LTQ Orbitrap XL™

Getting Started 2.4

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Read This First

Welcome to the Thermo Scientific, LTQ Orbitrap XL™ system! The LTQ Orbitrap XL is a member of the family of LTQ™ mass spectrometer (MS) detectors.

Who Uses This Guide

This *LTQ Orbitrap XL Getting Started* manual is intended for all personnel that needs to operate the LTQ Orbitrap XL, especially the key operator. This manual should be kept near the instrument to be available for quick reference.

Scope of this Guide

This *LTQ Orbitrap XL Getting Started* manual provides you with information on how to set up, calibrate, and tune the LTQ Orbitrap XL. Procedures in Chapters 1–4 can be performed from the Xcalibur® Tune Plus window.

LTQ Orbitrap XL Getting Started includes the following chapters:

- Chapter 1: "Introduction" provides general information about this manual.
- Chapter 2: "Tune Plus Window" provides information on the Tune Plus window.
- Chapter 3: "Calibrating the Orbitrap XL for FTMS Measurements" provides procedures to calibrate your LTQ Orbitrap XL for FT measurements.
- Chapter 4: "Performing Diagnostics/Checks" describes several diagnostic procedures.
- Chapter 5: "Instrument Setup" describes the FT relevant topics of the data dependent settings in the Instrument Setup.
- Chapter 6: "Instrument Configuration" gives instructions about configuring your instrument.
- Appendix A: "Miscellaneous Information" gives additional information about various topics.

Changes to the Manual

To suggest changes to this manual, please send your comments to:

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You are encouraged to report errors or omissions in the text or index. Thank you.

Typographical Conventions

Typographical conventions have been established for Thermo Fisher Scientific manuals for the following:

- Data input
- Admonitions
- Topic headings

Data Input

Throughout this manual, the following conventions indicate data input and output via the computer:

- Messages displayed on the screen are represented by capitalizing the initial letter of each word and by italicizing each word.
- Input that you enter by keyboard is identified by quotation marks: single quotes for single characters, double quotes for strings.
- For brevity, expressions such as "choose File > **Directories**" are used rather than "pull down the File menu and choose Directories."
- Any command enclosed in angle brackets < > represents a single keystroke. For example, "press **<F1>**" means press the key labeled *F1*.
- Any command that requires pressing two or more keys simultaneously is shown with a plus sign connecting the keys. For example, "press **<Shift> + <F1>**" means press and hold the <Shift> key and then press the <F1> key.
- Any button that you click on the screen is represented in bold face letters. For example, "click on **Close**".

Admonitions

Admonitions contain information that is important, but not part of the main flow of text.

Admonitions can be of the following types:

- **Note** – information that can affect the quality of your data. In addition, notes often contain information that you might need if you are having trouble.
- **Caution** – information necessary to protect your instrument from damage.
- **Warning** – hazards to human beings. Each Warning is accompanied by a Warning symbol.

Topic Headings

The following headings are used to show the organization of topics within a chapter:

Chapter Name

The following headings appear in the left column of each page:

Second Level Topics

Third Level Topics

Fourth Level Topics

Safety and EMC Information

In accordance with our commitment to customer service and safety, these instruments have satisfied the requirements for the European CE Mark including the Low Voltage Directive.

Designed, processor and tested in an ISO9001 registered facility, this instrument has been shipped to you from our manufacturing facility in a safe condition.

Caution This instrument must be used as described in this manual. Any use of this instrument in a manner other than described here may result in instrument damage and/or operator injury. ▲

Identifying Safety Information

The *LTQ Orbitrap XL Getting Started* contains precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed. Warning symbols which alert the user to check for hazardous conditions appear throughout the manual, where applicable, and are defined in Table i.

Table i. Warning Symbols

Symbol	Description
	**General** This general symbol indicates that a hazard is present, which if not avoided, could result in injuries. The source of danger is described in the accompanying text. ▲
	**Electric Shock** High voltages capable of causing personal injury are used in the instrument. The instrument must be shut down and disconnected from line power before service or repair work is performed. ▲
	**Hot Surface / Heat** Allow heated components to cool down before servicing them! ▲

Instrument-Specific Hazards

Every instrument has specific hazards, so be sure to read and comply with the following precautions. They will help ensure the safe, long-term use of your system.

1. Before plugging in any of the instrument modules or turning on the power, always make sure that the voltage and fuses are set appropriately for your local line voltage.

2. Only use fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.

3. The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that the cord also has an earth contact.

4. Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the system.

Caution The instrument is properly grounded in accordance with regulations when shipped. You do not need to make any changes to the electrical connections or to the instrument's chassis to ensure safe operation. ▲

5. Never run the system without the housing on. Permanent damage can occur.

6. Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact a service representative for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage may have occurred if the system shows visible signs of damage, or has been transported under severe stress.)

7. Damage can also result if the instrument is stored for prolonged periods under unfavorable conditions (e.g., subjected to heat, water, etc.).

8. Always disconnect the power cord before attempting any type of maintenance.

9. Capacitors inside the instrument may still be charged even if the instrument is turned off.

10. Never try to repair or replace any component of the system that is not described in this manual without the assistance of your service representative.

Contents

Figures

Tables

Chapter 1 Introduction

This manual describes only the FTMS detector relevant settings and procedures of the LTQ Orbitrap XL software (Tune plus version 2.4). For ion trap relevant settings and procedures, refer to the *LTQ XL Getting Started* manual.

In addition to this manual, the LTQ Orbitrap XL Tune Plus Online Help gives information to specific topics. Nevertheless, it is recommended to read this manual entirely.

Chapter 2 Tune Plus Window

This chapter provides LTQ Orbitrap XL specific information about the Tune Plus window. It contains the following topics:

Preliminary Remarks

The Tune Plus window shows the schematic view of the LTQ Orbitrap XL and the instrument name. See Figure 2-1.

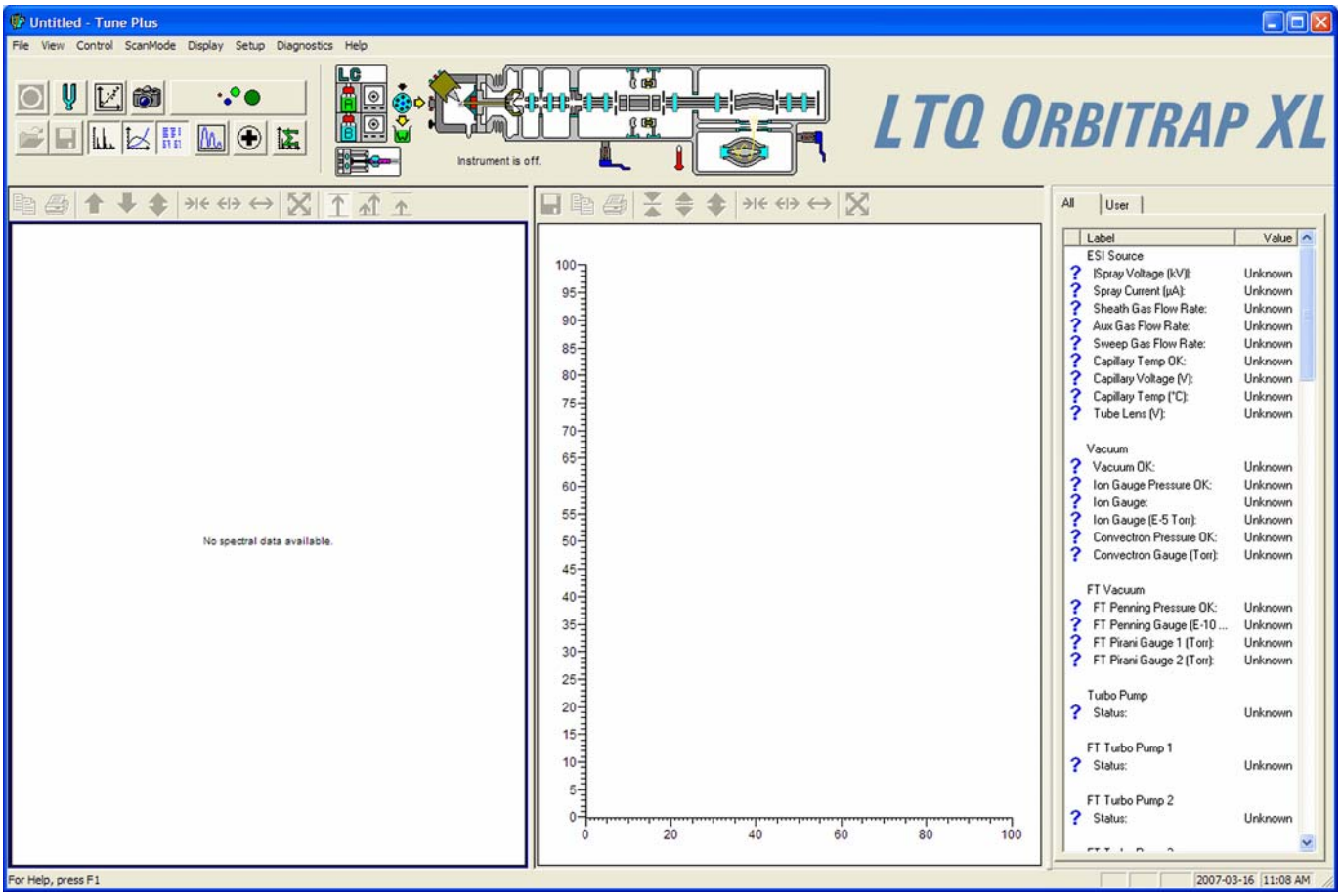


Figure 2-1. Tune Plus window

Note The Tune Plus window needs a minimum screen resolution of 1152×864 to be fully displayed. ▲

To access the functions of the Tune Plus window, use the menu commands, toolbar buttons, and display views. The FT relevant changes or additions of the menu commands, toolbar buttons, and display views are explained in the following chapters.

View Menu

This section describes those elements of the View menu that are different from the LTQ XL version of the Tune Plus window.

Spectrum View

The Spectrum view displays real-time ion trap or FT mass spectra depending on the analyzer type selected in the Define Scan dialog box. See Figure 2-2.

