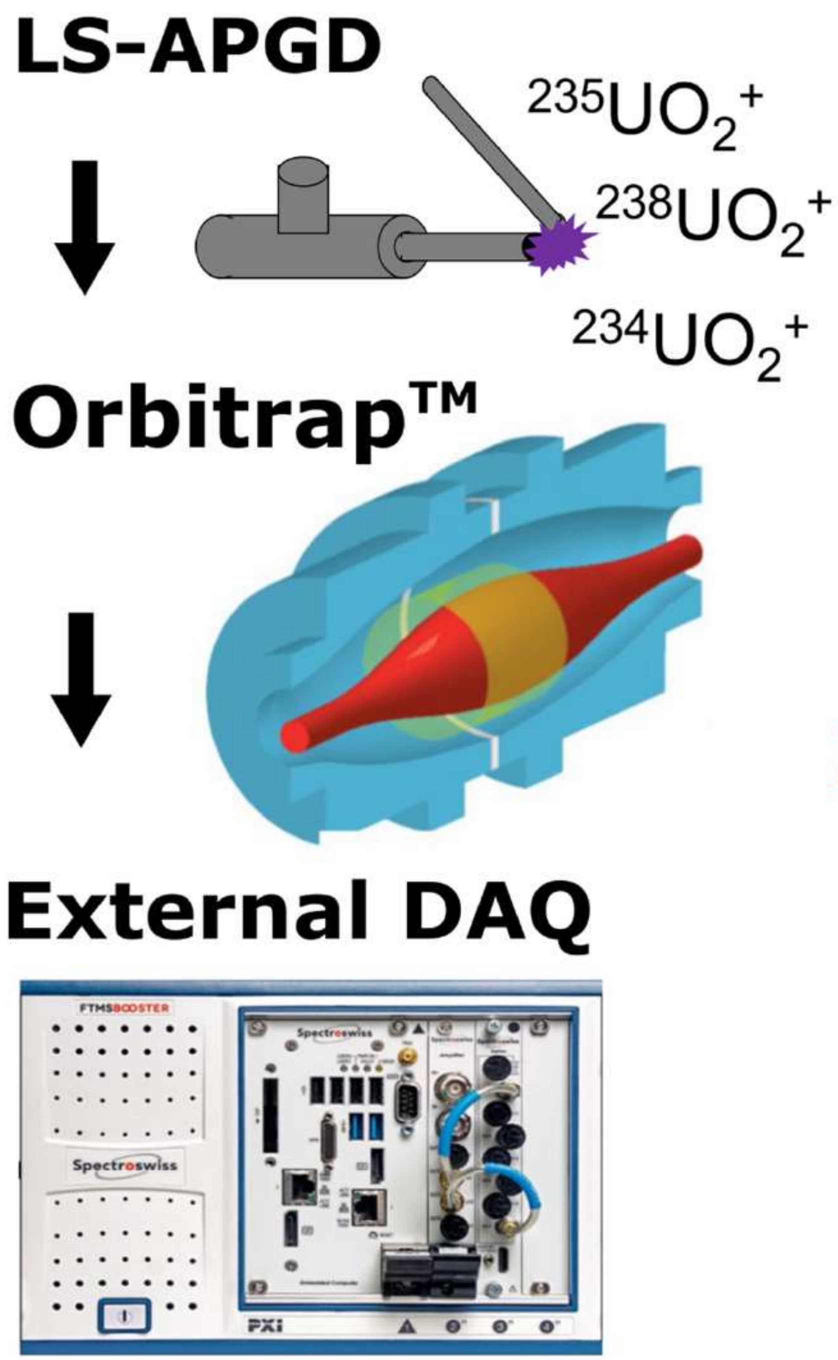


$$\frac{I(^{238}UO_2)}{I(^{234}UO_2)} = \frac{100}{0.0055} = \mathbf{18\ 200}$$

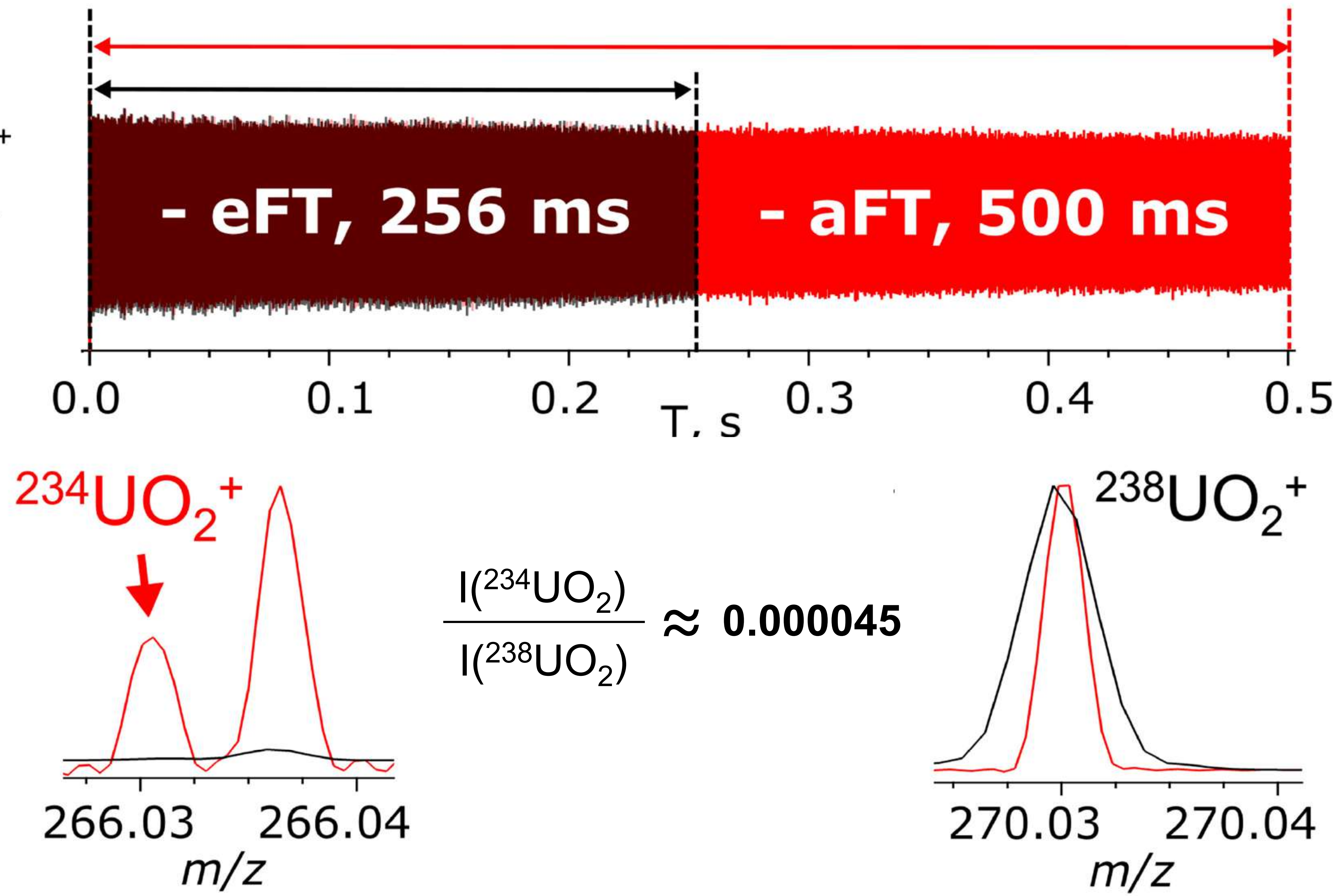
Simulation of FTMS data:
DOI: 10.1021/jasms.0c00190

