These release notes introduce the features of the Thermo Scientific™ Orbitrap™ Tribrid™ Series 3.3 instrument control application and known issues that still exist in the application.

You can use the Orbitrap Tribrid Series instrument control software to collect high-quality mass spectrometry data on the Thermo Scientific Orbitrap Tribrid Series mass spectrometers (MSs), including the Orbitrap Fusion™, Orbitrap Fusion Lumos™, Orbitrap ID-X™, and Orbitrap Eclipse™ MS systems. Control of the instrument is through two application packages: Tune and Method Editor.

- The Tune application displays acquired mass spectra in a continuous loop and continuously reports the observed values of various instrument parameters that indicate instrument status. Tune is used not only to view spectra but to provide tools to tune and calibrate the instrument for maximum performance with a variety of scan types, scan modes, ion polarities, scan rates, and resolution settings.

  The Tune application provides a host of diagnostic functions for easy troubleshooting. You can also use features to manage the USB-connected devices, for example, the external divert valves and the syringe pump. Finally, this application supports report generation so that you can document the outcome of various diagnostics, calibrations, and optimizations.

- In the Method Editor application, you can set up experiments by using the entire complement of scan types, advanced filters, and conditional logic, designing customized sequences of scans to interrogate complicated samples. For example, one method might have a full scan followed by one or more filters and then a data-dependent MS^n level scan on the reduced mass list. You have the choice of using your preferred fragmentation technique for MS^n scans.

  Using the Method Editor application, you can also specify peripheral device controls as part of an experiment. Methods constructed in the Method Editor can be executed in high-level applications such as the Thermo Xcalibur™ data system.

With the Thermo Foundation™ Instrument Configuration options, you can set up conditions specific to your instrument and the experiment run.

Contents

- Features
- Minimum Requirements
- Important Information
- Resolved Issues
- Known Issues
- Trademarks

For information about installing the Orbitrap Tribrid Series instrument control software, refer to the DVD insert or the installation instructions provided with the application. For information about configuring and using the Orbitrap Tribrid Series systems, refer to the manuals available as PDF files or the Help.
The Orbitrap Tribrid Series instrument control software version 3.3 incorporates the following new and improved features:

- Support for the Orbitrap Eclipse mass spectrometer
- (Orbitrap Eclipse only) Support for Real-Time Search
- (Orbitrap Eclipse only) Support for Proton Transfer Charge Reduction (PTCR) option
- (Orbitrap Eclipse only) Support for High Mass Range MS^n (HMR^n) option
- (Orbitrap Eclipse only) Support for TurboTMT scan feature
- Support for Precursor Fit Filter
- Improved ETD calibration implementation, eliminating the need for negative mode calibration
- Support for Auto, Dynamic (previously referred to as “inject for all available parallelizable time” check box), and Custom Maximum Injection Times
- Support for Normalized AGC Targets
- Support for low-resolution Advanced Peak Determination for intact protein analysis
- Support for Dynamic Retention Time Scheduling
- Support for normalized HCD collision energy for high charge state ions
- Support for more scan range options in all scan types (Auto, Scan range, First Mass)
- Support for variable isolation window and collision energy with multiplexed targeted scans
- Support for Dynamic Exclusion across experiments
- Support for TMTpro™ isobaric tag loss filter
- Support for Xcalibur-based alerts when new instrument control software is available (requires Xcalibur version 4.3)
- Support for Apex Detection Filter on a subset of outcomes when multiple outcomes are present
- Usability enhancements:
  - Improvements for DIA scan settings
  - New check box for “Do data-dependent scan if no targets are found” for targeted inclusion lists
  - Updated templates, including the SureQuant, DIA, and BoxCar templates
  - Aligned maximum injection and AGC target settings for multiplexed scans with Q Exactive™ and Orbitrap Exploris™ 480 MS systems

These are the minimum hardware and software configurations required for Orbitrap Tribrid Series 3.3 operation.

### System Requirements

<table>
<thead>
<tr>
<th>Computer</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 3.4 GHz dual core processor with 4 GB RAM</td>
<td></td>
</tr>
<tr>
<td>• 500 GB hard drive</td>
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</tr>
<tr>
<td>• DVD drive</td>
<td></td>
</tr>
<tr>
<td>• Resolution display 1280 × 1024 (SXGA)</td>
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</tr>
<tr>
<td>• NTFS file format</td>
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</tr>
</tbody>
</table>

**Note** To expedite processing time for some AcquireX workflows, Thermo Fisher Scientific recommends 32 GB of memory, even though the minimum requirement for Instrument Control Software version 3.3 is 16 GB.

