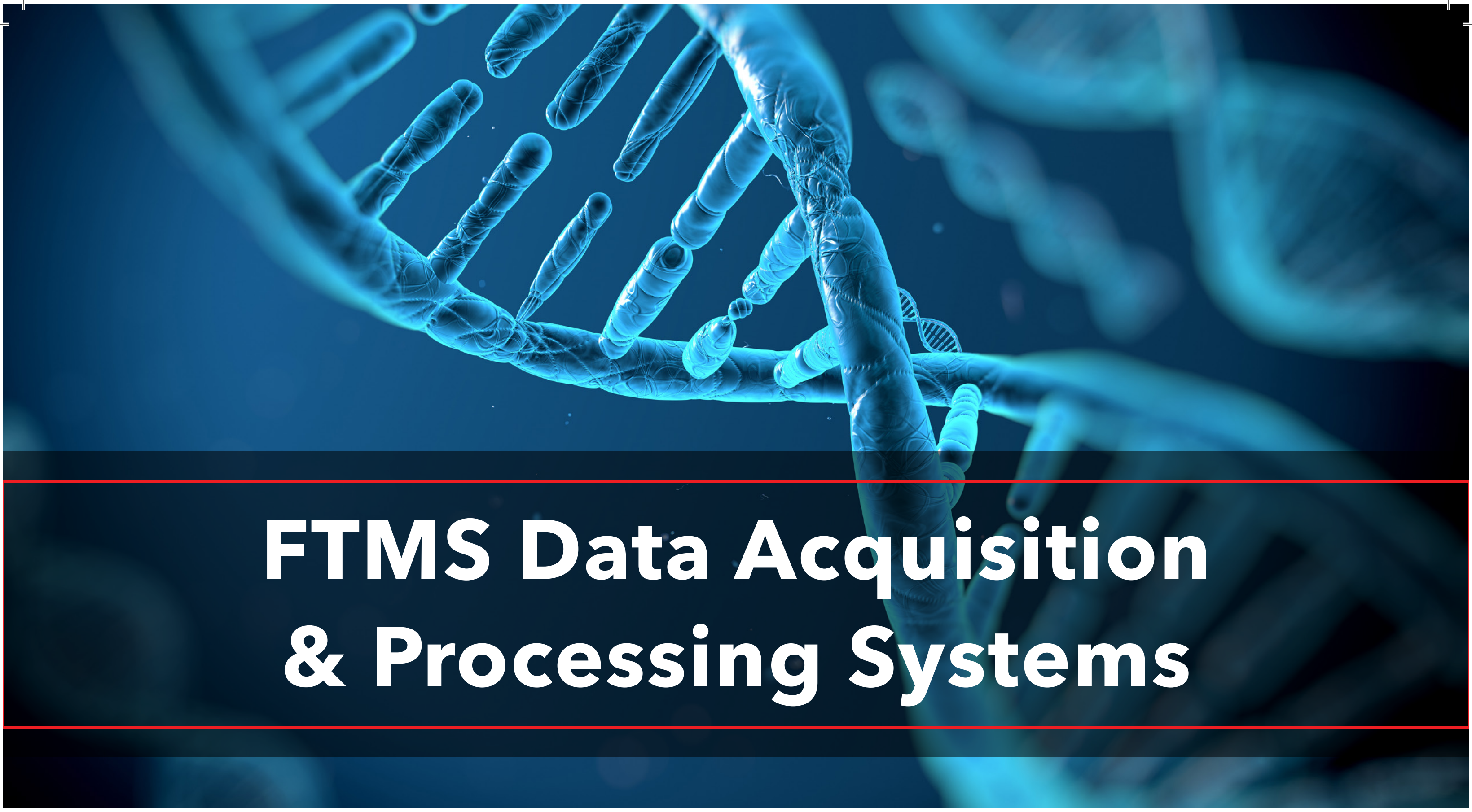
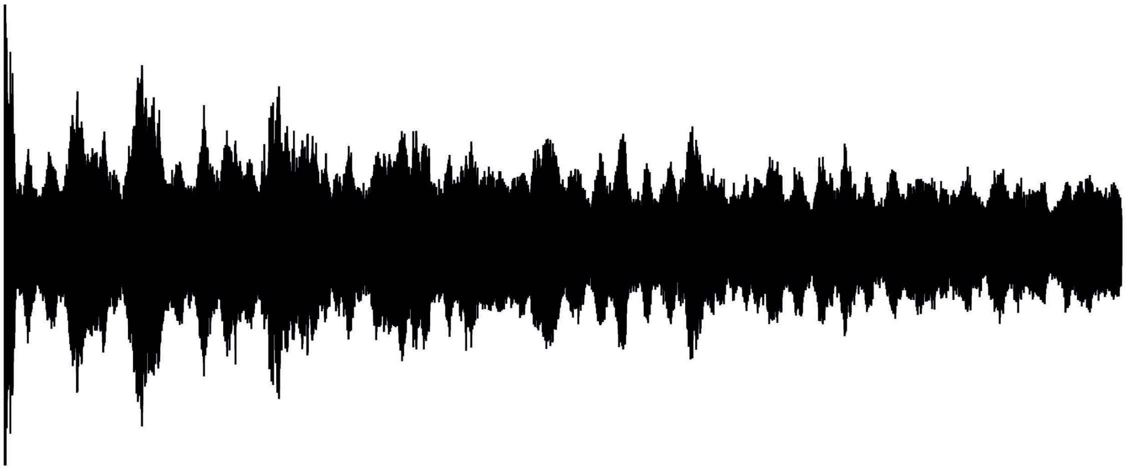


FTMS Data Acquisition & Processing Systems



Spectroswiss

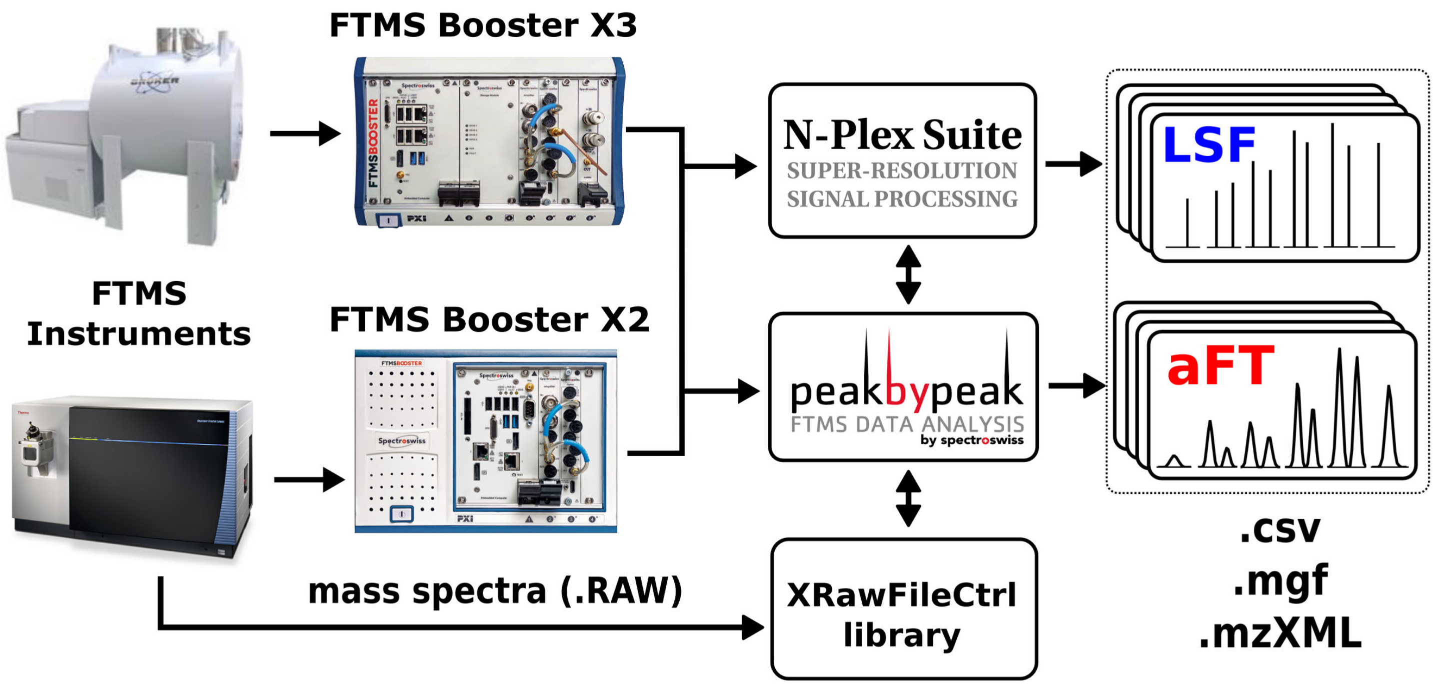
FTMS Data Acquisition & Processing Technologies

Most FTMS instruments provide only processed and noise-reduced data (mass spectra). The availability of the true raw data (FTMS transients) allows for the most efficient data processing including powerful time-frequency conversion using Fourier transform and super-resolution algorithms (e.g., least-squares fitting).

