FT-ICR MS with In-Hardware Absorption-Mode Capability: Evaluation in High-Resolution and High-Throughput Applications

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Introduction

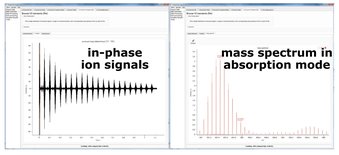
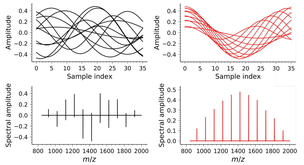


Figure 1. A specialized data acquisition and processing system for FT-ICR instruments. The system is based on the technology for detection of ICR transients with in-phase ion signals (generation of mass spectra in absorption mode), that we described in the previous work [1]. Here, we combine the developed electronics and the software-side processing in a single device (system), interface it to numbers of FT-ICR instruments, and benchmark it in high-resolution/high-throughput applications including petroleomics, imaging, and proteomics.

Methods

A. Transients with in-phase ion signals



B. Absorption-mode peakshape in FT-ICR MS

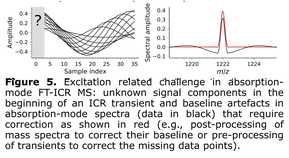


Figure 2. FT of a usual ICR transient whose components have diverse phases (left, top) yields a mixed-mode spectrum (left, bottom) that requires post-processing for it to be represented in absorption mode. Right panels: FT of transients with in-phase ion signals generates mass spectra directly in absorption mode.

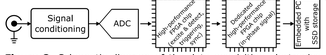


Figure 3. Schematic diagram of the data acquisition electronics of this work. The system runs in guest mode, acquiring data in parallel with the host instrument operation. Excite and detect analog signals are sampled simultaneously [1-4] and in-line processed to generate an output binary "transient" that contains signals corresponding to ions trapped in the ICR cell, with phases that are all close to zero. The software-side processing integrates the acquired data with metadata from vendors' data formats, decodes the binary transient, generates mass spectra, and exports data into H5, mzML, mzXML, imzML, MGF, TXT files.

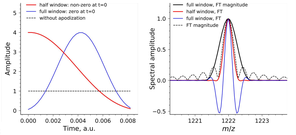


Figure 5. Excitation related challenge in absorption-mode FT-ICR MS: unknown signal components in the beginning of an ICR transient and baseline artefacts in absorption-mode spectra (data in black) that require correction as shown in red (e.g., post-processing of mass spectra to correct their baseline or pre-processing of transients to correct the missing data points).

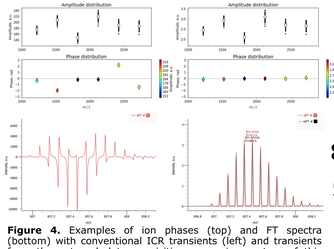


Figure 4. Examples of ion phases (top) and FT spectra (bottom) with conventional ICR transients (left) and transients from the external data acquisition-processing system of this work (right). The 2 datasets were acquired in parallel in the analysis of a ubiquitin sample on a commercial FT-ICR MS.

Figure 6. Two major classes of apodization windows (left panel), full windows (all data in blue) and half windows (all data in red), and corresponding peakshapes in FT mass spectra (right panels), as usually employed in absorption-mode FT data processing. All data in black are shown as a reference: a magnitude mode FT peakshape with standard (full) apodization window and a magnitude-mode FT peakshape without apodization.

C. Locked phase function: φ=0

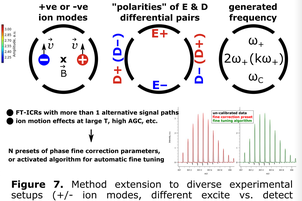


Figure 7. Method extension to diverse experimental setups (+/- ion modes, different excite vs. detect electrode arrangements, and detection frequency modes), and specific experimental conditions (presets with calibrated phase fine correction parameters, automatic fine tuning algorithm).

SIM stitching petroleomics workflow (7 T LTQ FT-ICR MS, ESI)

Petroleomics SIM stitching approach on a 7 T LTQ FT-ICR instrument (Ultra cell, Thermo Scientific). Data were acquired in parallel using (black) original electronics of the instrument and processed in magnitude mode (RAW files) and (red) using the external data acquisition-processing system (FTMS Booster X3, Spectroswiss) and processed in absorption (aFT) mode. Data courtesy of Wolfgang Schrader laboratory at Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany.