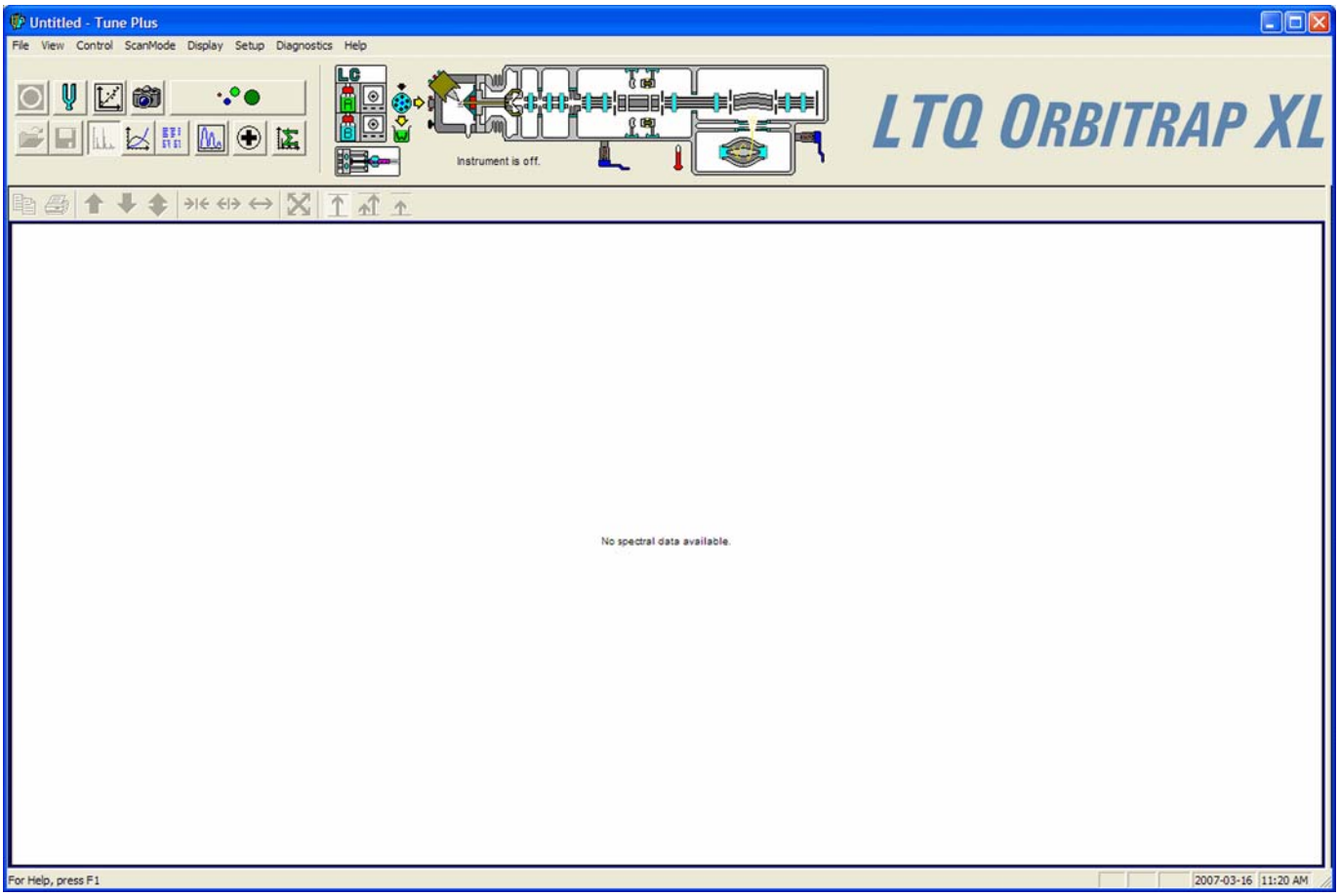


Figure 2-2. Spectrum View page

The Spectrum view page has a shortcut menu that is displayed when you right-click anywhere on the page. To open the Spectrum Display Options dialog box, choose **Display Options**. The dialog box has two pages, Ion Trap and FT.

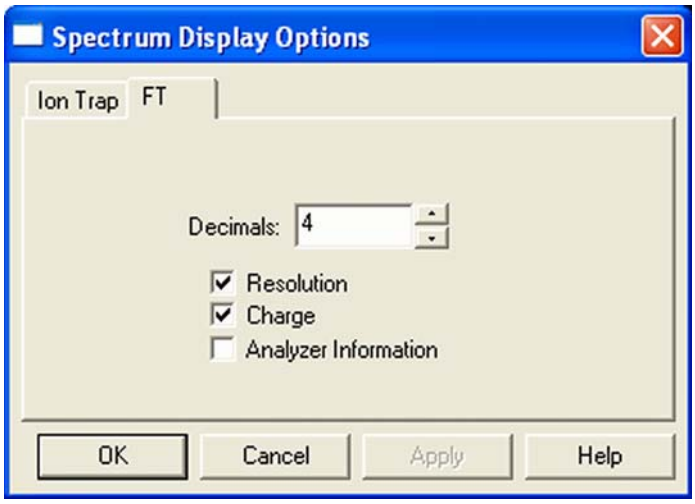


Figure 2-3. Spectrum Display Options dialog box - FT page

On the FT page, you can determine the number of decimals shown on peak labels. See Figure 2-3. To change the number of decimals, click on the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set the number of decimals to any value from 0 to 5. Alternatively, you can enter a value in the spin box text field. The LTQ Orbitrap XL changes the number of decimals when you click on **Apply** or **OK**.

A check box allows you to show additional analyzer information for FTMS scans. This information will be displayed above the spectrum graph if the box is checked. See "FT Analyzer Messages" on page A-3 for a list of items that may be displayed as analyzer information.

You can also decide whether or not to show the resolution and/or the charge state of peaks in the FT spectrum by clearing or checking the corresponding check boxes.

If the FTMS analyzer is used, it is possible to display different diagnostic views in the Spectrum view. See Chapter 4: "Performing Diagnostics/Checks" for diagnostic features that involve the Spectrum view.

Graph View

The Graph view displays, in a variety of traces, real-time data generated during calibration, tuning, and diagnostic tests. For example, Figure 2-4 shows the progress of the FT transfer multipole frequency calibration.

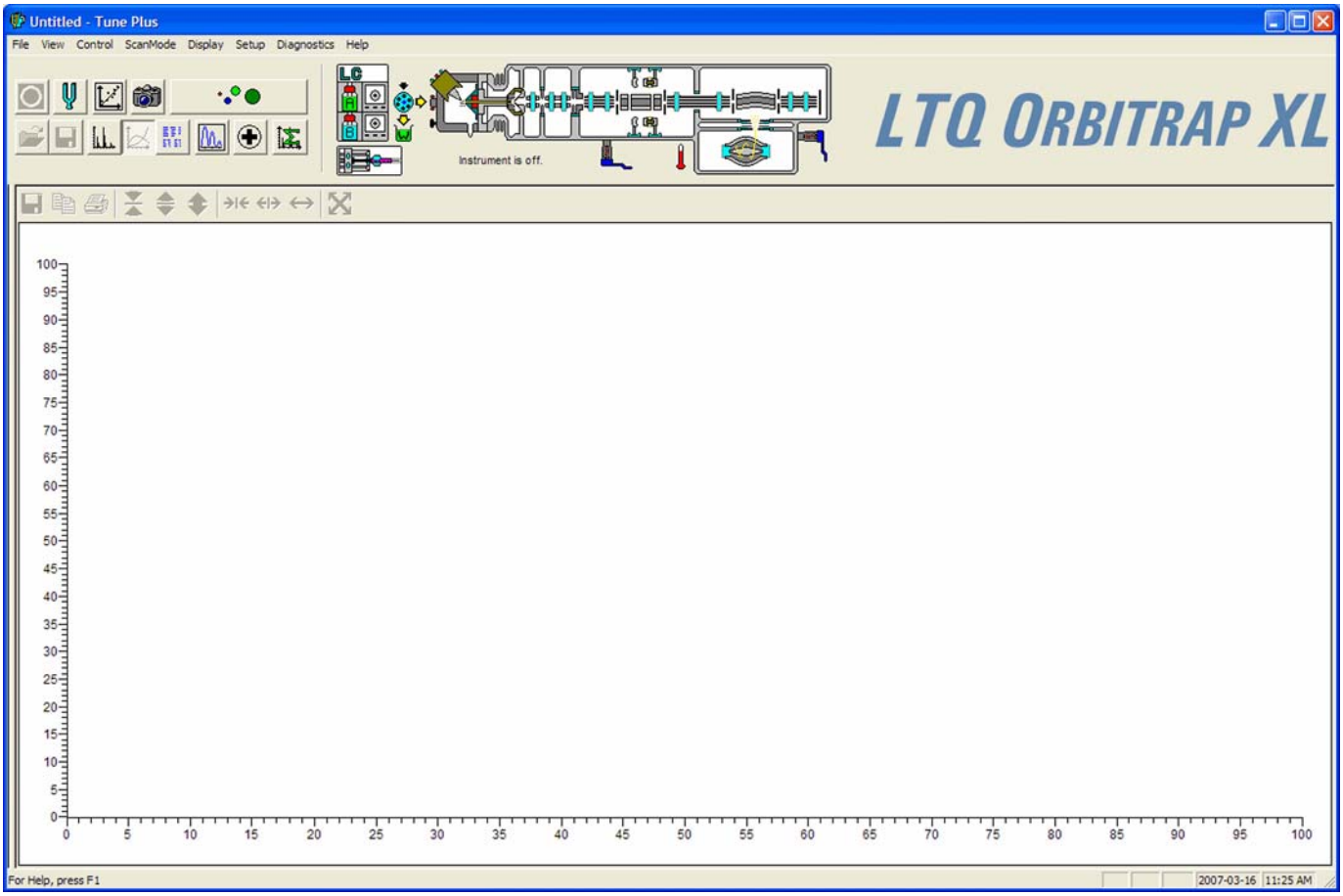


Figure 2-4. Graph view page

Status View

The Status view displays real-time status information for the LTQ Orbitrap XL. See Figure 2-5 on page 2-6. The Status view has two pages, the All page and the User page. The Status view - All page displays the real-time status information for about 80 parameters of the LTQ Orbitrap XL. You can scroll through the list to observe the status of the parameters. The Status view - User page displays real-time status information for LTQ Orbitrap XL parameters that you have selected in the User Status Display Configuration dialog box. (See page 2-6.)

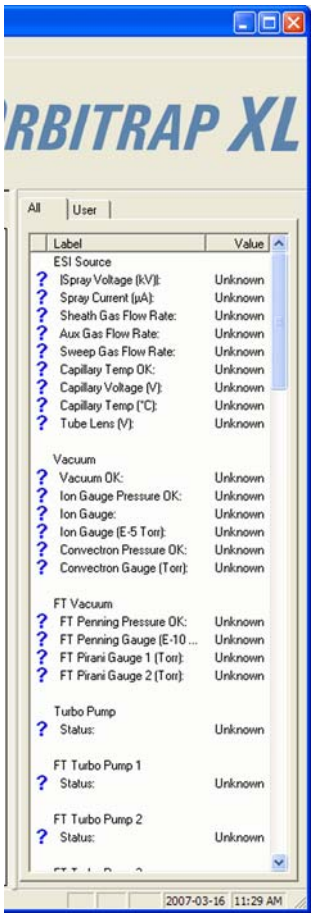


Figure 2-5. Status view page - All page

User Status Display Configuration Dialog Box

Figure 2-6 on page 2-7 shows the User Status Display Configuration dialog box. Use the following procedure to configure the user page:

1. Choose View > **Display Status View**.

2. Click on the User tab. Right-click on the User page to display the shortcut menu.

3. Choose **Configure**.

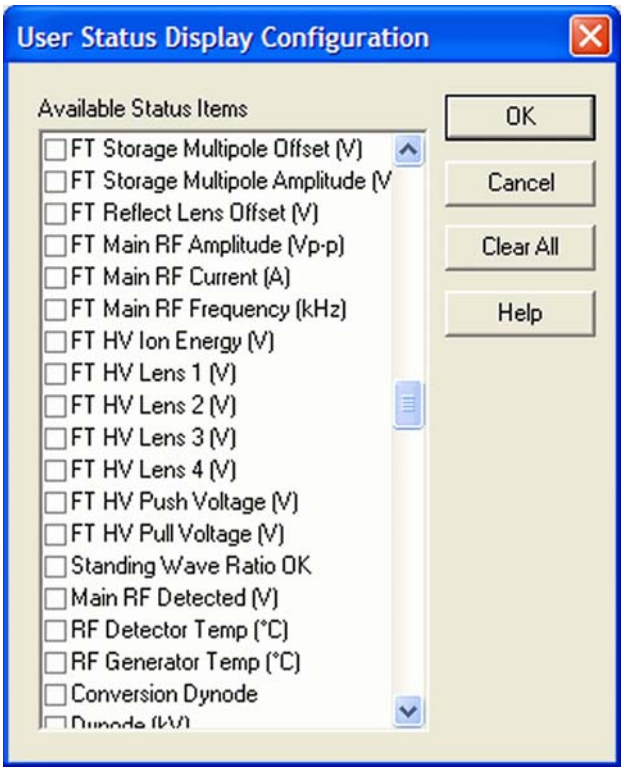


Figure 2-6. User Status Display Configuration dialog box

Control Menu

This section describes the elements of the Control menu that are different from the LTQ XL.

Advanced Calibration Features

Use the Advanced Calibration Features command to display advanced features on the Semi-Automatic page of the Calibrate dialog box, such as storage transmission and the FT transmission (See Figure 3-6 on page 3-14.):

- If the command is displayed as “Advanced Scan Features” this indicates that the command is disabled. Normal features will be displayed.
- If the command is displayed as “✓ Advanced Scan Features” this indicates that the command is enabled. Advanced features will be displayed.

Activate/deactivate this command by choosing Control > **Advanced Calibration Features**.

