High Resolution Mass Spectrometry

Feng Xian,† Christopher L. Hendrickson,*‡† and Alan G. Marshall*‡†

†Department of Chemistry and Biochemistry, Florida State University, 95 Chieftain Way, Tallahassee, Florida 32310-4390, United States
‡Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310-4005, United States

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■ MASS RESOLUTION, MASS RESOLVING POWER

This review focuses on recent advances in instrumentation, techniques, and applications of the highest resolution mass analyzers (multipass time of flight (TOF), orbitrap, and Fourier transform ion cyclotron resonance (FTICR)) since a prior similar Analytical Chemistry review in 2008.1 We begin with a brief discussion of the need for high mass resolution, and then proceed to mass analyzer types and their development and applications for bio-analysis (e.g., proteomics, lipidomics, drug discovery) and complex organic mixtures (environmental, petroleum, metabolomics).

Mass resolution is the minimum mass difference, $m_2 - m_1$, between two mass spectral peaks such that the valley between their sum is a specified fraction (e.g., 50%) of the height of the smaller individual peak. For two peaks of equal height, $m_2 - m_1 \equiv \Delta m_{50\%}$, in which $\Delta m_{50\%}$ is the full width at half-maximum height of either peak alone. Mass resolving power is $m_2/(m_2 - m_1)$, in which $(m_2 - m_1)$ is mass resolution; for an isolated peak of mass, $m$, mass resolving power is $m/\Delta m_{50\%}$. For a multiply charged ion, $m$ is replaced in the above definitions by $m/z$, in which $z$ is the ion charge in multiples of the elementary charge. The ability to distinguish ions of different elemental composition is determined by mass resolution (e.g., $m_2 - m_1 = 0.0034$ Da for ions differing in composition by C$_3$ vs SH$_4$). However, mass analyzer performance is usually expressed in terms of mass resolving power. Thus, higher resolving power is required to distinguish the same mass difference for ions of higher mass. In practice, high resolution designates a mass analyzer with resolving power $m/\Delta m_{50\%} > 10,000$, thereby excluding quadrupole mass filter, triple quadrupole, and quadrupole ion trap mass analyzers. Finally, mass measurement accuracy is usually expressed as the rms difference (in ppm) between measured and exact (based on elemental composition) mass. In the absence of systematic error, mass accuracy is the same as mass precision.

■ MASS RESOLUTION AND ACCURACY

In a mass analyzer, the detected ion signal (in this case, voltage, current, or time-of-flight) is digitized. Peak height and position are then determined by some sort of interpolation or fitting procedure.2−4 Mass measurement precision, namely, $m/\delta m$, in which $\delta m$ is the rms error from a large number of repeated measurements, may in general be predicted from the product of signal-to-noise ratio and the square root of the number of data points per peak width,5 depending on the nature of the noise,6 from a single spectrum. Thus, for a given mass resolution, mass measurement precision can be increased by sampling more data points per peak width, accounting in a significant part for the increase in TOF mass accuracy as faster digitizers became available. Once the peak positions have been precisely established, the spectrum is calibrated, on the basis of the exact masses of two or more ions of known elemental composition. External calibration (performed for a different sample than for analyte) is typically 2−3× less accurate than internal calibration (based on multiple ions of known $m/z$ values in the analyte sample).

As the number of atoms increases, the number of possible elemental compositions for a given experimental mass measurement increases.7 For proteins, the number of amino acid compositions also increases.8,9 Moreover, high resolving power is needed to resolve closely spaced mass doublets in complex mixtures. For example, resolution of two equally abundant ions of the same nominal mass but differing in elemental composition by C$_3$ vs SH$_4$ (separated by ~3.4 mDa at $m/z$ 700) requires a resolving power of $m/\Delta m_{50\%}$ higher than 200 000.

■ TIME-OF-FLIGHT MASS ANALYZERS

The time-of-flight mass analyzer, initially proposed by Stephan in 194610 and reduced to practice in 1948,11 has evolved through several stages. Delayed ion extraction addressed the
problem of kinetic energy spread among ions of the same m/z.\textsuperscript{12} The advent of fast digitizers; matrix-assisted laser desorption/ionization (MALDI), and MALDI imaging; orthogonal ion introduction; and kinetic energy focusing by use of a reflectron brought TOF MS to its present level of popularity.

