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New Tribrid MS ion optics and electronics improve sensitivity, duty cycle, and extend the MS range

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ABSTRACT

Mass spectrometer sensitivity and versatility have been steadily improving with each successive instrument generation. Older generations of Tribrid mass spectrometers could routinely collect FTMSn spectra at 40 Hz and measure *m/z* values out to 8,000. However, the sophistication and demands of the experimental workflows have increased in parallel with these instrument advances (e.g., native analyses that require >8,000 *m/z*, 18-plex TMT experiments with very short gradients). As such, MS instrument sensitivity and functionality must continually improve. Herein, we describe the hardware, electronics, and software changes included in the new Thermo Scientific™ Orbitrap Ascend™ Tribrid MS, which are meant to address some of the limitations of the previous generation of Tribrid MS, including improvements to FTMSn duty cycles and scan rates and extending the mass range to 16,000 *m/z*.

INTRODUCTION

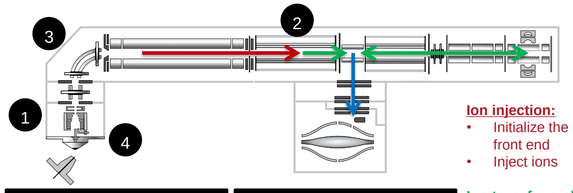
Our goal from the outset of this project was to increase the sensitivity of the Tribrid MS for FTMSn applications while also updating the instrument to extend the Orbitrap mass range to 16,000 *m/z*. With regards to the former goal, a major change in the Ascend MS is the addition of another ion routing multipole in front of the CTrap. This additional ion routing multipole allows parallel ion injection and accumulation during FTMSn ion manipulation and Orbitrap injection. We also updated ion optics and electronics to enable transmission and analysis of ions out to 16,000 *m/z*. These modifications included altering some of the critical dimensions in the ion inlet region to improve transmission and cooling of very large native ions and new RF electronics that extended the ion guide mass ranges to 16,000 *m/z*.

MATERIALS AND METHODS

MS timing experiments were performed using the Thermo Scientific™ FlexMix™ calibration solution. The peptide standard ALELFR was purchased and diluted in 50/50 Methanol/Water with 0.1% Formic Acid to a concentration of approximately 10 pmol/µl. The samples were infused into the MS with a mass range or 150-2000 Th, source RF set to 60% and the capillary temperature of 300ºC.

1 µg of Thermo Scientific™ Pierce™ HeLa protein digest standard was analyzed using a 50cm Thermo Scientific™ EASY-Spray™ column with 4 amu isolation window for FTMS2 scans. Data was processed in Thermo Scientific™ Proteome Discoverer™ software 3.0 include CHIMERYS™ by MSAID™ @1% false discovery rate (FDR).

A GroEL sample was diluted to 1.25 µM in 100 mM ammonium acetate and infused via static nanospray. Tune parameters were optimized to in-source CID of 225 V, source compensation of 0.15, and an MS resolution of 7,500.



Ion injection:
- Initialize the front end
- Inject ions

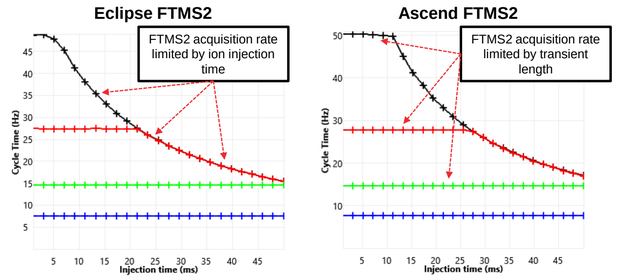
Ion transfer and manipulation:
- Manipulate the precursor ions (HCD, CID, ETD, etc).
- MSn >= 2.