Scan Mode Menu

This section describes the elements of the Scan Mode menu that are different from the LTQ XL.

Define Scan

The Define Scan dialog box allows you to define a scan in various ways depending on the scan mode and scan type combination. Also, the Define Scan dialog box allows you to choose the ion trap or the orbitrap analyzer as analyzer. Figure 2-7 shows the Define Scan dialog box showing the Advanced Scan features. The Advanced Scan features can be activated in the Scan Mode menu of Tune plus.

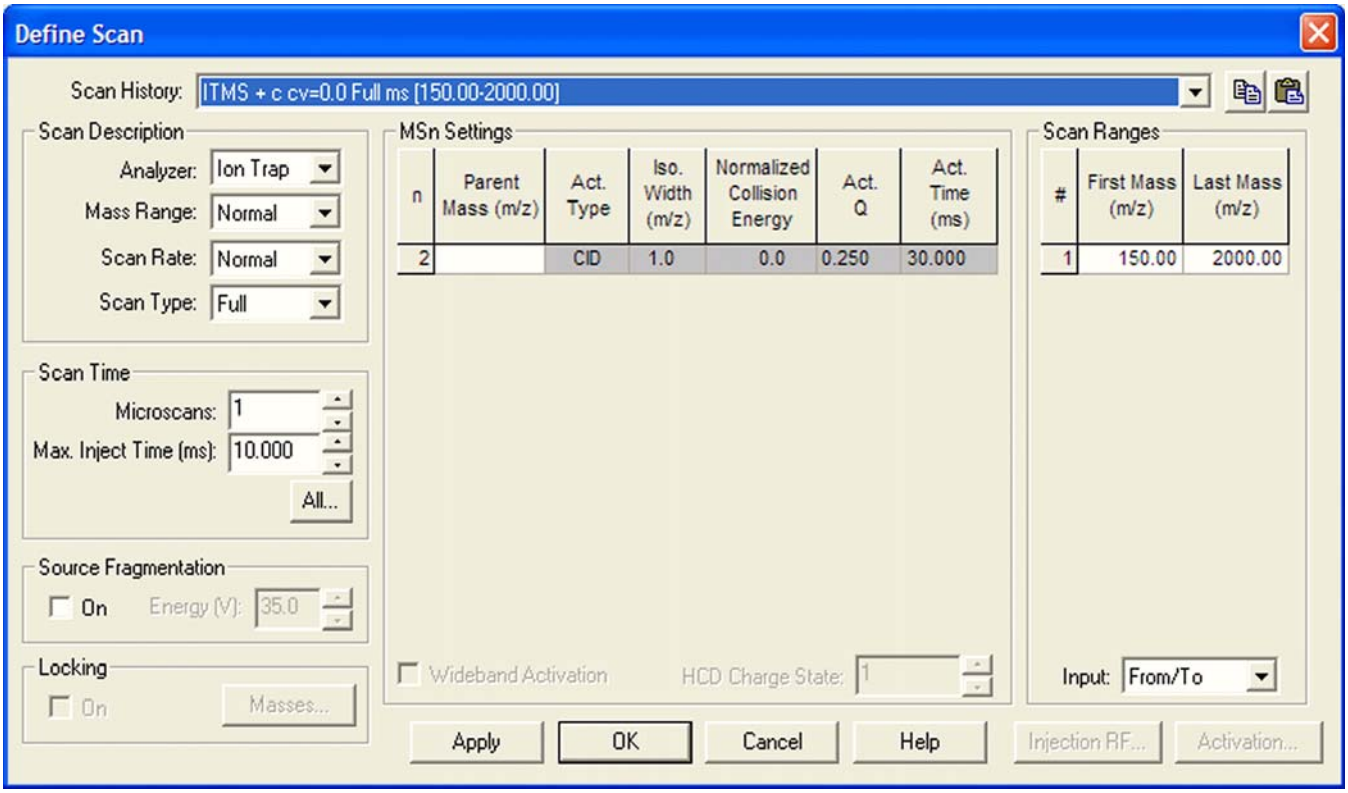


Figure 2-7. Define Scan dialog box

Scan Description

The Scan Description group box contains the following elements:

Analyzer

At the top, the Analyzer list box allows you to select the analyzer type used during the currently selected scan event. The options are as follows:

- **FTMS** selects the Orbitrap detector.
- **Ion Trap** selects the ion trap detector.

Mass Range The following mass ranges are available:

- Low: 15–200 for ion trap analyzer only
- Normal: 50–2000 for ion trap and FTMS analyzer
- High: 100–4000 for ion trap and FTMS analyzer

Scan Rate / Resolution When you have selected the entry ion trap in the Analyzer list box, this list box allows you to set the scan rate (Normal, Enhanced, Turbo, Zoom, UltraZoom).

When you have selected the entry FTMS in the Analyzer list box, this list box allows you to set the resolution of the FT mass spectra. The mass resolution is selectable between several options. Available resolution settings are 7500, 15000, 30000, 60000, and 100000.

Scan Type Usage of the scan types Full MS, SIM, SRM, or CRM is analogous to the ion trap with the exception that only one scan range is available for FTMS SIM, FTMS SRM, and FTMS CRM scans.

Scan Time

The Scan Time group box contains the following elements:

Microscans The number of microscans determines how many spectra are averaged in one analytical scan. If the FTMS is chosen as the analyzer, transients are averaged for one analytical scan.

The number of microscans can be set individually for FTMS, Ion Trap MS, FTMS SIM, Ion Trap SIM, FT MS^n, Ion Trap MS^n, and Ion Trap Zoom.

Max Inject Time The inject time is automatically controlled by the automatic gain control (AGC). The entry in this spin box limits the inject time to a maximum value. To ensure the high mass accuracy of the LTQ Orbitrap XL, the maximum inject time should not be reached. Otherwise, the number of ions does not correspond to the AGC target value.

The maximum inject time can be set individually for FTMS, Ion Trap MS, FTMS SIM, Ion Trap SIM, FT MS^n, Ion Trap MS^n, and Ion Trap Zoom.

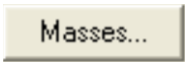
Note If the maximum inject time is reached the number of ions may not correspond to the current AGC target value. This may harm the mass accuracy of FTMS spectra. ▲

Scan Time Settings

By pressing the **ALL...** button of the Define Scan dialog box, the Scan Time Settings dialog box is displayed. It allows displaying and setting all scan time settings for all scan types at the same time for both the ion trap and the FT analyzer.

Locking

The locking feature allows using one or more peaks in the spectrum as internal reference in order to improve mass accuracy. Locking is available for FTMS analyzer type.



The locking feature can be enabled by activating the check box. When the Locking check box is activated, the **Masses...** button brings up a dialog box to enter and edit lock mass lists.

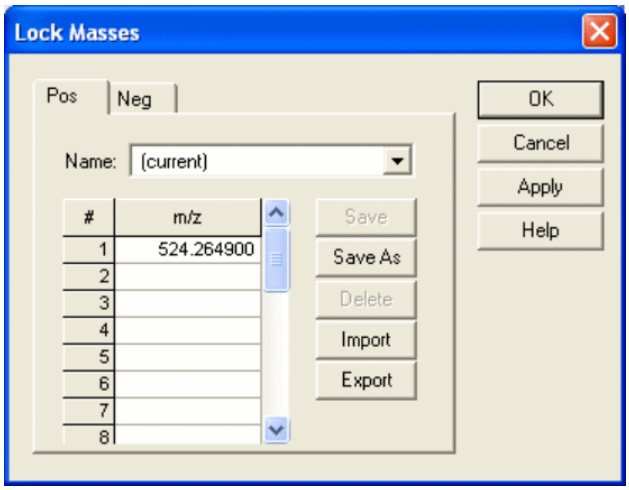


Figure 2-8. Lock Masses dialog box

Lock mass lists can consist of one or more lock masses. If the list contains lock masses that are (temporarily) not found in the spectrum, these lock masses are ignored (temporarily). For standard full scan experiments, it is expected that at least one of the lock masses is visible in the spectrum.

There are two situations where the instrument makes use of a special mode to artificially mix the lock mass into the spectrum:

1. If none of the given lock masses is found in the full spectrum, the instrument tries to improve the abundance of the lock mass by performing additional SIM-injections of the specified lock mass.

2. If the given lock mass cannot be found in the spectrum because the instrument runs in MS^n or SIM scan type, the instrument adds the lock mass by using SIM-injections.

This way, lock masses can be used for all FTMS scan types and for varying lock mass abundances. There is no need for user interaction other than specifying a list of reference peak candidates.

See "FT Analyzer Messages" on page A-3 on how to view information about the instruments locking state. See "Using Locking in Automated Runs" on page 5-2 on how to set FTMS locking in Instrument Setup.

MSn Settings

The table in this group box allows you to specify the parameters for each segment of an MSn experiment.

Act. Type

The Act. Type list box becomes available when you enter a parent mass. It allows you to specify how the ion is activated for fragmentation and has the following options:

- CID (Collision-induced dissociation)
- PQD (Pulsed-Q dissociation)
- HCD (high-energy CID)

  HCD is available only as last step in an MS^n experiment – it is not be possible to set up an experiment where the first activation method is HCD, and second is CID. If you enter a new step below an HCD experiment, the TunePlus program will change it to a CID experiment.

Act. Q

If the Advanced Scan features are enabled, the Activation Q input box is disabled for HCD type fragmentation.

HCD Charge state The HCD Charge state spin box is available only if HCD is selected as activation type, regardless of the status of the Advanced Scan features. See Figure 2-9.

To change the displayed value, click on the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. Alternatively, you can enter a value in the spin box text field. You can set the HCD charge state to any value from 1 to 99. The default value is 1.

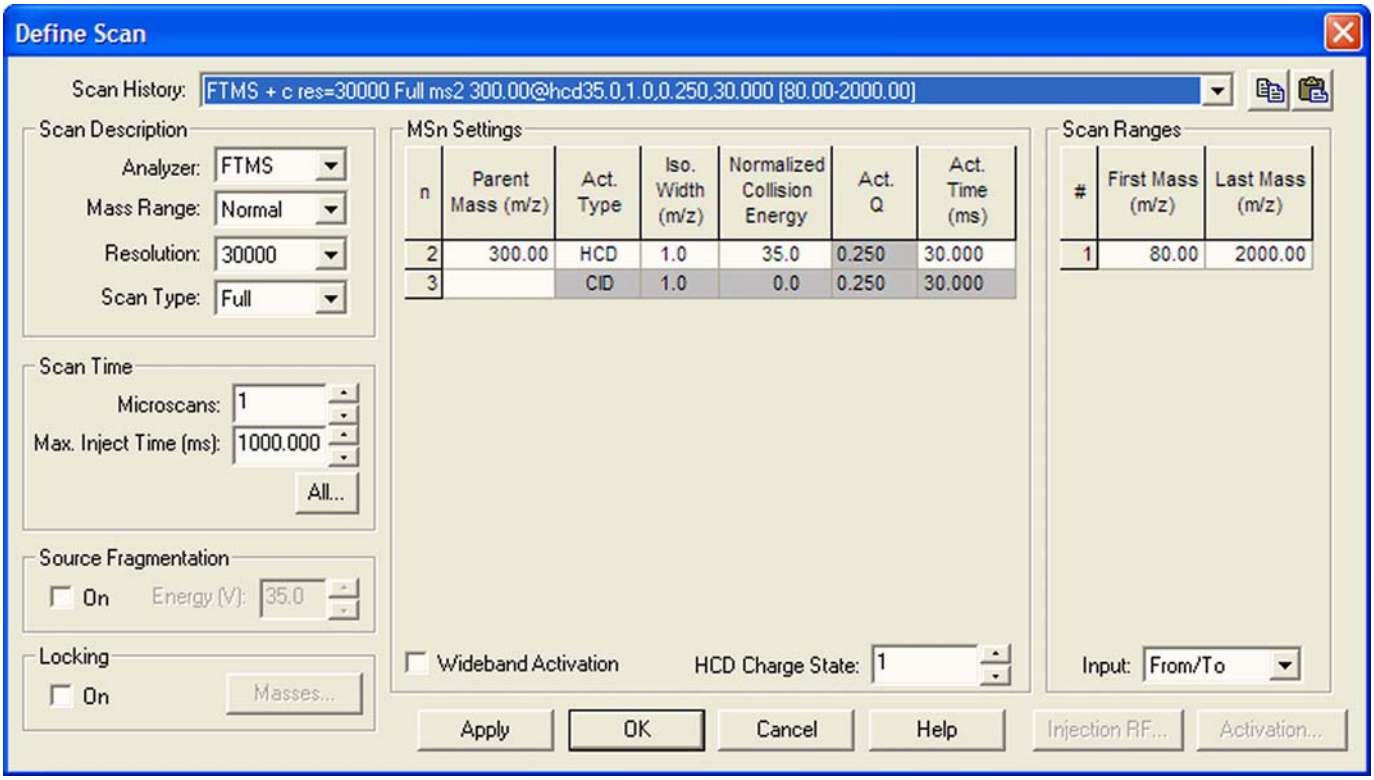
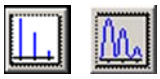


Figure 2-9. Define Scan dialog box with HCD selected as activation type

Scan Ranges

When HCD is selected as the activation method in the MSn Settings group box, the First Mass (m/z) is set to either 0.05×LastMass or 50, whichever is higher.

