Journal of the American Society for Mass Spectrometry

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TherMIDAS: An Integrated Data Acquisition System for FT-ICR Mass Spectrometry

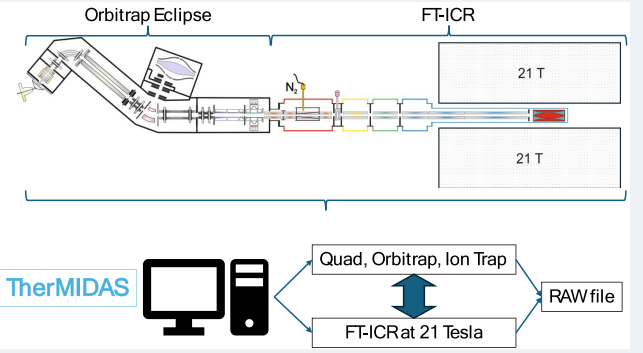
Published as part of Journal of the American Society for Mass Spectrometry special issue "Advances in Fourier Transform Mass Spectrometry".

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Cite This: https://doi.org/10.1021/jasms.6c00273 Read Online

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ABSTRACT: Over 30 years ago, the Modular ICR Data Acquisition System (MIDAS) was assembled, developed, and deployed on the then-new Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) instruments at the National High Magnetic Field Laboratory. The system was based on National Instruments electronics and software, with interface electronics and control code built and written in-house. Capable of controlling all aspects of FT-ICR MS data acquisition and data processing, the system formed the basis of MagLab instrument control for the following three decades. Here, we report on recent progress in data acquisition and analysis capabilities, motivated by the coupling of an Orbitrap Eclipse Tribrid mass spectrometer to the 14.5 and 21 T FT-ICR instruments. The new system is based on more recent National Instruments PXIe control boards, and leverages and extends the vendor instrument control hardware and software for a seamless integration of the FT-ICR MS instrumentation with the Eclipse front-end. The entire instrument is controlled from Tribrid Series Tune, Method Editor, and Xcalibur software. All mass analyzers, including the ICR analyzer, can be operated in parallel, exploiting the existing Tribrid architecture, and resulting in duty cycle improvements over previous implementations.



INTRODUCTION

Instrument control systems in mass spectrometry have evolved considerably since Comisarow and Marshall's 1974 publication of the first Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum,[1] which referenced Cooley and Tukey's original fast Fourier transform (FFT) work[2] from 1965 in the process: "the first 256 data points were then recorded on magnetic tape and a discrete Fourier transformation of that array was carried out by a digital computer." At that time, the state-of-the-art mass spectrometer instrument control was the Finnigan 1015 GC/MS/DS gas chromatography mass spectrometer; the "DS" in the name meant "data system," the control system built independently of the instrument hardware.[3] Eight years later, C.G. Enke published an overview of the use of computers in scientific instruments,[4] and used his triple-quadrupole mass spectrometer as one of several examples. By that time, control systems had grown in sophistication, carrying out not only signal processing and data analysis, but also more complex scripted experiments involving many cycles of mass spectral acquisitions across different dimensions of analysis. Specific to the FT-ICR subfield, the Nicolet Corporation used its experience in FT analysis in the context of NMR and IR to first commercialize FT-ICR MS instrumentation, with a data system employing in-house hardware and software: the FTMS-1000 powered by the Nicolet 1180 computer. At that time it was not unusual for the data system to be completely separate from the mass spectrometer; Gross and co-workers described assembling an FT-ICR instrument from Varian ICR-9 hardware and the Nicolet control and data system.[5−7] In 1992, EXTREL FTMS (later acquired by Finnigan) introduced the Odyssey data system, and it was this data system that served as the control and acquisition platform for the FT-ICR instruments initially

Received: July 10, 2026
Revised: September 25, 2026
Accepted: September 29, 2026

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built at the National High Magnetic Field Laboratory (NHMFL, MagLab) when Alan Marshall became the director of its ICR facility in 1993.

Due to the high cost and closed nature of the available proprietary commercial data systems, interest developed in less expensive, open solutions using off-the-shelf components. Beu and Laude introduced a simplified but flexible scheme built around the general purpose interface bus (GPIB) and employing well-known components (arbitrary waveform generator and digital storage oscilloscope).[8] A few years later, Senko and co-workers built MIDAS, a modular FT-ICR data acquisition and analysis system.[9] MIDAS leveraged several innovations by National Instruments (NI), which by the late 1980s had started to specialize in easily implemented and flexible research-grade electronics solutions. Control of the ICR instrument was accomplished by a combination of commercial and custom electronics: a pattern generator, an arbitrary waveform generator, and a high-speed digitizer, all housed within a VXI (VMEbus Extensions for Instruments) chassis. The VXI-based hardware was interfaced to instrument components via custom-built electronics that converted the pattern generator output to DC voltages and TTL trigger lines. MIDAS hardware was controlled with software written in NI's LabWindows/CVI (C for Virtual Instrumentation), an extension of the popular LabView software, that allowed users to write code in C incorporating common C-language libraries. MIDAS served as a less expensive alternative data system solution for many FT-ICR laboratories for years, and was in use in its original form at least as late as 2010.[10]

