

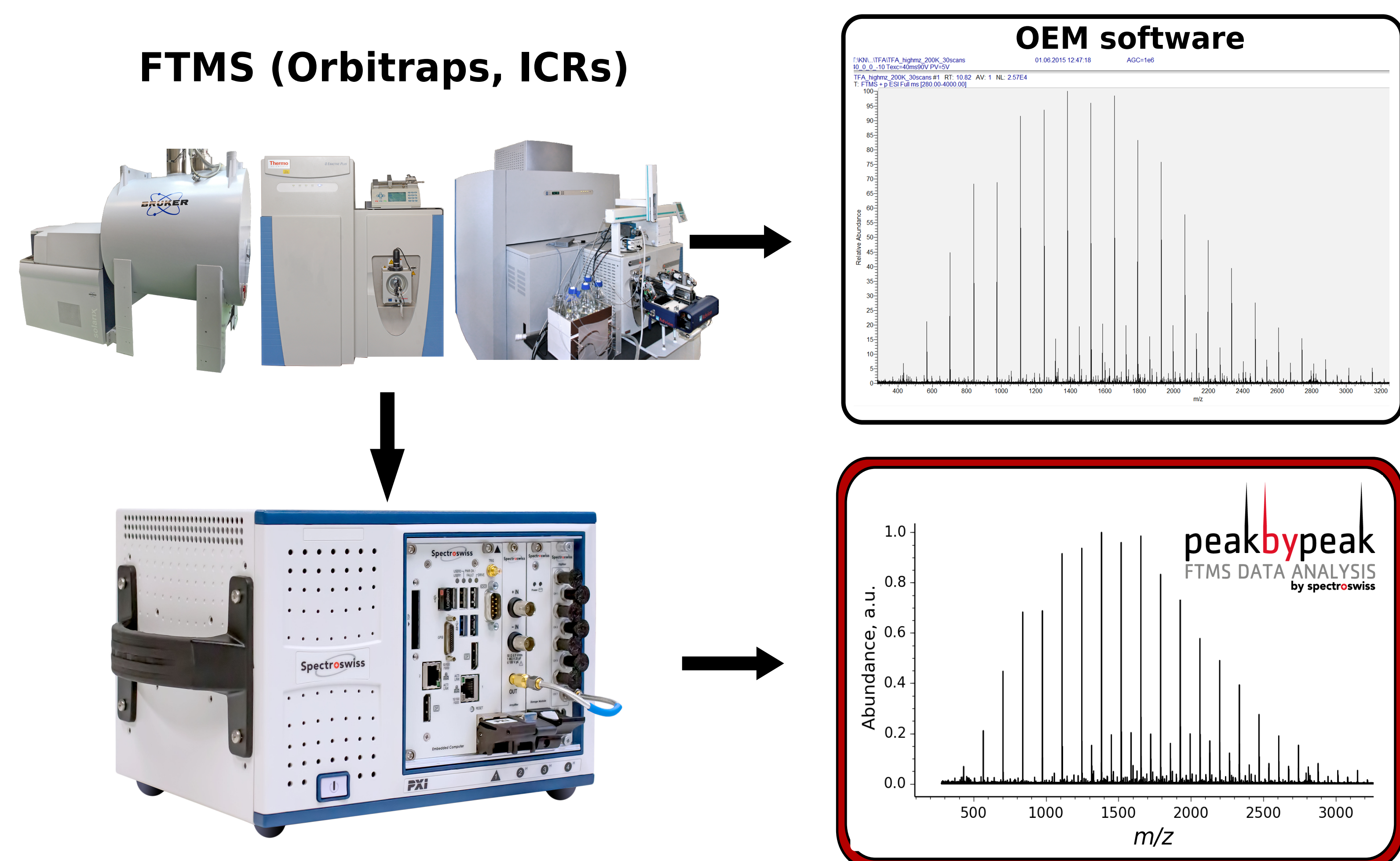
## 1. Overview

FTMS instruments that are currently in use worldwide employ data acquisition electronics whose front-ends are either built on analog circuits only or utilize cost-effective, yet small, field-programmable gate arrays (FPGAs). With high-throughput FPGAs that have recently become commercially available, a new hardware architecture with *in-line* digital signal processing is enabled, with which time-domain data can be acquired in a more intact and complete form compared to traditional architectures, *viz.* with improved performance characteristics and without data-correction post-processing requirements.

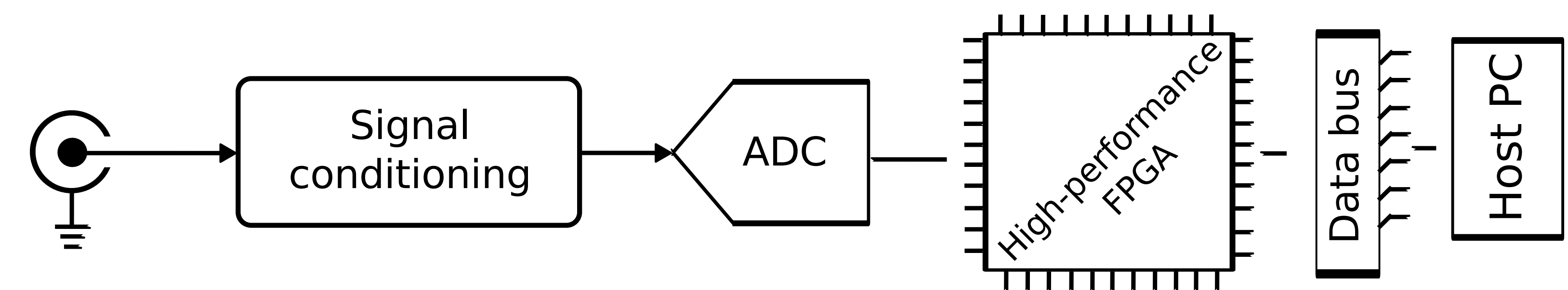
Here, based on the state-of-the-art FPGA technology, we implement an advanced data acquisition (DAQ) system for FTMS. The system is designed to operate parallel to existing data acquisition electronics and software, providing complementary data sets w.r.t. to the standard data acquisition workflow. The system's analytical values are in data acquisition during complete periods of ion trapping in mass analyzers, reduced digital and thermal noise components, and substantially reduced phase distortions along the analog/digital signal path during data acquisition.