**Orthogonal Acceleration (see ref 13).** Ideally, a TOF MS experiment should begin with all ions with the same initial position and velocity. Orthogonal ion introduction, in which ions are accelerated in a direction perpendicular to a collimated ion beam, effectively achieves that condition and thus improves mass resolving power. Another advantage of orthogonal introduction is that ions can be accumulated in the acceleration region while previously accumulated ions fly toward the detector, thereby more efficiently enabling coupling of continuous ion sources with TOF mass analysis. Further optimization of ion guides and use of an ion funnel\textsuperscript{14} with automatic gain control (AGC) improves the ion beam quality, for higher sensitivity and mass accuracy.\textsuperscript{15}

**Reflectron/Multipass TOF.** The resolving power for a TOF mass analyzer is \( m/\Delta m_{\text{res}} = (T/2\Delta t) \), in which \( T \) is the ion total flight time and \( \Delta t \) is the mass spectral peak width. Increasing the flight time for improved resolving power is made possible by the reflectron, introduced by Mamyrin in 1973.\textsuperscript{16} After an initial drift region, ions are subjected to a spatially quadratic potential that acts as an ion mirror. When ions of a given \( m/z \) but different kinetic energy fly through the reflectron region, the faster ions penetrate farther into the reflectron (and therefore travel a greater distance to reach the detector) than slow ions, and ions of all speeds arrive at the detector at the same time. The reflectron thus provides increased ion flight path, without defocusing due to spread in initial velocity, thereby increasing mass resolving power. Broadband mass resolving power of 10 000 and rms mass error of 5–10 ppm may now be routinely attained. With high field pusher and dual stage reflectrons, commercial TOF mass analyzers can now reach mass resolving power of 40 000\textsuperscript{17} (Figure 1, top). If the ion spatial focusing can be maintained with minimal ion loss at each reflection, the ion flight path may be extended by multiple reflections. Multipass (Figure 1, middle)\textsuperscript{18} and spiral (Figure 1, bottom)\textsuperscript{19} TOF mass analyzers now attain mass resolving power of 50 000 or higher.

Recently, an optimized multipass time-of-flight mass analyzer has been combined with MALDI for imaging mass spectrometry. The multipass time-of-flight mass analyzer consists of four electric sectors, with cylindrical side electrodes and Matsuda configuration to generate a toroidal electric field, in which focusing is maintained by quadrupole triplet lenses at the entrance and exit of each toroidal segment. Mass resolving power of 130 000 has been achieved for angiotensin II [M + H]\textsuperscript{+} ions (m/z 1046.542) after 500 turns and 654.8 m total flight path.\textsuperscript{20} In a typical multipass time-of-flight mass analyzer, the power supply for ion injection/ejection is very large and complicated in order to reduce instability of voltage switching between ion injection and ejection events. A more recent multipass TOF design adds two more sectors for ion injection and ejection only, thereby minimizing the size of the power supply.\textsuperscript{21}

Although a cyclic multipass TOF mass analyzer offers potentially high mass resolution, the observable mass range is limited, because low-m/z ions eventually overtake higher-m/z ions so that the effective m/z range is reduced by a factor of N for N passes. An elliptical flight path composed of four toroidal electric sectors works with a new segmentation method to identify the number of laps transited by ions of a given m/z.\textsuperscript{22}

**Figure 1.** High resolution time-of-flight mass analyzers. Top: Dual stage reflectron. Reproduced with permission from Waters Corporation. Copyright 2009 Waters Corporation. Middle: Multipass reflectron with linear segments. Reprinted with permission from ref 223. Bottom: Multipass spiral configuration. Reproduced with permission from JEOL USA. Copyright 2006–2011 JEOL Ltd.

The most recent multipass designs include doughnut-shaped ion optics,\textsuperscript{23} a spiral path electric reflector\textsuperscript{24} and multiple angle reflecting electrostatic ion mirrors\textsuperscript{25} (Figure 1, middle). The latter two designs have achieved broadband mass resolving power of 60 000.

**Recent Advances in TOF Mass Analyzers.** Detection. The most commonly used TOF ion detector is the microchannel plate (MCP) detector, which converts ions to secondary electrons, with large active area and rapid response time. However, secondary electron generation efficiency varies directly with incident ion velocity, and ion velocity varies inversely with the square root of accelerated ion mass, so that MCP detection efficiency for high-mass ions is low. The mechanical nanomembrane detector can detect the time-varying field emission of electrons from mechanical oscillation without mass discrimination and thus improve high-mass detection sensitivity. For example, singly charged immunoglobulin G (150 kDa) has been experimentally observed by TOF MS with nanomembrane detection.\textsuperscript{26} A different nanomechanical resonator,\textsuperscript{27,28} whose resonance frequency is perturbed by surface adsorption of ions, was also recently reported as a sensitive mass sensor, but further investigation for its practical use in TOF MS is needed. When applied to TOF MS, cryogenic detectors measure low-energy solid-state phonon excitation created by a particle impact. The energy of the phonons is less than a few meV, much smaller than the energy in the electronvolt range needed to produce secondary electrons in a conventional
ionization detector. The cryodetector is capable of detecting high m/z, slow-moving ions. For example, a strong signal at 510 kDa from a disulfide-linked dimer has been experimentally observed in a MALDI TOF mass spectrometer coupled with a cryodetector.

