

Theory and Performance of a Novel High-Resolution Mass Analyzer

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Introduction

Mass analyzer design inevitably involves compromise between competing performance characteristics. Resolution, transmission, mass accuracy and scan frequency play against one-another. Fourier transform mass analyzers, for example, compromise between scan rate and resolution, whilst time-of-flight analyzers suffer low ion transmission from coupled fast orthogonal extractors.

A new class of high-resolution accurate-mass analyzer, the Thermo Scientific™ Orbitrap™ Astral™ analyzer¹, has been developed that surpasses prior limitations. Ions are first accumulated within a high-speed extraction trap² and ejected into the space between two opposing ion mirrors. The beam is then directed and shaped by a pair of letterbox lenses and prism deflectors. Ions oscillate 23-25 times between two converging five-electrode ion mirrors, and drift orthogonally down the length of the mirrors, with expansion of the packet allowed to minimize space charge effects. ~300µm mirror convergence and the shape function of compensating stripe electrodes combine to slow and eventually reverse drift at 335mm depth, then refocus ions to the detector with very high transmission. As the perturbation upon ion oscillation frequency of mirror convergence and compensation electrode shape have the opposite sign, they compensate for one another, allowing high time focal quality and resolution.

Figure 1 shows the basic layout of the analyzer and principal ion trajectory, along with a MASIM3D generated simulation of m/z 200 ion arrival time distribution, with a resolving power of 138K. Further simulation results show the ~10% energy acceptance of the opposing ion mirrors, and the rotation of phase space induced by the drift reflection process.

