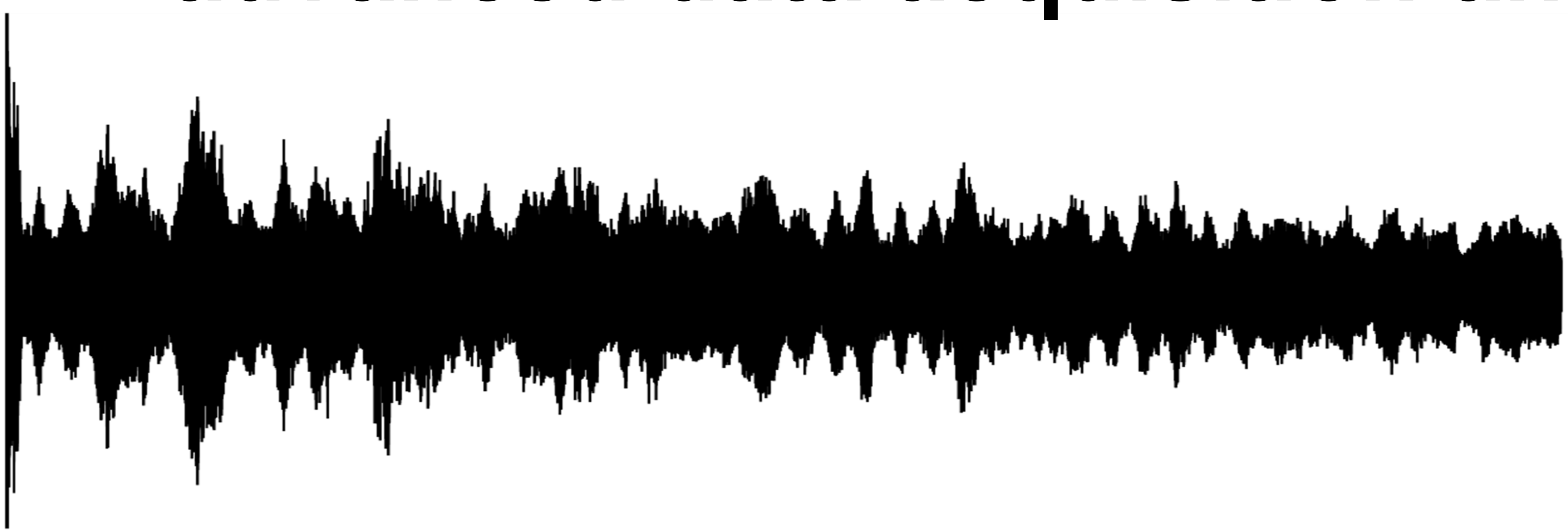
Spectroswiss
FTMS Science and Technology

Analytica 2020

Maximizing the value of Orbitrap FTMS through advanced data acquisition and processing



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Spectroswiss Sàrl
EPFL Innovation Park
Lausanne, Switzerland

21 October 2020 @ 13:30

Orbitrap FTMS Data: Transients & Mass Spectra

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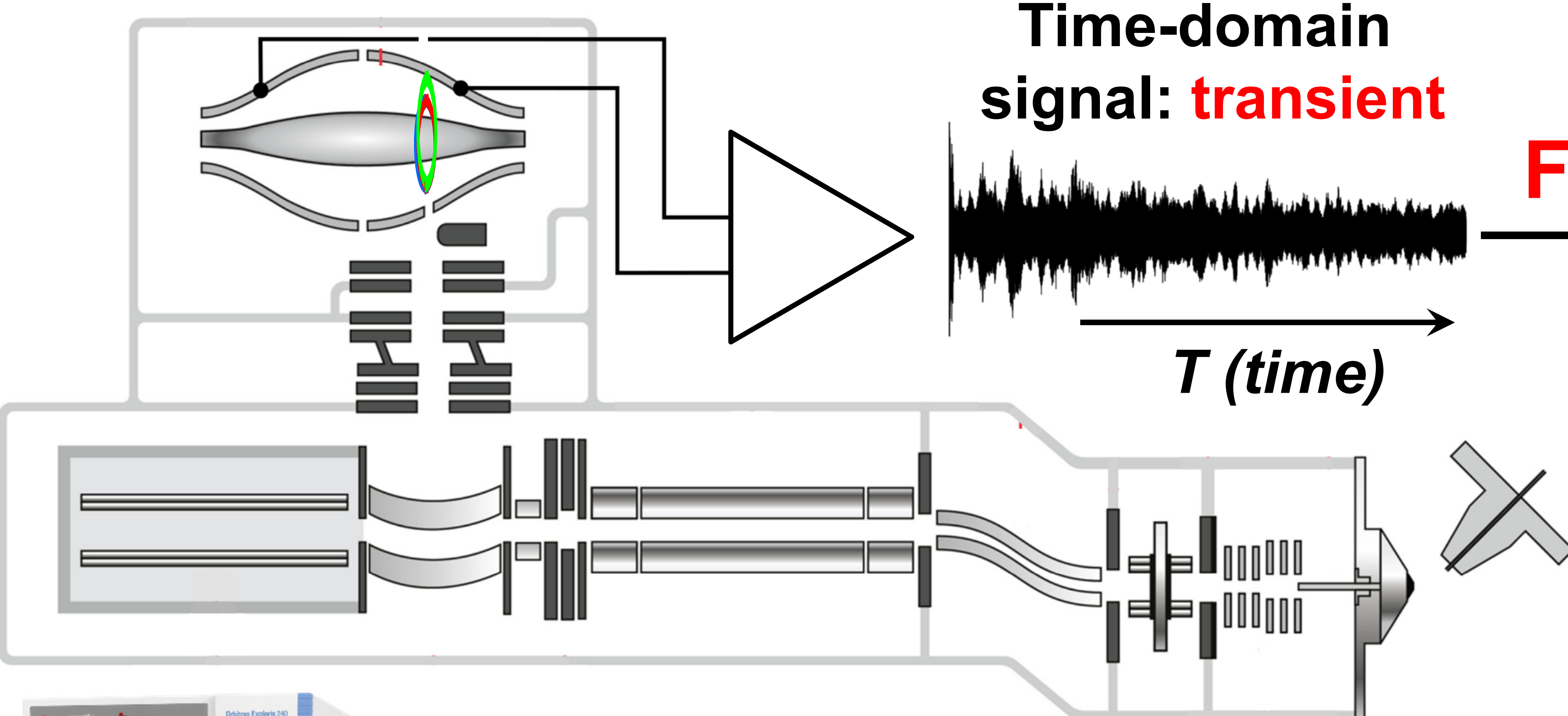
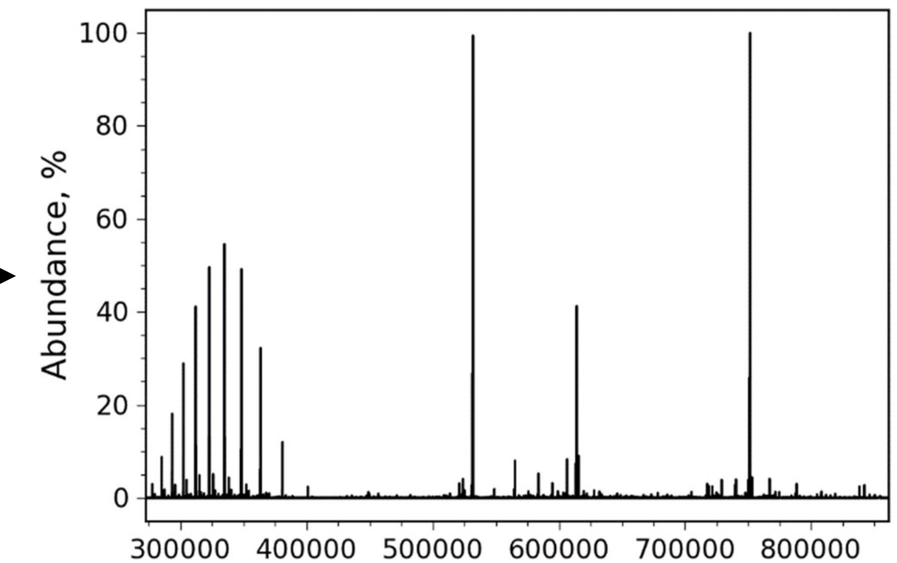
Time-domain signal: transient

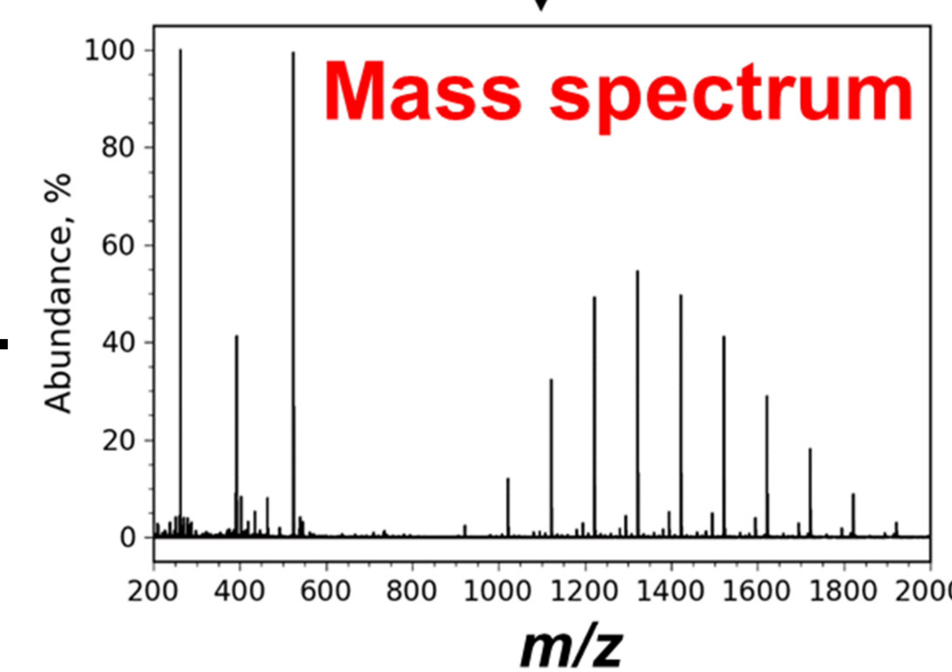


FT

Frequency spectrum

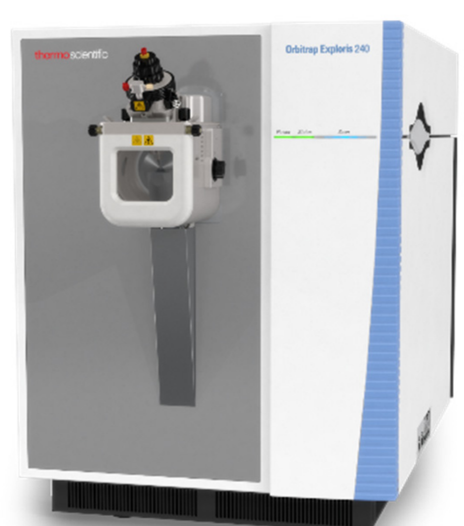


Calibration $\omega \sim \left(\frac{m}{z}\right)^{-\frac{1}{2}}$



Data reduction

.RAW



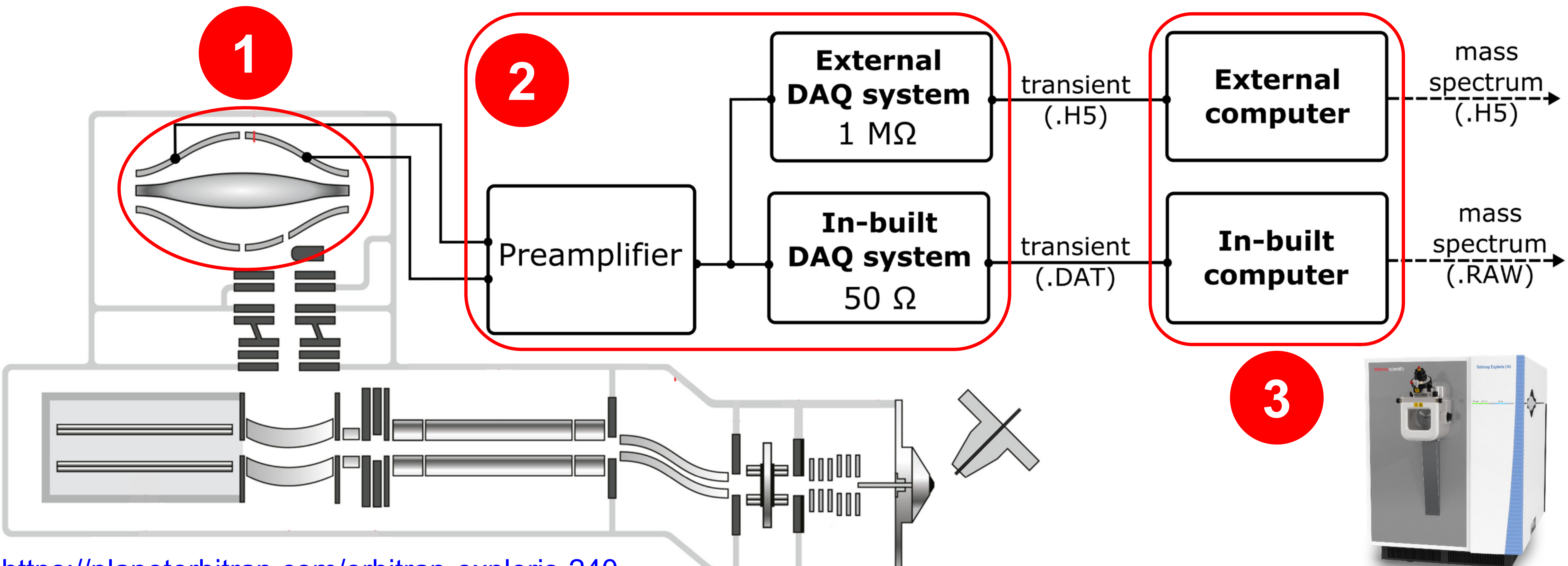
metabolites/proteins identified & quantified

% sequence coverage in protein structural analysis

https://planetorbitrap.com/orbitrap-exploris-240

How to Maximize the Information Output from Orbitraps

1. **Ion physics:** number of charges (space charge), injection (phase function)
2. **Electronics:** sampling (digitization), detection period and *m/z* range
3. **Software:** data processing (data reduction, noise thresholding, *etc.*)



https://planetorbitrap.com/orbitrap-exploris-240

How to Maximize the Information Output

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I. Ion Physics

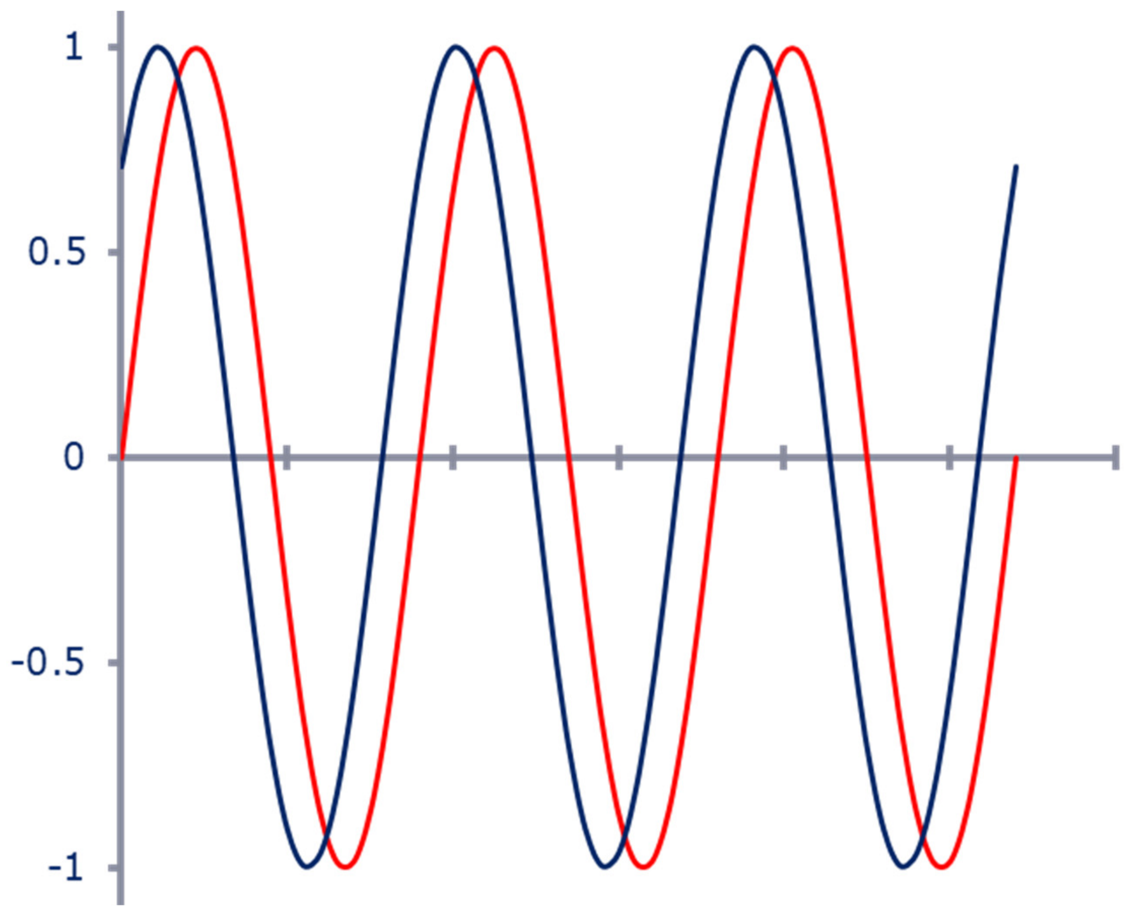
II. Electronics: Ion Signal Recording

III. Software: Data Processing

IV. Examples

Initial Ion Phases in FTMS

- Example: 2 signals in a transient
- Same amplitude & frequency
- Different **initial phases**

