

Thermo Scientific [™] Orbitrap [™] Tribrid [™] MS Series: Instrument Control Software v.4.2 QF1

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The world leader in serving science



Software Release

Flexera

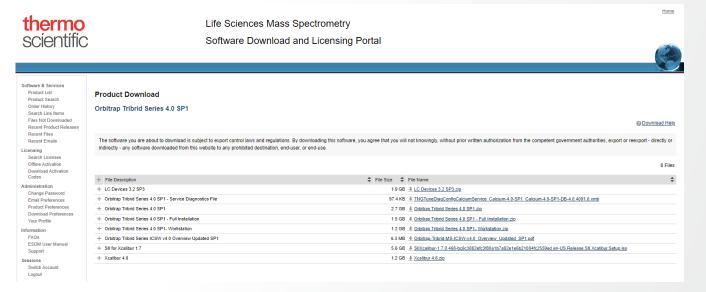
Orbitrap Tribrid MS Series ICSW 4.2 QF1 is available to customers using Flexera software distribution site. It is no longer possible to order a physical DVD. Instrument Control Software will be a digital distribution only.

Customers new to the Flexera site should use the following link:

https://thermo.flexnetoperations.com/control/thmo/RegisterMemberToAccount

After setting up an account, customers can access the site using the following link: <u>https://thermo.flexnetoperations.com/control/thmo/login</u>

In the 'Product List' page, find 'Instrument – Orbitrap Tribrid Series' and identify Orbitrap Tribrid Series 4.2 QF1 in the subfolder



Orbitrap Tribrid Series Instrument Control Software v 4.2 QF1

Thermo Fisher

Orbitrap Ascend Editions, Orbitrap Eclipse, Orbitrap Fusion Lumos, Orbitrap Fusion, Orbitrap IQ-X, Orbitrap ID-X MS Systems



Thermo Scientific[™] Orbitrap Ascend Editions Tribrid[™]



Thermo Scientific[™] Orbitrap Fusion Lumos[™] MS



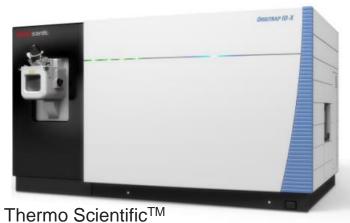
Thermo Scientific[™] Orbitrap Eclipse[™] MS



Thermo Scientific[™] Orbitrap IQ-X[™] MS



Thermo Scientific[™] Orbitrap Fusion Lumos[™] MS



Orbitrap ID-X[™] MS

Installation Directory Changes

Upgrading from CA 3.5 – CA 4.0 to CA 4.2 QF1

- When upgrading to CA 4.2 QF1 from legacy builds between 3.5 & 4.0 (inclusive) there is a new folder structure. These changes are already applied in ICSW 4.1, 4.1 SP1, & 4.2
 - New folder structure will be established as described on next slide
 - Content will be moved from important folders in the previous folder structure to the new folder structure
 - Content from MSI and msx folders *will* be transferred
 - Previous folders will remain in the old folder structure under the previous build number location
- Downgrading from Calcium 4.2 QF1, to a version using the older folder structure (4.0 or earlier)
 - Customer will not be allowed to downgrade from 4.2 QF1 without having to interact manually.
 First to uninstall and then re-install (this is for downgrading from CA 4.2 QF1)
 - Uninstall 4.2 QF1 control panel
 - Then run earlier version of software from that version's installer
 - User will have to *manually* back up MSI and msx folders and transfer to corresponding folder structure in downgraded software

Installation Directory Changes

Folder Structure for CA 4.2 QF1, CA 4.2, CA 4.1 & CA4.1SP1

- Clean install of ICSW CA 4.2
- C:\ProgramData\Thermo Scientific\Instruments\
 - Data
 - Reports
 - Folders
 - Backup
 - Images
 - DB Backup created only when database backup is performed
 - TNG
 - "Product name"
 - 4.2
 - Data
 - Systems
 - Logs
 - msx
 - Installer Logs
 - Archive
 - MongoDB

- C:\ProgramFiles\Thermo Scientific\Instruments\
 - TNG
 - "Product name"
 - 4.2
 - System
 - Programs
 - ICL
 - MSI
 - Help
 - Manuals