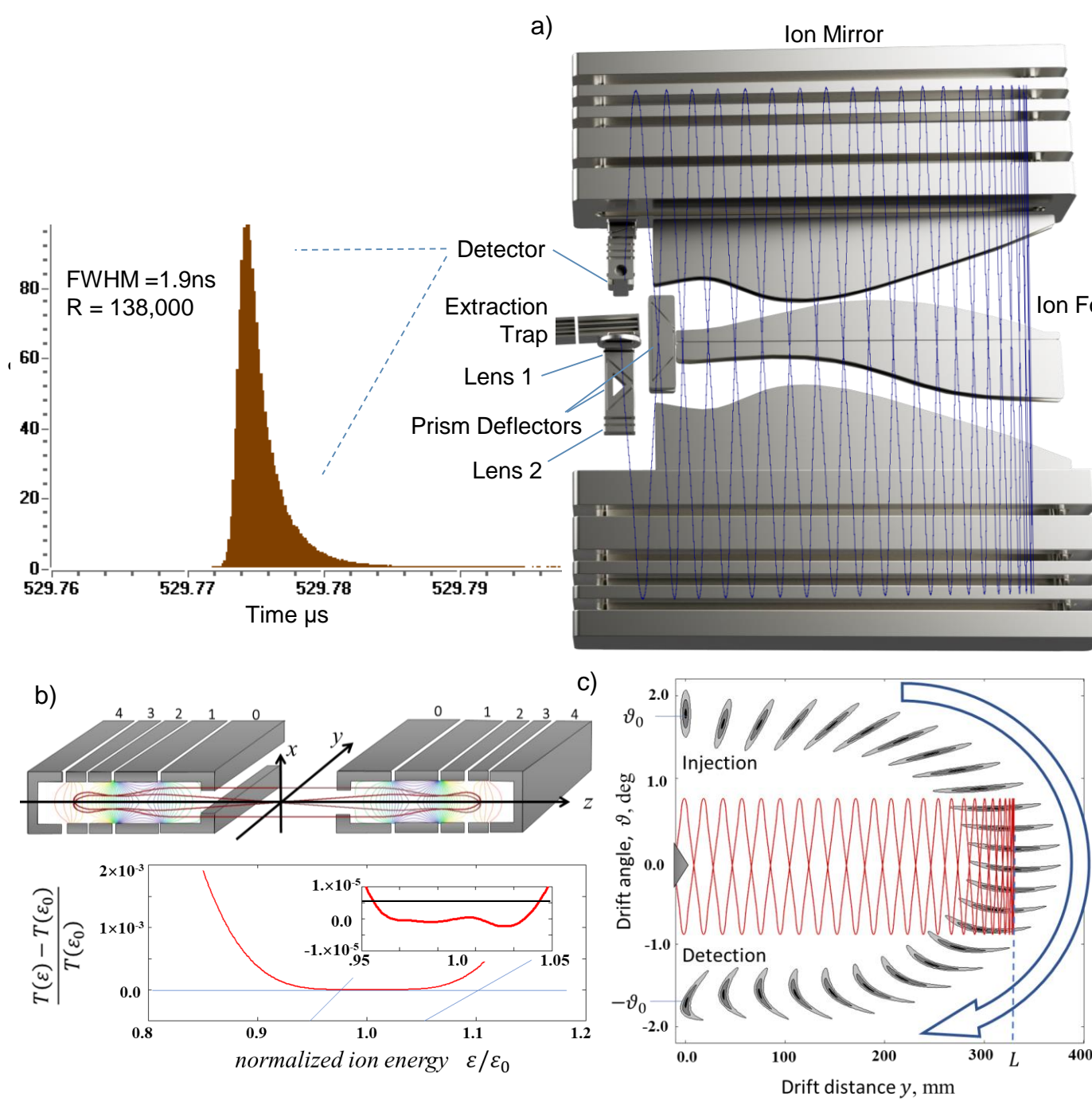


Figure 1. a) Diagram of the Astral analyzer layout, plus MASIM simulated arrival peak of m/z 200 ions at the detector surface. b) Mirror design and energy acceptance. c) Phase space of ion drift energy and position during transit through analyzer.

Experimental

A prototype instrument was constructed based on an Thermo Scientific™ Orbitrap Exploris™ platform mass spectrometer coupled to the Astral analyzer. Shown in Figure 2, this was intended for synergistic operation whereby the Orbitrap analyzer carried out high quality full-MS scans whilst the Astral analyzer made many fast, sensitive MS/MS acquisitions.

Astral analyzer performance characteristics were measured with electrosprayed ions of infused Thermo Scientific™ Pierce™ FlexMix™ calibration solution, intact proteins and TMT10plex™ reagent. Ions could be first isolated and fragmented, or sent to the analyzer unimpeded. Stability was investigated via changes in time, temperature, and the shift of applied potentials. Tolerance to space charge and saturation was measured by varying the mass range and numbers of admitted ions. Overall transmission was compared against a Thermo Scientific™ Orbitrap™ analyzer mounted upstream of the analyzer.

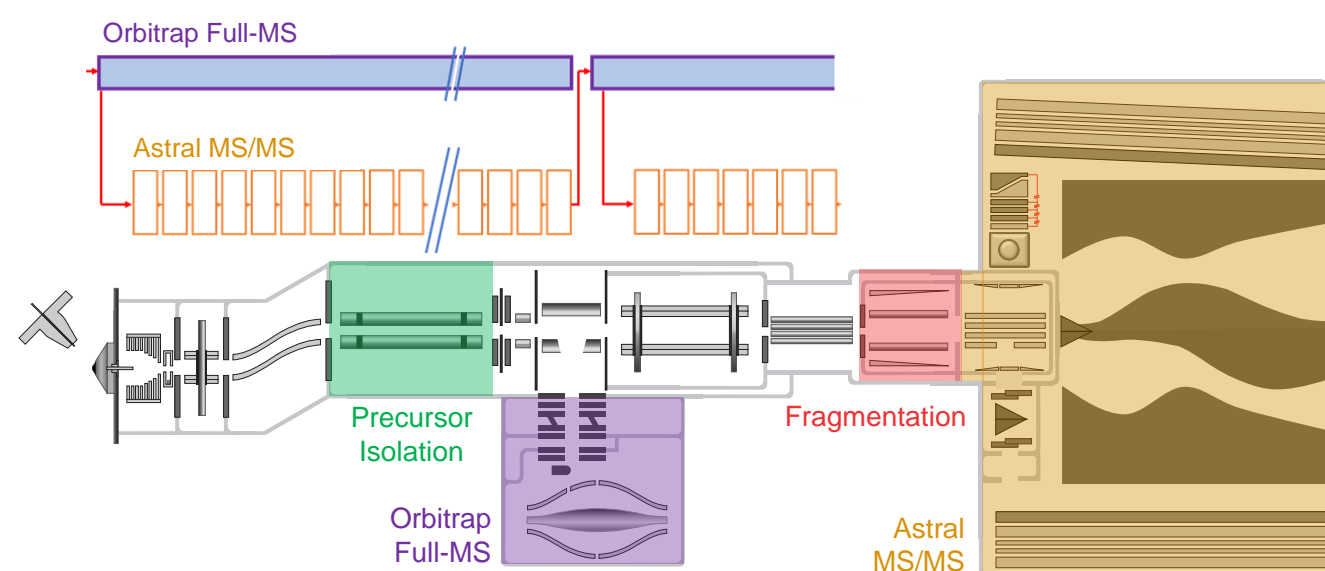


Figure 2. Experimental instrument incorporating Orbitrap and Astral analyzers.