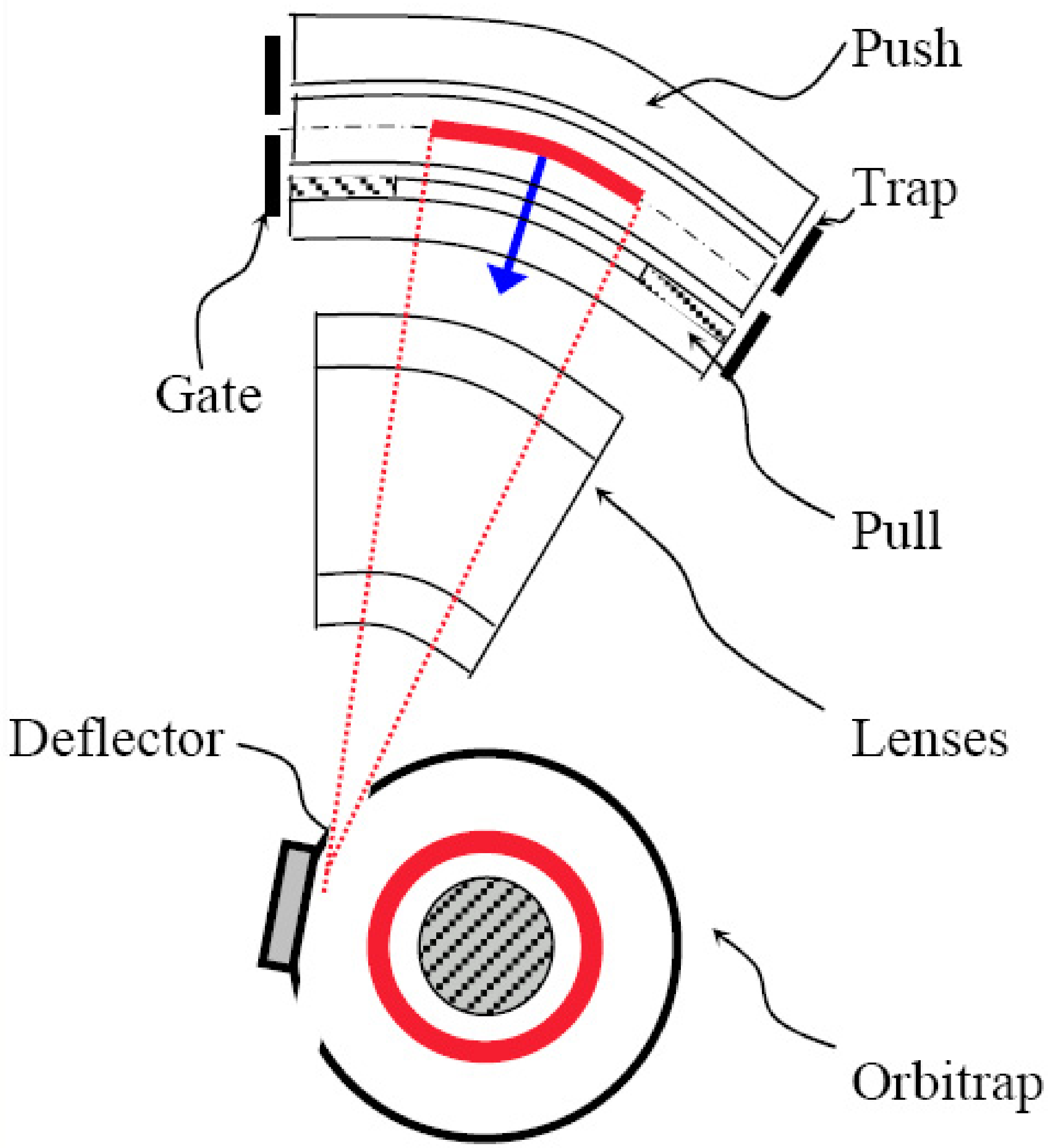


- Optimum FT processing of transients in FTMS requires signals from ions of different *m/z* to have the same initial phases (zero angle).
- But: at the time when ion detection starts, individual signals from ions of different *m/z* have different initial phases, in general.
- Phase correction sets the same value of initial phase (zero) for the ion signals from different *m/z*. Could be realized as post-processing.

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

Fourier transforms in FTMS:
http://www.kilgourlab.com/absorption-mode-for-ft-ms
https://www.youtube.com/watch?v=spUNpyF58BY

Ion (Initial) Phases: a Key to High Performance



DOI: 10.1021/ac4001223

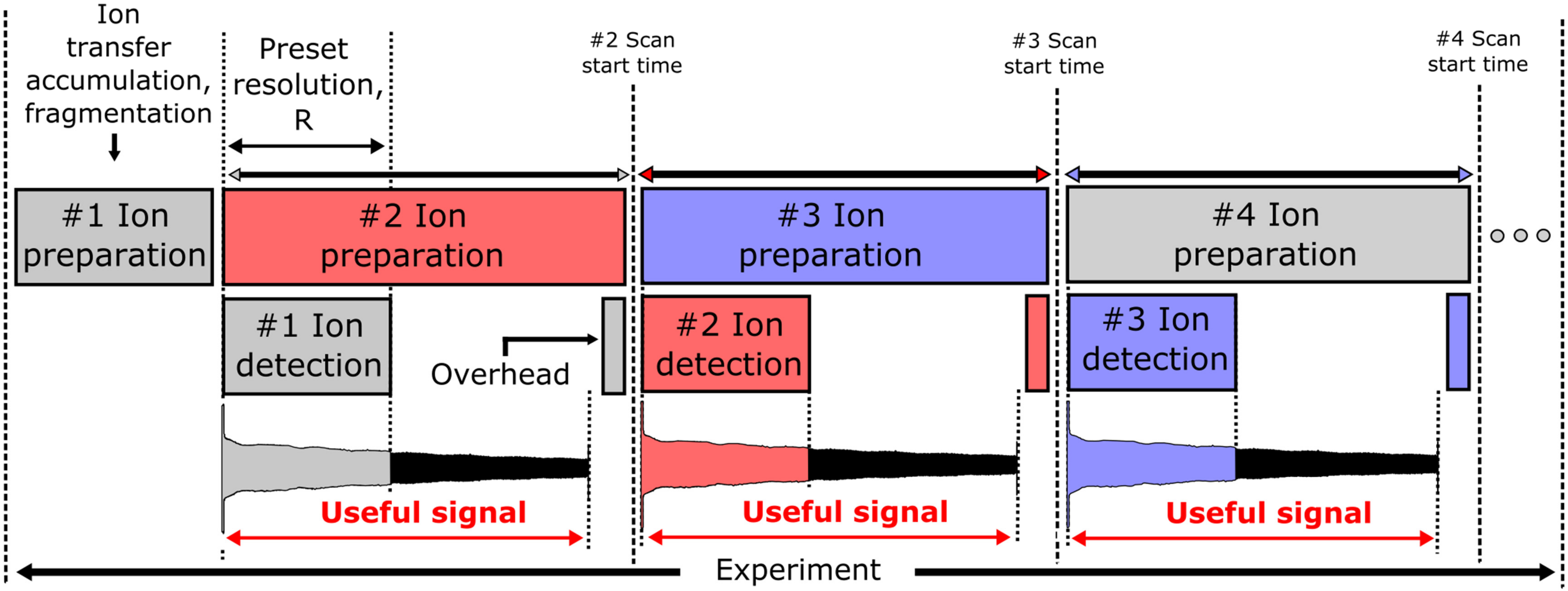
- Ions are ejected from the C-trap (a pulse event) towards the orbitrap: ion excitation by injection
- Ions reach the orbitrap and start oscillations at different times for different *m/z* (a TOF effect)
- At a time T1 when all *m/z* are eventually in the orbitrap, different *m/z* have **different phases**
- There exists time T0 prior to T1, when the ions of different *m/z* have (nearly) the **same phase**

Objective: acquire PHASED transients (all initial phases = 0)

DOI: 10.1016/B978-0-12-814013-0.00002-8

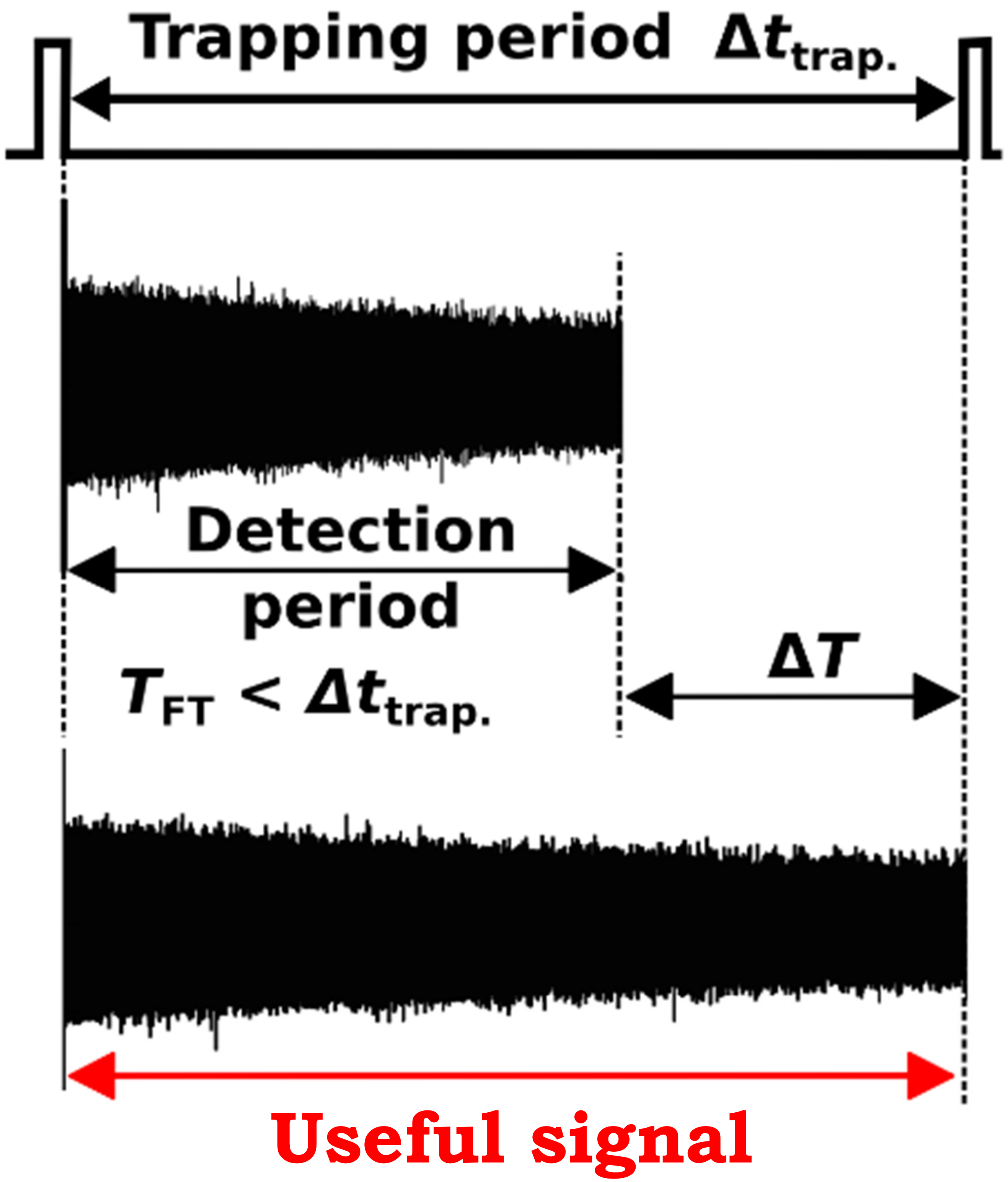
Orbitrap Logic: Parallel Ion Detection/Accumulation

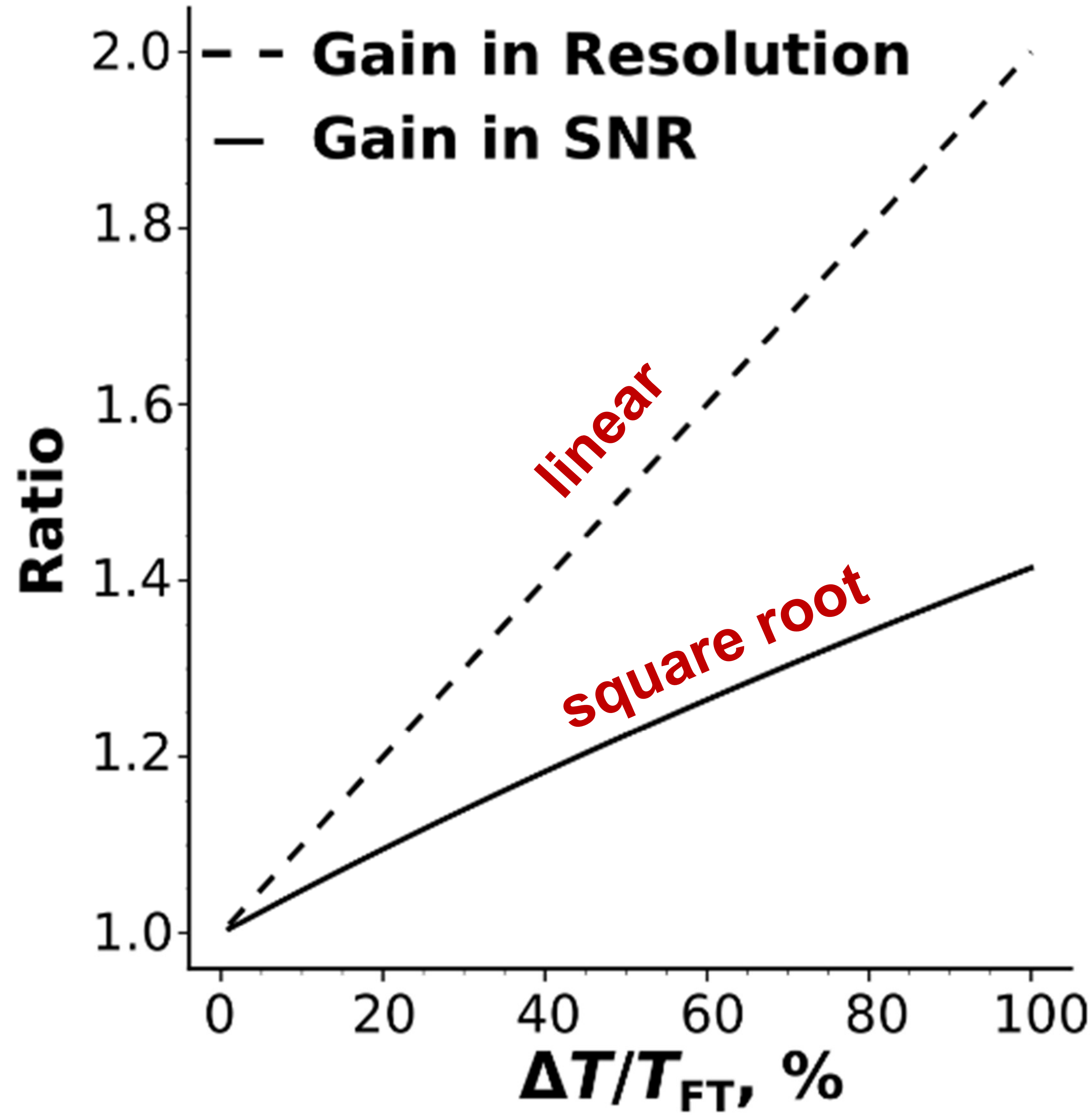
- Ion oscillations in Orbitrap for scan *N* take place during ion accumulation and fragmentation for scan *N*+1 (longer transients provide higher resolution/sensitivity)



Built-in DAQ system: detection period (# of datapoints to acquire) has to be preset prior to acquisition and to be equal to a power of 2