Our high-performance data acquisition systems (**FTMS Boosters**), alone or empowered by our software (**Peak-by-Peak**), provide a step change in FTMS data quality and processing sophistication, helping you to take on the most complex and challenging applications.

The accurately **phased transients** uniquely provided by FTMS Boosters improve performance of all signal processing algorithms. Disruptively, they enable in-hardware absorption mode FT spectral representation from any Orbitrap™ and ICR FTMS instrument.

In turn, **Peak-by-Peak** enables efficient processing of practically any size datasets of FTMS transients and mass spectra (represented in full or reduced profile, or centroided modes). Peak-by-Peak generates output files for a deep-dive analysis with the end-user software.



Workflows

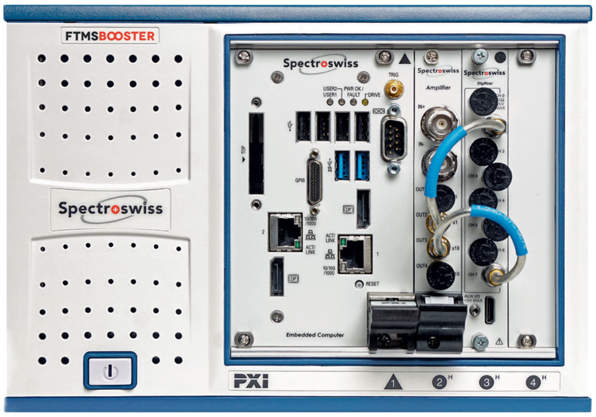
- Metabolomics
- Petroleomics
- GC applications
- Lipidomics
- Top-down MS
- Spectroscopy
- Imaging
- Proteomics
- On demand

FTMS Booster: Data Acquisition System

FTMS Booster X2

For Orbitrap™ transient acquisition

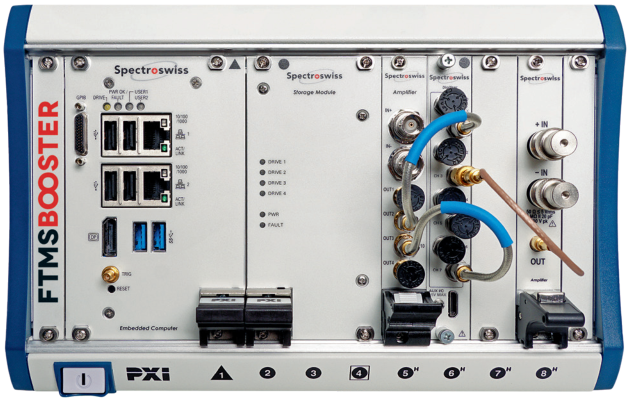
- Easy add-on to any Orbitrap™
- Works in parallel with FTMS manufacturer data acquisition
- Open file format of output data
- Unlocks absorption mode FT
- Full transient detection for enhanced sensitivity, resolution, and duty cycle
- Detection of signals from all ions contributing to the signal (full *m/z* range)
- Enables ultra-high resoluttion capability
- Maximizes sensitivity by acquiring two transients at a time, with different gains
- Allows transparent post-processing, data post-validation and re-analysis
- Rapid data transfer for measurements of large data sets and high duty cycles
- High-capacity (4TB) fast SSD buffer



FTMS Booster X3

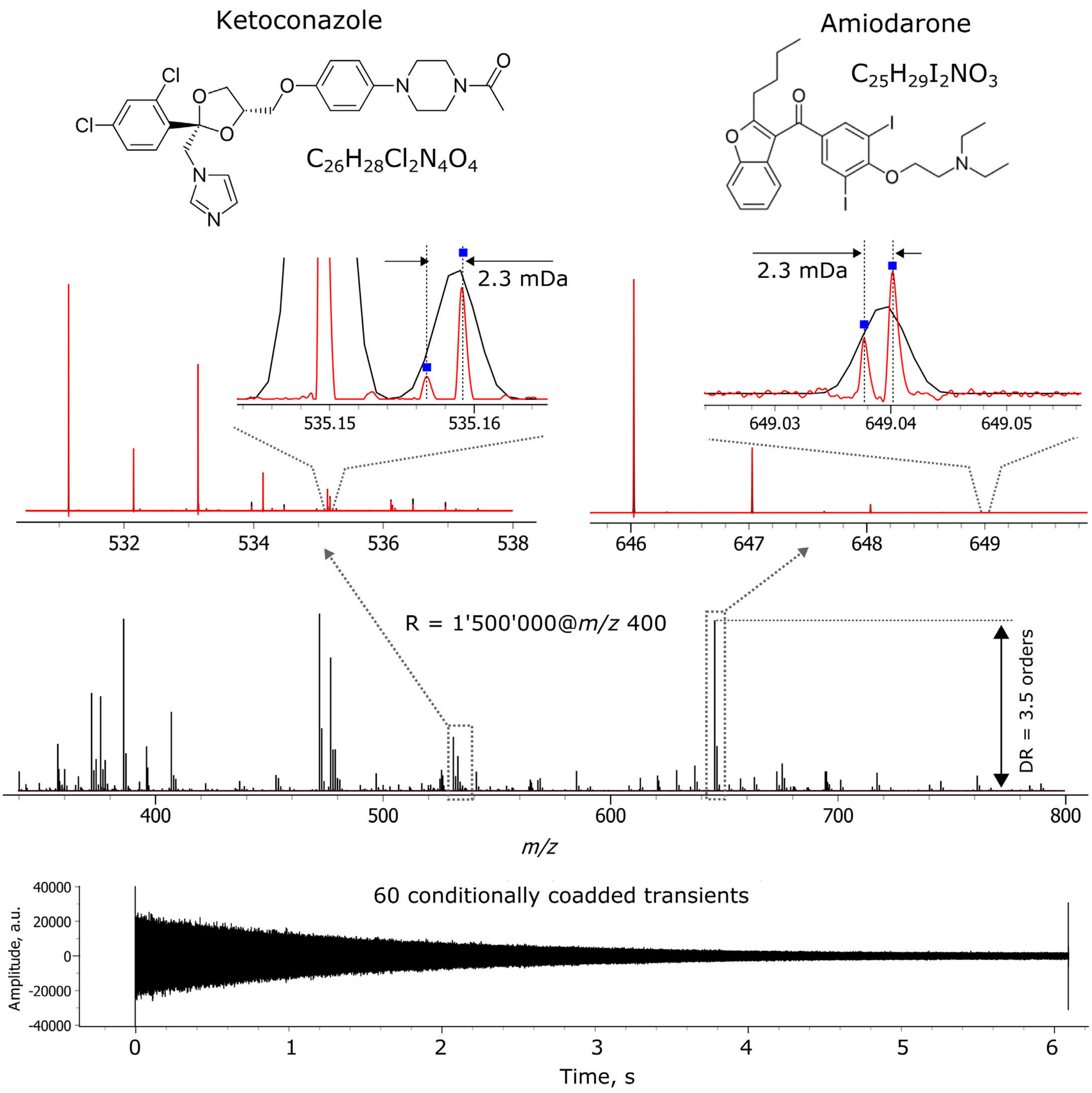
For FT-ICR MS transient acquisition

- All capabilities of FTMS Booster X2 technology
- Easy add-on to any FT-ICR MS instrument
- Acquisition of both excite and detect waveforms in FT-ICR MS
- Uniquely enables in-hardware absorption mode FT (aFT) for FT-ICR MS
- Powerful on-board data processing
- High-capacity (16 TB) fast SSD buffer for extended duration measurement campaigns for both ICR and Orbitrap™



Ultra-High Resolution Orbitrap™ FTMS

Ultra-high resolution can be enabled by acquisition and processing of **1-10 s** transients. Isotopic fine structure of lipids & drugs can be obtained with a **benchtop** Orbitrap™ FTMS. Complex mixture analysis (petroleomics, biofuels, top-down MS) can be enabled and facilitated



The isotopic fine structure **broadband** measurements of a mixture of metabolites, including ketoconazole and amiodarone, on a benchtop Q Exactive™ HF™ Orbitrap™ FTMS with FTMS Booster. Transient length is 6 s. Isotopic fidelity can be further improved via conditional co-adding of transients (Peak-by-Peak). Experimental settings : AGC=5e5 ; R=240’000 @ 200 *m/z*.