Results

Spectral Quality: Figure 3 shows a typical 10,000 ion FlexMix full-MS spectrum, acquired in a single shot. The full profile from m/z 150-2000 was observed with an average resolution >90K, excepting the most intense peaks. Also shown is the ability to isotopically resolve 6mDa separated TMT reporter ions, and a comparison of low intensity, 1000 ion, MS/MS spectra of the m/z 524 MRFA peptide obtained within both Astral analyzer and conjoined Orbitrap analyzers. The Astral MS/MS spectra are far more sensitive, with destructive ion detection catching many more low-intensity species, and may be recorded within a 5ms cycle with far higher resolution.

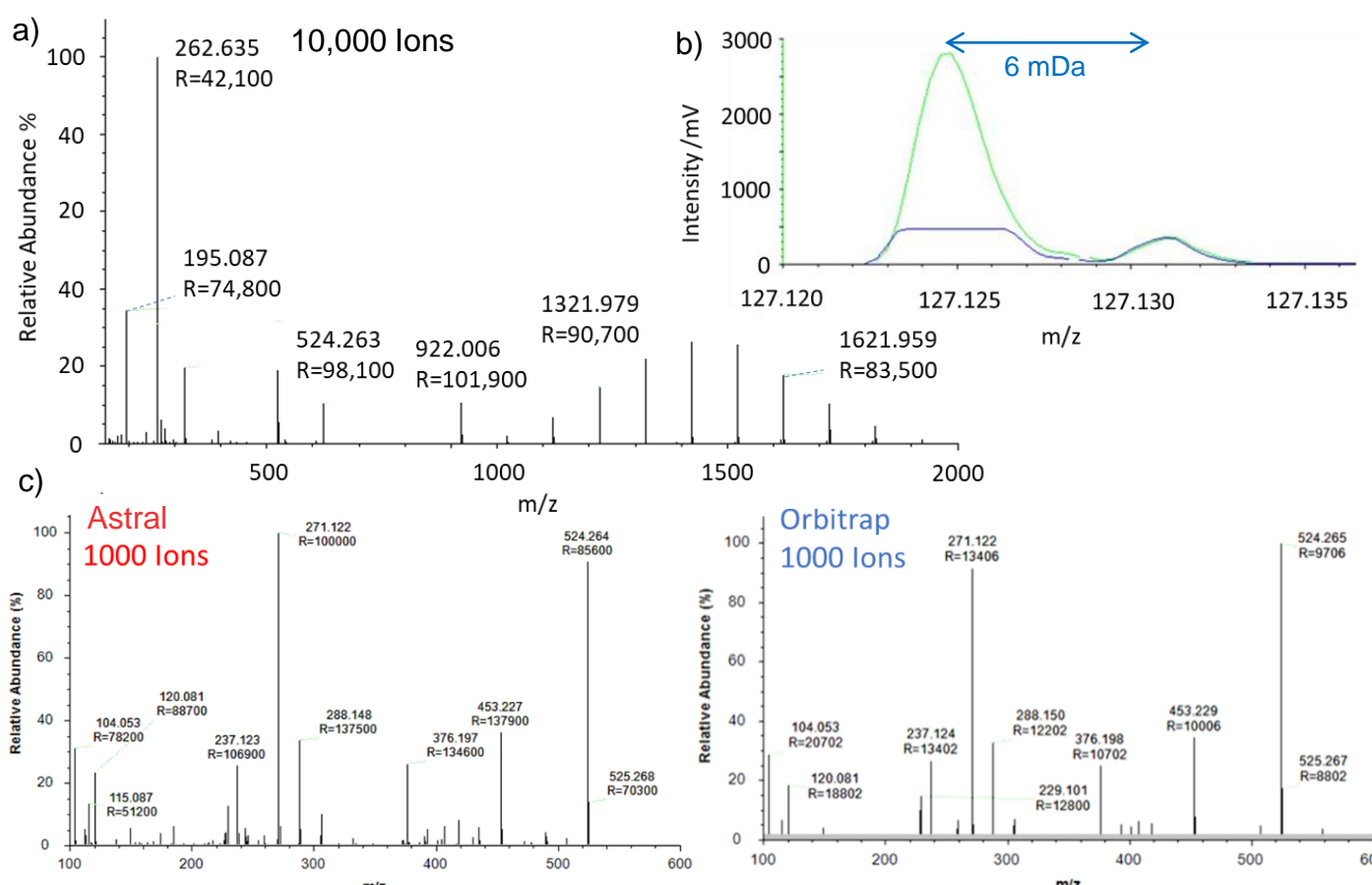


Figure 3. a) 10,000 ion FlexMix mass spectrum. b) Resolved TMT reporter doublet profile. c) Comparison of 1000 ion MRFA MS/MS in Orbitrap and Astral analyzers.