The impact of the system's integration with diverse contemporary, both current and past generation, FTMS instruments on their performance is evaluated in common analytical workflows, including commercial and custom-upgraded FT-ICR MS and Orbitrap FTMS.

## 2. Methods

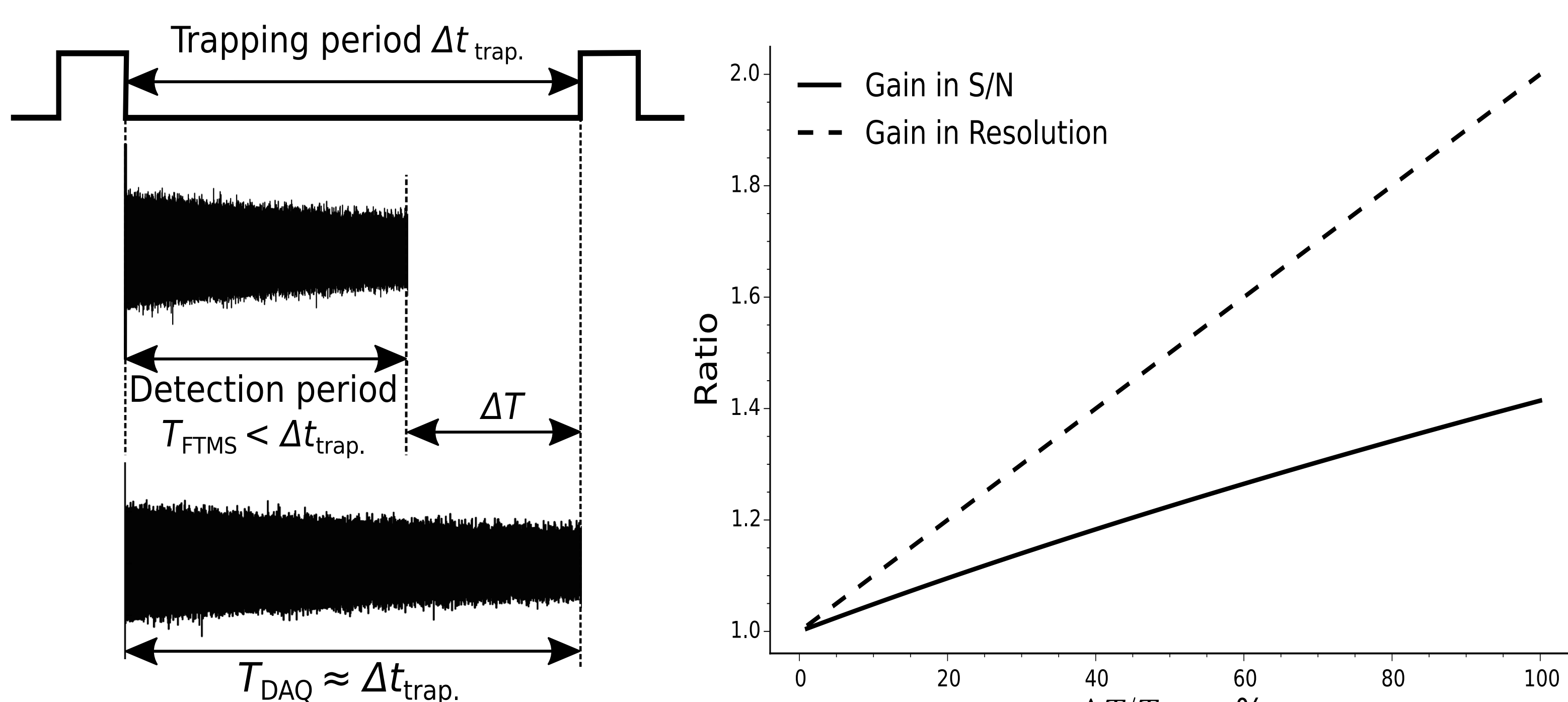


**Figure 1.** Schematic flow diagram representing parallel data acquisition with two complementary data sets obtained with built-in acquisition tools and with the described data acquisition system. MS data is acquired via the system's analog and digital interfaces to the host FTMS instrument's pre-amplifier and instrument control electronics, and parallel to the host's data acquisition tools.