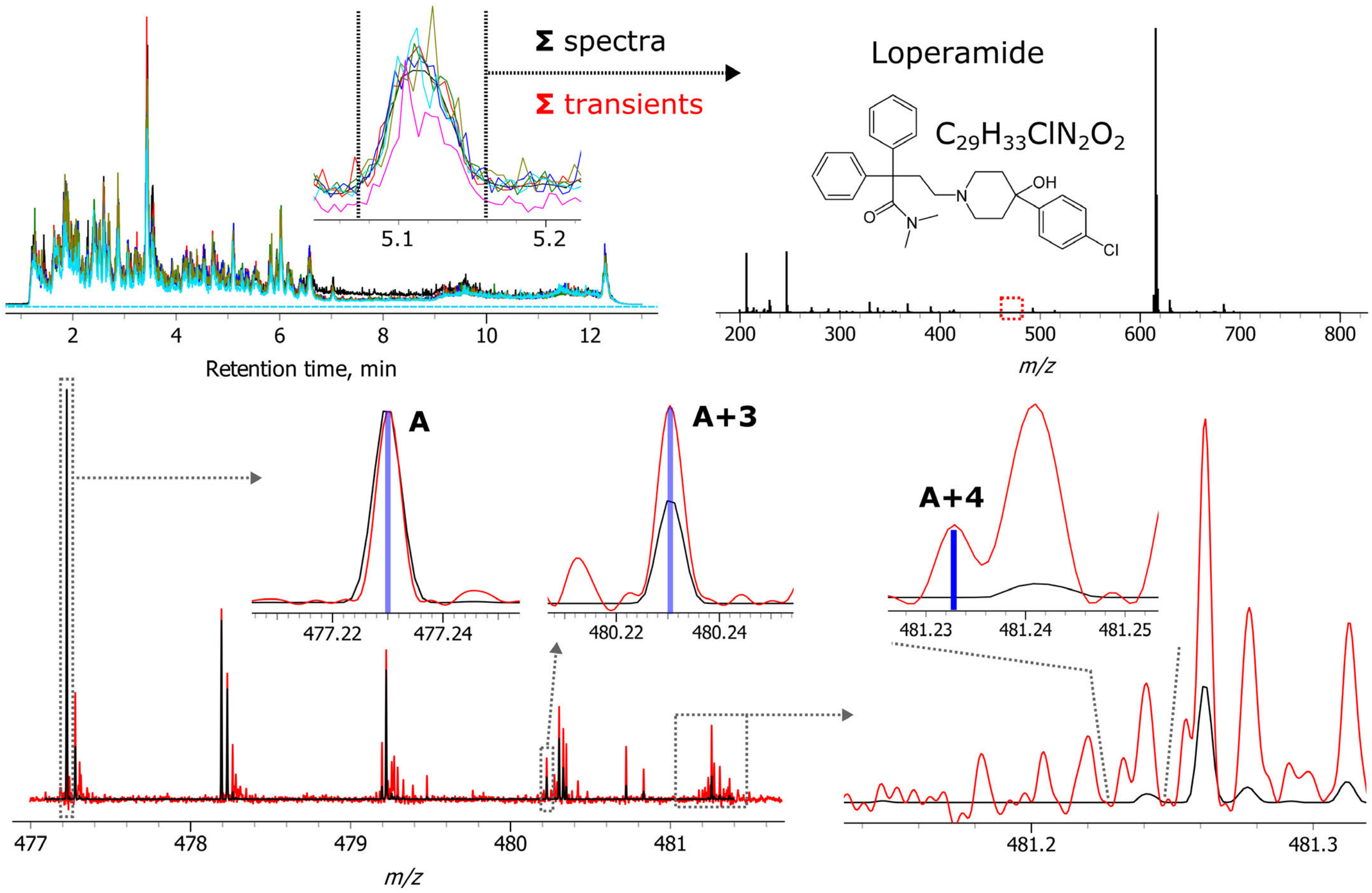
FTMS Sensitivity Increase: Multiplexed LC-MS

Peak-by-Peak enables **multiplexed transient and spectral averaging** from a number of separate LC-MS experiments (technical repetitions), offering an attractive opportunity for improving the sensitivity for precursor and product ion detection in LC-MS applications.

The provided increase in sensitivity (signal/noise ratio, S/N) is proportional to the square root of a number of LC-MS runs (or scans) when transients or full profile aFT mass spectra (without data reduction) are available for processing.

The use of mFT and eFT (.RAW) mass spectra in either reduced or full profile modes can also provide sensitivity benefits using this approach.

The multiplexed approach realized in Peak-by-Peak offers a targeted elution peak selection, chromatogram alignment and conditional co-adding of transients upon data averaging.

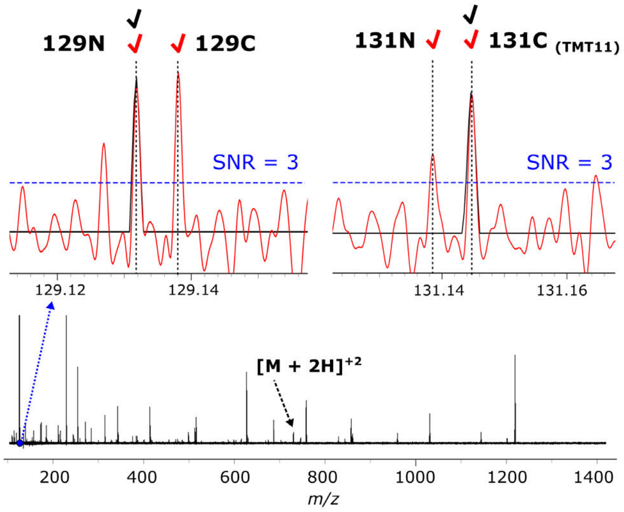


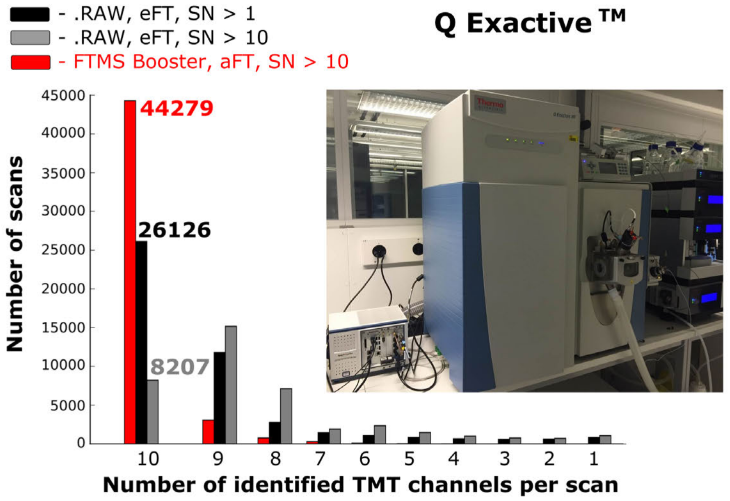
Metabolite mixture analysis using LC-MS on a Q Exactive™ Orbitrap™ FTMS. Seven chromatograms are first aligned by using non-linear binomial averaging functions (Peak-by-Peak). Spectral averaging (reduced profile eFT, .RAW, shown in **black**) vs. transient (full profile aFT, .H5, shown in **red**). The multiplexed LC-MS approach shows an increase in sensitivity, thus more confident metabolite isotopic structure analysis.