In 2004, Mize and co-workers built and demonstrated a system using NI's newer PCI eXtensions for Instruments (PXI) platform, with faster hardware and more memory.[11] Their system included home-built implementations of an arbitrary waveform generator, a signal digitizer, and processing units, and was controlled by an application built in C++ using Visual Studio. During the same time period, Blakney and co-workers at the MagLab upgraded MIDAS with PXI-based electronics, updates to the control scheme, and software updates in LabWindows. This modernized system was renamed Predator and became the MagLab's data system for all subsequent development.[12]

In 2004, an LTQ (Thermo Scientific) was installed as part of the front-end for the 14.5 T ICR instrument at the MagLab,[13] analogous to the commercially marketed LTQ-FT instrument. The LTQ platform provided capabilities for fast MSn, ion trap isolation, and most importantly for the mass accuracy of the FT-ICR spectra, automatic gain control (AGC), which offered a reliable way to regulate ion populations using the linear ion trap (LIT).[14,15] In this instrument, the ICR analyzer and all associated hardware were controlled by the LTQ embedded system. Existing control code was modified and new code developed to manage the expanded hardware; existing data pipelines from the LTQ-FT instrument were used to process the incoming ICR data. This work resulted in a seamless design from a user's perspective, in which all aspects of instrument control were determined by the LTQ data system, and returned data were processed and stored in the Xcalibur raw file. However, this instrument suffered from the limitations of the excitation and detection electronics. The LTQ-FT was designed around a 7 T ICR magnet, and therefore used a digitizer sampling rate sufficient to resolve the highest expected ion frequency, corresponding to the lowest anticipated m/z. Since cyclotron frequency increases linearly with magnetic field,[16] the frequency of an ion in a 14.5 T magnetic field approximately doubles relative to its frequency in a field of 7 T, thus requiring an approximate doubling of the digitizer sampling rate in order to properly represent the sinusoidal time-domain signal above the Nyquist limit. Doubling the sampling rate also doubles the number of data points acquired for a given acquisition duration, leading to increased memory requirements. It was not possible to modify this hardware, which effectively limited the available mass range and resolving power of this instrument.

In 2015, MagLab personnel reported on one of the more momentous upgrades in the lab's history, an advanced FT-ICR MS instrument operating at a magnetic field of 21 T.[17] The front-end system on this instrument was upgraded from the LTQ to the LTQ-Velos platform, following work at the Bruce lab where they replaced the LTQ on their LTQ-FT instrument with an LTQ-Velos.[18] The Velos-based 14.5 T system at the MagLab offered increased sensitivity resulting from the inclusion of the S-lens in the source, increased scan speed of the dual linear ion trap, and the opportunity to perform ion−ion reactions via a front-end ETD (fETD) interface.[19] However, this system retained the LTQ-FT ICR electronics and thus inherited the aforementioned drawbacks of the 14.5 T ICR system.

Then in another major upgrade on April 8, 2024, the day of a total solar eclipse, an Orbitrap Eclipse mass spectrometer (Thermo Scientific) replaced the LTQ-Velos on the front end of the 21 T system,[20] followed shortly by the same upgrade for the 14.5 T magnet instrument. These upgrades improved upon the same features the LTQ-Velos provided, such as front-end ETD, CID, MS^n, and ion population control, while adding capability for quadrupole isolation, higher-energy collisional dissociation (HCD), ion-trap based multinotch isolation, and an additional mass analyzer in the high-field Orbitrap.[21] Important aspects of this platform, as will be seen below, were improvements in instrument control that expanded on existing capabilities for parallelizing ion manipulation with mass analysis and signal processing.