<table>
<thead>
<tr>
<th>Mass spectrometer</th>
<th>Orbitrap Tribrid Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software</td>
<td>Microsoft™ Windows™ 10 Enterprise Long Term Service Branch (LTSB) 2016</td>
</tr>
</tbody>
</table>

**Note** Windows 7 Professional (64-bit) with Service Pack 1 was not tested with Orbitrap Tribrid Series 3.3 software but should be functional for all Tribrids, except the Orbitrap Eclipse, which requires Windows 10.
Important Information

If there are network, connectivity, or security issues, contact Thermo Fisher Scientific Technical Support to ensure that the security settings, firewalls, and anti-virus software are compatible with Thermo Scientific specifications.

Installation

Installing Orbitrap Tribrid Series 3.3 software requires that you have the Xcalibur 4.3 (or later) data system that includes the Foundation 3.1 SP7 and FreeStyle 1.6 applications.

If you are upgrading the software, check all calibrations. Run calibration tests to ensure they pass the “check.” If the system does not pass these tests, contact Technical Support.

Note: For a new installation, install Xcalibur 4.3 or later, and install your LC software. Then, go to “To launch the Orbitrap Tribrid Series 3.3 instrument control software installer.”

To upgrade to Xcalibur 4.3 or later

1. (For a new installation only) Install the Xcalibur data system version 4.3 and later, install your LC software, and then go to step 3.

2. (For an upgrade to Xcalibur 4.3 and later) Do the following:
   a. Using the Foundation Instrument Configuration window, remove all configured instruments.
   b. Uninstall Xcalibur and Foundation using Windows™ Add and Remove Programs in the following order:
      - Xcalibur
      - Foundation
   c. If SII for Xcalibur software (version 1.3 and earlier) is installed, uninstall it.
   d. Click ThermoLauncher.exe, and then click Adobe™ Reader™ 10.1 to install the Adobe Reader application.
   e. Click Xcalibur 4.3 and proceed as follows:
      - Follow the installer instructions to have the installer to install Foundation 3.1 SP7, Xcalibur 4.3, Almanac 1.3, and FreeStyle 1.6.
      - If prompted, restart the data system computer.
   f. (Optional) Install SII for Xcalibur 1.5 and later.

3. Start the Orbitrap Tribrid Series 3.3 installer to install the software, view the documentation, and download the release notes.

4. In the Foundation Instrument Configuration window, add and configure the system devices.

<table>
<thead>
<tr>
<th>System Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Microsoft SQL Server™ 2008 R2 Express Advanced Series (x86)</td>
</tr>
<tr>
<td>- Thermo Scientific software:</td>
</tr>
<tr>
<td>- Foundation 3.1 SP7, or later</td>
</tr>
<tr>
<td>- Xcalibur 4.3</td>
</tr>
<tr>
<td>- FreeStyle™ 1.6</td>
</tr>
</tbody>
</table>
5. Open the Tune application and follow the Upgrade Diagnostic prompts:
   a. Run **Upgrade Diagnostic - No Sample.**
   b. Infuse the calibration solution.
   c. With a stable spray of calibration solution, run **Upgrade Diagnostic - Calmix.**
6. After completing the upgrade diagnostics, run the **Check Calibration** tests. For any failures, run the applicable calibration procedure.

**Note** If the system does not pass all calibrations, contact Thermo Fisher Scientific Technical Support.

To launch the Orbitrap Tribrid Series 3.3 instrument control software installer
1. Launch the Orbitrap Tribrid Series 3.3 installer to install the software, view the documentation, and download the release notes.
2. In the Foundation Instrument Configuration window, add and configure the system devices.
3. Open the Tune application and follow the Upgrade Diagnostic prompts:
   a. Run **Upgrade Diagnostic – No Sample.**
   b. Infuse the calibration solution.
   c. With a stable spray of calibration solution, run **Upgrade Diagnostic – Calmix.**
   d. Once all upgrade diagnostics are complete, check all calibrations and ensure that they pass. Run calibrations that do not pass the “check.”

**Note** Contact Technical Support if the system does not pass the calibrations.

Licensing for Advanced Peak Determination (APD) and 1M Resolution

In the Tune application, click the **Options** icon, and choose **About Tune.** In the dialog box, you can obtain the Instrument Identification (ID), apply and remove licenses, and check for existing licenses.

To obtain new licenses through email, send the following information to ThermoMSLicensing@thermo.com. You can also obtain a 60-day trial license for APD on Orbitrap Fusion and Orbitrap Fusion Lumos MS systems.

- Company name
- Instrument serial number
- Instrument ID
- Trial or Permanent license: For a permanent license, provide the original Thermo Fisher Scientific sales order number.

Table 1 lists defects that were resolved between the Orbitrap Fusion Series 3.1 and Orbitrap Tribrid Series 3.3 applications.

The tables exclude Help issues and any cosmetic fixes. In some cases, the abstract has been amended or extended from the original to better describe the reported issue. Both an engineering fix and follow-up testing (verified by our product evaluation department) have resolved these issues.

