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GRANT APPLICATION RESOURCE

Orbitrap Fusion Tribrid Mass Spectrometers

Top 5 reasons to upgrade from a Thermo Scientific[™] Hybrid Orbitrap[™] to a Thermo Scientific[™] Tribrid[™] Mass Spectrometer System

Goal

This document is intended to provide conclusive arguments to justify upgrading from an Orbitrap Hybrid MS to an Orbitrap Tribrid mass spectrometer system.

Summary

The Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers are an essential tool for high-end life science research. Robustly designed, they come equipped with a quadrupole mass filter as well as an Orbitrap and linear ion trap mass analyzer. This hardware combination (exclusive to Orbitrap based mass spectrometers) provides superior analytical performance that enables multiple complex modes of analysis. This is due to the parallel isolation and detection mechanisms achievable with the Tribrid architecture, which was previously unattainable with Thermo Scientific™ Orbitrap hybrid MS. The most difficult analyses, including multiplexed quantitation of low-abundance peptides in complex matrices, characterization of positional isoforms of intact proteins, protein structure characterization using chemical crosslinking, and the deepest mining of challenging posttranslational modifications may be performed on the highly sensitive and versatile Thermo Scientific Orbitrap Fusion family of mass spectrometers. The Tribrid system can perform multiple fragmentation techniques that prove useful in terms of experimental flexibility for applications such as quantitation using isobaric tags, low level PTM analysis, data independent acquisition (DIA), and top-down proteomics. The Tribrid MS provides the highest resolution, heightened sensitivity, rapid acquisition rates, improved ETD fragmentation and exclusive analytical techniques (e.g. multiNotch SPS MS³) for accurate relative quantitation experiments. These instruments are intended to push the limits of detection, characterization and quantitation and are able to achieve proteome-wide coverage, by combining the versatility of a Tribrid system with the selectivity of Orbitrap technology, and the sensitivity and speed rivaling that of a triple quadrupole instrument.

Introduction

The Thermo Scientific™ Orbitrap™ Fusion™ and Thermo Scientific[™] Orbitrap Fusion[™] Lumos[™] Tribrid[™] mass spectrometers are a new class of mass spectrometry instrumentation engineered with a revolutionary Tribrid architecture- combining the best of quadrupole, Orbitrap and linear ion trap mass analyzers. This Tribrid design enables scientists to meet their analytical challenges by offering unprecedented depth of analysis especially for highly complex, low abundance, or difficult-to-analyze biological samples. The Tribrid based mass spectrometers are equipped with well-designed hardware features and a user-friendly software interface allowing for easy method development and instrument operations. The innovations of the Orbitrap Fusion MS and Fusion Lumos MS systems make them the most sensitive, most selective and most versatile mass spectrometers available to date.



Both Orbitrap Fusion MS and Fusion Lumos MS have the following capabilities:

- Sensitivity comparable to a triple quadrupole mass spectrometer is achieved through improved ion transmission with a brighter ion source, advanced quadrupole technology and detection with the most sensitive detector.
- Selectivity of an Orbitrap analyzer allows the lowest detection limit to be achieved due to the highest resolution and highest mass accuracy available.
- Versatility of a Tribrid mass spectrometer provides multiple dissociation techniques (CID, HCD, ETD, EThCD) and full experimental flexibility due to its unique Tribrid architecture.

The Tribrid MS systems are equipped with the following:

- Ultra-high field Orbitrap mass analyzer Offers resolution exceeding 500,000 resolving power at m/z 200 and scan speeds up to 20 Hz at 15,000 FWHM.
- Ion Routing Multipole Facilitates parallel analysis and performance of HCD at any MSⁿ stage.
- Dual-Pressure Linear Ion Trap Performs MSⁿ and sensitive mass analysis of four fragmentation types (CID, HCD, ETD HD and EThcD HD).

Table 1. Fundamental features and benefits of Tribrid technology.

Features	Benefits
Ultra high resolution	High selectivity, ability to resolve analytes down to a few mDa
Sub ppm mass accuracy	High selectivity and confidence in molecular formula
Dynamic scan management	Intelligent scan scheduling allows for efficient operation at all times
Speed	Sequencing of low abundant components in complex mixture, fast scanning MS ⁿ
	compatible with UHPLC
Synchronous precursor selection	Ability to carry out highly sensitive and accurate protein quantitation using TMT reagents
Easy to use software	Novel drag-n-drop flexible user interface makes it easy to build complex methods
Experimental flexibility	Use of multiple analyzers and dissociation techniques (HCD, CID, ETD) for any molecule at any MS ⁿ stage

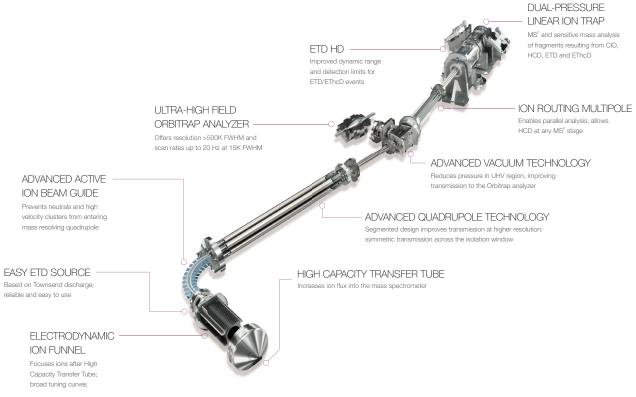


Figure 1. Hardware benefits.

