A Robust C-Trap Ion Injection Method Incorporating Electrodynamic Squeezing

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ABSTRACT

A new ion injection method was developed for C-Trap-Orbitrap based mass analyzers such as the Thermo Scientific™ Orbitrap Exploris™ series of instruments. Ions were first pre-accumulated within the collision cell ("Ion Routing Multipole", or IRM) conjoined to the C-Trap, before admission into the C-Trap. During the ion transfer period the DC voltages applied to the IRM and the C-Trap exit lens were ramped by several volts, deepening the trapping well so that reflected but poorly cooled ions were not lost on the lens.

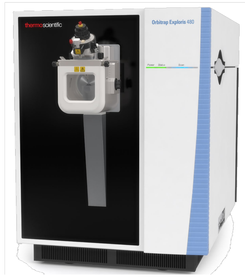
C-Trap lens performance and robustness were characterised by operating the instrument under a continuous infusion of concentrated ubiquitin over long periods to replicate heavy instrument usage and induce contamination. Optimum lens voltages for transfer of ions into the C-Trap were measured during this period.

Implementation of C-Trap/IRM voltage ramp, a weak form of electrodynamic squeezing, was observed to both widen the range of C-Trap lens voltages over which ions are transmitted, and also to reduce the shift in this window under prolonged ubiquitin exposure. An additional step was tested where a brief -20V pulse was applied to the IRM before ion transfer, to pull ions away from the lens and thus allow better focusing during injection. This was observed to greatly widen the acceptance window of C-Trap lens voltages, reducing the contamination (and deleterious effects of contamination) on the outer wall of the C-Trap lens.

INTRODUCTION

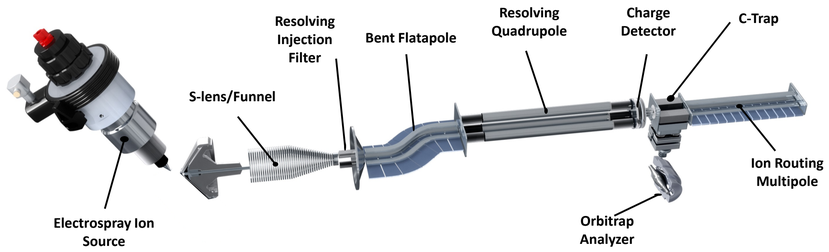
Commercial Orbitrap™ instruments first trap and accumulate ions in a curved linear ion trap (C-Trap) before pulsed extraction into the Orbitrap analyzer. The C-Trap is a gas filled curved linear ion trap, terminated by apertures that control gas flow through the trap and whose applied potentials control the admission of trapping of ions. It is preferred that the C-trap be very compact for a suitably compressed ion packet, but also have minimized pressure as buffer gas leakage into the Orbitrap or resolving quadrupole must be limited. Ions injected into the C-Trap must however be cooled sufficiently by buffer gas within the first axial oscillation or they may be lost striking the terminal apertures, causing contamination and charging effects that may snowball to greatly reduce trapping efficiency. As ion cooling is a product of trap length and gas pressure it can be seen that C-Trap must balance these competing factors.

An important advance over direct trapping of ions in the C-Trap was to first pass the ions through the C-Trap and trap them in the Ion Routing Multipole (IRM), a collision cell conjoined to the C-Trap. As this utilizes higher buffer gas pressure and greater length it could more efficiently trap energetic or high m/z ions from the source, and after thermalization return them to the C-Trap with a lower, better defined (~5eV) energy. This also allowed the C-Trap entrance lens to operate as an accelerating lens, with a stronger applied voltage, improving ion focusing through it and thus improving transmission and protecting the lens from contamination and charging effects.



Ions injected into the ultra-high vacuum Orbitrap analyzer can not be cooled by buffer gas, but are instead trapped by a process of *electrodynamic squeezing*, whereby a rising electrode voltage causes the creation of a trapping potential around the ions. Here this concept was applied to the C-Trap.

Figure 1. Ion optical layout of a Thermo Scientific™ Orbitrap Exploris™ mass spectrometer .