Mass Accuracy: Figure 4 shows mass drift of m/z 922 Ultramark ions over several days. Raw m/z shifts slightly with thermal drift of the aluminum chamber and mirror electrodes. A regular recalibration with a shot of fluoranthene ions supplied from an internal calibrant source eliminates this slow drift with negligible impact on instrument performance. Also shown is the mass measurement jitter, good performance for which requires very stable and reproducible applied voltages, and high resolution. For isolated ions, standard deviation σ of mass measurement without averaging was generally <0.5ppm, with <1.5ppm 3σ .

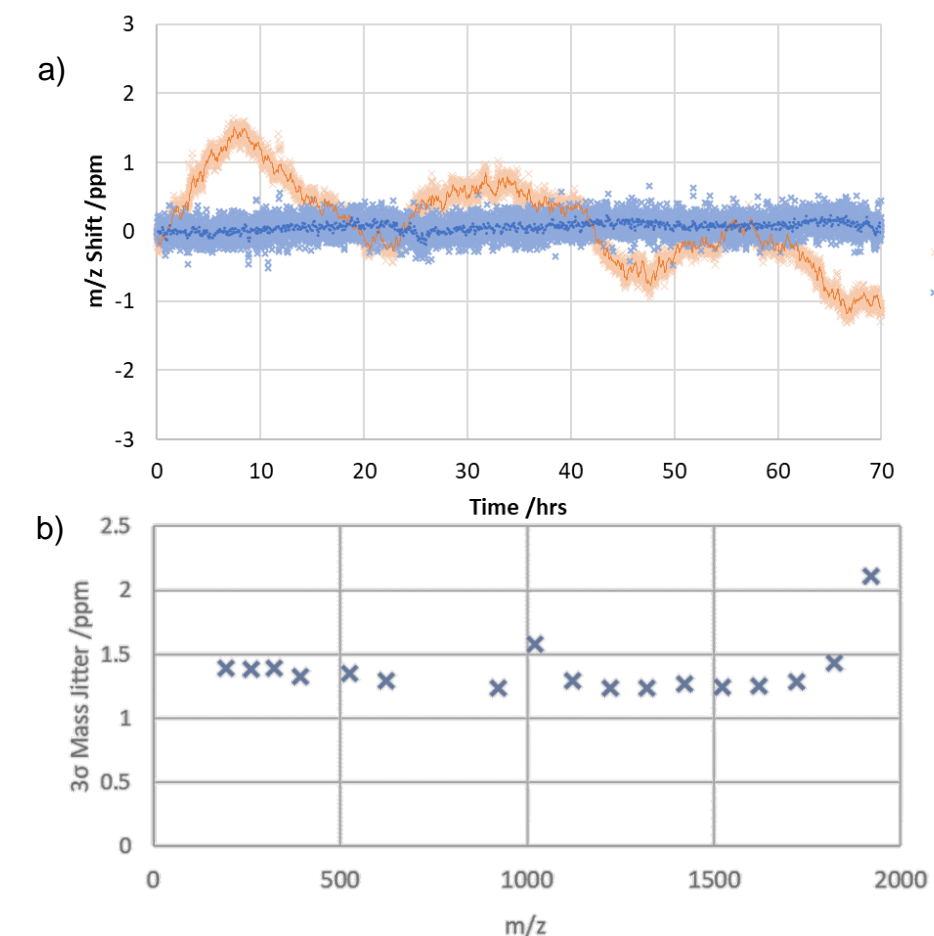


Figure 4. a) Mass drift of Ultramark m/z 922 over 70 hours, as raw unmodified signal and with intermittent internal calibrant correction. b) Shot to shot mass measurement jitter.

