

## Mass spectrometry

## Realize a universe of possibilities

### Orbitrap Astral Zoom mass spectrometer

**Benefits**

- High quality data with faster throughput due to higher sensitivity, dynamic range, and scan rate
- Higher sensitivity measurements for low input samples, including single-cell proteomics and immunopeptidomics experiments
- Deeper coverage and increased dynamic range for label-free quantitation (LFQ) and higher resolution to support multiplexing with Tandem Mass Tag™ (TMT™) 32-plex analysis
- Orbitrap and Astral analyzer BioPharma option for comprehensive protein characterization
- Full experimental flexibility with revolutionary instrument control software
- Common user interface with Thermo Scientific™ Orbitrap™ Astral™, Thermo Scientific™ Orbitrap Exploris™, Thermo Scientific™ Orbitrap Tribrid™, and Thermo Scientific™ TSQ™ Triple Quadrupole mass spectrometers

The Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer delivers novel capabilities for faster throughput, deeper coverage, and higher sensitivity with accurate and precise quantitation and expanded flexibility. The next generation Orbitrap Astral MS platform includes a Fast Ion Filter, Bent Trap for Pre-Accumulation, High-speed Quadrupole, Faster Stepped Collision Energy, Enhanced Dynamic Range mode, TMT HR mode, low input mode, Enhanced Spectral Processing, support for Hybrid DIA experiments, and an expanded BioPharma option including Thermo Scientific™ Astral™ analyzer support for top-down and middle-down measurements. Taken together, these features extend the performance capabilities for proteomics, metabolomics, and biopharma applications to more fully characterize complex samples from single-cells to bulk samples with higher speed and sensitivity.

## Key capabilities

- Next generation architecture including a quadrupole mass filter and Thermo Scientific™ Orbitrap™ and Astral high resolution accurate mass (HRAM) analyzers operating in parallel, enabling high acquisition rates and maximum experimental flexibility
- Novel front end enabling pre-accumulation of ions in Bent Trap to increase ion flux at higher acquisition rates
- Fast Ion Filter and High-speed Quadrupole maximize ion transmission with faster isolation switching times
- Modified Ion Routing Multipole enables Higher-energy Collisional Dissociation (HCD), including Faster Stepped Collision energy
- Parallel handling of six ion packages to maximize instrument utilization
- Improved MS<sup>2</sup> acquisition rates of up to 270 Hz in the Astral analyzer with resolution of 80,000 FWHM at  $m/z$  524
- Low input mode for Astral analyzer for significantly improved sensitivity
- Astral analyzer resolution of at least 100,000 FWHM at  $m/z$  138, enabling resolution of TMTpro™ 32-plex reporter ions
- Enhanced spectral processing for improved spectral deconvolution with the Astral analyzer
- MS/MS acquisition rates of up to 40 Hz with 7,500 resolution at  $m/z$  200 for the Orbitrap analyzer
- Maximum Orbitrap analyzer resolution of up to 480,000 FWHM at  $m/z$  200
- Enhanced Dynamic Range (eDR) mode Orbitrap scan to five orders of magnitude intrascan dynamic range
- Enhanced Vacuum Technology improves Orbitrap and Astral analyzer performance
- Thermo Scientific™ Precursor Fit enables precursor ion selection for a defined specificity
- Advanced Peak Determination (APD) improves precursor annotation in data-dependent experiments
- Intuitive, flexible drag-and-drop instrument method editor simplifies method development and creation of unique analytical workflows
- Thermo Scientific™ EASY-IC™ internal calibrant source generates ions for real-time mass calibration of Orbitrap analyzer and automated calibration of the Astral analyzer in both positive and negative modes
- Extensive pre-built and optimized method templates that are fully customizable for a variety of application workflows
- Streamlined calibration routines with improved transmission for ions with  $m/z$  <200 to Orbitrap and Astral analyzers
- Low energy consumption instrument featuring a low maintenance dry pump
- Optional Thermo Scientific™ BioPharma option provides expanded mass range capabilities for native analysis of intact proteins and protein complexes using the Orbitrap analyzer and support for top-down and middle-down MS<sup>2</sup> experiments using the Astral analyzer