TOF/TOF. Two time-of-flight (TOF) mass analyzers in succession enable MS2 experiments. Usually, the first TOF mass analyzer selects the precursor ion for injection into a high energy collision cell for collision induced dissociation (CID). The second TOF mass analyzer then records the fragment ions. The most common TOF/TOF configuration is a linear TOF mass analyzer followed by a reflectron TOF mass analyzer. However, low resolution in MS1 can render product ion identification difficult.

Multiplex TOF/TOF has been achieved with a MALDI ion source, a multiturn TOF mass analyzer (TOF), a collision cell, and a quadratic-field ion mirror, providing resolving power of 5000 for precursor ion selection and 1000 resolving power for product ions throughout the mass range. The identification of lyso-phosphatidylcholine (LPC) and phosphorylation has been demonstrated. Recently, a spiral ion optical TOF mass analyzer for MS1 was combined with an offset parabolic ion reflectron TOF as MS2, providing high resolution MS3 precursor monoisotopic ions up to m/z 2500.

Selected Applications. Although TOF mass analyzers have lower mass resolution than FT mass analyzers (FTICR and orbitrap), they have no upper m/z limit in principle and are thus particularly useful for identifying singly charged ions of high molecular weight (as from MALDI). Fast response/scan rate is also advantageous for applications requiring short acquisition period, e.g., analysis by liquid or gas chromatography mass spectrometry.

A method for screening doping agents in human urine consists of solid-phase extraction followed by HPLC-TOFMS. Coupled with orthogonal electrospray ionization, 124 sub-compounds, including stimulants, narcotics, agonists, diuretics, etc., are identified in less than 30 min. Further development of the extraction method enabled identification of 40 more doping compounds. The utility of the LC-TOF method was assessed by parallel analysis of 30 authentic urine samples by use of rapid emergency drug identification and led to identification of twice as many drugs. Compared to prior methods, the author concluded that UPLC-TOFMS offers an attractive alternative toxicological screening technique. Because the mass accuracy tolerance for broad toxicology screening has historically been quite wide (20 ppm or more), TOF mass accuracy less than 5 ppm provided substantial improvement in the number and confidence of identifications.

Mass spectrometry imaging (MSI) provides rapid detection, localization, and identification of organic components of complex biological mixtures, to establish chemical correlations with biological function or morphology. MSI experiments are typically performed with a TOF mass analyzer, due to its small sensitivity and speed. Localization of peptides by MALDI TOF imaging reveals the distribution of their respective proteins. Comparison of protein profiles from normal and tumor tissue can lead to biomarker candidates. Lipid imaging in rat brain tissues during focal cerebral ischemia reveals dynamic conversion from phosphatidylcholine (PC) to lyso-phosphatidylcholine (LPC) in brain areas with ischemic injury. As for protein profiling, a different lipid distribution between normal and cancer cells could diagnose disease.

In aerosol analysis, it is necessary to track changes in aerosol size and chemistry with subsecond time resolution. A TOF mass analyzer collects high resolution aerosol mass spectra at rates exceeding 1 kHz for determination of both aerosol size and mass. Alternately, two-dimensional gas chromatography (GCxGC) mass spectrometry requires fast scanning because the final GC peak width is typically 20–200 ms, making TOF the MS method of choice. GCxGC TOF MS applications include identification of naphthenic acids in oil samples and high throughput metabolic profiling and analysis of organic nitrogen compounds in aerosol samples. Moreover, fast transient temporal analysis of catalyst kinetics targets TOF mass analysis for its sensitivity, detector response, and time resolution.

The TOF mass analyzer has also been applied to polymer analysis by virtue of its high m/z range and MS/MS capability. Evaluation of top-down TOF MS/MS for poly(α-peptide) synthesized by N-heterocyclic carbine (NHC)-mediated zwitterion ring-opening polymerization showed that electrospray ionization (ESI) enabled detection of the intact molecular species, whereas MALDI resulted in elimination of the NHC initiator in the presence of cationizing salts. Coupled with MALDI, TOF MS can address the mechanism of polymer backbone degradation via free radical chemistry and quantify residual polyethylene glycol (PEG) in ethoxylated surfactants. Cation adducts generated more informative fragment ions under CID and TOF MS has characterized various polymers with ammonium adducts generated by ESI. Miscellaneous other applications include characterization of C4 explosives by TOF secondary ion mass spectrometry (SIMS), qualitative and quantitative analysis of herbal medicines (HMs) by LC/TOF MS, and identification of different contaminants in the human body by GC/TOF MS.

FOURIER TRANSFORM MASS ANALYZERS

The FTICR mass analyzer, introduced in 1974, has the highest mass resolving power and best mass measurement accuracy among current mass analyzers. The orbitrap, another Fourier transform mass analyzer, invented in 1999, has been widely distributed since its commercial introduction in 2004.