Key improvements have been made to various hardware components on the Orbitrap Fusion Lumos MS to give enhanced analytical performance:

- Brighter ion source increases overall signal by 2–5 times and consists of a High Capacity Transfer Tube (HCTT) and an Electrodynamic Ion Funnel (EDIF) to achieve increased ion flux and lower limits of detection. It also comes optional with an Easy ETD Source (based on Townsend discharge) which provides greater reliability and ease of use due to its ability to produce extremely stable anion flux and retain reagent longevity.
- Advanced Active Ion Beam Guide has been a proven technology that prevents neutrals and high velocity clusters from entering the mass resolving quadrupole, therefore keeping the quadrupole cleaner and reduces background contamination.
- Advanced Quadrupole technology Segmented design improves transmission efficiency at higher resolution allowing for symmetric transmission across the isolation window.
- Advanced Vacuum Technology reduces pressure in UHV region, improving transmission of high molecular weight species to the Orbitrap analyzer.
- Novel ETD HD gives improved dynamic range and detection limit of ETD analyses, significantly increasing the fragment ion coverage.

Detailed product specifications for the Orbitrap Fusion Tribrid MS and Orbitrap Fusion Lumos Tribrid MS systems can be found on www.thermofisher.com or www.planetorbitrap.com.

Table 2. Specifications.

Feature	Orbitrap Fusion MS and Orbitrap Fusion Lumos MS
Scan rate Orbitrap MS ²	20 Hz
Scan ratelon Trap MS ²	20 Hz
Max resolution	>500,000 at <i>m/z</i> 200
Quad isolation	Down to 0.4 amu
Ion trap isolation	Down to 0.2 amu
Mass accuracy	3 ppm (external); 1 ppm (internal)
	Source CID, CID, HCD, ETD, EThcD
Dissociation	(Orbitrap Fusion MS)
Diocoolation	Source CID, CID, HCD, ETD HD, ETh-
	cD HD (Orbitrap Fusion Lumos MS)
MS ⁿ capability	Up to MS ¹⁰ in ion trap or Orbitrap
Analyzers	Quadrupole, Orbitrap, Ion Trap
Detectors	Ion Trap, Orbitrap

Detailed product specifications for the Thermo Scientific Orbitrap Fusion Tribrid Mass Spectrometer.

Detailed product specifications for the Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer.

For sole source specifications, kindly contact your local sales representative or contact us at Grant Central.

Top 5 reasons for upgrading from Hybrid MS to Tribrid MS technology

- Enabling Multiplexing with TMT technology
- Faster scanning MS detectors for increased throughput
- Improved sensitivity for extended coverage of low abundant proteins
- Multiple fragmentation techniques for the elucidation of PTMs
- User-friendly data acquisition software for non-MS experts

Reason 1: Multiplexing with tandem mass tag (TMT) technology

About TMT

TMT reagents (Proteome Sciences®, commercially available from Thermo Fisher Scientific) are isobaric chemical tags consisting of a signature reporter group, a spacer arm, and an amine reactive group. The reagents covalently bind to the *N*-terminus of a peptide or to lysine residues. Upon MS/MS fragmentation, each version of the tag fragments and produces a unique reporter ion. In an experiment that compares several experimental conditions in a single analysis, the protein digest from each experimental condition is labeled with one of the isobaric versions of the TMT reagent. Afterwards, all the samples are pooled together and analyzed with LC-MS/MS. During the first dimension separation (RPLC), the same peptide labeled with different versions of the tag will elute together as they have the same chemical properties. On subsequent MS¹ analysis, these peptides will be detected simultaneously as a single indistinguishable precursor ion peak. Following MS/MS fragmentation of the precursor, the relative levels of each version of the tag used to label the peptide can be quantified by comparing the intensities of the unique reporter ion generated from each tag. Additionally, protein identification is achieved by matching the peptide fragment ions in the MS/MS spectrum to sequences in the appropriate database.

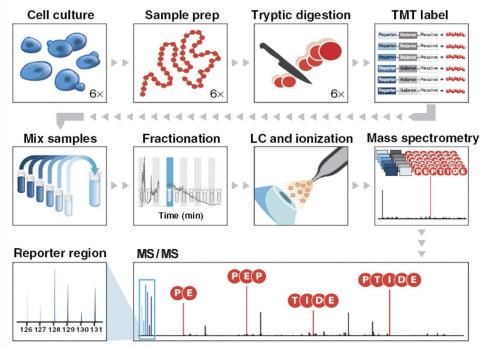


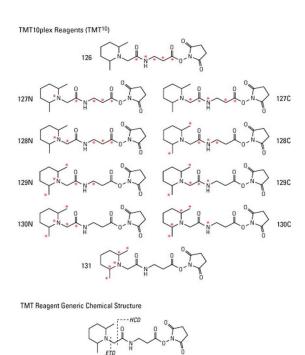
Figure 2. Overview of a tandem mass tag workflow.¹ The Orbitrap Fusion Tribrid mass spectrometers truly differentiates the TMT workflow from Orbitrap hybrid MS and quadrupole time-of-flight mass spectrometers by providing robust multiplexing capabilities and increasing throughput by over 10 folds.