New Features & Usability Enhancements

Orbitrap Tribrid Series Instrument Control 4.2 QF1

- New Hardware Support (Orbitrap Ascend Editions only)
 - *New* High-Quad, for controlled isolation up to 8000m/z
 - *New* High mass calibration support (High-Quad only)
 - *New* Quadrupole frequency switching support (High-Quad only)
- *New* Tune Alert Window
- **Enhanced** Unclogging routine for Autoready source emitter
- **Enhanced** Input for HCD in Tune supports multiple entries to control single or stepped collision energy mode
- New Fragment Ion Indexing for RTLS for faster searching
- **Extended** Enzyme lists for RTS
- New Exclude Isotopes for Targeted Mass Exclusion and Targeted Loss Exclusion filters
- *New* Support for TMT 32plex
- *New* Resolving powers for Orbitrap scans 90k & 75k (not IDX/IQX)

New Features & Usability Enhancements

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Orbitrap Tribrid Series Instrument Control 4.2 QF1

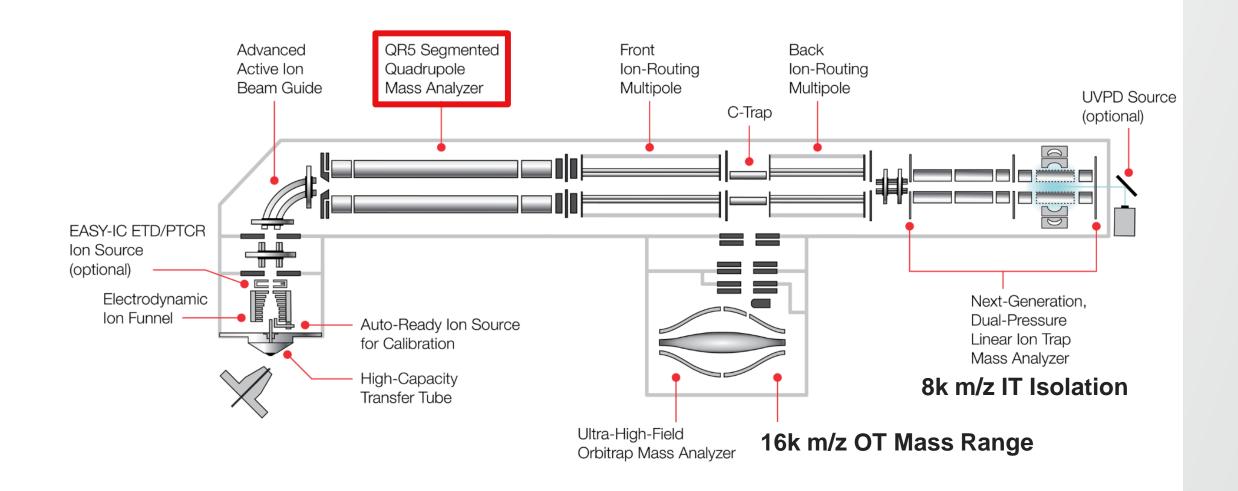
Usability Enhancements

- Improved injection time parallelization
- Improved SPS calibration routines
- Improved template management (Ascend Editions Only)

New High-Quad supports isolation range to 8000m/z

- Ascend Editions only (additional license required)
 - New quadrupole coil box that is cable of "frequency switching" to enable quadrupole isolations beyond 2000 m/z.
 - When the quadrupole mass filter precursor is > 2000 m/z the quadrupole RF coil box will switch from "high frequency" to "low frequency" mode. At this lower frequency we can isolate out to 8000 m/z.
 - The high RF frequency will be ~1080 kHz and the low RF frequency will be ~500 kHz.
 - To enable this new mode of operating the quadrupole mass filter, we require
 - New RF dip
 - New coarse calibration
 - New fine calibration routines.

- **1. Extended** the mass filter range to <u>8,000 m/z</u> to enable native MS workflows
- 2. Preserved the quadrupole performance in the normal mass range (2,000 m/z) [0.4 1,500 Th isolation widths]



New Coarse Calibrations

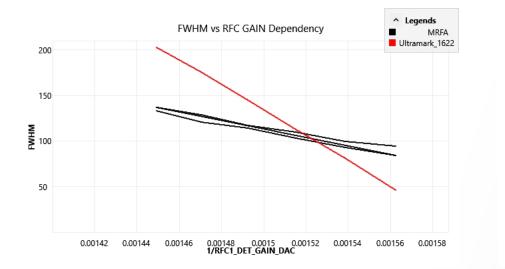
• Ascend Editions only (additional license required)

