Developments in Orbitrap mass spectrometry on a modified Tribrid mass spectrometer

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ABSTRACT

Purpose: Improved instrument performance at elevated pressures and for higher m/z analytes; improved speed of analysis for TMT workflows

Methods: We modified a standard Thermo Scientific™ Orbitrap™ Fusion Lumos™ Tribrid™ mass spectrometer. Modifications included software and hardware changes, as described below.

Results: (1) Sensitivity for larger analytes is improved by raising the pressure in the ion routing multipole (IRM) and slightly modifying transmission characteristics. (2) Resolution or speed for TMT work can be improved by employing TurboTMT, which uses the ΦSDM algorithm, delivering higher resolution than normal FT methods for the same transient length.

INTRODUCTION

Improving the acquired information per unit time is an important goal for any LC-MS workflow. Information flux is improved as the breadth and quality of data collected on a single instrument expands. In this work, we describe modifications to a Tribrid system for the purpose of extending the overall utility of the system in a variety of applications, from shotgun proteomics to large molecule analysis. These modifications include increasing the mass range, enabling a wider range of system pressures, and deploying new spectral annotation algorithms.

MATERIALS AND METHODS

Sample Preparation. In most cases, characterization was done using Thermo Scientific™ Pierce™ FlexMix™ calibration solution. For the protein measurements in Figure 4, carbonic anhydrase from bovine erythrocytes (Sigma-Aldrich, St. Louis) was dissolved in 50:49.9:0.1 water:methanol:formic acid to a concentration of 1 µM. TMT work was done with the Thermo Scientific™ Pierce™ TMT11plex yeast digest standard.

Instrument Modifications. We modified a standard Orbitrap Fusion Lumos Tribrid mass spectrometer as described below, and shown in Figure 1. Hardware modifications included elevating the pressure in the IRM as well as Enhanced Vacuum Technology, a proprietary modification of the vacuum system. Software modifications included integration of the ΦSDM algorithm [1] into the instrument control software.

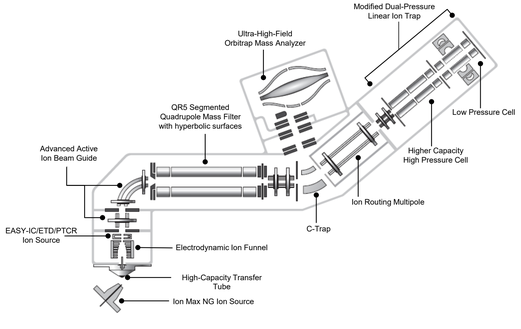


Figure 1. Modified Orbitrap Fusion Lumos Tribrid mass spectrometer. Changes discussed in this work are in the IRM and C-trap/Orbitrap area.

RESULTS

Operation at higher IRM pressure

The IRM is filled with ultra-high-purity nitrogen gas. The typical pressure setting is 8 mtorr. For some experiments involving heavier analytes, a higher pressure is sometimes required. To obtain higher pressures in the IRM, we modified the gas inlet with wider-diameter tubing, allowing more gas into the cell.

Figure 2. As shown at right, IRM pressure can be accurately regulated across a wide range of pressures, from 1 mtorr up to 20 mtorr.

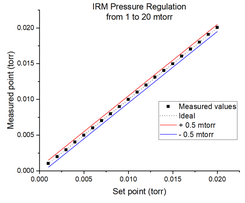


Figure 3. As shown at right, UHV pressure increases by about 1.5x from 2 mtorr up to 20 mtorr, an increase of only about 1.8x10^{-11} torr. Therefore, operating the instrument at elevated pressure does not compromise Orbitrap resolution.

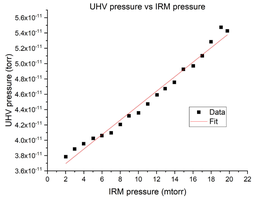
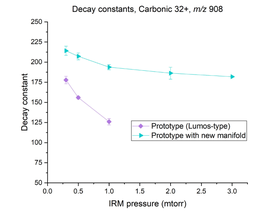


Figure 4. The above improvements have positive consequences for the decay of large molecules in the Orbitrap. At right is the decay of the 32+ charge state of carbonic anhydrase, plotted versus IRM pressure. For the "Prototype (Lumos-type)" configuration, decay measurement was not possible above a pressure of 1 mtorr, because only two beats were visible.

In contrast, on the new instrument, decay is nearly constant out to 3 mtorr.



Capturing larger molecules

Adjusting transfer settings

During transmission of ions from the C-trap to the Orbitrap, the central electrode is held about 1.5kV below its final value.

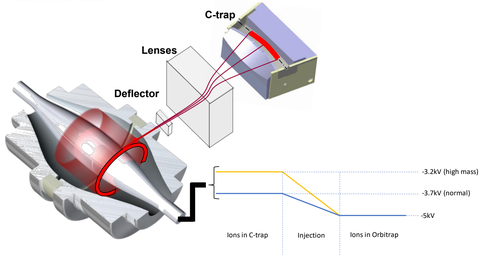


Figure 5. For enhanced performance for high-mass ions, we decrease the magnitude of central electrode voltage during injection – termed CE-inject – by about 500V. This allows more efficient capture of those larger, slower-moving ions.