Centroid/Profile



With this pair of buttons, you switch between the Centroid and the Profile format. The profile format for FTMS data is a compressed profile format. "FT Profile Mode" on page 4-8 describes how to switch to full profile format for FTMS data for diagnostic purposes.

For further information, see also topic "Data Size of FT Raw Files" on page A-4.

Positive/Negative

With this pair of buttons, you can toggle between positive ion and negative ion polarity. Different FT transfer, storage, and mass calibration parameters are used for the different polarities.

Display Menu

This section describes the elements of the Display menu that are different from the LTQ XL.

Spectrum Averaging

With this toggle, spectrum averaging can be switched on or off. If spectrum averaging is enabled the displayed spectrum is the moving average of several spectra before. Averaging FTMS scans is actually an averaging of transients.

Use this functionality in analogy to ion trap scans:

1. In the Tune Plus window, choose Display > Spectrum Averaging > **Settings…** to display the Spectrum Averaging dialog box. See Figure 2-10.

2. Enter the number of transients to average into the spin box.

3. Click on **OK** to save your changes and close the dialog box.

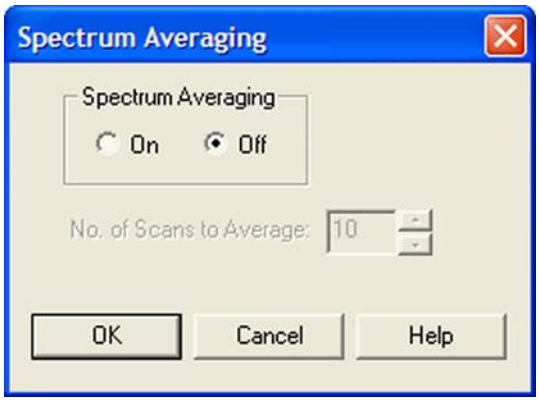


Figure 2-10. Spectrum Averaging dialog box

Setup Menu

This section describes the elements of the Setup menu that are different from the LTQ XL.

FT Transfer Optics

The FT transfer parameters are only changed by an FT transmission calibration, which is usually only necessary when the hardware of the system is modified somehow. This dialog box displays the actual FT readback values for the current scan mode. See Figure 2-11.

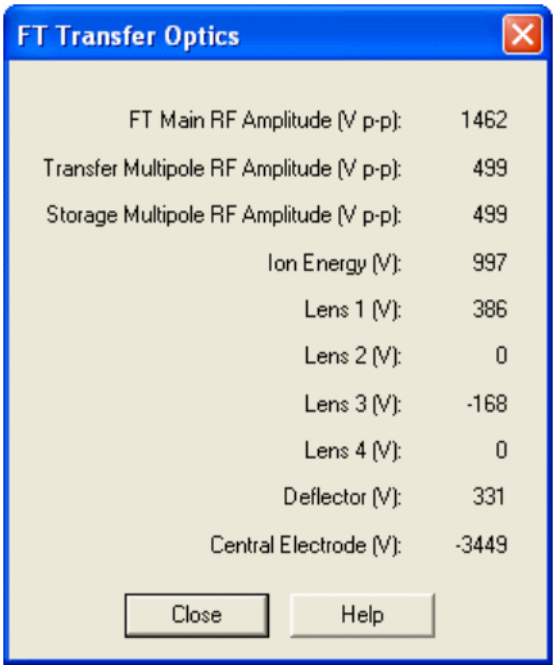
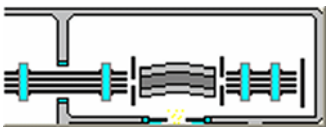


Figure 2-11. FT Transfer Optics dialog box

To open this dialog box:

- From the Tune Plus window, choose Setup > **FT Ion Optics**, or
- click on  in the Instrument Setup toolbar.

FT Injection Control

The Injection Control dialog box allows you to set the automatic gain control (AGC) target values. In addition, the Injection Control dialog box allows you to enable or disable the injection waveforms.

To open this dialog box:

- From the Tune Plus window, choose Setup > **FT Injection Control**, or

- click on  in the Instrument Setup toolbar.

The Injection Control dialog box has two tabs to enable an independent selection of target values for Ion Trap and FT scans.

Ion Trap Recommended target values for the ion trap:

Full MS Target: 3e+04

SIM Target: 1e+04

MS^n Target: 1e+04

Zoom Target: 3000.0

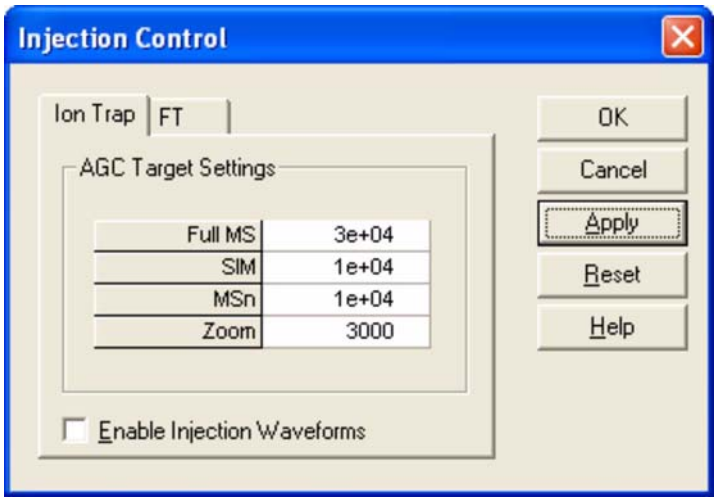


Figure 2-12. Ion Trap page of the Injection Control dialog box

FT For FTMS measurements, only the Full MS target, the SIM target, and the MS^n Target are used.

Recommended target values for the FT analyzer:

Full MS Target: 5e+05

SIM Target: 5e+04

MS^n Target: 2e+05

Note Lower target values than those listed above may be used to obtain shorter inject times. For MS^n scans, lower target values may also improve the isolation/fragmentation efficiency. Higher target values than those listed above can be used to improve the dynamic range. However, target values far above the recommended settings may affect isolation/fragmentation efficiency and mass accuracy for the FTMS analyzer. ▲

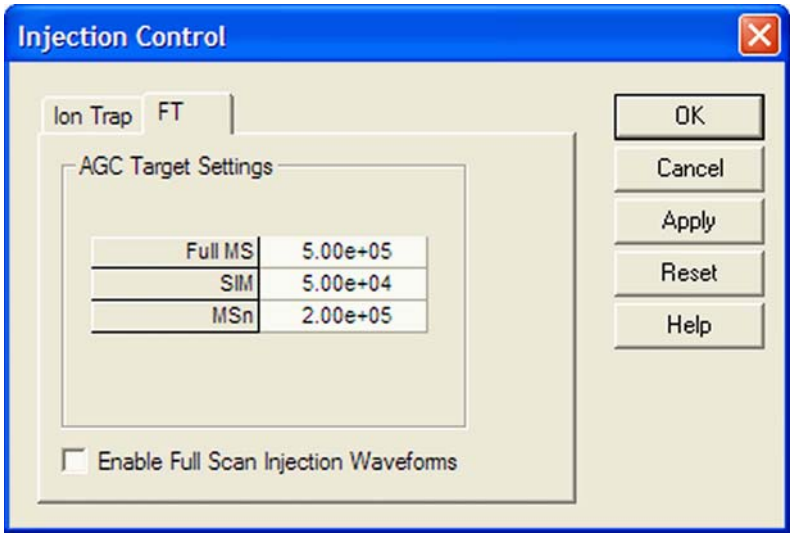


Figure 2-13. FT page of the Injection Control dialog box

Enable Full Scan Injection Waveforms

You can enable or disable the injection waveforms independently for ion trap and FT scans.

If the injection waveforms are enabled, a filter on the ions injected into the ion trap is applied. The ions above and below the selected ion or ion range selected are rejected. This option is often useful if the ion trap is being filled with ions of greater or lesser mass than the ion mass or ion mass range of interest. For example, this option can be used to remove high mass ions that are not of interest and ensures that more target ions can enter the trap before the trap is full.

Note The FT injection waveforms option only applies to full scan MS scans performed with the orbitrap mass analyzer. In FT SIM and FT MS^n scans, the injection waveforms are automatically enabled. ▲

FT Vacuum

The Vacuum dialog box allows you to monitor the vacuum system parameters. The Vacuum dialog box has two pages to enable an independent selection of displaying the vacuum data of the ion trap or the FT part.

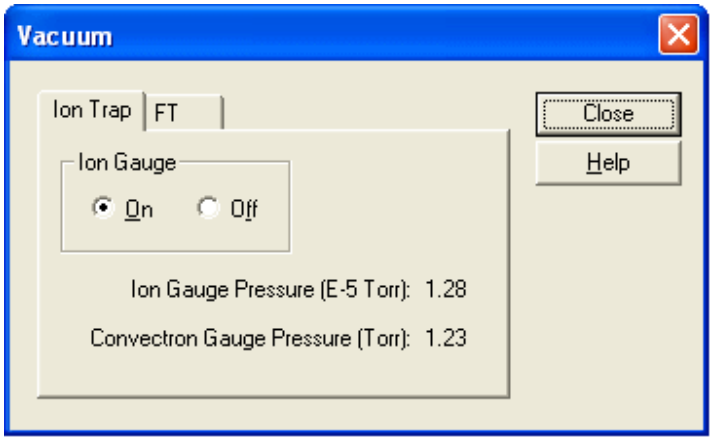


Figure 2-14. Ion Trap page of the Vacuum dialog box

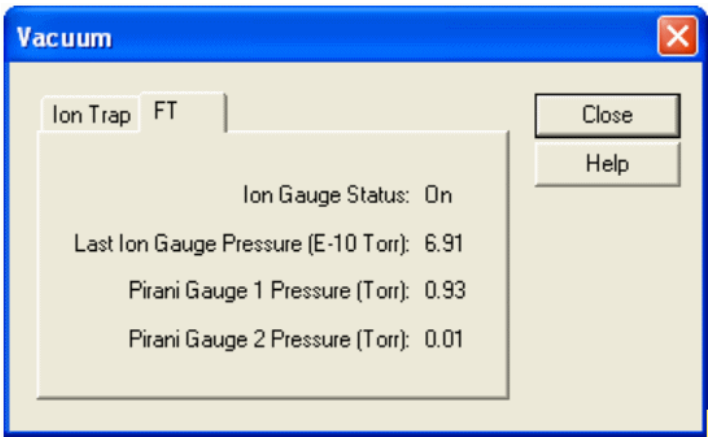


Figure 2-15. FT page of the Vacuum dialog box

To open this dialog box:

- From the Tune Plus window, choose Setup > **Vacuum…**, or
- click on  in the Instrument Setup toolbar.