Srzentić, et al., Multiplexed middle-down mass spectrometry as a method for revealing light and heavy chain connectivity in a monoclonal antibody. Analytical Chemistry, (2018) 90, 12527−12535

Improved Multiplexed Quantitative Proteomics

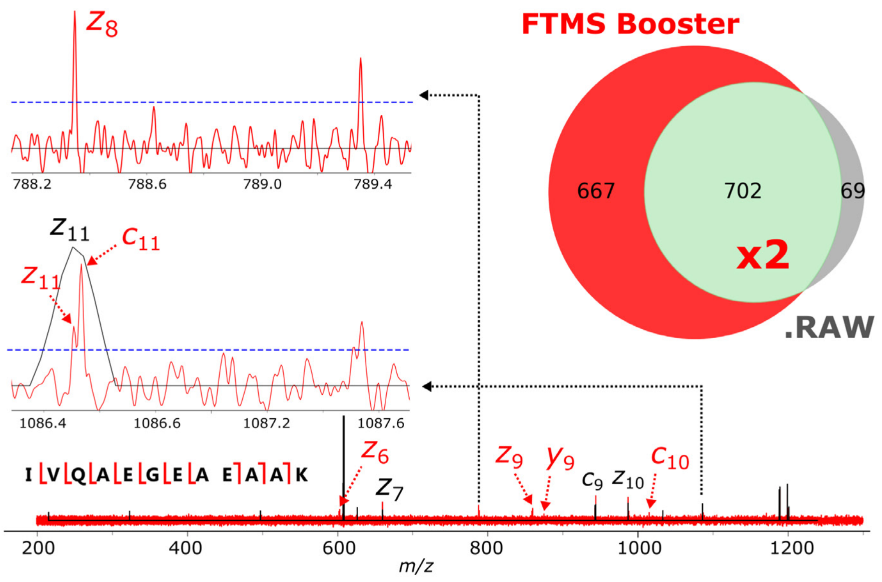
FTMS Booster and Peak-by-Peak deliver post-validated absorption mode FT (aFT) mass spectra in parallel to the regular mass spectral acquisition with an Orbitrap™ FTMS in enhanced FT mode (eFT, .RAW) to increase productivity and performance of Orbitrap™ FTMS for a multiplexed protein quantitation, e.g., using isobaric tandem mass tags, including the N-plex TMT™ technology.



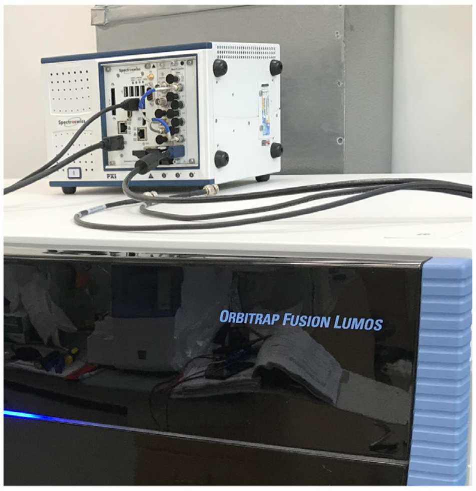


Example of an LC-MS/MS analysis of multiplex TMT™-labeled single-cell sample set on a Q Exactive™ Orbitrap™ FTMS, with FTMS Booster X2 and absorption mode FT (aFT, **red**). The aFT approach demonstrates quantitatively-accurate performance, even for very low S/N peaks. Data in **black** are from the original data acquisition electronics and software (Thermo Scientific).

Improved Protein Identification with ETD MS/MS



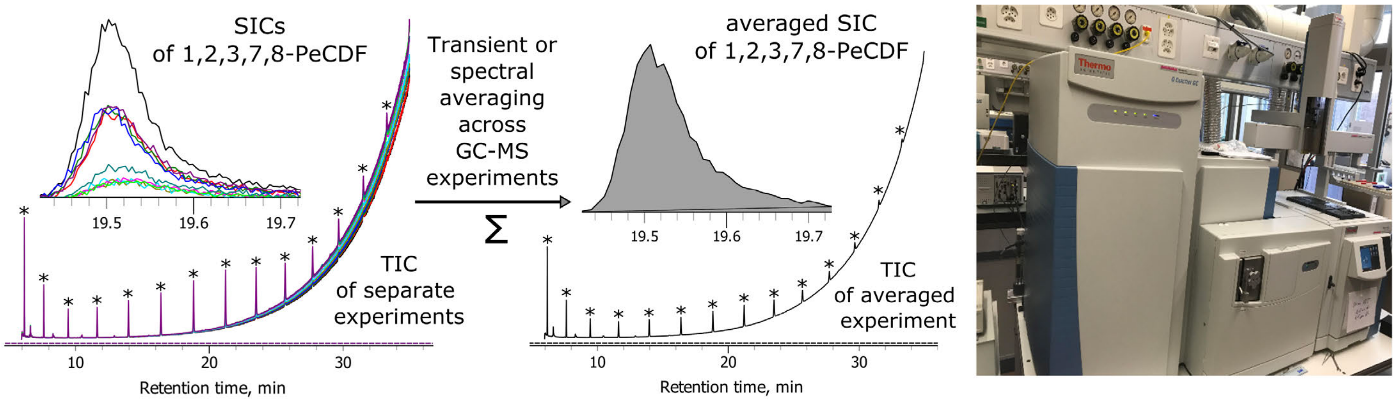
ETD Fusion™ Lumos ™



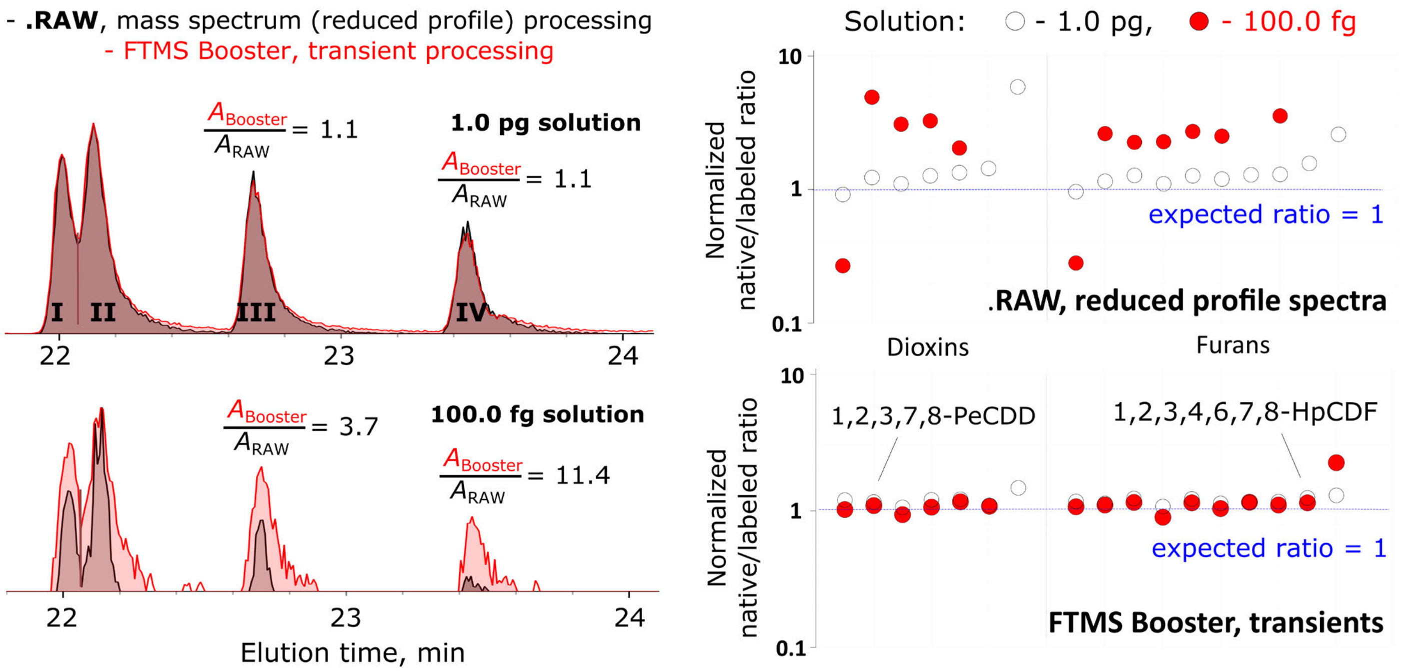
Bottom-up proteomics of a HeLa tryptic digest using LC-MS/MS with ETD on a Fusion™ Lumos™ Orbitrap™ FTMS. Enhanced duty cycle for ion detection enabled by FTMS Booster X2 results in improved resolution and product ion detection sensitivity. In turn, the number of identified proteins can be doubled in a single LC-MS/MS run in a considered here experiment.