Figure 6. With the central electrode injection voltage lowered in magnitude (less negative for positive ions), peak areas for larger ions at m/z 2122 and 2722 are enhanced.

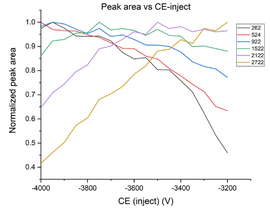
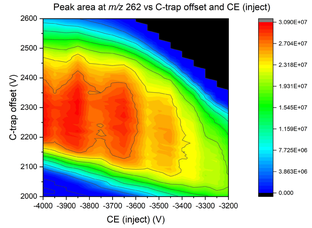


Figure 7. The downside to lowering the magnitude of the central electrode injection voltage is potential loss of ions at lower m/z. At a given value for the C-trap offset, which sets ions' initial energy, m/z 262 may be lost when the central electrode injection voltage is lowered (black region at upper right).



TurboTMT: Integration of the ΦSDM algorithm

The phase-constrained spectrum deconvolution method (ΦSDM) is one of a class of so-called super-resolution algorithms that seek to extract more resolution from a given time domain signal than is possible with conventional Fourier transform methods.

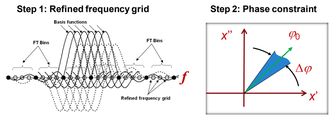
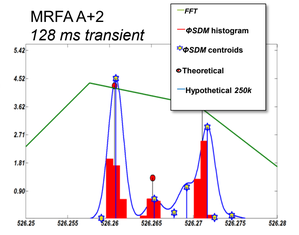
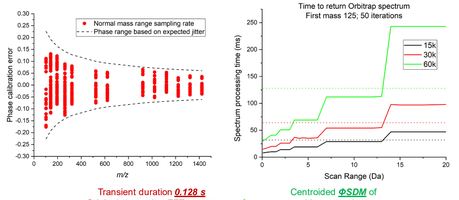


Figure 8. (Above) ΦSDM discretizes the signal frequency distribution with basis functions spaced more closely than FT bins. Evaluation of the transformed distribution is then aided by a constraint on the signal phases, which is calibrated (see below). The full machinery is explained in Reference 1.

(Right) A 128 ms transient would normally leave the MRFA A+2 fine structure completely unresolved. Application of ΦSDM yields resolution in excess of 200,000 for the same time domain signal.





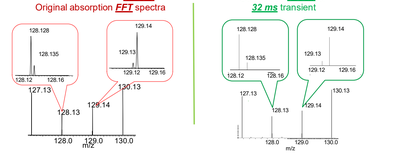


Figure 9. (Above left) Prior to using ΦSDM in real analyses, phases of calibrant ions are measured, in order to provide the phase constraints discussed above. (Above right) Due to onboard computational limitations, ΦSDM processing is limited to windows of about 12 Da, which is appropriate for analyzing TMT reporter ion signal. We increase the speed from this base number by selecting smaller windows about each TMT reporter channel.

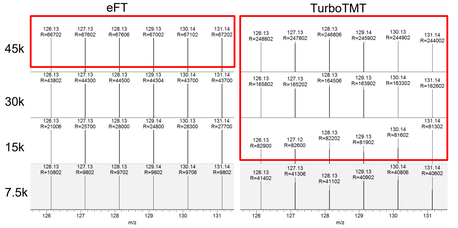
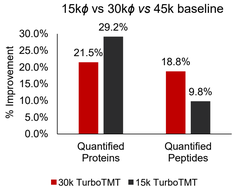


Figure 10. Applying the ΦSDM algorithm specifically to the TMT reporter ions increased the resolution sufficient to baseline resolve TMT isotopologues even when using transients that produce a 30k or 15k resolving power MS2 scan.

(Above) ΦSDM algorithm applied to 0.22 Da windows is sufficient to baseline resolve TMT isotopologues.

(Right) This approach increases the number of identifications for a 50 min gradient with 1 µg of TMT11plex yeast digest standard.



CONCLUSIONS

After modifying hardware and software on a Tribrid mass spectrometer, the instrument's versatility and utility is extended. In particular:

- By raising the IRM pressure and changing the settings for injecting ions into the Orbitrap, sensitivity for higher m/z ions has been increased.
- By integrating the ΦSDM algorithm into the instrument control software, it is now possible to acquire high-resolution spectra in a small percentage of the time required for traditional FFT methods.
- Although TurboTMT is limited to smaller windows in m/z due to available computational hardware, important benefits for TMT experiments are available.

REFERENCES

1. Grinfeld *et al.*, *Analytical Chemistry* **89,** 2, 1202-1211 (2016).
2. Scheffler *et al.*, ASMS 2016, poster MP312.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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