Trying to understand the complexities in biology requires understanding the functional aspects of biology, and that necessitates more sophisticate levels of mass spectrometric performance. The Orbitrap Fusion Lumos MS and Orbitrap Fusion MS are suitable instruments as they combine the analytical capabilities equivalent to three instruments, all within the usability of a single instrument platform.

As proteomics becomes more quantitative, the ability to perform relative quantitation for many samples accurately is absolutely critical. Typical experimental designs require running a separate LC-MS/MS analysis for each individual experiment, which results in the depletion of precious samples, the demand for long instrument analysis times, and introduction of run-to-run variations. Adopting the TMT isobaric tagging approach permits multiple time points, cell lines, or experimental conditions to be analyzed simultaneously (Figure 2). In addition to conserving samples while taking only a fraction of the time to run, a TMT workflow ensures reproducibility by analyzing all experiments under the exact same conditions. A straightforward workflow also allows the inclusion of "technical replicates" by using duplicate labels for the same condition for enhanced confidence and statistical analysis, all accomplished within a single LC-MS/MS analysis.

The Tribrid systems are capable of carrying out accurate quantification of proteins on a massive scale through high-throughput multiplexing. The combination of isobaric labeling technology, tandem mass technology and the novel scan functionality in the Orbitrap Tribrid instruments enable over one hundred thousand protein quantifications in a single day. This performance is heavily driven by the new capabilities in the unique hardware architecture on the Orbitrap Tribrid based MS systems. Parallelization of complex modes of analysis is accomplished by the concurrent isolation of ions with one analyzer and detection with the two other analyzers.

Quantification with higher plexing isobaric tags requires the highest resolution for all reporter ion channels and unexpected interferences in the reporter ion region of the mass spectrum. The multiplexing capabilities of the TMT tags have been extended by simply using a more complex isotopologue design (dependant on the 6.32 mDa mass difference between 13C and 15N) in which the heavy atoms are strategically placed in different positions of the mass reporter to provide 10 different distinct masses to report out each channel (Figure 3). High resolution (at least 50K at m/z 200) is absolutely crucial to obtain complete resolution of all 10 mass reporter channels which differ by a very small mass difference.



Mass

Reporter

Normalizer

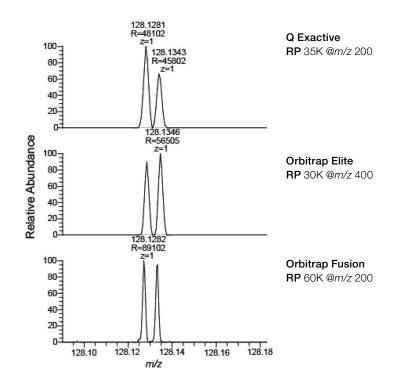


Figure 3. The TMT10-127 (13C and 15N labeled) and TMT10-129 (13C and 15N labeled) reagents differ only by a mass difference of ~6.32 mDa. High resolution Tribrid mass spectrometers have the unique ability to resolve these neighboring isobaric istopologues distinctly.²

All Orbitrap instruments are capable of resolving the closely-spaced reporter ions (Figure 3). It is evident, however, that the Tribrid-based MS systems yield the highest resolution in the shortest time due to faster scanning speeds and the ultra-high field Orbitrap analyzer. Although quadrupole time-of-flight mass spectrometers are known for their fast scan rates, they can be limited in achieving high resolution to resolve the tightly-spaced TMT reporter ions with good detection sensitivity. Additionally, improvements of the ion source and ion optics on the Orbitrap Fusion Lumos MS increase the number of quantifiable peptides, which is especially beneficial at lower sample concentrations (Figure 4).

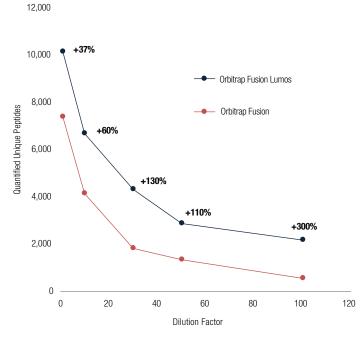


Figure 4: Standard HeLa digest labeled with TMT0 analyzed with an 85 min gradient using SPS MS³ on both Fusion and Fusion Lumos tribrids. The increase in number of quantifiable unique peptides across varying dilution factors becomes apparent at lower concentrations.

Achieving greater accuracy in quantitation using Synchronous Precursor Selection (SPS MS³) capability

The Orbitrap Fusion MS and Orbitrap Fusion Lumos MS offer the ability to perform "Synchronous precursor Selection (SPS)" for added experimental throughput while providing the depth and coverage needed for a TMT experiment. A common problem affecting the accuracy and precision in TMT quantitation experiments is the co-isolation and co-fragmentation of interfering ions. This causes reporter ion ratio distortions and inaccurate reporting of true fold changes or reporter ion intensities. While the MS³ method was found to be effective in mitigating the interference issue and restoring accuracy and precision of TMT quantitation³, an overall drop in sensitivity was observed.