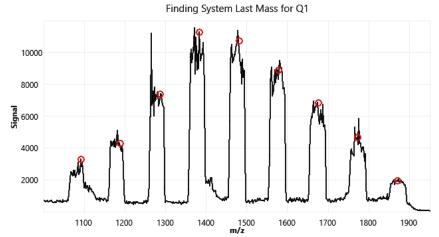
UI Changes:

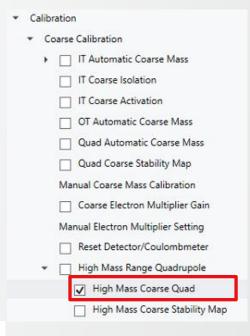
• On an Ascend Editions *with* the High-Quad license, there is a new tab under "coarse calibration" for the new "High Mass Coarse Quad" calibration.

Brief calibration description:

- The new coarse calibration procedure employs a 2 step process.
- First the instrument measures the relationship between FWHM and RFC1 detected gain dac. The intention is to produce a flat quadrupole scan line
- Second the instrument ramps the quadruple along the scan line to calculate the quadrupole last mass.







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New Fine mass & Resolution Calibrations

• Ascend Editions only (additional license required)

UI Changes:

- On an Ascend Editons with the High-Quad license, there is a new tab under "Positive Extended, Quadrupole High Mass Range" for the new quadrupole high mass range calibrations.
- There are two sets of calibrations
 - Mass and Resolution FlexMix and AHFP
 - Wide Isolation Window FlexMix and AHFP

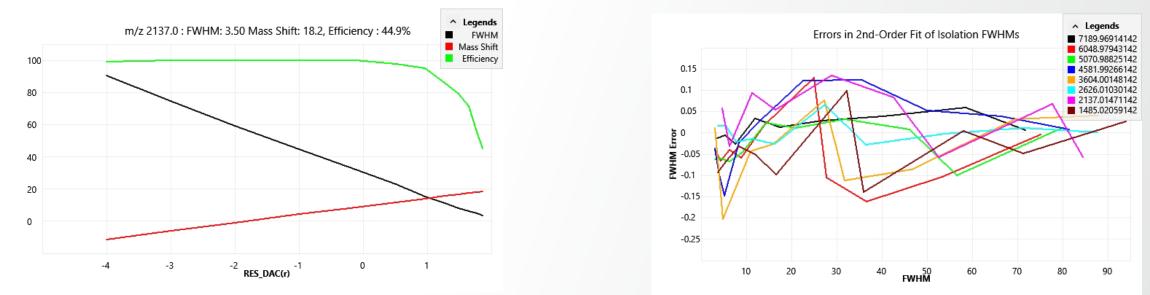
 Standard Calibration
Positive
 Positive Extended
Intact Protein Mode
 High Mass Range
 Quadrupole High Mass Range
Mass and Resolution - FlexMix
Mass and Resolution - AHFP
Wide Isolation Window - FlexMix
Wide Isolation Window - AHFP

New Fine mass & Resolution Calibrations

• Ascend Editions only (additional license required)

Brief calibration description:

- The new fine mass and resolution calibrations calibrate the quadrupole narrow isolation setting.
- The two versions of the fine mass and resolution calibrations are identical except for the calibrant masses that they use.
- In both cases the calibration measures the relationship of the mass shift and isolation width at various res DAC settings and calculates parameters which define the fine isolation region (5 Th - ~ 100 Th in the high mass range).
- The AHFP calibration is given priority because it gives much better results across the entire quadrupole mass range (2000 8000 m/z). With FlexMix, extrapolation from the last mass of 2722 m/z can cause isolation mass and width errors.

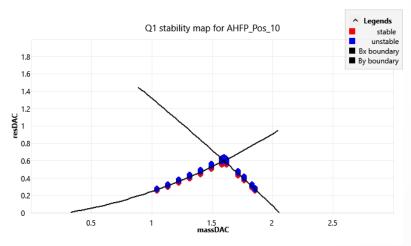


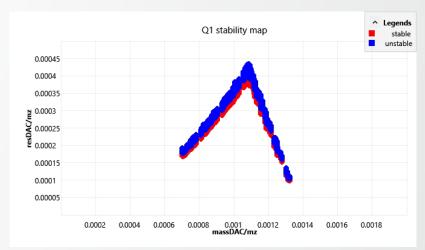
New Wide Isolation Window Calibration

• Ascend Editions only (additional license required)

Brief calibration description:

- The Wide Isolation Window calibration maps out the stability diagram of the quadrupole over a wide range of massDAC and resDAC settings for the calibrants of interest.
- It does this by scanning each of the calibrants through many massDAC and resDAC settings while monitoring the stability
 of the calibrant.
- It then uses a logistic regression model (gradient descent) to model the Bx and By stability boundaries. These fit coefficients are stored in the cal file and used when calculating wide isolation windows (~100 Th 3,000 Th in the high mass range).