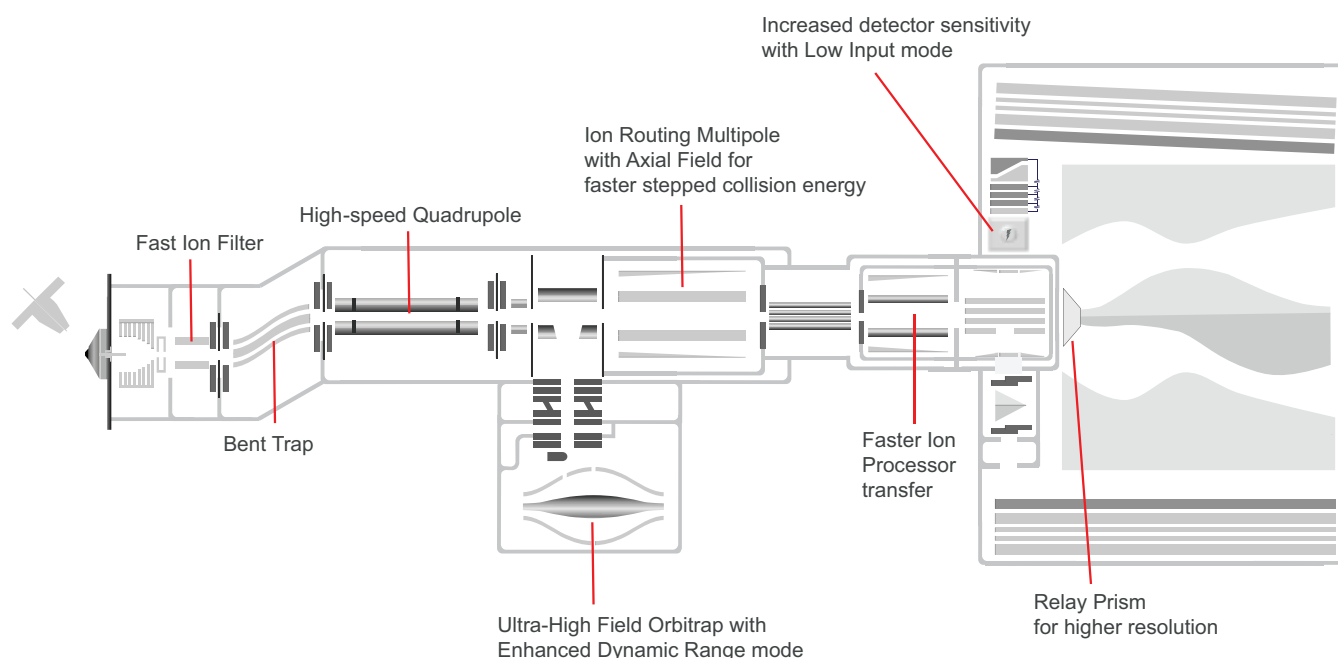


Figure 1. Orbitrap Astral Zoom mass spectrometer instrument schematic.

## Hardware features

### Next generation instrument architecture

- Orbitrap Astral platform unique architecture includes a quadrupole mass filter and Orbitrap and Astral HRAM analyzers, allowing for high acquisition rates and maximum experimental flexibility for dissociation and detection of fragment ions
- Full parallelization of Orbitrap analyzer MS and Astral analyzer MS<sup>2</sup> analyses through simultaneous handling of 6 ion packages for maximum instrument utilization

### Active Ion Management (AIM+) technology

AIM+ maximizes ion transmission and utilization, from injection to detection, using a novel hardware design for precise management of electrical fields and removal of noise to achieve unprecedented levels of quantitative performance

