Ion Pre-Accumulation for High Speed Orbitrap Exploris Operation

Hamish Stewart, Tabiwang N. Arrey, **Markus Kellmann** and Alexander Harder
Thermo Fisher Scientific, Bremen, Germany

Abstract

Purpose: Implementation of an ion trapping and accumulation stage in parallel to the regular C-Trap operation of a modified Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, allowing 100% duty cycle at high repetition rates.

Methods: Sensitivity comparisons were made between standard operation and pre-accumulation modes, both for infused Thermo Scientific™ Pierce™ FlexMix™ calibration solution and chromatographic separations of HeLa digest.

Results: Instrument sensitivity doubled at the normally allowed maximum repetition rate, and repetition rate could be increased from 40 Hz to above 80Hz without loss of duty cycle, whilst 90-100Hz sacrificed stability. Increases in peptide ID rates were observed for proteomics applications

Introduction

Orbitrap instruments have played a major role in advancing MS driven scientific research. However, these instruments have been hitherto limited to maximum acquisition rates <50Hz, primarily due to time constraints imposed not by the Orbitrap analyzer itself, but timing overheads imposed by the operation of the C-Trap and its conjoined Ion Routing Multipole (IRM) that prepare and inject ions into the analyzer.

Figure 1 shows the layout of ion optical devices within an Orbitrap Exploris 480 mass spectrometer, along with timings of operations running in series and in parallel. Electrosprayed ions are transferred into vacuum, quadrupole isolated, and injected into the IRM, before being cooled and transferred to the C-Trap for orthogonal ejection to the Orbitrap. The preparation of ions within the IRM and the C-Trap, as well as the ejection process and reset, takes ~10ms, a dead time during which no further ions may be accumulated. The ion beam is normally dumped at the charge detector during this period and lost, though a proportion of the period is used to adjust voltages of the ion guides and switch to the next target ion.

This fixed 10ms operation time runs in series with ion injection time, and faster operation rapidly leads to loss of duty cycle. For the fastest normally allowed Orbitrap acquisition cycles, resolution setting 7500 (16ms transient), the overall cycle time is >20ms but only 10ms may be used for ion beam acquisition; a loss of >50%. Faster operation, preferred for high throughput applications causes the duty cycle penalty to grow explosively and renders such a method non-viable.

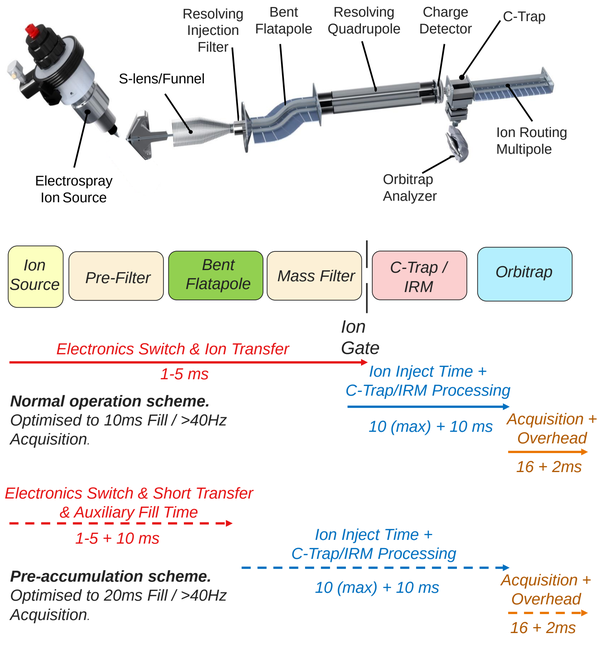

Figure 1. Ion optical layout of an Orbitrap Exploris mass spectrometer, and Illustration of in-series and in-parallel instrument operations in 40Hz standard and pre-accumulation modes.

One of the ion guides that serves to transfer ions across vacuum stages, between the ion source and C-Trap/IRM, is called the Bent Flatapole. This has a quadrupole structure, curved to separate ions from neutral gas, and incorporates a superimposed DC gradient, generated by a series of PCB printed DC electrodes. An exit lens aperture with an independent voltage separates the device from the quadrupole mass filter. Structurally this makes for an excellent ion trap, where trapping or release of ions may be controlled by switching the DC voltage applied to the exit lens.

A suitable method to circumvent the C-Trap dead time is to trap ions within the bent flatapole during this period, performed by switching the bent flatapole exit lens to a trapping potential (+10V) during the dead period, and back to a transmitting potential (-10V) at the start of ion injection. This way ions may be accumulated in parallel to the C-Trap/IRM ion processing, and the stored ions transmitted through after it becomes available. . This process is also shown in Figures 1, and the key operations drawn as a function of time in Figure 2.

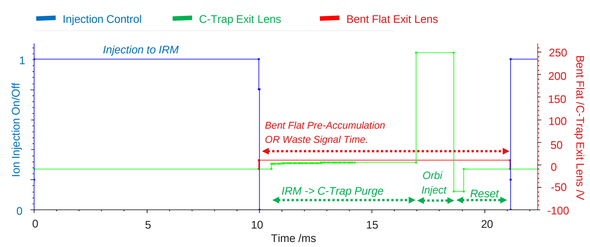

Figure 2. Pseudo-oscilloscope plot during operation showing instrument event timing, including implementation of bent flatapole pre-accumulation.