Advancing GC Orbitrap Applications

Recent introduction of GC Orbitrap™ FTMS (Thermo Scientific) is a welcome addition of a high-resolution and high mass accuracy GC-MS platform to support the most complex GC-MS applications. Our data acquisition (FTMS Booster) and data processing (Peak-by-Peak) technologies can aid in advancing these applications even further.



FTMS Booster X2-interfaced Q Exactive™ GC Orbitrap™ FTMS analysis of standard and labeled dioxins, in collaboration with Prof. Davide Bleiner (Empa, Switzerland). The 10 acquired chromatograms (total ion current, electron impact ionization) are aligned and areas under the curve are calculated for selected ion chromatograms using Peak-by-Peak.



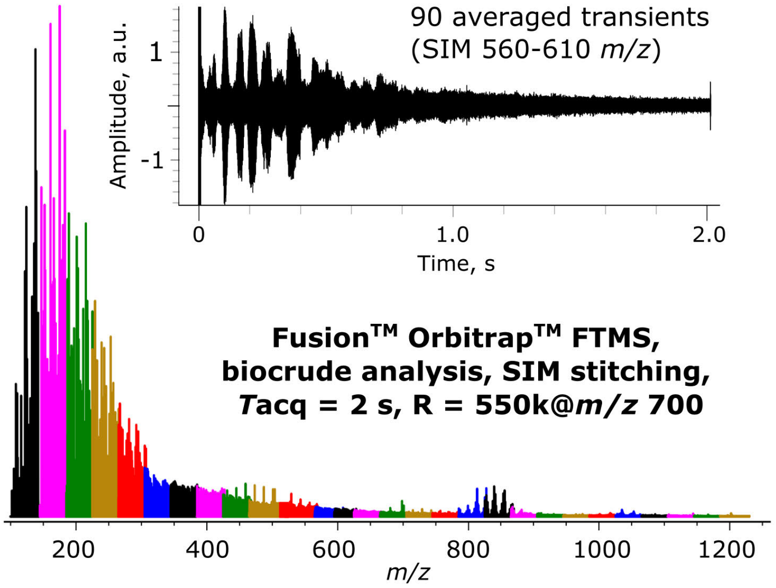
Quantitative measurements of dioxins in two samples: 1 pg and 100 fg concentration. Comparison of normalized peak areas obtained for light and heavy (labelled) dioxins shows substantially higher accuracy when transient averaging is performed (red filled circles and red trace, 10 GC-MS runs) versus spectral averaging (open circles and grey trace, 10 GC-MS runs).

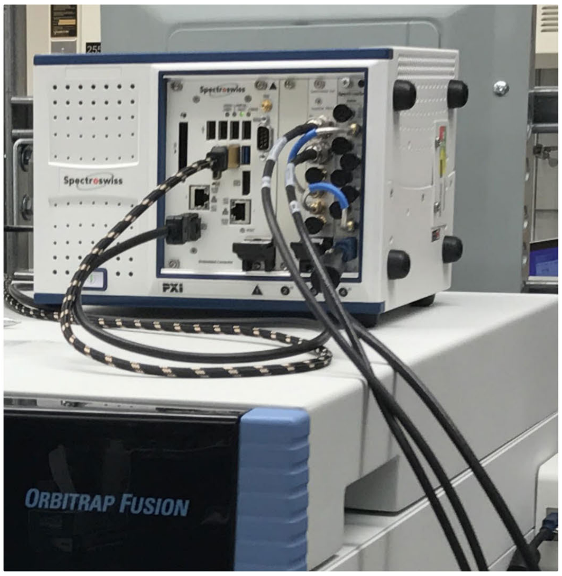
xCDD: polychlorinated dibenzo-para-dioxin
xCDF: polychlorinated dibenzo-para-furan

Empa
Materials Science and Technology

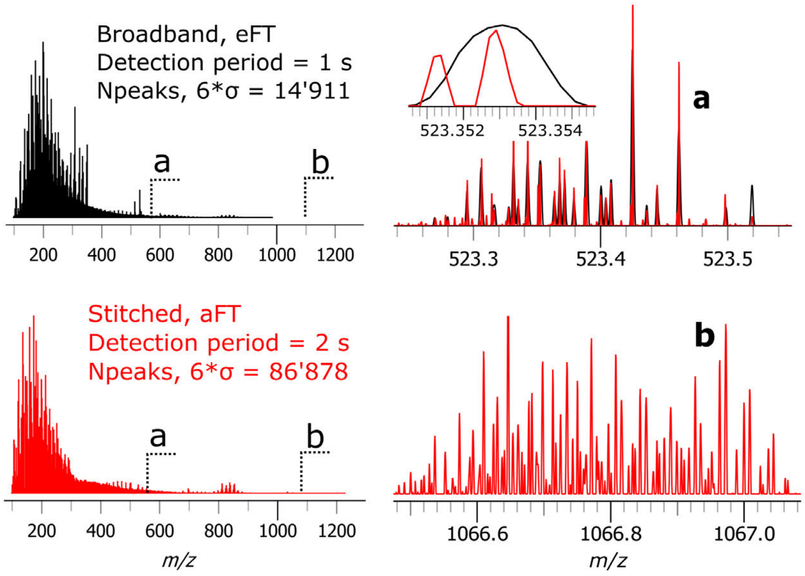
Nagornov, et al., Overcoming the sensitivity versus resolution trade-off in gas chromatography Orbitrap mass spectrometry by multiplexed digital processing, Submitted

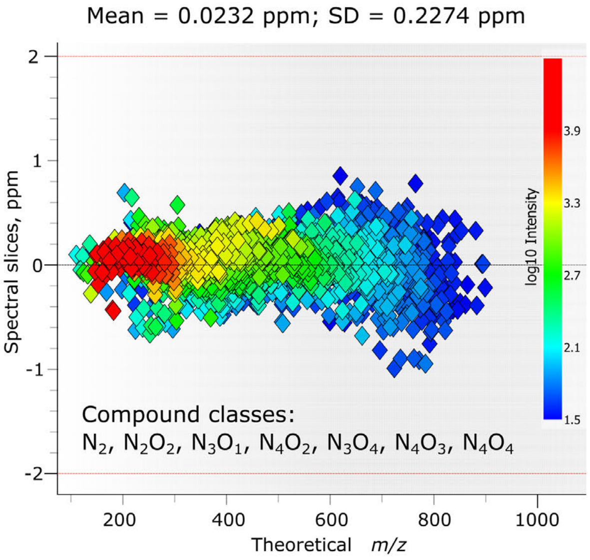
Improved LC-FTMS Petroleomics with Orbitraps





A biocrude analysis using SIM stitching procedure on a Fusion™ Orbitrap™ FTMS. Separate 50 *m/z*-wide SIM windows were acquired using FTMS Booster X2 and processed in aFT mode. Transient-averaged adjacent mass segments were stitched together on the spectral level. A representative transient for the *m/z* range 560-610 for 90 averaged time domain signals shows 2 second duration.