Benefits of Longer Transients: Resolution & Sensitivity

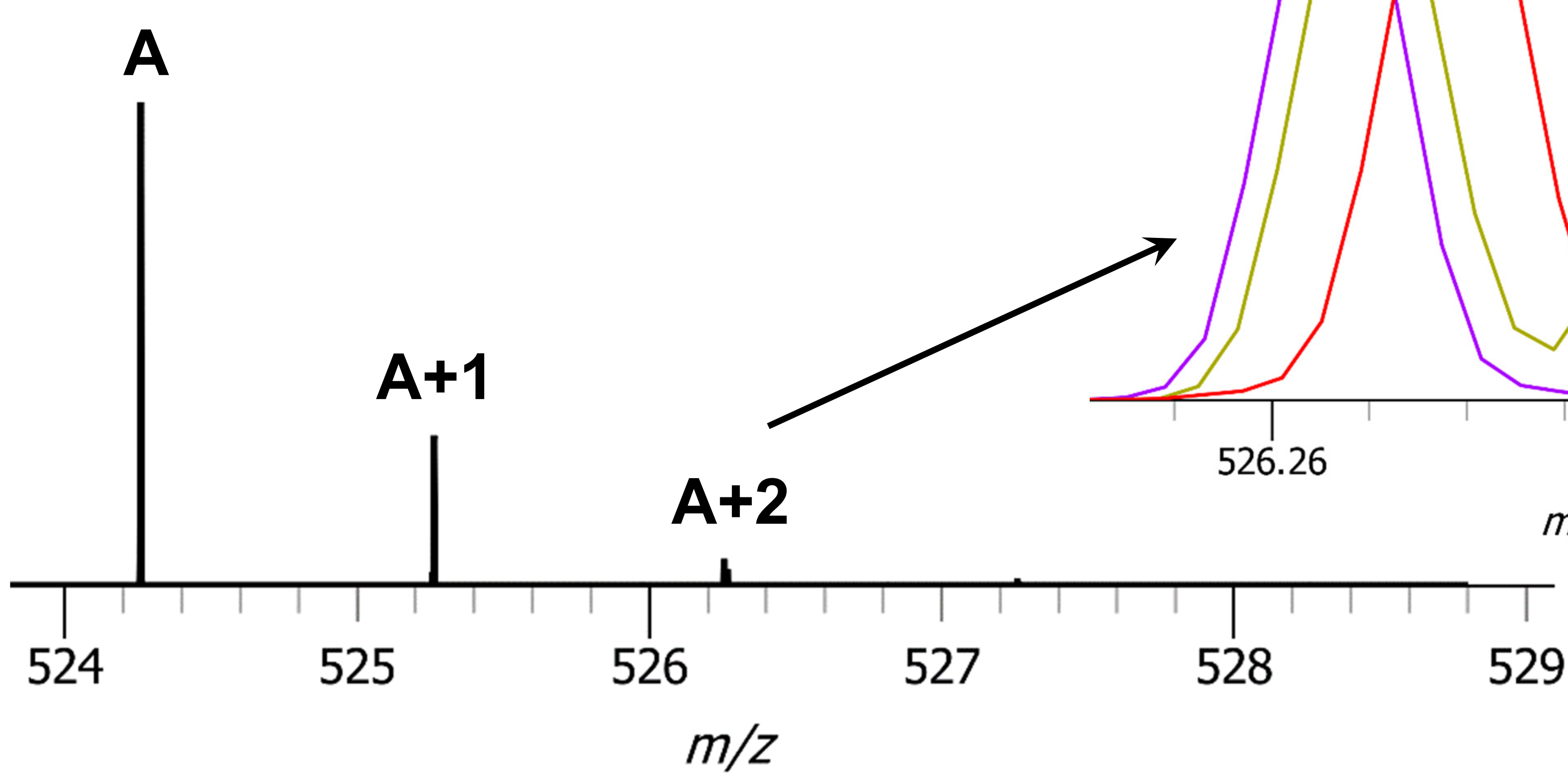


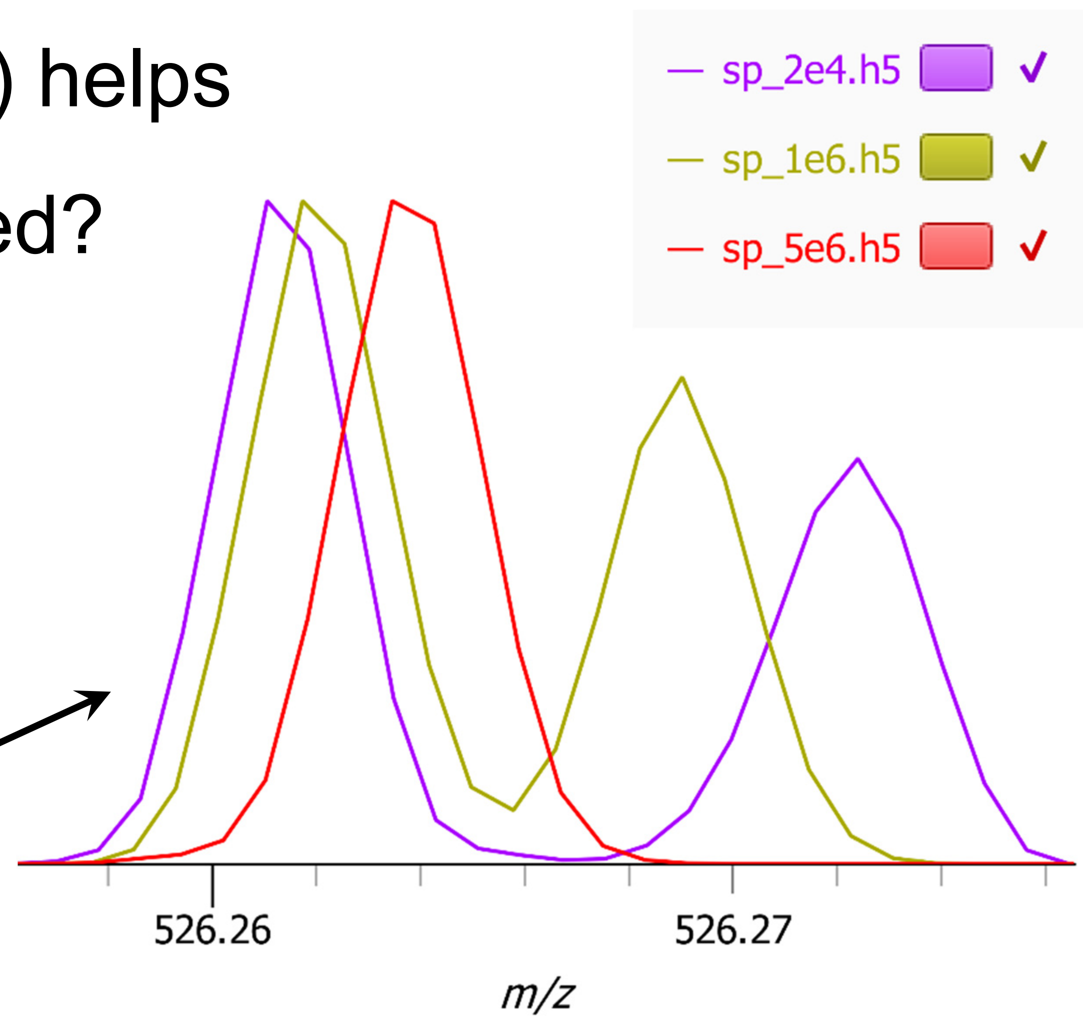


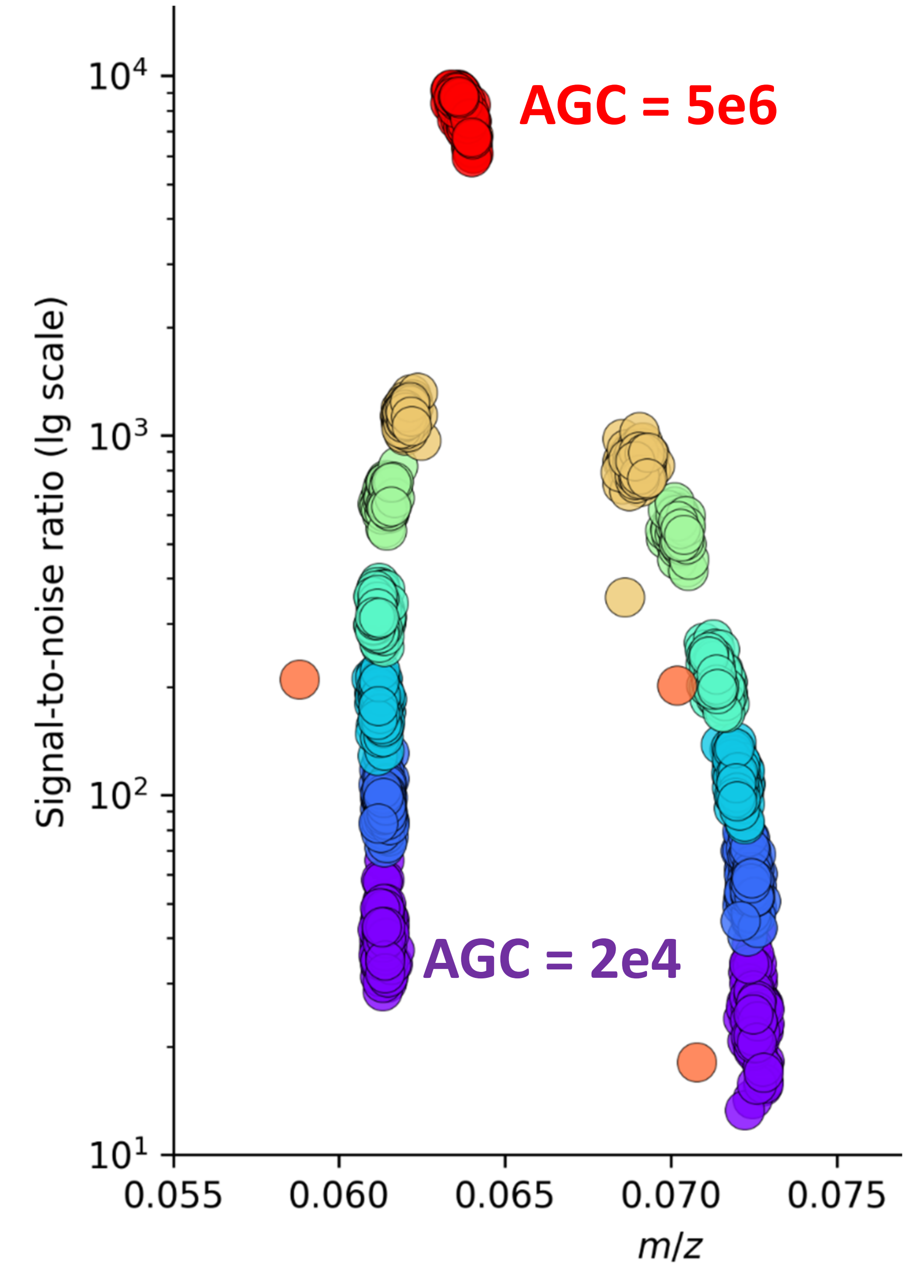
Space Charge Effects: Peak Artifacts

- Too many charges (ions): peak artifacts (*m/z*, abundance errors), coalescence
- AGC (automatic gain control) helps
- AGC logic: how is it calculated?

Q Exactive Orbitrap of MRFA, 1+







Take space charge effects into account for experiment design

DOI: abs/10.1002/rcm.6289; DOI: 10.1021/acs.analchem.7b03437

1 How to Maximize the Information Output: **Ion Physics**

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- **Take into account space charge effects**
  - Monitor peak shape (peak coalescence, artifacts): ion injection, pressure
  - Work with lower AGC values, acquire (many) technical replicates
  - Use SIM (narrow *m/z*) windows approaches for data acquisition
- **Consider Orbitrap logistics**
  - Parallel ion detection/accumulation: maximize the duty cycle
  - Optimize timing: AGC *vs*. IT_{max} *vs*. Resolution (ion detection period)
  - How AGC is calculated/measured? Take into account AGC pre-scan
- **Determine what performance is needed**
  - Define the required resolution & dynamic range wisely (simulate output)

JASMS 2020, 31, 1927-1942

How to Maximize the Information Output

I. Ion Physics

II. Electronics: Ion Signal Recording

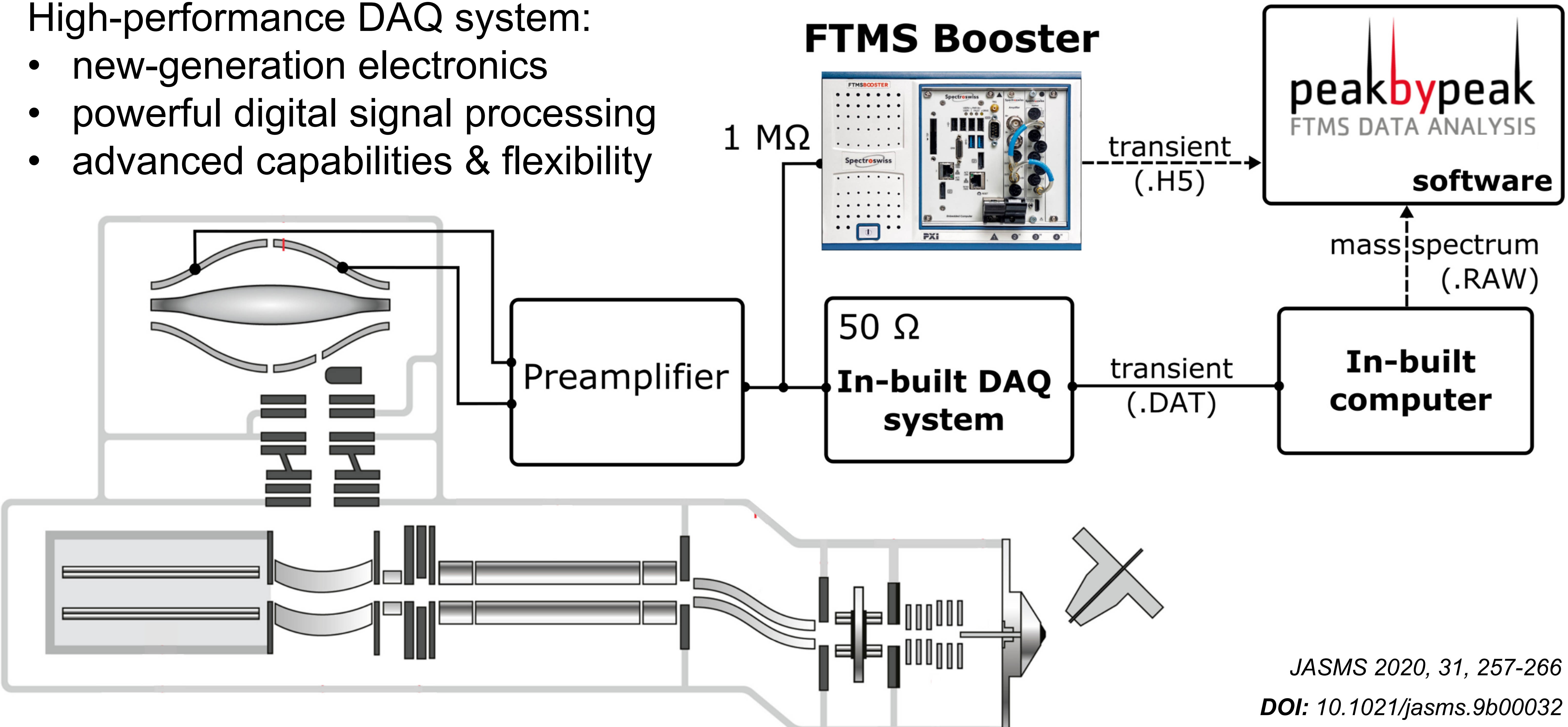
III. Software: Data Processing

IV. Examples

External **High-Performance** DAQ System: Example

High-performance DAQ system:

- new-generation electronics
- powerful digital signal processing
- advanced capabilities & flexibility