For the new Eclipse-ICR quaternary hybrid system—to our knowledge the first such four-analyzer instrument— control and data acquisition were consolidated into a new hybrid data acquisition system, which we describe here. In this system, the National Instruments hardware (the arbitrary waveform generator and the high-speed digitizer) are controlled by a new application written in C#. This application controls the timing and synchronization of the excitation and detection sequence, and functions as an entirely separate module, under control of the central Eclipse data acquisition system. In this design the advantages of the advanced ICR hardware, such as the dynamically harmonized cell and the world-record 21 T magnet, are not limited by the data station as was the case for the LTQ-Velos-based system. Meanwhile, all control is centralized under the Eclipse control software. The FT-ICR module is interfaced to the Eclipse data station by a custom library which encapsulates all of the proprietary data processing and packing functionality. This configuration allows incoming ICR data from the NI cards to be passed to the Thermo software for use in online methods, data-dependent operations, display in Tune and Xcalibur, and ultimately storage in the RAW file and/or MagLab transient DAT formats (MIDAS format). This implementation includes full real-time control of ICR spectral processing tasks such as zero filling, apodization, FFT, and magnitude-mode calculations prior to packing into

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the RAW file format. The entire instrument control scheme thus provides a seamless and flexible control system, resulting in data files that can be analyzed with any software that supports the RAW file format, an important upgrade for users of the ICR facility. Since this new control system is a combination of Thermo's software and the software that originated with MIDAS, we name the combined system TherMIDAS.

METHODS

Coupling a Tribrid Instrument to the MagLab ICR Systems

The Orbitrap Eclipse system provides important new capabilities for the MagLab ICR instruments, most notably quadrupole ion isolation prior to mass analysis and an ion source that is 3x more sensitive due to the letterbox-shaped inlet and the inclusion of an electrodynamic ion funnel. The Eclipse also retains the critical advantage of the previous LTQ-Velos-based system: precise control of the incoming ion population. The Eclipse includes an additional powerful mass analyzer in the high-field Orbitrap and offers many options for multimodal analysis: front-end ETD, HCD and CID fragmentation, synchronous precursor selection, MS^n, high and low-pressure modes, mild-trapping capability, and more. These options serve a wide variety of possible experiments and applications, from small molecule analysis to petroleomics and top-down proteomics.

The Eclipse mass spectrometer was mated to the ICR's first vacuum stage by rotating the instrument 19.5 degrees from the ICR cell axis to accommodate the ion-optical bend introduced by the presence of the C-trap. The back plate of the Eclipse's linear ion trap chamber was replaced with a custom flange, connecting a chamber containing a specially designed 302 mm quadrupole ion guide. This ion guide serves as the link between the Eclipse and the ICR instrumentation. From the low-pressure cell of the dual linear ion trap in the Eclipse, ions travel down the transfer quadrupole to the multipole storage device (MSD). From the MSD, ions are ejected and captured in the ICR cell, a dynamically harmonized cylindrical (DHC) design of the type pioneered by Nikolaev.[17,22] Figure 1 shows

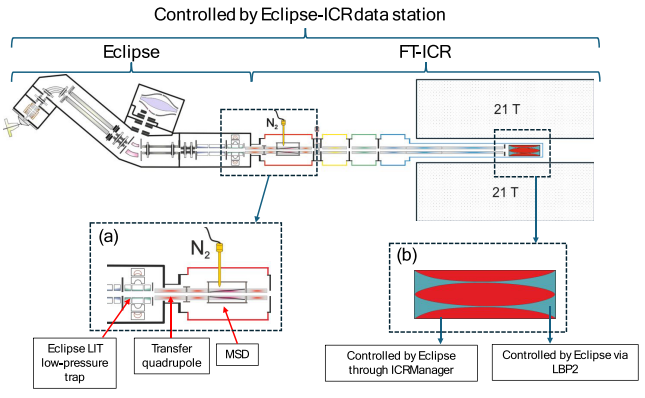


Figure 1. Instrument architecture. The top half of the figure shows a top-down schematic of the Orbitrap Eclipse mass spectrometer mated to the ICR instrumentation and the 21T magnet. Inset (a) shows the 302 mm transfer quadrupole that links the two sections; inset (b) shows the DHC and the control sources for the cell plates.

a schematic of the combined instrument. Figure 1(a) on the lower left shows the 302 mm transfer quadrupole between the low-pressure trap of the Eclipse's dual LIT and the MSD of the ICR instrumentation. Figure 1(b) shows the DHC. Both the excite/detect plates and the DC plates are controlled by the Eclipse: the excite/detect plates are controlled through the ICRManager interface (described below) to the NI electronics, while the DC plates are controlled directly by the Eclipse.

The Eclipse uses a lens control module, termed a lens backplane (LBP), to control its own myriad lenses and ion guides. The LBP consists of 32 individual DACs, each providing ±300 V DC, with 1500 V/ms slew rates and RMS noise <100 microvolts peak-to-peak. An additional LBP (LBP2) was connected to the Eclipse system to provide control of the 32 devices contributed by the ICR instrumentation. As mentioned above, the excite/detect plates of the DHC are controlled using the ICRManager interface to FTPC2, which controlls the NI electronics; all other ICR devices are controlled directly by the Eclipse using LBP2.

