Modifying the Ion Optics and Scan Sequences on a Tribrid MS to Improve Sensitivity, Duty Cycle, and Overall Instrument Ease-of-Use

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ABSTRACT

Purpose: We modified the ion optics on a Tribrid mass spectrometer to improve its quadrupole selectivity, its high-mass range transmission, and its overall FTMS^n duty cycle.

Methods: We assessed these ion optics and scan cycle changes using a standard calibration solution, an intact protein standard, and an LC-MS analysis of a complex cellular lysate.

Results: All together these ion optics modifications improve the sensitivity and selectivity of the mass spectrometer while increasing its effective analytical mass range.

INTRODUCTION

Mass spectrometer sensitivity has steadily increased with each successive generation of instrumentation. Various hardware and software advances have contributed to these improvements (e.g., high-capacity transfer tubes and parallel ion processing). However the sophistication and demands of the applied experimental workflows have increased in lock step with the instrumentation advances (e.g., single cell proteomics with very low sample loads). As such, MS instrument sensitivity is an area that requires constant advancement. Furthermore, multiuse mass spectrometers like the Tribrid instruments are now being used in wholly original workflows that involve interrogating previously unexamined classes of compounds (e.g., biopharma analysis of very large biomolecules). These new ion classes require significant changes to the ion optics to enable sensitive and robust analysis.

MATERIALS AND METHODS

In this study we modified a Thermo Scientific™ Tribrid™ mass spectrometer, and we characterized its resulting performance. This study was specifically focused on changes we made to the ion optics of this instrument and to the scan sequences run on this modified hardware.

With regards to the previously mentioned ion optics hardware changes, these included an advanced quadrupole mass filter and a re-designed bent ion guide after the source. In the context of these hardware modifications, we carefully studied and optimized all the times we allot to all the scan events.

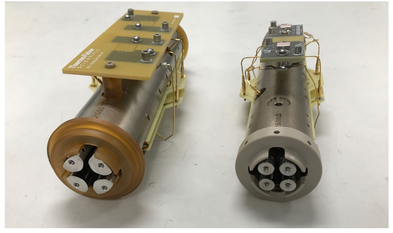
We assessed the overall performance and utility of these hardware and scan sequence changes using a variety of samples and methods that ranged from direct infusion of calibration solutions (Thermo Scientific™ Pierce™ FlexMix™) and intact protein standards (Herceptin from Genetech) to LC-MS analysis large intact proteins (K562 cells grown and prepared by collaborators).

Figure 1. All of the ion optics modifications were performed on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer. These modifications have been bundled with a suite of other changes under the new commercially available Tribrid mass spectrometer, the Orbitrap Eclipse.

Improved quadrupole transmission efficiency

Our goal from the outset of this project was to increase the sensitivity and selectivity of the Tribrid MS. To that end, we tested an advanced segmented quadrupole mass filter with a larger r^0 dimension. Increasing r^0 enlarges the area of acceptance for the quadrupole mass filter, which in turn improves the transmission efficiency at narrower isolation windows and higher *m/z*. The Orbitrap Fusion and Orbitrap Fusion Lumos mass spectrometers both used quadrupole mass filters with an r^0 of 4 mm, while herein we tested a quadrupole with an r^0 of 5.25 mm. This larger mass filter also utilized pre- and post-filters similar to the ones currently used with the smaller quadrupole mass filter in the Orbitrap Fusion Lumos. The entire quadrupole vacuum manifold had to be removed and reworked to accommodate the larger mass filter.

Figure 2. We modified a Tribrid mass spectrometer at accept a much larger quadrupole mass filter with an r^0 dimension of 5.25 mm. All the other dimensions on the quadrupole mass filter we scaled up accordingly (e.g., effective radius of the hyperbolic rods and the outer diameter of the metal casing).



For typical shotgun proteomics experiments, where the precursor ions typically cover an *m/z* range of 400-1200 and where the isolation windows start at 0.4 *m/z*, we observe a 10-50% increase in isolation efficiency with this new quadrupole over the segmented quadrupole used in the Orbitrap Fusion Lumos MS.