Analysis:
- OT analysis

WHAT IS NEW?	HOW?
1. Reduced in-source fragmentation	1. Softer ion funnel
2. Optimized ion transfer to improve sensitivity and spectrum acquisition rate	2. Front ion routing multipole before the CTrap for ion trapping and MS² HCD *
3. Improved protein characterization including native mode.	3. New optics for better high *m/z* transmission
4. Higher uptime, ease-of-use	4. Automated calibration source
	* Back IRM: OT MSⁿ CID, ETD, UVPD, MS³⁺ HCD

Alterations in the Orbitrap Tribrid Architecture Improve Ion Management

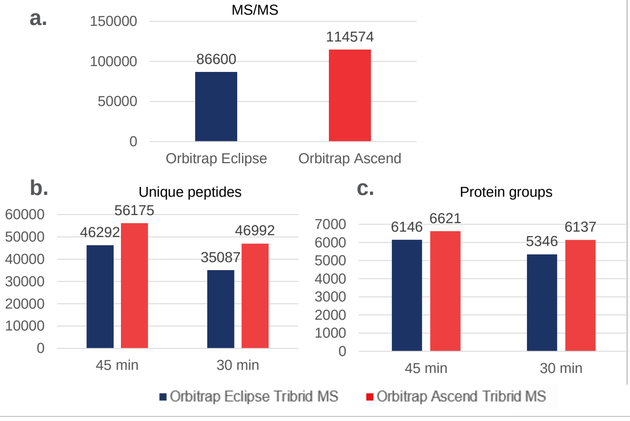
Figure 1. Depending upon the FTMS2 and FTMSn conditions, this new IRM improves the instrument duty cycle, the instrument scan rate, or both. For standard FTMS2 with HCD the maximum scan rate at a resolving power of 7.5k and an injection time of 10 ms increased to 50 Hz. For FTMS3 the spectral acquisition rate increases 20% (50k RP with max IT 65 ms).



The new Ascend Tribrid MS layout allows three discrete packets of ions to move throughout the instrument: injection, manipulation, and analysis. This is true for all scan types and both analyzers (e.g., ITMS2 and FTMS3). The spectral acquisition rate and duty cycle improvements from this change increase as the scan complexity grows (e.g., ETD with FTMS2).

Faster Scan Speed Leads to More Identifications

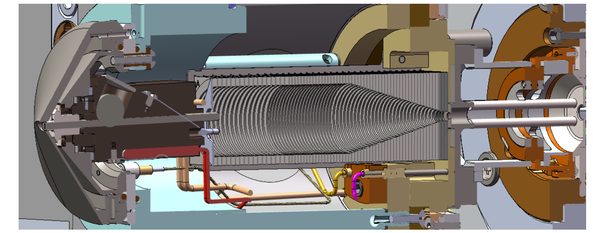
Figure 2. Comparative data from a HeLa protein digest standard run on the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ MS (Blue) and the Orbitrap Ascend Tribrid MS (Red). The amount of FTMS2 spectra acquired within a 45 min run **(a.)** gives nearly a 33% increase in possible identifications. We see a proportional increase in the number of unique peptides **(b.)**, with a 30 min run giving a 34% increase in identifications. In terms of protein groups **(c.)**, we can see that the 30 min run on the Ascend results in a 15% increase. The 30min Orbitrap Ascend Tribrid MS data, and the 45 min Orbitrap Eclipse Tribrid MS data have nearly identical number of IDs, thus enabling increased sample throughput on Orbitrap Ascend Tribrid MS with sacrificing coverage.



Alterations to the API inlet for Higher *m/z* Transmission and Gentler Ion Introduction

Recently, many Tribrids are used to analyze very large ions (e.g., native complexes) and very fragile ions (e.g., lipids and phosphopeptides). To better serve these workflows we made a few changes to the API inlet region of the new Tribrid MS.

Figure 3. The apertures for the last few lenses in the new Tribrid ion funnel were expanded from 2 to 3 mm. The ion funnel exit lens aperture was also increased from 2 to 2.5 mm. These changes reduce the axial forces felt by the ions during injection. The ion guide downstream of the ion funnel was also lengthened from ~20 mm to 30 mm. The longer length is compounded by an increase in pressure due to the larger ion funnel exit lens. These later changes help improve very high m/z transmission (>8k *m/z*) through additional ion cooling.



