Enabling up to 270Hz acquisition speed on the Orbitrap Astral Zoom MS

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Introduction

High-throughput experiments demand shorter gradients to analyze more complex samples in a short amount of time. The Thermo Scientific™ Orbitrap™ Astral™ MS acquires up to 200 MS/MS scans per second [1], allowing for sufficient points across the LC peak even at a throughput of 180 samples per day. To further increase this number while maintaining quantitative accuracy, an even higher acquisition rate is highly desired. The Astral analyzer is hereby mostly limited by the switching times of ion optics and the transfer of ions through the instrument.

We present a new version of the Orbitrap Astral MS, the **Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer** (Figure 1) with optimized ion filter and quadrupole components, as well as faster ion transfer to enable higher scan speeds for data independent acquisition (DIA) and data-dependent acquisition (DDA) applications. Additional pre-accumulation in the Bent Trap is utilized to improve the overall duty cycle of the instrument (Figure 2).

Methods

The switching and transfer times of the high-speed quadrupole and fast ion filter are determined utilizing transitions between two ion species, i.e. jumps between two isolation windows with varying center *m/z* and isolation width. The settling time is determined as the time required for the second ion species to asymptotically reach full transmission. Data has been recorded over from *m/z* 69 – 1422 using electrosprayed ions from Thermo Scientific™ Pierce™ FlexMix™.

The ion transfer is optimized at multiple instrument stages by determining the minimum required transfer times without compromising ion transmission or residual ions. The following transfer stages have been optimized: releasing ions from the ion routing multipole (IRM); transferring ions to the ion processor; and moving ions inside the ion processor (IP) from high-pressure/collision to low-pressure/extraction region.

Scan rate and duty cycle measurements have been performed for DIA and DDA using FlexMix as function of maximum injection time.

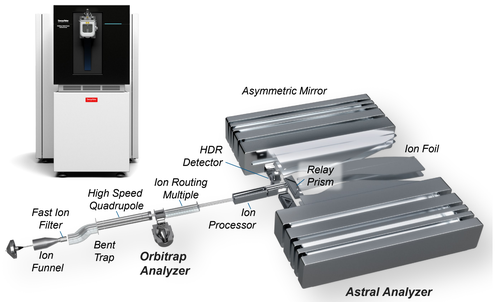

Figure 1. Ion optical layout of the Orbitrap Astral Zoom mass spectrometer.

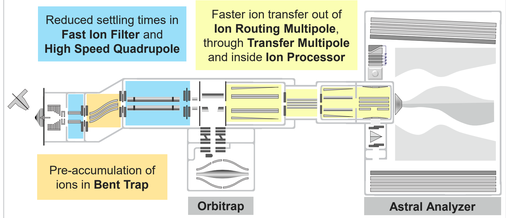

Figure 2. Schematics of the Orbitrap Astral Zoom mass spectrometer highlighting the different components and improvements to increase the acquisition speed and overall duty cycle.

Results – Settling Times

The settling times of the ion optical components selecting the precursor mass could be reduced for larger mass jumps from 3 ms (going to higher isolation center) and 4 ms (going to lower isolation center), down to 2.5 ms settling time independent of the direction of the isolation center change (Figure 2). Additionally, a slightly broader region around very narrow variations now benefit from the minimum settling time of 0.6 ms. For Thermo Scientific™ Orbitrap™ Astral™ MS, this minimum settling time could be utilized for *m/z* jumps < 10 Th while the Orbitrap Astral Zoom MS now utilizes the shortest settling time for window changes up to 15 Th with a linear interpolation in between 15 to 30 Th isolation center changes. The improvements will mostly benefit DDA and targeted applications, and also DIA runs with wider isolation windows.

The required settling time depends also on the target isolation center where smaller isolation centers require less time to settle. This could be utilized by adding a *m/z*-dependent settling time.

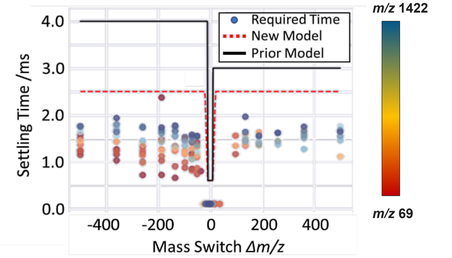

Figure 3: Settling times of the injection ion optics components as function of *m/z* jump. Experimental data was recorded on several Orbitrap Astral Zoom MS instruments to derive the new settling time model.

Results – Transfer Times

Ion transfer times out of the IRM towards the ion processor and within the ion processor have been characterized using FlexMix ions of *m/z* 195 – 1722 (Figure 4). A *m/z*-dependency is visible for the transfer of ions from the IRM to the ion processor (Figure 4 a) which is partly result of the time-of-flight through the ion optical components. While *m/z* 195 requires around 0.3 ms to reach the 90% transmission level, *m/z* 1722 needs with 0.8ms more than double. With the improved IRM HW like the stronger axial gradient on the Orbitrap Astral Zoom MS, this transfer time is nearly halved to 0.8 ms (Figure 4 c) vs the ~1.5 ms used on the Orbitrap Astral MS. For precursors with *m/z* above 1000, the transfer time is slightly increased to leave sufficient margin.

The ion transfer within the ion processor (Figure 4 b) requires around 1.6 ms to fully transmit ions during the voltage lift from high-pressure to low-pressure part. This suggests that ions are well located at the connection point of both parts of the ion processor which is guaranteed by the wedge electrodes inside the high-pressure part. For the Orbitrap Astral Zoom MS, a transfer time of 1.8 ms is used for precursor masses up to *m/z* 1000 with increased transfer time for higher *m/z* (Figure 4 d). While this seems counter-intuitive with the *m/z*-independency, a slightly increased transfer time will make the ion transfer less dependent on pressure variations inside the low-pressure part of the ion processor, thus enhancing robustness of the system.