Therefore, the SPS MS³ method is the ideal solution to address the above-mentioned issues observed from a TMT experiment. This method uses isolation waveforms with multiple frequency notches to co-isolate and co-fragment multiple MS² fragment ions (up to 20) (Figure 5), effecting increased number of reporter ions in the MS³ spectrum over standard MS³ method.⁴ The dynamic range of reporter ion quantitation is better, signal variance decreases and higher quality quantitative measurements are obtained with the SPS MS³ method. This dramatically

increases the signal intensity, improves the ratio accuracy (due to counting statistics) and boosts overall quantitative sensitivity by leveraging on such intense multiplexing capabilities obtainable only with the Tribrid technology mass spectrometers. Sensitivity and interference ion issues are eliminated to bring back accuracy and precision to the TMT experiment. To demonstrate this capability, we performed an SPS MS3 experiment on the Orbitrap Fusion Lumos. Human HeLa cells were spiked with yeast samples labeled with four TMT channels in equal amounts, to simulate interference effects in TMT labeled samples (Figure 6). The true benefits of SPS MS3 in recovering accurate ratios in TMT experiments are evident from this example. Results from the analysis show that the MS2 acquisition (in blue), even at 0.7 amu isolation width, produced significant reporter ion ratio distortions compared to theoretical ratio values. The implementation of the SPS MS3 method greatly reduced the ratio distortion effect and gave a minimal difference from the expected values. The SPS Multi-notch MS3 functionality can be implemented only on Tribrid systems and is advantageous over the previous generation top-tier hybrid instruments which can only perform single notch MS³ methods without the multiple precursor isolation (SPS). This workflow, unique to the Orbitrap Tribrid systems, cannot be achieved with TOF-based analyzers.

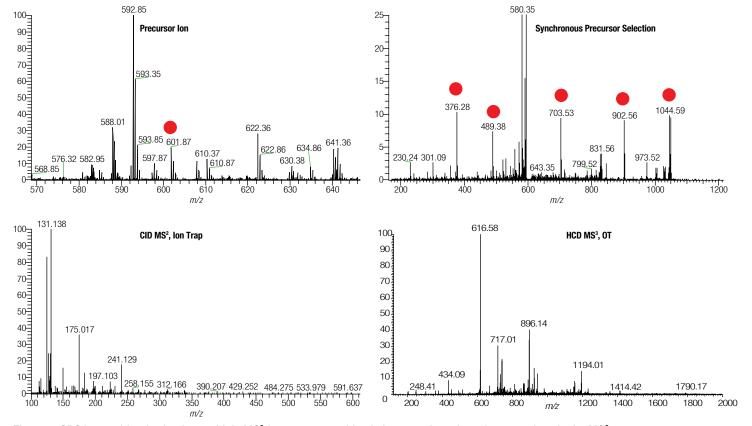


Figure 5. SPS is capable of selecting multiple MS² fragments, resulting in increased number of reporter ions in the MS³ spectrum.

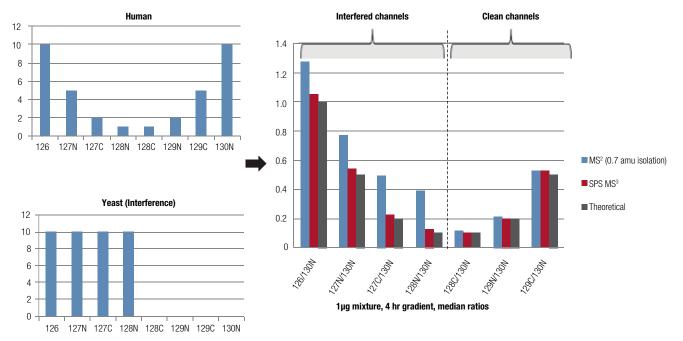


Figure 6. Benefits of the SPS MS³ method on the Orbitrap Fusion Lumos MS are demonstrated to provide accuracy and precision to the TMT quantification experiment and to the resultant observation of true reporter ion intensities. Since the yeast samples only had 4 channels labeled, there is no data for the remaining 4 columns.

Reason 2: Faster scanning speeds give more data points for more accurate relative quantitation

The improvements in speed for Tribrid instrumentation give more data points across the liquid chromatographic (LC) peak. From figures 7 and 8, the Orbitrap Fusion Tribrid MS produces more data scans compared to the Thermo Scientific™ Orbitrap Elite™ Hybrid mass spectrometer. This provides better MS-based quantitation and potentially increases the number of sequencing attempts and identifications. The ultra-high field Orbitrap analyzer is

capable of fast acquisition rates of up to 20Hz, thereby fueling the possibilities for various quantitative experiments and greater instrument throughput. The number of protein groups in 1ug of HeLa cell lysate was also determined to be more in the Tribrid MS system. In half the analysis time, the Tribrid-based MS identified more protein groups over the Orbitrap Elite hybrid MS (Figure 9). This brings greater success when it comes to carrying out identification and characterization studies, providing depth in analysis and understanding of the biological significance of the results.