Extreme spectral dynamic range is required (with high IR precision): 1: 20 000

Averaging of the Unreduced Data: UO_2



8000 scans data averaging

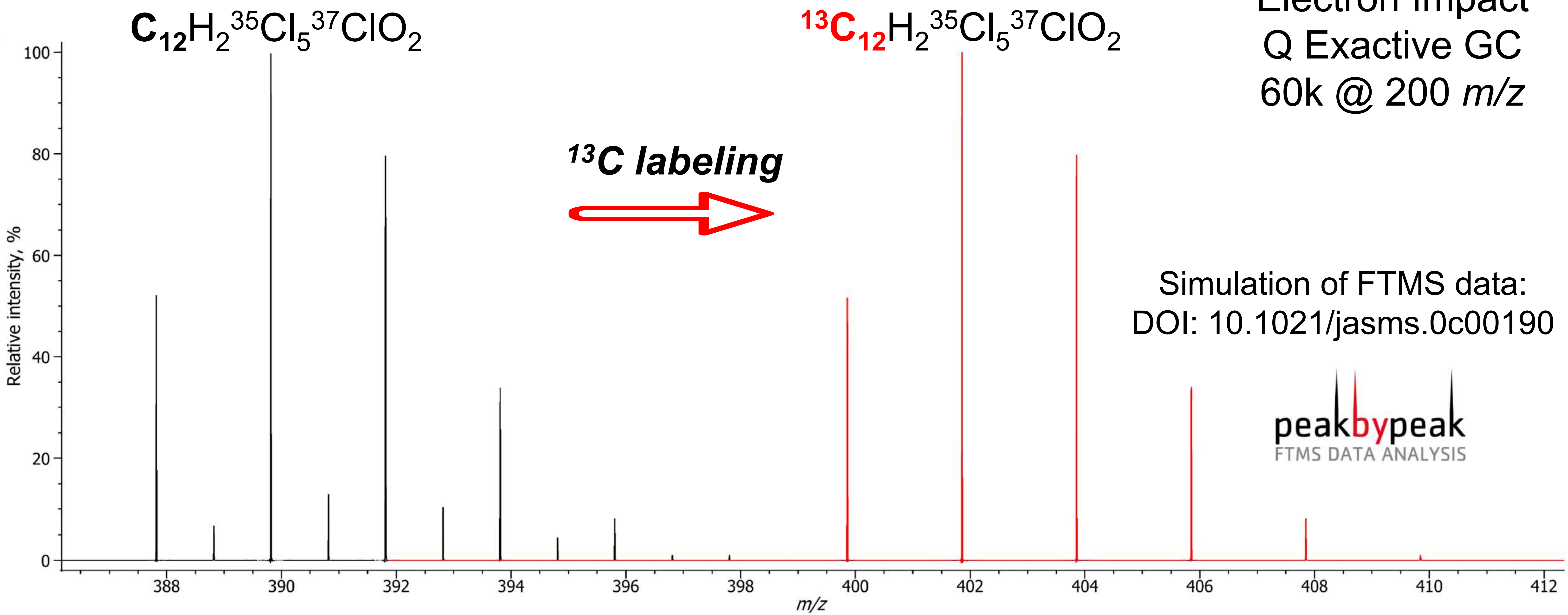


Markus et al., JASMS, in print

Example: Quantitation of Trace-Level Compounds

2, 3, 4, 7, 8 – hexachlorodibenzo-dioxin

Electron Impact
Q Exactive GC
60k @ 200 *m/z*



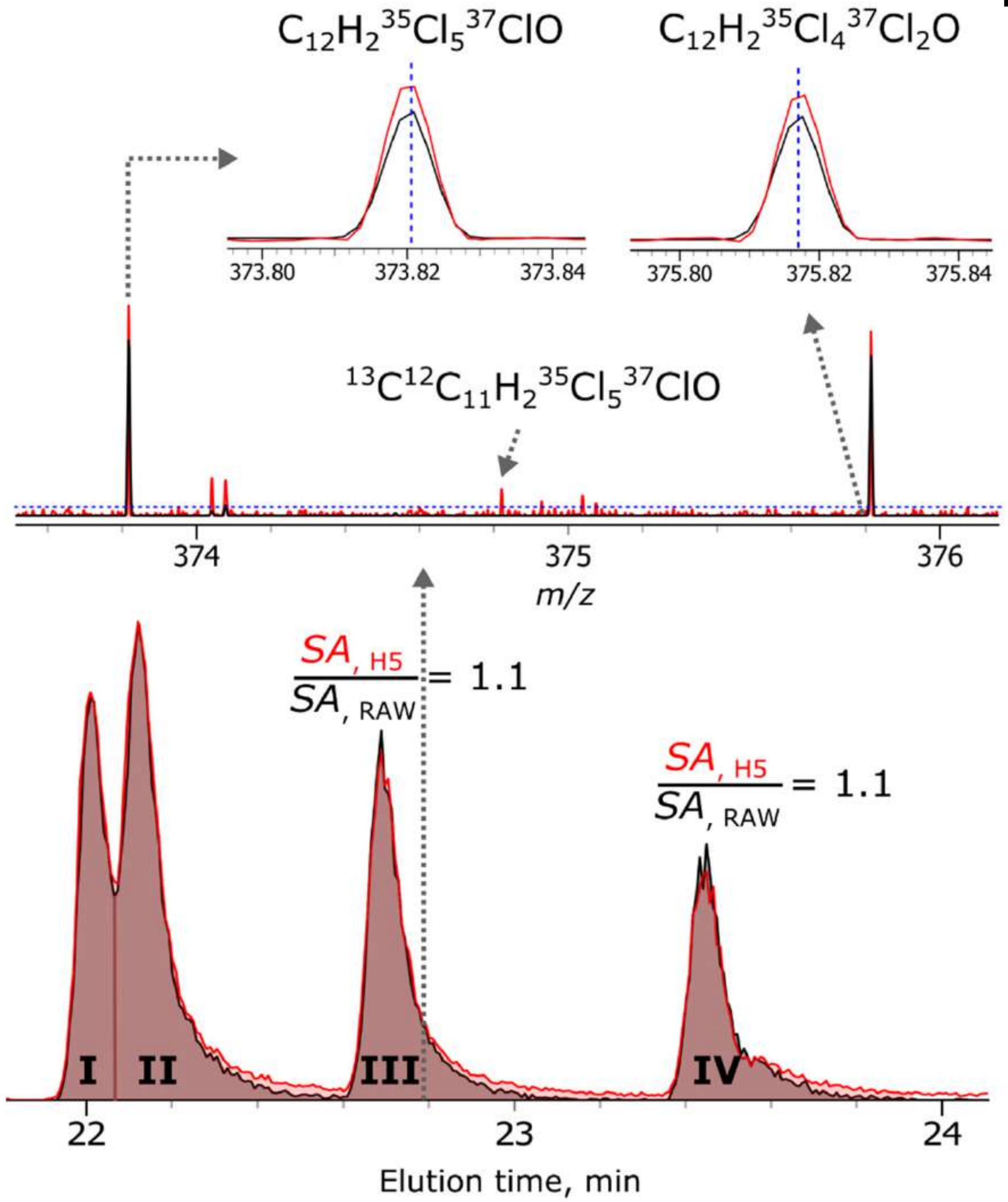
Simulation of FTMS data:
DOI: 10.1021/jasms.0c00190

Challenge: quantitatively accurate analysis of diluted samples (low abundance)