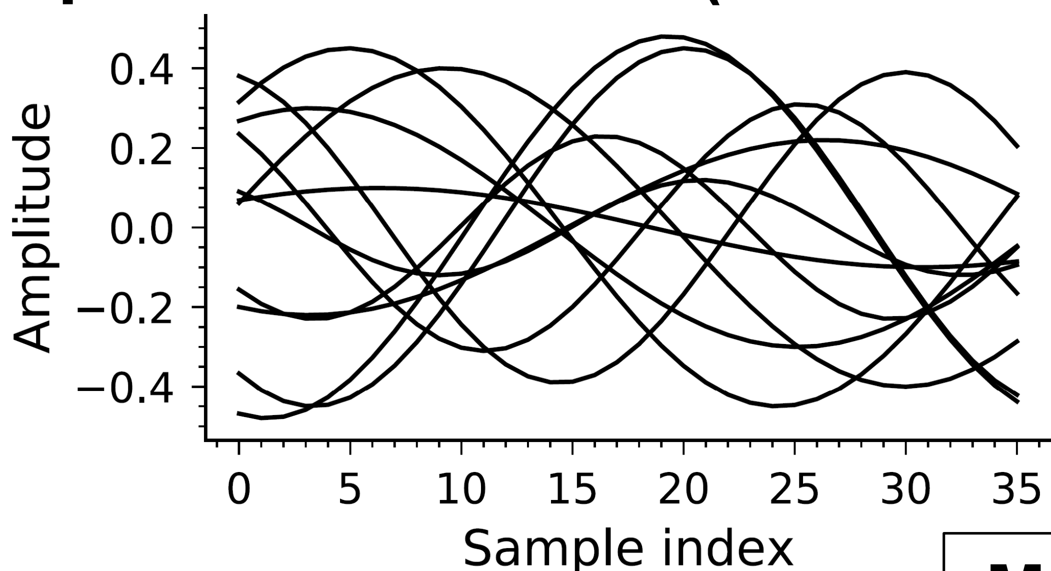
JASMS 2020, 31, 257-266

***DOI:** 10.1021/jasms.9b00032*

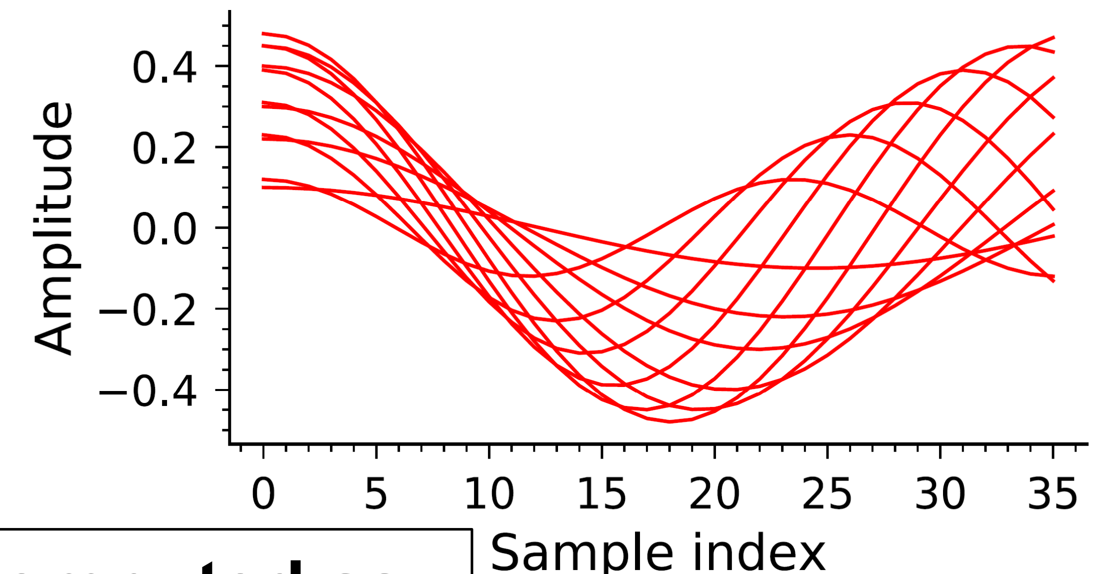
FPGA: field programmable gate array (chip)

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

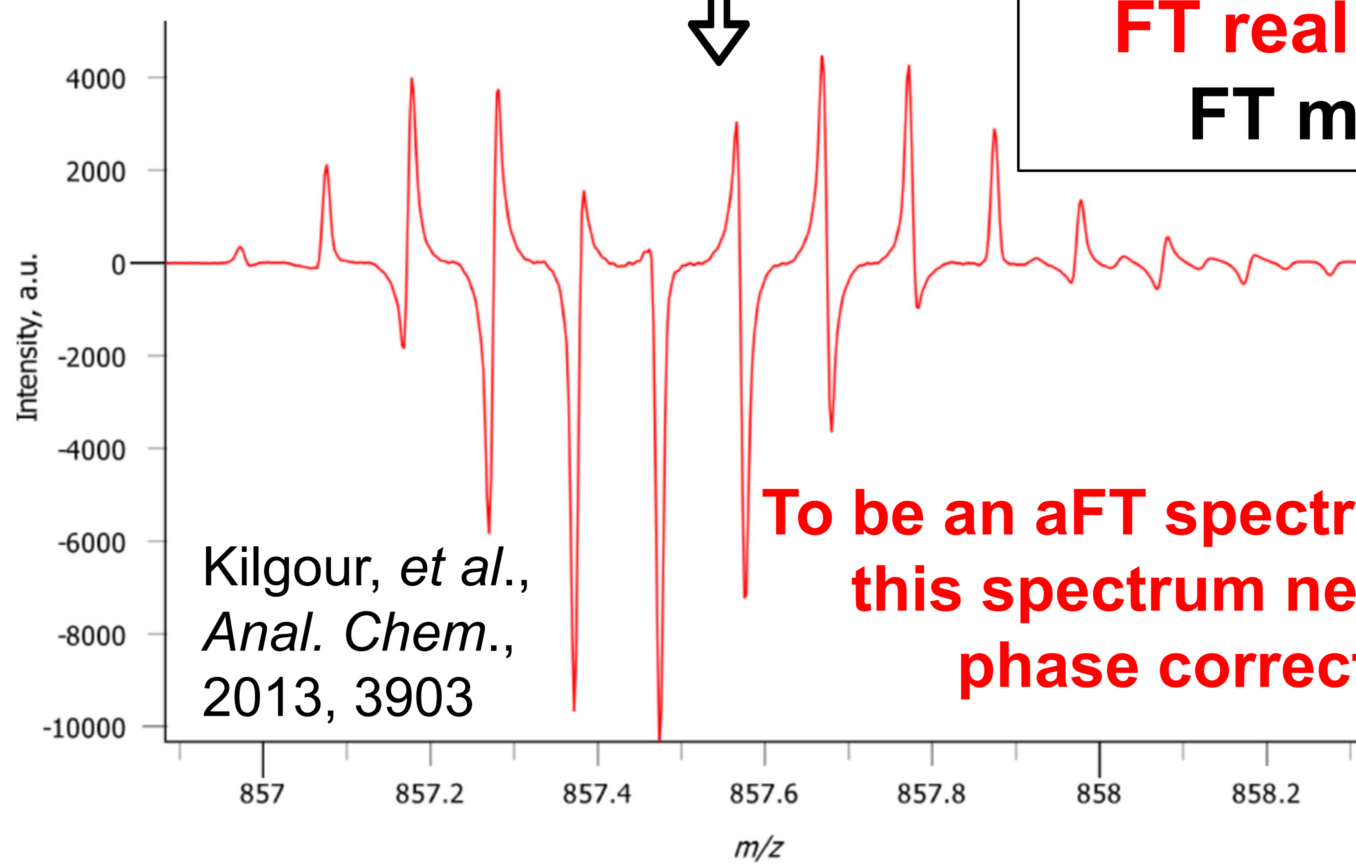
Un-phased transients (state of the art)



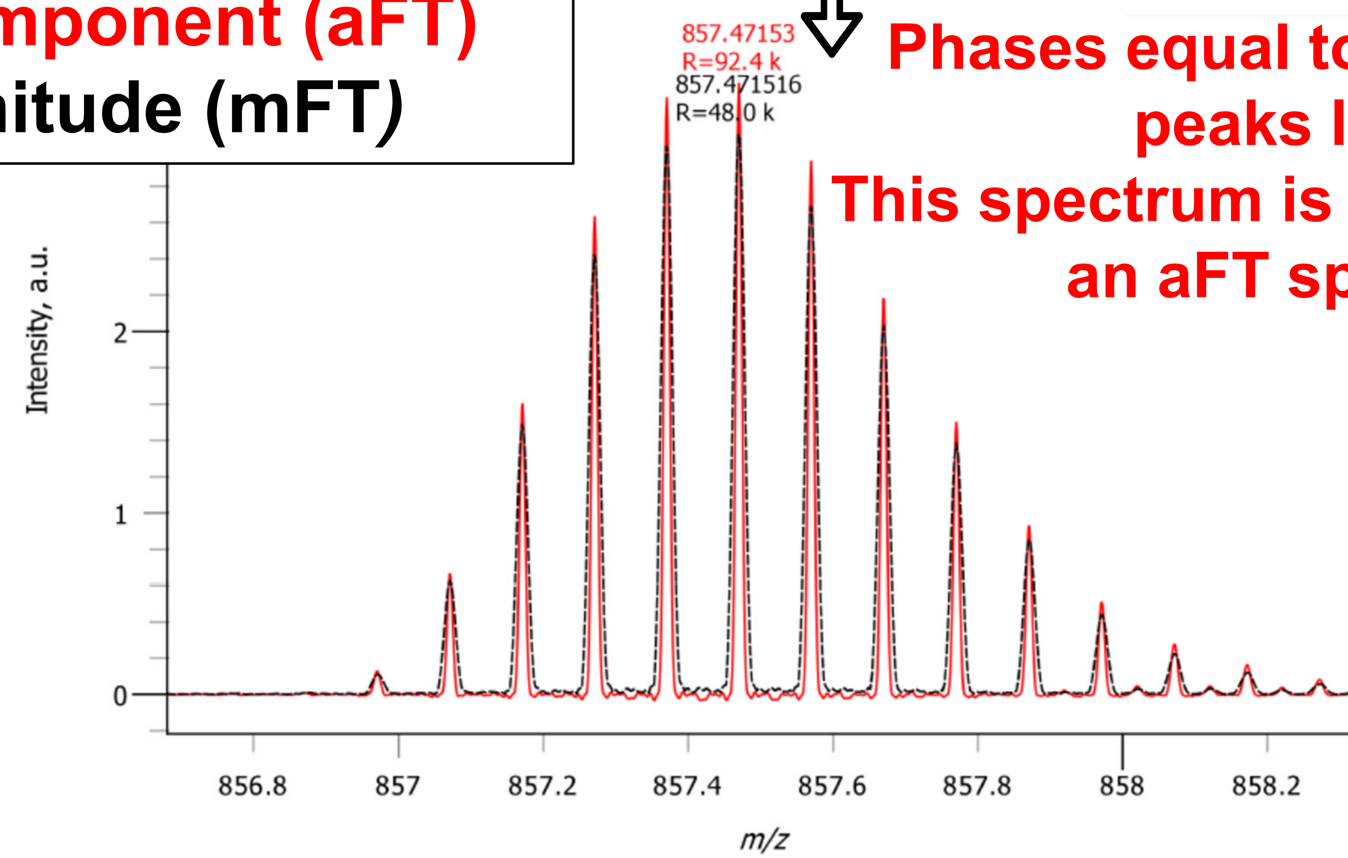
Phased transients (data in this work)



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



To be an aFT spectrum, this spectrum needs phase correction



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

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

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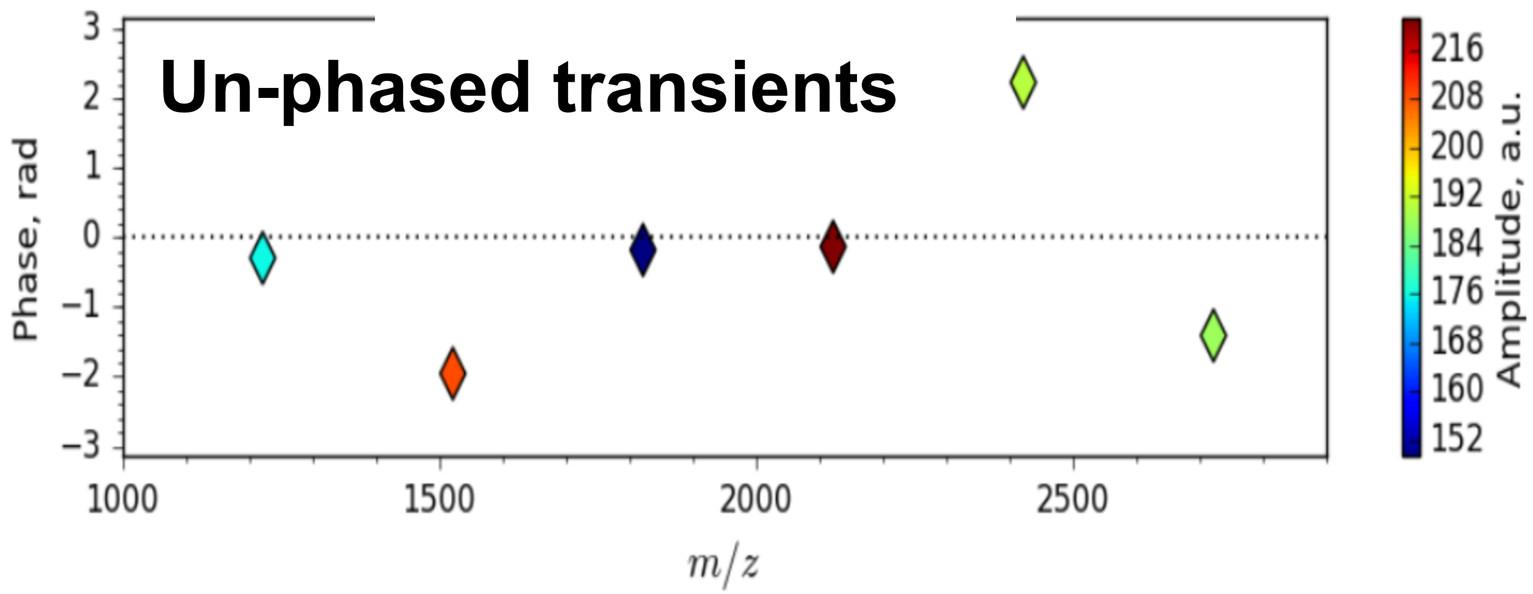
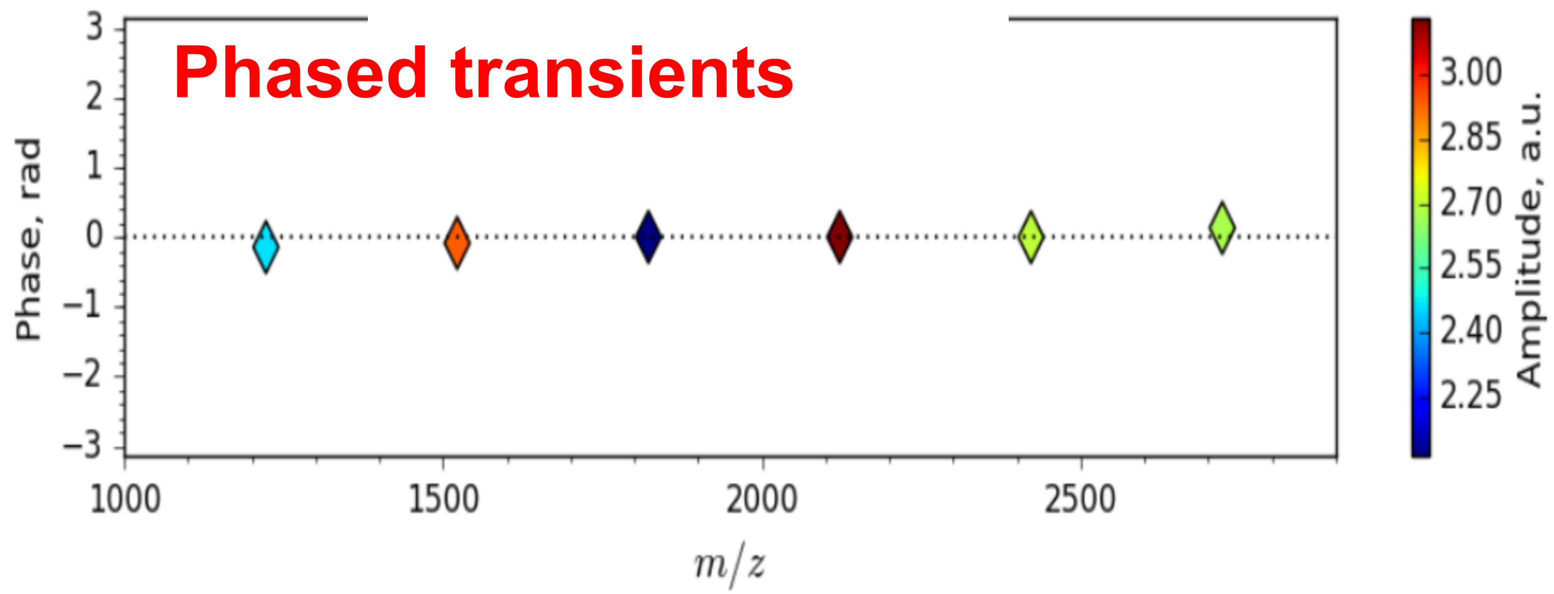
Comparison of phase distributions (initial phase vs. *m/z*), an example





Phased transients can be obtained from any Orbitrap model

How to Acquire Longer Transients on LTQ Orbitraps?

