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Parallel Spectral Acquisition with an ICR Cell Array

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Abstract

Mass measurement accuracy is a critical analytical figure-of-merit in most areas of mass spectrometry application. However, the time required for acquisition of high resolution, high mass accuracy data limits many applications and is an aspect under continual pressure for development. Current efforts target implementation of higher electrostatic and magnetic fields because ion oscillatory frequencies increase linearly with field strength. As such, the time required for spectral acquisition of a given resolving power and mass accuracy decreases linearly with increasing fields. Mass spectrometer developments to include multiple high resolution detectors that can be operated in parallel could further decrease the acquisition time by a factor of n , the number of detectors. Efforts described here resulted in development of an instrument with a set of Fourier transform ion cyclotron resonance (ICR) cells as detectors that constitute the first MS array capable of parallel high resolution spectral acquisition. ICR cell array systems consisting of three or five cells were constructed with printed circuit boards and installed within a single superconducting magnet and vacuum system. Independent ion populations were injected and trapped within each cell in the array. Upon filling the array, all ions in all cells were simultaneously excited and ICR signals from each cell were independently amplified and recorded in parallel. Presented here are the initial results of successful parallel spectral acquisition, parallel MS and MS/MS measurements, and parallel high resolution acquisition with the MS array system.

Introduction

Mass spectrometry (MS) technologies have advanced dramatically over the past decades enabling evolution of entirely new research fields including proteomics^{1–4} and metabolomics^{4,5} and others⁴. For successful analysis of complex biomolecular samples, high resolving power and mass measurement accuracy are critical parameters of MS and multiple (MSⁿ) stages of analysis to allow accurate identification and quantitation of hundreds of thousands of possible analytes.^{6–9} For example, early application of high performance mass analyzers revealed that accurate mass analysis of even a single peptide together with other constraints can in some cases, enable unambiguous identification from an entire proteome.¹⁰ Generally however, accurate mass measurements of peptide and fragment ion masses are needed to enable accurate identification and quantitation.^{2,7} Because proteome samples typically contain 10⁵ or 10⁶ possible peptide analytes that cover a wide dynamic range in

Supporting Information

Supporting Information materials available include: Supplemental Text describing methods and Supplemental Figures 1–5.

concentration, on-line chromatographic separations are required to spread these species out over time and increase the chance for ion detection. This separation step creates additional demands on mass spectrometry performance since each analyte is only available for investigation during a brief period of time, limiting how many spectra and what stages (MS or MSⁿ) can be acquired with high resolution. Because of this constraint, significant MS instrumentation development efforts and resources are and have been focused on increasing mass accuracy and reducing the time required to obtain each mass spectrum.^{6,9,11}

Fourier transform mass spectrometers, including FT-ICR and Orbitrap mass analyzers achieve the highest resolving power and mass accuracy measurements of peptides.^{2,6,9,12–16} In recent years, hybrid-type mass spectrometers, which consist of two coupled mass analyzers, each capable of independent data acquisition, offer a compromise between these two constraints (mass resolution and ion signal analysis time) by combining rapid, low mass accuracy MS/MS analyzers in parallel with slower, high mass accuracy analyzers for precursor mass determination. However, recent studies suggest that high mass accuracy measurements in the MS/MS stage may also help resolve co-isolation of multiple precursors that can adversely affect peptide identification.¹⁷

Higher mass resolving power and mass accuracy with all FT-based methods require longer ion signal acquisition times. One approach to minimize required signal analysis time for high resolution mass spectral acquisition is to use stronger electrostatic or magnetic fields.^{9,15} Currently, 10 to 15 T FTICR-MS instruments are commercially available^{15,18–20} and high-end 21 T FTICR-MS instruments are being developed in National Laboratory settings. Increased magnetic or electric fields result in increased detected frequencies with ICR and Orbitrap mass spectrometers and therefore, increase the achieved resolving power for signal acquisition of the same duration. However, another way to increase detected frequency is to utilize multiple detection electrodes and record frequency multiples of the fundamental frequency, or harmonic frequency detection. Since the detected frequency of each ion is increased by the order of the harmonic (i.e., the detected frequency doubles or triples for the first, or second harmonic), the time required to achieve a given resolving power is divided by 2 or 3. Furthermore, the frequency difference between peaks increases with the order of the harmonic as well. Thus, recent efforts in ICR cell development involve incorporation of detection geometries that can enhance harmonic signals and improve acquisition rates.²¹

