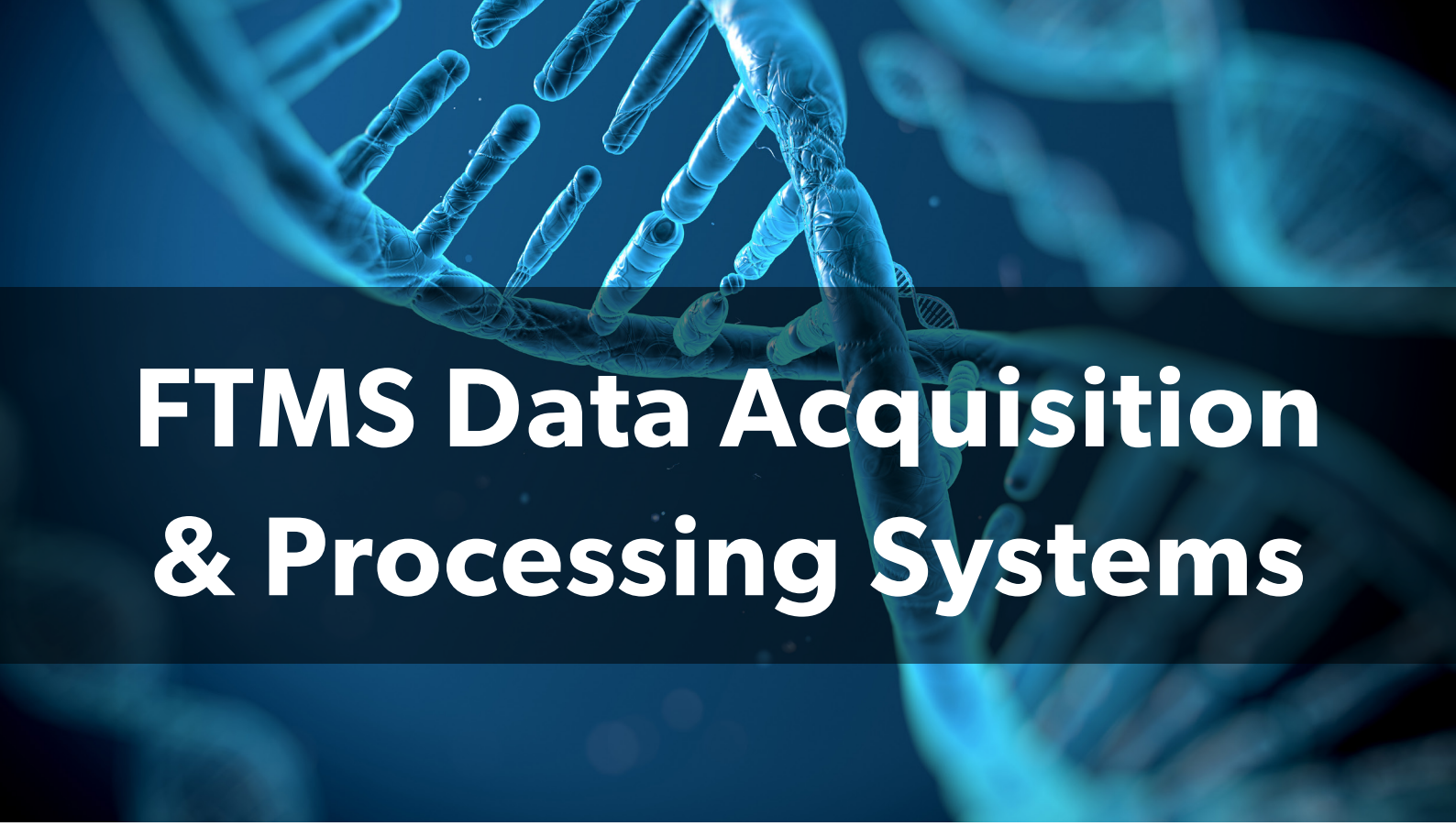
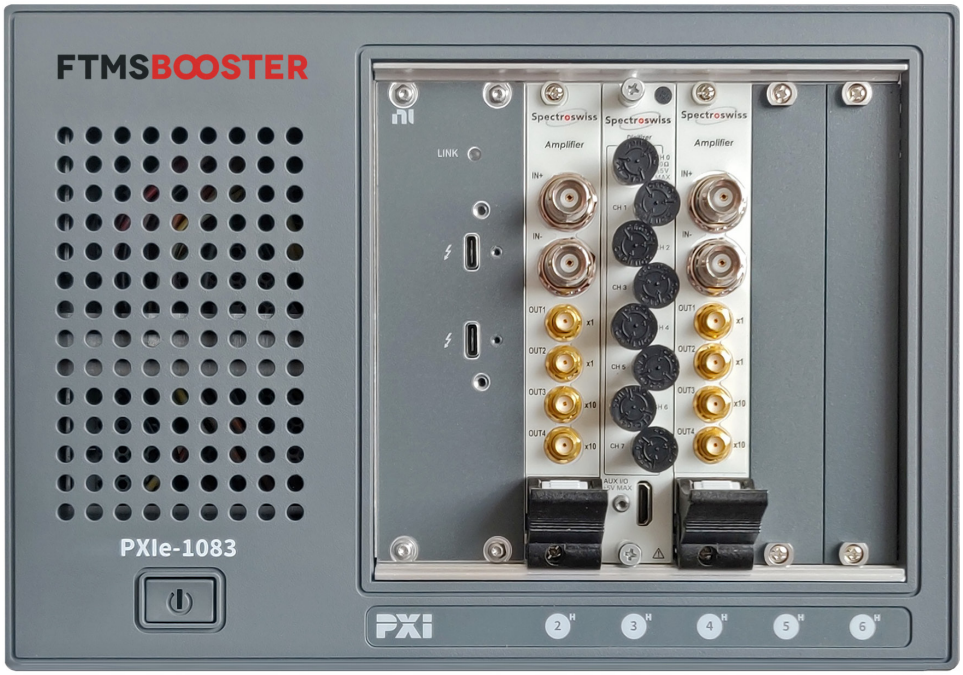
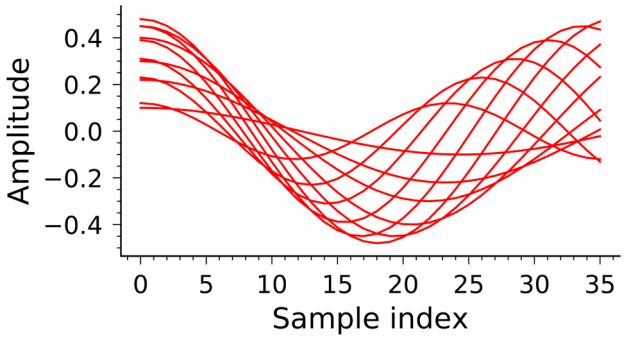