Common Features of Fourier Transform Mass Analyzers. The ion cyclotron and orbitrap each produce a spatially coherent packet of ions of a given m/z. Coherence in FTICR is achieved by rf excitation at the ion cyclotron frequency, whereas coherence in the orbitrap is achieved by injecting ions of a given m/z into the mass analyzer in a time short compared to the ion oscillation frequency. In both devices, the periodic motion (rotation in ICR, oscillation in orbitrap) is detected from the oscillating current induced in opposed detection electrodes, as ions pass near each electrode. The signal from ions of a given m/z is linearly proportional to the number of those ions and to the radius of amplitude of the ion motion. Linearity is especially important for FTICR, because ions of different m/z may be selected/excited in any desired combination by a time-domain excitation waveform produced by inverse Fourier transformation of the desired frequency-domain excitation profile ("stored waveform inverse Fourier transform" (SWIFT) excitation). Both devices inherently detect ions of a wide m/z range simultaneously. The time-domain signal is then digitized, apodized, zero-filled (to improve digital resolution), and subjected to discrete Fourier
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transformation to yield mathematically “real” and “imaginary” frequency-domain spectra, Re(\(\omega\)) and Im(\(\omega\)), which are typically combined to yield a “magnitude” spectrum, M(\(\omega\)) (see below). Spectral frequency is then converted to \(m/z\) by an appropriate calibration equation (see below).

FTMS resolving power is ultimately limited by the acquisition period, \(T\), for the time-domain transient. However, the time-domain transient duration is in turn limited by magnetic and electric field imperfection, ion-neutral collisions, and ion–ion interactions. Apodization functions help to detect small peaks near large peaks by reducing the magnitude of auxiliary maxima and by narrowing the peak width near the peak base (at the cost of broadening the peak width at half-maximum peak height).

**Ion Accumulation and Detection.** Both ICR and orbitrap mass analyzers are pulsed detectors. Because ion introduction is often temporally continuous (e.g., ESI and APPI), ions are typically accumulated externally during detection of ions from the preceding accumulation period. In FTICR, ions are externally accumulated in a multipole electric ion trap and simultaneously ejected toward the ICR cell, whereas in the orbitrap, ions are collected in a “C” trap and injected simultaneously toward the orbitrap. In ICR, ion spatial coherence is maintained by the confining magnetic and electrostatic fields and by ion–ion interactions, whereas in the orbitrap, ion axial coherence is achieved by short pulsed injection from the C trap and radial coherence is lost as ions spread out to form a rotating ring (actually, an advantage, by reducing space charge repulsions and enabling increased dynamic range), and the ring for ions of a given \(m/z\) oscillates axially to produce an image current on the detection electrodes. The cyclotron frequency in ICR signal varies as \((m/z)^{-1/2}\), whereas the orbitrap axial frequency (like the ICR “trapping” oscillation) varies as \((m/z)^{-1/2}\). As a result, ICR mass resolving power varies as \((m/z)^{-1}\) vs \((m/z)^{1/2}\) for the orbitrap.

**Advances in Fourier Transform Mass Analyzers. High Field Strength.** The simplest way to improve FTMS performance is to operate at higher magnetic field, \(B\) (ICR) or higher electric field (orbitrap). ICR mass resolving power or scan rate increases proportional to \(B\), whereas mass accuracy, dynamic range, and upper \(m/z\) limit increases as \(B^2\). The highest field FTICR instrument is currently 15 T, and 21 T systems are under construction at the USA National High Magnetic Field Laboratory and Pacific Northwest National Laboratory.

The orbitrap axial oscillation frequency can be expressed as:

\[
\omega = \frac{e}{mz} \sqrt{\frac{2U_z}{R_m} \left( R_z \frac{R_i}{R_z} \right) - \frac{1}{2} \left( R_z^2 - R_i^2 \right)^2}
\]  

(1)

in which \(e\) is the elementary charge \((1.602 \times 10^{-19} \text{C})\), \(R_z\) is the maximum radius of the outer barrel-like electrode, \(R_i\) is the radius of the central spindle-like electrode, \(U_z\) is the voltage applied to the central electrode, and \(R_m\) is the “characteristic” radius at which \(dU(r)/dr = 0\). From eq 1, it is clear that orbitrap resolving power may be increased by increasing the central electrode voltage or decreasing the \(R_i/R_z\) ratio. The newest orbitrap exhibits increased \(U_z\) and optimized \(R_z/R_i\) ratio to provide \(\sim 2\)-fold improvement in resolving power (for a given data acquisition period) or similar reduction in data acquisition period (for faster online LC sampling, for a given mass resolving power). With careful balancing of construction tolerances and experimental parameters, the new orbitrap resolved an isotopic distribution at a resolving power in excess of 600 000 at \(m/z\) 195.90

**Electric Field Optimization.** The orbitrap mass analyzer is composed of a spindle-like central electrode and a barrel-like outer electrode (Figure 2). Application of a dc voltage to the two axial electrodes produces an electrostatic potential that ideally is the sum of a quadrupole ion trap potential and a logarithmic potential of a cylindrical capacitor. For ICR, a strong homogeneous static magnetic field confines ion motion transverse to the magnetic field, and an ideally three-dimensional quadrupolar electrostatic potential confines ion motion parallel to the magnetic field.