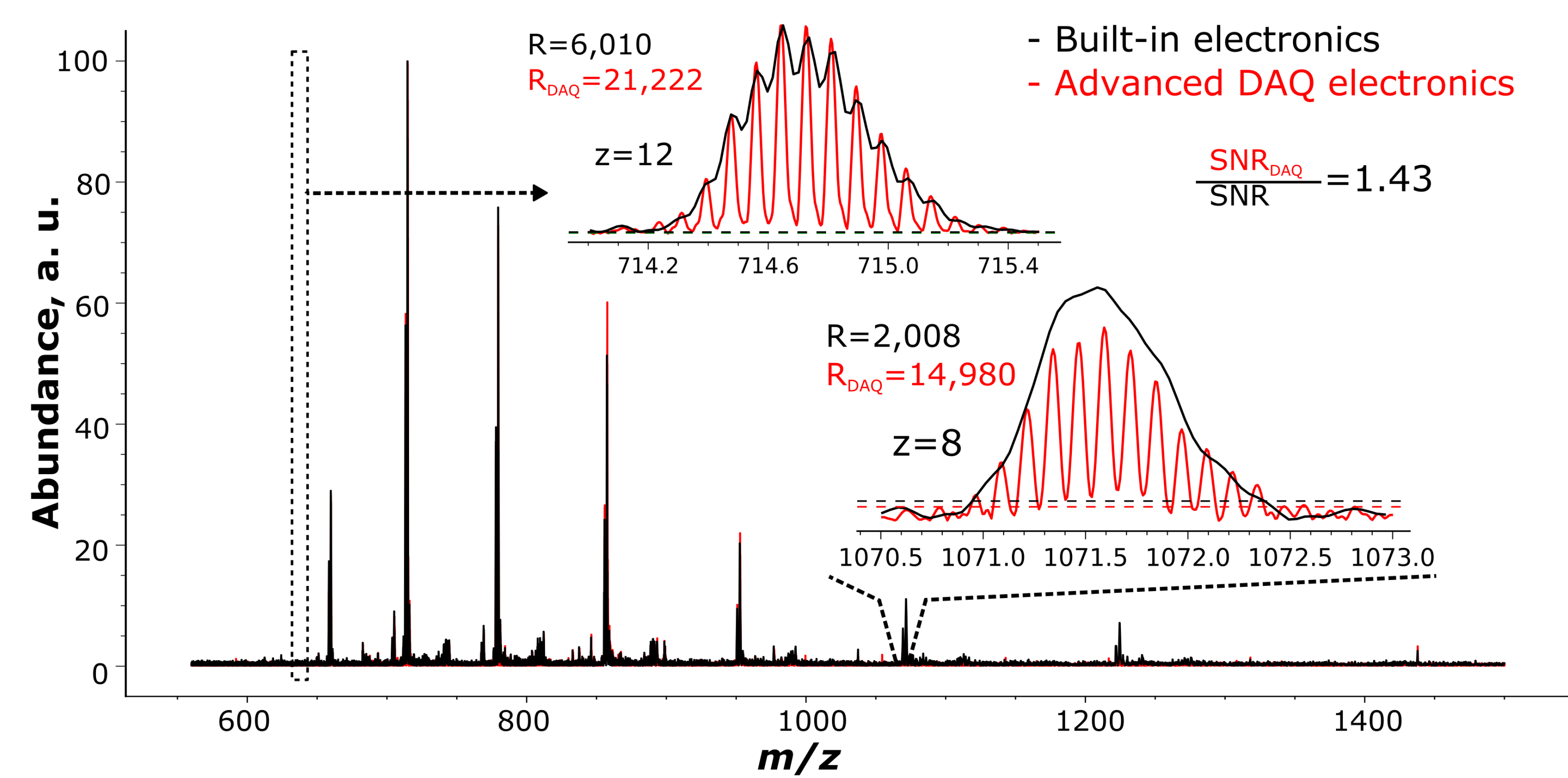


**Figure 2.** Schematic block diagram of the data acquisition system. An analog signal that, as outputted by the host instrument's pre-amplifier, is routed to an analog input of the system, passes through a signal conditioning sub-system, and reaches an analog-to-digital conversion (ADC) sub-system. Next, a continuous stream of digital data produced by the ADC sub-system is sent to a high-performance FPGA chip with custom firmware for in-line signal processing. Finally, individual digitized transients are transferred to an embedded (host) computer for further signal processing, e.g. FT and frequency-to- $m/z$  conversion.

## 3. Duty cycle of transients recording

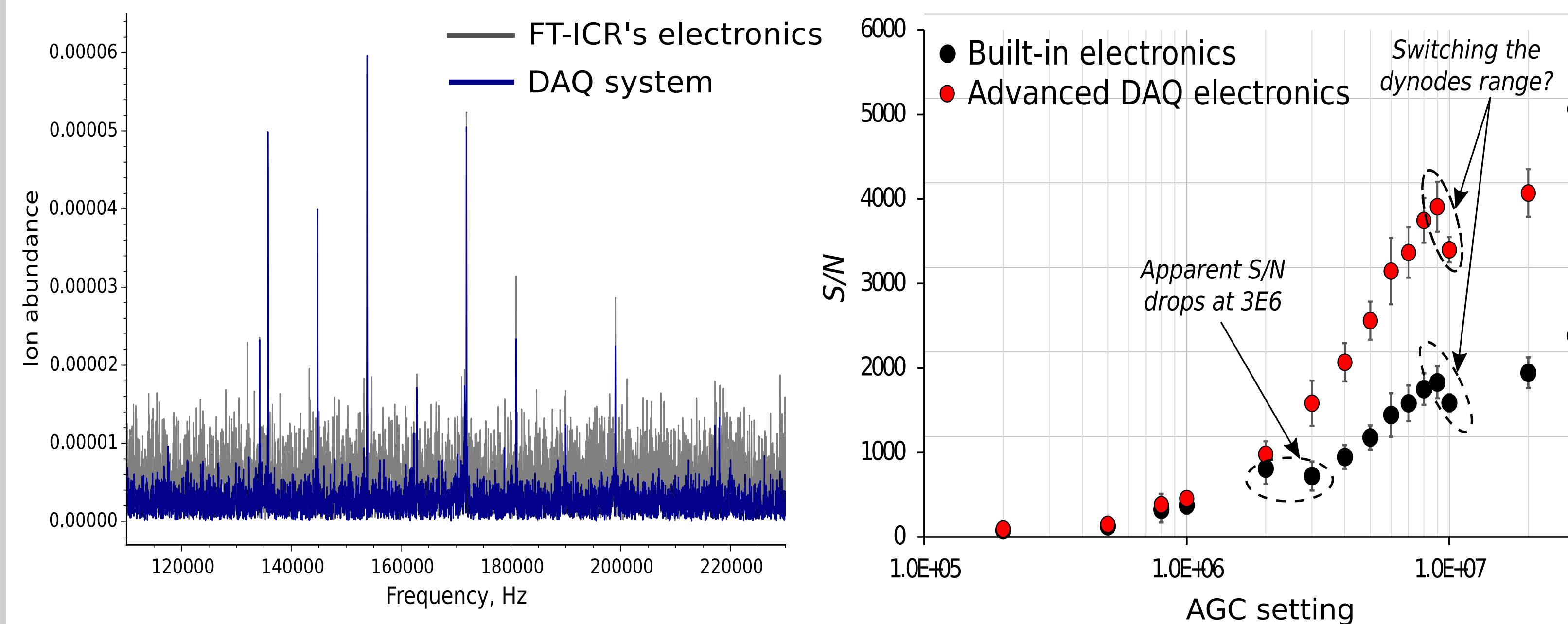


**Figure 3.** Increased duty cycle of transient data acquisition. Left panel: schematic flow diagram of time-domain data acquisition within a measurement scan. Detection periods of built-in data acquisition systems are usually pre-selected before signal digitization. Hence, detection periods are usually shorter than a period of time within which ions are trapped in a mass analyzer, resulting in sub-optimal duty cycles of transients recording. With the data acquisition system of this work, the detection period is not fixed in advance so that maximized duty cycles are achieved. Right panel: estimated increase in  $S/N$  and resolution as functions of relative increase in detection periods.

