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Orbitrap Mass Spectrometry

Orbitrap is the newest addition to the family of high-resolution mass spectrometry analyzers. With its revolutionarily new, miniature design, Orbitrap combines high speed with excellent quantification properties, ranking favorably in many analytical applications.

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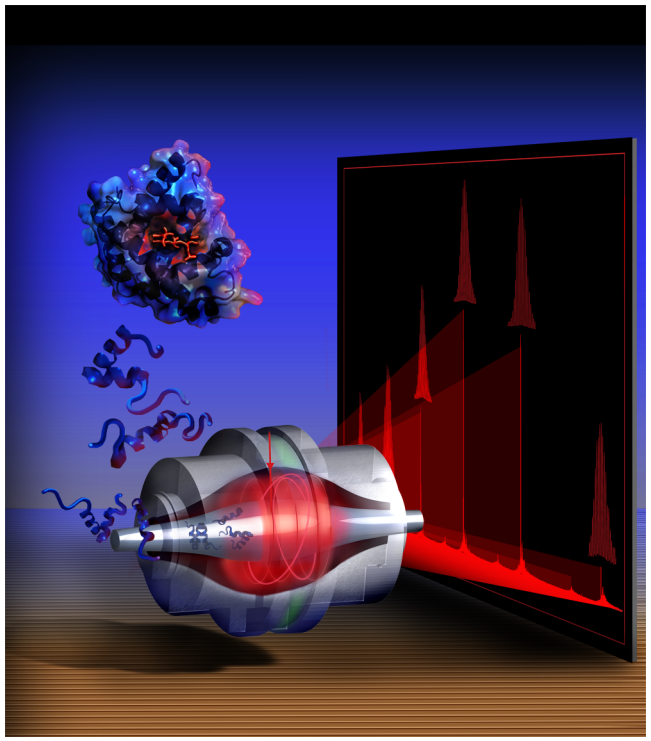

WHY ARE MORE ANALYZERS NEEDED IN MASS SPECTROMETRY?

Newcomers in mass spectrometry (MS) usually get baffled by the sheer variety of different ionization, mass analyzing, and detection methods. While ionization methods determine the classes of substances available for measurement, it is a combination of the mass analyzer with the detector that ultimately determines the quality and reliability of analysis. Depending on the physics of mass analysis, analyzers could belong to quadrupole, magnetic sector, ion trap, time-of-flight (TOF), or Fourier transform (FT) generic types. They could be further combined together to allow analysis of both analytes and their fragments (MS/MS), most popular combinations being triple quadrupole and quadrupole/time-of-flight hybrids. Alternatively, the same analyzer can perform MS and MS/MS (MS^2) analysis, sometimes to a high MS^n stage, such as a radiofrequency ion trap (Paul trap) or a static electromagnetic trap (Penning trap).

Until a decade ago, FTMS was used just as another term for Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. In such instruments, ions are trapped in a strong magnetic field combined with a weak electric field. Image current from coherently excited trapped ions is detected, digitized, and converted using Fourier transform into the frequency domain and then mass spectra.[1] Inherent stability and field uniformity of superconducting magnets in synergy with the very high accuracy and dynamic range of frequency measurements made this technique an ultimate champion in mass resolving power and mass accuracy. This ability to discern ions with closely located mass-to-charge ratios (m/z), e.g., isotopic peaks of high-mass ions, and thus to determine their charge z, allowed researchers to measure their masses m with relative accuracies at the level of $<10^{-7}$–10^{-6}. This ability became increasingly important over the last 2 decades as the advent of electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) resulted in ever increasing acceptance of MS for analysis of biological samples. This process was accompanied by escalation of complexity of analyzed mixtures and acceleration of preceding liquid separations. This in its turn became an impetus for improving existing mass analyzers and searching for new, even more efficient ones. Numerous attempts were made over decades to develop FTMS on the basis of radiofrequency ion traps, electrostatic traps, and multireflection systems,[2] but numerous technical issues prevented them from entering mainstream mass spectrometry.

Such conditions created fertile soil for the appearance and growth of a new member of the FTMS family: the Orbitrap analyzer. Appearing first as a cute scientific curiosity in patent literature and presented first to the general MS public at a conference of the American Society for Mass Spectrometry in 1999, it quickly made its debut in mainstream MS in 2005 as an accurate and compact mass detector. The first commercial implementation was in a hybrid instrument (LTQ Orbitrap) featuring a linear ion trap front-end. Since then thousands of Orbitrap-based instruments were produced and became a common sight in analytical laboratories and facilities worldwide.

The latest newcomer to the noble family of mass analyzers inherited some features from its older relatives. For example, it borrowed the principle of image current detection from

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FTICR, the use of ion trapping in precisely defined electrode structures—from the radiofrequency ion trap, pulsed injection and the use of electrostatic fields—from the TOF analyzers. Altogether, these features resulted in a powerful and unique combination of analytical features. At the same time, they allowed one to address some of the major limitations of the older relatives, such as necessity for a superconducting magnet in FTICR, severe limitations on space charge in the radiofrequency ion trap, or on dynamic range of detection in TOF analyzers.

HOW DOES IT WORK?