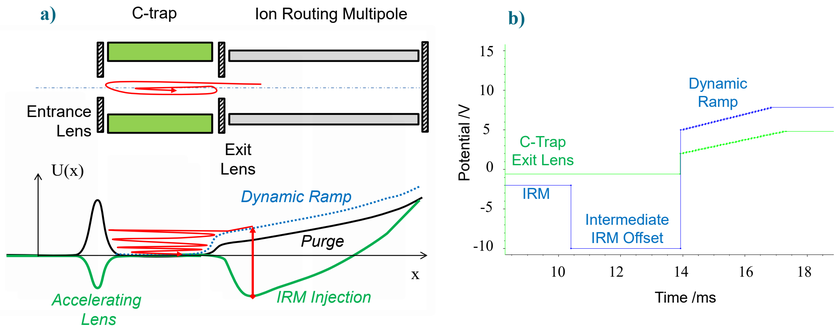


Ions were injected first through the C-Trap and into the IRM as normal, but during the purge of ions from IRM to C-Trap, the voltage applied to the IRM and aperture was ramped over time. Ions passed through the Exit Lens and C-Trap until they were reflected by the relatively strong DC barrier applied to the Entrance Lens. Upon returning to the Exit Lens, the Exit Lens potential was raised higher (dynamic ramping) so that even poorly cooled ions would not be able to escape the trapping well. As this ramp was applied over time then ions could enter the C-Trap from the IRM without having to be a finely compressed packet, and allows for the differing velocities of different masses of ions.

It was considered that the static IRM axial DC would push ions so close to the Exit Lens that focusing through the aperture could be compromised. A step was implemented before ion purge where a small 10-20V intermediate IRM offset was briefly set to pull ions away from the lens.

Figure 2. a) Illustration of C-Trap/Ion Routing Multipole configuration, and applied potential gradients during injection into IRM and subsequent purge of ions into C-Trap (intermediate DC offset step not shown).

b) . Voltages and timings of the Ion Routing Multipole and C-Trap Exit Lens offset, with electrodynamic ramping and intermediate offset applied.



METHOD

A prototype orbitrap instrument with identical layout to the Thermo Scientific™ Orbitrap Exploris™ Mass Spectrometer shown in Figure 1 was operated under prolonged (up to 97 hours) 10ml/min infusion of 3 µmol ubiquitin dissolved in Acetonitrile:Water to induce contamination. During exposure the instrument was operated at 50Hz without quadrupole isolation and a target m/z range of 150-2000.

Contamination effects were measured by regularly scanning the C-Trap Exit Lens voltage during the purge to C-Trap, and measuring the signal intensity of ions against this. This time all ubiquitin ions were fragmented with 35V normalised collision energy, to produce a wide range of mz ions to target. The mass range was set to 40-1400. This mass range results in the C-Trap RF amplitude being set to a very low level (150V), which resulted in very poor trapping of the higher m/z ions, greatly exacerbating any negative effects of aperture charging. This extreme treatment was necessary to observe relatively small charging effects over short experimental cycles.

The overall signal and background pressures were monitored to ensure consistent exposure rates, and lenses were cleaned between experimental runs. A scheme for dynamic ramping of the IRM and Exit Lens was written to give a 0.8V/ms ramp for 3ms. Experimental runs were carried out with and without the dynamic ramping process applied.

RESULTS

Figure 3 shows a comparison of the Exit Lens tuning curves for a higher m/z ubiquitin fragment with and without dynamic ramping applied. It can be seen that not only does the dynamic ramp increase the acceptance range (and shift it slightly to negative potential), it also reduces the shift of the curve over time. This indicates that the rate of contamination is successfully being reduced.

The effect is less overt for the better trapped lower m/z ions as shown in Figure 4. These suffer less from charging effects, as the trapping RF is sufficient to produce a trapping well even close to the apertures for this m/z, but nevertheless the curve can still be seen to be widened.

Figure 5 shows the impact upon the tuning curve of several masses of the implementation of a -20V intermediate IRM offset. The curve is effectively widened as the tolerance to low exit lens voltages increases, and ions in such circumstances are not simply sucked onto the lens instead of being transmitted to the C-Trap.

As different m/z ions have different tuning curves, it is highly desirable to use methods that increase the width of the curve, and maximize overlap across the mass range.

Figure 3. Dependence of ion transmission on C-Trap exit lens voltage (tuning curve) for higher m/z ubiquitin fragment with/without electrodynamic squeezing.