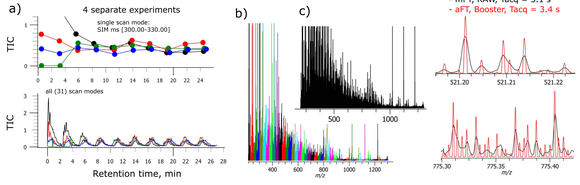


Figure 8. Experimental design: (a) an asphaltene sample was directly infused for 27 minutes, and data were acquired using 31 narrow SIM windows of 30 Th each. The experiment was replicated 4 times. (b) all scans of each individual SIM window were averaged, spectra (RAW) or transients (Booster), within single and across separate replicates. (c) averaged and noise thresholded spectra were further stitched together and processed via petroleomics tool (Peak-by-Peak, Spectroswiss).

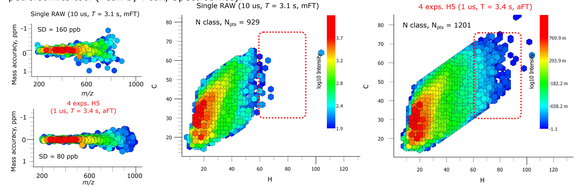


Figure 9. Prior to the data analysis, stitched mass spectra were re-calibrated using the non-linear iterative algorithm (Peak-by-Peak). The left panel shows mass error distributions for identified monoisotopic peaks of the N class (DBE: 4 - 41) after the re-calibration. The middle and right panels show C/H plots for the N1 class compounds identified in the mass spectra obtained via SIM stitching workflow using spectral (1 run, mFT, RAW) and transient (4 runs, aFT, Booster) averaging.

Intact protein native analysis, ESI (12 T SolariX^{TM} XR FT-ICR MS)

Direct infusion analysis of Robo/Arixtra protein-glycan complex (~23 kDa) on a 12 T SolariX XR FT-ICR MS (Bruker Daltonics) equipped with Paracell. Data were acquired in parallel using (black) original electronics of the instrument and processed in magnitude mode (D folder) and (red) using the external data acquisition-processing system (FTMS Booster X3, Spectroswiss) and processed in absorption mode. Data courtesy of Jon Amster laboratory at UGA, Athens.

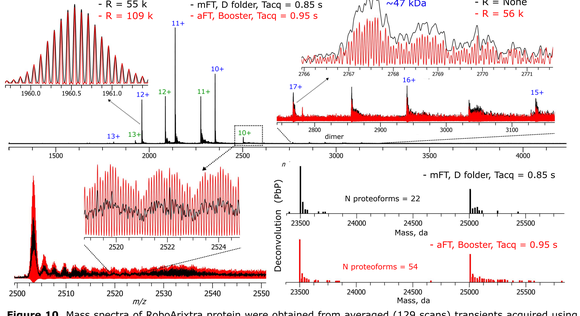


Figure 10. Mass spectra of RoboArixtra protein were obtained from averaged (129 scans) transients acquired using (black) built-in electronics of the instrument and (red) the external DAQ. The mass spectra were deconvolved using the high-resolution deconvolution tool (Peak-by-Peak). Deconvolved features were grouped by their charge state. Only proteoforms presented in at least three different charge states are considered and grouped.

Intact mAb native analysis, ESI (15 T SolariX^{TM} FT-ICR MS, Infinity cell)

Analysis of an intact mAb (NIST) was performed under native conditions on a 15 T FT-ICR MS (SolariX, Bruker Daltonics). Data were acquired in parallel using (black) original electronics of the instrument and processed in magnitude (mFT) mode (D folder) and (red) using the external data acquisition-processing system and processed in absorption (aFT) mode. The results show the implemented aFT acquisition and processing workflow increases the performance of an ICR instrument equipped with the previous generation ICR cell (Infinity). Data courtesy of Joe Loo laboratory at UCLA, Los Angeles.

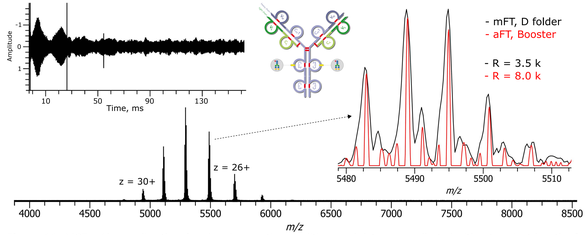


Figure 11. NIST mAb was directly infused under a native conditions and data were collected with a transient length of 175 ms (512 k). 100 scans were averaged and processed in (black) mFT and (red) aFT mode.

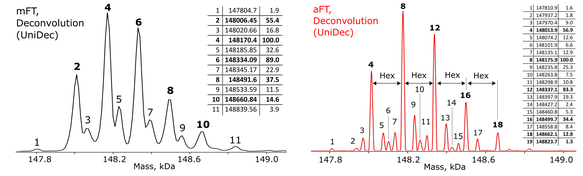


Figure 12. Averaged mass spectra generated in (black) mFT and (red) aFT mode were deconvolved (low-resolution) using UniDec software. The results show almost twice more number of identified proteoforms for (right) aFT data in comparison with (left) mFT one.