The Orbitrap mass analyzer consists essentially of three electrodes as shown in Figure 1. The cut-outs represent both

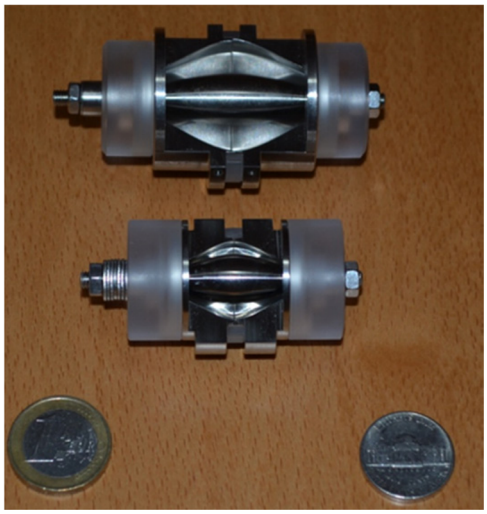

Figure 1. Cut-outs of a standard (top) and a high-field (bottom) Orbitrap analyzer. Reprinted with permission from Thermo Fisher Scientific. Copyright 2012 Thermo Fisher Scientific.

the standard trap as introduced commercially in 2005 and the so-called high-field compact trap introduced in 2011.[3,4] Outer electrodes have the shape of cups facing each other and electrically isolated by a hair-thin gap secured by a central ring made of a dielectric. A spindle-like central electrode holds the trap together and aligns it via dielectric end-spacers. When voltage is applied between the outer and the central electrodes, the resulting electric field is strictly linear along the axis and thus oscillations along this direction will be purely harmonic. At the same time, the radial component of the field strongly attracts ions to the central electrode.

Ions are injected into the volume between the central and outer electrodes essentially along a tangent through a specially machined slot with a compensation electrode (a "deflector") in one of the outer electrodes. With voltage applied between the central and outer electrodes, a radial electric field bends the ion trajectory toward the central electrode while tangential velocity creates an opposing centrifugal force. With a correct choice of parameters, the ions remain on a nearly circular spiral inside the trap, much like a planet in the solar system. At the same time, the axial electric field caused by the special conical shape of electrodes pushes ions toward the widest part of the trap initiating harmonic axial oscillations. Outer electrodes are then used as receiver plates for image current detection of these axial oscillations. The digitized image current in the time domain is Fourier-transformed into the frequency domain in the same way as in FTICR and then converted into a mass spectrum.

HISTORY OF THE TECHNOLOGY

The roots of the Orbitrap analyzer can be traced back to 1923 when the principle of orbital trapping was realized by Kingdon[5] by placing a charged wire inside an enclosed cylindrical metal can. The ions formed by discharge inside the can were attracted toward the wire but "missed" it if they had sufficiently high tangential velocity, starting to orbit around the wire for prolonged periods of time.

Experiments performed with the Kingdon trap over the subsequent half a century and reviewed in 2008[6] proved the efficiency of electrostatic trapping but offered no hint as to how to use this device for mass analysis. Because of advances in charged particle optics, new electrostatic fields began to be used, e.g., a quadro-logarithmic potential distribution was employed by Knight for orbital trapping of laser-produced ions.[7] Crude mass analysis was performed by means of axial resonant excitation of trapped ions, with ions being detected by a detector placed near the axis outside of the trap. As such a device was not capable of separating even the simplest mixtures, this attempt made it clear that there is still a very long way to a high-performance analyzer. Considerable improvements in all key areas were necessary, most notably a more accurate definition of the quadro-logarithmic field, an ion injection into the analyzer from an external ion source, and ion detection matching the features of the trap.

These crucial issues have been successfully addressed in the seminal work of Makarov.[8] Unlike in the previous attempts, the central electrode was implemented not as a thin wire but rather as a massive metal electrode manufactured with the accuracy of machining at the limit of the present-day technology. Outer electrodes followed the equipotential surface matching the shape of the central electrode; they were split into two halves to work as receiver plates for image current detection. Geometry of the trap was optimized to improve the sensitivity and reduce higher harmonics.

Following the first proof-of-principle experiment with a laser ion source, a number of very significant technological advances were implemented to bring the analyzer to practice. One of the most important advances was the development of pulsed injection from an external ion storage device of the C-trap type.[3] Such a storage device (Figure 2) effectively decouples the Orbitrap analyzer from any preceding ion source, ion transmission device, or analyzer. Therefore, any device capable of selecting or transmitting precursor ions as well as any fragmentation technique could be interfaced to the Orbitrap.

The first commercial instrument to utilize this capability, LTQ Orbitrap Classic, was introduced by Thermo Electron (currently Thermo Fisher Scientific) in 2005 and was followed by important extensions of the same family: (i) Addition of a collision cell after the C-trap in LTQ Orbitrap XL (2007) has opened a route to utilizing higher-energy collisions (with energies higher than those achievable in the linear ion trap), hence the term higher collision energy dissociation (HCD).[9] (ii) Addition of electron transfer dissociation (ETD) capabilities to that instrument made it possible to expand the range of post-translational modifications amenable for analysis in proteomic applications.[10] (iii) MALDI source operating at reduced pressure became the basis of high-end LTQ Orbitrap XL MALDI instrument.[11] (iv) A stacked ring rf ion guide (so-called S-lens) brought about 10-fold higher transfer efficiency in