FTMS Data Acquisition & Processing Systems





Spectroswiss

FTMS Data Acquisition and Processing

Most Fourier transform mass spectrometers (FTMS) provide only processed and noise reduced data (mass spectra). Having access to the true raw data (FTMS time-domain transients) permits the most informative and efficient data processing possible. Our **FTMS Boosters**, compatible with all FTMS instruments on the market, enable time-domain transient acquisition and access to high quality unreduced data. Combine them with our **Peak-by-Peak** software for advanced data processing and data analysis to take full advantage of your FTMS platforms!

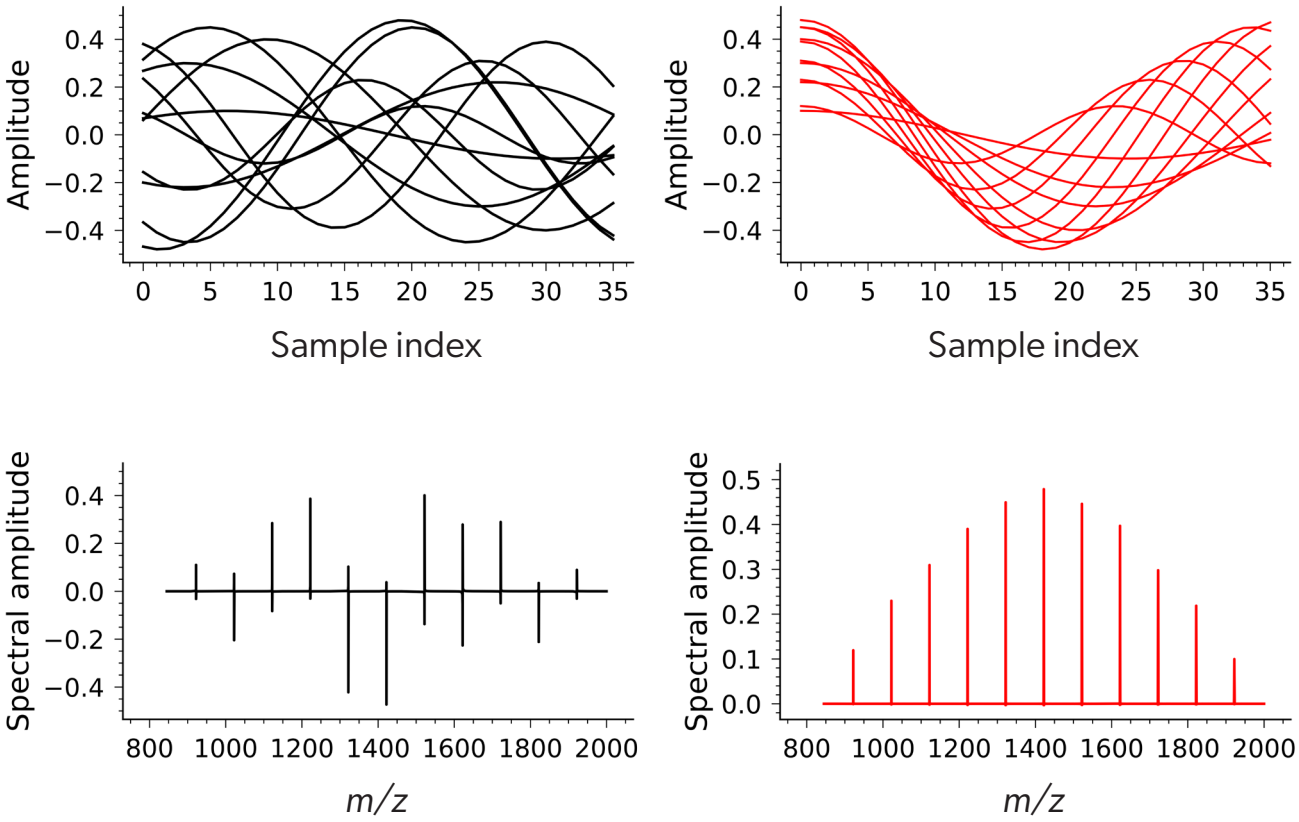
In-hardware phased transients and aFT mass spectra