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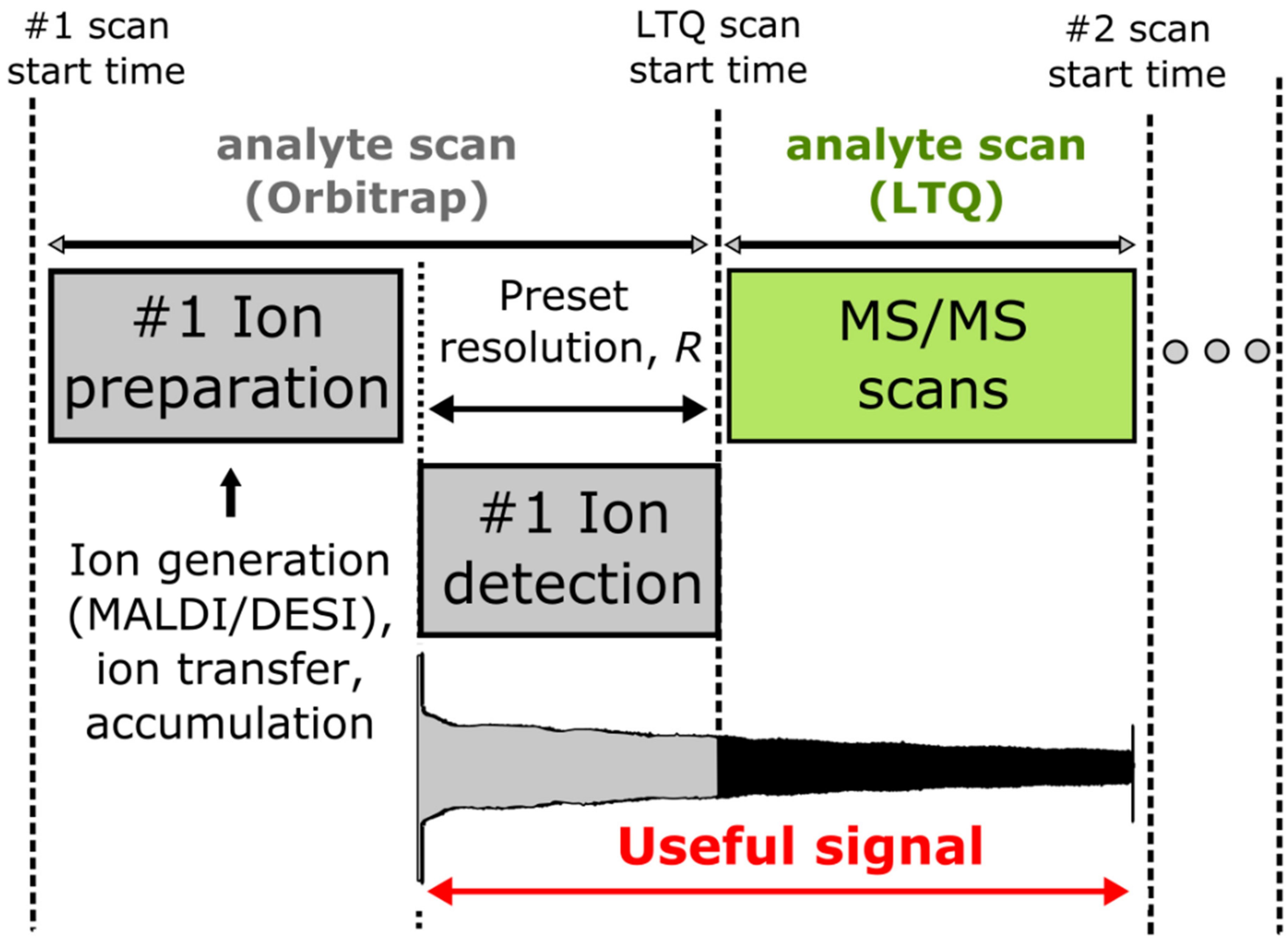
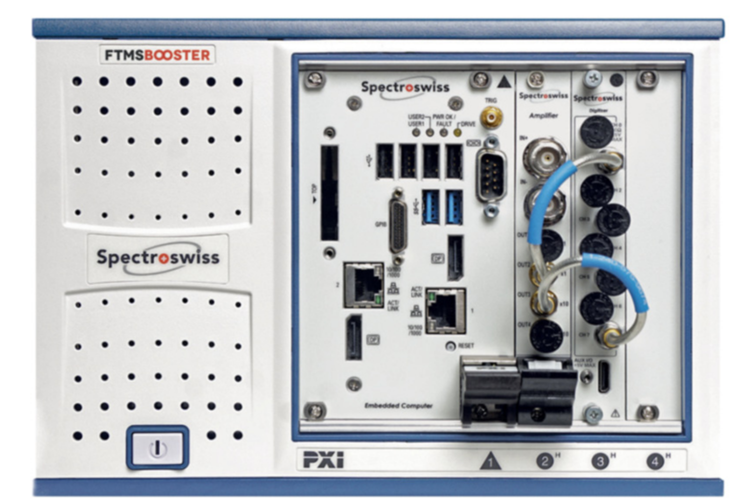


up to 5 s transients were acquired

Example of implementation on LTQ FT:
DOI: 10.1038/s41598-018-36957-1

External DAQ system



New-generation DAQ system: detection period (# of datapoints to acquire) does NOT have to be preset prior to acquisition or be equal to a power of 2

2 How to Maximize the Information Output: **Electronics**

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- **Enable artifact-free transient recording**
  - Phased transient acquisition (enabling FT in true absorption mode)
  - Artifact-free ion signal detection (such as phase function dispersion)
  - Data-dependent transient amplification prior to digitization
  - Provide (save) phased transients for post-processing (any file size)

- **Enhance flexibility of experiment design**
  - Reveal experiment logistics: visualize AGC pre-scans & full detection periods
  - Enable acquisition of extra-long transients: full ion detection time
  - Maximize duty cycle: ion detection during MS/MS events (ETD, UVPD, *etc.*)

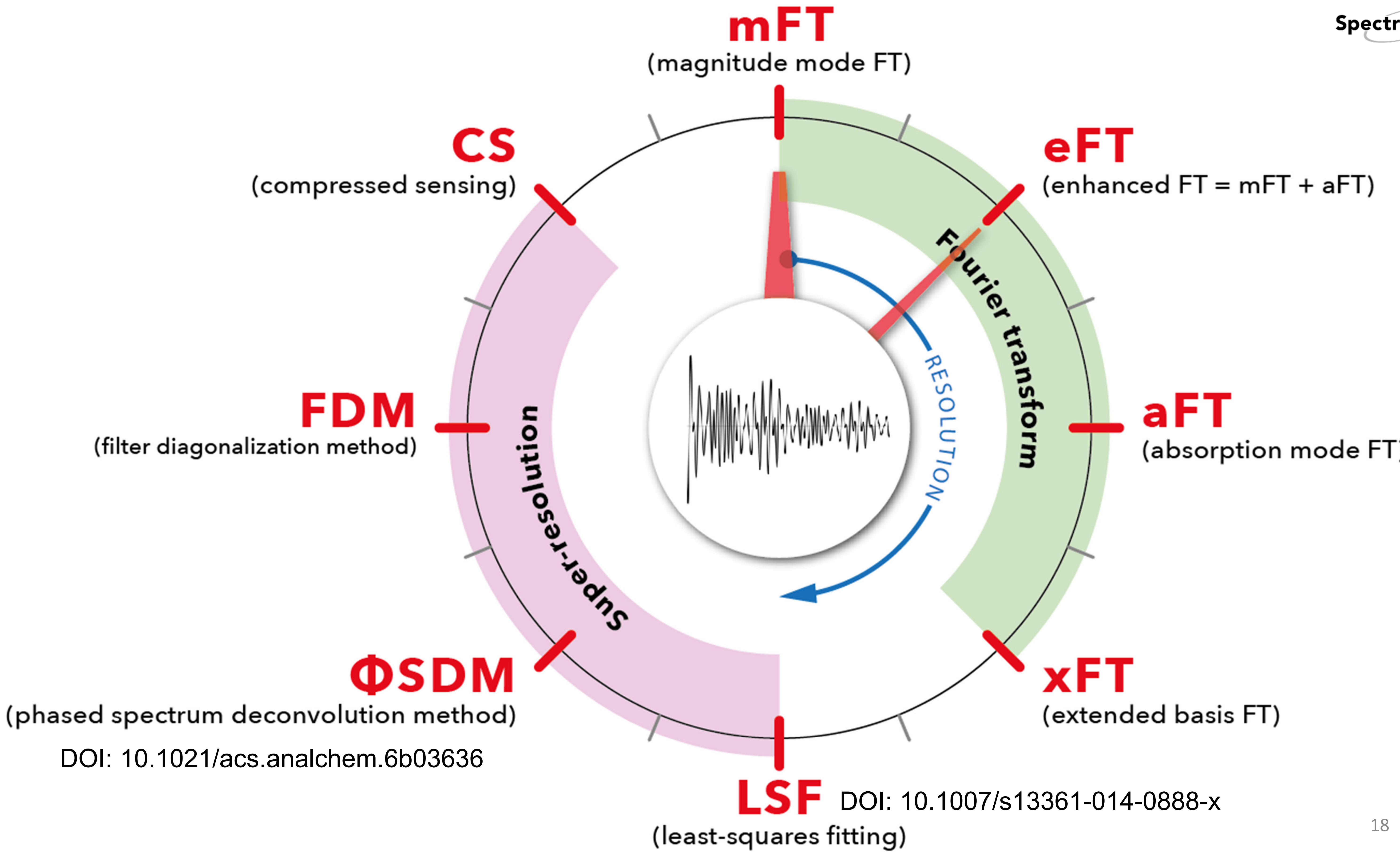
How to Maximize the Information Output

I. Ion Physics

II. Electronics: Ion Signal Recording

III. Software: Data Processing

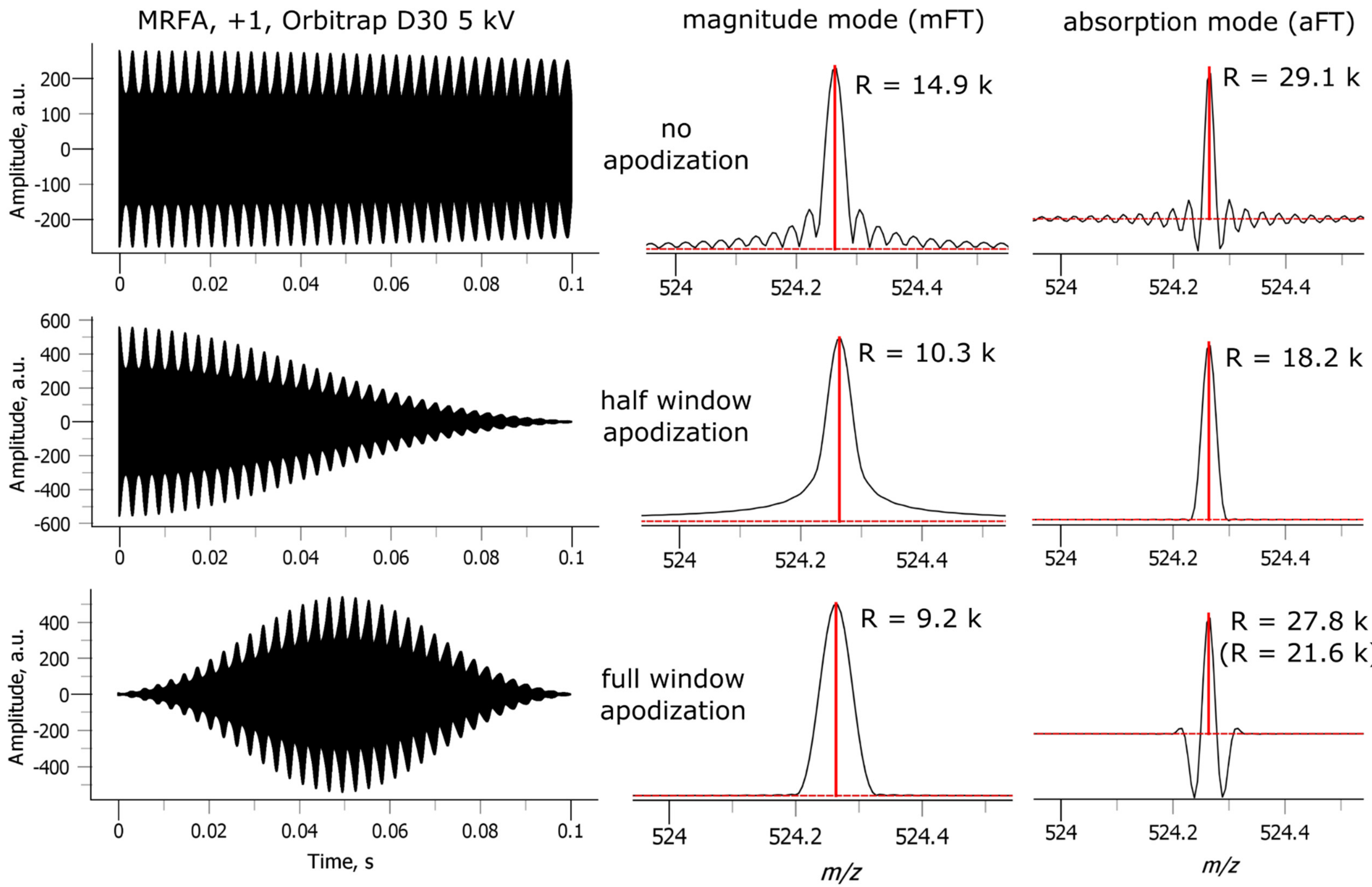
IV. Examples



DOI: 10.1021/acs.analchem.6b03636
DOI: 10.1007/s13361-014-0888-x

Fourier Transforms in FTMS: mFT and aFT

- **aFT** provides (twice) higher resolution than **mFT** (a function of apodization window)
- **aFT** mass spectra (full profile) have both positive and negative data points



Orbitrap data simulation

ACS Partner Journal

Journal of the American Society for Mass Spectrometry

pubs.acs.org/jasms — Research Article

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*

Cite This: https://dx.doi.org/10.1021/jasms.0c00190 Read Online

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ABSTRACT: Fourier transform mass spectrometry (FTMS) applications require accurate analysis of extremely complex mixtures of species in wide mass and charge state ranges. To optimize the related FTMS data analysis accuracy, parameters for data acquisition and the allied data processing should be selected rationally, and their influence on the data analysis outcome is to be understood. To facilitate this selection process and to guide the experiment design and data processing workflows, we implemented the underlying algorithms in a software tool with a graphical user interface, FTMS Isotopic Simulator. This tool computes FTMS data via time-domain data (transient) simulations for user-defined molecular species of interest and FTMS instruments, including diverse Orbitrap FTMS models, followed by user-specified FT processing steps. Herein, we describe implementation and benchmarking of this tool for analysis of a wide range of compounds as well as compare simulated and experimentally generated FTMS data. In particular, we discuss the use of this simulation tool for narrowband, broadband, and low- and high-resolution analysis of small molecules, peptides, and proteins, up to the level of their isotopic fine structures. By demonstrating the allied FT processing artifacts, we raise awareness of a proper selection of FT processing parameters for modern applications of FTMS, including intact mass analysis of proteoforms and top-down proteomics. Overall, the described transient-mediated approach to simulate FTMS data has proven useful for supporting contemporary FTMS applications. We also find its utility in fundamental FTMS studies and creating didactic materials for FTMS teaching.

Simulated transient — FT mass spectrum (MHz, Time, FT, m/z)

KEYWORDS: *Orbitrap, ion cyclotron resonance, Fourier transform, absorption mode Fourier transform, magnitude mode Fourier transform, apodization, top-down proteomics, isotopic fine structure*

■ INTRODUCTION

To address the growing measurement accuracy requirements of modern Fourier transform mass spectrometry (FTMS) applications, revealing and explaining artifacts in FT mass spectra are among the primary topics of fundamental studies reported in the past years.[1−4] Luckily, in the prior decades, the nuclear magnetic resonance (NMR) spectroscopy and ion cyclotron resonance (ICR) FTMS communities have developed a solid knowledge base and understanding of the underlying physical phenomena, including fundamentals of data acquisition and data processing in FT-based spectroscopy and spectrometry.[1,5−11] Recently, these have been projected onto the principles of data acquisition and processing with Orbitrap FTMS.[12−14]

In a comprehensive study related to understanding FT artifacts, Rockwood and Erve described reasons why and how the peaks observed in FT mass spectra can differ from those expected from a linear processing of information encoded in transients on the isotopic fine structure (IFS) level of the corresponding compounds.[15] While their report focused exclusively on analysis of artifacts in mass spectra represented in magnitude FT (mFT) mode and as a result of apodization with a full (Hann) window, a number of conclusions and predictions they draw extend to the broader field of FTMS, addressing also modern data processing approaches. Their findings, among others, shed light on the earlier FT-ICR MS studies which discussed in depth relationships between the transient length, beat patterns in transients, and the resulting mass spectra. Although not commonly described, the beat pattern is an important characteristic of FTMS transients.[1,4,14,16,17] It arises due to a constructive and destructive interference from multiple sinusoidal ion signals with closely separated and nearly equidistant frequencies. Such ion signals can be produced by isotopic envelopes. For example, Easterling and co-workers reported that, when analyzing broad distributions of polymers, a transient length that is acceptable for analysis of high mass polymers is not the same as the one

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J. Am. Soc. Mass Spectrom. XXXX, XXX, XXX−XXX

JASMS 2020, 31, 1927-1942

.RAW Mass Spectra: Enhanced FT (**eFT**)