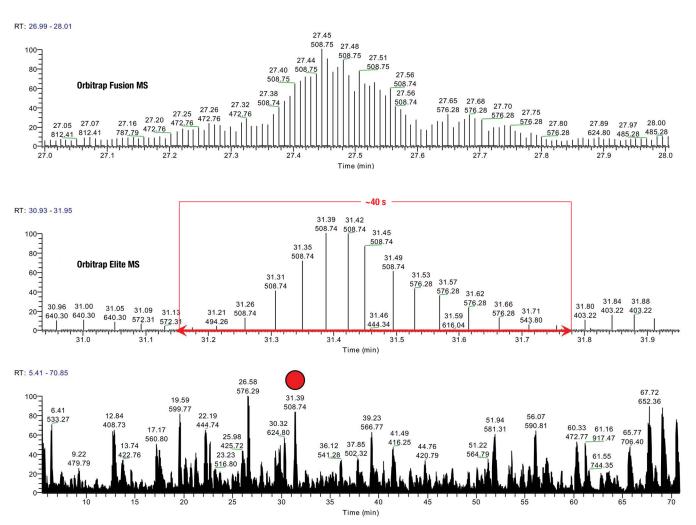


Figure 7. Many more data points are observable across the chromatographic peak for Orbitrap Tribrid MS systems compared to the Orbitrap Hybrid MS systems (1 µg HeLa, 140 min run).

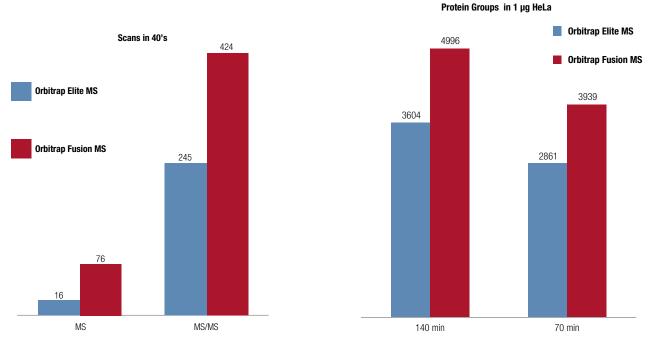


Figure 8. Number of MS and MS/MS scans across the Hybrid and Tribrid platform.

Figure 9. Number of protein groups identified from the Hybrid and Tribrid MS systems at 70 and 140 min gradients.

Reason 3: Most sensitive and fastest performing Orbitrap mass spectrometer guarantees increased productivity and reliable experimental results.

Detection of low abundance proteins is the key to understanding biological systems. Most of the peptides and proteins at low concentration have important biological functions such as protein biomarkers for disease markers, proteins involved in cellular signaling and cancer processes. Therefore it is vital to attain a lower detection limit to identify these significant proteins. Scientific research needs and technological demands have driven the development of more sensitive and faster scanning mass spectrometers to push the limits of performance. The Orbitrap Tribrid mass spectrometers can achieve better low-level detection, particularly for low abundance proteins compared to the Orbitrap Elite and Q Exactive hybrid mass spectrometers (Figure 10, 12). This difference in detection limits is attributed to the revolutionary hardware enhancements that are found only in the Tribrid hardware architecture: increased sensitivity due to brighter ion source design; improved ion optics and segmented quadrupole for better ions transmission; fast acquisition rates and high resolution of the ultra-high field Orbitrap analyzer which gives more detection. The fold change in the number of peptides identified of the Orbitrap Fusion MS versus other Orbitrap hybrid platforms was tremendous and is of great scientific significance when it comes to the identification of important protein biomarkers and transcription factors (Figure 11).

Unique peptides as a function of the protein cellular abundance 5000 Orbitrap Elite MS 4445 Q Exactive MS ■ Orbitrap Fusion MS 3576 3750 3119 Peptides Detected 2500 2208 1226 1250 500 181 47 29 0 15 20 2.5 3.0 3.5 40 4.5 5.0 5.5 6.0 Protein Molecules Per Cell, Log 10

Figure 10. Unique peptides identified across three different Orbitrapbased MS systems.

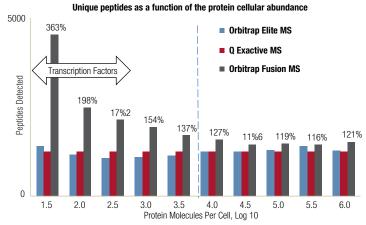


Figure 11. Fold improvement determined for all three Orbitrap-based MS systems.

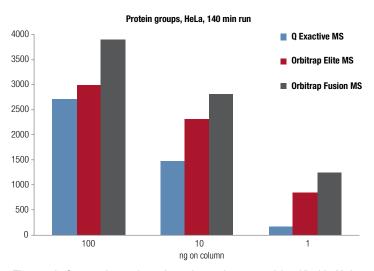


Figure 12. Comparison of number of protein groups identified in HeLa digest across different MS platforms.

A complete yeast proteome analysis is accomplished in half the analysis time for the Orbitrap Fusion Tribrid MS compared to the Q Exactive hybrid mass spectrometer (Figure 13). A comparable number of unique peptides and protein groups identified on the Orbitrap Fusion MS in a 2 hour gradient means faster analysis time, improved experimental efficiency and greater throughput. To further demonstrate the benefits of a Tribrid instrument on whole proteome analysis, high pH fractions of the K562 cell line were analyzed on the Orbitrap Fusion MS and Orbitrap Fusion Lumos MS (Figure 14). Not only did the Orbitrap Fusion Lumos MS identify 20% more protein groups per run, it showed a 2× improvement in experimental throughput. Fewer fractions (2× less) were needed on the Orbitrap Fusion Lumos MS to get the same level of coverage as the Orbitrap Fusion instrument, potentially reducing precious analysis time and providing higher throughput.