Operation of the ICR cell, including excitation, detection, synchronization, and retrieval of the ion time-domain signal, is controlled by a separate Windows PC connected to a National Instruments PXI chassis, containing two PXI cards: an arbitrary waveform generator and a signal digitizer. The PC connects to the PXI chassis via a NI PXIe-8381 PC-to-PXI interface card. The arbitrary waveform generator (AWG) is a NI PXIe-5442, with a vertical resolution of 16 bits and a maximum update rate of 100 MS/s. The digitizer is a NI PXIe-5122 two-channel digitizer with a vertical depth of 14 bits, a maximum sampling rate of 100 MS/s, and memory per channel option of 512 MB (1024 MB total on board).

Software Architecture

Native software control for the Orbitrap Eclipse is implemented on three major components: the data system PC (DS), a main embedded CPU board controlling everything except the Orbitrap subsystem, and a subordinate embedded CPU, termed the FTPC, controlling the Orbitrap subsystem. The DS communicates with both embedded CPUs over ethernet via TCP/IP. An individual spectral acquisition proceeds as follows. First, a scan is defined by a user, either through Tune or via a method definition. Then, a scan definition is assembled from the various user interface (UI) elements, packaged, and sent to the main board. Code on the main board parses the scan definition and converts it into a scan matrix, which is essentially a translation of a scan definition from user-interpretable values like mass ranges into precisely timed device-specific settings like DC voltages and RF amplitudes. If the scan definition and its matrix contain instructions for Orbitrap analysis, a separate set of data is sent to the FTPC, where it is translated into an FTPC-specific scan matrix. When the execution command is given, all parts of the instrument trigger their specific matrix events at times synchronized via a high-speed serial bus (HSSB). After the scan has been executed, mass spectral data are returned to the DS where the instrument manager service publishes the spectrum for consumption by the Tune application (for display), Xcalibur (for display in the acquisition manager), and RAW file writing. A reduced peak list is also returned to the Eclipse main board for use in data-dependent experiments.

We followed this same basic model in adding ICR control to the Eclipse-ICR system. As mentioned above, a separate PC controlls the National Instruments PXI hardware. This PC hosted a C# application called FTPC2, which controlls all aspects of configuration and control of the AWG and digitizer. FTPC2 incorporats a dynamically linked library (DLL) called ICRManager, through which FTPC2 connects to the DS computer via TCP/IP. For ICR spectral acquisition, as with Orbitrap acquisition described above, ICR-specific scan parameters are received by ICRManager from the Eclipse main board, translated to a scan sequence by FTPC2, and then downloaded to the NI AWG and digitizer cards. The NI hardware executes this sequence upon receipt of an external trigger from the Eclipse, synchronized with other Eclipse hardware. This on-demand acquisition approach differs significantly from both MIDAS and Predator predecessors, for which the desired number of acquisitions were required upon execution.

After execution of the scan sequence, time-domain data are received from the digitizer by FTPC2, which applies apodization and zero filling, performs the fast Fourier transform (FFT), and computes the magnitude-mode spectrum. Transformed data are then processed by ICRManager, which replicates Thermo's standard algorithms for peak determination, noise reduction and thresholding, and peak annotation. ICRManager also provides methods for packaging data and sending it to the Eclipse DS, as well as sending a reduced feature

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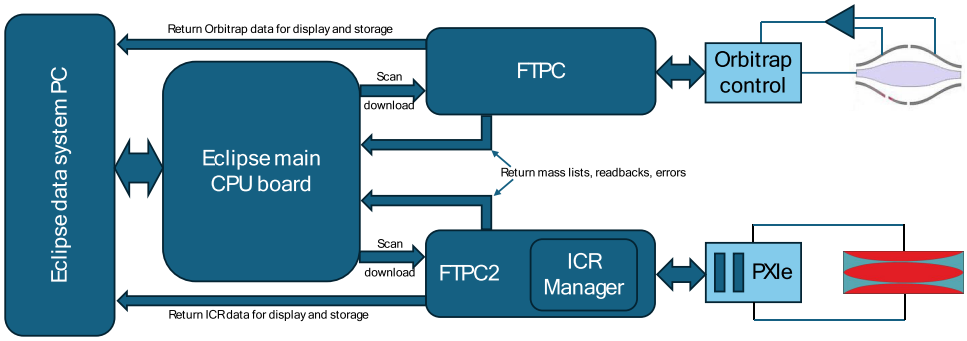


Figure 2. Instrument control architecture. The Eclipse data system PC sends instructions to the Eclipse main board, which then sends scan information to the FTPC (in the case of Orbitrap scans) and to the FTPC2 (in the case of ICR scans). FTPC and FTPC2 then send scan execution data to their respective control modules. After acquisition completes, data are returned to FTPC and FTPC2, which then return data back to the main board and the data system PC.

list to the Eclipse main board for use in data-dependent experiments. Data stored in RAW files includes all standard metadata (like injection time, base peak abundance, calibration data, and so forth). This storage format is ∼120× smaller than the full MIDAS DAT format, although this factor scales with sample complexity somewhat. The mass spectral data type was restricted to reduced profile data in this initial implementation. The overall architecture is summarized in Figure 2.