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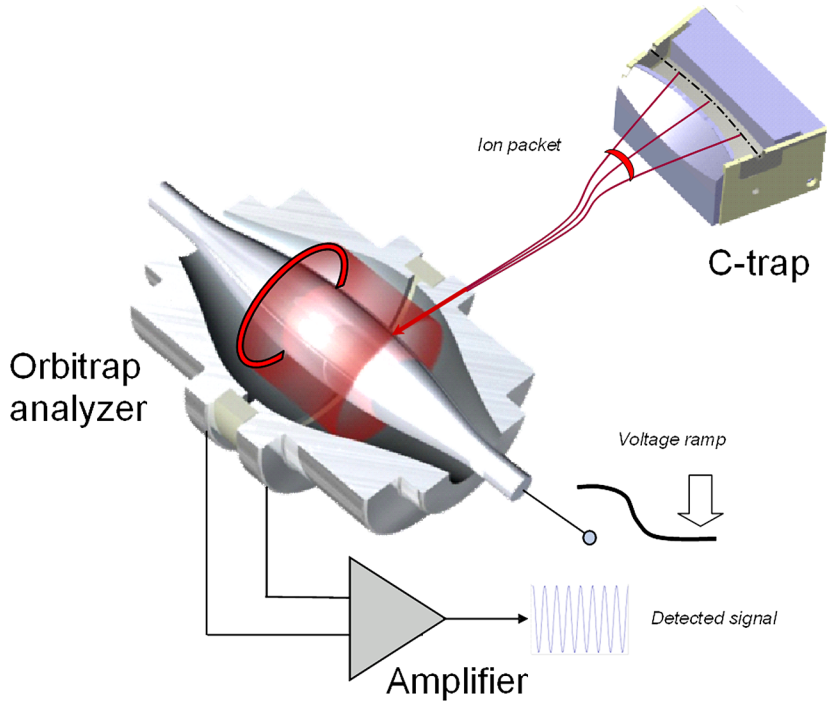

Figure 2. Cross-section of the C-trap and Orbitrap analyzer (ion optics and differential pumping not shown). Ions are stored in the rf-only bent quadrupole of the C-trap, then the rf is ramped down and a high-voltage pulse is applied across the trap, each m/z being ejected in a short packet. The packets from the C-trap enter the analyzer during the voltage ramp and spread into oscillating rings that induce current detected by the differential amplifier. Reprinted with permission from Thermo Fisher Scientific. Copyright 2012 Thermo Fisher Scientific.

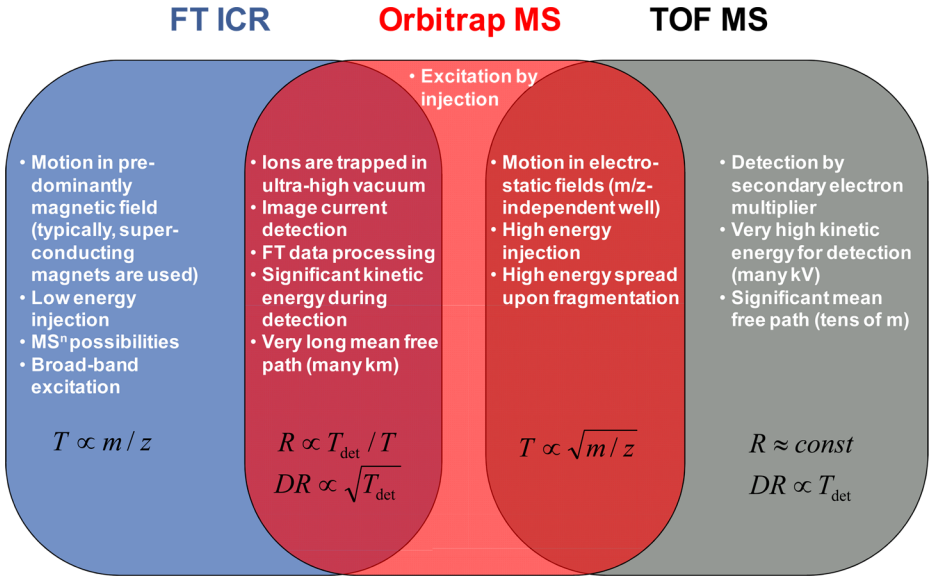

Figure 3. Comparison of physical and analytical features for high-resolution, full mass range techniques in mass spectrometry (T is period of oscillation, R is resolving power, DR is in-spectrum dynamic range, T_{det} is duration of detection per one spectrum).

the MS/MS mode and a 3−5-fold increase in full scan mass spectra in LTQ Orbitrap Velos (2009), while a dual ion trap ramped up the speed of MS/MS.[12] (v) In the Orbitrap Elite instrument (2011),[4] the resolving power of the analyzer has been increased almost 4-fold to 240 000 at m/z 400 for a 768 ms transient. Both Orbitrap and FTICR instruments can accumulate longer transients, with FTICR being able to detect transients lasting several minutes and thus achieving hyper-resolution.[13] The maximum resolution on an Orbitrap has been achieved by employing a compact, high-field analyzer (as shown at the bottom of Figure 1) and an enhanced Fourier transform (eFT) algorithm. This algorithm incorporates information about the phases of ion oscillations which are precisely defined in the Orbitrap due to the built-in "excitation-by-injection" mechanism (described below).