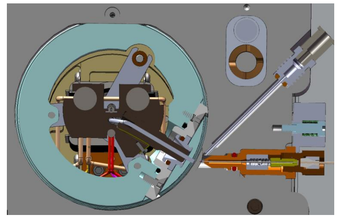
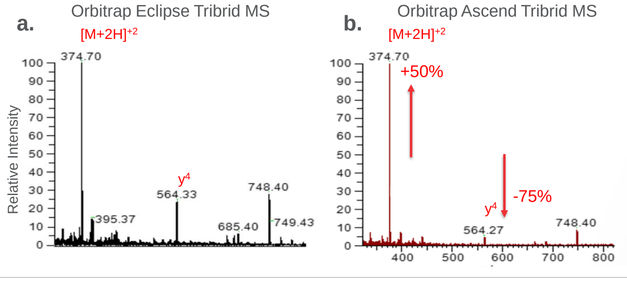


Figure 4. We also incorporated a dedicated calibration source into the new Tribrid MS. This dedicated calibration source is already standard on the Tribrid IQ-X. This source allows regularly scheduled calibrations to occur automatically with minimal user intervention.

Figure 5. MS1 spectra of the fragile peptide ALELFR where 374.70 Th is the +2 charge state of the precursor, 748.40 Th is the +1 charge state of the precursor, and 564.33 Th is the most abundant fragment (y^4) of the peptide. MS1 spectrum from the Orbitrap Eclipse Tribrid MS **(a.)** compared to MS1 spectrum from the Orbitrap Ascend Tribrid MS with the new API inlet **(b.)** shows a gentler transmission. We see a 50% increase in the intact precursor and 75% decrease in the fragment on the Orbitrap Ascend Tribrid.



Extended *m/z* Range Allows Analysis of Large Complexes

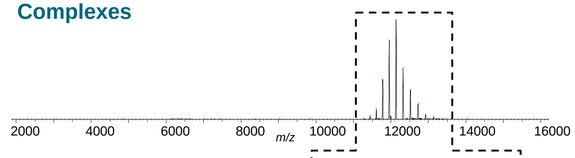
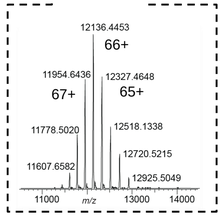


Figure 6. The Orbitrap Ascend Tribrid MS has an extended mass range double that of the Orbitrap Eclipse. In addition to the source changes already discussed, the Ascend also has frequency switching ion guide boards that allow for more favorable potentials when transmitting these very large ions. This allows for the analysis of larger complexes, including GroEL, shown below. GroEL has a theoretical native intact mass as a 14mer of approximately 801 kDa.



CONCLUSIONS

- **The new Ascend layout allows for 33% faster LC-MS methods for same number of IDs as old architecture and a 34% increase in the number of IDs for the same method length.**
- **The softer ion source shows a 50% increase in the intact precursor for ALEFFR peptide and 75% decrease in the y^4 fragment ion compared to usual ion funnel. While maintain the high throughput of the Eclipse transfer tube.**
- **Extended *m/z* range of the Ascend Orbitrap Tribrid MS allows the instrument to scan up to 16,000 *m/z*, seeing the 14mer complex GroEL in native state with clear, distinct charge state distribution and signal to noise.**

POSTER/PRESENTATIONS on ASCEND at ASMS

ThOB 8:50 Benefits of dual ion routing multipoles for glycoproteomics
MP 714 Improved characterization of the intact mammalian proteome >30 kDa using targeted proton transfer charge reduction (tPTCR)
TP 111 A Comparison of Tribrid Mass Spectrometer Architectures for Deep Blood Plasma Proteomics
WP 317 Evaluation of a novel Tribrid Orbitrap Ascend system for in-depth high-throughput proteomics and phosphoproteomics
ThP 251 High-Depth Multiplexed Drug Profiling with the Orbitrap Ascend
ThP 683 Increasing the depth of single shot proteomics with enhanced data acquisition and processing strategies using a new Orbitrap Tribrid MS
ThP 696 Extending coverage in multiplexed single-cell proteomics
Plus many more: **TP 676, WP 635, MP 416, Thp 251**

TRADEMARKS/LICENSING

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