An independent approach to help overcome the limitations imposed by high resolution signal acquisition periods includes multiple high resolution mass analyzers within a single mass spectrometer operated in parallel. With other goals in mind, FTMS instruments with two analyzers were previously introduced, such as the Finnigan model 2001 FTMS dual-cell FT-ICR mass spectrometer and its predecessor, the Nicolet model 2000 FTMS.^{22,23} In those systems, each dual-cell is aligned with the magnetic field, differentially pumped, and ions were generated in the high-pressure “source” cell and then transferred to the low-pressure “analyzer” cell for detection. The high pressure cell was advantageous for study of ion/molecule reactions while the low pressure ICR cell could be used for high resolution single acquisition. These instruments demonstrated that two ICR analyzers could be implemented within a single magnet, although not for use as parallel ion detectors. Here, we present the

results of our efforts to implement multiple ICR cells within a single superconducting magnet so as to allow parallel or MS array operation to increase high resolution spectral acquisition rates n -fold, where n is the number of ICR cells in the array.

Experimental Section

LTQ FT-ICR MS

All experiments were performed using a modified hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometer (LTQ FT-ICR MS; Thermo Scientific, Bremen, Germany) originally equipped with a cylindrical Ultra ICR cell and 7 T actively shielded superconducting magnet as shown in Figure 1. After the removal of the Ultra cell and the installation of an ICR cell array, a preamplifier array, a custom multi-pin feedthrough flange on the source side of the vacuum system and wiring, the system was pumped and baked out overnight. Electrospray ionization (ESI) was used as an ion source for all spectra presented here with syringe pump infusion of samples at rate of 3.0 $\mu\text{L}/\text{min}$. A spray voltage of 3.5 kV was applied to the spray solution through a metal union for ionization. The ions were accumulated in LTQ and were then transferred into an ICR cell array through the original equipment octapole ion guide. The pressure indicated on the ion gauge in the cell region during all experiments was approximately 2×10^{-10} Torr.

ICR cell array design

The concept of an ICR cell array with three independent analyzers was first explored using the cell design illustrated in Figure 2 and was constructed using printed circuit boards (PCBs). This open cell design was selected because it would require only 4 identical PCBs to comprise the cell array, with two additional identical boards that serve as entrance/exit lenses. The boards that contained the excitation and detector plates were segmented into seven sections, which produce an open cell geometry with a square cross-section, three excitation/detector electrodes and four trapping electrodes printed on the PCBs using gold-coated copper as shown in Fig. 2a. Electrical connections to the electrodes were made through the pads on a cell outer surface (opposite board surface) as shown in Fig. 2b. Excitation/detector electrodes were each 1×1 inch. The width of the trapping electrodes was 0.1 inch. The gap between the excitation/detection electrodes and the trapping electrodes was 0.01 inch. The entrance and exit lens plates are shown in Fig. 2c. These electrodes have 0.2 inch hole at the center of the plates through which the transferred ions from LTQ come into the ICR cell array. All individual PCB components for preparing an ICR cell array were designed using a circuit board layout program EAGLE ver. 7.3.0 (CadSoft Computer, Pembroke Pines, FL) and manufactured by OSH Park (Advanced Circuits, Aurora, CO). To form an ICR cell array shown in Fig. 1d, excitation and detector plates were soldered to entrance and exit lens plates. After that, the trapping electrode segments were electrically coupled together by soldering 22 AWG copper wires on the pads on a cell outer surface to make trapping ring electrodes. The excitation coupling of three cells to excite ions trapped in all three cells at the same time was performed through the connection of the three excitation electrodes on excitation plates using 22 AWG copper wires as shown in Fig. 1d. The initial ICR cell array assembly used for this study had four trapping ring electrodes and three cells and later designs had 6 trapping rings and five cells.

The entire length of the 3-ICR cell array was 3.6 inch. The 3-ICR cell array was placed at the end of an octapole ion guide in the place of a ThermoFisher Ultra ICR cell. Therefore, these developments are in principle adaptable to existing LTQ FT or any other ICR instruments.