In practice, the potentials in both mass analyzers deviate from ideal, because the electrodes are truncated, imperfect in shape and alignment, and must have apertures to admit ions (both) and electrons or photons (ICR). In ICR, an imperfect (anharmonic) quadrupolar electrostatic potential produces an axially dependent, nonlinear radial electric field \((E_z)\) that makes the observed cyclotron frequencies depend on ion radius and axial amplitude. Different approaches to the cell anharmonicity problem have been investigated since the 1980s. One approach is to insert compensation rings into an open cylindrical cell (O-trap), with separate compartments for ICR excitation and detection, but has been experimentally demonstrated. The potential benefit from that design is enhancement of resolving power equal to detected frequency multiple order that could alternatively yield a shorter detection period for correspondingly higher throughput. (e.g., for LC/MS).

Compared to a standard orbitrap geometry, the outer electrode dimensions of the compact high-field orbitrap analyzer are scaled down by a factor of 1.5, whereas the central electrode dimensions are scaled down by a factor of 1.2 (Figure 2). The entrance aperture cross-section is reduced by more than a factor of 2; thus, to avoid a corresponding loss in sensitivity, a new miniature lens system focuses ions to a much smaller spot. The capacitance of the orbitrap drops in proportion to size, which results in improved image current detection sensitivity. New transistors in the preamplifier further increase sensitivity. In spite of higher ion density, the relatively thicker central electrode results in smaller space charge shifts than for the standard orbitrap.

![Figure 2. Standard orbitrap (left) and compact high field orbitrap (right) mass analyzers. Reproduced with permission of ThermoFisher Scientific.](image-url)
Addition of an ion abundance-dependent term calibration for each of up to $\sim 30$ individual mass spectral segments. $113,71$ Systematic error has been reduced by a factor of up to 3 (and multiple ions of known mass spectra, yield magnitude-mode (also known as absolute-value) and phase spectrum. For FTICR, calibration typically relies on a two-conversion of a frequency-domain spectrum to an $m/z$ spectrum. For ICR, orbitrap mass calibration may be improved by considering the effect of space charge, leading to a one term calibration equation. As for ICR, orbitrap mass calibration may be improved by the short ion injection period from the C trap but due to the short ion injection period from the C trap but

$$A(\omega) = \cos[\phi(\omega)]\text{Re}(\omega) - \sin[\phi(\omega)]\text{Im}(\omega) \quad (3a)$$

$$D(\omega) = \sin[\phi(\omega)]\text{Re}(\omega) + \cos[\phi(\omega)]\text{Im}(\omega) \quad (3b)$$

An absorption-mode spectral peak is inherently narrower than its corresponding magnitude-mode spectral peak (Figure 4, top) by a factor that depends on the presence and mechanism of signal damping. $81-84$ For a high vacuum gas oil sample with $\sim 5200$ peaks, FTICR rms mass error for an absorption-mode spectrum (see below) drops from 107 to 27 ppb by essentially eliminating systematic error. For the orbitrap, $m/z$ is first-order proportional to $(\omega)^{-1/2}$, leading to a one term calibration equation. As for ICR, orbitrap mass calibration may be improved by considering the effect of space charge, leading to a one term calibration equation. As for ICR, orbitrap mass calibration may be improved by the short ion injection period from the C trap but due to the short ion injection period from the C trap but

$$\omega = \frac{\text{Im}(\omega)}{\text{Re}(\omega)} \quad (2a)$$

$$\phi(\omega) = \arctan[\text{Im}(\omega)/\text{Re}(\omega)] \quad (2b)$$

If the “phase spectrum”, $\phi(\omega)$, is known, absorption- and dispersion-mode spectra, $A(\omega)$ and $D(\omega)$, may in turn be obtained as linear combinations of $\text{Re}(\omega)$ and $\text{Im}(\omega)$.
complicated by the change in central electrode voltage (and therefore resonant frequency) during ion injection. A “pseudo”-phase correction for the orbitrap combines absorption-mode display for the top part of the peak with an increasing magnitude-mode component for the bottom part. Although the peak width at half peak height is thus narrower, improvement vanishes for low abundance peaks that are near the base of a high abundance peak, and the mass measurement accuracy does not improve.