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Tune Alert Window

New Tune Alert Window

• All Models Supported

- Implementation
 - Tune
 - The system alerts the user to Info such as, Errors & Warnings. These are then displayed in the Alerts window of Tune
 - The user can set the preferences of the alerts to be displayed in the preferences

Ø Orbitrap Ascend Tune Application	4.2.4310			– 🗆 ×
thermoscientific Orbitrap Ascend		Application Mode Peptide -	Positive Profile OFF	Valve NC Syringe NC
ION SOURCE DEFINE SCAN	CALIBRATION			¢,-
Ion Source Optimizatio	0	Get Default	90	Preferences Load Diagnostics 90 Load Standard Diagnostics
lon Source Type Pos Ion Spray Voltage (V)	Heated ESI		80	80 View Calibration Reports View Other Reports
Neg Ion Spray Voltage (V)	2500		70	70 View Instrument
Sheath Gas (Arb) Aux Gas (Arb)	2		60	60 Tune Help Instrument Manuals
Sweep Gas (Arb)	2		50	50 Instrument Web Page
lon Transfer Tube Temp (°C)	350		40	 40 About Tune
Vaporizer Temp (°C)	350		30	 30

*minimized alert window



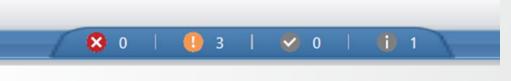
Setup Tune Alert Window

- Tune Preferences
 - In the Tune preferences menu there is a new section "Alerts Console Options"
 - By default, Errors and Warnings will be displayed
 - If items aren't selected the Tune Alert Window will not display the corresponding message type
 - The log file will automatically include 300 minutes of information before and after the alert is detected.
 - This information is then exported to the desired location when generating the logs.

Alerts (Console Options
Sho	w warnings
🖌 Sho	w recovered errors and warnings
🖌 Sho	w information
300	Minutes included in the log file before alert detected
300	Minutes included in the log file after alert detected

Expanding & Using Tune Alert Window

- Alert Window control minimized
 - Minimized Alerts window visible if there are any errors/warnings/info
 - Displays the count of the errors/warnings/info
 - Toggles the view between displaying/hiding alert lists
 - Button to maximize the table view



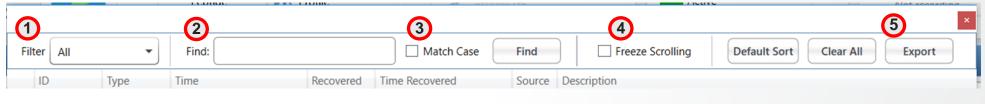
- Alert Window control expanded
 - Maximized Alerts window
 - Displays the table view of the error/ warnings/info

Filt	ter All	•	Find:		Match Case	Find	Freeze Scrolling Default Sort Clear All Export
	ID	Туре	Time	Recovered	Time Recovered	Source	Description
Ð	12020	Warning	12/5/2023 2:00:06 PM	False		PPC	analyzer temperature is out of range: -50.000 (20.0~35.0)
Ð	2000	Warning	12/5/2023 2:00:06 PM	False		PPC	raw progress 12800/58126
Ð	207	Warning	12/5/2023 2:00:02 PM	False		GH	For best results, please use standard sweep cone.
Ð		Information	12/5/2023 2:03:45 PM			GH	Source not mounted : IsRecovered = True
Ð		Information	12/5/2023 2:03:45 PM			GH	GH >>> System ready□
Ð		Information	12/5/2023 2:03:45 PM			GH	Error/Warning Cleared: ERROR_SOURCE_NOT_MOUNTED
Ð		Information	12/5/2023 2:03:45 PM			GH	Enclosure opened : IsRecovered = True
Ð		Information	12/5/2023 2:03:44 PM			GH	Error/Warning Cleared: ERROR_ENCLOSURE_OPENED
Ð		Information	12/5/2023 2:03:44 PM			GH	GH >>> Gateshead system errors cleared.D
Ð		Information	12/5/2023 2:02:50 PM			GH	Source not mounted : IsRecovered = False
Ð		Information	12/5/2023 2:02:50 PM			GH	Error/Warning Set: ERROR_SOURCE_NOT_MOUNTED
9	100	Error	12/5/2023 2:02:50 PM	True	12/5/2023 2:03:45 PM	GH	Source not mounted
Ð		Information	12/5/2023 2:02:50 PM			GH	Enclosure opened : IsRecovered = False
Ð		Information	12/5/2023 2:02:49 PM			GH	GH >>> Gateshead system error: 1010
Ð		Information	12/5/2023 2:02:49 PM			GH	Error/Warning Set: ERROR_ENCLOSURE_OPENED
9	101	Error	12/5/2023 2:02:49 PM	True	12/5/2023 2:03:44 PM	GH	Enclosure opened