Methods

Instrument Set-Up: The Orbitrap Exploris 480 mass spectrometer's firmware files were modified so that when performing an injection to the IRM, the bent flatapole exit lens would be set to transmitting mode, but switch to trapping mode at the end of the injection. This gave a crude implementation of the pre-accumulation method, incompatible with automatic gain control and forcing use of a fixed injection time instead.

For >70Hz operation, the Orbitrap transient was reduced to 8ms, equivalent to resolution of 3750 at m/z 200, and the injection time reduced from 10 to 3ms. Over 100Hz operation was achieved by further reducing transient to 4ms, inject time to the stable minimum of 2ms, and IRM->C-Trap purge time to ~2.5ms. Sensitivity measurements and optimisations were performed via infusion of FlexMix calibration solution.

To enable tandem MS methods, the method control file was altered to allow 8ms Orbitrap transients, and a legacy C-Trap only injection matrix applied for full mass scans, which bypassed the pre-accumulation mode. This way pre-accumulation could be applied specifically to MS/MS spectra, where the added sensitivity mattered most, and not for full MS spectra where ion population control was essential.

Sample and Method: Pierce™ HeLa Digest Standard (20 µg/vial) was reconstituted in 200µL 5% ACN/0.1% FA to 100 ng/µL. 2µL (200ng) of sample was injected via autosampler onto a trapping column and separated on a 15cm PepMap Reversed Phase using a Vanquish™ Neo UHPLC or Easy-nLC™ system. Different gradients lengths were used to separate the samples. The mass spectrometer was operated in a TopN Data Dependent Acquisition (DDA) mode. Raw data files were processed via Proteome Discoverer™ software 3.0 with Sequest HT search engine and PSM/Peptide validation using Percolator.

Results

40Hz Pre-Accumulation On/Off Comparison: FlexMix Calibration Solution was directly infused at 5 ul\min. An MS/MS spectrum of the isolated MRFA peptide (524 Da) was acquired, using a fixed injection time of 10 ms, a 16ms Orbitrap transient, and a mass range from 150-600 Da. Spectra were measured with pre-accumulation active and inactive, both of which are shown in Figure 3. The results show a >2x improvement in signal intensities with pre-accumulation active, and preservation of relative intensities between fragments. The "normalized largest" (NL) measurement of signal current more than doubles, a consequence of the doubled effective injection time.

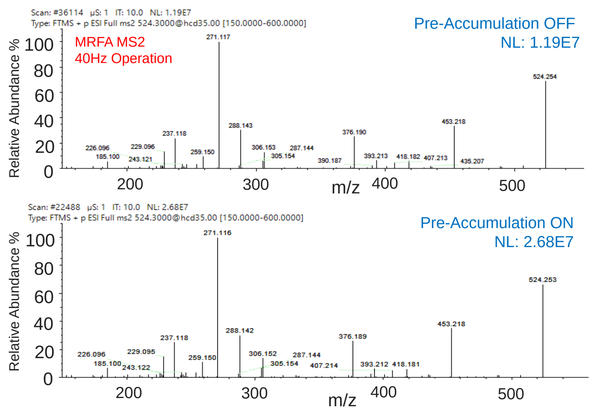

Figure 2) Comparison of MRFA MS/MS spectra with pre-accumulation disabled (top) and enabled (bottom).

Sensitivity and Repetition Rate: An experiment was performed whereby the orbitrap acquisition was set to 8ms, so that inject time limited the repetition rate. The inject time was scanned from 10 down to 2 ms, varying the repetition rate from 47 to 76 Hz, and the signal to noise ratio of the isolated MRFA peak recorded for pre-accumulation on and off. A further scan was made with a 4ms transient, fixed 2ms inject time, and variation in scan rate by reducing the IRM->C-Trap purge time, normally set far longer than required. The same shift allowed 80Hz to be reached with an 8ms transient without obvious issue.

The ion current was calculated, and the proportional losses determined. This is plotted in Figure 4 and shows the collapse in duty cycle without pre-accumulation, as inject time becomes a smaller and smaller proportion of the instrument cycle. Conversely when pre-accumulation was active, no clear losses were observed. Unfortunately, although spectra at high repetition rate looked generally good, albeit with low signal/noise due to the 4ms transient, as in Figure 5, occasional signal instabilities were observed at >90Hz.

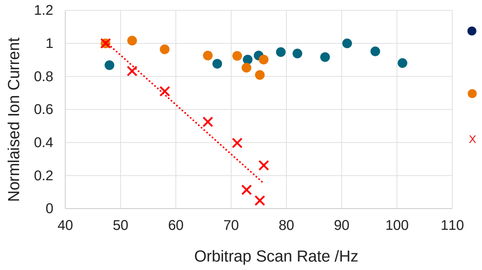

Figure 4. Relative MRFA ion current with increasing repetition rate (decreasing inject time), for pre-accumulation active and disabled, for 8ms and 4ms orbitrap transients.