Ion signals in FTMS are recorded in the time-domain as **transients**, where each ion signal is defined by its frequency, amplitude, and phase. The **absorption mode FT** (aFT) mass spectra preserve all the information contained in the transients, providing equal information.

The unreduced (full profile) aFT mass spectra maximize the FTMS performance, including resolution and sensitivity, and thus the information output.

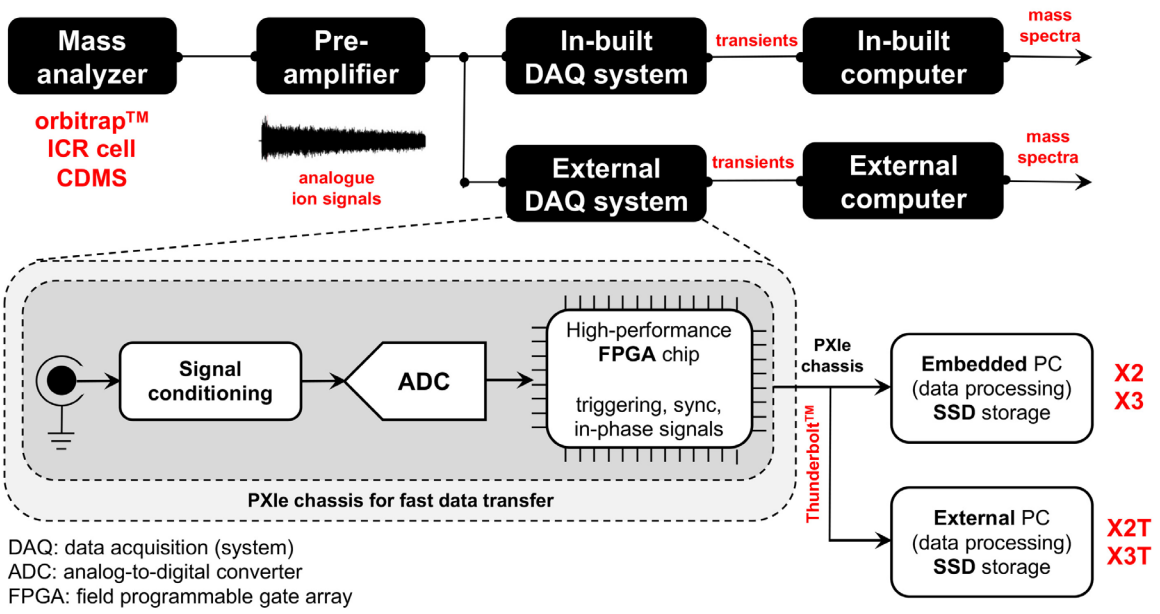
The **in-hardware phased transients**, where all ion signals have the same initial phase, as provided by the firmware of the FTMS Boosters, greatly facilitate access to the aFT mass spectra.