### Ion source

#### Thermo Scientific™ OptaMax NG™ ion source

- Adjustable heated electrospray ionization (HESI) probe provides ultimate sensitivity and ease of use
- Flow rates 1–1000  $\mu\text{L}/\text{min}$
- Designed for maximum performance for a broad range of molecular ions
- Enhanced ruggedness
- Reduced chemical noise with sweep gas
- Optional APCI probe compatible with liquid flow rates of 50–1000  $\mu\text{L}/\text{min}$  without splitting; the APCI probe can be upgraded to optional APPI capability

### Ion optics

#### The Atmospheric Pressure Ionization (API) interface consists of:

##### *High-capacity ion transfer tube (HCTT)*

- Increased ion flux into the vacuum system for improved sensitivity and desolvation
- Vent-free maintenance

##### *Electrodynamic ion funnel (EDIF)*

- A radio frequency (RF) device that efficiently captures and transmits ions as they leave the HCTT
- Automatic tuning results in broad ion transmission curves with reduced ion losses, increasing sensitivity

##### *EASY-IC internal calibrant source*

- Generates internal calibrant ions for real-time mass calibration of spectra in positive and negative polarity modes for the Orbitrap analyzer

- Provides <1 ppm RMS drift over 24 hours, measured with FlexMix Calibration Solution for the Orbitrap analyzer
- Automatic single point recalibrations for the Astral analyzer to reduce mass drift

##### *Fast Ion Filter*

- Rapid ion filtering reduces background noise

##### *Bent Trap*

- Double bent geometry removes neutrals and high velocity clusters
- Automatic gain control (AGC) modulated pre-accumulation of ions when needed to increase duty cycle and sensitivity

##### *High-Speed Quadrupole*

- Segmented quadrupole mass filter for precursor ion selection with variable precursor isolation width from 0.4 to 2,000 u
- MS/MS precursor ion selection with high transmission from  $m/z$  40 to 2500
- Improved robustness with patented RF- switching mode
- Faster quadrupole isolation switching down to 0.6 ms for ion selection

##### *Ion-routing multipole (IRM)*

- Efficient ion trapping and storage
- Performs higher-energy collisional dissociation (HCD) with nitrogen collision gas for acquisition of Orbitrap and Astral analyzer stepped energy HCD MS<sup>2</sup> spectra
- Additional axial field for faster ion transfer and improved electronic switching for faster fragmentation speed when using two or three stepped HCD energies
- Selection of absolute collision energy (CE) or normalized collision energy (NCE) for precursor fragmentation
- Efficient and reliable ion transfer between the ion-routing multipole, Orbitrap analyzer, and Astral analyzer
- Variable pressure control with BioPharma option, optimizing performance for small and large molecule workflows

##### *Automatic Gain Control (AGC)*

- Dynamic adjustment of ion injection time based on real-time measurement of ion flux

##### *Orbitrap analyzer*

- Ultra-high-field Orbitrap mass analyzer
- Low noise detection pre-amplifier
- 4 kV central electrode voltage
- Enhanced dynamic range mode

## Next generation high-resolution accurate mass analyzer

- Ion processor with dual pressure regions to efficiently trap, store, and fragment ions with HCD, and prepare ion packet for Astral analyzer MS<sup>2</sup> acquisition at rates up to 270 Hz
- Injection optics maximize ion transmission and alignment to minimize losses
- Gridless asymmetric ion mirrors direct ions into transversal asymmetric oscillations
- Ion foil shapes and maintains ion packet to maximize transmission and resolution and provide a higher space-charge capacity
- Relay prism controls asymmetric track length for increased resolution
- High dynamic range (HDR) detector assembly aligns and post-accelerates ion packet for single-ion sensitivity HRAM detection over a wide  $m/z$  range with toggleable low input mode for increased sensitivity
- High linear dynamic range two-channel detection system
- Unique HDR detector design for low noise and extended lifetime