FT Temperature Monitor

The FT Temperature Monitor dialog box allows to view the status of the FTMS analyzer temperature regulation. Deviations of the actual temperature from the temperature setpoint can affect instrument performance. It is not possible to operate the instrument when the bakeout procedure is active.

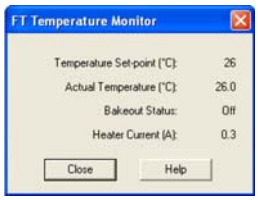


Figure 2-16. FT Temperature Monitor dialog box

To open this dialog box:

- From the Tune Plus window, choose Setup > **FT Temperature Monitor…**, or
- click on  in the Instrument Setup toolbar.

Tune Methods

Several parameters, like the ion source parameters, ion trap optics parameters, AGC target values etc., are stored in tune methods. This topic points out for which parameters a differentiation between ion trap and FTMS is made. The Tune Plus title bar displays the name of the current tune method. If you are not currently editing a preexisting tune method, the title bar displays the word Untitled.

Parameters with Differentiation between Ion Trap and FT Scans

A differentiation between Ion Trap and FTMS scans is made for the following tune parameters.

AGC Target Values

They can be set and saved independently for these experimental modes (no differentiation between positive and negative ion polarity mode):

- Ion Trap Full MS Target
- Ion Trap SIM Target
- Ion Trap MS^n Target
- Ion Trap Zoom Target
- FT Full MS Target
- FT SIM Target
- FT MS^n Target

Microscans and Maximum Inject Time

They can be set and saved independently for these experimental modes:

- Ion Trap Full MS, positive ion mode
- Ion Trap SIM, positive ion mode
- Ion Trap MS^n, positive ion mode
- Ion Trap Zoom, positive ion mode
- FT Full MS, positive ion mode
- FT SIM, positive ion mode
- FT MS^n, positive ion mode
- Ion Trap Full MS, negative ion mode

- Ion Trap SIM, negative ion mode
- Ion Trap MS^n, negative ion mode
- Ion Trap Zoom, negative ion mode
- FT Full MS, negative ion mode
- FT SIM, negative ion mode
- FT MS^n, negative ion mode

Inject Waveform Flags

The flag whether the inject waveform is enabled or disabled can be set and saved independently for

- Ion trap scans
- FT full scans.

Parameters without Differentiation between Ion Trap and FT Scans

No differentiation between Ion Trap and FT scans is made for all ESI parameters, and for all ion source and ion optics parameters.

Parameters not saved in a Tunefile

All parameters which can be set in an instrument method are not saved in the tune method. Thus the following parameters are not saved in a tune method:

- Analyzer (Ion Trap or FTMS)
- Mass Range (Low, Normal, or High)
- Scan Rate
- Resolution
- Scan Type (Full, SIM, SRM, CRM)
- Scan Range
- Polarity* (positive or negative)
- Data type* (centroid or profile)

* Only the data format (centroid or profile) and the ion polarity are saved in a tunefile that are set after a new start of TunePlus.

Chapter 3 Calibrating the Orbitrap XL for FTMS Measurements

This chapter provides procedures to calibrate your LTQ Orbitrap XL for FTMS measurements. It contains the following topics:

Preliminary Remarks

There are no specific tune procedures for the FTMS. All FTMS ion transfer and excitation parameters are treated as calibration parameters and are determined in automatic calibration procedures.

In the automatic calibration, the FT transmission calibration and the FT mass calibration are automatically performed for all calibration ranges. In the semi-automatic calibration, it is possible to decide whether the transmission and/or mass calibration are performed only for the positive ion mode, only for the negative ion mode or for both polarities. See topics "Automatic Calibration Page" on page 3-11 and "Semi-Automatic Calibration Page" on page 3-12 for further details.

On the FT Manual calibration page, you can select your own calibration masses for FT ion transmission, storage transmission, and FT mass calibration. See topic "FT Manual Calibration Page" on page 3-18 for further details.

Note It is recommended to use the semi-automatic calibration. ▲

Calibration Files and their Backups

After a successful or partly successful calibration, the ion trap and FT calibration parameters are saved automatically. All ion trap and FT calibration parameters are stored in the calibration file `master.LTQCal`, which is located in the folder:

`C:\Xcalibur\system\ltq\msx`

Backup Current Calibration

It is possible to create a backup of the current calibration file manually or by choosing File > **Backup Current Calibration** in the Tune Plus window. The Backup Current Calibration and Restore Backup Calibration items work by copying the `master.LTQCal` to `user.LTQCal` and vice versa.

If a backup calibration `user.LTQCal` was already generated, the old `user.LTQCal` will be backed-up to a file named `userXYZ.LTQCal`. If you perform backup calibrations at regular intervals, then a history of your calibration files is generated in the folder:

`C:\Xcalibur\system\ltq\msx`

Using the Backup Calibration command regularly allows to return to previous calibrations in case a new calibration is suspected to worsen instrument performance.

Restore Backup Calibration

Upon **Restore Backup Calibration**, the calibration values saved in `user.LTQCal` are automatically downloaded to the instrument. Therefore, it is recommended to generate a current backup after a successful calibration.

It is also recommended to use the **Restore Backup Calibration** command instead of the **Restore Factory Calibration** command since the backup calibration file is newer than the factory calibration file.

Calibration Solutions

This section provides information about preparing the calibration solutions for the LTQ Orbitrap XL.

Sollte man die Namen der Loesungen nicht beibehalten. Sonst werden Leute verwirrt die neue und alte Orbitraps - und LTQ FTs - im Labor stehen haben.

LTQ Calibration Solution

The LTQ calibration solution consists of caffeine, MRFA, and Ultramark 1621 in an acetonitrile:methanol:water solution containing 1% acetic acid.

Note Vials of caffeine, MRFA, and Ultramark 1621 are included in the API accessory kit. To order more of these compounds, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
Phone (800) 325-3010 (in the USA or Canada)
(314) 771-3750 (outside the USA or Canada) ▲

Caution AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS.

Always wear protective gloves and safety glasses when you handle solvents or corrosives. Also contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for proper handling of a particular solvent. ▲

To prepare the LTQ calibration solution, use the following procedure:

1. Stock Solution: MRFA

   Prepare a 1.5 mL stock solution of 166.7 pmol/µL MRFA in 50:50 methanol:water as follows:

   a. Obtain the vial of L-methionyl-arginyl-phenylalanyl-alanine acetate × H_2O (MRFA) in your accessory kit. In this form, the MRFA sample has an average molecular weight of 607.7 u. Carefully weigh 3.0 mg of the MRFA sample.

   b. Dissolve the MRFA sample in a total volume of 1.0 mL of 50:50 methanol:water. Mix the solution (5.0 nmol/µL) thoroughly.

   c. Transfer 50 µL of the 5 nmol/µL solution into a clean polypropylene tube.

   d. Add 1.45 mL of 50:50 methanol:water to the tube. Mix this solution (166.7 pmol/µL) thoroughly.

e. Label the tube *MRFA stock solution* and store it in a refrigerator until it is needed.

2. Ultramark 1621 stock solution

   Prepare a 10 mL stock solution of 0.1% Ultramark 1621 in acetonitrile as follows:

   a. Obtain the vial of Ultramark 1621 in your accessory kit.
   b. Using a syringe, measure out 10 µL of Ultramark 1621, and dissolve it in 10 mL of acetonitrile. Mix the solution thoroughly.
   c. Label the vial *Ultramark 1621 stock solution* and store it in a refrigerator until it is needed.

3. LTQ calibration solution

   Prepare 10 mL of the LTQ calibration solution as follows:

   a. Obtain the 1 mg/mL stock solution of caffeine in 100% methanol that is provided in your accessory kit.
   b. Pipet 200 µL of the caffeine stock solution into a clean, dry 10 mL volumetric flask.
   c. Pipet 100 µL of the MRFA stock solution into the flask.
   d. Pipet 100 µL of the Ultramark 1621 stock solution into the flask.
   e. Pipet 100 µL of glacial acetic acid into the flask.

Note Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Using plastic pipet tips causes contamination of acid stock solutions that can introduce contaminants into the calibration solution. ▲

   f. Pipet 5 mL of acetonitrile into the flask.
   g. Bring the volume of the solution up to the 10 mL-mark on the flask with 50:50 methanol:water.
   h. Mix the calibration solution thoroughly.
   i. Transfer the solution to a clean, dry vial.
   j. Label the vial *LTQ Calibration Solution* and store it in a refrigerator until it is needed.

LTQ Orbitrap XL Calibration Solution

In contrast to the ion trap calibration, the FT calibration of the LTQ Orbitrap XL requires both positive and negative ions. The LTQ ESI calibration solution does not provide applicable negative ions below m/z 1000. Thus, sodium dodecyl sulfate (gives anion at m/z 265) and sodium taurocholate (gives anion at m/z 514) is added to the LTQ ESI calibration solution to generate the LTQ Orbitrap XL calibration solution. In addition, the ratio of MRFA is increased versus the LTQ calibration solution.

Note Vials of sodium dodecyl sulfate and sodium taurocholate are shipped with the instrument. Refer to the Material Safety Data Sheet (MSDS) for proper handling of these substances. To order more of these compounds, contact Sigma-Aldrich. The product number of sodium dodecyl sulfate is L4509-10G, the product number of sodium taurocholate is T4009-250MG. Note that sodium dodecyl sulfate and sodium taurocholate are not included in the standard LTQ API accessory kit. ▲

Caution AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS.

Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for proper handling of a particular solvent. ▲

To prepare the LTQ Orbitrap XL calibration solution, use the following procedure:

1. Stock Solution: Sodium Dodecyl Sulfate

   a. Obtain the vial of sodium dodecyl sulfate. In this form the sample has an average molecular weight of 288.4 u.

   b. Prepare the stock solution of sodium dodecyl sulfate by dissolving 2.88 mg in 10 mL of 50:50 methanol:water.

   c. Mix the solution (1.0 nmol/µL) thoroughly.

   d. Label the vial *Sodium Dodecyl Sulfate stock solution (1 nmol/µL)*.

2. Stock Solution: Sodium Taurocholate

   a. Obtain the vial of sodium taurocholate. In this form the sample has an average molecular weight of 537.7 u.

   b. Prepare the stock solution of sodium dodecyl sulfate by dissolving 5.38 mg in 10 mL of 50:50 methanol:water.

   c. Mix the solution (1.0 nmol/µL) thoroughly.

d. Label the vial *Sodium Taurocholate stock solution (1 nmol/µL)*.

3. Stock Solution: MRFA

   To prepare the MRFA stock solution (5.0 nmol/µL or 166.7 pmol/µL in 50:50 methanol:water), refer to step 1 of topic "LTQ Calibration Solution" above.

4. LTQ Orbitrap XL Calibration Solution

   Prepare 10 mL of the LTQ Orbitrap XL calibration solution, as follows:

   a. Pipet 10 mL of the standard LTQ ESI calibration solution into a vial.
   b. Add 100 µL of the Sodium Dodecyl Sulfate stock solution.
   c. Add 100 µL of the Sodium Taurocholate stock solution.
   d. Add 6.6 µL of the 5.0 nmol/µL MRFA stock solution or 200 µL of the 166.7 pmol/µL MRFA stock solution, respectively.
   e. Mix the solution thoroughly.
   f. Label the vial *LTQ Orbitrap XL calibration solution* and store it in a refrigerator until it is needed.

Applicable Calibration Solutions for Automatic Calibration

In an automatic calibration of the LTQ Orbitrap XL, both the ion trap and the FT calibrations (positive and negative ion mode) are performed. Thus for an automatic calibration, the LTQ Orbitrap XL calibration solution has to be used.

Applicable Calibration Solutions for Semi-Automatic Calibration

For the ion trap calibrations, the LTQ ESI calibration solution or the LTQ Orbitrap XL calibration solution can be used. For the FT calibrations of the positive ion mode, the LTQ ESI calibration solution or the LTQ Orbitrap XL calibration solution can be used. For the FT calibrations of the negative ion mode, the LTQ Orbitrap XL calibration solution has to be used. Thus, for a complete FT calibration the LTQ Orbitrap XL calibration has to be used, too.