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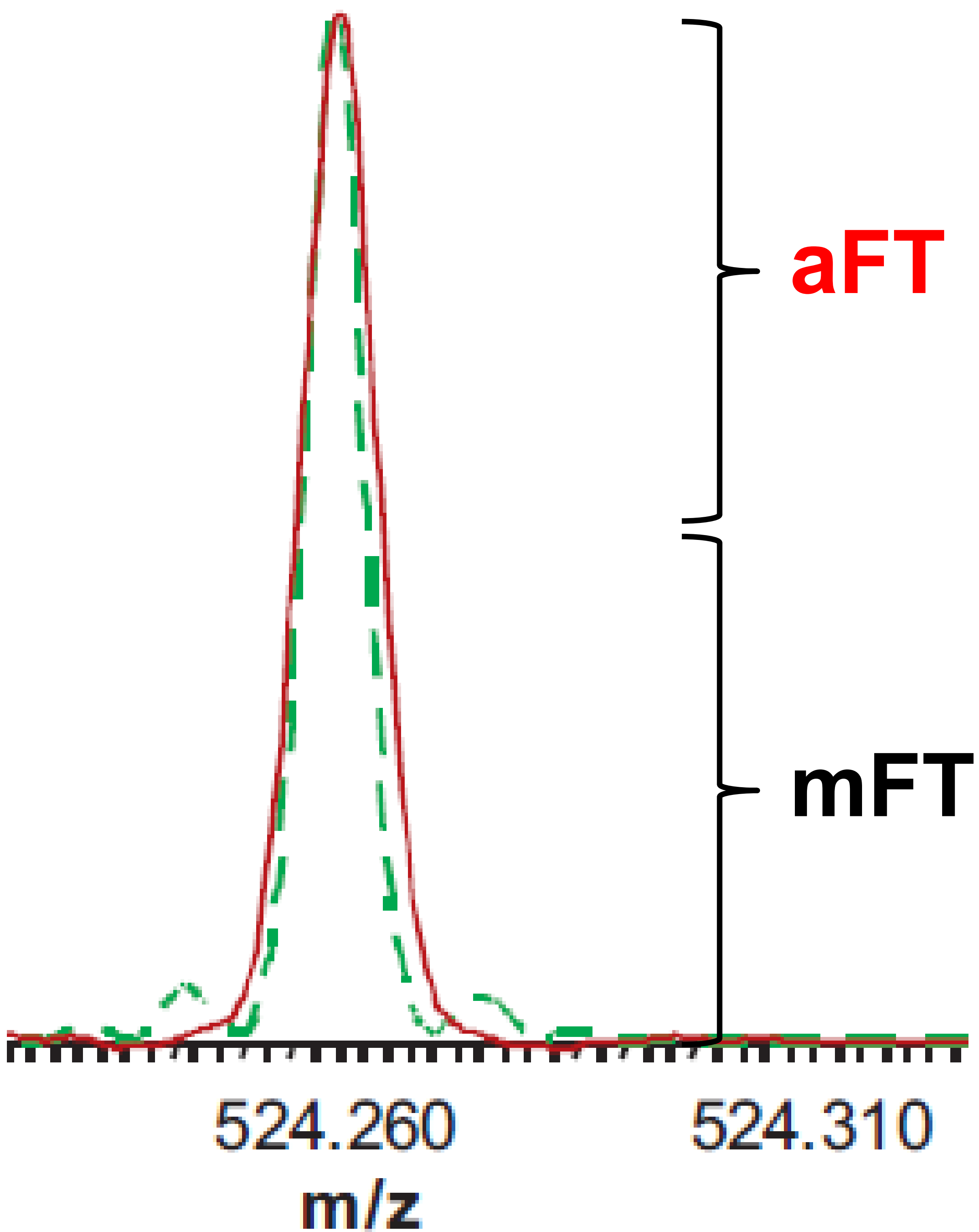


- **aFT** (especially with half window) requires exceptional performance of data acquisition electronics (transient quality)
- **eFT** is an efficient & fast algorithm to plot mass spectra matching the (moderate) quality of transient data
- **eFT** mass spectra have only positive data points (a limitation for sensitivity & dynamic range upon spectral averaging)

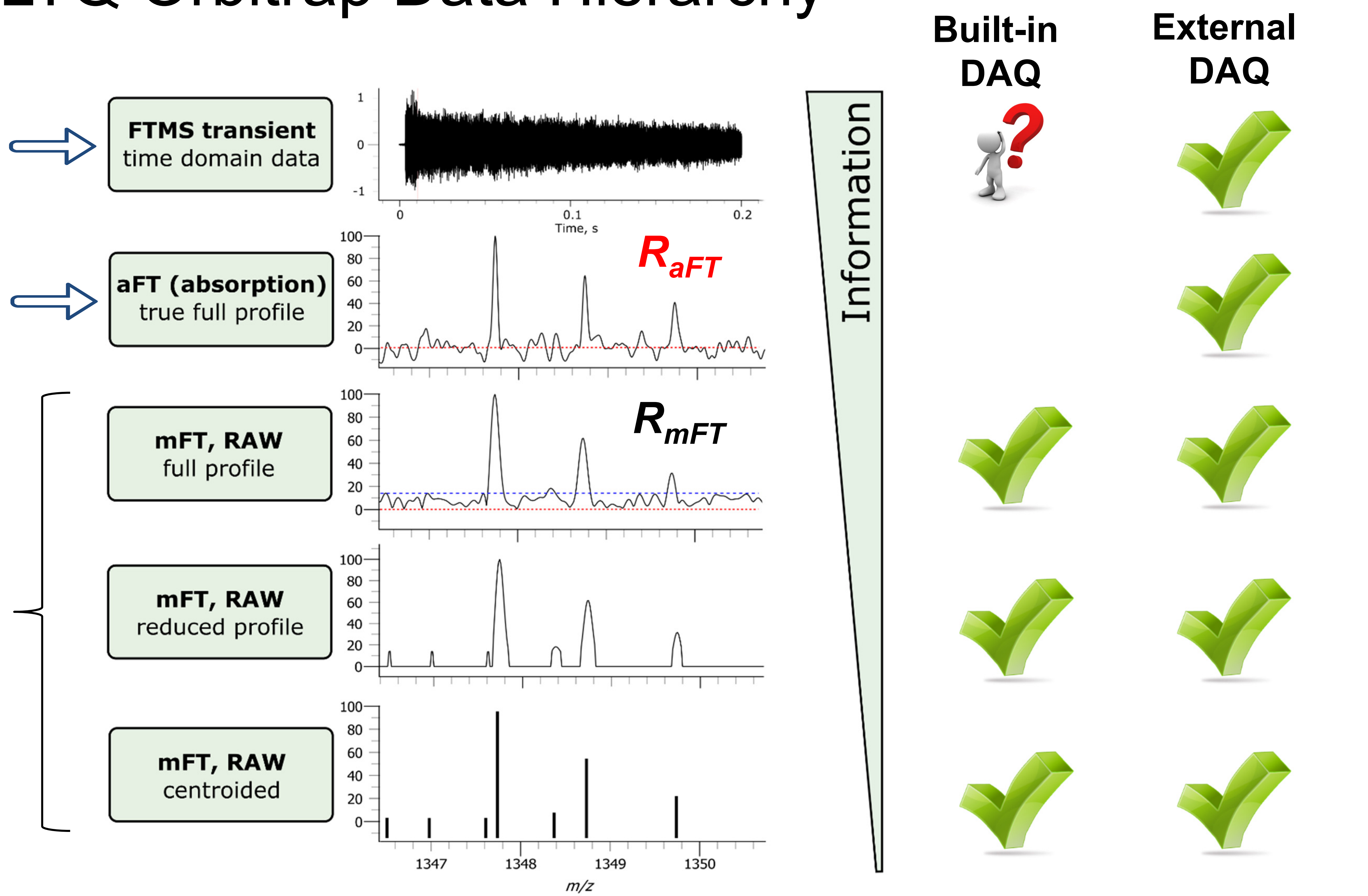
Lange, Makarov, et al. IJMS 369 (2014) 16–22

LTQ Orbitrap Data Hierarchy

aFT - absorption FT
with external DAQ system
or via post-processing
(AutoPhaser, *etc.*)

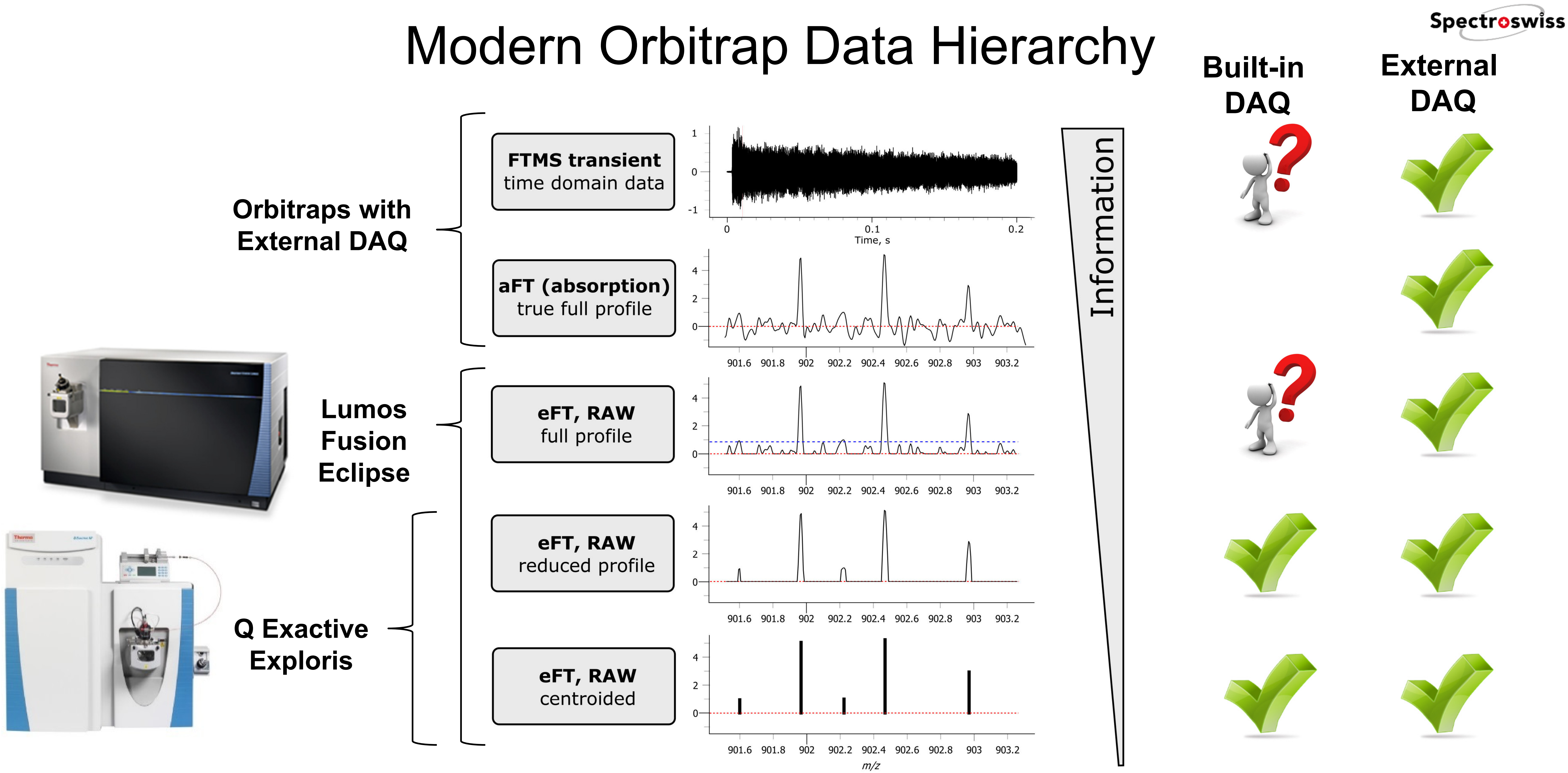
mFT - magnitude FT
(LTQ Orbitrap XL, Velos)

$R_{aFT} > R_{mFT}$



External DAQ unlocks superior performance of original LTQ Orbitraps

Modern Orbitrap Data Hierarchy



External DAQ unlocks superior performance of modern Orbitraps

3 How to Maximize the Information Output: **Software**

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- **Toward full data processing**
  - Transient processing (*e.g.*, data-dependent transient averaging)
  - Mass spectra representation in absorption mode FT (pos & neg values!)
  - Full profile aFT/eFT mass spectra processing (*e.g.*, spectral averaging)
  - Single ion transient-level analysis: charge state determination (STORI)
  - Super-resolution processing of transients (least-squares fitting, FDM, ΦSDM)
- **Advanced data processing approaches**
  - Noise thresholding, peak picking, recalibration, *etc*.
  - SIM stitching approaches (including noise thresholding for each SIM window)
  - Data averaging across multiple technical replicates (MS, LC/GC MS, mobility)
  - Rational selection of FT processing parameters (apodization window, *etc*.)
  - Compare experimental and simulated (via transients!) Orbitrap data

How to Maximize the Information Output

I. Ion Physics

II. Electronics: Ion Signal Recording

III. Software: Data Processing

IV. Examples

aFT performance @ Q Exactive HF

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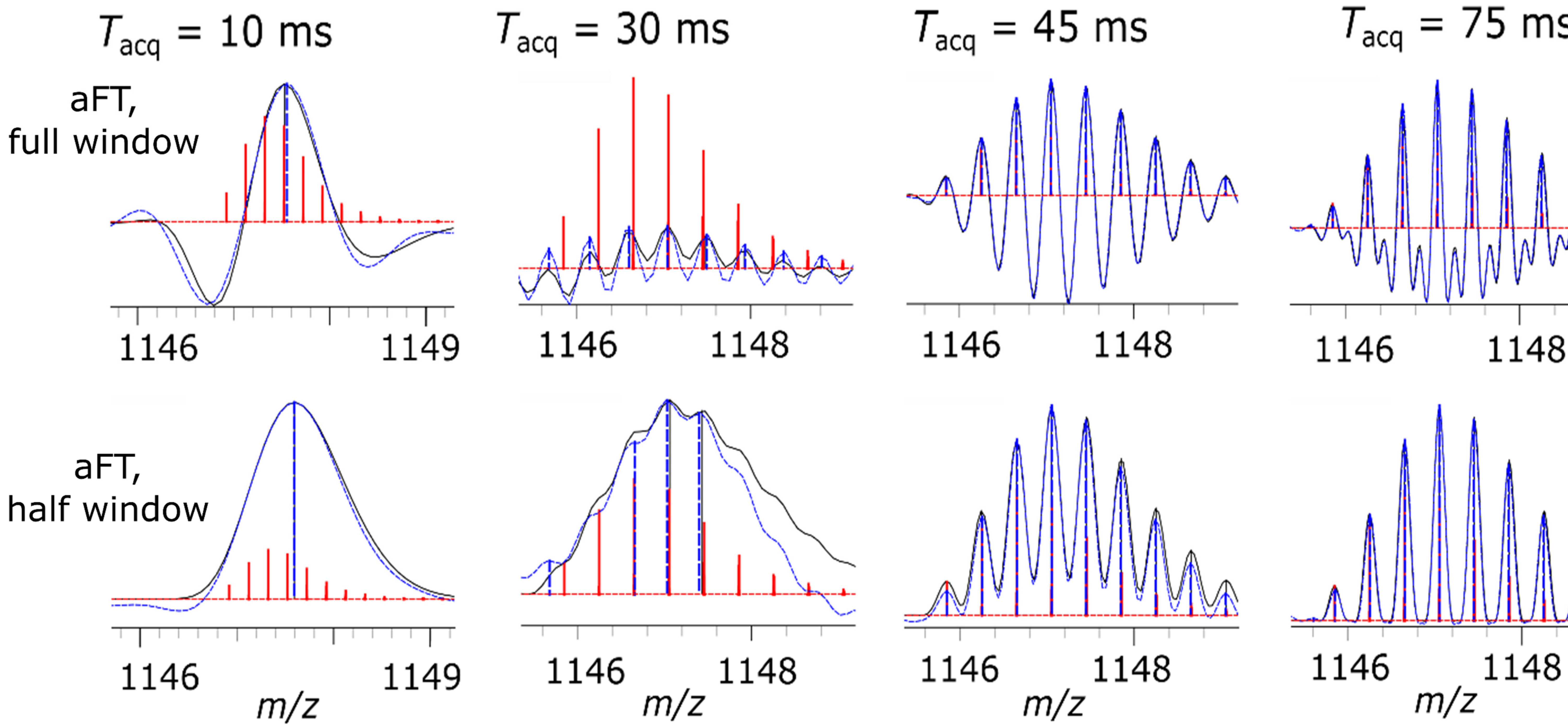
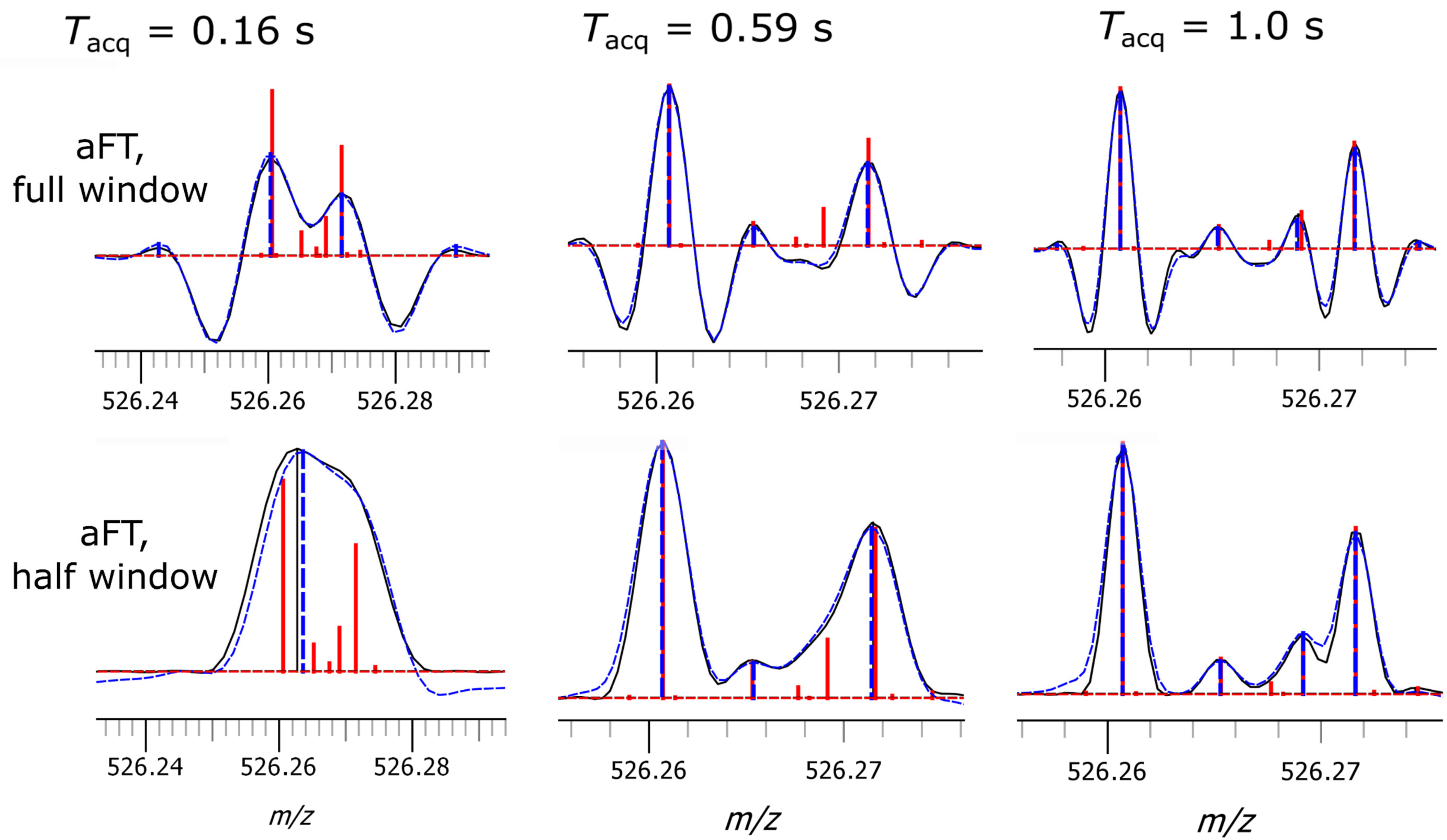