## Vacuum system

- Three turbomolecular pumps and a single dual-stage oil-free roughing pump for reduced energy consumption
- Two split-flow turbomolecular pumps for ultra-high vacuum in Orbitrap and Astral analyzers
- Stainless steel and aluminum high-vacuum analyzer chambers
- Enhanced vacuum technology reduces the pressure in the ultra-high vacuum region of the Orbitrap analyzer to  $<10^{-9}$  Torr and to  $<10^{-8}$  Torr in the Astral analyzer
- Improved Orbitrap analyzer performance at a wider range of collision gas settings

## Analog inputs

- Channel 1 analog input (0–10 V); Channel 2 analog (0–2 V)

## Optional hardware

### BioPharma option

- Provides additional capabilities for the analysis of large molecules, including intact proteins and protein complexes sprayed under denatured or native conditions
- Extends mass transmission and detection up to  $m/z$  8,000 in the Orbitrap analyzer
- Enables acquisition of top-down and middle-down Astral analyzer MS<sup>2</sup> spectra and spectral averaging of up to 100 Astral analyzer  $\mu$ scans

## Thermo Scientific™ Ardia™ Platform

The Ardia Platform is designed for chromatography and mass spectrometry users with an intuitive web-based interface that enables them to work with their instruments and data, both in and out of the lab

- Automatically stores data in a secure, central location for all connected Thermo Scientific chromatography and mass spectrometry systems
- Enhances connected Thermo Scientific and third-party data processing software with additional capabilities such as automation and connectivity to streamline processing and simplify data sharing
- Delivers core functionality that enables online scheduling of instruments, visualization and electronic recording of instrument-related activities, viewing MS and chromatography data in a single location, and dashboarding to provide key lab insights

The Ardia Platform is delivered pre-installed on a dedicated appliance to support on-premise central data storage with transfer, archival and backup capabilities and data processing using the CHIMERYS™ intelligent search algorithm.

## Thermo Scientific™ OptiSpray™ ion source

- Maximum low-flow electrospray ionization performance and reproducibility using automated spray optimization with integrated sheath gas control
- Simple installation, use, and usage tracking of cartridge nano and capillary columns
- Enhanced robustness with exchangeable emitters and inactive spray position between injections
- Supports Thermo Scientific™ OptiSpray™ cartridge columns
- Compatible with flow rates from 50 to 10,000 nL/min

## Thermo Scientific™ EASY-Spray™ ion source

- Easy-to-use nano-ESI source
- Supports Thermo Scientific™ Easy-Spray™ and Thermo Scientific™  $\mu$ PAC™ columns
- Compatible with flow rates from 50 to 4,000 nL/min

## Thermo Scientific™ NanoSpray Flex NG™ ion source

- High nano-ESI flexibility and probe position adjustment
- Supports widest range of column formats and static and dynamic electrospray ionization
- Compatible with flow rates from 50 to 4,000 nL/min



### Thermo Scientific™ FAIMS Pro Duo interface

- Performs online gas-phase fractionation based on differential ion mobility
- Reduces noise and substantially increases system robustness
- Optimized performance for 100 nL/min to 1 mL/min flow rates

### APCI probe for OptaMax NG ion source

- Compatible with flow rates of 50-100  $\mu\text{L}/\text{min}$  without splitting
- The APCI probe can be upgraded to APPI

## Software features

### Orbitrap Astral Zoom MS instrument control software

- Tune application for instrument calibrations and checks, diagnostics, and manual data acquisition
- Method editor with a comprehensive application-specific template library, method setup supported by tooltips, and a drag-and-drop user interface to facilitate method development

### Instrument Control PC

- High-performance PC with Intel® microprocessor
- High-resolution LED color monitor
- Microsoft® Windows® 10 Enterprise (Long Term Service version) operating system