Table 1. Resolved issues between Orbitrap Fusion Series 3.1 and Orbitrap Tribrid Series 3.3 (Sheet 1 of 2)

<table>
<thead>
<tr>
<th>Item ID</th>
<th>Software section</th>
<th>Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE81325</td>
<td>Calibration</td>
<td>Addressed an issue where Orbitrap calibrations fail for high mass ions at 5e5 targets, primarily in negative mode.</td>
</tr>
<tr>
<td>DE78228</td>
<td>Acquisition</td>
<td>Addressed an issue causing a slight overhead in MS2 scanning.</td>
</tr>
<tr>
<td>DE78591</td>
<td>Acquisition</td>
<td>Addressed an issue where scanning is delayed when UVPD is configured.</td>
</tr>
</tbody>
</table>
Known Issues

Suggested recovery actions

- For some issues like connectivity problems, restarting the application is the appropriate recovery action.
- In some cases (particularly issues that arise during data acquisition), restarting applications such as the Xcalibur data system might not ensure complete recovery. Typically, restarting the data system computer resolves issues, but some devices with error conditions might require power cycling.
- As a fix we generally do not recommend reinstalling the software or the operating system, which more commonly occurs after you install a new hard drive.

Feature requests and other removed items

- We do not include issues where there is insufficient information logged to successfully reproduce the reported problem.
- We do not list feature requests as software issues, regardless of the reported significance or severity of the request. Product managers evaluate logged feature requests for future releases.
- We report only discrepancies in the documented software as known issues.

Terminology

<table>
<thead>
<tr>
<th>Severity</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crash/Data Loss</td>
<td>A problem that renders the system unusable because either an entire function is unusable and no workaround exists, or use of the current system compromises data integrity or results in data loss. Catastrophic problems also include significant and non-obvious quantitative errors, and all human and instrument safety issues.</td>
</tr>
<tr>
<td>Major Problem</td>
<td>A serious issue that does not affect data integrity (meaning data loss, corruption of data, or the wrong answer), but affects the customer's ability to use the product as designed. It can be a failure, design issue, or documentation error or omission. A workaround might or might not exist.</td>
</tr>
<tr>
<td>Minor Problem</td>
<td>A minor error or poor behavior of a product feature. There is probably a workaround.</td>
</tr>
<tr>
<td>Cosmetic</td>
<td>An issue that has a limited effect on customer usage of the product; for defects with visibility so low that a customer might never see it; or for ease of use issues or other items not causing any performance degradation.</td>
</tr>
<tr>
<td>Risk</td>
<td>Interpretation</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>High</td>
<td>Occurrence is likely to happen and can compromise operation.</td>
</tr>
<tr>
<td>Medium</td>
<td>Occurrence is uncommon, but could compromise operation if it occurs.</td>
</tr>
<tr>
<td>Low</td>
<td>Issue is minor; however, the software could operate differently from a user's expectations. A workaround might be available.</td>
</tr>
<tr>
<td>No Risk</td>
<td>This issue causes no problem but is commonly an inconsistency or cosmetic issue.</td>
</tr>
</tbody>
</table>

**Known defects**

Table 2 contains known defects in the software, categorized by software section, with a brief abstract and information related to the defect’s severity and risk, if applicable. The Item ID is the internal number assigned to the issue, if applicable. Product management assesses risk, which can differ significantly from the reported severity.

**Table 2. Known software issues**

<table>
<thead>
<tr>
<th>Software section</th>
<th>Severity</th>
<th>Abstract</th>
<th>Risk</th>
<th>Item ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tune</td>
<td>Minor Problem</td>
<td>With Windows 10, the View Raw File button does not open the raw file properly, nor can you double-click the raw file to open it. Instead, navigate to the raw file, right-click it, and browse to the appropriate application (FreeStyle) to open the file. Select the check box to always open a raw file with the selected application, which then becomes the default.</td>
<td>Low</td>
<td>DE57094</td>
</tr>
<tr>
<td>Tune</td>
<td>Minor Problem</td>
<td>When system is set to Orbitrap mode and SIM scan when the electron multiplier calibration is started, the Orbitrap analyzer is used and might lead to a failed calibration. Instead, set the system to full scan mode before initiating calibration.</td>
<td>High</td>
<td>DE84513</td>
</tr>
<tr>
<td>Acquisition</td>
<td>Minor Problem</td>
<td>EASY-IC is not compatible with Orbitrap last mass greater than 4000 m/z, however, the method editor does not check this validation. Using EASY-IC with last mass above 4000 m/z may hinder sensitivity for the high mass species.</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Orbitrap Eclipse, Eclipse, Orbitrap ID-X, ID-X, Q Exactive, Orbitrap Exploris, AcquireX, TMTpro, FlexMix, Foundation, and FreeStyle are trademarks; and Orbitrap, Orbitrap Fusion, Orbitrap Fusion Lumos, Thermo Scientific, Tribute, and Xcalibur are registered trademarks of Thermo Fisher Scientific Inc. and its subsidiaries in the United States.

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