Figure 3. The larger quadrupole mass filter has better transmission efficiency at narrower isolation windows and an improved quadrupole peak shape. This reduction in quadrupole peak "tailing" translates into better quadrupole S/N.

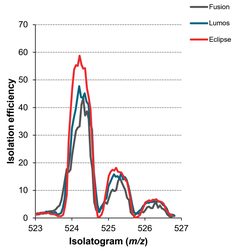
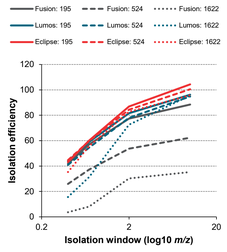


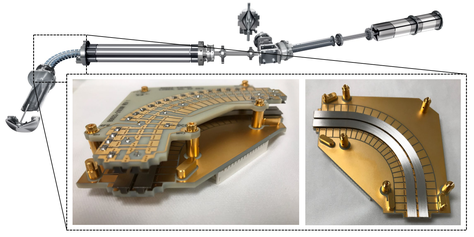
Figure 4. The largest transmission efficiency improvements are at higher *m/z* and narrower isolation window widths. We observe a 2x increase in isolation efficiency at 1622 *m/z* with a FWHM of 0.4.



Development of a new flat-rod bent ion guide

We updated the bent ion guide located immediately downstream of the the source region. The bent ion guide is after the ion funnel but before the quadrupole mass filter. By bending the ion beam 90 degrees, this ion guide discriminates against neutral species in favor of ions that are capable of making the turn. This bent ion guide is the "first line of defense" when it comes to preventing contamination of the downstream mass analyzers (second line if you consider in-source filtering).

Figure 5. This modified bent ion guide utilizes flat rods that move the critical electrode surfaces away from the central axis of the ion beam. This flat rod profile increases the clearance between the electrode surfaces and the neutral ion beam this ion guide is designed to reject. The new design is also designed to "self align" when reconnecting the two halves, which simplifies the process of disassembling and reassembling the electrode surfaces during cleaning.



Similar to the ion guides we've used before in the Tribrid MS, this one utilizes a drag field to actively convey the ions through this higher pressure region in a reasonable amount of time. The two plots below demonstrate some of the measurements we collected when optimizing the drag field for this bent ion guide.

The drag field needs to be strong enough to quickly convey ions through the device. However the drag field can't be too strong, or else the fringe fields created by the drag potential will start to reject ions at the entrance to the ion guide. This fringe field can be overcome by increasing the offset between the bent ion guide and the preceding multipole. Unfortunately this approach would negatively impact fragile ions and larger species. So the best solution is a modest drag voltage.

Figure 6. We varied the drag field applied to the bent ion guide and measured the flight time through the instrument. With a positive voltage the ions will experience a "downhill" potential through the bent ion guide, while a negative voltage is equivalent to an "uphill" climb.

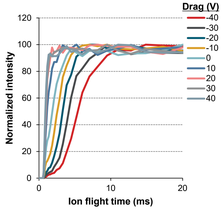
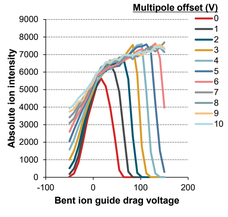


Figure 7. As the drag potential increases, so does the fringe fields the ions encounter as they enter the bent ion guide. These fringe fields can be overcome by increasing the offset for the upstream multipole.

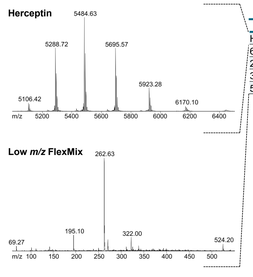


Improved high *m/z* ion transmission

We configured this modified Tribrid mass spectrometer to transmit and analyze high *m/z* ions (4000-8000 *m/z*). The modifications for high *m/z* transmission included optimizing the RF and DC voltages applied to this new bent ion guide. Optimizing these RF and DC potentials entailed reworking the DC fields we use to convey the ions through this bent ion guide, and tailoring the RF frequency to optimize the pseudopotential well for these large species.