Instrument Control Software Modifications

The current Tribrid instrument control software does not support mass analyzers beyond the Orbitrap, linear ion trap, and quadrupole mass filter, and previous support for legacy instruments like the LTQ-FT is no longer available. However, because there is support for FTMS in general (via the Orbitrap), adding ICR support mainly consisted of replicating much of the existing Orbitrap functionality, with modifications made as necessary to account for different analyzer properties. An additional analyzer (called FT-ICR) was added, and an additional instrument model name was created, which allows for MagLab- and ICR-specific UI elements in Tune and elsewhere. Additional software ports were added to fully support communication and data transfer between FTPC2 and the Eclipse.

The Eclipse main board firmware consists of two layers. A C++ layer controls communication, low-level device control, and scan matrix execution. A Lua scripting layer operates in this C++ environment for higher-level control of method execution, scan definition handling, matrix building, and instrument parameter control. ICR-specific code was added to these areas in the Lua layer, allowing the ICR part of the instrument to be fully integrated into the instrument control software. This included the execution pipeline, thus enabling fully parallelized operation of the ICR analyzer in combination with all three of the existing tribrid mass analyzers. ICR-specific Lua diagnostics were also added to the instrument diagnostic menus, where parameters specific to the NI hardware could be set if necessary.

Experiments

The added functionality was demonstrated by conducting a variety of experiments using FlexMix (Pierce) as the sample. FlexMix is a mixture of 16 components spanning a m/z range from 50 to almost 3000 and is used for calibration of Thermo Scientific mass spectrometers. FlexMix was delivered to the mass spectrometer inlet via the heated ESI (HESI) source using a syringe pump, generally set at a flow rate of 2−5 μL/min.

To demonstrate the parallelization of mass analyzers, liquid chromatography (LC) was coupled with the Eclipse front-end for the separation of per- and polyfluoroalkyl substances in the 3 M Peterson Bucket aqueous film-forming foam (AFFF). The LC solvents were 10 mM ammonium acetate (Fisher Scientific, Optima) in water adjusted to a pH 7.5 with ammonium hydroxide (Fisher Scientific, Trace Metal grade) and 10 mM ammonium acetate in a 90/10 v/v mixture of acetonitrile/water for solvents A and B, respectively. PFAS were separated on an Ultimate 3000 (Thermo Scientific) using a two-step gradient from 5 to 20% solvent B over 6 min and 20−70% solvent B over 26 min, followed by a 3 min isocratic hold at 100% solvent B at a flow rate of 30 μL/min. The column oven was held at 40 °C. The solvent delay column was an Agilent Zorbax XDB-C18 (0.5 mm × 35 mm, 3.5 μm particles) and the analytical column was an Agilent Zorbax SB-C18 (0.5 mm × 150 mm, 3.5 μm particles). To retain polar PFAS with symmetric peak shapes, a trap column (Agilent Zorbax SB-C18 0.5 mm × 35 mm, 3.5 μm particles) was installed on a 10-port switching valve. A 5 μL injection was loaded onto the trap column for 2 min at 40 μL/min of 5% solvent B, with the eluent flowing to waste. After 2 min, the valve switched to flow over the analytical column, the flow rate was decreased to 30 μL/min, and the gradient began. A heated electrospray ionization source was used in negative mode at −3000 V. The ion transfer tube and vaporizer temperatures were set to 275 and 75 °C, respectively. The sheath gas was set to 25, the auxiliary gas to 8, and the sweep gas to 3 (arbitrary units). MS^1 spectra were collected in the ICR mass analyzer at a resolving power of 2.5 M (at m/z 200; 3.35 s transient duration) over a m/z range of 200−1500. Hanning apodization was used. The RF lens was set to 35% and data were collected in profile mode. The AGC target was set to 400,000 charges. Ion trap MS^2 spectra were collected in data-dependent mode with an isolation window of 1.6 Da. The fragmentation mode was HCD with normalized collision energies of 18, 53, and 87%. The sample was run in both parallel and serial modes in order to assess the benefits of parallelization. Data files were processed using a nontargeted PFAS workflow in Compound Discoverer (Thermo Scientific, 3.4 SP4) and PFAS identity was confirmed by comparing measured and reference MS^2 spectra.