REGULAR TRANSIENTS | **PHASED TRANSIENTS**



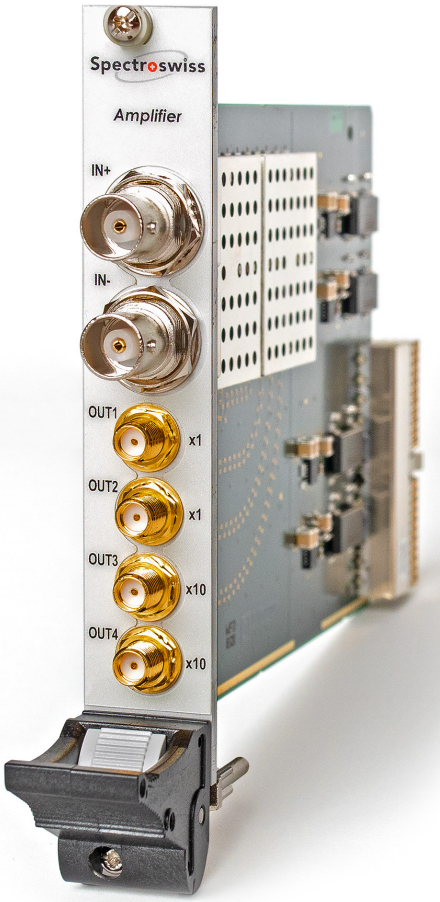
Transients vs. aFT: Fourier transform (FT) of a regular FTMS transient whose components have diverse phases (left, top) yields a mixed-mode mass spectrum (left, bottom) that requires post-processing for it to be represented in absorption mode (aFT). Right panels: FT of transients with in-phase ion signals (right, top) generates mass spectra directly in absorption mode (right, bottom).

FTMS Booster systems architecture



DAQ: data acquisition (system)
ADC: analog-to-digital converter
FPGA: field programmable gate array

Key Features



- Works in parallel with the in-built data acquisition electronics: original mass spectra (e.g., RAW) & transients are acquired in parallel without noticeable influence on each other
- Takes care of the heavy data, removing the technical challenge away from the built-in DAQ system to acquire large datasets
- Employs own patented* technology to maximize sensitivity through simultaneous acquisition of low/high gain transients via proprietary PXIe amplifier (manufactured by Spectroswiss in Switzerland)
- Uniquely provides in-hardware phased transients for direct and phase artifact-free aFT mass spectra generation
- Supports advanced triggering options – recognizes both stop and start triggers, increasing experimental design flexibility and sophistication
- Allows flexible length (at the ms scale) transients – beyond the conventional two-fold increase in the data points (resolution)
- Maximizes the ion detection duty cycle, allowing extended transient recording during all the time ions ring in the mass analyzer
- Ensures full transient detection for enhanced sensitivity, duty cycle, and (ultra-high) resolution
- Detects and reports ion signals across the full *m/z* range (all ion detection)
- Removes the need for microscans in FTMS data acquisition by enabling post-processing transient averaging
- Offers transients for post-processing capabilities, including the use of advanced signal processing approaches (advanced FT and non-FT methods, such as super-resolution signal processing, e.g., least-squares fitting)
- Enables charge detection mass spectrometry (CDMS) by providing individual transients, including ultra-long transients, for post-processing

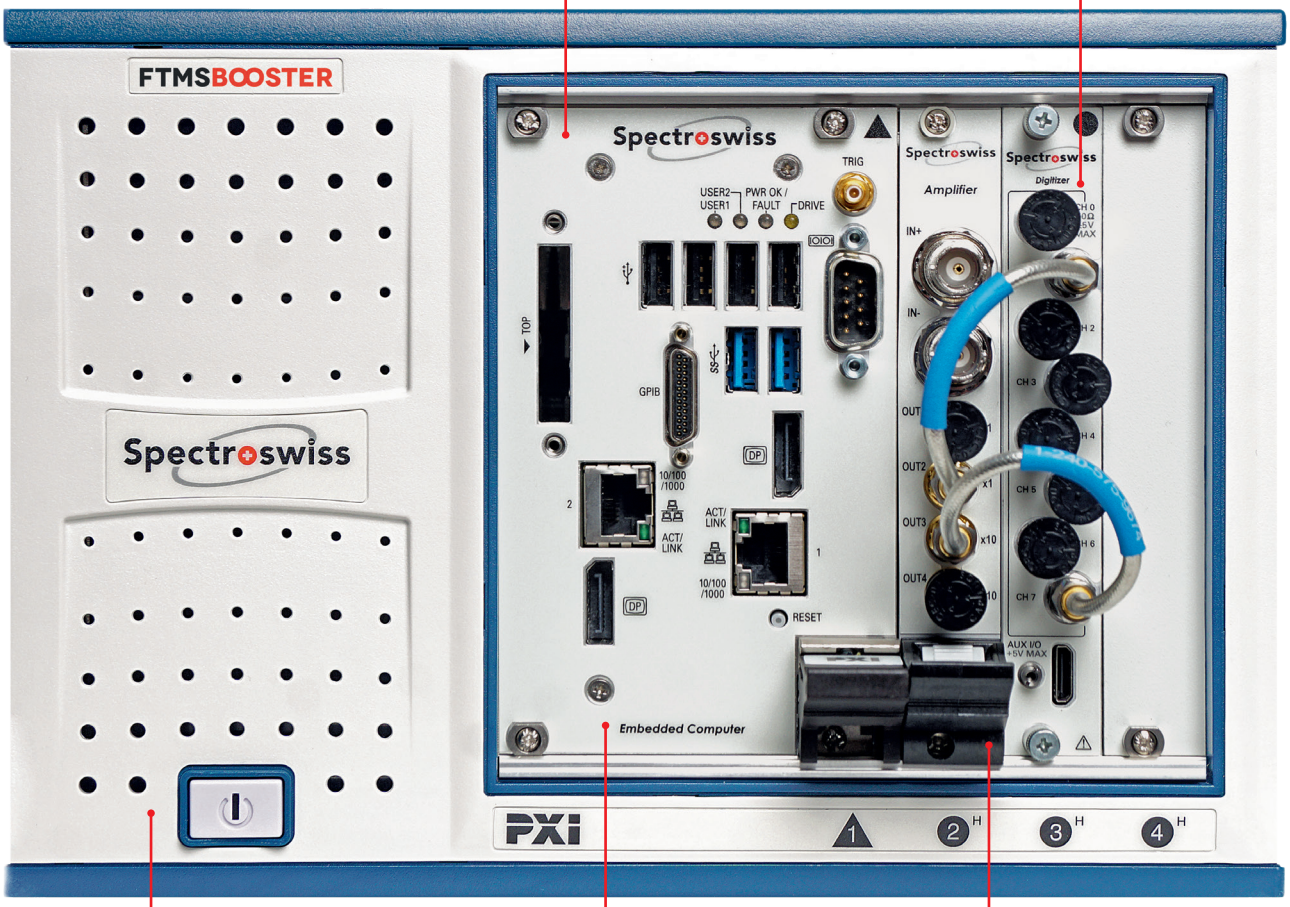
** Data acquisition apparatus and methods for mass spectrometry, by Anton Kozhinov, Yury Tsybin, and Konstantin Nagornov, Spectroswiss, Patent US 11,222,774 from January 2022*