Applicable Calibration Solutions for FT Manual Calibration

Since the FT Manual Calibration page allows using your own calibration masses, it is possible to use custom calibration solution here. However, there are some requirements for the calibration masses. The scan ranges of the instrument need to be covered properly by the given masses.

Calibration and Tuning of the Ion Trap

This chapter describes the calibration and tuning of the ion trap for FT measurements. It contains the following topics:

- Calibration of the Ion Trap
- Tuning the Ion Trap for Positive Ion Mode
- Tuning the Ion Trap for Negative Ion Mode

Calibration of the Ion Trap

To perform an FT calibration, the ion trap has to be successfully calibrated before. It is very important that the electron multiplier gain is correctly calibrated since the AGC prescan is performed in the ion trap. Thus, the electron multiplier gain calibration should be checked before an FT calibration is performed.

Note It is not necessary to use the ESI standard solution to perform a multiplier gain calibration. It is sufficient to use an MRFA solution (for example 5×10^{-6} M in 100% methanol, 1% acetic acid). ▲

For the ion trap calibrations, the LTQ ESI calibration solution or the LTQ Orbitrap XL calibration solution can be used.

Tuning the Ion Trap for Positive Ion Mode

For the positive ion mode, it is recommended to perform an automatic tune of m/z 524 at a Full MS Target of 1e4–3e4. Use the LTQ ESI calibration solution or the LTQ Orbitrap XL calibration solution with the following settings in the Define Scan dialog box. See Figure 3-1 on page 3-9.

After the automatic tune, a manual adjustment of the tube lens should be used to get an ion trap spectrum in the scan range 130–2000.

The spectrum should look similar to the spectrum shown in Figure 3-2 on page 3-9. Make sure the peaks at 138, 195, 524, and the highest Ultramark peaks are all present, ideally above 30% of the base peak.

Note The peak at m/z 138 should have a height of more than 10%. Run an automatic ion trap tune on 138 if this signal is too weak. ▲

The inject time should be stable and less than 1 ms (if a Full MS target of 1e4 is used). Do not forget to save the tune method after a successful tuning.

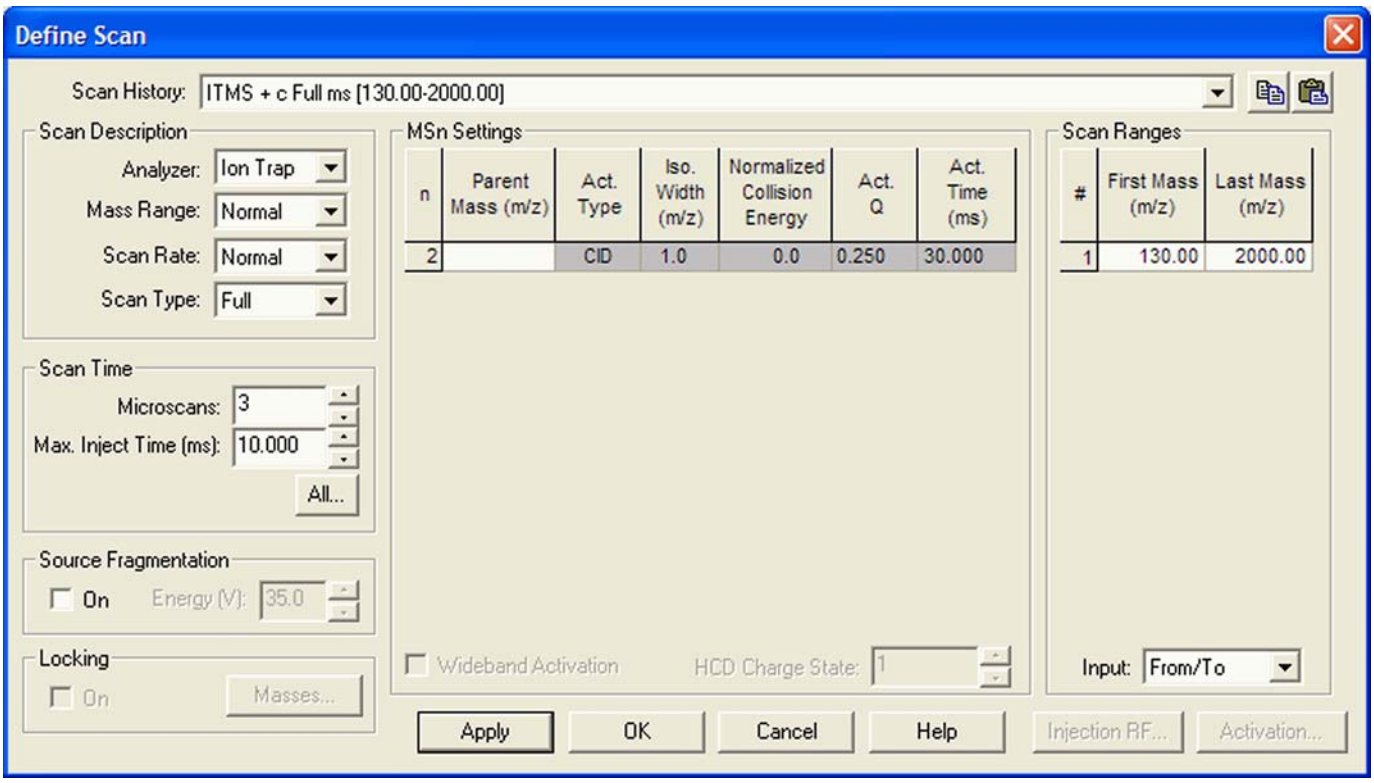


Figure 3-1. Recommended settings in the Define Scan dialog box for an automatic tune of the ion trap

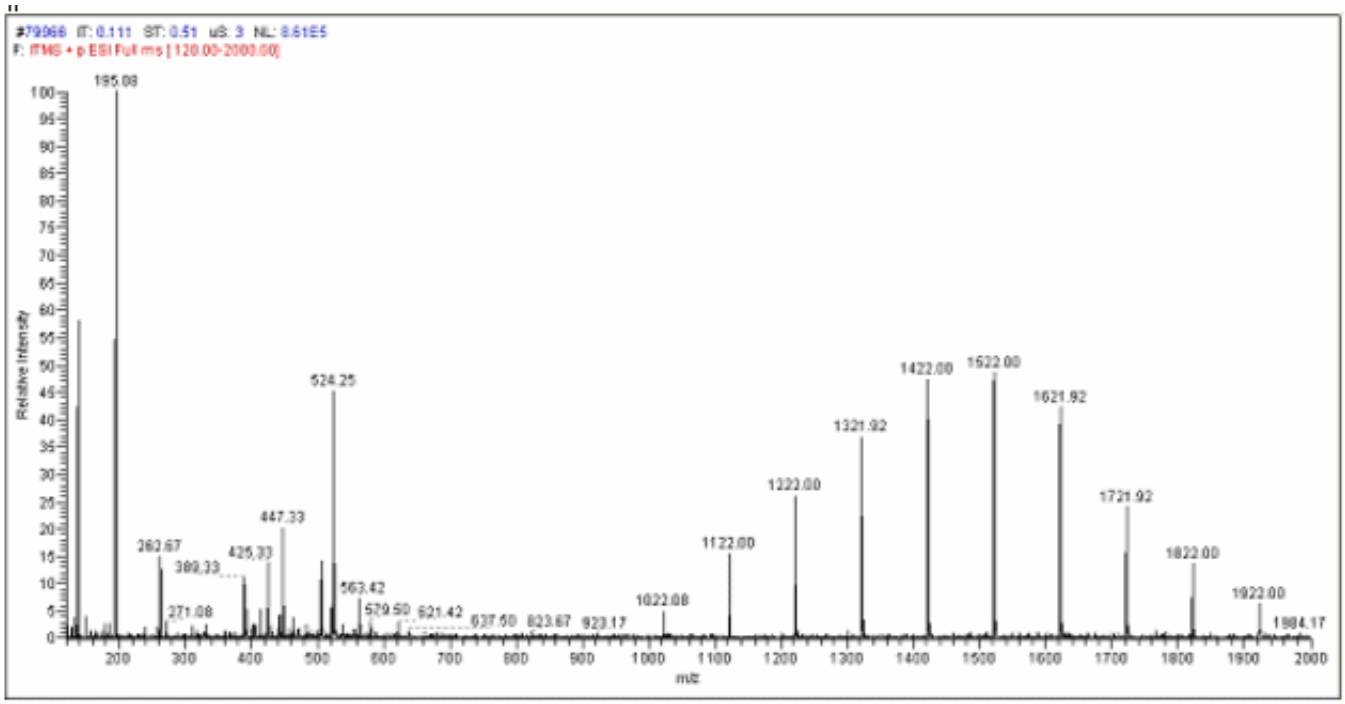


Figure 3-2. Ion trap spectrum of the LTQ Orbitrap XL calibration solution, scan range 120–2000, positive ion polarity mode

Tuning the Ion Trap for Negative Ion Mode

For the negative ion mode, it is recommended to perform an automatic tune of m/z 514 at a Full MS Target of 1e4–3e4. Use the LTQ Orbitrap XL calibration solution with the settings shown in Figure 3-1 on page 3-9.

After the automatic tune, a manual adjustment of the tube lens should be used to get an ion trap spectrum in the scan range 150–2000. At m/z 265 is the base peak (100%) and the highest Ultramark adduct ion peaks are at about 80%, as shown in Figure 3-3 on page 3-10.

If the tube lens is set to a very high negative voltage, in-source fragmentation of the Ultramark adduct ions may occur. Thus, if you observe rather ions at m/z 906, 1006, … than ions at m/z 1280, 1380, … the tube lens setting has to be decreased. The inject time should be stable and less than 1 ms (if a target of 1e4 is used). Do not forget to save the tune method after a successful tuning.

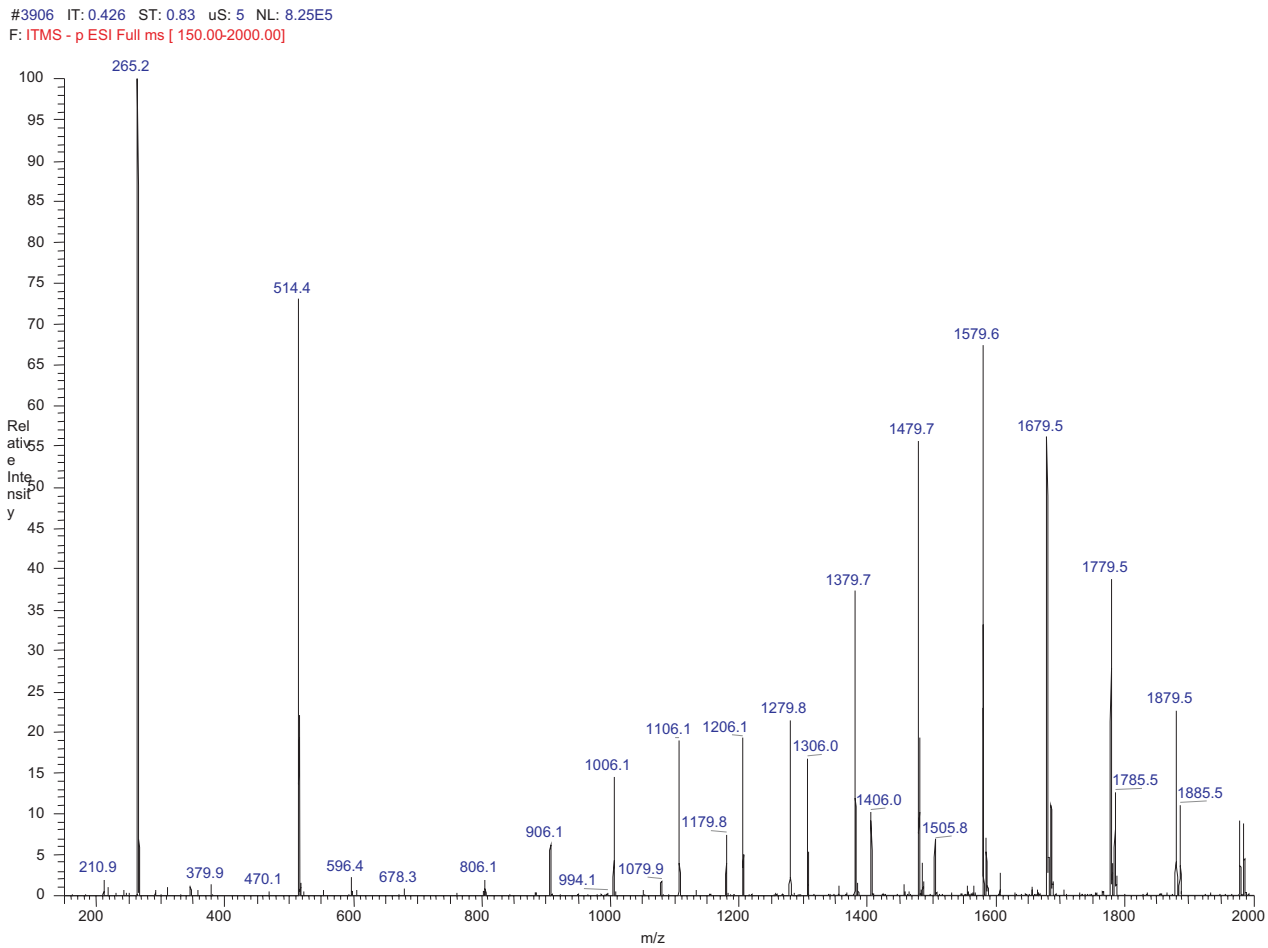


Figure 3-3. Ion trap spectrum of the LTQ Orbitrap XL calibration solution, scan range 150–2000, negative ion mode