### Thermo Scientific™ Xcalibur™ software

- Xcalibur software is the control software for the next-generation Thermo Scientific mass spectrometer portfolio
- Shared instrument control software interface with Orbitrap Astral MS, Orbitrap Tribrid MS, Orbitrap Exploris MS, TSQ MS, and Stellar MS platforms to accelerate familiarization and reduce training needs
- Optional integration with the Thermo Scientific™ Ardia™ Platform for automated data transfer and secure central storage

## Operation modes

### Resolution settings

- Orbitrap analyzer ranging from 7,500 to 480,000 at  $m/z$  200
- Astral analyzer is operated at 80,000 FWHM at  $m/z$  524 or with TMT HR mode at 100,000 FWHM at  $m/z$  138

### High Resolution-Data Independent Acquisition (HR-DIA)

- Acquisition logic in which the scan event execution follows a predetermined pattern set by the experimental settings
- Enables acquisition of user-defined full scan using the Orbitrap analyzer and parallel acquisition of Astral analyzer DIA MS<sup>2</sup> scans

### Data-Dependent Acquisition (DDA)

- Acquisition logic in which a decision for a scan event execution is made in real-time, based upon certain criteria being met in a master scan
- Top speed: Maximizes the number of high-quality MS<sup>2</sup> spectra in each cycle by intelligently scheduling MS and data-dependent MS<sup>2</sup> scans based on a user-defined time between adjacent survey spectra
- TopN: Enables a user-defined maximum number of high-quality MS<sup>2</sup> spectra from each cycle

### Hybrid DIA

- Acquisition logic in which the scan event execution follows a predetermined pattern set by the experimental settings of user-defined full scan using the Orbitrap analyzer and parallel acquisition of Astral analyzer DIA MS<sup>2</sup> scans
- Adds intelligent acquisition of directed data-dependent MS<sup>2</sup> scans on endogenous targets of interest in the Orbitrap or Astral analyzer based on the detection of corresponding synthetic isotopically labeled targets in survey scan and confirmatory data-dependent MS<sup>2</sup> scan

### Enhanced Dynamic Range (eDR) Mode

- Multiplexed isolation of  $m/z$  sub-ranges and acquisition of Orbitrap full scans to maximize the number of detected analytes

### TMT HR mode

- Higher resolution Astral analyzer mode enabling dual scan acquisition for peptide ion fragment detection and higher resolution TMT reporter ion measurement, for optimal support of TMTpro 32- and 35-plex applications

### Enhanced spectral processing

- Spectral deconvolution algorithm to improve Astral analyzer peak assignment and resolution

### Advanced Peak Determination (APD)

Precursor annotation algorithm for improved charge state assignment to increase the number of precursors available for data-dependent analysis

### Dynamic scan management

Enables intelligent, real-time scheduling, parallelization, and prioritization of acquisition events, and selection, sorting, and routing of ions to different analyzers based on user-selected parameters, including precursor  $m/z$ , intensity, and/or charge

### Automatic gain control

Ensures that the optimum number of ions are utilized within a given ion injection time range for any acquisition type, including the use of pre-accumulation in the bent trap

## Thermo Scientific™ SureQuant™ internal standard (IS) targeted protein quantitation workflow

Intelligent acquisition of directed high-quality data-dependent MS<sup>2</sup> scans on endogenous targets of interest in the Orbitrap or Astral analyzer based on the detection of corresponding synthetic isotopically labeled targets in survey scan and confirmatory fast data-dependent MS<sup>2</sup> scan.