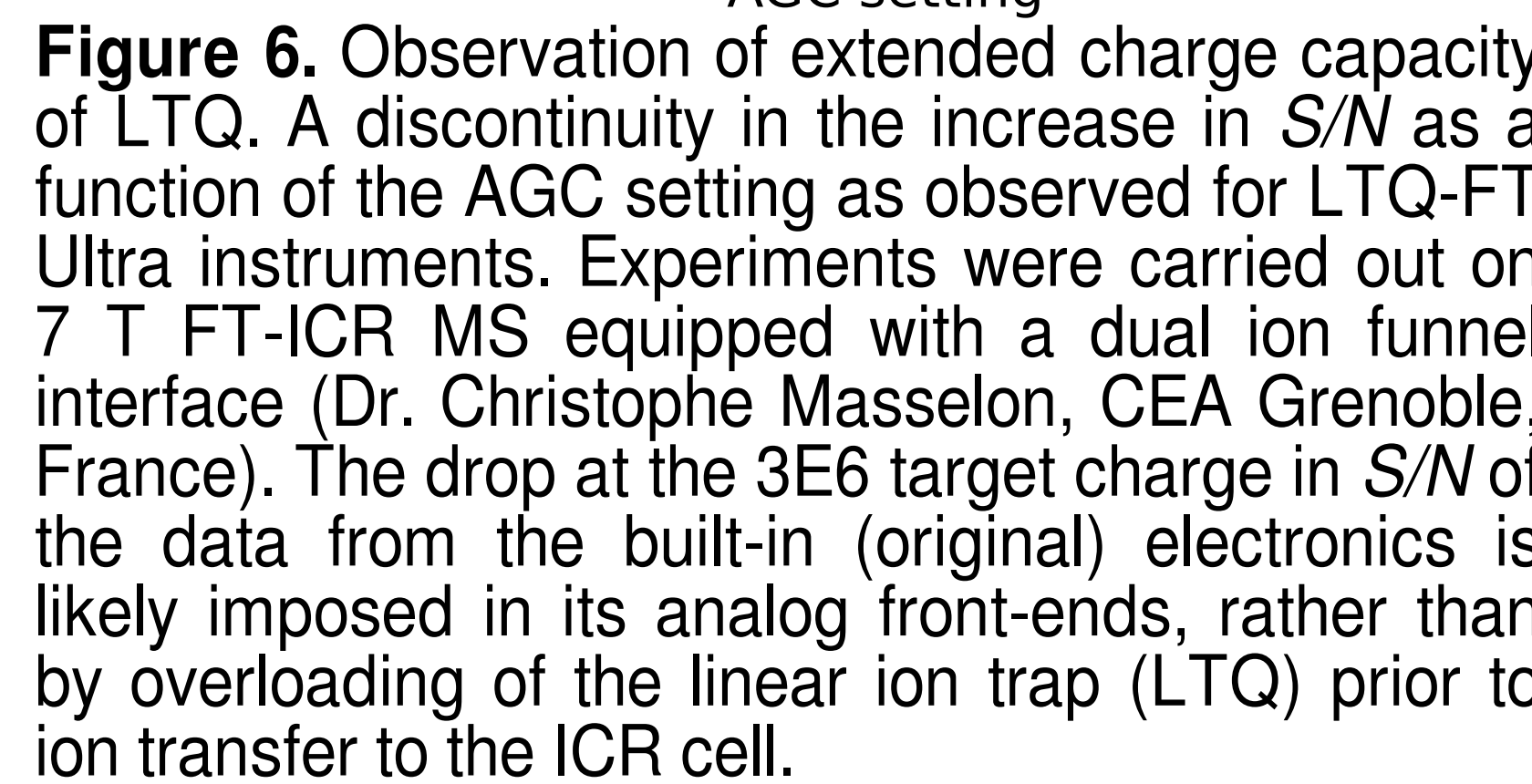


**Figure 4.** Increased resolving power in the MS analysis of ubiquitin on 10 T FT-ICR MS (Thermo Scientific) due to extended duty cycles of transient data acquisition. The data was processed in magnitude mode FT. Shown mass spectra are normalized to their base peaks. Dashed lines represent six standard deviation of noise. The resolution setting was 12,500, ion accumulation time 100 ms, the AGC function off.