Automatic Calibration Page

The Automatic page of the Calibrate dialog box (see Figure 3-4) allows you to perform an automatic calibration of all the calibration parameters including all ion trap calibrations and all FT calibrations.

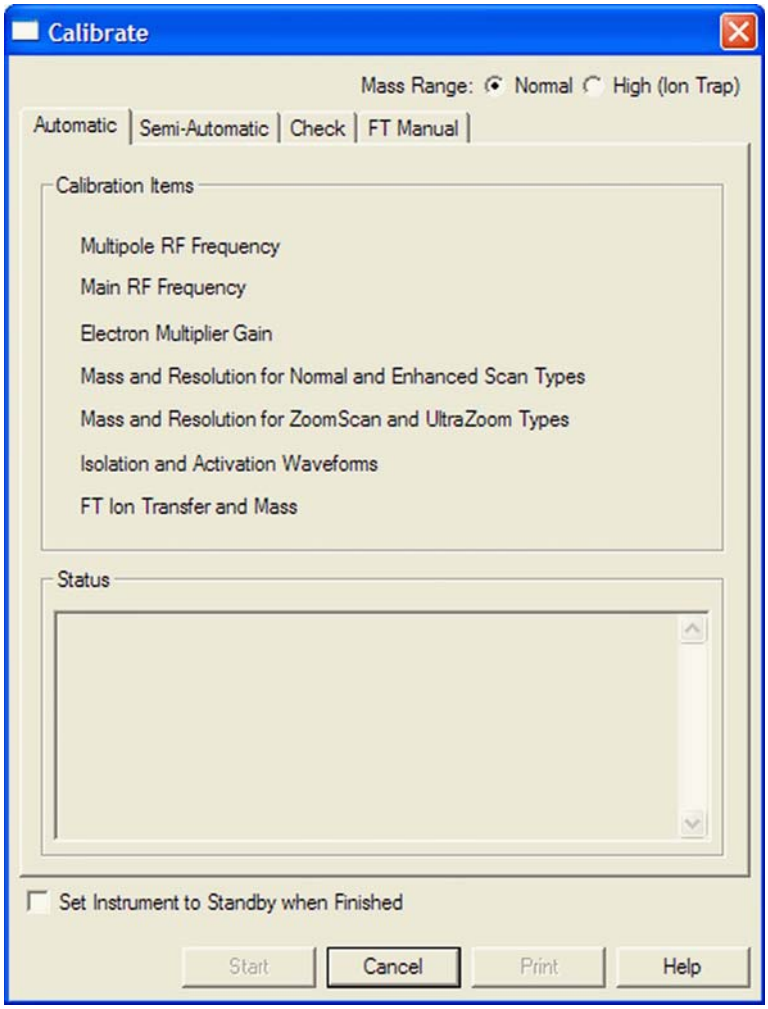


Figure 3-4. Automatic page of the Calibrate dialog box

In an automatic calibration, these four FT calibration procedures are performed automatically one after another subsequent to the ion trap calibration. To perform an automatic calibration, the LTQ Orbitrap XL calibration solution has to be used.

The calibration masses and all experimental parameters like target values, scan ranges, resolution settings, etc. are set automatically and cannot be influenced by the user.

Note Usually you do not need to perform a complete ion trap calibration or an FT ion transmission calibration unless the hardware is modified in some way. However, it is necessary to repeat the electron multiplier calibration and the FT mass calibration on a regular basis. Thus, in the most cases it is not recommended to perform an automatic calibration of the LTQ Orbitrap XL since all calibrations are performed which takes about 1 hour. To run a multiplier gain calibration or an FT mass calibration (which takes only some minutes), it is recommended to use the semiautomatic calibration. ▲

Semi-Automatic Calibration Page

The Semi-Automatic page of the Calibrate dialog box (see Figure 3-5) allows you to select specific calibration parameters to calibrate, for example only the ion trap calibrations or only the FT calibrations. For FT calibrations, it is also possible to differentiate between positive and negative ion mode.

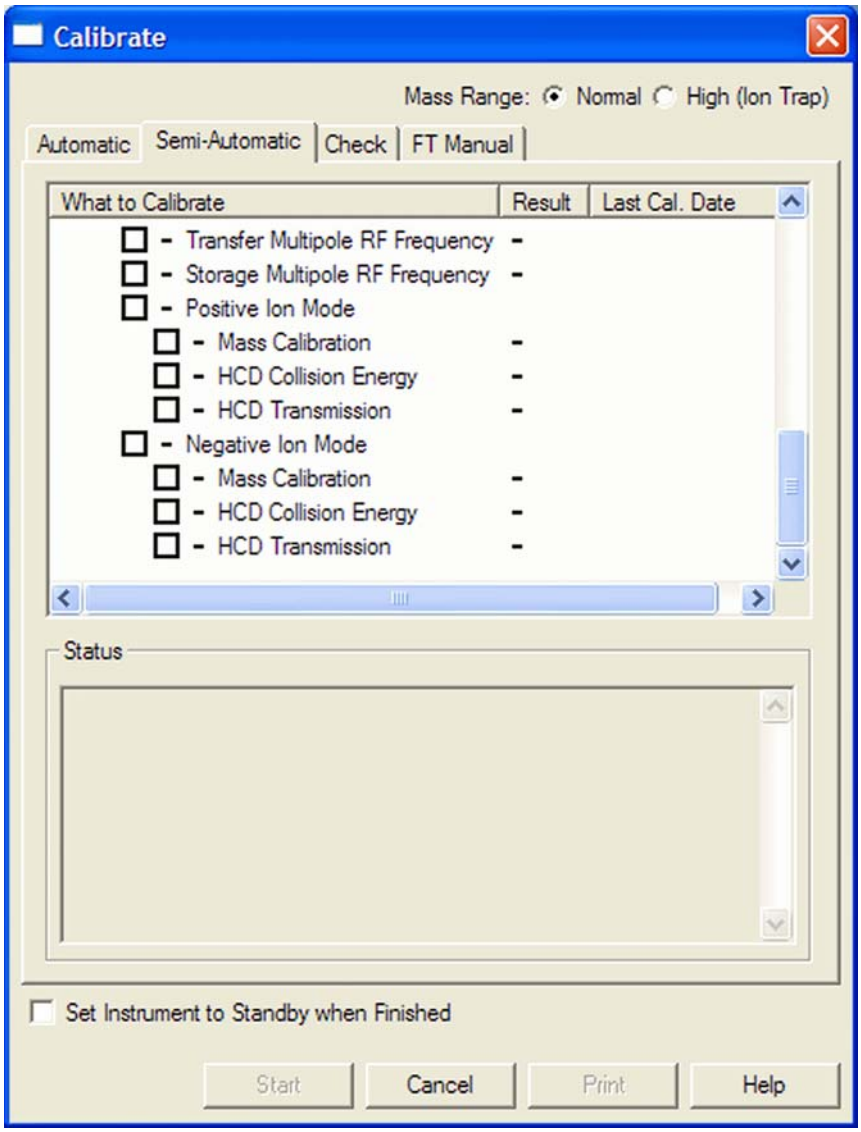


Figure 3-5. Semi-Automatic page of the Calibrate dialog box

To calibrate one or more selected parameters, clear the Select All check box to make the individual calibration parameters available. Select the parameter(s) you want to calibrate, then click on **Start**.

For example:

- To run a complete automatic calibration (ion trap and FT), tick the Select All check box. Then, click on **Start**. This is analogous to the automatic calibration. As already described before, it is not recommended to perform an automatic calibration of the LTQ Orbitrap XL if not necessary since all calibrations are performed, which takes about 1 hour.
- To run an automatic calibration of the ion trap, tick the Select All - Ion Trap check box. Then, click on **Start**.
- To run an automatic calibration of the FT part, tick the Select All - FT check box. Then, click on **Start**.
- To run a FT mass calibration, tick the Mass Calibration check box. Then, click on **Start**.

In a semi-automatic calibration, the selected FT calibration procedure(s) are performed automatically one after another.

All calibrations apart from the FT calibrations for the negative ion mode can be performed with the LTQ ESI calibration solution or with the LTQ Orbitrap XL calibration solution. To run the FT ion transmission and/or mass calibration for the negative ion mode, the LTQ Orbitrap XL calibration solution has to be used.

The calibration masses and all experimental parameters like target values, scan ranges, resolution settings, inject waveforms etc. are set automatically and cannot be influenced by the user.

HCD Calibration

The Semi-Automatic page of the Calibrate dialog box allows calibrating the HCD collision energy and the HCD transmission for the positive and negative ion mode as well.

Advanced Calibration Features

When the Advanced Calibration Features in the Control menu are enabled, the Semi-Automatic page of the Calibrate dialog box additionally allows calibrating the storage transmission and the FT transmission for the positive and negative ion mode as well. See Figure 3-6.

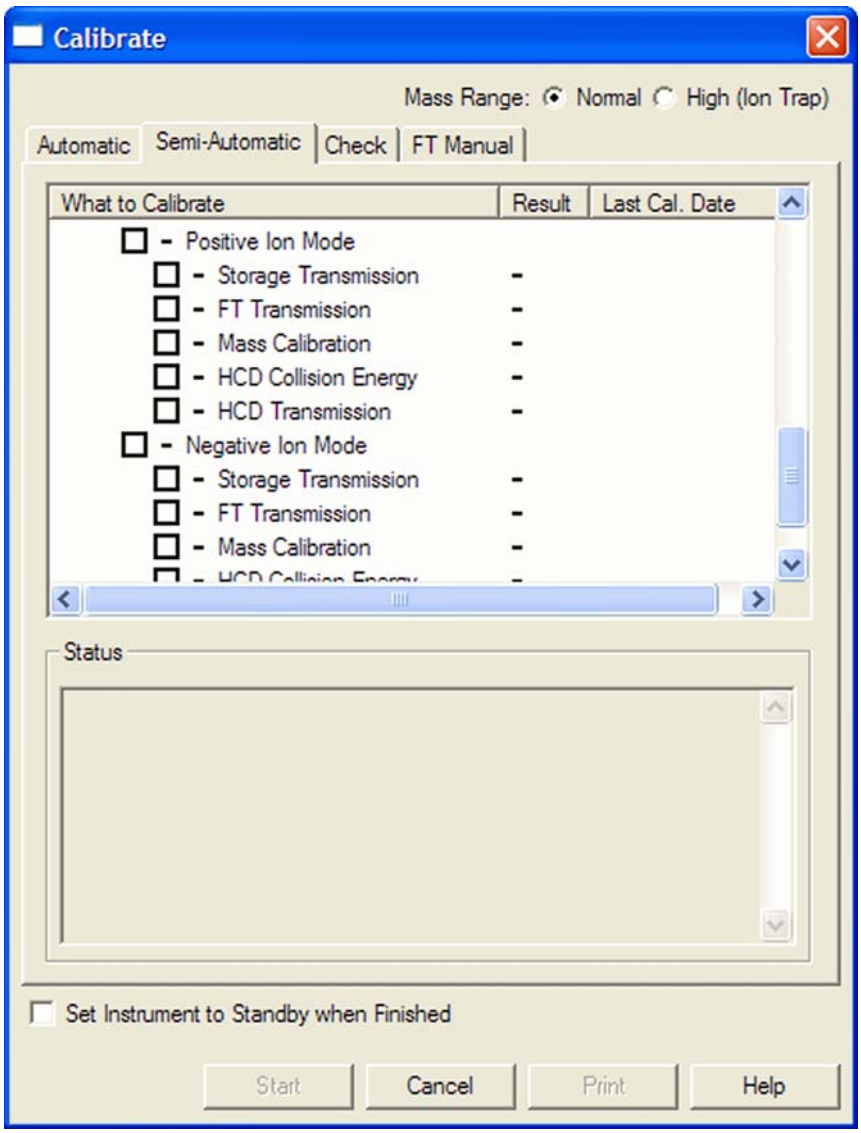


Figure 3-6. Semi-Automatic page of the Calibrate dialog box (Advanced Calibration Features enabled)

Check Calibration Page

The Check page of the Calibrate dialog box allows you to automatically check several calibration settings. See Figure 3-7.