### Precursor fit filter

Allows selection of precursors with defined specificity

## Application-specific operation modes and associated system templates

- Peptide application mode
  - Used for the analysis of complex peptide mixtures and includes extensive pre-built method templates for data-dependent acquisition (DDA), data-independent acquisition (DIA), TMT, and SureQuant for multiple application areas including plasma and single-cell proteomics
- Small molecule application mode
  - Pre-built method templates for small molecule analysis including the option to enable 'Mild Trapping' as a global setting that applies to the entire run for Orbitrap analyzer scan events to reduce unwanted fragmentation of particularly labile compounds
- Intact protein application mode (included with BioPharma option)
  - Pressure regimes (low, medium, and high) can be selected for best performance with native and denaturing conditions
- Low input application mode
  - Astral analyzer operation mode that increases the sensitivity of ion detection for low input samples

## Scan Functions

- Full MS using Orbitrap analyzer
- Selected Ion Monitoring (SIM)
  - in DDA experiment (discovery)
  - in targeted SIM experiment based upon a sample-dependent, user-defined mass list
  - with Targeted Mass Filter applied (confirmation)
- MS<sup>2</sup> scan using Orbitrap or Astral analyzers in DDA experiments following a master scan (e.g., a full scan):
  - using TopN or Top Speed logic (discovery)
  - using Targeted Mass Filter (confirmation)
  - by combining TopN or Top Speed and a Targeted Mass Filter to include confirmation in discovery experiments

- Targeted MS<sup>2</sup> (tMS<sup>2</sup>) scan using Orbitrap or Astral analyzer, based upon a sample-dependent, user-defined target mass list
- MS<sup>2</sup> scan using Orbitrap or Astral analyzer by data-independent acquisition (DIA)
  - With full MS scan using the Orbitrap analyzer (e.g. parallel acquisition of Full MS scan in Orbitrap analyzer with simultaneous acquisition of DIA MS<sup>2</sup> scans in the Astral analyzer) with adjustable, experiment-dependent widths
- Hybrid-DIA
  - Acquisition of Orbitrap analyzer Full MS scans and simultaneous Astral analyzer DIA MS<sup>2</sup> scans
  - Intelligent triggering of directed data-dependent MS<sup>2</sup> scans on endogenous targets of interest using the Astral analyzer based on detection of corresponding synthetic isotopically labeled targets in a survey scan and confirmatory fast data-dependent MS<sup>2</sup> scan
- AIF scan: All Ion Fragmentation

## Filters for data-dependent experiments

Filters are used to optimize data-dependent decisions in real time based on the goals of the experiment. To achieve optimum results when applying application-dependent filter settings, the user is guided by the application-dependent default parameter settings and tool-tip text.

Below are some of the available filters that can be used in a data-dependent experiment:

- Dynamic Exclusion
- Intensity
- Precursor Fit
- Charge State
- Targeted Mass Inclusion
- Targeted Mass Exclusion
- Monoisotopic Precursor Selection (MIPS)
- Precursor Selection Range
- Apex Detection
- Isotope Exclusion

## Other method capabilities

### Alternate precursor sorting

Modifies the logic in which precursor order is selected for fragmentation

### Targeted mass trigger

Performs data-dependent scans upon the detection of a defined product ion for targeted, confirmational experimental set-ups

## Isolation offset

Custom centering of the precursor isolation window, optimized for broad isotopic distributions or SureQuant acquisition support

## Quanfirmation workflow

Synchronous acquisition of HRAM SIM in the Orbitrap analyzer with MS<sup>2</sup> in the Astral analyzer for precursor identity confirmation

## Optional data acquisition and analysis software

### Thermo Scientific™ Proteome Discoverer™ software

- Connects with the Ardia Platform
- Flexible, expandable software for the qualitative and quantitative analysis of proteomics data
- Simplifies a wide range of proteomics workflows, from protein and peptide identification to PTM analysis to isobaric mass tagging and label-free quantitation with both DDA and DIA data
- Includes the CHIMERYS intelligent search algorithm to enhance DDA, DIA, TMT, and phosphoproteomics data analysis

### Thermo Scientific™ ProSightPD™ software

- Processing node within Proteome Discoverer software that allows execution of ProSight searches of top-down and middle-down data