FTMS Booster X2: empowering Orbitrap™ FTMS

FTMS Booster X2

Embedded Computer
Acquisition and visualization of FTMS data, control of the system, data processing.

High-Performance Digitizer / FPGA chip
High sample rate analog-to-digital conversion, in-line digital signal processing and trigger decoding.



High-bandwidth Chassis
PXI Express backplanes for high-speed data transfer.

Data Storage Buffer (SSD)
High-speed data streaming. The buffer's volume is 4 TB.

Signal Amplifier
Impedance interface to FTMS. Low noise, high bandwidth amplification with multiple outputs (different gains).

Key Features

- Easy add-on to any* Orbitrap™
- All capabilities of FTMS Booster X2T technology
- Rugged single-chassis system, small footprint
- High-capacity (4TB) fast SSD buffer

*Implemented on

LTQ Orbitrap™
LTQ Orbitrap XL™
LTQ Orbitrap Velos™
LTQ Orbitrap Elite™
Exactive EMR™

Q Exactive™
Q Exactive Focus™
Q Exactive Plus™
Q Exactive HF™
Q Exactive GC™
Q Exactive UHMR™

Exploris 480™
Fusion™
Fusion Lumos™

(all from Thermo Fisher Scientific)

Orbitrap™ Transients Acquisition and Processing

FTMS Booster X2T

- Easy add-on to any* Orbitrap™
- **Thunderbolt™** technology for remote control and rapid data transfer
- Unlocks absorption mode FT (aFT) mass spectra representation
- **[NEW] User-defined first *m/z* value** to minimize data sizes and maximize processing speeds
- **[NEW]** Acquires transients of any length, e.g.,> 25 s
- Option: stand-alone **workstation** with powerful data processing and data storage capabilities

Selected Applications

Isotopic ratio analysis

Certain chemical elements and compounds require isotopic ratio measurement performance, namely dynamic range, that is beyond the state-of-the-art in Orbitrap™ FTMS. Acquisition and averaging of multiple time-domain transients can help to overcome these limitations. Even the isotopic ratio of $^{238}UO_2$ to $^{234}UO_2$, requiring exceptional dynamic range, can be precisely measured in a single mass spectrum, following averaging of 8'000 transients acquired with FTMS Booster X2. See *Bills et al., JASMS, 2021, 1224.*

Quantitative metabolomics

Accurate quantitation of small molecules, e.g., metabolites, in LC/GC-MS requires the corresponding levels of sensitivity. When the required sensitivity levels cannot be provided, the sensitivity can be enhanced by averaging the time-domain transients across a single LC/GC-MS experiment or across multiple technical replicates of LC/GC-MS experiments. FTMS Booster X2/X2T can provide access to the time-domain transients and, thus, to enhance sensitivity and quantitation accuracy in such cases. See *Nagornov et al., JASMS, 2020, 257.*