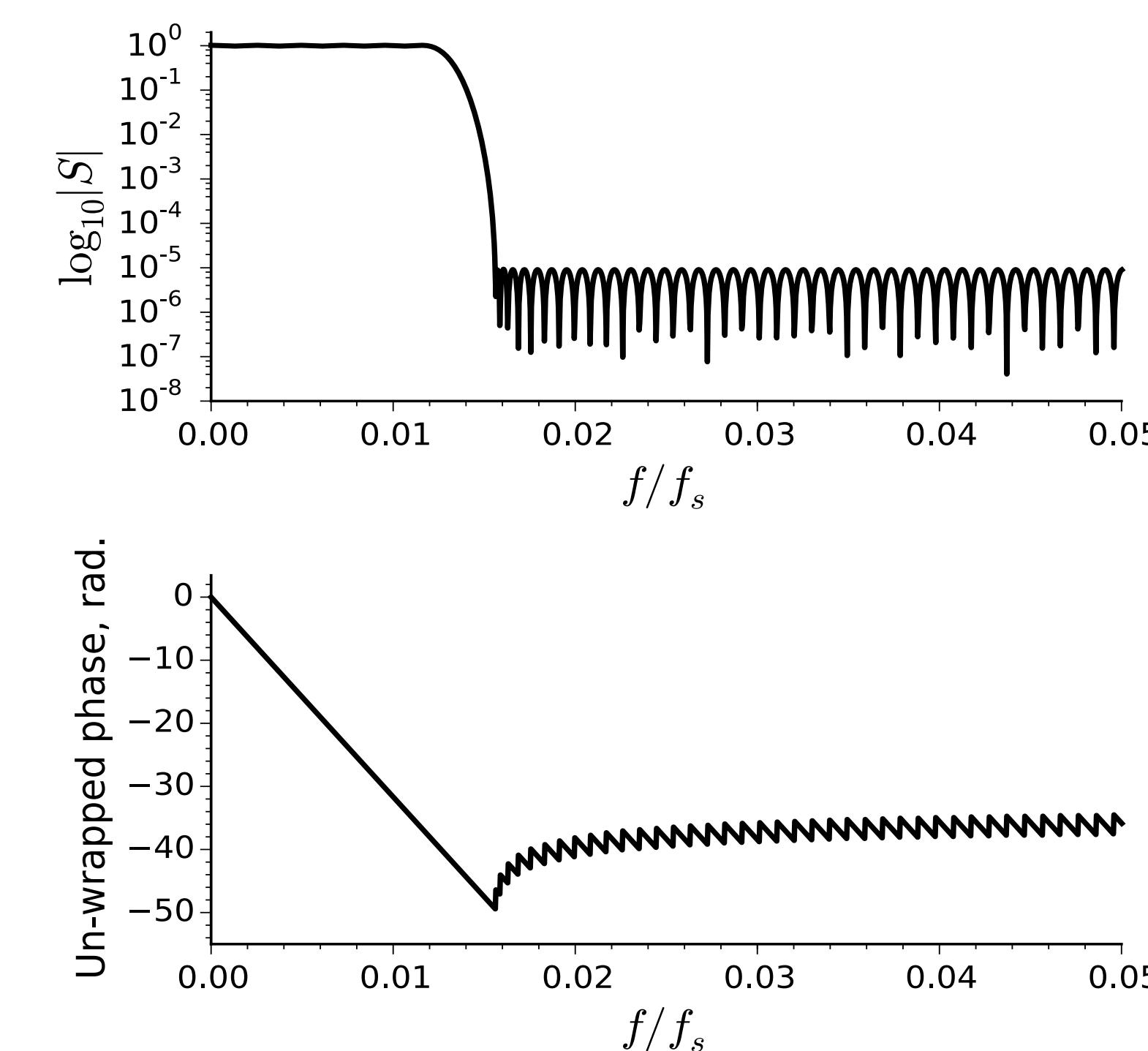
## 4. Analog & digital noise performance



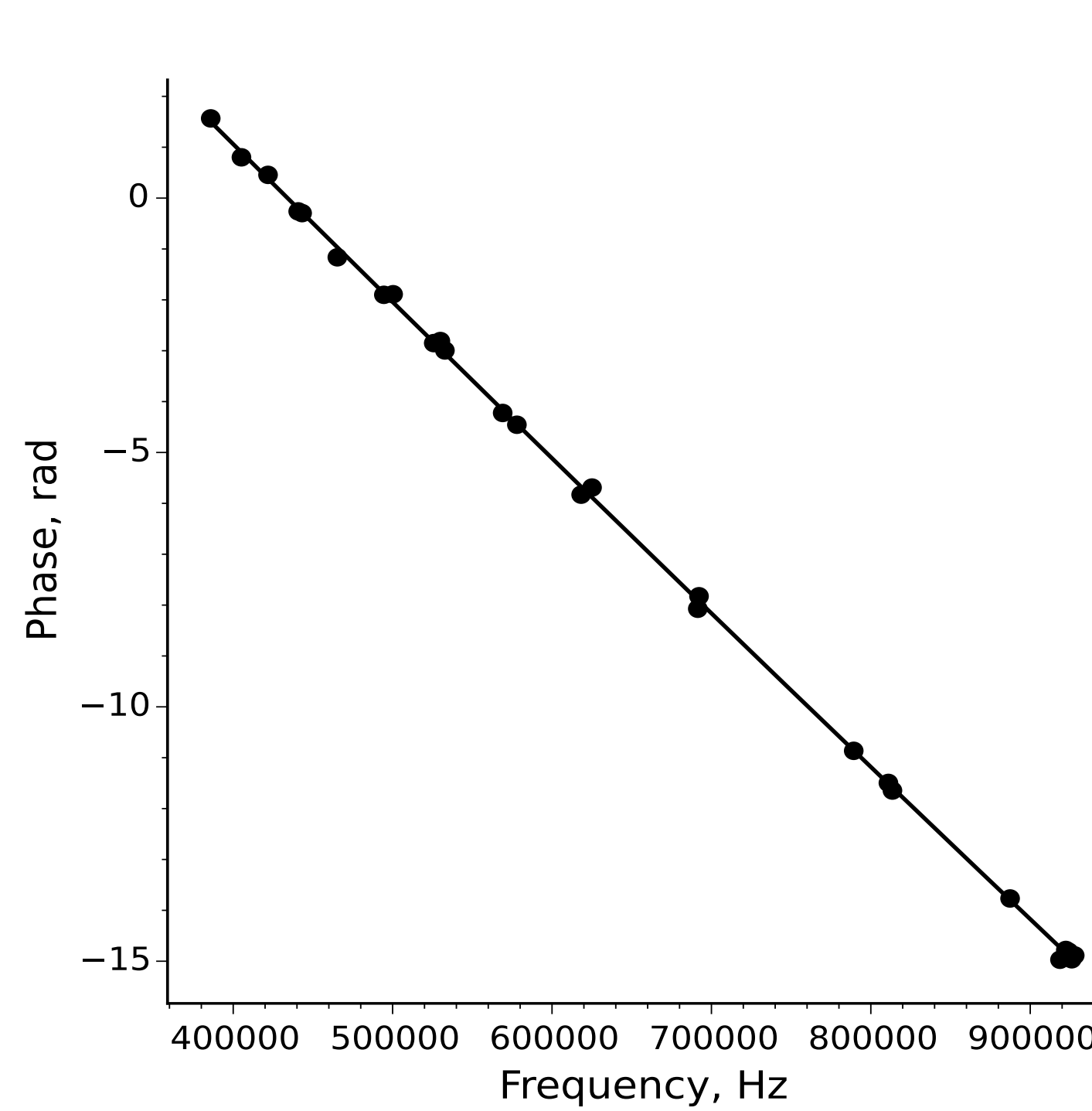
**Figure 5.** Comparison of noise levels, including contributions of both analog and digital noise components, in FT spectra obtained from two transient signals that were acquired in parallel using the external data acquisition system and the original electronics in the analysis of low-abundance ions of a calibration mixture on 10 T FT-ICR MS (Thermo Scientific). From 20% to 4-fold reductions in noise levels (for the same transient lengths) are observed as a function of selected parameters of the original electronics.