Expanding & Using Tune Alert Window

Filters the results in the

table based on the selection. Default is All



1. Filter

All	•
All	
Errors	
Warnings	
Recovered	
Information	

2. Find



Filters the table with the matching value entered in the find input field

3. Match case

Match Case

Filters the table with the matching value entered in the find input field with a matching case

4. Freeze Scrolling

When freeze scrolling is enabled, the new messages in the table will not scroll automatically to the latest message in the display window.

5. Export

Exports alert messages along with the log files information preceding and succeeding the error or warning to the selected folder

×

Expanding & Using Tune Alert Window

Filter All Find: Match Case	Find Image: Freeze Scrolling Default Sort Clear All Export
ID Type Time Recovered Time Recovered	Source Description
Alert Window Features	Description
Default Sort	 Default sort button defaults the contents in the table. Default order Error Warnings Information/ Recovered Information
Copy logs at time reported Generate service bundle for time reported GH Error/Warmin GH Enclosure op	Saves the logs at the time reported to the desired location.
Generate service bundle for time reported Copy logs at time reported Generate service bundle for time reported GH Enclosure opened : IsRe	Generates a zipped file of the services
Copy logs at time recovered Copy logs at time reported Copy logs at time recovered Generate service bundle for time reported T2/5/2023 2:15:43 PM GH Enclosure opened	Copy logs at time recovered feature is enabled when the log contains a recovered error/warnings. Upon click, it saves the logs to the desired location.

AutoReady Source Enhancements

NEW Unclogging routine

- Supported Models
 - Ascend
 - IQX
- Added an item to diagnostic tree under:
 - System
 - Auto-Ready
 - Fluidics
- This is a standalone unclogging routine, which can be invoked separately from the normal Auto-Ready startup process.
- It has parameters for a maximum duration and volume dispensed, allowing for more/less aggressive unclogging, as needed.



Diagnostics Check Only (Applies only to Standard Calibration) Hardware System Vacuum Dip RF Detector Ion Source Auto-Ready Start Spray Stop Spray Check Spray Stability Check Calibrant Solution Fluidics Flush Lines New Vial Installed Clean Emitter Calibrate Flow/Pressure Check Flow Paths Unclog Emitter High Voltage Utilities Ion Funnel Parameter Name Parameter Value Max Time (minutes) 5 Max Volume (uL) 50

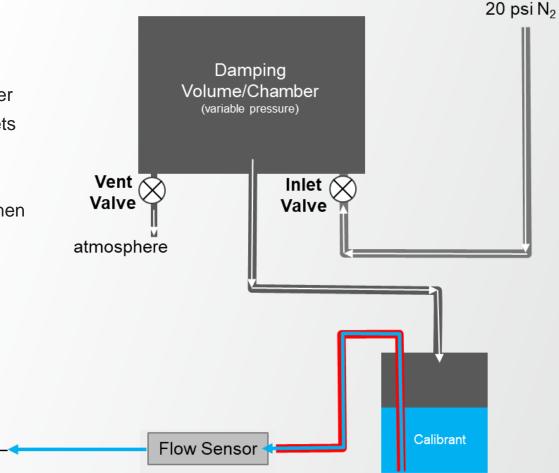
AutoReady Source Enhancements

NEW Unclogging routine

- Steps taken during execution of routine
 - Routine takes over the manual control of the CorSolutions valves
 - Puts the sheath gas to 20 psi, instead of the usual 10
 - Raises the sweep gas up to 18psi to help avoid droplet forming on sprayer
 - Fully opens the inlet valve and closes the vent valve, so the full 20 psi gets applied to the vial
 - Holds for several minutes
 - Checks if the expected maximum flow rate has been achieved. If so, it then checks that we have acceptable flow at the "analytical" rate (1 uL/min)