Since 2008, a stand-alone Orbitrap mass spectrometer, Exactive, has been built, wherein the ion source was directly linked to the C-trap.[14] In 2011, a combination of that device with a quadrupole mass analyzer was launched under the name

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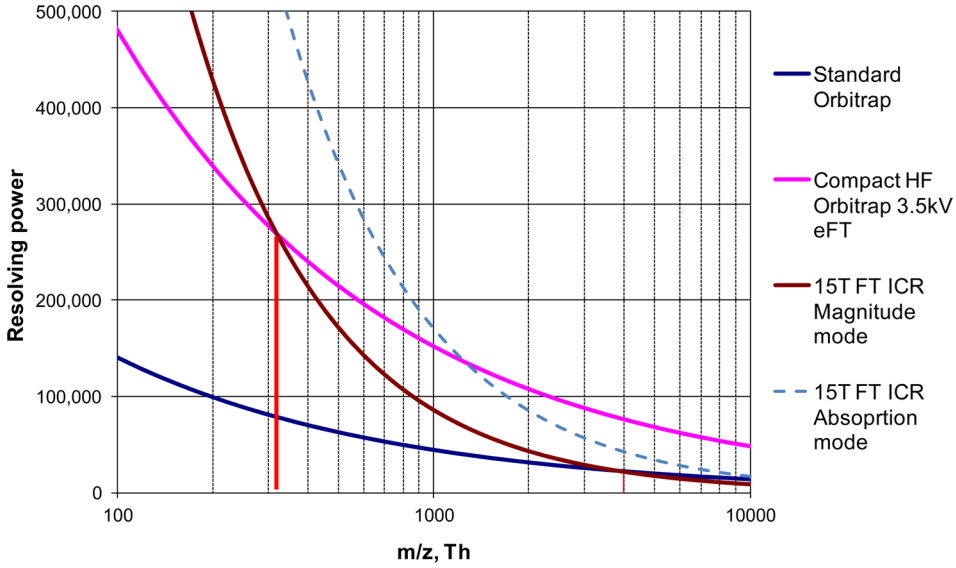

Figure 4. Dependence of resolving power on m/z for the following analyzers (all data are shown for a 0.76 s scan): (i) standard trap (magnitude mode, 3.5 kV on central electrode), (ii) compact high-field trap (eFT, 3.5 kV on central electrode), (iii) FTICR (magnitude mode, 15 T), (iv) FTICR (absorption mode, 15 T).

of Q Exactive and became popular for proteomics and high-throughput screening.[15]

■ ORBITRAP vs OTHER HIGH-RESOLUTION ANALYZERS

Figure 3 shows a comparison of physical and analytical features for all three high-resolution, full mass range m/z analysis techniques utilized in mass spectrometry. It is instructive to perform a pairwise comparison of the analyzers.

Orbitrap vs FTICR. Basic FTICR design precedes Orbitrap by almost three decades.[16] During this time, the progress has been driven by a string of ingenious innovations as well as the growing field strength of available superconducting magnets.[17,18] Although the attempts to employ the Fourier transform detection method in high-resolution mass analyzers have been made in other trapping devices as well,[19] Orbitrap is the first FT device after FTICR to reach commercialization. Disregarding the vast differences in size and cost, these two analyzers share a number of similar features. In both analyzers, the ions are trapped in ultrahigh vacuum to ensure very long mean free paths (of many tens or even hundreds of kilometers). Furthermore, the ions are detected based on their image current and FT data processing while they are moving at significant kinetic energies (of several hundred or few thousand volts). Thus for both of these analyzers, resolving power R is proportional to the ratio of the detection time T_{det} to the period of main oscillations T, and the in-spectrum dynamic range depends relatively weakly on T_{det}. However, in FTICR, ions move in the magnetic field of large superconducting magnets and therefore, appropriate mathematics being applied, T is directly proportional to m/z and R inversely scales with m/z. In the Orbitrap analyzer, the ion motion is determined by the electrostatic field, which leads to T being proportional (and R inversely proportional) to the square root of m/z. The consequence of this difference is that, for any FTICR and for any Orbitrap device, there is a critical m/z_c below which the resolving power achieved for the same T is higher for FTICR but above which the Orbitrap analyzer starts to show higher resolving power. An example is shown in Figure 4, with m/z_c around 300 for 15 T FTICR and a high-field compact trap and 4000 for a standard trap. This slower decrease of resolving power with m/z allows users to employ Orbitrap mass spectrometers also for very high m/z.[20]

The use of just electrical fields for ion trapping ensures a small size of the analyzer and makes it possible, at least in principle, to use it in benchtop and even portable instruments. However, the compact size has also important implications for its analytical parameters. Bigger size means lower axial oscillation frequency and thus lower resolution at the same T. Thus, counterintuitively, smaller trap can possess better analytical properties than the larger trap (Figure 1). This is not so in FTICR, where a larger size of the trap is considered beneficial, as the ion capacity increases and the space charge effects diminish, while the constant magnetic field throughput the trap ensures that the cyclotron frequency remains size-independent. The drive toward smaller size in the Orbitrap analyzer may limit its charge capacity or result in a larger mass shift due to space charge effects, but its central electrode gives it a massive advantage by shielding the ions on different parts of the near-circular trajectory from each other and thus greatly increasing the effective charge capacity compared to a device with no central electrode or with a thin, wire-type central electrode. The high charge capacity translates in high dynamic range, reaching 4 orders of magnitude or larger in a single mass spectrum.[21]

Perhaps the greatest advantage of the FTICR analyzer is its ability to accept ions with low and very low energies and to trap them practically indefinitely (many hours), subjecting to a variety of excitations (UV, IR, collisional, etc.) and ion−ion as well as ion−neutral reactions. Moreover, one can select a precursor ion in the FTICR analyzer with high resolution, e.g., isolating a single isotopomer of a large molecule, excite and analyze it, and then de-excite and reanalyze. This flexibility is practically absent in the Orbitrap analyzer, where high-energy ions colliding with neutrals or other ions, or undergoing unimolecular dissociation, are usually lost just within several seconds after injection.