2D Averaging in GC-MS: Sensitivity & Accuracy

10 GC-MS runs averaged data

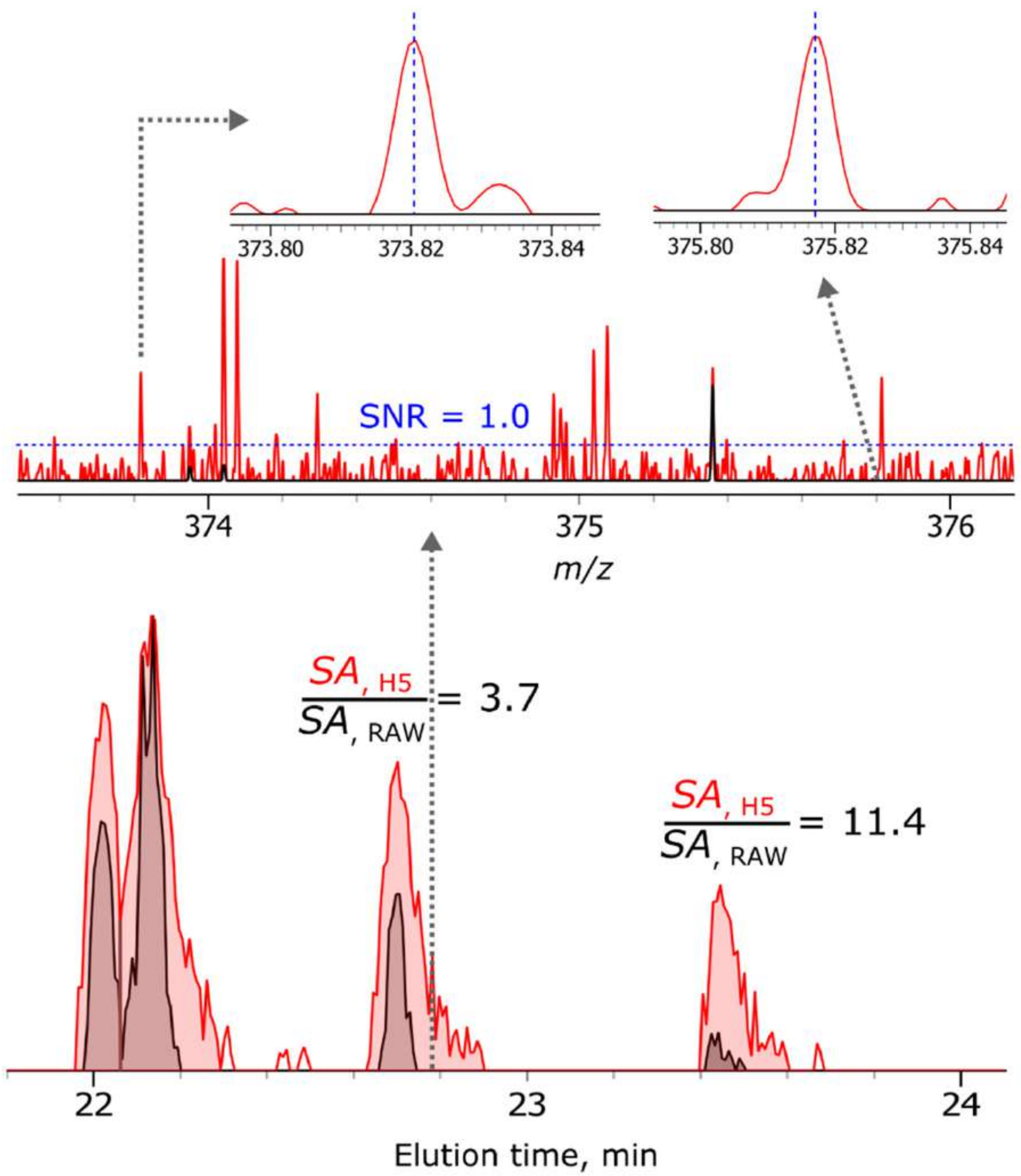
1.0 pg solution



- **Reduced data: .RAW mass spectra (eFT, reduced profile)**
- **Unreduced data: .H5 mass spectra (aFT, full profile), or transients**

JASMS 2020, 31, 257-266

100.0 fg solution



Increased sensitivity is provided by the unreduced data, improved LOD/LOQ

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

Transient post-processing: super-resolution algorithms

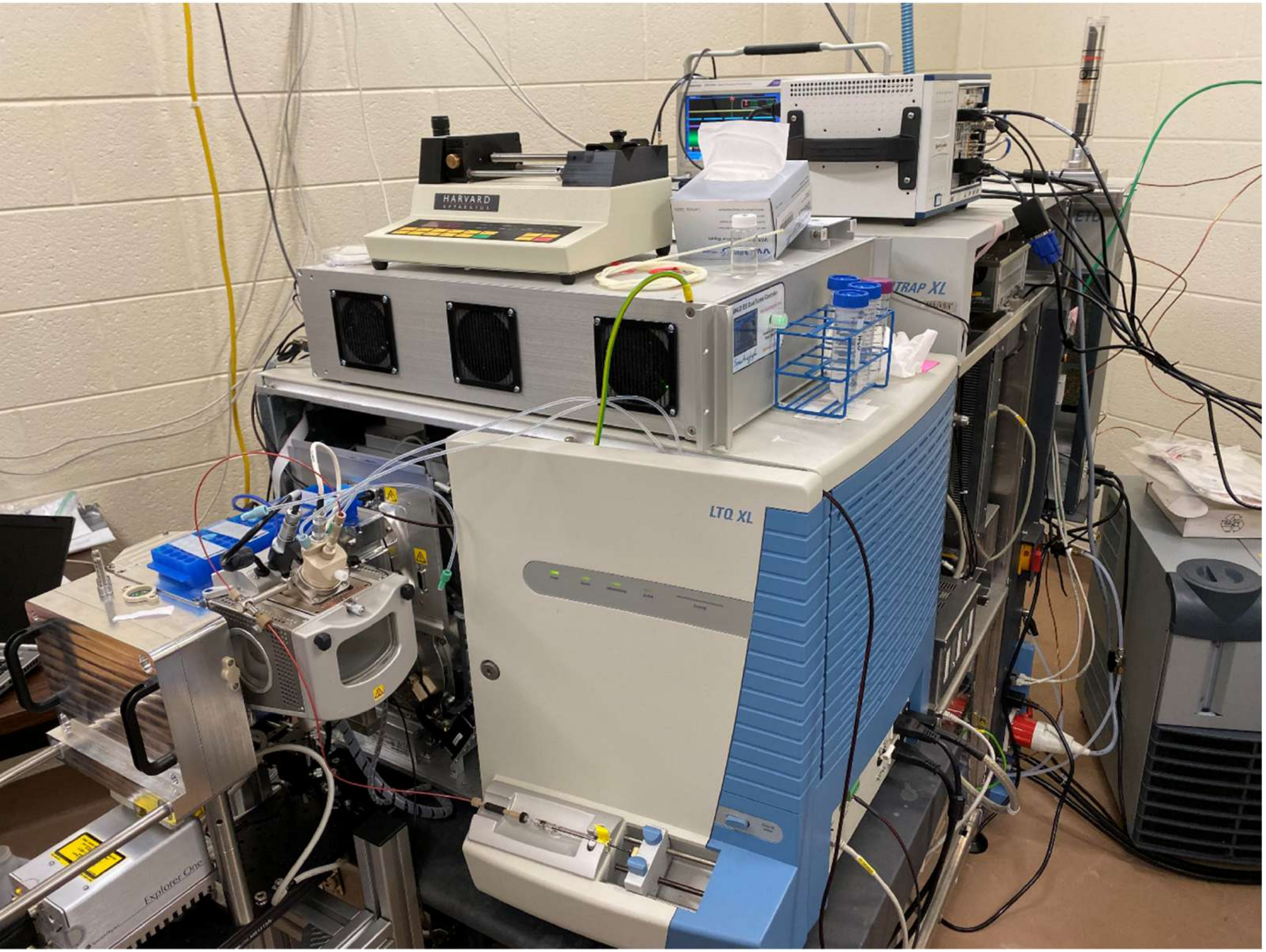
Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

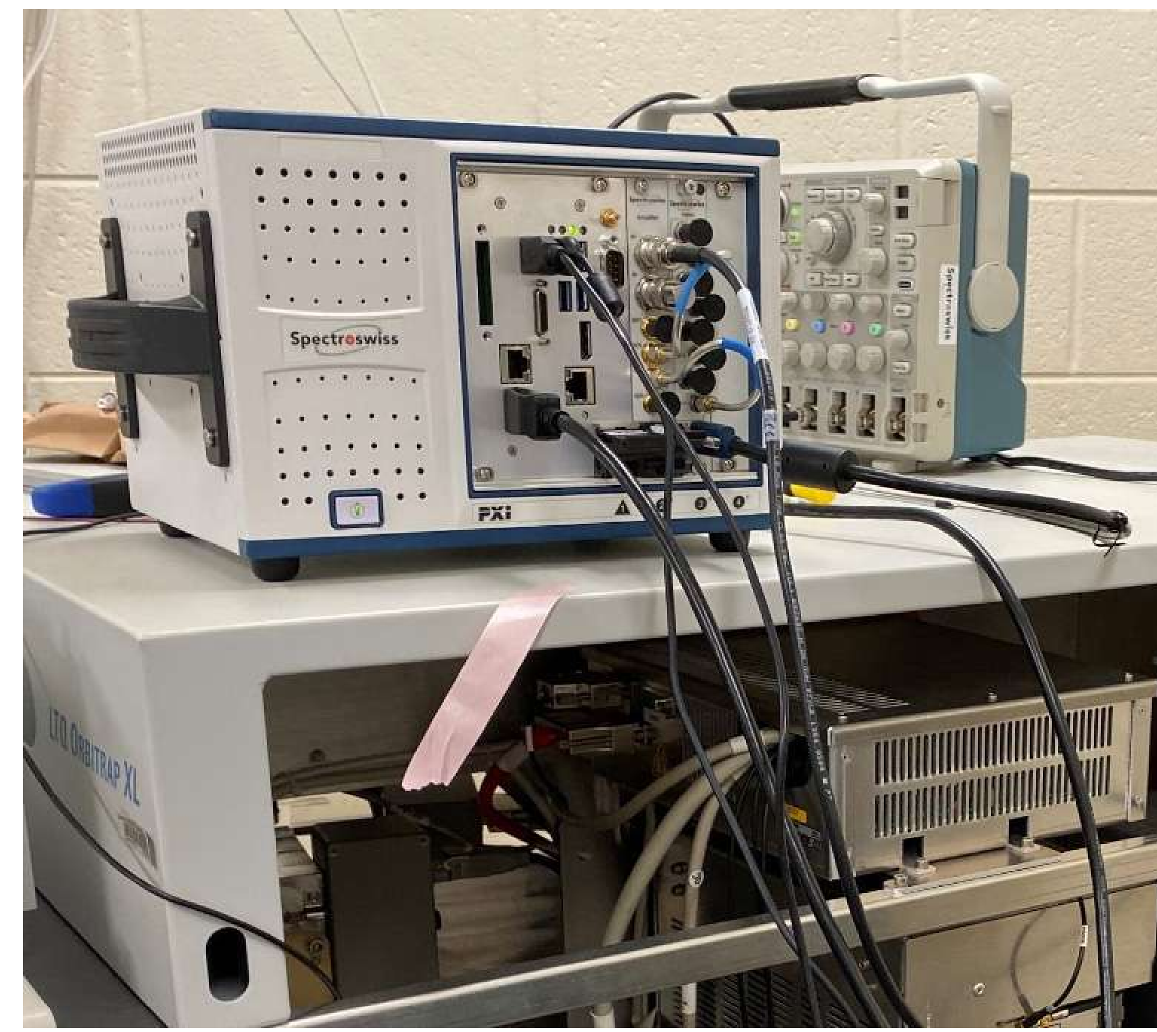
Transient post-processing: enhanced spectral quality