Middle-up mAb analysis, MALDI (15 T SolariX^{TM} XR FT-ICR MS)

MALDI mass measurements of IdeS digested and reduced mAb (Cetuximab) on a 15 T SolariX XR FT-ICR MS equipped with Paracell. MALDI generates low charge state (1+, 2+, etc.) species even for large proteins. Data were acquired in parallel using (black) original electronics of the instrument and processed in magnitude mode (D folder) and (red) using the external data acquisition-processing system and processed in absorption (aFT) mode. Data courtesy of LUMC, the Netherlands (Simone Nicolardi).

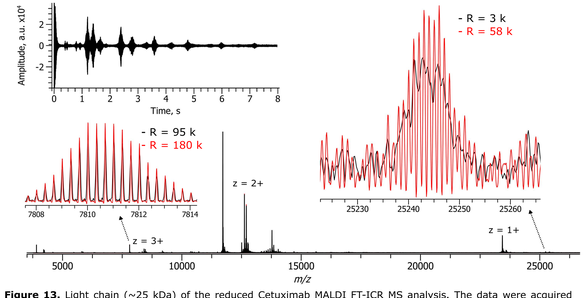


Figure 13. Light chain (~25 kDa) of the reduced Cetuximab MALDI FT-ICR MS analysis. The data were acquired with a transient length of 8 seconds. Averaged transients (20 conditionally co-added scans) were processed in (black) mFT and (red) aFT mode. The conditional co-adding allows to eliminate scans with substantial frequency shifts. More proteoforms were identified in deconvolved (Peak-by-Peak) aFT spectra in comparison with mFT one, presented in at least three different charge states (not shown).

DESI imaging (21 T FT-ICR MS)

NanoDESI imaging analysis of a rat brain on a custom 21 T FT-ICR instrument (EMSL, PNNL) equipped with the external data acquisition-processing system (FTMS Booster X3, Spectroswiss). Experimental parameters: transient length of 0.768 s, ~5 hours, spatial resolution: x~25 um and y~180 um. Data courtesy of Gregory Vandergrift at EMSL.

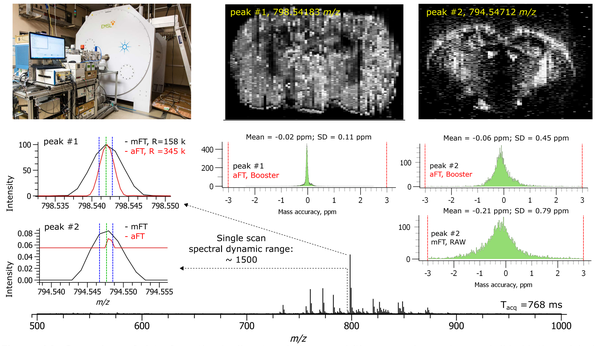


Figure 14. Comparison of data from the (red) external data acquisition-processing system and (black) the original electronics. Absorption (aFT) mode almost doubles the resolution and mass accuracy in comparison with magnitude (mFT) mode. Overall, a high spectral dynamic range in a single scan with more than 3 orders of magnitude was achieved.

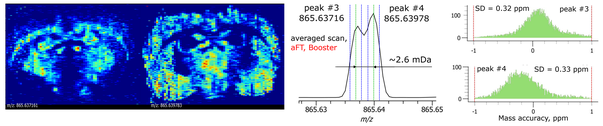


Figure 15. The instrument setup allows to construct complementary images of two target peaks with mass differences of only 2.6 mDa at a scan rate of < 1 s from mass spectra processed in aFT mode.

Conclusions

1. A specialized data acquisition-processing system has been developed, that enables to routinely use absorption-mode FT processing in real-life FT-ICR MS applications.
2. The in-hardware absorption-mode method demonstrates its fundamental properties: the method was found to be applicable to various commercial and customized FT-ICR instruments (e.g., 7 T LTQ FT-ICR, 12 T and 15 T SolariX^{TM} XR with ParaCell^{TM} and with Infinity^{TM} cells and the 21 T PNNL system).
3. Absorption mode, detection of full transients, and multiplexing of separate experiments (replicates) translate to expected gains in resolving power, S/N, mass accuracy, and sensitivity.

References

1. Kozhinov A.N. *et al*. Proc. 67th ASMS Conference, Atlanta, GA, USA, **2019**.
2. Marshall A.G. *Chem. Phys. Lett.* **1979**, *63*, 575.
3. Oppenheim, A.V, Schafer R.W. Discrete-Time Signal Processing. Pearson, **2013**.
4. Beu S. *et al*. *Anal. Chem.* **2004**, *76*, 5756-5761.
5. Kooijman P.C. *et al. Scientific Reports*, **2019**, 9, Article 8.

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