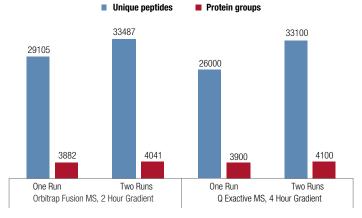


Figure 13. Performance of Orbitrap Fusion Tribrid MS compared to the Q Exactive MS in half the analysis time.

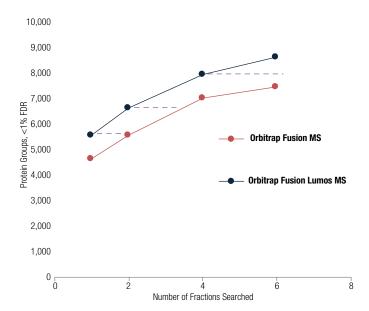


Figure 14. Difference in protein groups identified for Orbitrap Fusion Lumos vs. Orbitrap Fusion.

The Tribrid MS systems come equipped with an ultrahigh-field Orbitrap analyzer while the Hybrid platforms use the standard high-field Orbitrap analyzer. This upgraded Orbitrap detector on Fusion-based instruments has faster scanning speeds (20 Hz vs. 4 Hz) and higher resolving power (500,000 vs. 240,000 at *m/z* 200). With these enhanced features based on the innovative Tribrid architecture, more MS² scans are obtained on the Tribrid than Hybrids due to faster scan performance and the ability to basically detect more spectral features, therefore giving rise to better identification (Figure 15).

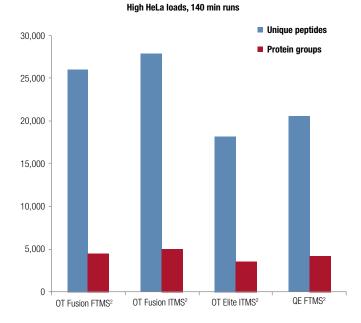


Figure 15. Enhanced hardware improvements gives better analytical performance on the Tribrids vs. Hybrids.

Reason 4: The Orbitrap Tribrid mass spectrometers have multiple fragmentation techniques available (CID, HCD, ETD, EThCD) that provides versatility to explore more experimental possibilities.

As the Orbitrap Fusion Tribrid MS and Orbitrap Fusion Lumos Tribrid MS are equipped with three mass analyzers, these platforms are capable of performing complex parallelization experiments by concurrent isolation of ions in one analyzer and detection in the remaining two analyzers.5 Another dimension of experimental flexibility is added through multiple fragmentation techniques that are available exclusively on the Tribrid mass spectrometers. These include collision induced dissociation (CID), higher-energy collisional dissociation (HCD) and optional electron transfer dissociation (ETD, ETD HD, EThcD and EThcD HD). Each of these fragmentation techniques can be performed at any stage of MSⁿ, with detection of the fragment ions in either the dual-pressure linear ion trap or ultra-high-field Orbitrap mass analyzer. These multiple fragmentation capabilities on the Orbitrap Tribrid mass spectrometers unlock new experimental approaches to determine and quantify PTMs, including phosphorylation, acetylation and glycosylation. This capability is uniquely exploited in the Tribrid architecture through product ion dependent scanning functions.

ETD fragmentation is one such example that is especially useful for glycan structure characterization in glycan analysis or for glycoproteomics.6 The ETD ion source used in Orbitrap Fusion MS is based on Townsend discharge ion source which generates a highly stable reagent ion flux with minimal user input for optimization and tuning as was required on previous ETD sources equipped on the hybrid platforms. Additionally, the Orbitrap Tribrid instruments have been implemented with intelligent, automated precursor ion sorting routines, reagent filtering using the quadrupole mass filter, and charge-statespecific calibration of ETD reaction times that maximize the quality of ETD spectra and increase the number of glycopeptides identified compared to previous generation mass spectrometers. A comparison of Orbitrap Elite MS to Orbitrap Fusion MS for the identification of human serum glycopeptides is shown here (Figure 16). The Orbitrap Elite MS selects precursors based on intensity while Orbitrap Fusion MS can acquire data with intelligent precursor selection giving priority to highest charge precursors which are optimal for ETD fragmentation.

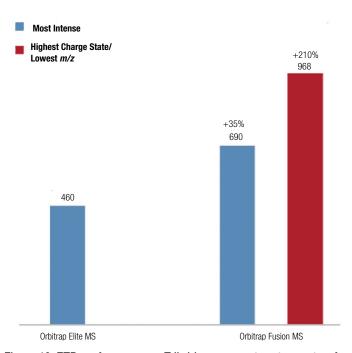


Figure 16. ETD performance on Tribrid mass spectromters outperform Hybrid mass spectrometers due to utilization of intelligent precursor selection feature implemented on Orbitrap Fusion MS and Orbitrap Fusion Lumos MS.