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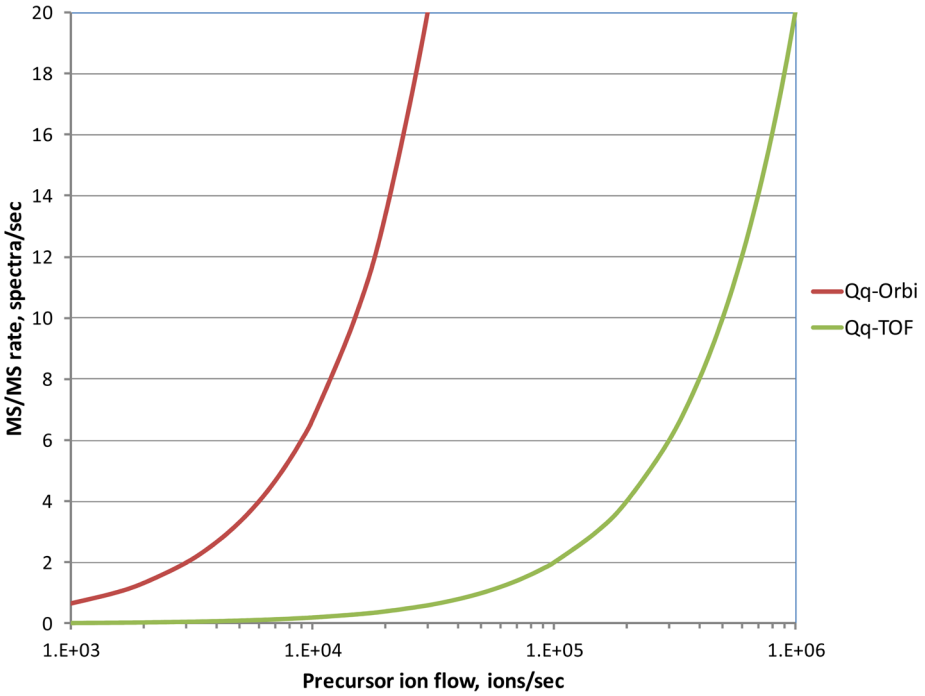

Figure 5. MS/MS spectra acquisition rate as a function of precursor ion flow for Orbitrap and TOF hybrids with a quadrupole analyzer. Higher transmission of the Orbitrap analyzer allows one to achieve a detection system-limited rate at much lower precursor ion abundances. The graph is shown for effective oaTOF transmission of 4% at a resolving power of 20 000 or 1% at resolving power 40 000, Orbitrap transmission 40% and 64 ms transient (standard trap) or 32 ms (high-field compact trap). It is modeled that the precursor dissociates into 20 fragments of equal abundance with a required fragment mass tolerance of 10 ppm (4σ-band).

On the other hand, the Orbitrap analyzer possesses a unique feature in relation to the method of exciting axial oscillations. This is achieved by injecting pulsed ion packets into the trap tangentially through a slot in the outer electrode. The slot position relatively to the center of the trap defines the amplitude of axial oscillations ("excitation by injection"). This approach minimizes perturbations of the quadro-logarithmic field when an additional electrode (so-called "deflector") is positioned above the injection slot with compensation voltage applied to it. It should be noted that accurate control of this voltage is crucial for ensuring similar decay rate for different ion abundances and hence correct measurement of isotope ratios. "Excitation by injection" requires a very fast ejection of large ion population from an ion source or an external rf storage device. More importantly, it provides an unequivocal link between the phase of oscillations and m/z. This allows one to use not only standard (so-called magnitude-mode) Fourier transform but also a more refined version of it (eFT)[4] that utilizes phase information. This allows double resolving power (or, alternatively, increase the speed of analysis by the same factor while keeping the resolution constant). In contrast, magnetic trapping demands for efficient ion detection broadband excitation, where the phase-m/z link is much harder to recover, and thus only standard FT is typically available for commercial FTICR instruments. Simultaneous excitation and detection allows one to achieve broadband detection in the so-called absorption mode (the corresponding improvement in resolving power is shown in Figure 4 by a dashed line), but so far this mode was limited to research or noncommercial instruments only.[17]

Orbitrap vs Quadrupole Ion Traps. High resolution ($R \geq 30\,000$) is relatively easily achievable on a quadrupole ion trap (QIT) even without FT detection.[22] In principle, such resolution should immediately yield ppm mass accuracy, but QITs are notorious for poor conversion of high resolving power into mass accuracy. Various effects leading to mass scale nonlinearity and grotesque distorted spacing between the isotopic peaks are mostly related to ion−ion interaction in the QIT analyzer[23] and can in principle be reduced by having few ions trapped at any given time. However, there is a more fundamental reason for mass shifts in QIT, which are often hard to predict for a novel ion, the gas pressure. Intense interaction of the trapped ion with the background gas, often kept in a QIT at a mTorr pressure, leads to the ion shape and polarizability creeping into the equation of motion, combining the feature of mass spectrometry and ion mobility. FT detection, although possible in QIT,[24] does not bring about the benefit of high mass accuracy. The highest mass accuracy on peptide molecular ions reported with a high-resolution QIT is ∼12−15 ppm,[25] which is far below the subppm accuracies routinely achieved in FTMS.