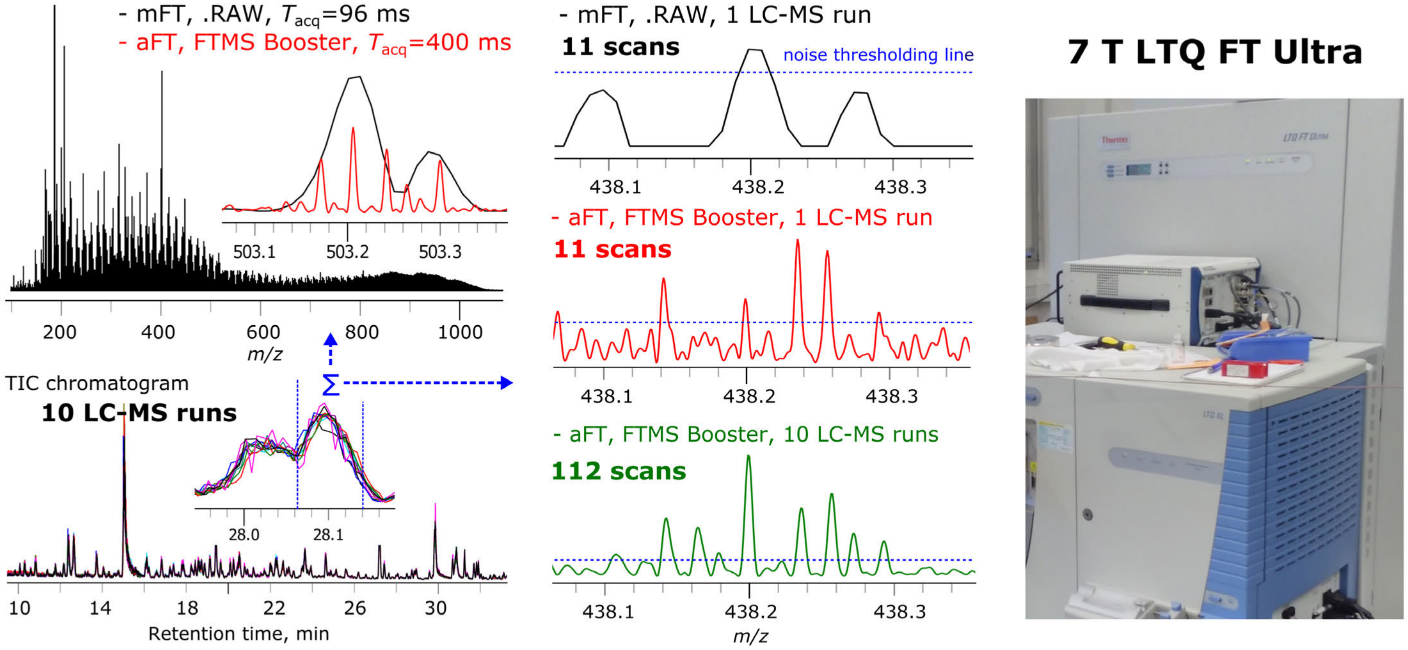
Example shows (**black**) conventional (.RAW) and (**red**) stitched (aFT) mass spectra illustrating the impact of improved resolution and sensitivity of extended duration transient averaging followed by mass segment spectral stitching for a biocrude (complex mixture) analysis. Transients were acquired with FTMS Booster X2 operating in aFT mode from Fusion™ Orbitrap™ FTMS. Notably, the profiles of ion signals for the stitched and conventional mass spectra are comparable.

Mass error distribution for Nn and NnOm compound classes obtained from the stitched mass spectrum after internal mass calibration (Peak-by-Peak capabilities). The annotated mass peaks of the targeted classes spread out for 3 orders of magnitude in the range 100 – 900 *m/z*.

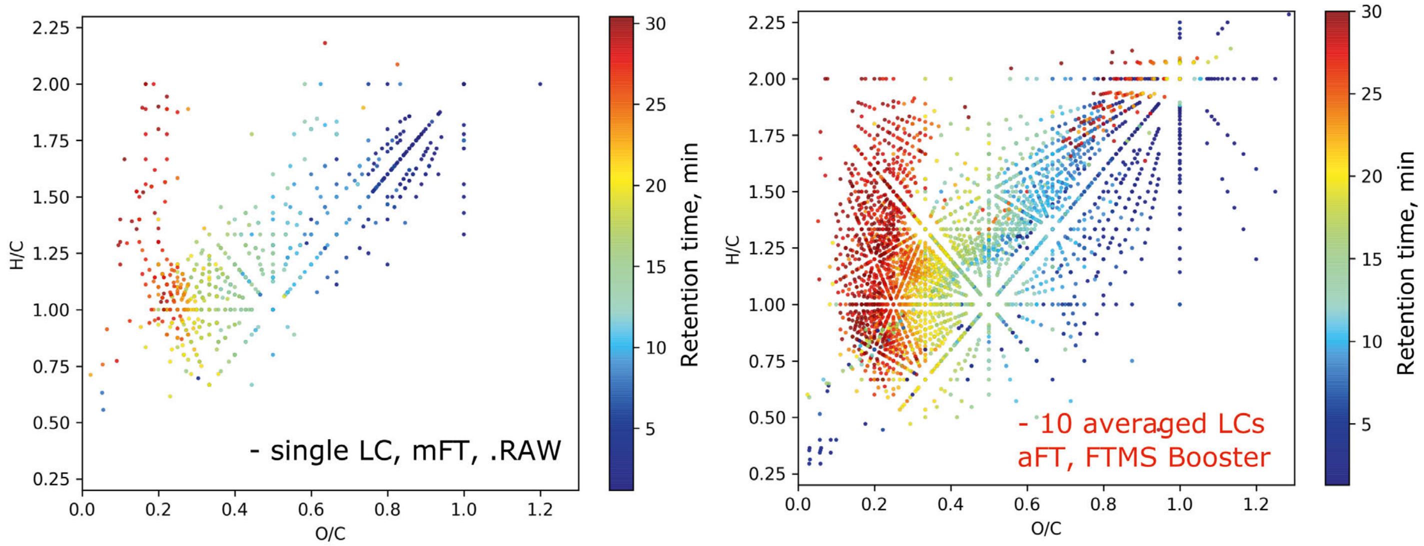
Krajewski, et al., Characterization of Biocrude Oils with an Orbitrap Fusion Mass Spectrometer with Increased Resolution and Dynamic Range, Submitted

Improved LC-FTMS Petroleomics with FT-ICR MS

FTMS Booster uniquely enables **in-hardware** absorption FT (aFT) spectral representation for FT-ICR MS, including for LC-MS experiments. The aFT doubles the peak resolution compared to the magnitude FT (mFT) mass spectra provided by conventional FT-ICR MS instruments.



Multiplexed LC-MS analysis of a complex mixture on a **7 T LTQ FT Ultra** (Thermo Scientific). 10 LC-MS replicates were acquired in parallel using built-in electronics (mFT) shown in **black**, and using **FTMS Booster X3** (aFT) shown in **red**. Spectral or transient averaging across separate LC-MS experiments significantly improves analysis sensitivity (**Peak-by-Peak** data processing capabilities).

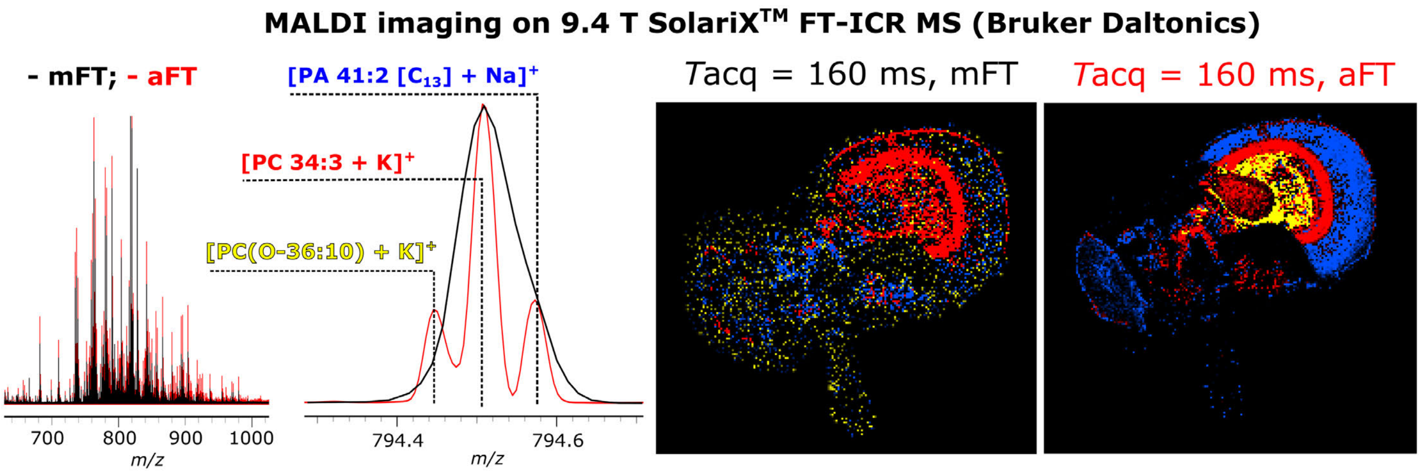


Results of a multiplexed LC-MS analysis of a complex molecular mixture on a **7 T LTQ FT Ultra** (Thermo Scientific). Van Krevelen diagrams for (left) a single experiment, .RAW data in mFT mode (reduced profile spectra) and (right) for an averaged experiment (**FTMS Booster X3** data in aFT mode with transient averaging). The averaged experiment was generated from 10 separate LC-MS technical replicates using the advanced data processing capabilities of **Peak-by-Peak** software.