Transient post-processing: single ion counting

Upgraded Imaging Platform @ Leach Labs



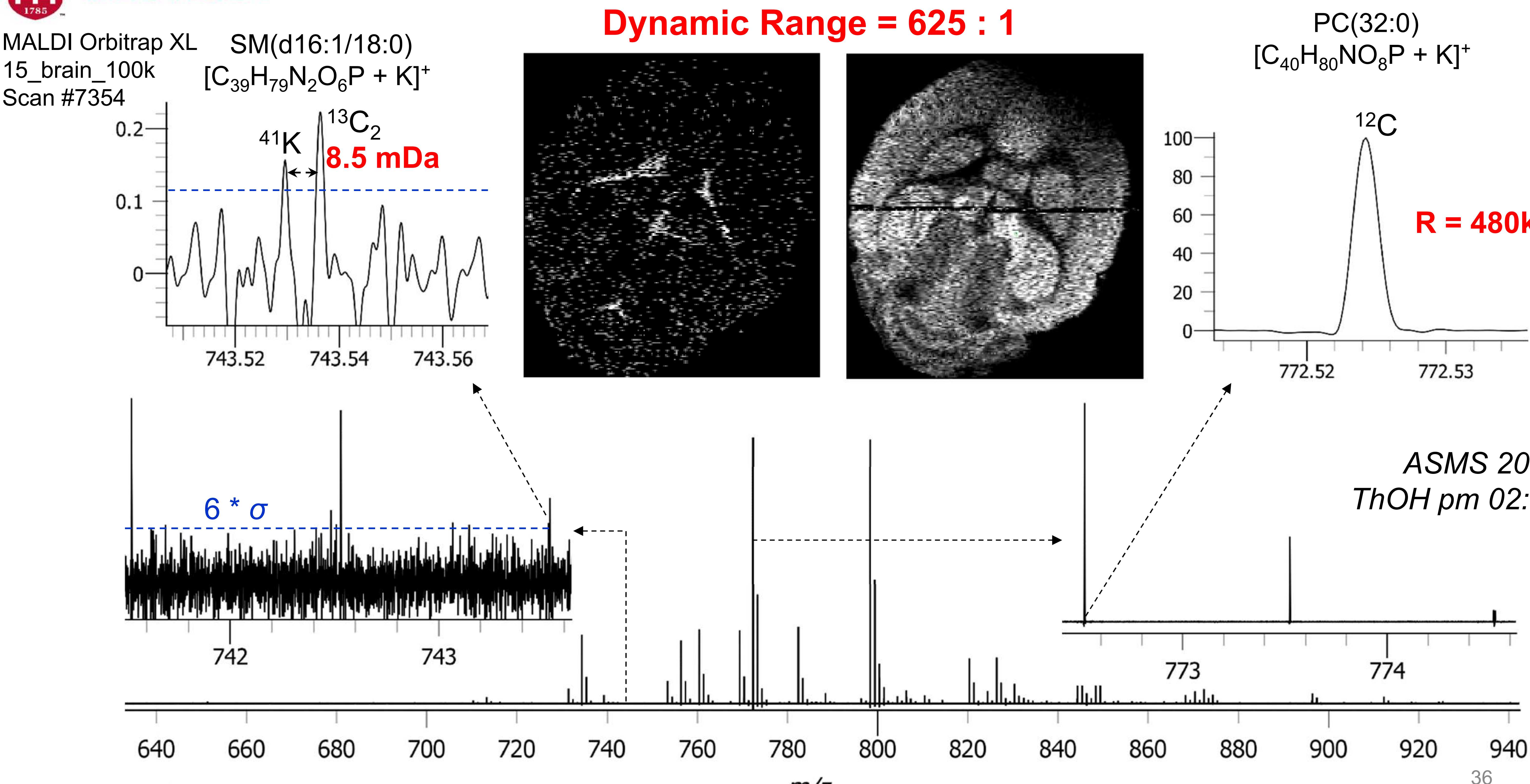
- LTQ Orbitrap XL (mFT)
- MALDI ion source Spectroglyph
- Data acquisition system



UNIVERSITY OF GEORGIA

Spectroswiss

Benefits of Longer Transients and aFT



ASMS 2020
ThOH pm 02:50

See 21 T FT-ICR MS data: Smith et al., Anal. Chem. 2020

Spectroswiss

Examples of Applications

Small molecules (metabolites/peptides)

Data averaging: sensitivity & quantitation accuracy

Full transients: ultra-high resolution, throughput

Transient post-processing: super-resolution algorithms

Large molecules (proteins)

Data averaging: top-down & middle-down sequencing

Transient post-processing: enhanced spectral quality

Transient post-processing: single ion counting

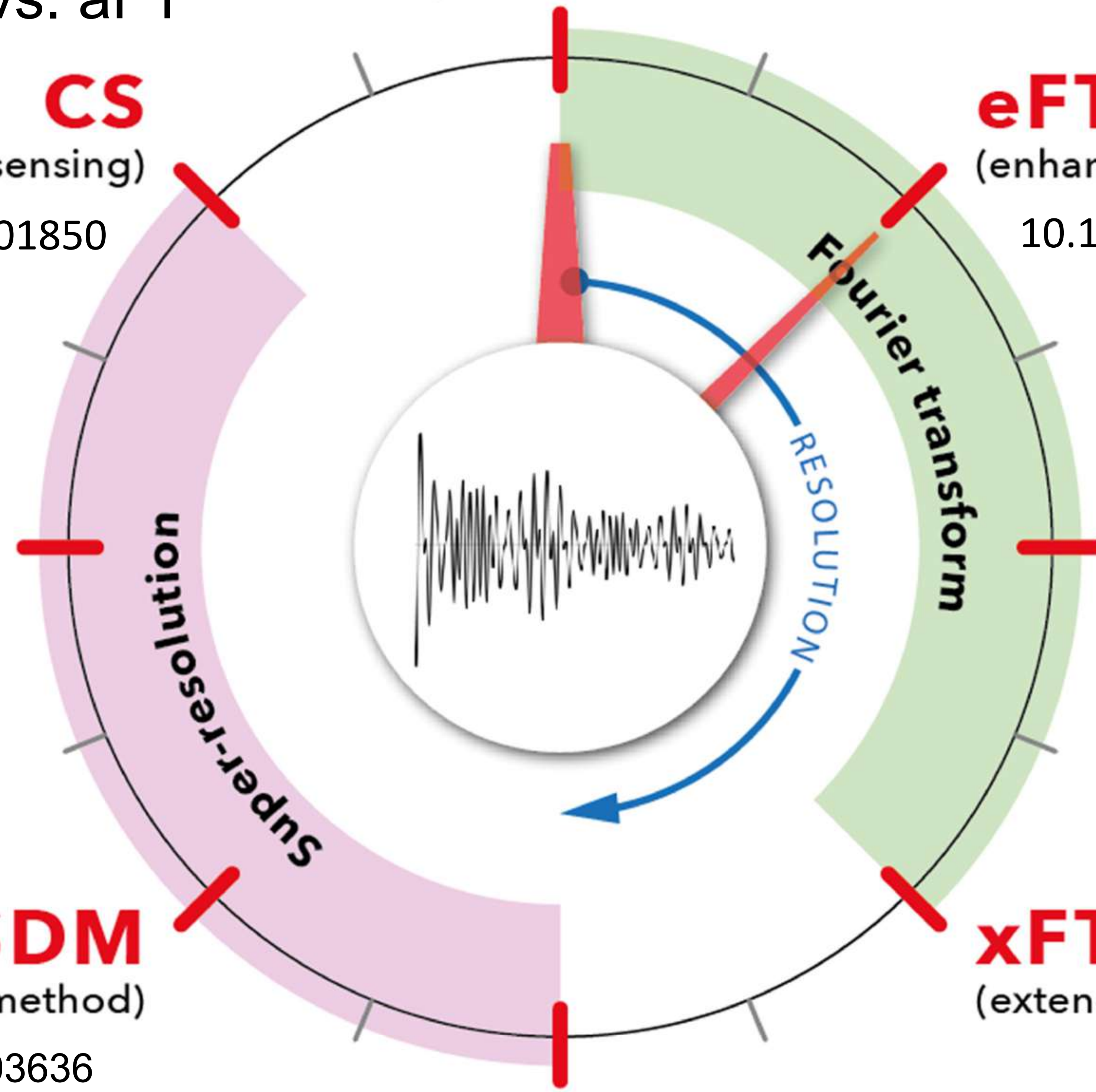
Super-resolution algorithms:
2-4 fold shorter transients vs. aFT