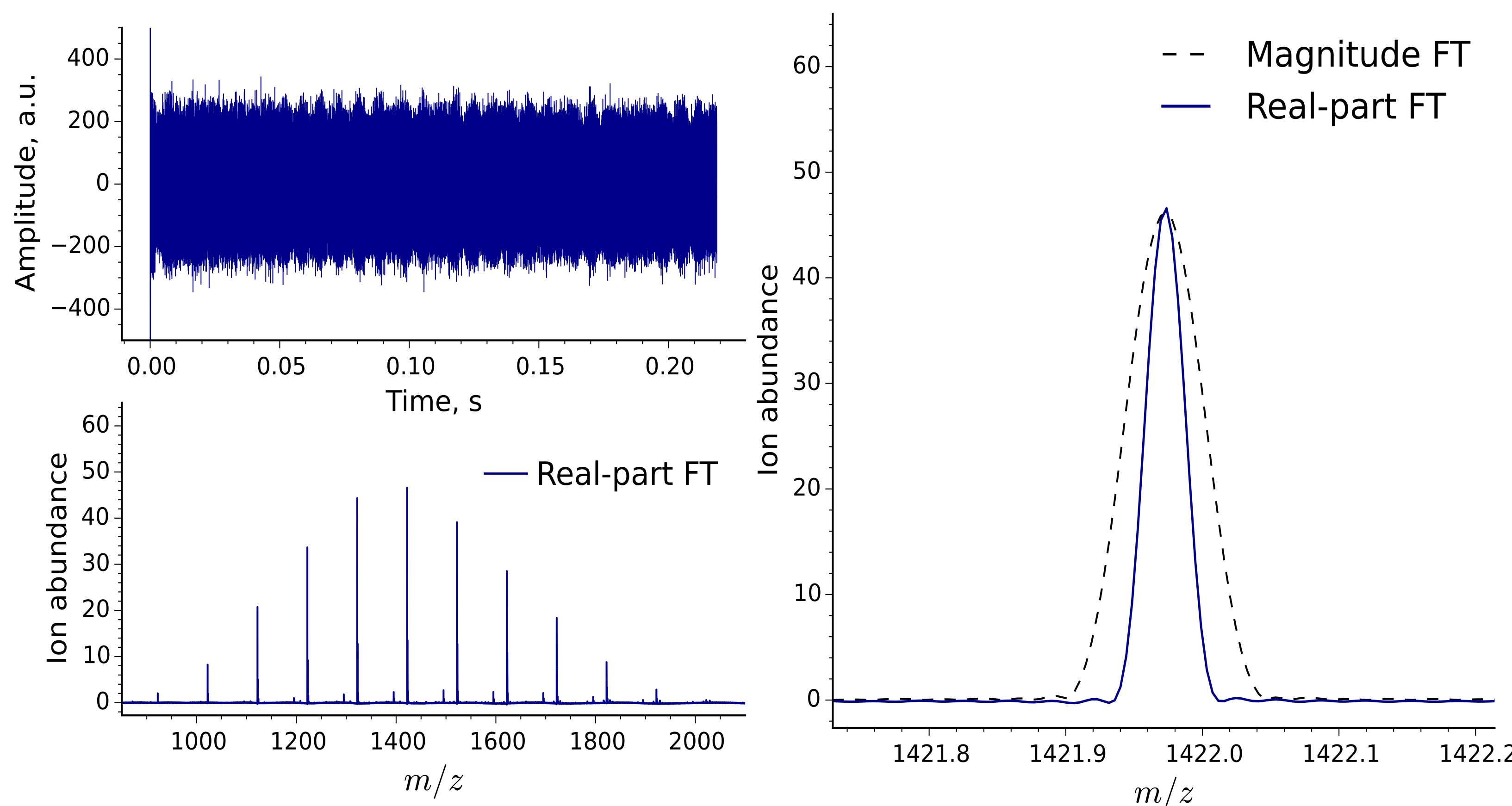
## 5. Absorption mode FT-ready transients