Ions transferred from the LTQ were serially injected into each detector cell of the MS array by filling the cell furthest away from an octapole (Cell 1) first. Then Cell 2 and Cell 3 were filled. Each ICR cell in the array requires at least one independently controlled DC voltage to allow formation of trapping wells in each cell to restrain ions. All trapping voltages were supplied with a multi-channel programmable DC power supply (Modular Intelligent Power Source (MIPS), GAA Custom Engineering, Benton City, WA, USA). MIPS is programed with a PC through communication via USB and provides up to 16 channels of ± 50 volt DC outputs with sub-microsecond switching capability. Independent control of each trapping ring electrode, entrance and exit lens potentials was achieved by connecting each electrode to separate MIPS DC outputs. DC voltage profiles produced by MIPS for trapping and parallel detection of ions in the MS array are provided in Supporting Information (Figure S-1). All connections between the cell and vacuum feedthrough were made using Kapton coated wires (22 AWG, Accu-Glass Products, Inc. Valencia, CA).

Excitation of ion cyclotron motion with the ICR cell array was achieved using ThermoFisher excitation waveforms as normally used for ICR excitation with the Ultra Cell. In the present case, however, a single ion excitation event was applied to all cells simultaneously following the final injection of ions into the last cell in the array. The amplitude of the excitation setting within the ThermoFisher Excalibur software was set to 0.1.

After ion excitation, parallel ICR signals from all three cells were simultaneously amplified using a custom vacuum-compatible preamplifier array (GAA Custom Engineering) as shown in Fig. 2e. The preamplifier array was mounted close to the ICR cell array to reduce detection capacitance and noise and increase sensitivity. Each single preamplifier occupied approximately 1×1 inch on a PCB. 22 AWG copper wires insulated with glass tubing were used to connect detection electrodes from each cell to a single preamplifier. Each amplifier output signal was connected with kapton-coated wire to a single vacuum feedthrough pin and then to a four channel oscilloscope (DS 1074, RIGOL Technologies, Inc.). Sample rate and memory depth of the oscilloscope were set up 500k Samples/s and 300K pts, respectively. The power ± 5 V DC voltage to operate the preamplifier was supplied by an external power supply. The obtained time domain signals from the oscilloscope were transferred to a computer through a USB interface and were processed with the program called ICR-2LS (<http://omics.pnl.gov/software/icr-2ls>) without zero-filling or apodization.

Sample Preparation

All peptides and Ultramark 1621 (a mixture of fluorinated phosphazenes) were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol, acetic acid and dimethyl sulfoxide were obtained from Fisher Scientific (Pittsburgh, PA, USA). 10 μ M standard peptide solutions were prepared by dissolving them in 1:1 (v/v) water/methanol solvent mixtures containing 0.1% (v/v) of acetic acid. An Ultramark 1621 stock solution was prepared by dissolving 10 μ L of Ultramark 1621 in 10 mL of acetonitrile. A 10 mL solution

of Ultramark 1621 was prepared by dissolving 100 μ L of the stock solution of Ultramark 1621 in a solution of 1% acetic acid in 50:50 methanol:water.

Since a major focus of research in our lab involves proteome and protein interaction studies, we chose to test parallel spectral acquisition with the MS array using Protein Interaction Reporter (PIR) cross-linked peptides.²⁴ To prepare cross-linked peptides, the PIR cross-linker BDP-NSP was synthesized with solid phase 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry using an Endeavor 90 system (APPTec, Louisville, KY), and esterified to produce activated n-hydroxyphthalamide esters just prior use as described by Chavez et al.²⁵ The purified BDP-NHP ester was dissolved in dimethyl sulfoxide to a concentration of 333 mM and stored at -80°C until use. A stock solution of 0.01 M bradykinin peptide was prepared in 0.17 M of Na_2HPO_4 (pH 8) buffer. For cross-linking reaction, 100 nmols of the peptide solution was incubated with $\times 10$ molar excess BDP-NHP overnight at room temperature with constant mixing. The cross-linked peptide standard was stored at -20°C until use. 10 μM working solution of the cross-linked bradykinin peptide was prepared in 1:1 (v/v) water/methanol solvent mixtures containing 0.1% (v/v) of acetic acid. For parallel acquisition of multiple stages of MS analysis, all peptide standard solutions were mixed together to prepare a 1 μM peptide standard mixture solution.