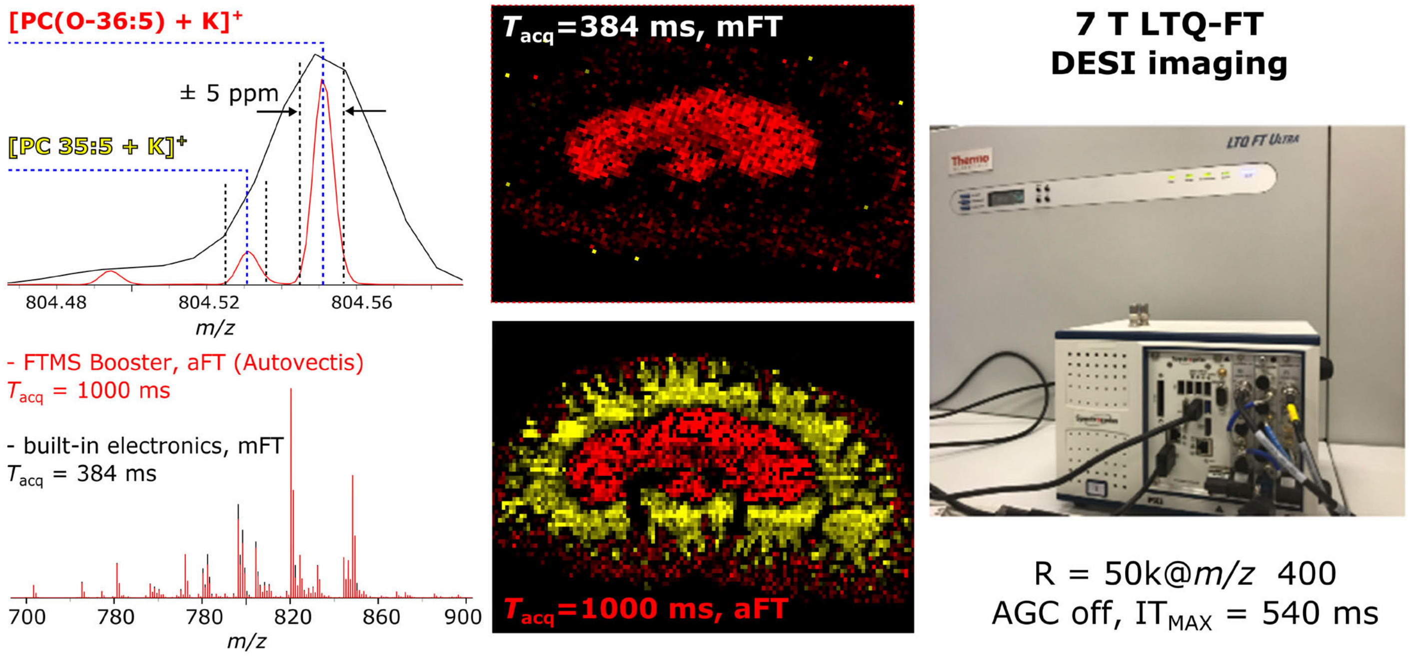
The results clearly show a benefit from a substantially improved resolution and sensitivity performance for the averaged aFT data, drastically facilitating identification of compound classes.

Improved Imaging Applications on FT-ICR MS

High-performance data acquisition system (FTMS Booster) and allied data processing software (Peak-by-Peak) increase productivity and performance of MALDI/DESI FT-ICR MS tissue imaging.



Example of a **MALDI imaging** analysis of a butterfly head's cross section on a 9.4 T SolariX™ (Bruker Daltonics). Transients were acquired with FTMS Booster and processed (**black**) in magnitude mode FT (mFT, Peak-by-Peak) and (**red**) absorption mode FT (aFT, Peak-by-Peak). Clearly, the aFT mass spectra provide superior quality to the mFT data. Image construction performed with Peak-by-Peak.

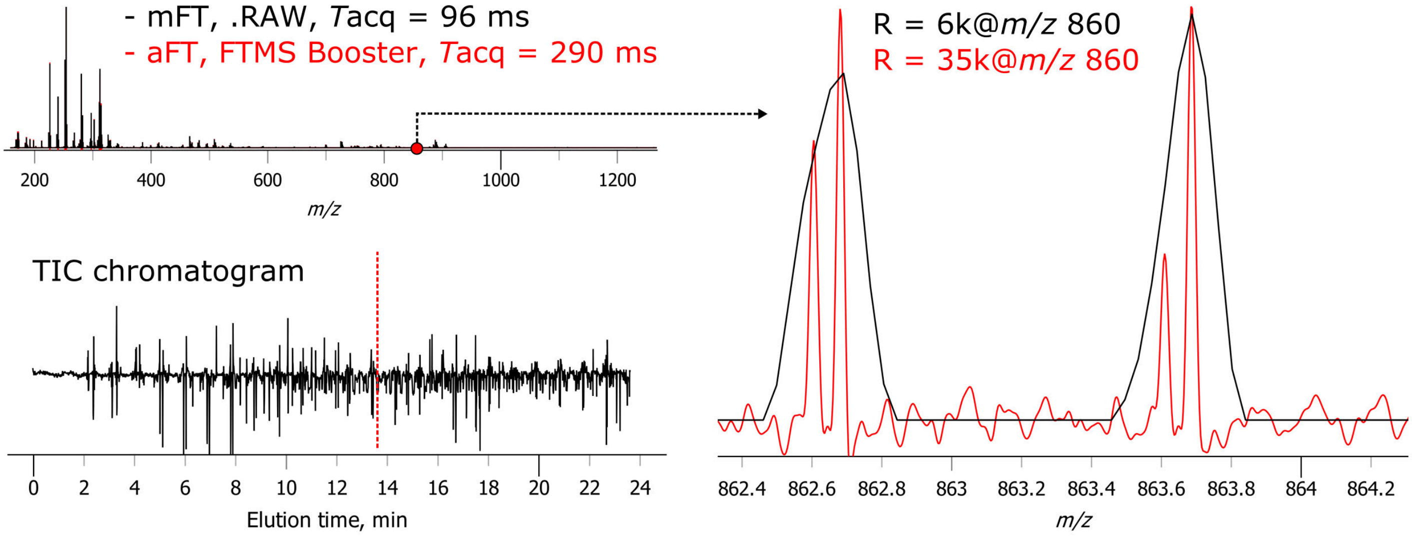


Example of a **DESI imaging analysis** of a rat kidney section on a 7 T LTQ-FT (Thermo Scientific). (**Black**) the original mass spectra (mFT, .RAW) were acquired using built-in electronics of the instrument. (**Red**) transients were acquired in parallel using the FTMS Booster X1 and processed in absorption mode (aFT, AutoVectis). Almost twice more lipids (35 vs. 66) were identified using METASPACE against the HMDB-v4 database in the dataset acquired with the FTMS Booster.