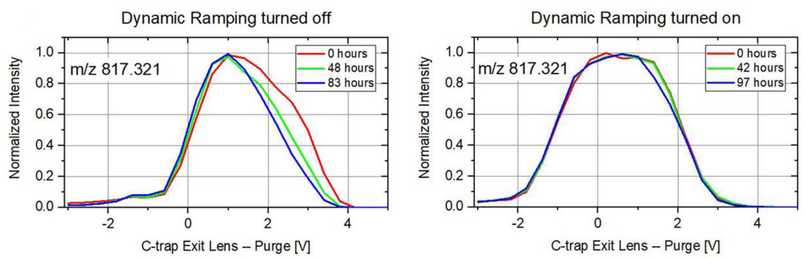


Figure 4. Dependence of ion transmission on C-Trap exit lens voltage (tuning curve) for low m/z ubiquitin fragment with and without electrodynamic squeezing, over prolonged exposure of system to ubiquitin sample.

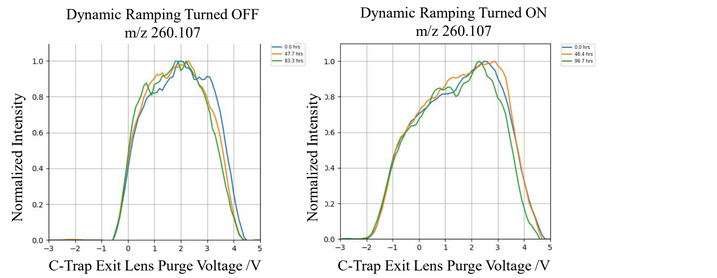
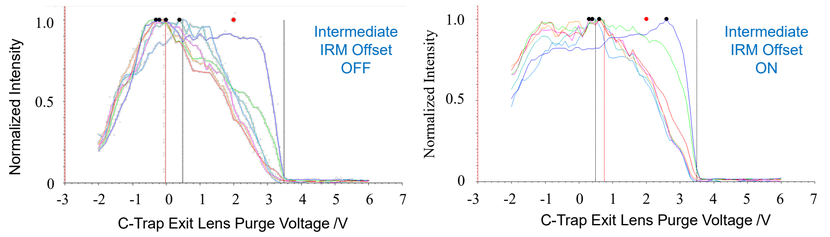


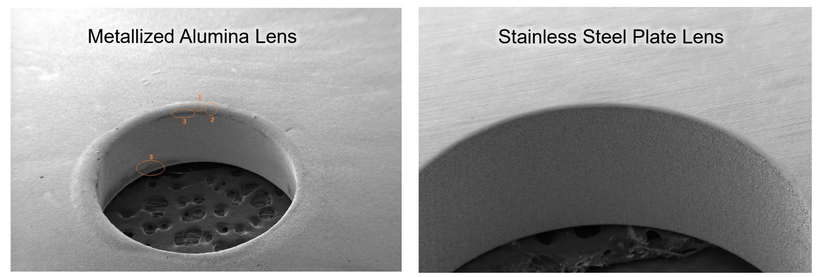
Figure 5. Dependence of ion transmission on C-Trap exit lens voltage (tuning curve) for ubiquitin fragments of m/z 659-1224 where intermediate voltage on HCD quadrupole was either disabled or set to -20V.



Another related matter affecting lens charging is that whilst contamination by sample may create a base for charging effects by coating metal with non-conducting material, such contamination may also be present from dust or imperfections in the lens surface. For lenses that are formed from a thin metal coating on a non-conductive substrate such as ceramic or PCB, additional risk occurs from scratches during handling or compromised coating.

Figure 6 shows scanning electron microscope images of aperture surfaces for a lens formed via a metallized ceramic substrate and one from a stainless steel plate. The coated ceramic lens can be seen to have dark patches on the inside, indicating differences in conduction under the electron beam, or charging. The steel plate lens is by comparison extremely even, and such design has been adopted into Orbitrap Exploris™ mass spectrometers.

Figure 6. Scanning electron microscopy images of different models of C-Trap lenses. Older style metal coated ceramic lens shows regions of charging (dark patches), which are not present on the newer steel plate design.



CONCLUSIONS

A method for injecting ions into the C-Trap incorporating electrodynamic squeezing was implemented into a prototype C-Trap Orbitrap instrument, and shown to improve robustness of the C-Trap lenses to contamination.

An additional intermediate voltage step was also implemented to improve transmission through the C-Trap exit lens, and shown to further improve the voltage tolerance and robustness of the exit lens.

TRADEMARKS/LICENSING

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