On the Orbitrap Fusion Tribrid MS, an intelligent acquisition strategy termed HCD product-dependent ETD workflow (HCD-pd-ETD) that enables on-the-fly identification of glycopeptides was implemented which improves overall productivity of glycopeptide analyses. In this approach, the Orbitrap Fusion mass spectrometer acquires HRAM HCD spectra in a data-dependent fashion. The instrument identifies glycan oxonium ions on the fly in the HCD spectra and triggers ETD spectra on the glycopeptide precursors only (Figure 17). This results in streamlined data analysis and improvements in dynamic range and duty cycle. The HCD-pd-ETD method is provided within the instrument control software for Orbitrap Tribrid based mass spectrometer. In addition to HCD-pd-ETD, the Tribrid instruments can trigger any fragmentation based on oxonium ion presence including CID and HCD (HCD-pd-CID, HCD-pd-HCD). Triggering CID fragmentation based on the detection of oxonium ions is useful for elucidating glycan composition information as CID tends to produce more detailed glycan backbone fragmentation (Figure 17). This approach is useful as glycans are heterogeneous PTMs; multiple glycans can be present at a single amino acid site and requires complete characterization of all detected compositions.

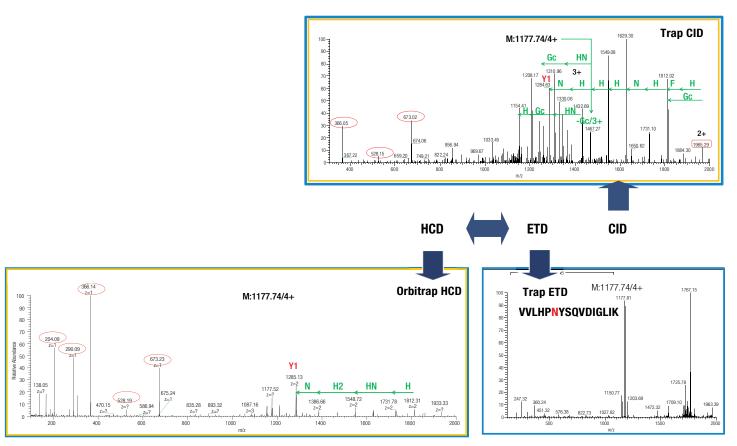


Figure 17. Representation of HCD-pd-ETD and HCD-pd-CID acquisition methods. The HCD spectrum shows diagnostic glycan oxonium ions in the low m/z region which are used to trigger ETD and/or CID spectrum. The ETD spectrum gives important information about the peptide and glycosylation site. The CID spectrum provides glycan composition information.

Reason 5: Availability of universal methods – Intelligence built into a new software interface gives non-MS experts access to highly complex technology.

The technological developments in mass spectrometers have grown more powerful and sophisticated, but at the same time more difficult to operate. The Orbitrap Fusion Tribrid mass spectrometer delivers higher-quality information from more sample types, at a rate faster than any mass spectrometer available today. The intelligence built into the Orbitrap Fusion Tribrid instruments and software makes it possible to achieve exemplary results with far less effort than required by previous generations of mass spectrometers. This built-in intelligence provides researchers with greater experimental flexibility, allowing them to focus more on their science instead of intensive method development and instrument operation.

Built-in intelligence features include:

 Dynamic Scan Management schedules scan events to maximize MS efficiency, as well as intelligently prioritizing precursors for data dependent analysis with their optimum fragmentation mode and mass analyzer.

- A library of method templates with application specific defaults is available for common experiments allowing you to run guided methods with less effort. For unique experiments, customized method development is available for maximum flexibility.
- Automated Synchronous Precursor Selection (SPS) for MS³ significantly increases the number of peptides and proteins identified and quantified by TMT isobaric mass tagging workflows.
- Top-speed (Top S) mode efficiently schedules MS and data-dependent MSⁿ scans based on user-definable parameters and maximizes the number of high-quality MSⁿ spectra acquired.
- Simultaneous identification, quantitation, and confirmation are achieved by a combination of high-resolution, accurate-mass, low-detection-limit SIM quantification with the Orbitrap mass analyzer and sensitive full-scan MS/MS confirmation with the ion trap.

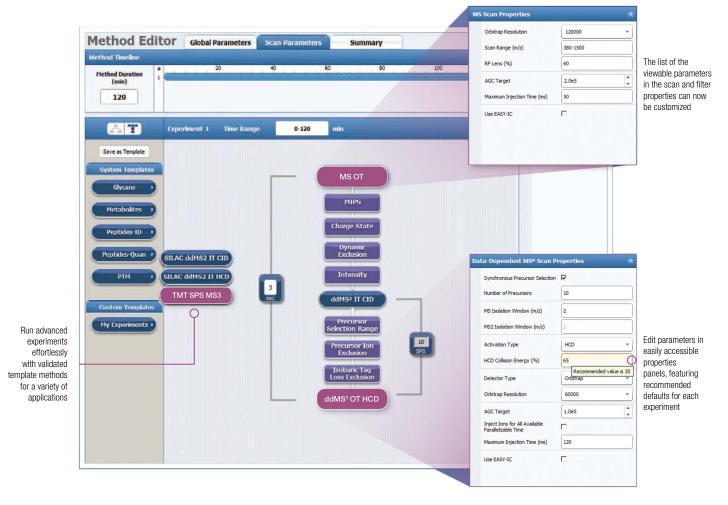


Figure 18. Instrument method set-up interface on Orbitrap Fusion based MS instruments, displaying a simple and easy-to-use guided method editor.

Why choose Orbitrap Mass Spectrometry?