Spectroswiss

mFT
(magnitude mode FT)

CS
(compressed sensing)
DOI: 10.1021/acs.analchem.7b01850

eFT
(enhanced FT = mFT + aFT)
10.1016/j.ijms.2014.05.019



FDM
(filter diagonalization method)
DOI:10.1016/j.jasms.2006.02.018
DOI: 10.1021/ac203391z

aFT
(absorption mode FT)

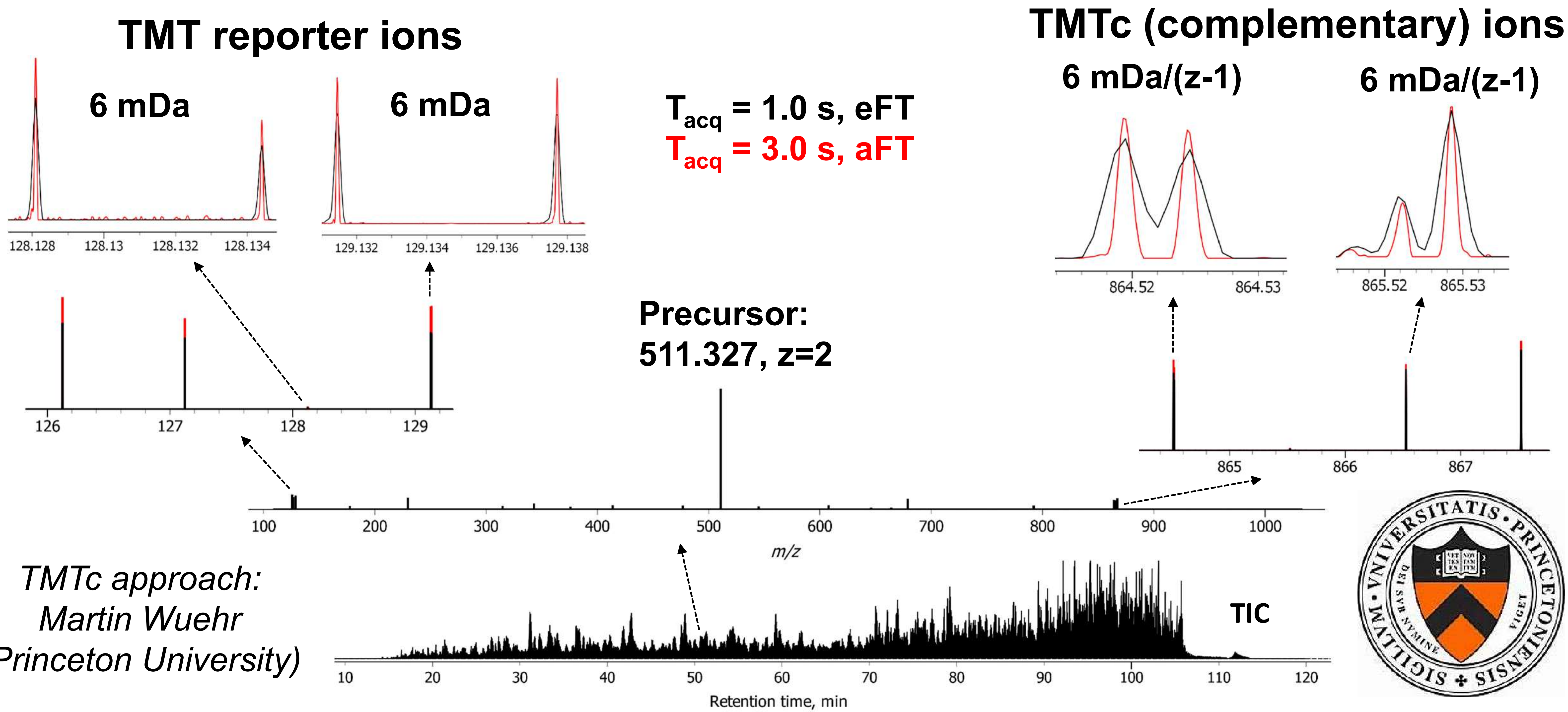
ΦSDM
(phased spectrum deconvolution method)
DOI: 10.1021/acs.analchem.6b03636

xFT
(extended basis FT)

LSF
(least-squares fitting)
DOI: 10.1007/s13361-014-0888-x **targeted**
DOI: 10.1002/mas.21681

Protein Quantitation: TMT Complementary Ions (TMTc)

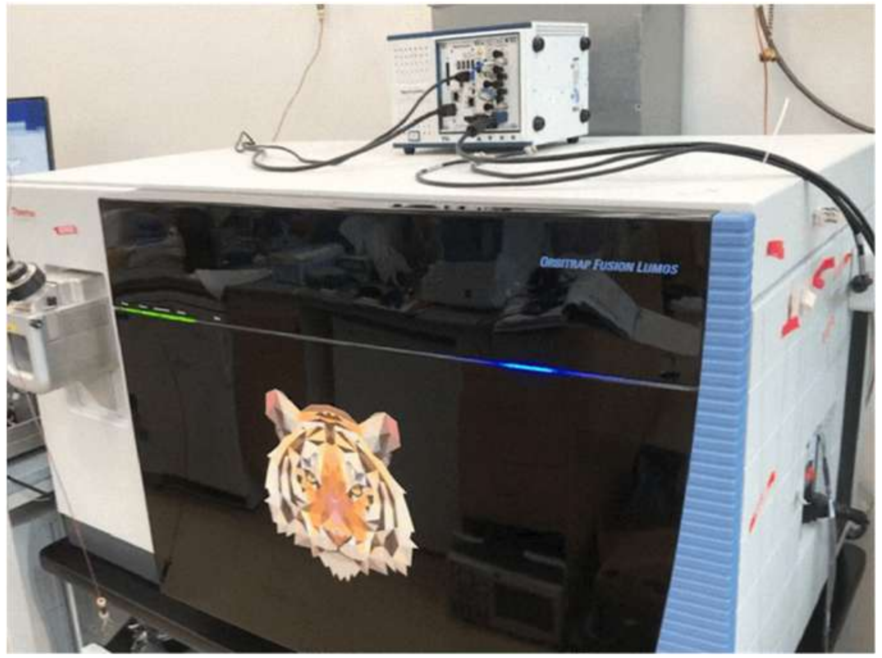
Multiplexed Tandem Mass Tag (TMT) proteomics on Fusion Lumos (HCD)



TMTc approach:
Martin Wuehr
(Princeton University)

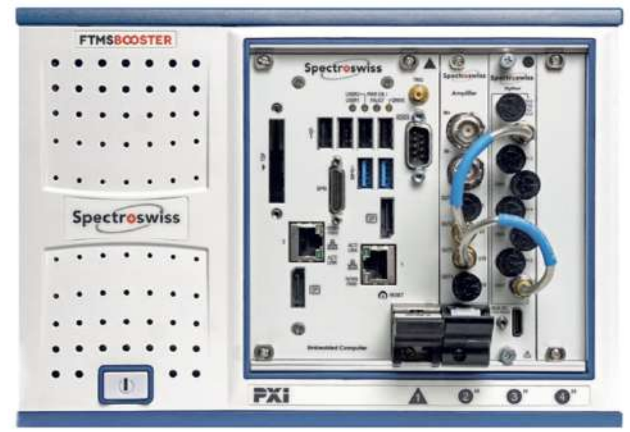
Wühr et al., ChemBioChem. (2019) 20:1210-1224; doi:10.1002/cbic.201800650

Speed Up TMTc with Least-Squares Fitting (**LSF**)