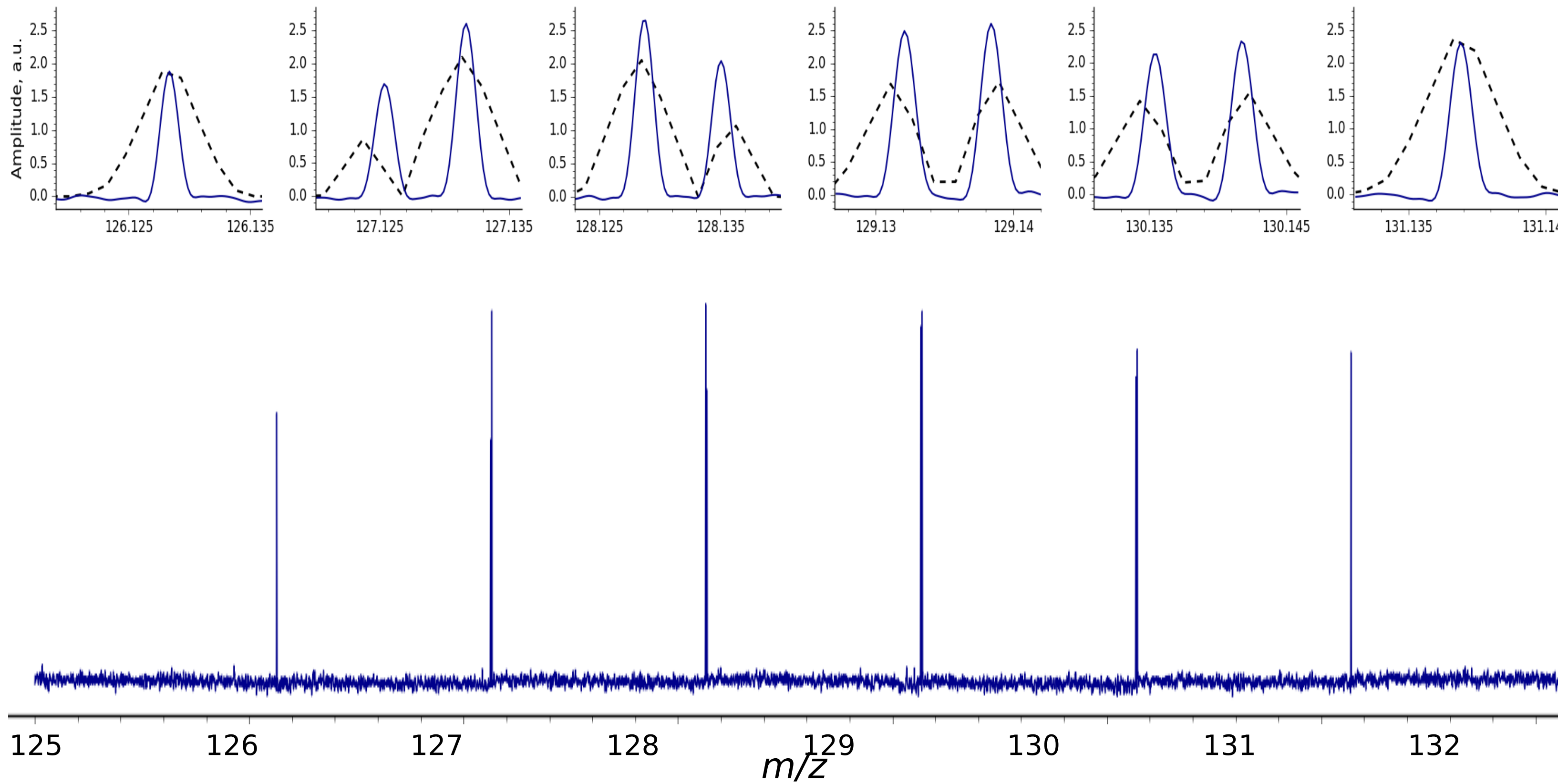
**Figure 7.** Simulation of amplitude (top panel) and linear phase (bottom panel) characteristics for the data acquisition system's design with low phase distortions in the analog/digital signal path during signal digitization.



**Figure 8.** Ion signal phase (un-wrapped data) as a function of ion frequency, obtained with the data acquisition system of this work in the analysis of a calibration mixture on Orbitrap™ FTMS instrument.

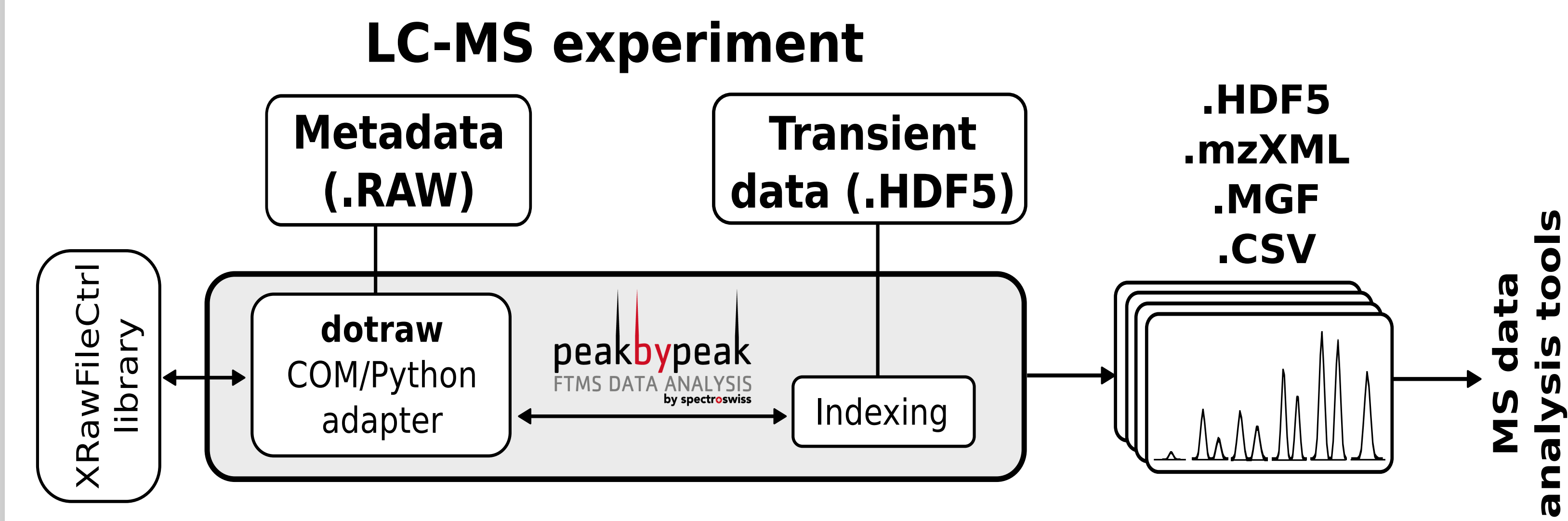


**Figure 9.** FT absorption-mode display without data-correction requirements. Transient signals are digitized in a form suitable for straightforward use of absorption-mode FT. Real-part of (complex-valued) FT appears as a pure absorption-mode display with correct peak shape representation. A mixed-mode FT spectrum and its phasing post-processing are thus avoided. The data was acquired in the analysis of a calibration mixture on Fusion™ Lumos™ Orbitrap™ FTMS. FT processing included signal apodization with Kaiser function and 3-fold zero padding.