Orbitrap vs TOF. Comparison with TOF shows that both analyzers are based on ion motion in electrostatic fields (with m/z-independent forces acting on ions) that results in relatively slow dependence of the oscillation period or time-of-flight T from m/z (as a square-root). Also, in both analyzers ion injection involves strong acceleration and hence large kinetic energy spread upon fragmentation if ions happen to fragment within the analyzer. The latter consequence severely compromises the quality of MS^n in both analyzers and makes them more effective as accurate-mass detectors for an external source of fragmented ions. This explains why most Orbitrap and TOF analyzers are employed in hybrid rather than stand-alone configurations. On the other hand, TOFs typically utilize detection by secondary electron multipliers that reach their maximum detection efficiency at a very high kinetic energy of

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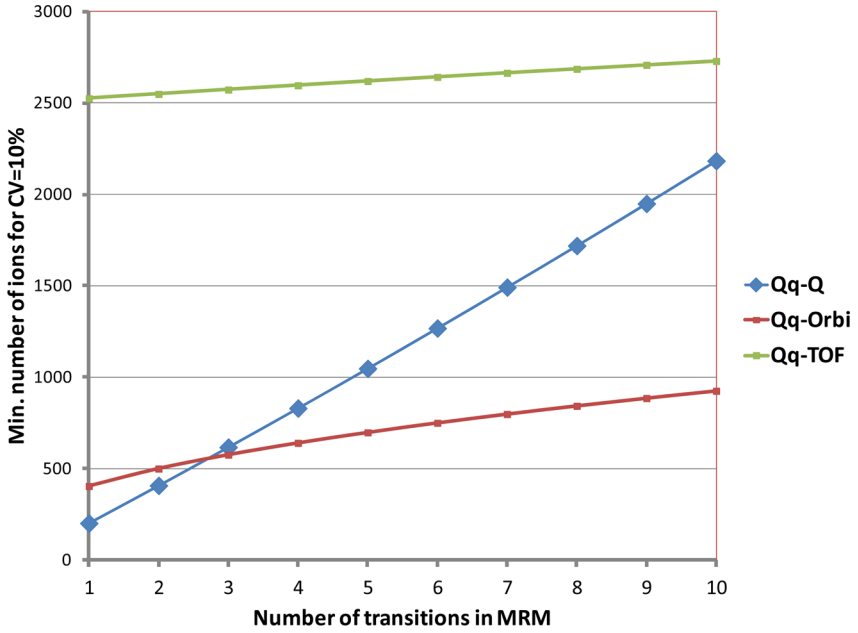

Figure 6. Minimum total number of precursor ions needed to be delivered from the ion source for quantification at CV = 10% as a function of fragmentation channels in a model multiple reaction monitoring (MRM) experiment. The graph includes three hybrids of the same quadrupole front-end with different analyzers for fragments: quadrupole, oaTOF, and Orbitrap. It is modeled that the precursor dissociates into fragments of equal abundance and different mass and quantification is done on the sum of fragment ion abundances. Assumptions for oaTOF and Orbitrap analyzers are the same as in Figure 5, while for quadrupole analyzer transmission of 50% and no isobaric interference in QqQ is assumed. Even under this generous assumption, Orbitrap hybrids require less ions starting from the number of MRM transitions around three.

ions (up to tens of kilovolts). This makes resolving power R independent of detection time or m/z (except for lower m/z where it is normally reduced due to the data system) but makes in-spectrum dynamic range directly dependent on T_{det} (the shorter the acquisition time or higher spectrum acquisition rate, the smaller is the dynamic range).

Typically, the highest resolving powers available in TOF devices are several times lower than the ultimate resolution in both Orbitrap and FTICR MS, although recent multipass TOF devices are capable of ultrahigh resolution ($R \geq 100\,000$ at m/z 400).[26,27] At the same time, TOF resolution is largely the same in the MS and MS/MS mode, which formally offers an advantage over the Orbitrap analyzer, where the resolution in MS/MS mode is often sacrificed for the sake of speed. However, in practice the higher resolution of TOFs in MS/MS does not necessarily translate into higher mass accuracy (which is frequently the most desirable analytical parameter) due to limited transmission of TOFs which usually employ orthogonal acceleration of continuously flowing ions. Thus the actual reported true positive identification rate achieved with Orbitrap mass analyzers is probably higher than with TOFs in a comparable experiment. Consistent with that suggestion, the highest rates of true positive protein identification reported for a single LC/MS experiment are achieved with an Orbitrap.[28] Figure 5 illustrates this for comparison with the most widely used configuration of TOF utilizing orthogonal acceleration of ions: only at the highest ion fluxes the actual acquisition rate approaches the specified rate of the instrument.