Emitter

· Returns a result, saying whether or not it was successful



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HCD in Tune

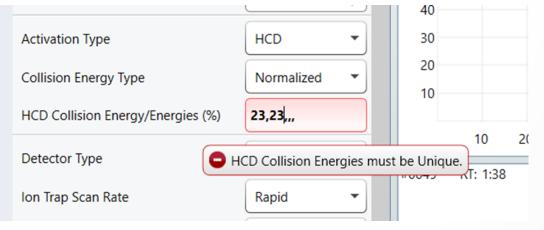
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Multiple values can be used for HCD input

UI Changes

- A user can now input up to 5 values for HCD separated by a comma
- Values should be in ascending order, variable order is not supported, and repeat values are not supported
- Collision Energy mode option has been removed from tune when using HCD

Error state example and tool tip



thermoso Orbitrap E			Applica Pej
ION SOURCE	DEFINE SCAN	CALIBRATION	
Scan Type		MS ² Scan	• Í
Precursor (m/	'z)	200	*
Precursor Cha	arge State	1	*
Isolation Mod	le	Quadrupole	•
Isolation Wid	th (m/z)	2	*
Activation Typ	be	HCD	•
Collision Ener	ду Туре	Normalized	•
HCD Collision	Energy/Energies (%	6) 30,,,,	
Detector Type	2	lon Trap	•
Ion Trap Scan	Rate	Rapid	•

Fragment Ion Indexing

NEW Fragment ion indexing to improve spectral library searching (for DIA)

Indexing overview: Each fragment ion that matches between the experimental data and the spectral library is indexed (catalogued). This index allows for quick reference and identification of the peptide or protein

Previously, spectral library entries were stored in a precursor mass binned based on precursor m/z and charge.

The new approach extends this by introducing a fragment ion index, which changes the query process. Instead of considering all candidates with a precursor m/z within a tolerance range, the fragment ion index filters candidates based on matching fragment ions in the experimental spectrum.

To create the fragment ion index, fragment ions are initially binned by fragment m/z, then each bin is sorted by the fragment's precursor m/z. This allows for quick matching of experimental fragment ions to library candidates by finding the appropriate bin and performing a binary search within it.

A Fragment Ion class is introduced to track information during searches, including LastQueryNumber to prevent repeated matches in the same scan, and is also able to access additional details like precursor m/z.

Rea	Real-Time Library Search Properties				
•	LIBRARY SEARC	H SETTINGS			
	Spectral Library				
		Browse			
	Collision Energy Tolerance	15			
	Similarity Search				
	Precursor Search Tolerance (ppm)	10			
C	Use Fragment Ion Index				

Fragment Ion Indexing

NEW Fragment ion indexing to improve spectral library searching (for DIA)

Real-Time Library Search Properties					
•	LIBRARY SEARCI	H SETTINGS			
	Spectral Library				
		Browse			
	Collision Energy Tolerance	15			
	Similarity Search				
	Precursor Search Tolerance (ppm)	10			
1	Use Fragment Ion Index	V			
2	Minimum Matched Peaks	4			
3	Minimum Library Fragment Ion Intensity Contribution	0.05			
4	Number of Experimental Peaks Considered	100			
5)	Maximum Search Time (ms)	150			
	Use Retention Time Filter				
	Isotope Error Correction	None (0) 🔹			
	Reverse Library Search				
	Use Multiple Precursors in Search				

- 1. **Check box** Select here to activate Fragment Ion Indexing and expose the associate parameters.
- 2. *Minimum Matched Peaks* This value controls the minimum number of peaks required to match between the library and the experimental data, the minimum peaks must also pass the intensity threshold set in the "Minimum Library Fragment Ion Intensity contribution" setting. Range 0-100
- **3.** *Minimum Library Fragment Ion Intensity Contribution* This value controls the minimum fragment intensity required for a library peak to be considered relevant for indexing. Range 0.0-1.0. Peaks with an intensity contribution above the threshold indicated are added to the candidate list for fragment ion indexing. The value specified in the "Minimum Matched Peaks" option will be impacted by the threshold specified here, as the matched peaks are counted and accumulated from the content of the fragment ion index.
 - **Number of Experimental Peaks** This value controls the number of experimental peak candidates to pull in and match to the library. The peak selection is based purely on intensity. Only the specified "Top N" peaks are taken form the spectra and processed using the library search settings parameters for a match.
- **5.** *Maximum Search Time* Set an upper limit on how long you want to allow for a search to take. Anything taking longer than the specified upper limit will return a failing result and move onto the next. Keep in mind your cycle time and top speed settings.