DISCUSSION

Parallelization of FT-ICR analyzer operation with external ion accumulation was demonstrated at the MagLab in 1997.[23] Subsequently, similar strategies were implemented for both the LTQ-FT and LTQ-Orbitrap (Thermo Scientific) series of instruments, in which the linear ion trap mass analyzer was operated in parallel with the FTMS analyzer. For these instruments, data-dependent acquisition (DDA) experiments employed a "preview mode" whereby a reduced-resolving-power spectrum could be returned and processed well before the complete spectrum was available. Upon receipt of the preview spectrum, data-dependent linear ion trap acquisition could be queued and executed while the complete survey spectrum was still being processed. In separate instrument development programs, the Q-Exactive system could parallelize ion injection with Orbitrap spectral acquisition.

The first Tribrid instrument, the Orbitrap Fusion (of which the Orbitrap Eclipse pictured in Figure 1 is a derivative), combined the above developments into the concept of an ion

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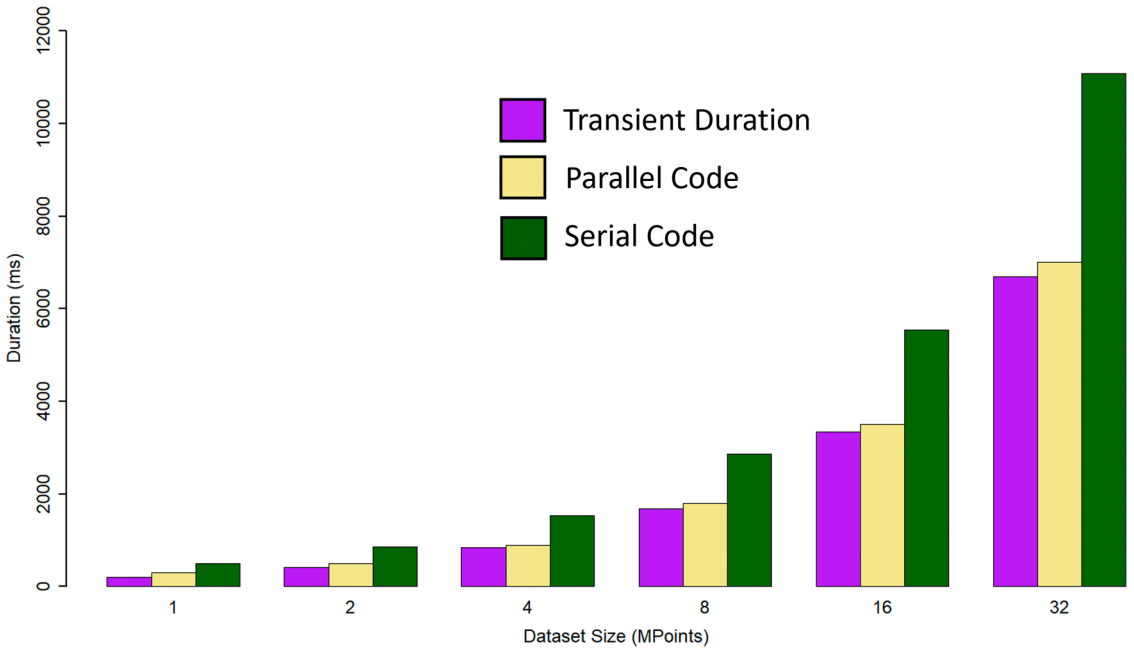


Figure 3. Transient acquisition and processing times for different data set sizes and processing modes. Parallelizing data processing (including FFT, noise determination, and peak-picking) with transient acquisition leads to a substantial duty cycle improvement, which becomes more important as the transient length increases. With parallelized processing, the time to acquire and process successive transients reduces the processing overhead by about 40%. Digitization rate was 5 MHz.

"pipeline". Pipelining ions simply means that after a given packet of ions has been injected, trapped, and sent to a mass analyzer, the next injection may commence immediately, before the mass spectral data have arrived back at the DS and even before mass analysis has begun. In fact, depending on the exact architecture and method sequence, injection of subsequent ion packets may commence before the previous packet even arrives at a mass analyzer.[24]

The Orbitrap Fusion also introduced the concept of *N-1 execution*, referring to the order of processing just-acquired mass spectra for data-dependent experiments. Instead of determining peak lists for following acquisitions after a given survey scan is completed, these peak lists are compiled based on the *previous* cycle's survey scan. This approach allows data-dependent spectra to be acquired in parallel with the acquisition of the next survey spectrum. Previously, data-dependent acquisitions could not be executed until the peaks to be interrogated were known, i.e., until the survey spectrum was complete, which could take hundreds of milliseconds for a single survey spectrum. The aforementioned preview mode provided a shortcut, but still spent time in processing and ultimately returned a reduced-resolving power spectrum.