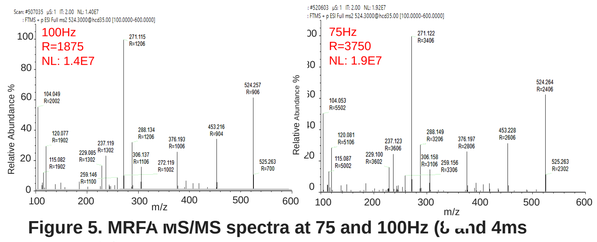

Figure 5. MRFA MS/MS spectra at 75 and 100Hz (8 and 4ms transients).

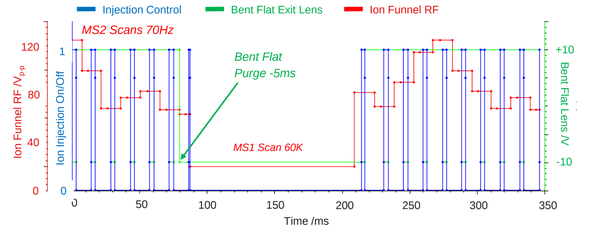

Figure 6. Pseudo-oscilloscope plot of key events during MS and MS/MS scanning of a 70Hz Pre-Accumulation DDA Method.

DDA Method: Figure 6 shows event timings measured on instrument during a DDA method at 70Hz. MS/MS timings were regular, and the bent flatapole correctly trapping outside of inject periods. The bent flatapole also correctly opened well in advance of the MS1 injection, normalising the ion flow. The only fly in the ointment was that front-end ion guides such as the ion funnel, inject filter etc delayed 5ms before switching. As there is a low-resolution inject filter prior to the bent flatapole, likely sensitivity in these experiments was adversely affected by this limitation.

60-minute gradients: Comparative tests were first performed with 200ng HeLa digest, for 60-minute 22Hz and 40Hz runs, with pre-accumulation on/off. Figure 7 shows the change in protein and peptide identifications. Whilst these were not small sample loads, and thus unflattering for a method that solely gives a sensitivity benefit, in both cases there were modest improvements in identified protein groups and peptides.

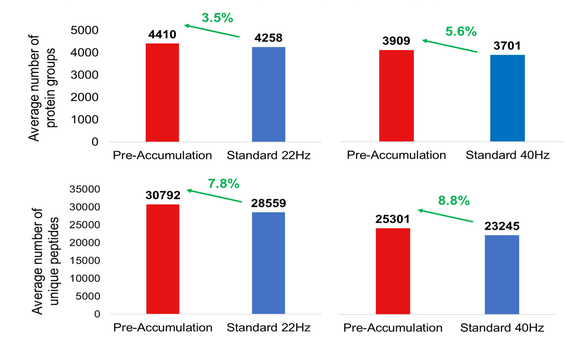

Figure 7. Pre-Accumulation vs Standard 60-minute 200ng HeLa DDA. Left) 22Hz, R=15K MS/MS, R=120K MS. Right) 40Hz, R=7.5K MS/MS, R=60K MS.

High throughput: Tests with short gradients were then made with pre-accumulation at 70Hz R=3750, and a 40Hz (really ~45Hz) R=7500 standard method for control. These results are shown in Figure 8. As expected, the 70Hz method gave consistently >50% more MS/MS spectra, although the proportional increase in peptide IDs depended on the gradient length. The 24-minute gradient even saw a decline with pre-accumulation, a likely consequence of the shorter transient. Oddly, the massive improvement in IDs for the 6-minute tests was out of proportion to the increase in spectra, so perhaps the lower quality counter-intuitively helped by hiding analytically harmful fragments. It remains to be seen if this improvement will be maintained with advanced processing algorithms such as the Chimerys™ method, that may make multiple IDs per spectrum.

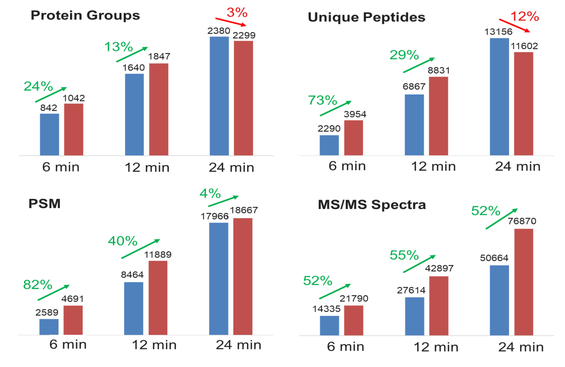

Figure 8. 70Hz Pre-Accumulation vs Standard 40Hz 200ng HeLa DDA Results.

Conclusions

- Pre-accumulation is a promising method to improve sensitivity at high scan rate, and to enable major increases in Orbitrap Exploris scan rate.
- Likely ~80Hz is the practical/stable limit but 100Hz has been shown operating.
- Real application gains are considerable for high throughput experiments but limited for longer gradients. However, it will likely regain utility at low sample concentrations.
- Performance with DIA, and optimized conditions/data processing and sample loads has yet to be assessed.

Trademarks/licensing