Exclude Isotopes for Targeted Mass Exclusion & Targeted Loss Exclusion filters

NEW isotopic exclusion option for Targeted Mass/Loss Exclusion

- Instrument Models Impacted:
 - All Orbitrap Models (IQ-X, ID-X, Fusion, Fusion Lumos, Eclipse, Titanium)
- Scans + Filters Impacted:
 - tMSn
 - Dynamic Exclusion
 - Isotope Exclusion
 - Targeted Mass Exclusion
 - Targeted Loss Exclusion
 - MS
 - Dynamic Exclusion
 - Isotope Exclusion
 - Targeted Mass Exclusion
 - SIM
 - Dynamic Exclusion
 - Isotope Exclusion
 - Targeted Mass Exclusion

Targeted Mass Exclusion

Targeted Loss

Exclusion

Exclude Isotopes for Targeted Mass Exclusion & Targeted Loss Exclusion filters

NEW isotopic exclusion option for Targeted Mass/Loss Exclusion



Tar	geted Mass Exclusion Proper	ties		
Þ		MASS LIST		
	Exclusion mass width	ppm		
	Low	25		Targeted M
	High	25		Exclusior
1	Exclude Isotopes		•	New excl
				now chor

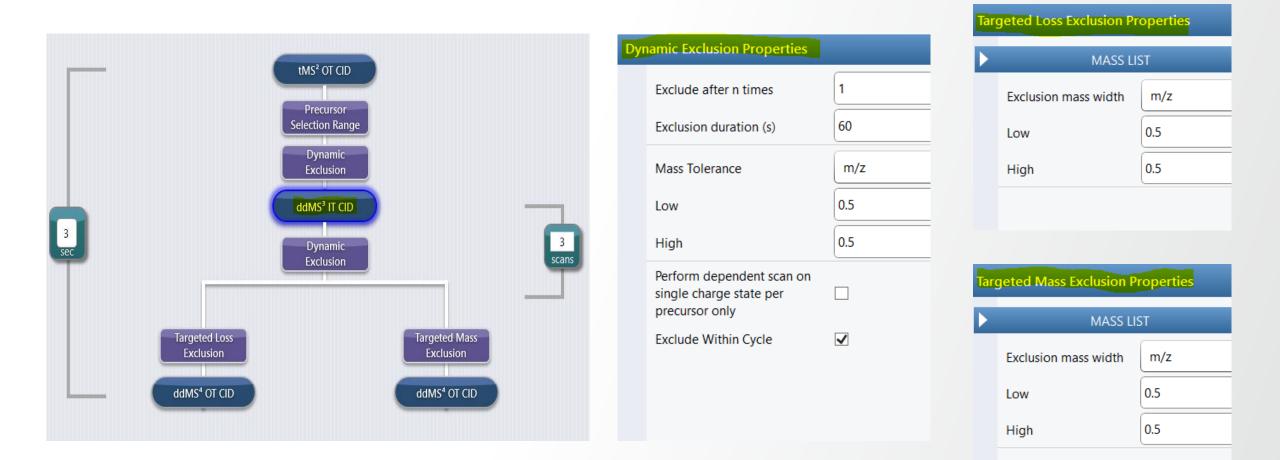
Targeted Mass	Targeted Loss
Exclusion	Exclusion

- New exclude Isotopes option within the properties of the filter a new check box appears to exclude isotopes for any given target.
- Parent Scan must be an Orbitrap Scan
- Same rules apply for Dynamic Exclusion

Tar	geted Loss Exclusion Proper	ties	
Þ		MASS L	IST
	Exclusion mass width		ppm
	Low		10
	High		10
2	Exclude Isotopes		