As an example of the improvement offered by N-1 execution, consider a data-dependent experiment with a 3-s cycle time (time between adjacent survey spectra). Assume a one-second survey acquisition (512 ms of time-domain data acquisition plus about the same time in processing) and a 50 ms linear ion trap acquisition. Under these conditions and with the old processing scheme, ion trap spectra can only be acquired for 2 s of the 3 s cycle time, leading to an acquisition rate of 2 s/0.05 s/3 s = 13 ion trap spectra per cycle. However, if ion trap spectra can also be acquired during the full time of transient acquisition and processing, then the rate becomes 3 s/0.05 s/3 s = 20 spectra per cycle.

Pipelining ions and N-1 execution both lead to much-improved acquisition rates for nearly all experimental schemes. Data-independent acquisition (DIA) experiments do not use the N-1 execution scheme, of course, but still benefit substantially from parallelized operation, which has been used to great effect in recent developments like the Astral mass analyzer.[25]

The greatest acquisition rate improvements from ion pipelining come from parallelizing ion injection and analysis with acquisition and processing of the time-domain data (i.e., the transient) for FTMS mass analyzers. In all current Tribrid instruments, the N-1 execution described above is mainly used to acquire as many MS/MS scans as possible while longer-duration acquisitions are performed in the Orbitrap analyzer. Other processing and analysis may be parallelized as well. Examples of other parallelizable operations include building scan matrices, calculating isolation waveforms, and more time-consuming activities such as photoactivation.

With the above strategies in mind, one of the first adjustments we made after adding the ICR analyzer to the software was to parallelize ICR data processing (including FFT, noise determination, peak picking, and data transfer) with ICR acquisition, so that processing the time-domain data does not force the analyzer to remain idle. This implementation resulted in a nearly 2× acquisition rate improvement as shown in Figure 3.

We also extended the pipelining scheme to include the ICR analyzer. Thus, up to 4 mass analyses may occur simultaneously: while packet A is being analyzed in the ICR cell, packet B may be injected and transferred to the Orbitrap for mass analysis. While packets A and B are spinning in the ICR cell and Orbitrap, respectively, packet C can be injected and transferred to the linear ion trap. Finally, while packets A and B are still spinning in their respective FTMS analyzers and packet C is being scanned out of the dual linear ion trap, packet D is

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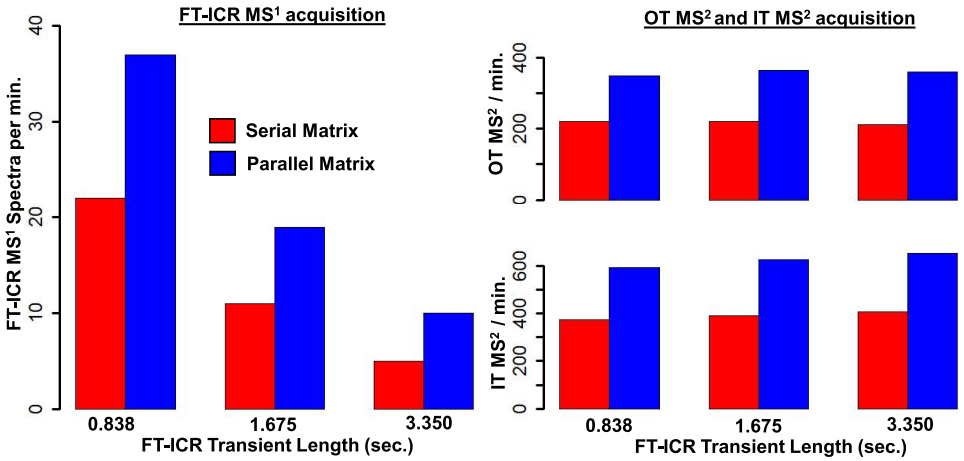


Figure 4. Impact of parallelization on mass spectral acquisition rates. For a given ICR transient length, acquisition rate was greatly improved by parallelizing ion manipulation and mass analysis among the different analyzers and trapping devices.