Though the mean free path is significant (a few to few tens of meters), it is orders of magnitude shorter than for other two analyzers and therefore vacuum requirements are not so stringent in TOF. Perhaps the biggest current advantage of TOF analyzers is the detection of individual singly charged ions, often with >50% probability, while induced-current detection in the FT analyzer requires several charges as a minimum (see below). The peak detection limit is determined by the signal/noise ratio, with noise arising in TOFs due to the dark current of the detector, stray ions as well as "chemical" background ions. The latter consists of a multitude of weakly bound complexes between analyte ions, solvents, and sometimes gas molecules, with charges often coming not only from protons but also from alkali metals and other adducts.[29] These noncovalent complexes give rise to annoyingly broad, unresolved peaks appearing at every m/z unit, with the valleys between the peaks filled by multiply charged species of similar origin as well as products of their metastable dissociation. The presence of this chemical background, appearing both in MALDI and electrospray ionization, is often the main factor limiting the detection threshold and dynamic range of TOF instruments.[30] Somewhat paradoxically, mass spectra acquired with FT analyzers (both FTICR and Orbitrap) are practically free of the chemical background. The likely explanation of this phenomenon is that, to be detected (even at the wrong m/z), a background ion has simply to enter the TOF analyzer, after which it can fragment or stray from its path due to a collision, with neither event affecting the detection probability significantly. At the same time, to give a sharp, software-identified peak in the FT mass spectrum, the ion has to survive intact inside the FT analyzer in a coherent motion with similar ions for a significant fraction of the transient duration, i.e., for many milliseconds. All stray, metastable, or incoherent ions are either not detected in FT or contribute to the broad, smooth background that is easily subtracted by the software. Therefore, although TOFs are formally more sensitive, the real detection limit in the FT analyzers can be comparable or lower.

Another aspect of analyzer comparison relates to fidelity of reproducing isotopic distributions, which is becoming increasingly important for confirmation of elemental compositions

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tentatively derived from accurate masses.[31] In TOFs and other beam machines isotopic fidelity is limited by statistics and frequently by detection artifacts (e.g., detector tailing, saturation, dead time and others), while in FTMS, by statistics, the noise of the preamplifier (for smallest peaks), interference effects, and different decay rates for different ion packets.

Interference effects could be addressed by using dedicated, FTMS-centric isotopic modeling tools to correctly predict the experimentally observed isotopic abundances for a given elemental composition. Meanwhile, the difference in decay rates could be reduced by tuning the trap electrodes to minimize field perturbations and hence eliminate undesirable nonlinear effects at the highest resolving power settings (done automatically during calibration in the latest Orbitrap instruments). With the issues above addressed for both FTMS and TOF, their fidelity of isotopic distribution could approach the statistical limit for most mass peaks.

Quantitative Analysis Using High-Resolution Mass Spectrometry. Quantitative analysis is traditionally considered to be one of the most important and universal applications of mass spectrometry, with triple quadrupole instruments being historically the most widely established.[32] For complex mixtures, the required specificity is achieved by detecting and adding together the abundances of specific diagnostic fragments of a precursor ion. The quality of quantitation is considered acceptable when the concentration variation (CV) is below, e.g., 10% (ideally, limited only by statistical variation). All high-resolution instruments offer an additional dimension of specificity due to reliable separation of previously overlapping isobaric peaks. However, even in the absence of such a dramatic advantage, high transmission of the Orbitrap analyzer allows it to achieve the required CV using fewer ions than even a triple quadrupole instrument, as soon as the number of required diagnostic fragments exceeds a certain threshold. For example, if precursor ion splits into N fragments of equal abundance, the breakeven point could be as low as $N \approx 3$ for theoretical model of Figure 6.

FUTURE OF ORBITRAP MASS SPECTROMETRY

As with every technology, the Orbitrap analyzer has its physical limitations, which will eventually confine its analytical applications to certain areas. However, at the moment these limitations are far from reached and this technology continues to expand its realm in the analytical sciences. Newly emerged applications include top-down proteomics, analysis of native protein complexes, quantitation, high-throughput screening, metabolomics, and others. When considering specific parameters of the Orbitrap analyzer, the potential improvements can be achieved in the following areas.

Speed. While a straightforward increase in the speed of m/z analysis requires a higher detection frequency and thus even smaller Orbitrap dimensions, which may compromise the space charge capacity, there are alternative means of increasing the effective speed, such as (i) MS/MS spectra multiplexing. It has been shown that the high mass accuracy of FTMS allows one to deconvolute multiple MS/MS scans "merged" together in the C-trap and analyzed in the Orbitrap analyzer once.[33] This method, when not limited by the ion current, effectively increases the speed of MS/MS analysis by more than 2-fold. Its limitation is the presence of a sufficient number of complementary fragment pairs in the MS/MS spectra, which can be hampered by excessive fragmentation of less stable b ions in HCD. However, further development of fragmentation techniques can find ways of preserving the b ions intact while providing abundant fragmentation, which will enhance the attractiveness of this multiplexing approach. Multiplexed MS/MS analysis can also enable the pseudo-MRM regime (but retaining the benefits of high resolution and high mass accuracy!) in a typical proteomics experiment.[34] Though this regime is somewhat similar to broad-band fragmentation in Q-TOF instruments,[35] it has the fundamental advantage of providing the ability to adjust as well as mix-and-match precursor ion intensities, thus making deconvolution more feasible. (ii) New processing methods, such as filter diagonalization method (FDM) could potentially be used to circumvent the Nyquist limit of Fourier transform and obtain higher resolving power over the same transient length.[36,37] However, notorious numerical artifacts of this and other non-FT methods still remain an obstacle to realizing this potential. Another limiting factor of many non-FT methods that they often require explicit assumptions on the number of the analytical form of the time-domain signal, number of oscillators, and signal-to-noise ratio, while FT is a general and hypothesis-free time-to-frequency transformation. (iii) Multielectrode or overtone trap. In FTICR, a multielectrode cell with M pairs of electrodes could be used to obtain M periods of ion oscillation in the detected signal for every period of physical cyclotron motion, thus effectively increasing the resolving power M-fold.[38] In the Orbitrap analyzer, implementation of multi-electrode detection is far more challenging, not least due to the ultrahigh accuracy required in the mechanical arrangement of the curve-shaped electrodes. (iv) Orbitrap multiplexing. With an appropriate layout, it is possible to combine several Orbitrap analyzers into a single integrated analyzer with several traps operating in parallel in an overlapping fashion.[39]