### Thermo Scientific™ BioPharma Finder™ software

- Connects with the Ardia Platform
- Offers complete workflows for peptide mapping, host cell protein analysis, and MAM development, ensuring high-confidence identification and quantification
- Powerful visualization tools for quality attributes, sequence variants, and post-translational modifications
- Simplifies and automates data processing, reducing manual intervention and increasing throughput for both expert and non-expert users

### Thermo Scientific™ Compound Discoverer™ software

- Connects with the Ardia Platform
- Streamlines small molecule unknown identification, determination of differences between samples, and elucidation of biological pathways with an integrated suite of data analysis tools

### Thermo Scientific™ Tracefinder™ software

- Acquire and process high-throughput screening and quantitation with built-in intelligence, driving productivity gains from data acquisition and processing to reporting

## Installation requirements

### Power

- 2 x 208V/230V 15/16A for the instrument, separately fused (tripping characteristics C).
- 1 x 200V/230V 16A for the Edwards nXL110iDE pump (Fuse tripping characteristics C)
- 1 x 100V/240V 15/16A for Instrument Control PC and accessories

### Gas

- High purity Nitrogen (99.5% pure, flow rate 15 L/min) gas supply for the API source and ultra-high purity nitrogen (UHP, 99.999%) with less than 1 ppm each water and oxygen for ion routing multipole (IRM) and Ion Processor

### Dimensions (w × d × h)

- 952 × 1962 × 1392 mm

### Weight

- 680 kg without data system, vacuum rough pump, and optional items

### Environment

- System averages power consumption of 1,400 W for low-flow and 1,500 W for high-flow operation
- Heat output of approximately 5,200 Btu/hr
- Operating environment must be 18–27 °C and relative humidity must be 20–80% with a non-condensing and non-corrosive atmosphere
- Maximum temperature fluctuation of 0.5°C/10 min
- Designed for indoor use at an altitude of up to 3000 m (10,000 ft) above sea level

### Optional FAIMS Pro Duo interface

- Nitrogen supply for FAIMS Pro Duo interface is 99.5% pure nitrogen at >20 L/min (100 psi)

## Performance specifications – Orbitrap analyzer

Orbitrap mass range	Standard mass range $m/z$ 40–6,000, $m/z$ 40–8,000 with BioPharma option
Orbitrap resolution	Standard resolution settings range from 7,500 to 480,000 (FWHM) at $m/z$ 200, with isotopic fidelity up to 240,000 FWHM
Acquisition rate*	Orbitrap MS <sup>2</sup> acquisition rates up to 40 Hz when measured using a data-dependent experiment with Pierce™ FlexMix™ Calibration Solution, MS and HCD MS <sup>2</sup> resolution setting of 7,500 @ $m/z$ 200
Orbitrap mass accuracy*	External calibration achieves <3 ppm RMS drift over 24 hours, measured with FlexMix Calibration Solution; internal calibration achieves <1 ppm RMS drift over 24 hours, measured with FlexMix Calibration Solution
MS/MS Electrospray Ionization (ESI) Orbitrap sensitivity	MS/MS: 50 fg reserpine on column S/N 100:1 SIM: 50 fg reserpine on column S/N 150:1
Dynamic range*	Five orders of magnitude intrascan dynamic range

## Performance specifications – Astral analyzer

Astral mass range	Standard mass range $m/z$ 40–6000
Astral resolution	80,000 FWHM at $m/z$ 524 or with TMT HR mode enabled 100,000 FWHM at $m/z$ 138
Acquisition rate*	Astral analyzer MS <sup>2</sup> acquisition rates up to 270 Hz
Astral mass accuracy*	External calibration achieves <5 ppm RMS drift over 24 hours, measured with FlexMix Calibration Solution
Dynamic range	>1,000 within a single microscan Astral analyzer mass spectrum

\* Under defined conditions

 Learn more at [thermofisher.com/orbitrapastralzoom](https://thermofisher.com/orbitrapastralzoom)

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