Antibody structure and DAR analysis

Orbitraps are widely employed for the structural analysis of antibodies (monoclonal antibodies, mAbs) and antibody-drug conjugates (ADCs). Time-domain transients processing helps to enhance the performance of these methods and to deliver additional information on the biotherapeutics. For example, sequence coverage of mAbs can be increased in top-down and middle-down FTMS via transient averaging-based product ion sensitivity gain. In addition, reducing the resolution to ultra-low levels can be beneficial for drug-to-antibody ratio (DAR) analysis. For these applications, FTMS Booster X2/X2T provides indispensable access to the transients. See *Nagornov et al., Anal Chem, 2021, 12930.*

Multiplexed Quantitative Proteomics

FTMS Booster X2/X2T provides transients that are processed with super-resolution signal processing approaches, such as least-squares fitting. It allows resolving the neutron-encoded peptide doublets that differ by 6.3 mDa in the whole mass range in quantitative proteomics. That increases the number of quantitation channels (level of multiplexing) in the TMT™-based quantitative proteomics with (heavy) complementary (TMTc) ions. See *Kozhinov et al., Anal Chem 2023.*

Charge Detection (Single Ion) Mass Spectrometry (CDMS)

CDMS implementation benefits from the ability to process the time-domain transients. The regular length transients can be provided by FTMS Booster X2/X2T for user-controlled post-processing. In addition, FTMS Booster X2/X2T devices can provide long (1-4 s) and ultra-long (4-25 s) transients, to improve the CDMS performance characteristics, such as mass and charge accuracy. See *Heck et al., ASMS 2023, WOA pm 2:50.*

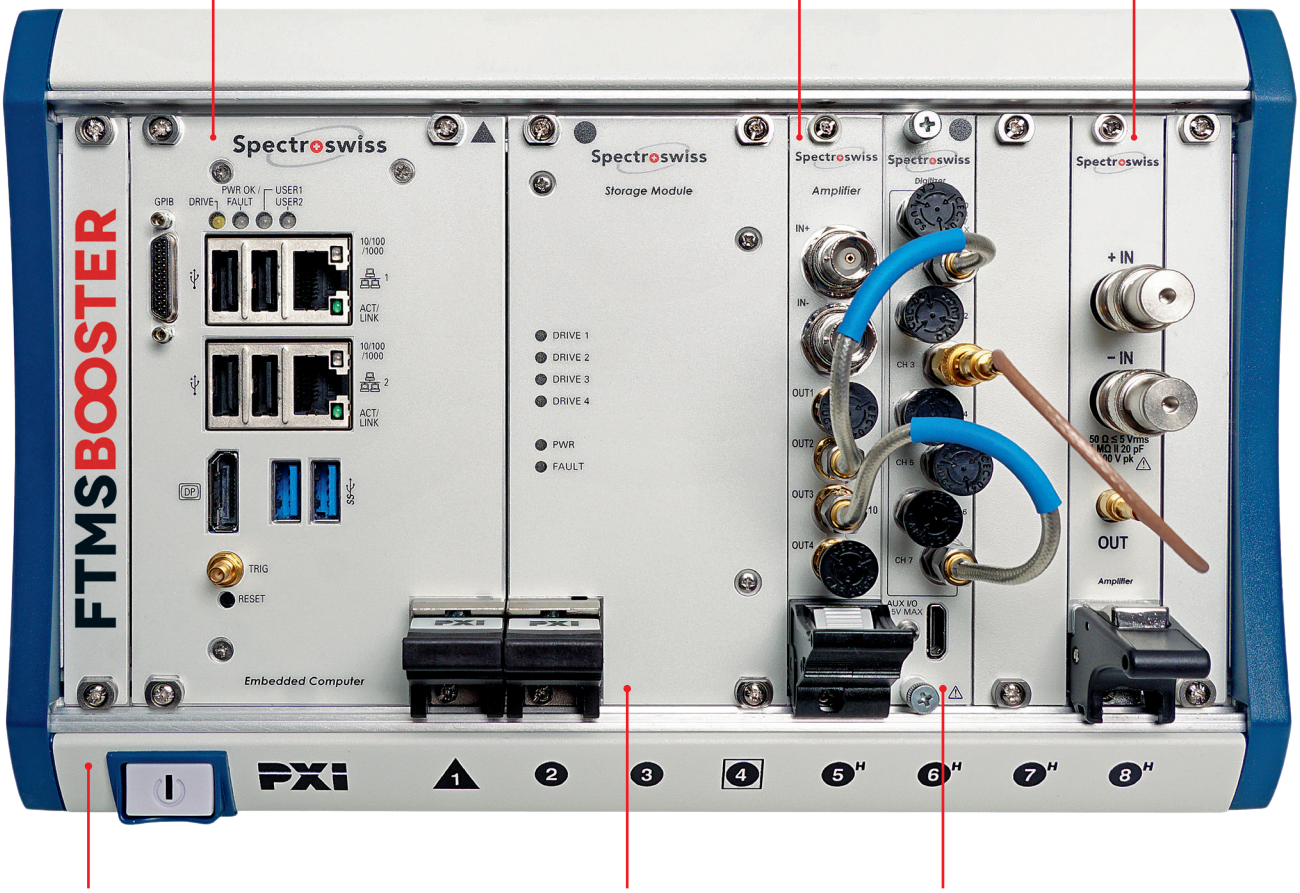
FTMS Booster X3: empowering FT-ICR MS

FTMS Booster X3

Embedded Computer
Acquisition and visualization of FTMS data, control of the system, data processing.