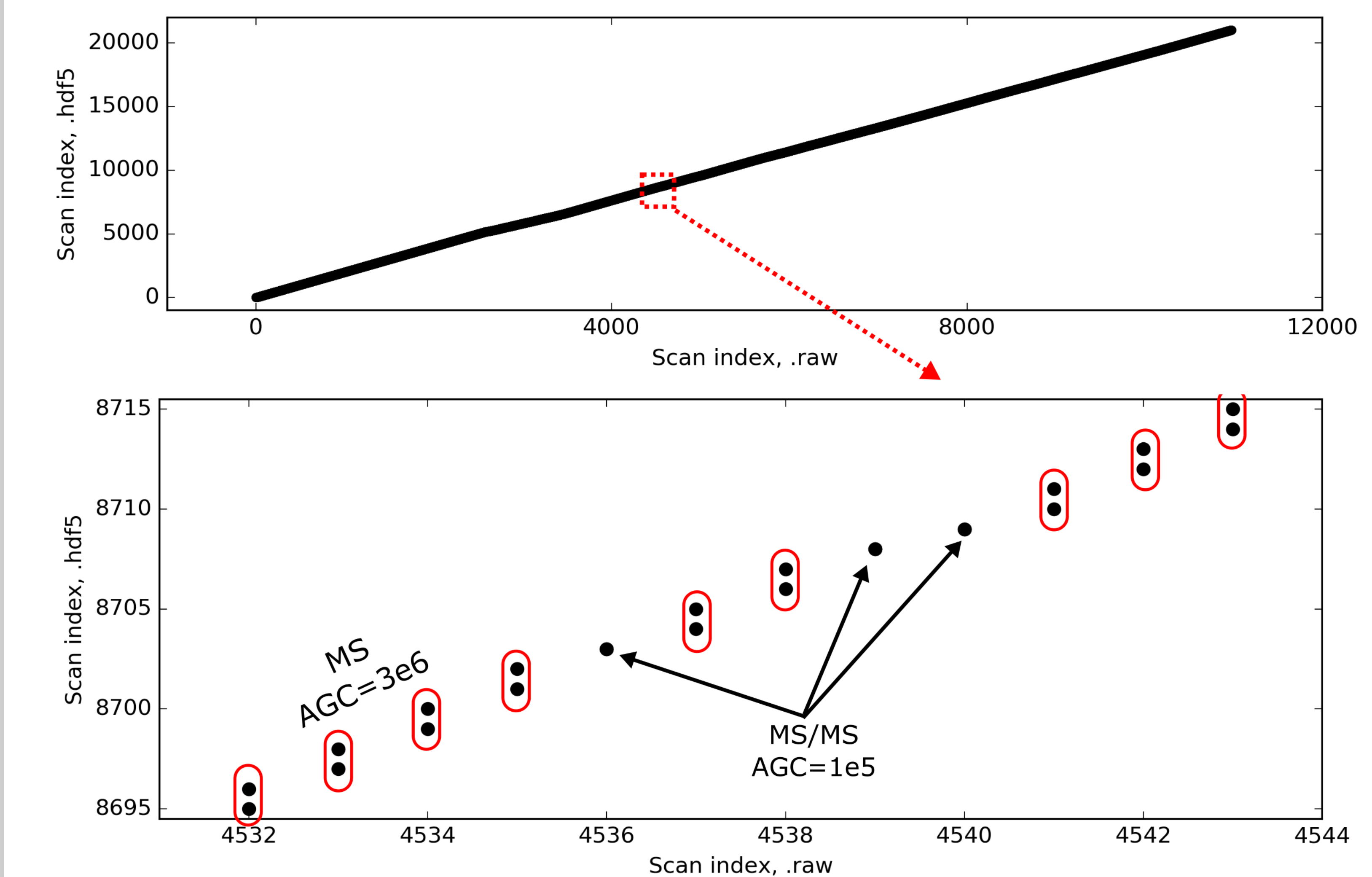


**Figure 10.** Evaluation of absorption-mode FT processing (with extended duty cycles) in the lower  $m/z$  range of mass spectra (blue color). The data was acquired in the LC-MS analysis of 10-plex TMT™-labeled yeast digest on Fusion™ Lumos™ Orbitrap™ FTMS. FT processing included signal apodization with Kaiser function and 3-fold zero padding. Bottom panel: a magnified view of an MS/MS spectrum into the reporter ions'  $m/z$  range. Top panel: magnified views of the MS/MS spectrum into individual  $m/z$  regions of the reporter ions. Data in black relates to original data acquisition electronics and software.

## 6. Integration with MS metadata



**Figure 11.** Schematic flow diagram of data and metadata integration between two complementary data sets obtained with the described data acquisition method and with standard data acquisition tools. A software interface to the standard data format (RAW files) is provided by a custom Python adapter that connects to the XRawFileCtrl library (Thermo Scientific) for retrieving pertinent experimental MS parameters from the RAW file (metadata).



**Figure 12.** An example of metadata mapping for an LC MS/MS measurement on Q Exactive™ Plus™ Orbitrap™ FTMS. Scan indices of an MS file acquired with the original electronics and software are mapped to indices of corresponding transient signals acquired with the data acquisition system of this work. The measurements were carried out with the following experimental settings: MS, AGC=3E6, 70,000 resolution setting; MS/MS, AGC=1E5, 17,500 resolution setting.

## 7. Conclusions

- Analog and digital noise components have been reduced, compared to previous-generation instruments, due to improved analog signal conditioning.

- Increased duty cycles of transient data acquisition have been achieved due to signal detection during complete periods of ion trapping in mass analyzers, translating to improved resolution performance, including for both FT methods, e.g. absorption-mode FT, and super-resolution methods, e.g. least-squares fitting (LSF), of signal processing, as well as for further increase in  $S/N$  of mass spectral data.

- Phase distortions arising along the analog-digital signal path during data acquisition have been substantially reduced, compared to current architectures of FTMS data acquisition systems, facilitating implementation and improving performance of those signal processing methods that benefit from phase information, e.g. absorption-mode FT, LSF, and other non-FT methods.

- In-line* signal processing has been implemented for acquiring transient signals in a form suitable for direct application of FT (without phase-correction post-acquisition processing) to generate absorption-mode display, including a potential for improving performance of past-generation instruments.