Experimental (Blue) vs. Simulated (Black) Mass Spectra of Insulin 5+

DOI: *10.1021/jasms.0c00190*

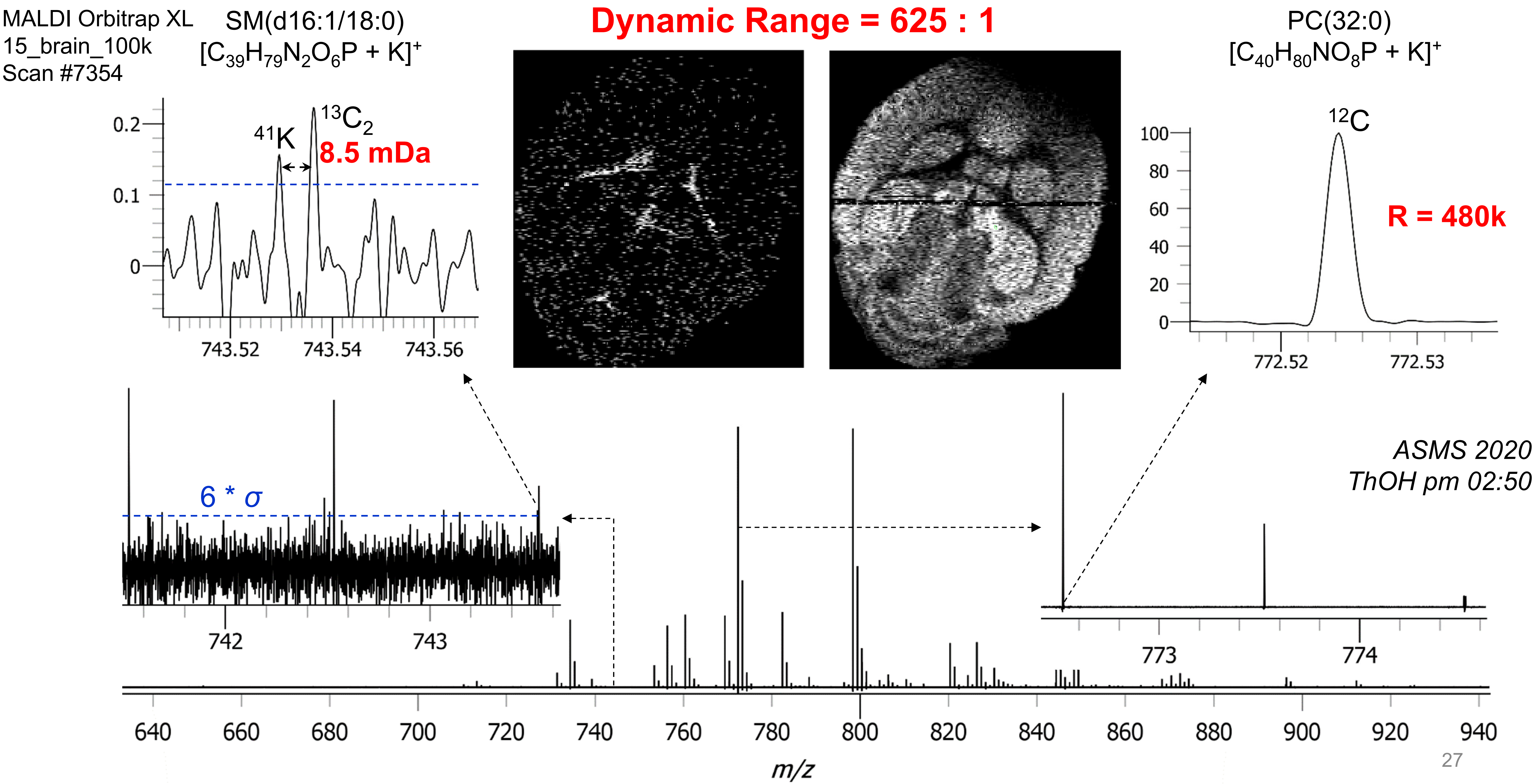
aFT performance @ Q Exactive HF



IFS: Experimental (Blue) vs. Simulated (Black) Mass Spectra of MRFA, A+2

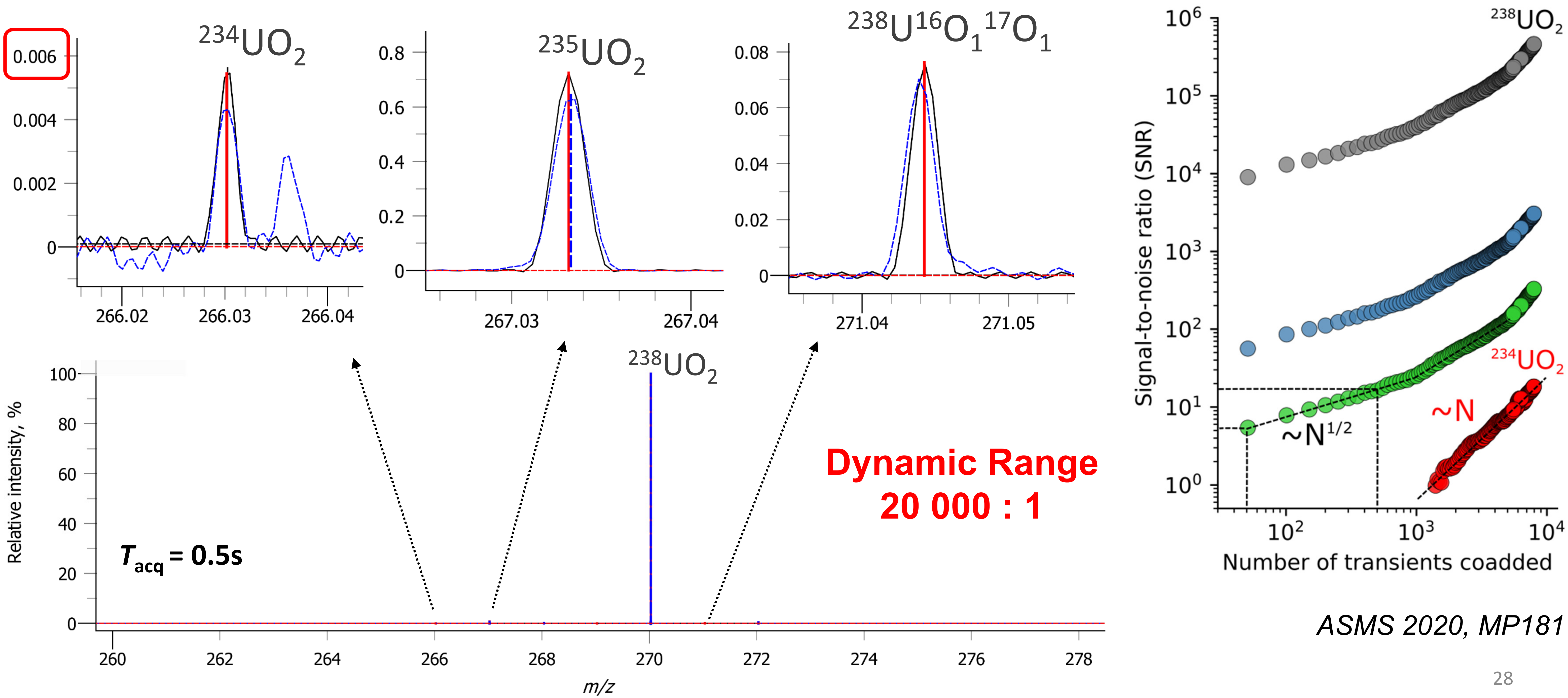
DOI: *10.1021/jasms.0c00190*

Benefits of Longer Transients and aFT on LTQ OT XL



Maximizing the Dynamic Range: Transient Averaging

- Uranium isotopes can be detected in a single broadband mass spectrum
- QE Focus experimental data (in blue) is compared with simulated data (in black)



ASMS 2020, MP181

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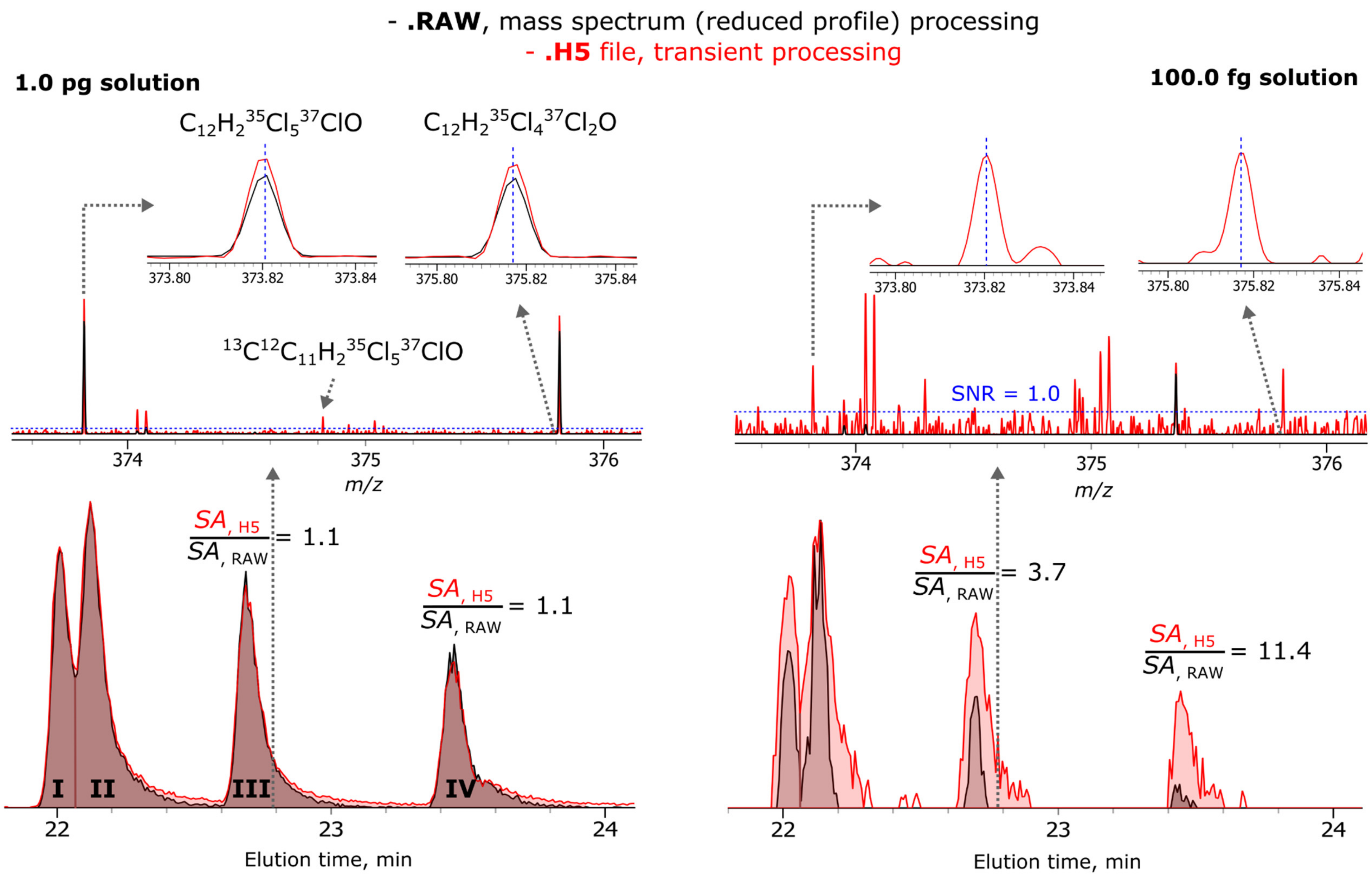
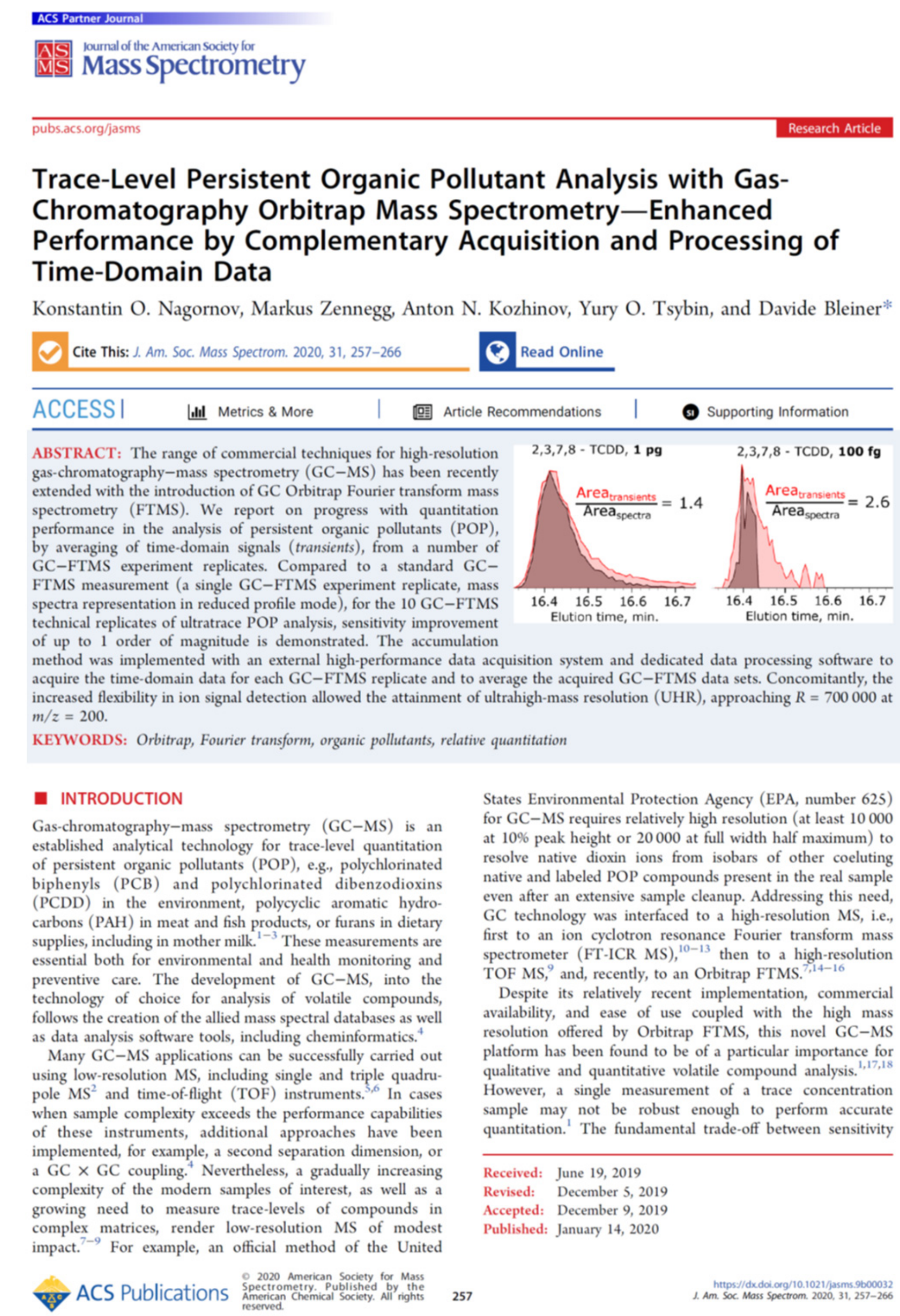
Data Averaging Across Technical Replicates: GC Orbitrap




ACS Partner Journal
Journal of the American Society for Mass Spectrometry
pubs.acs.org/jasms
Research Article

Trace-Level Persistent Organic Pollutant Analysis with Gas-Chromatography Orbitrap Mass Spectrometry—Enhanced Performance by Complementary Acquisition and Processing of Time-Domain Data

Konstantin O. Nagornov, Markus Zennegg, Anton N. Kozhinov, Yury O. Tsybin, and Davide Bleiner*

Cite This: J. Am. Soc. Mass Spectrom. 2020, 31, 257−266
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ABSTRACT: The range of commercial techniques for high-resolution gas-chromatography−mass spectrometry (GC−MS) has been recently extended with the introduction of GC Orbitrap Fourier transform mass spectrometry (FTMS). We report on progress with quantitation performance in the analysis of persistent organic pollutants (POP), by averaging of time-domain signals (*transients*), from a number of GC−FTMS experiment replicates. Compared to a standard GC−FTMS measurement (a single GC−FTMS experiment replicate, mass spectra representation in reduced profile mode), for the 10 GC−FTMS technical replicates of ultratrace POP analysis, sensitivity improvement of up to 1 order of magnitude is demonstrated. The accumulation method was implemented with an external high-performance data acquisition system and dedicated data processing software to acquire the time-domain data for each GC−FTMS replicate and to average the acquired GC−FTMS data sets. Concomitantly, the increased flexibility in ion signal detection allowed the attainment of ultrahigh-mass resolution (UHR), approaching R = 700 000 at m/z = 200.