Result and discussion

Parallel detection of equivalent ion populations

As an initial test, parallel detection of equivalent ion populations was demonstrated using a 10 μM solution of ultramark 1621. Each accumulated population of ions in the LTQ were injected, transferred and serially filled the ICR cell array by controlling DC voltages applied to the trapping ring electrodes using MIPS as described in the Experimental Section. The resulting spectra acquired in one third the time required for serial acquisition with a single detector from three cells are shown in Figure 3. ICR cell array and preamplifiers illustrated equivalent performance with no obvious discrimination in ion m/z values, S/N or intensity variations observed among all three cells. Additionally, no adverse effect on vacuum quality or ICR signal detection was observed from the PCB-based cell components or the in-vacuum preamp array after overnight bake out.

Revolving power

A major benefit of array development based on ICR technology is the potential for parallel acquisition of high resolution mass spectra. To investigate this potential, high resolution array acquisition from the three cells was evaluated in the analysis of standard peptide LH-RH ($M_w=1182$). The resulting mass spectra shown in Figure 4 were acquired by accumulation and injection of equivalent ion populations. Parallel acquisition of high resolving power spectra ($\text{FWHM} = 185,000$) was obtained for LH-RH ion at m/z 1183 from all three cells with parallel ICR time domain signals recorded for 4 seconds. These efforts resulted in spectra with measured resolving power matching that theoretically possible by magnitude mode FFT which would otherwise have taken 12 seconds to acquire using only a single detector. Thus, the number of high resolution spectra that can be acquired in a given time period increases n -fold with the MS array.

Detection of ions in selected cells

We further investigated the ability to detect different signals in each array cell by incorporating mock ion injection events, whereby the LTQ was set to accumulate in a region of the spectrum where no ions were expected to confirm independent cell function. Figure 5 shows the resulting mass spectra obtained by transferring a population of ions to Cell 1, a mock injection event in Cell 2 and then a second population of ions was injected into Cell 3. As is shown, expected ion signals were observed in Cell 1 and Cell 3, but only noise peaks and no ion signals were detected in Cell 2. The mass spectra shown in Figure S-2a, b, and c (Supporting Information) show additional demonstrations for ion population control in each cell with all other possible combinations of mock and ion population injections. These were important tests to be investigated with open cells since mixing of ions between cells is a potential problem with this design. These results demonstrate that ion control within the MS array is achieved and mixing of populations between cells does not occur to any appreciable extent under the conditions used here.

Multiple injections of different ion populations

Ideally, the MS array detectors will be used for simultaneous high resolution analysis of differing ion populations. Multiple injections of different ion populations into each cell in the array are demonstrated in Figure 6. For this demonstration, ions in specific mass ranges were selected in the LTQ, transferred to the selected cells and parallel ICR signals were acquired. Ions at m/z 1422, 1522, and 1622 were selected and injected into Cell 1, Cell 2 and Cell 3, respectively. As is shown in Fig. 6, an expected abundant single peak is observed in each cell.

Parallel acquisition of multiple stages of MS

A key rationale for development of an array mass spectrometer is that accurate mass determination could in principle be performed on multiple stages of mass analysis simultaneously. To investigate the MS array for this purpose, simultaneous acquisition of MS and MS/MS spectra from a peptide standard mixture solution including luteinizing hormone-releasing hormone (LH-RH) (Mw. 1182.3), angiotensin II (Mw. 1046.2), substance P (Mw. 1347.6), and PIR-cross-linked bradykinin (Mw. 3264.7) was demonstrated. The resulting data are shown in Figure 7. A full mass spectrum collected in Cell 1 shows the peaks for all four analytes as indicated. The mass spectra collected in parallel with Cell 1 using Cells 2 and 3 shown in Fig. 7 were obtained from MS/MS analysis of fragment ions from m/z 817 and 1183 ions, respectively. The mass spectrum obtained in Cell 2 resulted from selection and fragmentation of the cross-linked bradykinin peak at m/z = 817 and includes expected peaks for PIR reporter ions, released bradykinin peptides with expected residual “stump” mass, and so-called “long arm” peptides whereby only one of the two cleavable groups in the PIR cross-linker were cleaved. Detailed information about PIR fragmentation and assigned fragment ions in this spectrum is provided in Supporting Information (Figure S-3). Simultaneous acquisition of the MS/MS spectrum in Cell 3 resulted from selection and fragmentation of m/z = 1183 which is from singly-charged LH-RH peptide ions. Fragment ions consistent with the sequence of LH-RH are shown assigned in this spectrum. Figure S-4 (Supporting Information) shows additional demonstrations for

parallel data acquisition obtained from an ultramark 1621 standard solution using an ICR cell array.