Figure 5 gives an overview of the changes introduced on the Orbitrap Astral Zoom MS compared to the Orbitrap Astral MS. The shorter settling times of the fast ion filter and the high-speed quadrupole are shown falling from 3 ms and 4 ms to 2.5 ms settling time. With the improved axial gradient of the IRM, the opening time of the IRM exit lens could be halved from 1 ms to 0.5 ms. This also leads to faster transfer towards the ion processor. Lastly, the required time when moving ions inside the ion processor was reduced from 2.5 ms to 1.8 ms.

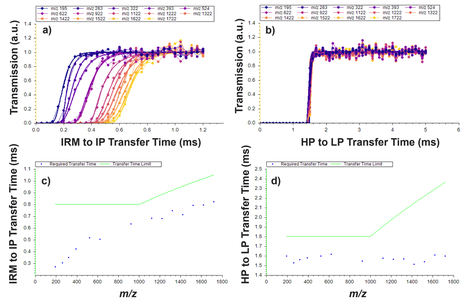

Figure 4: Required transfer time as function of *m/z*. Top: *m/z*-dependent transmission of ions from IRM to the ion processor (IP) shown in panel a) and inside the ion processor from high pressure (HP) to low pressure (LP) as shown in panel b). Bottom: Transfer times determined at the 90% transmission level as function of m/z, required transfer time and applied timing model from IRM to IP (panel c) and from HP to LP inside the ion processor (panel d).

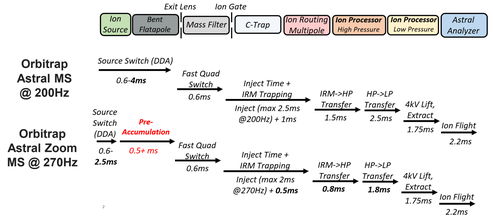

Figure 5: Parallel acquisition scheme of the Orbitrap Astral MS (top) and the Orbitrap Astral Zoom MS with all relevant settling and transfer times. Switching times of the fast ion filter and high-speed quadrupole have been improved. Additionally, transfer times through IRM and ion processor could be reduced.

Results – Scan Rate and Duty Cycle

Figure 6 shows the scan rate as a function of maximum injection time, measured both on the Orbitrap Astral Zoom MS and the Orbitrap Astral MS. Data was recorded for DIA runs with 2 Th and 10 Th isolation width (Figure 6 left) as well as a top100 DDA experiment (Figure 6 right). In the case of narrow DIA, the Orbitrap Astral Zoom MS reaches 270 Hz acquisition speed for injection times up to 2 ms. At the same injection time, the Orbitrap Astral MS reaches around 220 Hz. The scan rate difference is reduced at higher injection times. A larger gap is visible for 10 Th isolation window where the improved settling times have strong influence.

For DDA applications, the Orbitrap Astral Zoom MS is around 40 Hz faster at low injection times and still 20 Hz ahead for longer injection times. This is realized partly by the faster switching of the ion optics and the faster transfer times.

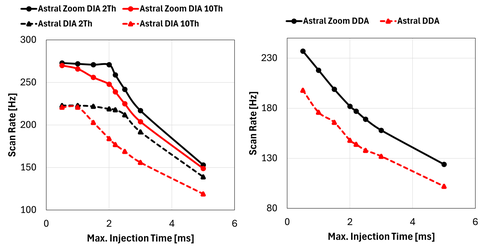

Figure 6: Scan rate as function of the maximum injection time. Left: Scan speed during DIA runs with 2 Th (black) and 10 Th (red) window width for the Orbitrap Astral Zoom MS (solid lines) and the Orbitrap Astral MS (red lines). Right: Scan speed for DDA top100 runs for the Orbitrap Astral Zoom MS (black) and the Orbitrap Astral MS (red).

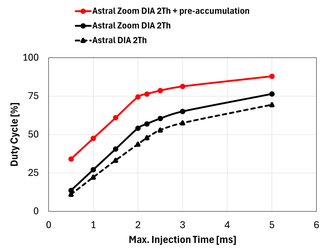

Figure 7: Duty cycle as function of injection time for narrow DIA methods

The measured scan rates have been further used to calculate the duty cycle of both instruments for narrow DIA. At 2 ms injection time, the Orbitrap Astral Zoom MS has around 10% higher duty cycle than Orbitrap Astral MS when pre-accumulation is disabled. However, when utilizing the additional average 0.75 ms pre-accumulation injection time, the duty cycle including OT scans and AGC fluxscans reaches more than 75 % for injection times above 2 ms. Astral-only MS2 scans reach a duty cycle above 80%.

Conclusions

The new Orbitrap Astral Zoom MS combines faster settling of ion optics as well as faster transfer of ions through the instrument. Additional pre-accumulation in the bent trap can further be utilized, realizing the following performance:

- Scan rate of 270 Hz for DIA at 2 ms injection time compared to 220 Hz on the Orbitrap Astral MS.
- Increase of scan speed for DDA of 20 – 40 Hz depending on the injection time
- Duty cycle of up to 75% for DIA methods when using pre-accumulation.

References

1. Stewart et al., **Parallelized Acquisition of Orbitrap and Astral Analyzers Enables High-Throughput Quantitative Analysis**; Analytical Chemistry 2023 95 (42), 15656-15664; DOI: 10.1021/acs.analchem.3c02856

Acknowledgements and Disclosure of Interests

The authors would like to thank the many colleagues from Thermo Fisher Scientific and collaborators around the world who made this work possible. All authors are employees of Thermo Fisher Scientific, a manufacturer of scientific instruments who funded this work.

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