Sensitivity: Relative transmission through the analyzer was measured by comparison to ion numbers recorded in the Orbitrap analyzers, and vs a single oscillation diagnostic mode whereby the second deflector prism reflected injected ions directly to the detector. Ion number was calibrated against measured single ion intensities, and the m/z dependency of the detector's conversion dynode separately corrected for. Relative transmissions are shown in Figure 5, with much greater ion flux recorded at the Astral detector than the Orbitrap analyzer. The drop-off at lowest m/z is thought a consequence of ion transfer to the extraction trap, as it is not reflected in MS/MS spectra or generation of low m/z TMT reporter ions.

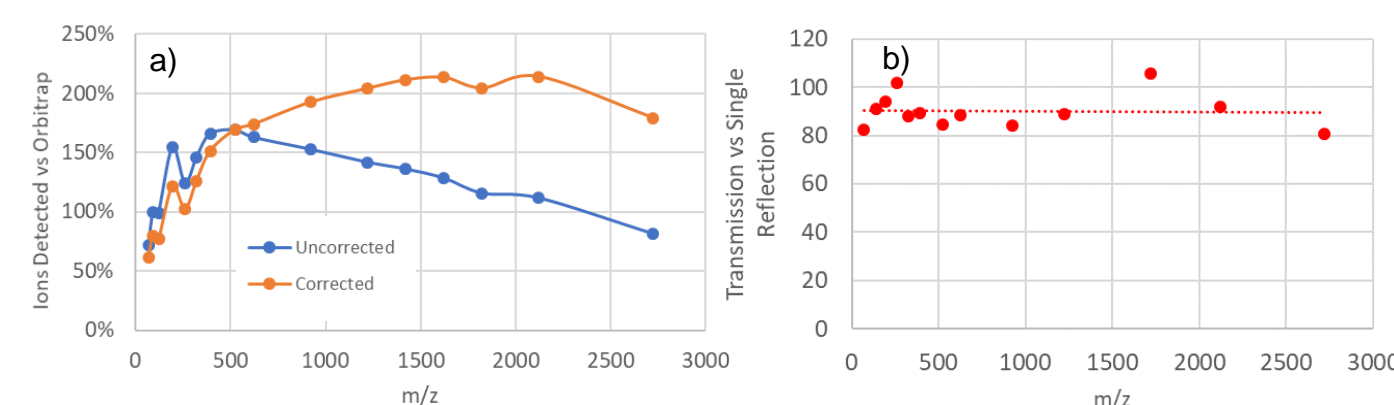


Figure 5. Relative number of ions detected in the Astral analyzer compared to a) Orbitrap and b) diagnostic single oscillation mode.

Space Charge: The main influence of space charge effects was to reduce peak resolution, as the ion focal plane position decouples from the detector surface. For isolated MRFA ions, resolution fell to ~50K at <2500 ions, as shown in Figure 6. Tolerance was far greater, 5000 ions, when a wide mass range of additional ions was introduced. This is believed due to broadening of the initial ion packet size within the extraction trap, acting to reduce the charge density of ions in flight. Figure 6 also shows FlexMix full-MS resolution at low and high ion loads, and even at 50K ions per shot, high ~80K resolution was observed. Space charge, and mechanical accuracy requirements may explain why resolution falls short of simulated limits.