**Improved Data Station.** Continued improvement in FTz mass analyzers requires optimized control of multiple experimental events, precisely timed data acquisition, and efficient data analysis to accommodate new hardware modifications and ion optics configurations. A recent Predator data station exploits improved PC bus speed to transfer data to and from the control PC in real time. Conditional data acquisition can evaluate data in real time and apply user-defined conditions to average and store data. This capability is primarily used to eliminate low quality data so as to improve the data S/N ratio and minimize the computer memory required to store data. Tool command language (Tcl) scripting has been applied for 17 voltages and 18 triggers and provides a fast and easy way to control the Predator data station and associated peripherals, e.g., LC, autosampler, laser source, and electron emitter. Another improved data control system combines PXI hardware with a workflow-based acquisition and control software to allow unique types of data-dependent experiments. Dynamic switching between different dissociation technologies may be achieved due to fast and precise hardware response.

**Selected Applications. Tandem Mass Spectrometry.** The main difference between ICR and orbitrap for MS/MS is that precursor ion dissociation can be performed in a Penning trap but not in an orbitrap. In principle, fragment ions can be brought to coherence in an orbitrap for subsequent excitation and detection, but MS/MS is typically performed externally (CID or ETD) for orbitrap detection. MS/MS can be performed in an ICR cell by collision-induced dissociation (CID), infrared multiphoton dissociation (IRMPD), and electron capture dissociation (ECD). However, high resolution requires low pressure in the ICR cell, so introduction of collision gas into the cell requires subsequent pumpdown before excitation/detection; ergo, CID FTICR experiments are now typically conducted with an external electric multipole ion trap. Because ECD (or ETD) can fragment a peptide but not dissociate the noncovalently bound fragments, infrared irradiation is commonly used to release the product ion. CID and IRMPD heat an ion and typically break the weakest bond to produce predominantly b- and z-type peptide fragment ions. However, post-translational linkages (e.g., phosphorylation, glycosylation) are often lost before peptide backbone cleavage, thereby eliminating knowledge of their location. In contrast, ECD (or ETD) produces mainly c- and z-type backbone cleavage fragment ions by breaking the backbone N-Cα bond without loss of phosphate or glycan. Thus, ECD or ETD is much preferred for locating post-translational modifications.

**Proteomics.** Proteomics encompasses identification and structural characterization of proteins and their complexes, determination of post-translational modifications, and quantitation of protein expression under different treatments. In “top-down” proteomics, a single gas-phase protein is isolated and subjected to fragmentation (e.g., CID, IRMPD, ECD, ETD, etc.) to generate fragment masses for comparison to an appropriate database for protein identification, as well as identity and location of each post-translational modification according to its characteristic additional mass relative to an unmodified amino acid residue. The top-down approach can in principle locate all post-translational modifications, even when several are present at the same time. High resolution and high mass accuracy are essential to resolve isotopic distributions from peptides of different charge state as well as to assign isotopic peptide compositions (e.g., lysine vs glutamine, differing in mass by 0.0364 Da). Examples of top-down post-translational identifications include: rhesus monkey cardiac troponin, integral membrane proteins, and human histones. Further, 99 proteins have been identified by top-down tandem mass spectrometry in *Methanosarcina acetivorans*, and capillary liquid chromatography coupled with FTICR identified with high confidence 102 endogenous peptides in the suprachiasmatic nucleus. Robust two-dimensional liquid chromatography combined with top down mass spectrometry increases efficiency for improved quantification and characterization. Further automated data processing can increase top-down throughput. In addition to conventional ESI, IRMALDESI has been coupled with an FTICR mass analyzer to perform top-down analysis of equine myoglobin (17 kDa). Discovery and characterization of endogenous peptide and candidate pharmacodynamic markers has been demonstrated. Recently, FTICR MS achieved unit mass baseline resolution for an intact 148 kDa therapeutic monoclonal antibody, the highest mass protein isotopically resolved to date.

In the “bottom-up” approach, proteins are typically separated by HPLC and/or electrophoresis, proteolytically digested into peptides, and then subjected to online LC/MS, and the most abundant peptides are subjected to MS/MS. Any of several algorithms then compare precursor ion mass and MS/MS peak spacing to an appropriate database for protein identification. Identification reliability may be improved by incorporating LC elution time. Alternatively, “shotgun” proteomics also relies on online LC/MS and MS/MS but without prior gel separation of the proteins. Shotgun proteomics has been applied to microorganisms, histones (especially post-translational modifications), and discovery of biosynthetic pathways, etc. For proteins too large for efficient gas-phase fragmentation, “middle-down” proteomics denotes LC/MS and MS/MS for large proteolytic fragments of the original protein and has identified 7454 peptides from 2 to 20 kDa after 23 LC MS/MS injections of Lys-C digests of HeLa-S3 nuclear proteins. Post-translationally modified histone H3 variants have also been characterized by that approach.