5 ICR cell array

To demonstrate that the concept of multiple analyzer arrays can be further extended, a 5-ICR cell array was developed using PCBs. This is a linear extension of the 3-ICR cell array shown in Fig. 2, including two additional detection and excitation electrodes. To make the 5-ICR cell array, the excitation/detection PCB boards were segmented into eleven sections. These included five excitation/detector electrodes and six trapping electrodes printed on the PCBs using gold-coated copper as was used with the 3-ICR cell array described in the experimental section. Accumulated ions in the LTQ were transferred to and serially filled in 5 cells by controlling DC voltages applied to the six trapping ring electrodes using MIPS. With the 5-ICR cell array, multiple injections of different ion populations and parallel detection of equivalent ion populations were demonstrated using a 10 μ M solution of ultramark 1621. The spectra in Figure 8 show the results of multiple injections of differing ion populations in each cell. In this experiment, full MS analysis was performed in Cell 1 by transferring all ions accumulated in the LTQ to Cell 1. Cells 2 – 5 were used to trap selected ions m/z 1622, 1522, 1422, and 1322 transferred from the LTQ, respectively. Mass spectra in Figure S-5 (Supporting Information) illustrate the parallel acquisition of equivalent ion populations using the 5-ICR cell array. Although much effort is still required for optimization needed to remove noise peaks and optimize trapping and signal intensities, these data show that five spectra can be acquired in the same time as required for a single acquisition. **Accompanying** supplementary video files are included illustrating parallel spectral acquisition with the 3- and 5-cell mass spectrometer arrays.

CONCLUSIONS

Most mass spectrometry development efforts in the past decade have focused on increasing mass measurement accuracy and decreasing the time required to acquire each spectrum. FT-MS is a powerful instrument for the study of complex biological samples due to its ability to acquire high resolution and mass measurement accuracy, but requires longer signal acquisition times to achieve high resolution. In this study, we demonstrated the first realization of an MS array concept based on advanced ICR technology. ICR MS array allowed acquisition of parallel high resolution spectra from all three cells at the same time and could be used for simultaneous acquisition of MS and MS/MS spectra from a mixture of peptides. These results indicate that the array can significantly extend high resolution analysis to parallel multi-stage analyses. MS array technology will significantly enable future data-dependent and data-independent modes of operation since each analyzer can be used for high resolution detection of fragment ions from selected precursor mass ranges.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Fourier transform ion cyclotron resonance mass spectrometer (LTQ FT-ICR MS) equipped with an ICR cell array.

Figure 2.

Individual PCB components and assembled 3-cell array. **A)** Cell inner surface showing detection and trapping electrode surfaces on one board. **B)** Cell outer surface showing pads used for electrical connections. **C)** Entrance and exit lenses board. **D)** 3-ICR cell array assembly. **E)** Vacuum-compatible preamplifier array.

Figure 3.

Initial MS array acquisition of spectra using calibrant ions. Each array cell was filled with an independent population of ions. All ions were simultaneously excited, ion signals were amplified, recorded and processed to yield parallel spectral acquisition in a 3 cell array.

Figure 5.

MS Array acquisition of spectra using ultramark ions where cells 1 and 3 were filled with ions, but a mock injection of ions event was used to inject no ions in cell.

Figure 6.

Multiple injections of different ion populations. Each array cell was filled with an selected ions. All ions were simultaneously excited, ion signals were amplified, recorded and processed to yield parallel spectral acquisition in a 3 cell array.

Figure 7.

MS Array parallel acquisition of multiple stages of mass spectral data. Cell 1 was used for full mass scan with a mixture of three peptides shown above. Cell 2 was used for MS/MS acquisition of fragment ions from m/z ion 817, while Cell 3 was used for MS/MS spectral acquisition m/z ion 1183.

Figure 8.

The initial successful parallel ICR signal acquisition using a 5-ICR cell array. A full spectrum of ions or selected populations of Ultramark ions were accumulated and injected into each of the 5 cells, followed by a single cyclotron excitation event and then array detection. Five parallel ICR time-domain signals were acquired using 2 digital oscilloscopes, downloaded to a PC and processed with ICR-2LS.