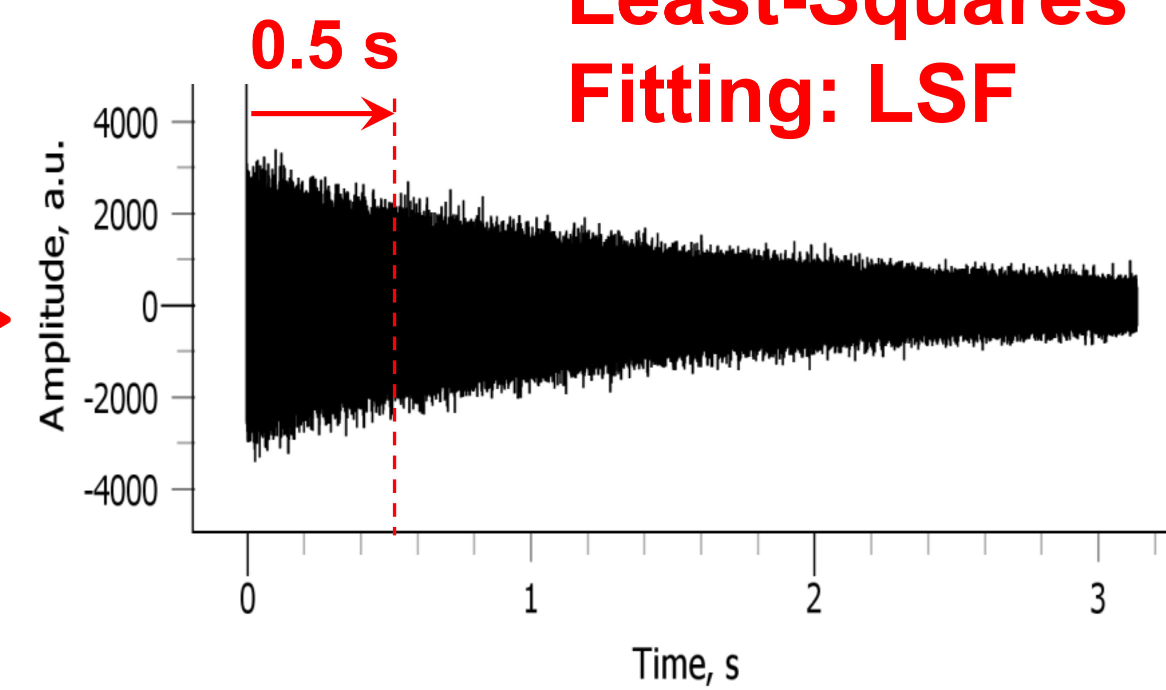


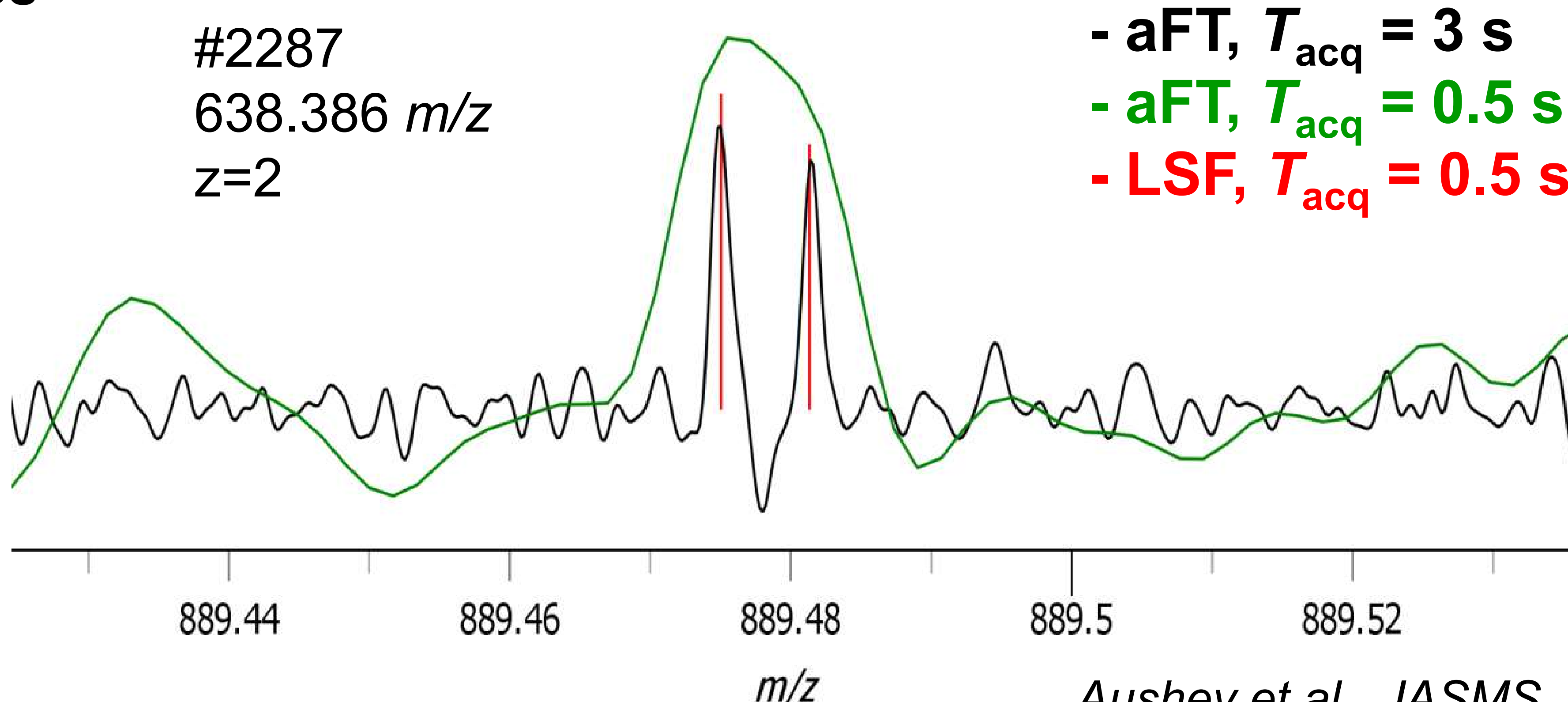
Orbitrap Fusion Lumos™

External DAQ system



Least-Squares Fitting: LSF

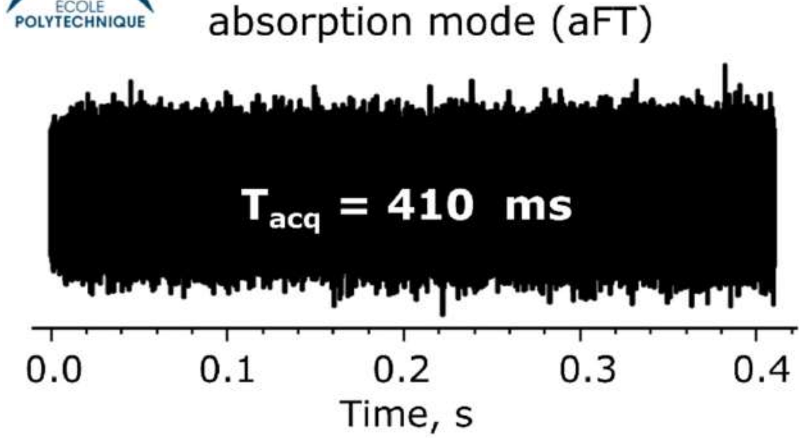


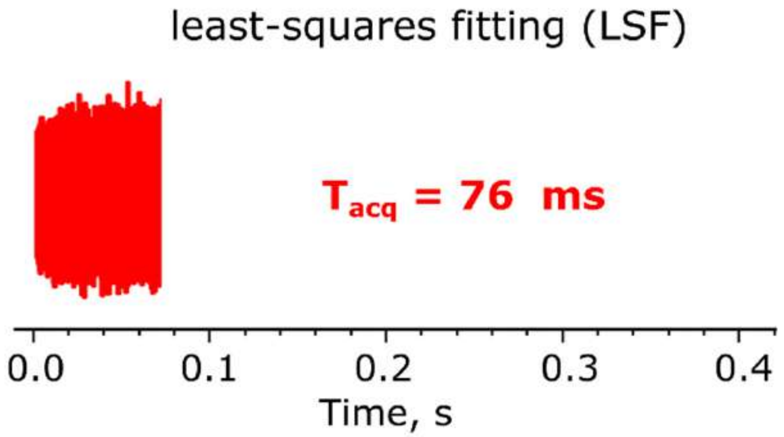


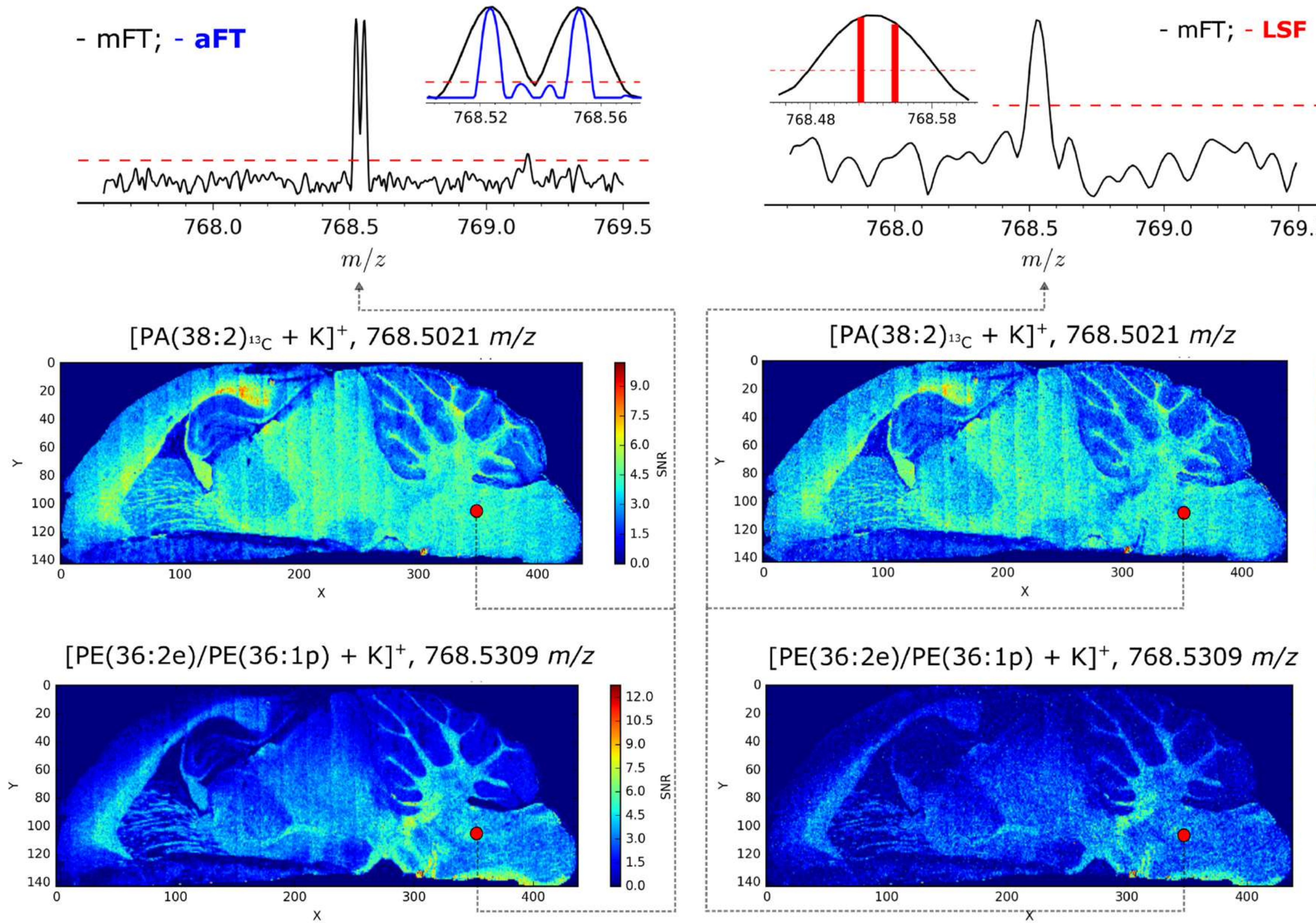
TMTc approach:
Martin Wuehr
(Princeton University)

Aushev et al., JASMS, 2014: 10.1021/jasms.8b04808

Speed Up Imaging: Targeted Metabolite/Drug Analysis



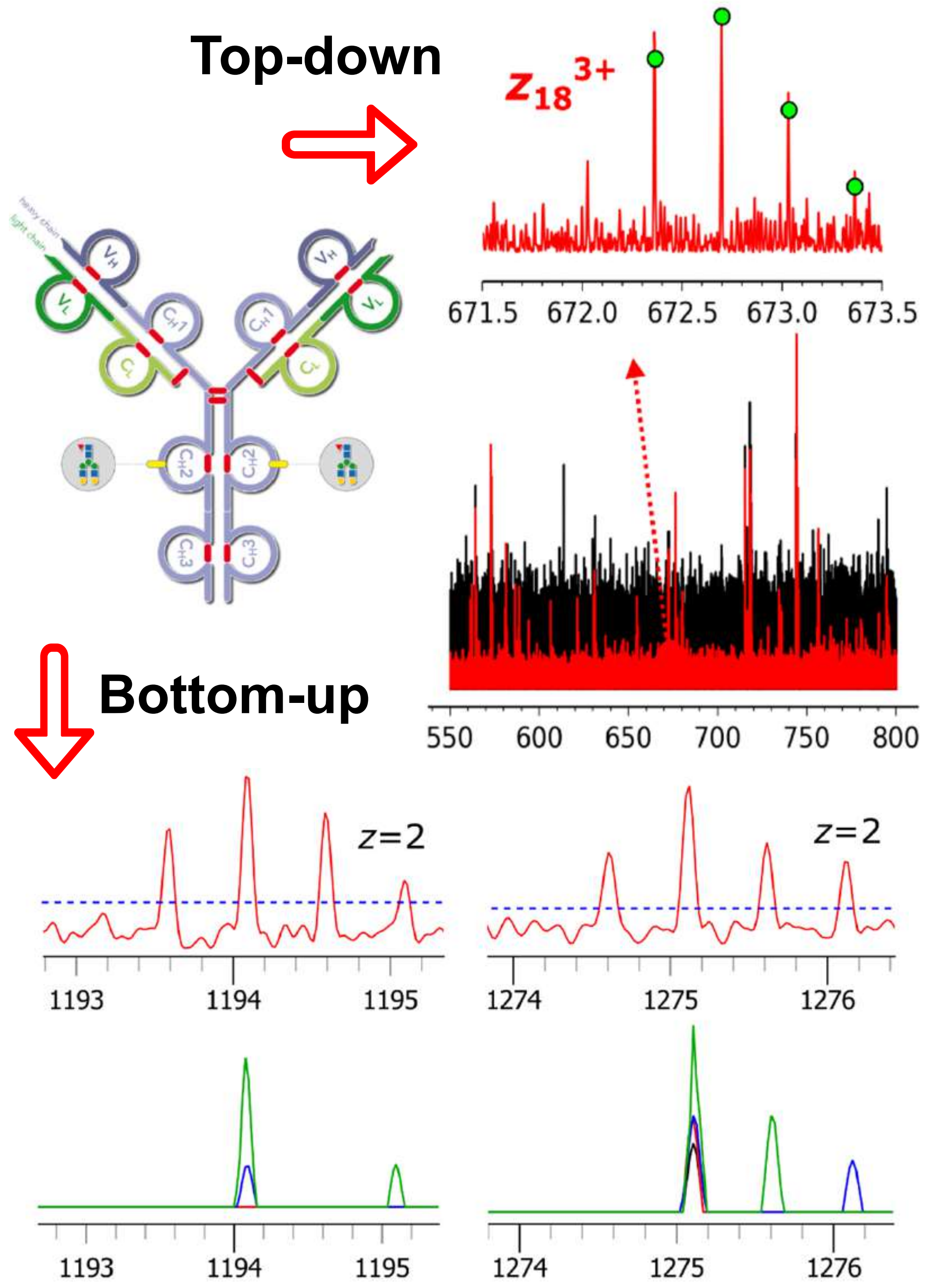




Tsybin et al., Mass Spec. Rev. (2021) 10.1002/mas.21681

Spectroswiss

So, When to Use the Unreduced Data?