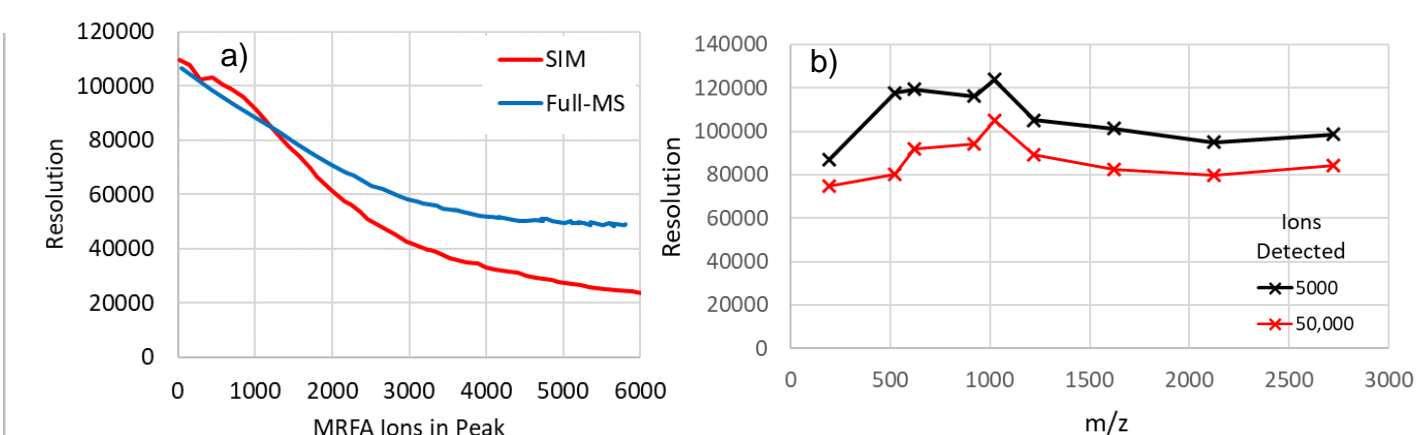


Figure 6. a) Resolution shift of MRFA ions with ion number, isolated (SIM) or Full-MS. b) Resolution of FlexMix full-MS ions at 5000 and 50,000 ions detected per shot.

Intact Proteins: The analyzer pressure of $<1 \times 10^{-8}$ mbar allows for transmission of larger ions, even over a 27-meter flight path. Figure 7 shows 100x averaged profile spectra of infused myoglobin (17KDa) over both a wide mass range and zoomed into a single charge envelope. Resolution was maintained, but due to space charge limitations and the influence of scattered stray ions, at least 10x averaging was required. As individual shots could still be acquired at 200Hz, this level of averaging still represented a great improvement in speed over the state of the art. MS/MS of intact proteins could also be performed, with a ProSight analysis of 4s acquisition of 29KDa carbonic anhydrase producing better sequence coverage in Astral than Orbitrap analyzers. The larger the ion however, the greater the scatter/chemical noise and the more averaging was required for isotopic resolution. Heavier proteins are a direction for future research.

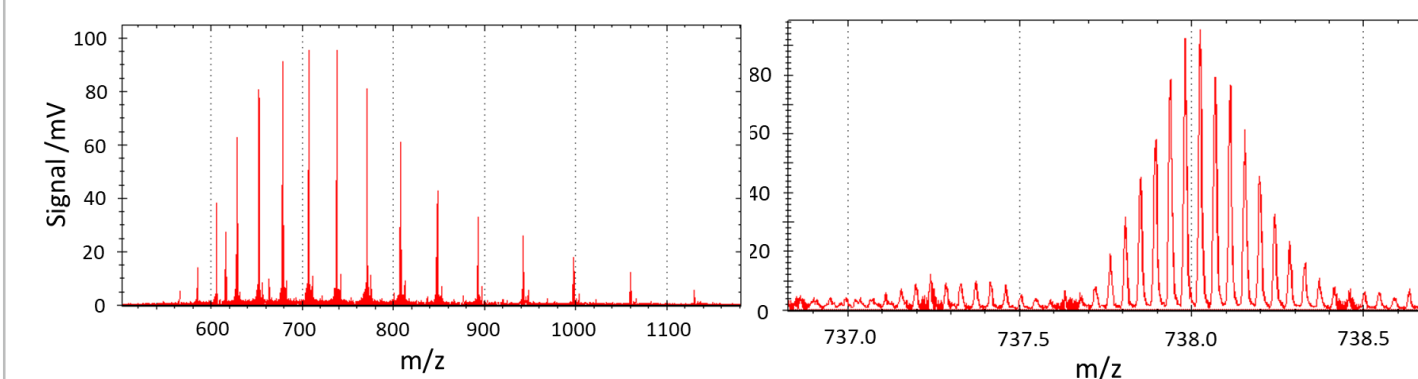


Figure 7. Myoglobin full-MS profile spectrum and zoom in of m/z 738 envelope

CONCLUSION

A powerful new mass analyser has been introduced that combines extremely high ion transmission, speed and resolving power with mass measurement stability and dynamic range. It is hoped that this unique technology will enable a wide range of scientific inquiry and contribute to the growth of mass spectrometry as a field.

REFERENCES

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ACKNOWLEDGEMENTS

The authors are grateful to acknowledge the enormous contributions of fellow scientists, engineers and other colleagues involved in the development of mass spectrometry at Thermo Fisher Scientific.

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