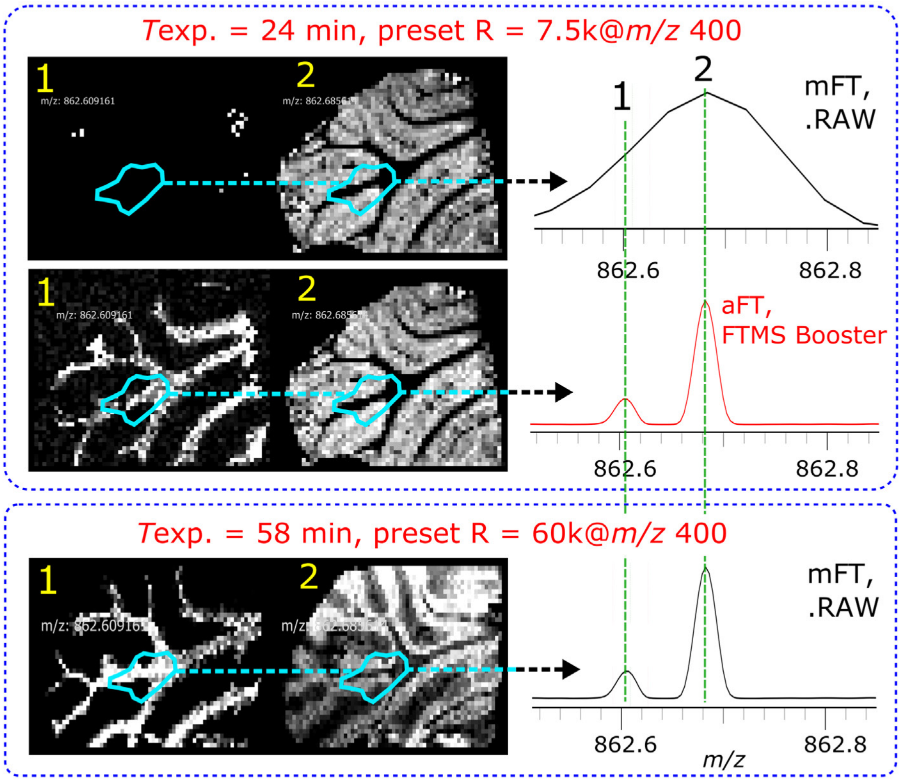
Kooijman, et al., Increased throughput and ultrahigh mass resolution in DESI FT-ICR MS imaging through new-generation external data acquisition system and advanced data processing approaches,

Improved FTMS Imaging Applications on Orbitraps

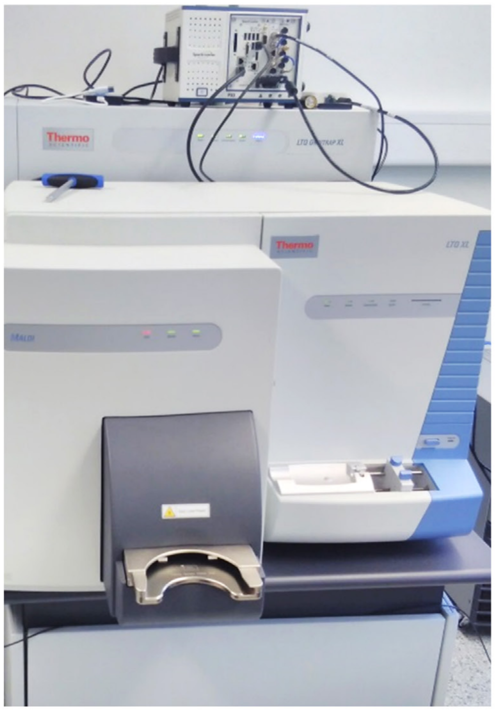
High-performance data acquisition system (FTMS Booster) and allied data processing software (Peak-by-Peak) increase productivity and performance of MALDI Orbitrap™ FTMS in tissue imaging.



Example of **MALDI imaging** analysis of a mouse brain on a MALDI LTQ™ Orbitrap™ XL™ (Thermo Scientific). (**Black**) the original mass spectra (mFT, .RAW) were acquired using built-in electronics of the instrument. (**Red**) phased transients were acquired in parallel with FTMS Booster X2 to deliver aFT mass spectra (FTMS Booster, Peak-by-Peak). Preset resolution 7’500 @ *m/z* 400.

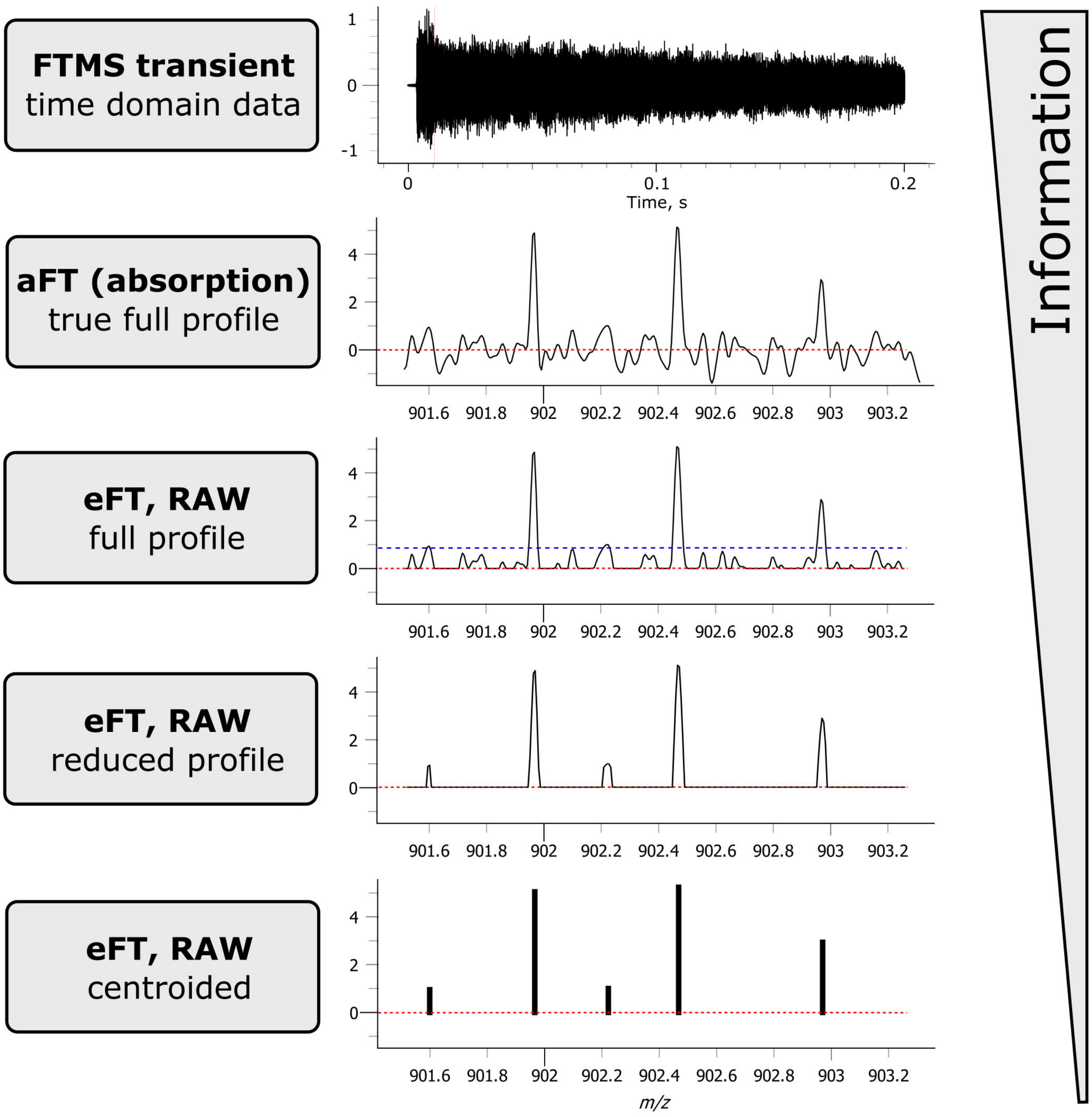


MALDI LTQ™ Orbitrap™ XL™



The results show an increased throughput for MALDI imaging experiment on a MALDI LTQ™ Orbitrap™ XL™ (Thermo Scientific). The comparable resolving power performance of ~40k @ *m/z* 800 can be achieved in the FTMS imaging experiment acquired 2.5 faster with FTMS Booster X2 and processed in absorption mode FT (aFT, Peak-by-Peak) in comparison with the standard workflow delivering .RAW mass spectra acquired using in-built electronics and processed in mFT mode.

FTMS Data Hierarchy



FTMS transient
time domain data

aFT (absorption)
true full profile

eFT, RAW
full profile

eFT, RAW
reduced profile

eFT, RAW
centroided

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FTMS Science and Technology

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