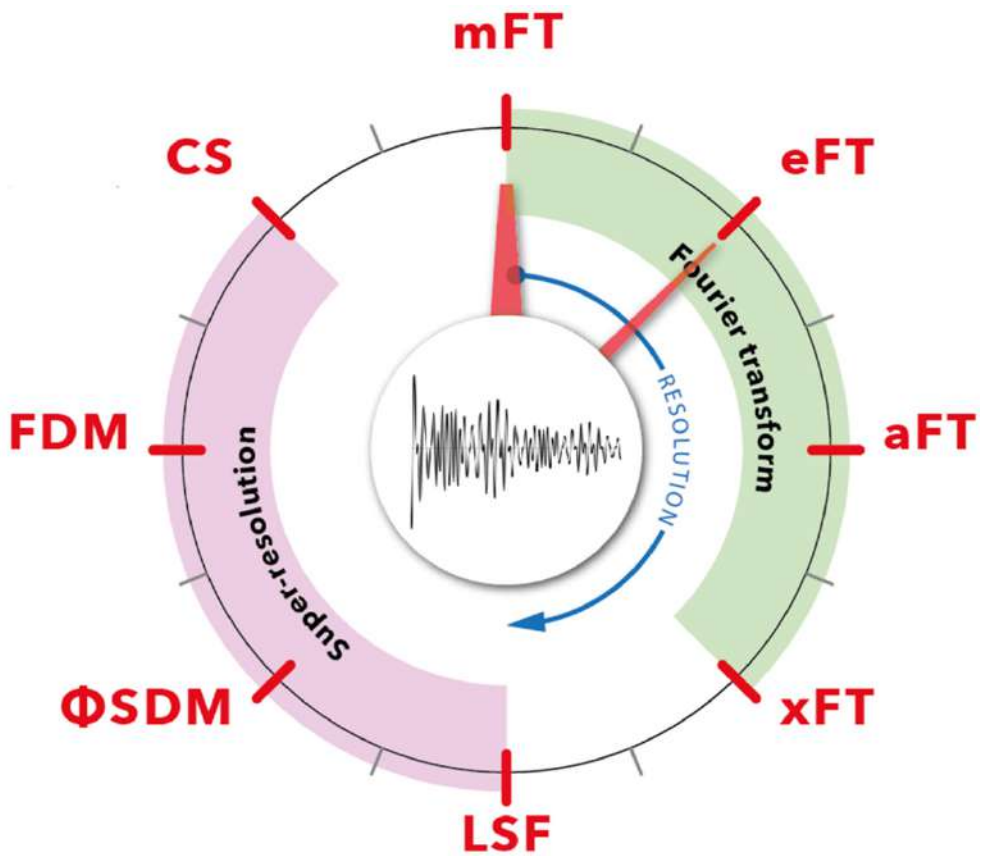


- Higher spectral dynamic range is required: data averaging across multiple scans
- Ultra-high resolution is required: ion detection during all the time ion signal rings
- Higher sensitivity is required: data averaging across multiple scans & multiple LC/GC-MS runs; data averaging of selected areas in single cell imaging
- Higher throughput is required: enhanced ion detection duty cycle due to parallel ion detection and ion manipulation (fragmentation, accumulation, *etc.*)
- Spectral complexity is beyond the conventional approaches: single ion counting, super-resolution

Always! But benefits differ between applications – to evaluate pros & cons

Summary: Empowering FTMS with The Unreduced Data

Full profile aFT > **Full profile eFT/mFT** > **Reduced profile eFT/mFT** > **Centroided**



- The two equal (!) types of the unreduced data in FTMS: **transients** and **absorption mode** mass spectra
- Advances in hardware and software enabled overcoming the technological challenges & creating the required tools for the unreduced data generation and handling
- Applied benefits: enhanced analytical characteristics for selected (especially multi-scan) applications & enabling the new ones: single ion counting, super-resolution MS, …
- Current inhibitors: storage space for the unreduced data; computational speed for super-resolution data processing

The unreduced data and tools are now available to advance FTMS applications

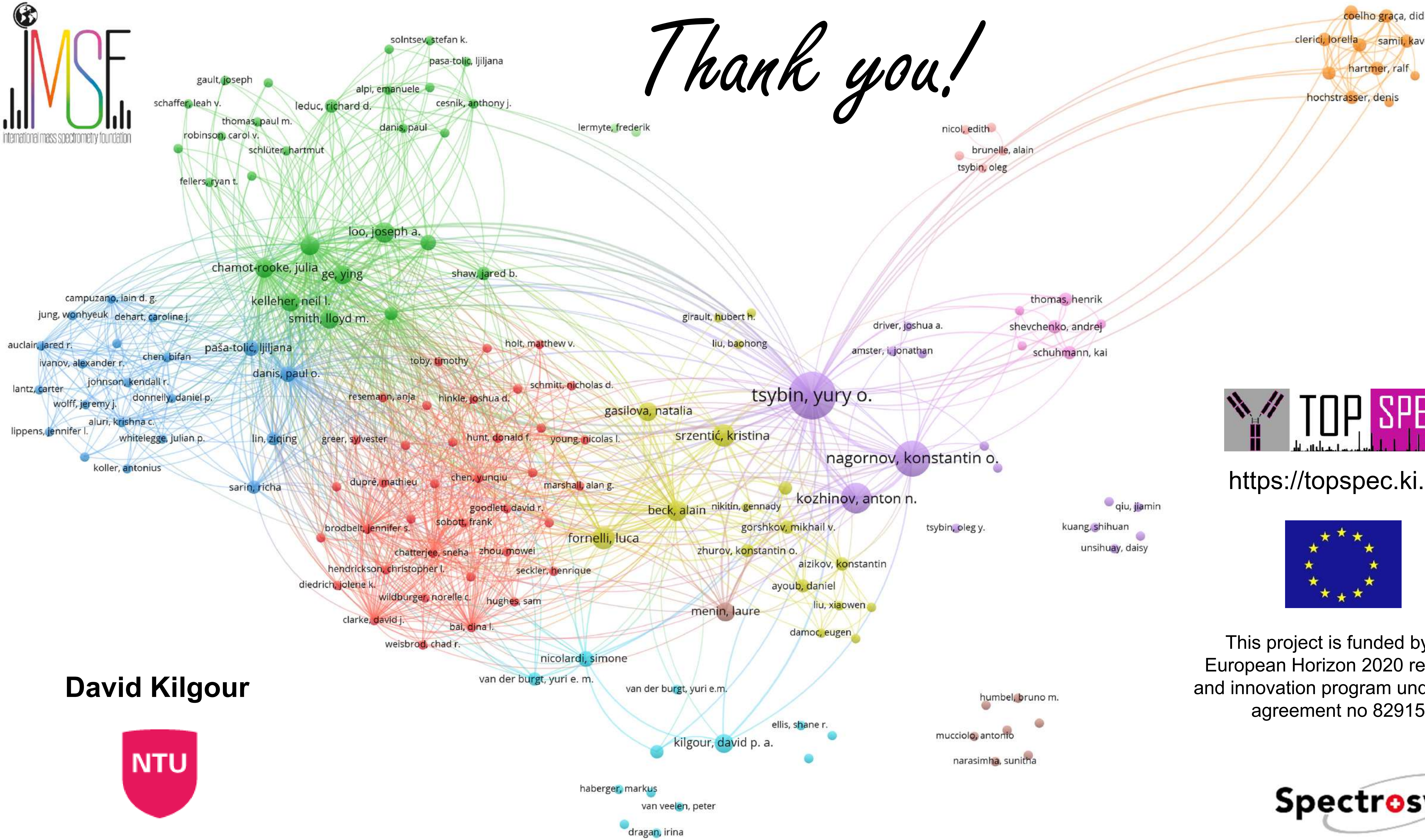
Empowering FTMS with The Unreduced Data

Suggested Reading

- Resource on Orbitrap models design and applications: https://planetorbitrap.com/
- Advanced fundamentals in Fourier transform mass spectrometry: https://doi.org/10.1016/B978-0-12-814013-0.00005-3
- Enhanced Fourier transform for Orbitrap mass spectrometry: https://doi.org/10.1016/j.ijms.2014.05.019
- Absorption mode Fourier transform for FTMS: http://www.kilgourlab.com/absorption-mode-for-ft-ms/
- Transient-Mediated Simulations of FTMS Isotopic Distributions and Mass Spectra to Guide Experiment Design and Data Analysis: https://doi.org/10.1021/jasms.0c00190
- Fourier transform mass spectrometry at the uncertainty principle limit for improved qualitative and quantitative molecular analyses (PhD thesis, Anton Kozhinov): https://infoscience.epfl.ch/record/205045
- Trace-Level Persistent Organic Pollutant Analysis with Gas-Chromatography Orbitrap Mass Spectrometry - Enhanced Performance by Complementary Acquisition and Processing of Time-Domain Data: https://doi.org/10.1021/jasms.9b00032
- Improved Uranium Isotope Ratio Analysis in the Liquid Sampling-Atmospheric Pressure Glow Discharge/Orbitrap FTMS Coupling Through the Use of an External Data Acquisition System: *by Marcus et al., JASMS, in print*

Questions? Ideas? Most welcome to discuss: tsybin@spectroswiss.ch

Thank you!



David Kilgour

https://topspec.ki.se/

This project is funded by the European Horizon 2020 research and innovation program under grant agreement no 829157