It was important to us that the changes we made to support higher *m/z* transmission didn't negatively impact the lower *m/z* ions. For example, decreasing the ion guide frequency will improve high *m/z* transmission by increasing the pseudopotential well depth for these larger ions (assuming a fixed amplitude). However this same frequency change can negatively impact lower *m/z* transmission because smaller ions will require weaker RF amplitudes at lower RF frequencies when those ions are transmitted at a constant q-value.

Figure 8. We varied the frequency of the RF voltage applied to the bent ion guide, and we measured the transmission efficiency of Herceptin (146 kDa) vs. a few low *m/z* calibration ions. A frequency of 2.1 MHz nicely balanced the need to transmit Herceptin ions without disturbing the transmission efficiency of the lower *m/z* species.



	2.9 MHz	2.1 MHz	1.85 MHz	1.6 MHz
Herceptin	<LOD	100%	200%	250%
69 *m/z*	100%	100%	100%	100%
262 *m/z*	100%	100%	85%	20%
322 *m/z*	100%	100%	40%	10%
524 *m/z*	100%	100%	10%	2%

Optimized FTMS scan sequence

In the context of all of these hardware changes, we carefully studied and optimized all the times allotted to all the scan events. As a result of this characterization and optimization work, we improved the maximum FTMS^n spectral acquisition rate, while simultaneously increasing the maximum parallelizable injection time.

Figure 9. FTMS^2 scan rate as a function of injection time and resolving power (HCD fragmentation with quadrupole isolation). Using the older scan event timing.

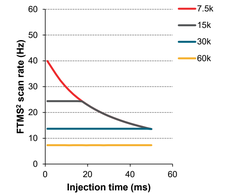
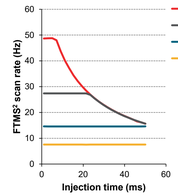


Figure 10. FTMS^2 scan rate as a function of injection time and resolving power (HCD fragmentation with quadrupole isolation). Using scan event timing optimized for the new hardware.

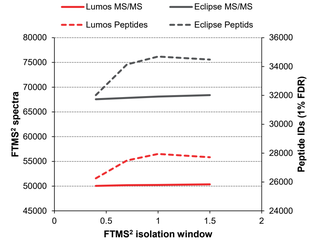


Large scale FTMS^2 results

All together, these changes to mass spectrometer ion optics and the timing of the scan events allow this modified Tribrid to collect higher quality spectra at a faster acquisition rate than any of the previously released Tribrid instruments.

To demonstrate the utility of these improvements we analyzed a K562 cell line lysate using a 60 minute LC-MS method. This data-dependent method targeted peptidic precursors using FTMS^2 analysis (15k resolving power with a maximum injection time of 22 ms). The resulting RAW files were searched using Proteome Discoverer and then trimmed to a 1% FDR. Between a standard Orbitrap Fusion Lumos MS and this modified Tribrid, we see 20% improvement in the number of peptide IDs.

Figure 11. We analyzed a K562 cell line lysate using a 60 minute LC-MS method. This data-dependent method targeted peptidic precursors using FTMS^2 analysis (15k resolving power with a maximum injection time of 22 ms). Across a series of acquisitions we varied the quadrupole isolation window size.



CONCLUSIONS

- The larger quadrupole mass filter enables better transmission efficiency, with better quadruple peak shapes that translates into improved precursor S/N.
- We installed a modified bent ion guide and we optimized the voltages applied to this device to transmit higher *m/z* species (without any cost to any lower *m/z* ions that may be of interest).
- The scan event timing was optimized for this new hardware. This enables FTMS^2 scans at up to 40 Hz (7.5k resolving power with a maximum injection time of 11 ms). During a typical 1 hour LC-MS/MS experiment this translate into 20% more MS/MS and peptide IDs than was capable with the previous generation of Tribrid instrumentation.

ACKNOWLEDGEMENTS

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