KEYWORDS: *Orbitrap, Fourier transform, organic pollutants, relative quantitation*

INTRODUCTION

Gas-chromatography−mass spectrometry (GC−MS) is an established analytical technology for trace-level quantitation of persistent organic pollutants (POP), e.g., polychlorinated biphenyls (PCB) and polychlorinated dibenzodioxins (PCDD) in the environment, polycyclic aromatic hydrocarbons (PAH) in meat and fish products, or furans in dietary supplies, including in mother milk.[1−3] These measurements are essential both for environmental and health monitoring and preventive care. The development of GC−MS, into the technology of choice for analysis of volatile compounds, follows the creation of the allied mass spectral databases as well as data analysis software tools, including cheminformatics.[4]

Many GC−MS applications can be successfully carried out using low-resolution MS, including single and triple quadrupole MS[2] and time-of-flight (TOF) instruments.[5,6] In cases when sample complexity exceeds the performance capabilities of these instruments, additional approaches have been implemented, for example, a second separation dimension, or a GC × GC coupling.[4] Nevertheless, a gradually increasing complexity of the modern samples of interest, as well as a growing need to measure trace-levels of compounds in complex matrices, render low-resolution MS of modest impact.[7−9] For example, an official method of the United States Environmental Protection Agency (EPA, number 625) for GC−MS requires relatively high resolution (at least 10 000 at 10% peak height or 20 000 at full width half maximum) to resolve native dioxin ions from isobars of other coeluting native and labeled POP compounds present in the real sample even after an extensive sample cleanup. Addressing this need, GC technology was interfaced to a high-resolution MS, i.e., first to an ion cyclotron resonance Fourier transform mass spectrometer (FT-ICR MS),[10−13] then to a high-resolution TOF MS,[9] and, recently, to an Orbitrap FTMS.[7,14−16]

Despite its relatively recent implementation, commercial availability, and ease of use coupled with the high mass resolution offered by Orbitrap FTMS, this novel GC−MS platform has been found to be of a particular importance for qualitative and quantitative volatile compound analysis.[1,17,18] However, a single measurement of a trace concentration sample may not be robust enough to perform accurate quantitation.[1] The fundamental trade-off between sensitivity

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JASMS 2020, 31, 257-266

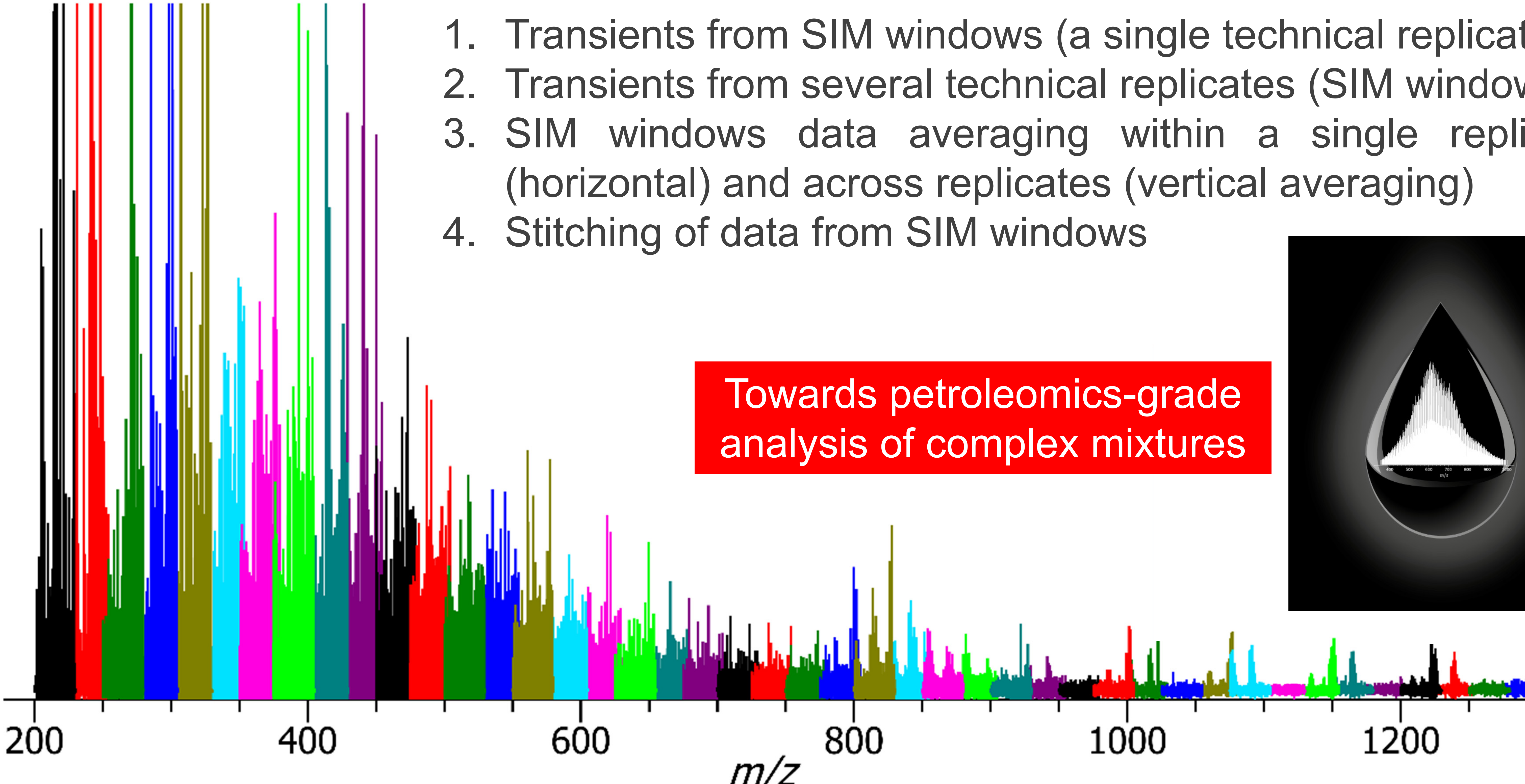
Enhanced sensitivity and quantitation accuracy for low-abundance compounds

Tackling the Space Charge & Sensitivity: SIM windows

Spectroswiss

1. Transients from SIM windows (a single technical replicate)
2. Transients from several technical replicates (SIM windows)
3. SIM windows data averaging within a single replicate (horizontal) and across replicates (vertical averaging)
4. Stitching of data from SIM windows

Towards petroleomics-grade analysis of complex mixtures

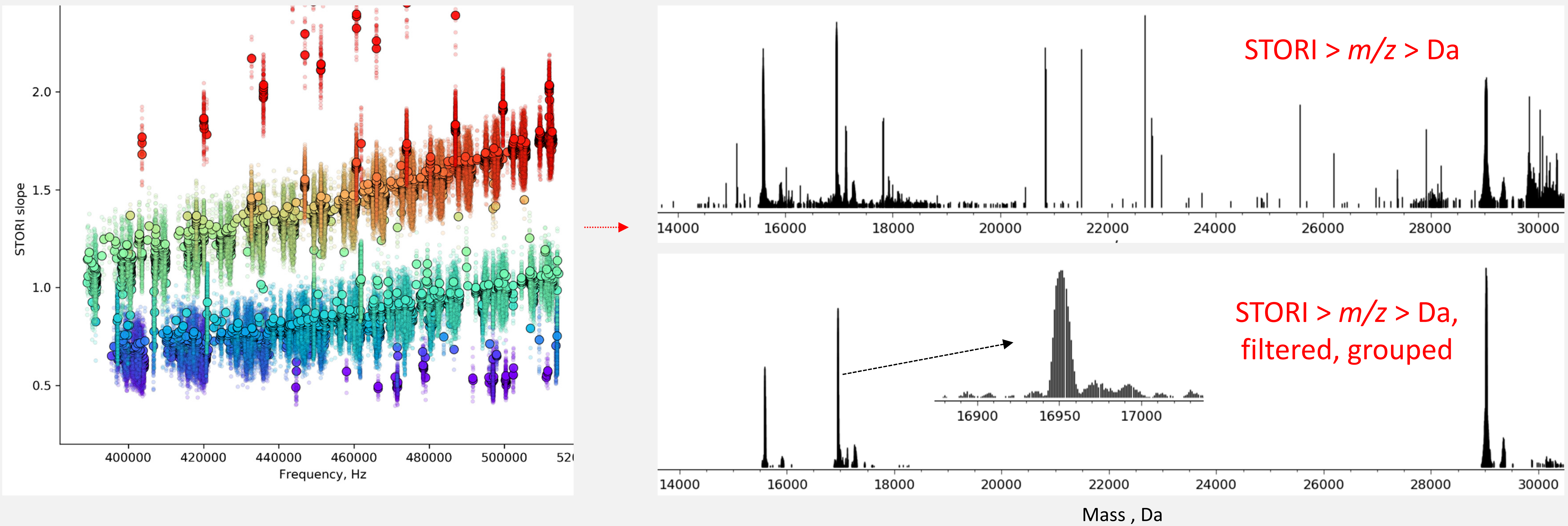


Single Ion Counting: Resolving Protein Complexity

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

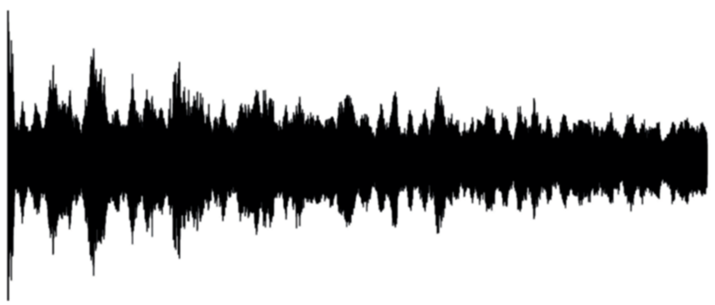
DOI: 10.1021/acs.analchem.9b01669; DOI: 10.1038/s41592-020-0764-5

Ex.: single ion counting on a Q Exactive HF for a mixture of model proteins (external DAQ acquisition)

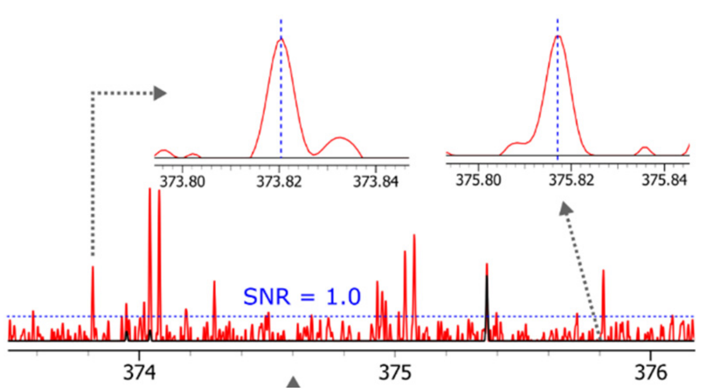


STORI: Selective Temporal Overview of Resonant Ions, DOI: 10.1021/jasms.8b06253

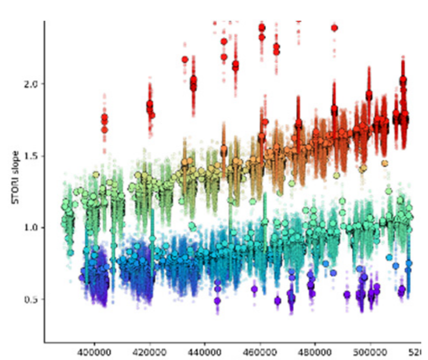
1 2 3 Summary



- Modern data acquisition electronics (new-generation, high-performance) allows to maximize information content encoded into time-domain signals (transients)

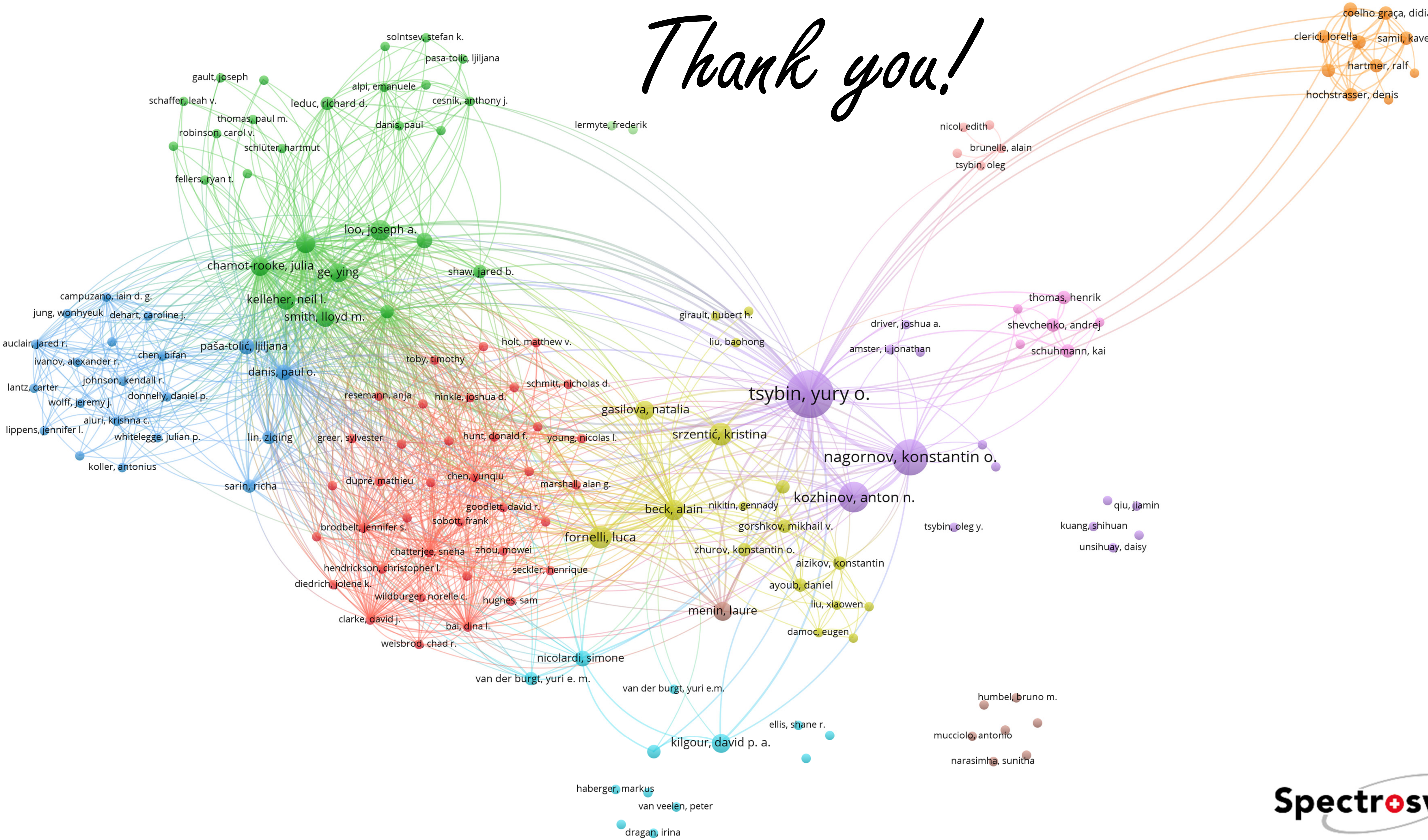


- Powerful data processing software (multi-processing, computationally efficient) allows to efficiently extract full information from transients and process any size data sets



- Novel transient-centered methods are gaining importance in challenging applications: single ion detection (I2MS), super-resolution and ultra-high resolution mass spectrometry

Thank you!



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