Signal Amplifier #1: Detect Signal
Impedance interface to FTMS.
Low noise, high bandwidth amplification with multiple outputs (different gains).

Signal Amplifier #2: Excite Signal
Impedance interface to FTMS.
Low noise, high bandwidth amplification with multiple outputs (different gains).



High-bandwidth Chassis
PXI Express backplanes for high-speed data transfer.

Data Storage Buffer (SSD)
High-speed data streaming.
User-defined buffer's volume.

High-Performance Digitizer / FPGA chip
High sample rate analog-to-digital conversion, in-line digital signal processing and trigger decoding.

Key Features

- Easy add-on to any* FT-ICR MS
- All capabilities of FTMS Booster X3T technology
- Rugged single-chassis system
- High-capacity (up to 16 TB) fast SSD buffer

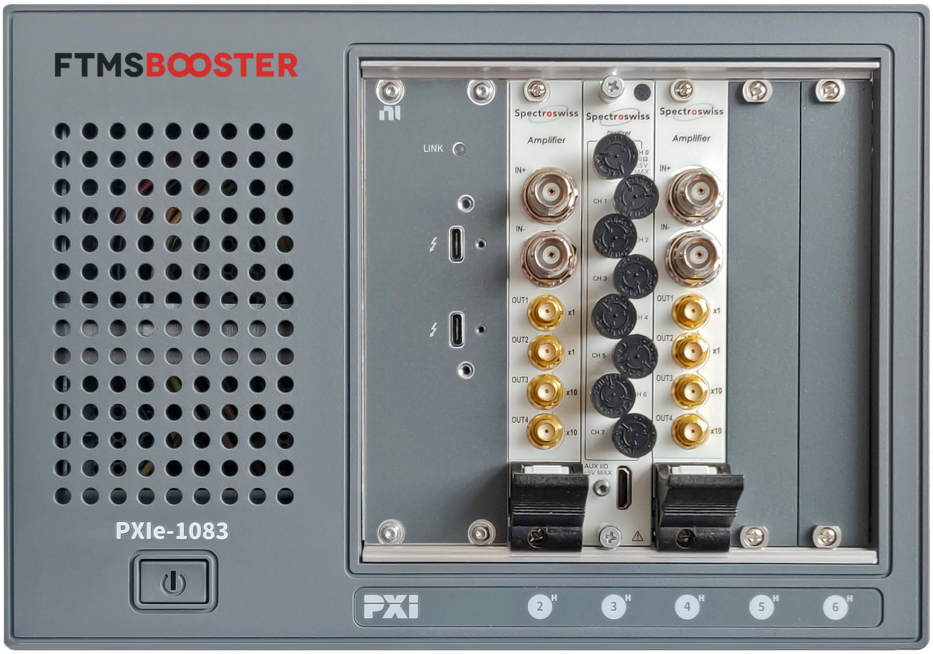
*Implemented on

7 T LTQ FT™ (Thermo Fisher Scientific)
10 T LTQ FT™ (Thermo Fisher Scientific)
21 T LTQ FT-ICR MS (PNNL)
9.4 T FT-ICR MS with Infinity™ ICR cell
15 T FT-ICR MS with Infinity™ ICR cell
9.4 T SolariX XR™ FT-ICR MS
12 T SolariX XR™ FT-ICR MS
15 T SolariX XR™ FT-ICR MS
7 T scimaX™ FT-ICR MS

(all from Bruker Daltonics)

Orbitrap™ Transients Acquisition and Processing

FTMS Booster X3T



- Easy add-on to any* FT-ICR MS, as well as any Orbitrap™ FTMS
- **Thunderbolt™** technology for remote control and rapid data transfer
- Acquisition of both excite and detect waveforms in FT-ICR MS
- Unlocks absorption mode FT (aFT) mass spectra representation
- **User-defined first *m/z* value** to minimize data sizes and maximize processing speeds
- Acquires transients of any length, e.g.,> 25 s
- Two reserved slots for hardware extension modules
- Option: stand-alone **workstation** with powerful data processing and data storage capabilities