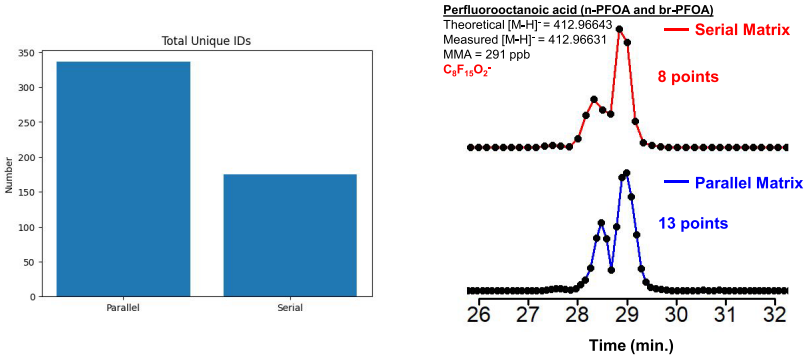


Figure 5. We show a comparison of parallel vs serial acquisition in an analysis of polyfluoroalkyl substances (PFAS). On the left, we show the number of unique IDs identified (analysis by Compound Discoverer). On the right, we show how the increase in acquisition rate led to better definition of chromatographic peaks, helping to separate isomers of perfluorooctanoic acid.

injected, analyzed in the quadrupole and accumulated in the ion-routing multipole (IRM).

As an initial demonstration of these developments, ion trap MS^2 and Orbitrap MS^2 spectral acquisitions were both parallelized with each other *and* with ICR MS^1 acquisitions. By analogy to the implementation on the native Tribrid instrument, the expanded instrument was able to acquire MS^1 data in the ICR cell while simultaneously acquiring a multitude of MS^2 acquisitions in both the linear ion trap and the Orbitrap. The difference between serial and parallel acquisition is shown in Figure 4, along with bar graphs showing improvements in acquisition rate for ion trap and Orbitrap MS^2 acquisitions.

As a real-world demonstration of the benefits of increased acquisition rate afforded by the developments just described, we performed an assay for polyfluoroalkyl substances (PFAS) that has been developed at the MagLab. As shown in Figure 5, this LC-MS/MS analysis yielded nearly twice the number of identified compounds (as determined by Compound Discoverer) in the assay relative to serial acquisition. Moreover, acquisition rate improvements led to an increase in the MS^1 repetition rate, leading to an increase in the number of points sampled per chromatographic peak. The increase in sampling frequency led to an improvement in the system's ability to resolve chromatographic peaks in mass spectral data, as shown by the improved separation of perfluorooctanoic acid (PFOA) isomers, PFOA was measured at m/z 412.96631, corresponding to a mass accuracy of 291 ppb.

CONCLUSIONS AND FUTURE WORK

We have developed a flexible and complete data acquisition system that leverages new instrument control modalities and preserves existing capabilities while introducing experimental flexibilities not previously available for the FT-ICR systems at the MagLab. While the system provides a large variety of capabilities, an assortment of features has yet to be implemented and will be the subject of future work. The ability to acquire microscans (i.e., mass spectra that are derived from averaged ICR transients, which are not individually processed and displayed) is one such feature that was not prioritized in initial development. However, the averager within the modified Tune software allows users to average transients and store these averaged transients in DAT format, while the resulting mass spectra can be viewed in Tune and stored in the RAW file.

With respect to the FFT data output, we have thus far restricted the data for display and RAW file storage to magnitude mode spectra, to which we alluded above. While absorption mode spectra can be displayed and saved in RAW files without issue, display software such as Tune and Freestyle has not been officially tested under these conditions, motivating our choice. We note that the data from the TherMIDAS system can be phased and presented in

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absorption mode postacquisition without further modification. Another future development could therefore involve implementation of automatic online absorption mode calculations. Such calculations will lengthen the amount of time needed for processing but should not substantially affect the benefits of parallelization described here. Beyond incremental progress in feature offerings, future work will draw upon the different combinations of mass analyzers and the variety of supported fragmentation techniques (ETD, PTR, UVPD, IRMPD, and combinations thereof) to enable the kinds of complex experiments that will help answer the scientific questions being asked by the interdisciplinary users at the MagLab's ICR facility.

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Notes

The authors declare the following competing financial interest(s): JDC and MWS are employees of Thermo Fisher Scientific, the manufacturer of one of the instruments used in this study.

ACKNOWLEDGMENTS

We gratefully acknowledge Raman Mathur, Hoa Truong, Lucas Szalwinski, and Philip Remeš at Thermo Fisher Scientific for assistance and helpful discussions. John Quinn of the MagLab was the major architect of the mechanical coupling of the Eclipse to the ICR instrumentation. Additionally, Lissa Anderson at the MagLab helped with the initial evaluation of the system as it was brought into working order. The authors also recognize the profound and lasting influence that Alan Marshall has had on this and preceding work, all our respective careers, and, of course, the entire mass spectrometry field. A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by the National Science Foundation Divisions of Chemistry and Materials Research through Cooperative Agreement No. DMR-2128556 and the State of Florida.

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