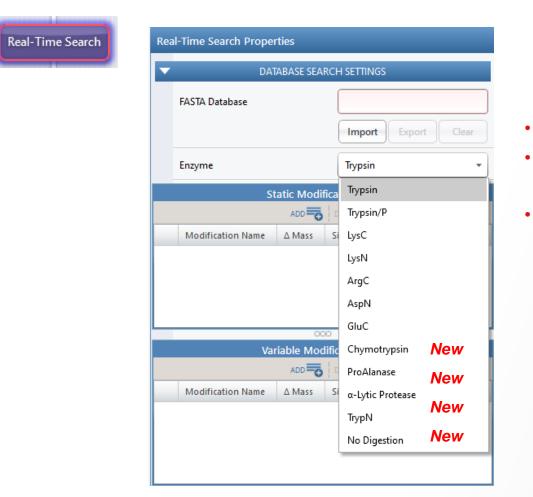
Exclude Isotopes for Targeted Mass Exclusion & Targeted Loss Exclusion filters

"Exclude Isotopes" checkbox hidden as preceding scan analyzer is Ion Trap



Extend Enzyme List for RTS

New enzyme and No digestion mode for RTS

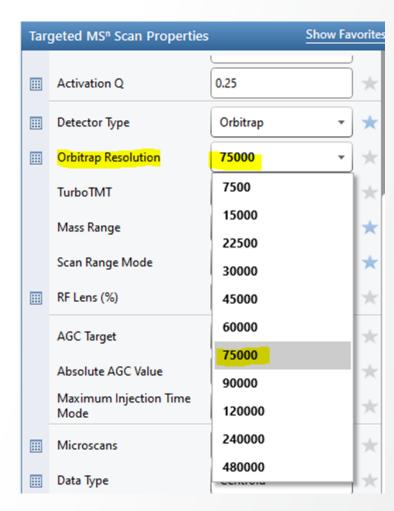


- Enzyme list for RTS has now been extended
- The no digestion option will allow for consideration of complete FASTA "Protein" entries without any additional digestion.
- No digest entries must stay within the peptide mass and length considerations imposed by Comet

New Orbitrap Resolving Powers

Instrument Models & Scan types supported

- Instrument Models Impacted:
 - Orbitrap Fusion
 - Orbitrap Fusion Lumos
 - Orbitrap Eclipse
 - Orbitrap Ascend
- Scans Impacted:
 - All Scans (MS, tMSn, tSIM, DDMSn, DDSIM, DIA)



- A new resolution setting between 60k and 90k is desired in order to balance resolution and speed for resolving the TMT reporter ions present in a TMT 35plex experiment.
- The new setting has a transient detect duration of 160ms, and is labelled 75000 (75k) in the Tune and Method Editor UI.

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New Turbo TMT supported Resolving Powers

Instrument Models

- Orbitrap Eclipse
- Orbitrap Ascend
- Orbitrap Ascend Series (all models)
- Resolving powers:
 - Eclipse 50k and 60k
 - Ascend (all) 45k and 60k

Tar	geted MS ⁿ Scan Properties		Show Favorite
	MS ⁿ Level (n)	2	• *
	Multiplex lons		*
	Isolation Mode	Quadrupole	• *
	Isolation Window (m/z)	1.6	*
	Activation Type	HCD	• *
	HCD Collision Energy Type	Normalized	• *
Ⅲ	HCD Collision Energy/Energies (%)	30	*
	Detector Type	Orbitrap	• *
	Orbitrap Resolution	<mark>45000</mark>	• *
	TurboTMT	All TMT Reagents	• *
		· · ·	

Targeted MS ⁿ Scan Properties Show Favorites				
	MS ⁿ Level (n)	2	• *	
	Multiplex lons		*	
	Isolation Mode	Quadrupole	• *	
Ⅲ	Isolation Window (m/z)	1.6	*	
==	Activation Type	HCD	• *	
	HCD Collision Energy Type	Normalized	- *	
==	HCD Collision Energy/Energies (%)	30	*	
Ⅲ	Detector Type	Orbitrap	• *	
⊞	Orbitrap Resolution	60000	• *	
	TurboTMT	All TMT Reagents	- *	

Targ	geted MS ⁿ Scan Properties	Show Favorites	
	MS ⁿ Level (n)	2 • ★	
	Multiplex lons	• *	
▦	Isolation Mode	Quadrupole 🔹 🖈	
Ⅲ	Isolation Window (m/z)	1.6	
Ⅲ	Activation Type	HCD •	
	HCD Collision Energy Type	Normalized	
▦	HCD Collision Energy/ Energies (%)	30 ★	
	Detector Type	Orbitrap 🔹 ★	
▦	Orbitrap Resolution	75000 - 🛧	
	TurboTMT	All TMT Reagents 🔹 ★	
	Mass R For TurboTMT option, Resolution must be 15000, 30000,		
	45000 or 60000 Scan Ra		

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