Quantitative proteomics typically requires isotopic labeling, either before (SILAC) or after peptide isolation. Quantification of human monkeypox virus and vaccinia virus confirmed the level required for pathogenesis with the accurate mass and time tag (AMT) method after peptide isolation. With stable isotope labeling of amino acids in cell culture (SILAC), it is possible to quantitatively evaluate phosphorylation changes in stable cell lines. Quantitative proteomics depends on fragmentation energy. When isotopic labeling is not applicable, label-free methods can provide a measure of quantitation.

**Metabolomics.** High throughput screening of chemical toxicity in *Daphnia magna* has been reported. Metabolic profiling facilitates biochemical phenotyping of normal and neoplastic colon tissue at high significance levels and annotates the ions originating from identical metabolites by fragmentation pattern and database searching analysis. Coupled with different chromatography platforms, higher dynamic
range and identification of more metabolites can be achieved. Autocorrelation of retention and ionization leads to more exhaustive metabolic fingerprints.168

**Lipidomics.** Characterization of nonpolar lipids and selected steroids by high resolution mass analysis has emerged as a powerful tool for lipidomics.169,170 Online LC separation can quickly narrow down the possible phospholipid and glycosphingolipid compositions and facilitate their identification.171,172 Unequivocal lipid elemental composition from isotopic fine structure has been used to validate the identification of sulfur-containing lipids in algae extracts (Figure 5).173 Direct infusion high resolution mass analysis with computer-assisted assignment is able to profile the 13C-isotopologues of glycerophospholipids (GPL) directly in crude cell extracts.174 High resolution MS/MS enabled identification of tricylglycerols archeological extracts175 and neutral lipids.176 Matrix-assisted laser desorption ionization combined with high-resolution MS enables confident identification and spatial localization of lipids in tissue sections.177,178

**RNA/DNA Analysis.** Most high resolution MS analyses of oligonucleotides have focused on synthetic DNA, RNA, aptamers, etc. Double-stranded small interfering RNA (siRNA) presents numerous fragment ions and multiple chemical modifications. The different metabolite patterns for synthetic siRNA in different biomatries (rat and human serum) provide insight into the stability and pharmacokinetic properties of therapeutic siRNA compounds.179 A method for sequencing single and double stranded RNA oligonucleotides by acid hydrolysis has been presented. From the mass differences between adjacent members of a mass ladder, the complete sequences of different siRNA 21-mer single and double strands could be verified. This simple and fast method can be applied to controlling sequences of synthetic oligomers, as well as for de novo sequencing.180 A high resolution mass spectrometry-based analytical platform for RNA, employing direct nanoflow reversed-phase liquid chromatography with a spray tip column predicted the nucleotide composition of a 21-nucleotide small interfering RNA, detected yeast tRNA post-transcriptional modifications, and analyzed the nucleolytic fragments of RNA at a subfemtomole level.181 Various other approaches, e.g., combing chemical modification of RNA with mass spectrometry,182 based on neomycin B183 or classical nucleic acid ligands,184 enable characterization of polyuridylic tract-containing RNA–DNA hybrids and show that the common structural features of lentiviral and retrotransposon polyuridylic tracts facilitate the interaction with their cognate reverse transcriptase. Structural elucidation of the HIV-1 virus has also been achieved by the combination of footprinting and cross-linking techniques with high resolution MS detection.184,185

**Synthetic Polymer Analysis.** The most obvious advantage of mass spectrometry for synthetic polymer analysis is the generation of the full molecular weight distribution, rather than simply number-average or weight-average molecular weight.186,187 High resolution MS can also reveal pathways of polymer synthesis, as for a sterically hindered alkoxyamine initiator for the nitroxide-mediated copolymerization (NMP) of methyl methacrylate (MMA);188 the styrene monomer was added as polymerization terminator, and the progress was monitored from the product oligomer distributions. Synthetic polymer structure has been probed by various fragmentation techniques, including electron capture dissociation (ECD), collision induced dissociation (CID), and electron detachment dissociation (EDD),189–194 e.g., polyamidoamine dendrimer characterization by ECD and EDD.

**Petroleomics.** From sufficiently complete characterization of the organic composition of petroleum and its products, it is becoming possible to correlate (and ultimately predict) their properties and behavior.195,196 FTICR MS is the preferred identification technique for petroleum analysis because of the need to resolve mass splits down to 3.4 mDa (32S1H4 vs 12C3) or ~0.5 mDa (for nickel-containing porphyrins) (Figure 6). Removal of contaminants in asphaltenes during the crude oil processing is necessary to avoid catalyst fouling and hence lower liquid yield. Also, asphaltenes can precipitate during production and refining. Thus, asphaltenes are one of the most discussed topics in petroleomics.198–202 Ion mobility mass spectrometry and FTICR MS have been combined to confirm the presence of asphaltene nanoaggregates in toluene at concentrations (10−4 mass fraction) below those in crude oils.197 Subsequently, in situ analysis of a 3000 ft vertical column of crude oil by downhole fluid analysis indicated that the asphaltenes in a black crude oil exhibit

![Figure 5](image1.png)

**Figure 5.** Positive electrospray ionization 14.5 T FTICR mass spectrum for Nannochloropsis oculata species lipid extract. Inset: Two peaks differing by 2.37 mDa are resolved and assigned. (Figure kindly provided by Huan He.)