Research trends and analytical needs have driven mass spectrometry innovation especially in the past decade. Mass spectrometers of today must be equipped with superior performance features such as high resolution, mass accuracy, dynamic range and fast scanning capabilities in order to fulfil rigorous experimental demands and handle extremely complex samples. In today's research, these same instruments have to provide the flexibility to carry out a variety of analytical techniques including multiplexing, multi-stage fragmentation and multiple dissociation techniques, in addition to being highly robust and giving consistent performance for high throughput analysis. Since its introduction in 2005, the Orbitrap technology has revolutionized mass spectrometry based research to meet these various challenges across multiple application fields of interest. The exceptional value of Orbitrap-based MS systems in delivering uncompromised analytical performance and

achieving greater experimental possibilities have been well recognized by the scientific community. Adoption of Orbitrap technology over the years has grown exponentially with the proven increase in numbers of Nature and Science family publications (Figure 19).

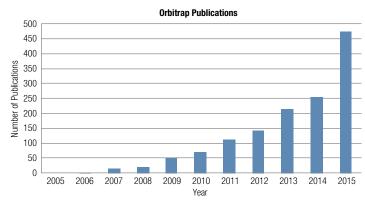


Figure 19. Rising trend in number of Orbitrap MS based research publications in *Nature* and *Science* journals since introduction in 2005.

Which Orbitrap system is right for my research?

Table 3. Orbitrap selection guide.

Instrument Attributes	Q Exactive Focus MS	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Orbitrap Elite MS	Orbitrap Fusion MS	Orbitrap Fusion Lumos MS
Analyzer	Orbitrap	Orbitrap	Orbitrap	Ultra High Field Orbitrap	Hybrid: Linear ion trap, Orbitrap	Tribrid: Quadrupole with dual pressure linear ion trap, Orbitrap D20	Tribrid: Quadrupole with dual pressure linear ion trap, Orbitrap D20
Mass Range	<i>m/z</i> 50–2000	<i>m/z</i> 50–6000	<i>m/z</i> 50–6000	<i>m/z</i> 50–6000	m/z 50–2000; m/z 200–4000	<i>m/z</i> 50–6000	<i>m/z</i> 50–6000
Maximum Resolution @ m/z 200	70,000	140,000	140,000	240,000	240,000	500,000	500,000
Scan Speed	12 Hz	12 Hz	12 Hz	18 Hz	4 Hz	20 Hz	15 Hz
Top N/MS ⁿ	Top 2 ddMS ²	Top 2 ddMS²	Top 2 ddMS ²	Top 2 ddMS ²	$MS^{n}, n = 1$ to 10	$MS^{n}, n = 1$ to 10	MS^{n} , $n = 1$ to 10
Mass Accuracy - Internal Calibration	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm
Polarity switching	<1 sec	<1 sec	<1 sec	<1 sec	No	<1 sec	<1 sec
Multiplex	No	Yes, up to 10 precursors	Yes, up to 10 precursors	Yes, up to 10 precursors	No	Yes, up to 10 precursors	Yes, up to 10 precursors
Intact Protein Mode	No	No	Yes	Yes	Yes	Yes	Yes
Enhanced Resolution	No	No	280,000 (Option)	N/A	N/A	N/A	N/A
Collision Energy	CE only	Normalized CE	Normalized CE	Normalized CE			
Dissociation	HCD	HCD	HCD	HCD	CID, ECD	CID, HCD, ETD, EThCD	CID, HCD, ETD HD, EThCD HD
ETD Option	No	No	No	No	Yes, efficiency > 15%	Yes, efficiency > 15%	Yes, efficiency > 15%

Table 4. Which Orbitrap system best suits my experimental requirements?

Performance Features	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Orbitrap Elite MS	Orbitrap Fusion MS	Orbitrap Fusion Lumos MS
Resolution	///	V V	////	////	////	////
Sensitivity	///	////	////	///	////	\ \ \ \ \ \
Speed	////	////	////	///	\ \ \ \ \ \	\ \ \ \ \ \
Dynamic Range	///	////	////	///	////	\ \ \ \ \ \
Mass Accuracy	////	////	////	////	////	////
Multiplexing	////	////	////	////	////	/ / / / /
Fragmentation	√ √	√√	✓✓	///	\ \ \ \ \ \	\ \ \ \ \ \
MS ⁿ Capability	-	-	_	/ / /	////	\ \ \ \ \ \ \
ETD	-	-	-	-	\ \ \ \ \ \	\ \ \ \ \ \ \
MultiNotch	-	-	_	_	////	////

Table 5. Which Orbitrap system best suits my area of research?

Application	Q Exactive MS	Q Exactive Plus MS	Q Exactive HF MS	Orbitrap Elite MS	Orbitrap Fusion MS	Orbitrap Fusion Lumos MS
Peptide IDs	///	///	////	///	////	////
TMT Quantitation	$\checkmark\checkmark\checkmark$	///	///	///	////	////
SILAC	///	///	////	///	$\checkmark\checkmark\checkmark\checkmark$	\ \ \ \ \ \ \
Label Free Quantitation	$\checkmark\checkmark\checkmark$	///	////	///	////	\ \ \ \ \ \ \
Top Down	///	///	////	///	////	\ \ \ \ \ \ \
Intact Analysis	$\checkmark\checkmark\checkmark$	///	////	///	////	\ \ \ \ \ \ \
PTM Phosphorylation	///	///	///	///	////	\ \ \ \ \ \ \
PTM Glycosylation	✓✓	√√	√√	///	////	////

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thermoscientific

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