Selected Applications

Complex mixture analysis

FT-ICR MS analysis of complex molecular mixtures, for example, in petroleomics, requires high-resolution, high mass accuracy, and high sensitivity. Modern analysis of complex mixtures may also involve on-line LC-MS separation. FTMS Booster X3/X3T technology uniquely provides the in-hardware phased transients that can directly lead to the absorption mode FT (aFT) mass spectra. Supported by transient averaging within a single experiment and across multiple technical replicates, as well as automated SIM stitching, the aFT capability helps to analyze extremely challenging complex mixtures. See *Kew et al., ASMS 2023, WP 319; Kozhinov et al., ASMS 2021, ThP 120.*

Imaging

Comprehensive imaging mass spectrometry relies on high-resolution and high mass accuracy. In turn, scan speed (experimental total time) becomes a critical parameter. FTMS Booster X3/X3T delivers the in-hardware phased transients (thus offers aFT mass spectra), increases ion detection duty cycle by recording transients during the overheads (thus increases scan speed), promotes data post-processing (reduction in the total experimental time), and supports the acquisition of ultra-long transients (thus offering ultra-high resolution mass spectra). Super-resolution processing of transients provides further increases in scan speed. See *Anderton et al., Anal Chem 2022, 8, 3629; Ellis et al., Scientific Reports, 2019, 9, 8; Kozhinov et al., ASMS 2021, ThP 120.*

Top-down protein analysis

FT-ICR MS instruments are employed for the structural analysis of proteins, including monoclonal antibodies, mAbs, using the intact mass and top-down approaches. Time-domain transients processing helps to enhance the performance of the methods and to deliver additional structural information. For example, sequence coverage of mAbs can be increased in top-down and middle-down FT-ICR MS via transient averaging-based product ion sensitivity gain. FTMS Booster X3/X3T provides the in-hardware phased time-domain transients and, thus, the corresponding aFT mass spectra. See *Kozhinov et al., ASMS 2021, ThP 120.*

Selected references

FTMS instrument	Reference
Q Exactive GC Orbitrap™	Trace-level persistent organic pollutant analysis with GC Orbitrap MS -enhanced performance by complementary acquisition and processing of time-domain data, *Bleiner et al., JASMS 2020, 31, 2, 257–266*
Q Exactive Focus Orbitrap™	Improved uranium isotope ratio analysis in LS-AP glow discharge / Orbitrap FTMS coupling through the use of an external data acquisition system, *Marcus et al., JASMS 2021, 32, 5, 1224–1236*
Q Exactive Plus Orbitrap™	Image-wide adjacent-pixel data averaging increases sensitivity toward dosed drugs of abuse and antiretrovirals in Q Exactive mass spectrometry imaging, *Desyaterik et al., ASMS 2023, TP337*
Q Exactive HF Orbitrap™	Drug-to-antibody ratio estimation via proteoform peak integration in the analysis of antibody–oligonucleotide conjugates with Orbitrap FTMS, *Nagornov et al., Anal. Chem. 2021, 38, 12930–12937*
Q Exactive UHMR Orbitrap™	Benefits of ultra-long transients in Orbitrap based charge-detection mass spectrometry ; *Heck et al. ASMS 2023, WOA pm 02:50*
Exploris 480 Orbitrap™	Omnitrap-Orbitrap performance enhancement via unreduced data processing, *Kozhinov et al. ASMS 2023, WOA am 08:30*
Fusion Lumos Orbitrap™	Super-resolution MS enables rapid, accurate, and highly multiplexed proteomics at the MS2 level, *Kozhinov et al., Anal. Chem. 2023, 7, 3712*
LTQ Orbitrap XL™	Performance evaluation of a MALDI LTQ Orbitrap XL imaging platform interfaced with a new-generation data acquisition system, *Nagornov et al. ASMS 2020, ThOH pm 02:50*
7 T LTQ FT-ICR MS	Increased throughput and ultra-high mass resolution in DESI FT-ICR MS imaging through new-generation external data acquisition system and advanced data processing approaches, *Ellis et al., Sci Rep 9, 8 (2019)*
21 T LTQ FT-ICR MS	Imaging and direct sampling capabilities of nanoDESI with absorption-mode 21 T FT-ICR MS, *Anderton et al., Anal. Chem. 2022, 8, 3629–3636*
12 T & 15 T SolariX XR™ FT-ICR MS	FT-ICR MS with in-hardware absorption-mode capability : evaluation in high-resolution and high-throughput applications, *Kozhinov et al., ASMS 2021, ThP 120*
7 T scimaX™ FT-ICR MS	Optimizing 7 Tesla FT-ICR MS equipped with frequency multiple detection and absorption mode processing for high throughput organic matter analysis, *Kew et al. ASMS 2023, WP 319*

Spectroswiss

Spectroswiss Inc. | 245 First Street | Riverview II | Cambridge, MA 02142 | USA
Spectroswiss Sàrl | EPFL Innovation Park | 1015 Lausanne | Switzerland
Spectrotech SAS | 132 rue Bossuet | 69006 Lyon | France
www.spectroswiss.ch