![Figure 6](image2.png)

**Figure 6.** Positive ion atmospheric pressure photoionization (APPI) 9.4 T Fourier transform ion cyclotron resonance (FTICR) mass spectrum of a petroleum crude oil. Upper inset: Mass scale expansion revealing 90 singly charged ions within a mass range of 0.32 Da. Lower inset: Baseline resolution of ions differing in mass by 1.1 mDa, made possible by ultrahigh mass resolving power of 1 000 000 at m/z 428. (Data kindly provided by Amy McKenna.)
gravitational sedimentation according to the Boltzmann distribution and that the asphaltene colloidal size is ~2 nm.203 The detailed characterization of a Middle Eastern heavy crude oil distillation series fully validated the Boduszynski model, namely, that petroleum is a continuum with regard to composition, molecular weight, aromaticity, and heteroatom content and that boiling point can be predicted from aromaticity and heteroatom content.204,205 Naphthenic acids (i.e., species containing non-aromatic hydrocarbon rings) can form harmful deposits and may be identified in crude oil before the deposits form206–208 as well as in oil sands processed water.209–211 Ultrahigh mass resolution also enables identification of biomarkers, such as nickel and vanadyl porphyrins.212,213

Environmental Analysis. Applications of high resolution MS technology in the fields of food safety214 and environmental analysis have recently been reviewed.215 High resolution helps to trace contaminants, as well as to identify analogs for which standards are not available. High-resolution full scan MS analysis with simultaneous targeted CID MS/MS has been applied to analysis of polyether toxin azaspiracid food contaminants in shellfish.216 High resolution is also useful in the high-throughput screening of micropolllutants in wastewater treatment. Both targeted and nontargeted screening have identified a variety of unreported and previously reported pesticide transformation products.216 Characteristic polar oil sand components (naphthenic acids and other related acid fraction components) spiked into plant tissue prior to extraction can be differentiated from coextracted endogenous plant components by high resolution MS.210 LC/high resolution MS showed that the industrial chemical, perfluorooctanoic acid, is microbiologically inert and hence environmentally persistent.217 The high production volume chemicals, benzo-triazoles and benzo(h)iazoles, have been extracted from environmental water by solid-phase extraction and identified by LC/high resolution MS.218 Induced metabolite changes in myriophyllum spicatum, an aquatic vascular plant, have been monitored by high resolution MS.219 The method has also provided molecular characterization as the level of elemental composition, for dissolved organic matter (DOM), e.g., from pore water,220 secondary-treated wastewater,221 and the Greenland ice sheet,222 because it is capable of resolving complex molecular mixtures and providing information about the exact elemental composition.

AUTHOR INFORMATION

Corresponding Author
*E-mail: marshall@magnet.fsu.edu (A.G.M.); hendrick@magnet.fsu.edu (C.L.H.).

Biographies

Alan G. Marshall completed his B.A. degree with Honors in Chemistry at Northwestern University in 1965 and his Ph.D. in Physical Chemistry from Stanford University in 1970. He is Robert O. Lawton Professor of Chemistry and Biochemistry at Florida State University and Director of the Ion Cyclotron Resonance (ICR) Program, an NSF national user facility for mass spectrometry. His current research spans FTICR instrumentation development, fossil fuels and environmental analysis, and mapping the primary and higher-order structures of biological macromolecules and their complexes.

Christopher L. Hendrickson, Associate Director of the Ion Cyclotron Resonance Program at the National High Magnetic Field Laboratory and Courtesy Professor of Chemistry at Florida State University, received his B.A. degree in chemistry from the University of Northern Iowa and his Ph.D. in analytical chemistry from the University of Texas at Austin. His research interests are in instrumentation, technique development, and applications of analytical Fourier transform ion cyclotron resonance mass spectrometry.

Feng Xian is a PhD candidate in the Ion Cyclotron Resonance program at the National High Magnetic Field Laboratory at Florida State University in Tallahassee, Florida. He received his Bachelor of Science in Chemical Engineering from the Xi’an Jiaotong University in 1996, and he anticipates his Ph.D. in Analytical Chemistry from Florida State University in 2012. His research focuses on Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry instrumentation development.

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(217) Liou, J. S.-C.; Szostek, B.; DeRito, C. M.; Madsen, E. L. Chemosphere 2010, 80, 176−183.