Resolving Power. The resolving power in the Orbitrap, as in any FT analyzer, relates to speed and is inversely proportional to it. Thus every improvement in either parameter offers a choice of sharing it with the related one. For instance, enhanced FT offers a choice of twice the speed at the same resolving power or twice the resolving power at the same speed. The only limitation is imposed by the physical duration of the transient, which is particularly stringent for high-mass ions, and is mostly determined by vacuum conditions in the trap and the ion optics immediately adjacent to it.[40] These conditions are greatly improved in the Elite version of the instrument, with the corresponding improvement in the performance for high-mass ions.[20] Further improvements in this area are possible, which, together with the high-m/z transmitting multipole, make the technology highly competitive with FTICR mass spectrometers in the top-down analysis of large proteins and protein complexes.

Detection Limit. The detection limit is mainly determined by the internal noise of the preamplifier, and it is currently at the level of 2−4 elementary charges in a 1 s acquisition. This means that, for charge states higher than 5−10, single-ion detection could be achieved.[20]

Dynamic Range. The dynamic range in a single spectrum is related to the ratio of the total number of trapped ions (charge capacity) to the detection limit, thus improving either of these two parameters increases the dynamic range. In human proteomics, the dynamic range is believed to be the most important parameter limiting the depth of the one-shot experiment,[41] and thus it is a focus of many development efforts.[42,43] The charge capacity of the Orbitrap is determined by its size and configuration (e.g., the gap between the central

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and detecting electrodes) and lies in the range of 0.5−1 million elementary charges. Typically, this charge is accumulated by allowing ions from a wide mass range to flow into the C-trap storage for the same duration. As a result, each mass peak is represented by a number of charges proportional to its abundance and that disadvantages low-abundance peaks. This is where the presence of a beam-type mass filter allows to break this link and accumulate ions intelligently by enhancing certain m/z ranges by "focused investment" and/or selecting peaks of interest in any desired combination. Similar selectivity could be also achieved by using ion traps with injection waveforms though at lower speed and numbers of ions. All selected peaks could be then acquired in a single Orbitrap spectrum (so-called "spectrum multiplexing"). This approach is particularly promising for targeted mass analysis, with multiple SIM or MRM acquired in a single Orbitrap spectrum. It could be also used to combine multiple fragmentation techniques and/or conditions in one spectrum (e.g., HCD energy scan). In future, it is expected to enable selection of multiple charge states of the same precursor, each fragmented at optimum conditions, in order to increase sequence coverage. Data-independent as well as multisegment wide mass range acquisition could be also based on this method.

OUTLOOK

The Orbitrap mass analyzer has become a powerful addition to the arsenal of mass spectrometric techniques for probing biological systems as well as increasing selectivity and confidence of routine analyses. Analytical performance of the trap can support a wide range of applications from routine compound identification to the analysis of trace-level components in complex mixtures, for example, in proteomics, drug metabolism, doping control, and detection of contaminants in food and feed.

The Orbitrap technology will continue to evolve toward increased acquisition speed, higher resolving power, mass accuracy, and sensitivity. This evolution will undoubtedly give rise to exciting new applications as the Orbitrap instruments are becoming more widespread and penetrating into new areas of research.

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Notes

The authors declare the following competing financial interest(s): The authors declare the existence of a financial interest in Thermo Fisher Scientific, the corporation that produces Orbitrap mass spectrometers.

Biography

Roman Zubarev is professor of Medicinal Proteomics at the Karolinska Institutet, Stockholm, Sweden. He has received a B.S. and M.Sc. from the Moscow Engineering Physics Institute, Russia, and a Ph.D. in Analytical Chemistry from the Uppsala University, Sweden. His research group is dealing with a wide range of research topics, from instrument and method development for mass spectrometry to biological and medical topics.

Alexander Makarov is Director of Research of Life Science Mass Spectrometry at Thermo Fisher Scientific in Bremen, Germany. He has received a M.Sc. and Ph.D. from the Moscow Engineering Physics Institute and was a postdoctoral fellow in the Warwick University, U.K., prior to joining a small start-up HD Technologies later acquired by Thermo Fisher. His research group focuses on development of instrumentation for high-resolution mass spectrometry.

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