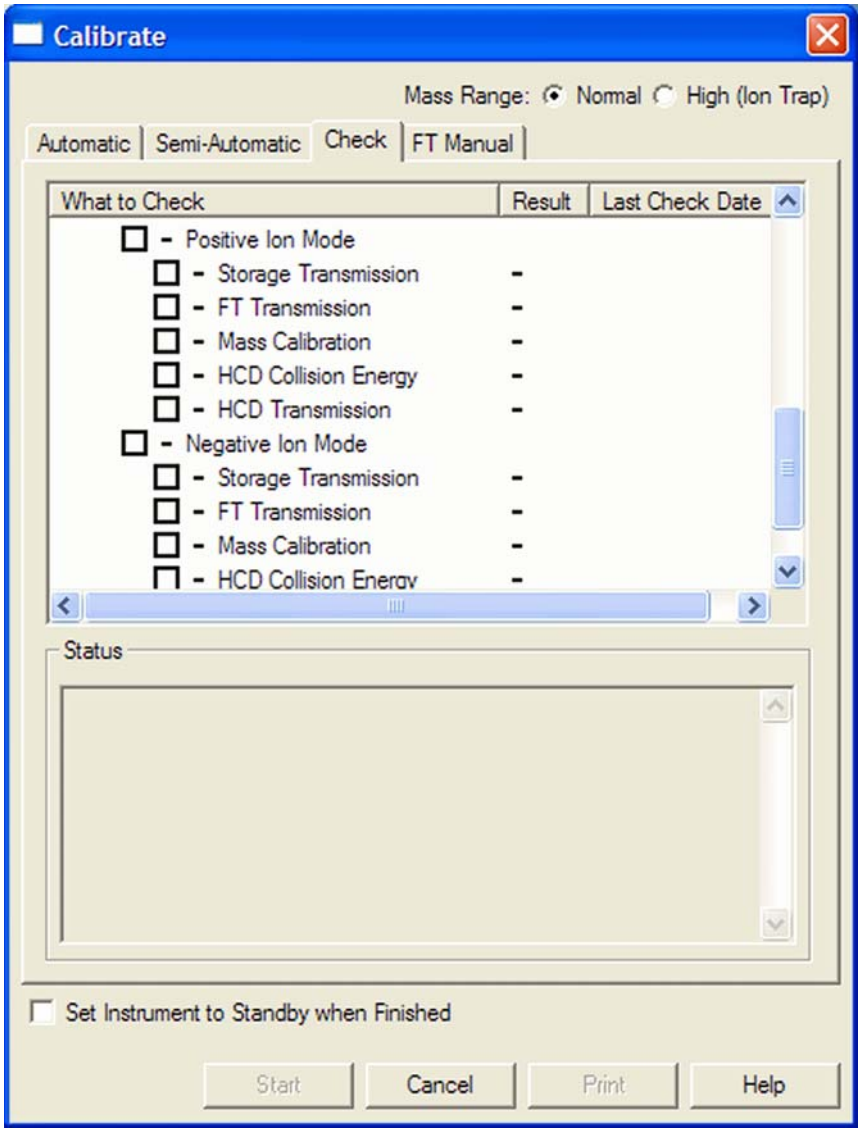


Figure 3-7. Check page of the Calibrate dialog box

All calibration checks apart from those for the FT negative ion mode can be performed with the LTQ Orbitrap XL calibration solution or the LTQ ESI calibration solution. To check the FT calibrations in the negative ion mode, the LTQ Orbitrap XL calibration solution has to be used.

The calibration masses and all experimental parameters like target values, scan ranges, resolution settings, etc. are set automatically.

At the conclusion of the check procedure, the LTQ XL MS detector displays a message that indicates whether the parameter(s) are calibrated properly or not.

Using the Check page of the Calibrate dialog box, you can select the following parameters:

Select All	This check box allows you to specify whether or not to check all of the calibration parameters. To check all calibration parameters, select the Select All check box. In this case, all ion trap calibration parameters and all FT calibration parameters are checked. You can also check each calibration parameter individually. To make the individual calibration parameters available, clear the Select All check box.
Select All Ion Trap	This check box allows you to specify whether or not to check the calibration of the linear ion trap parameters only.
Select All-FT	This check box allows you to specify whether or not to check the calibration of the Orbitrap ion transfer optics and mass analyzer only.
Transfer Multipole RF Frequency	This check box allows you to specify whether or not to check the frequency of the RF voltage of the transfer multipole in the FT transfer ion optics.
Storage Multipole RF Frequency	This check box allows you to specify whether or not to check the frequency of the RF voltage of the storage multipole in the FT transfer ion optics.
Positive Ion Mode	This check box allows you to specify whether or not to check the FT ion transmission calibration and FT mass calibration for the positive ion mode.
Negative Ion Mode	This check box allows you to specify whether or not to check the FT ion transmission calibration and FT mass calibration for the negative ion mode.
Storage Transmission	This check box allows you to specify whether or not to check the ion storage transmission calibration. The storage transmission is checked by transferring ions form the ion trap to the ion storage device and backward, then scanning in the ion trap. The FT storage transmission calibration can be checked for the positive and negative ion mode independently.

FT Transmission	This check box allows you to specify whether or not to check the FT ion transmission calibration. The ion transmission from the ion trap to the Orbitrap is checked by means of the calibration masses in SIM experiments at different AGC target values. The FT transmission calibration can be checked for the positive and negative ion mode independently.
Mass Calibration	This check box allows you to specify whether or not to check the mass calibration of the Orbitrap mass analyzer. In this check, the current mass calibration is checked, i.e it is a check of the external mass calibration. The FT mass calibration can be checked for the positive and negative ion mode independently.
HCD Collision Energy	This check box allows you to specify whether or not to check the HCD collision energy.
HCD Transmission	This check box allows you to specify whether or not to check the HCD transmission.

The Last Check Date readback column gives the date of the last successful check for each item. If a check is performed that fails, the last successful check date still appears in the Last Check Date readback column. The last successful check continues to be in effect in the instrument. However, the result column will show a red x mark indicating that the current attempt check has failed or was aborted.

FT Manual Calibration Page

The FT Manual page of the Calibrate dialog box allows to perform or to check an FT transmission calibration, storage transmission calibration, and an FT mass calibration with user-defined calibration masses. See Figure 3-8.

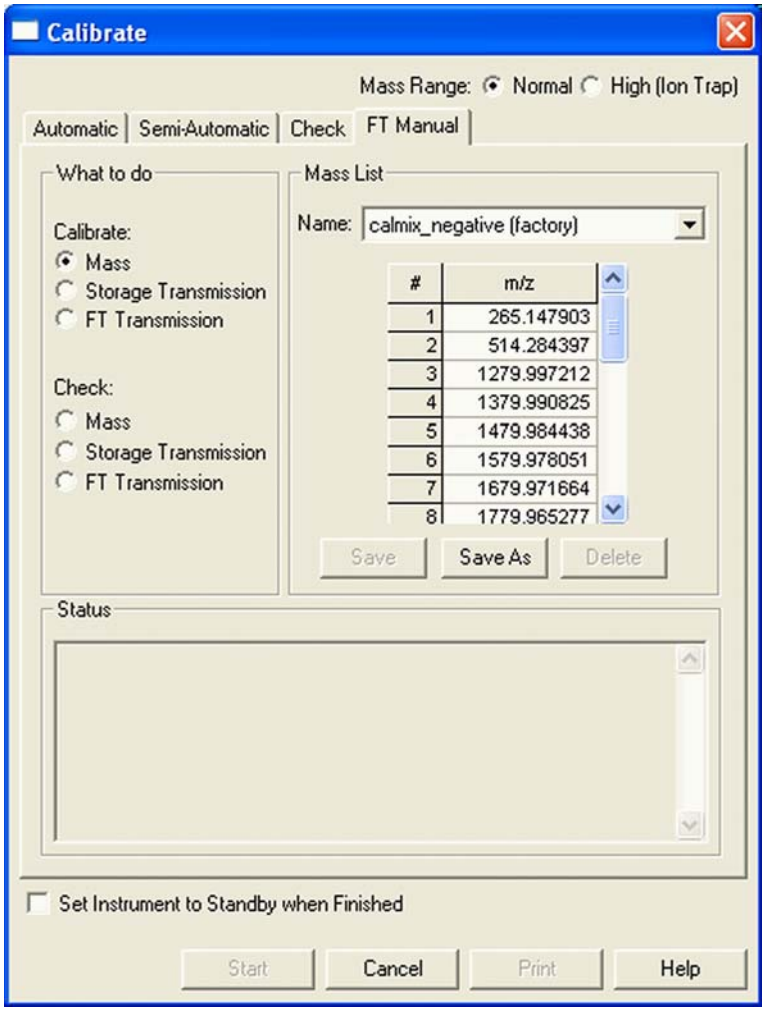


Figure 3-8. FT Manual page of the Calibrate dialog box

Note Starting from the FT Manual Page, the calibration is performed for the currently selected polarity only. ▲

Mass List Group Box

The calibration masses for the manual calibration can be defined in the corresponding mass list on the FT Manual page of the Calibration dialog box. Mass lists can be imported and exported by means of the Instrument Configuration page, see further details in Chapter 6: "Instrument Configuration".

Note Ensure that you use calibration masses of sufficient accuracy (sub ppm). ▲

Name This list box lists the names of the factory supplied and user created mass lists.

Mass List This table lists the mass-to-charge ratios of the ions that you are using to calibrate the orbitrap mass analyzer. You can select an existing mass list in the Name list box, or you can create or modify a mass list by clicking on it and editing the entries in the Mass List table.

Note Ensure that you use calibration masses of sufficient accuracy (sub ppm). ▲

Save Click on **Save** to save the mass list with the name that is selected in the Name list box.

Save As Click on **Save As** a to save the mass list with a new name.

Delete Click on **Delete** to delete the mass list that is selected in the Name list box.

Factory-Supplied Mass Lists

There are also two factory supplied mass lists, *calmix_positive* (factory) and *calmix_negative* (factory). They contain the exact masses of all main ion peaks, which should appear if the LTQ Orbitrap XL calibration solution is used in positive or negative ion mode, respectively.

Chapter 4 Performing Diagnostics/Checks

This chapter describes several diagnostic procedures for the LTQ Orbitrap XL. It contains the following topics:

System Evaluation Procedures

The System Evaluation page in the Diagnostics dialog box allows you to evaluate system performance. See Figure 4-1.

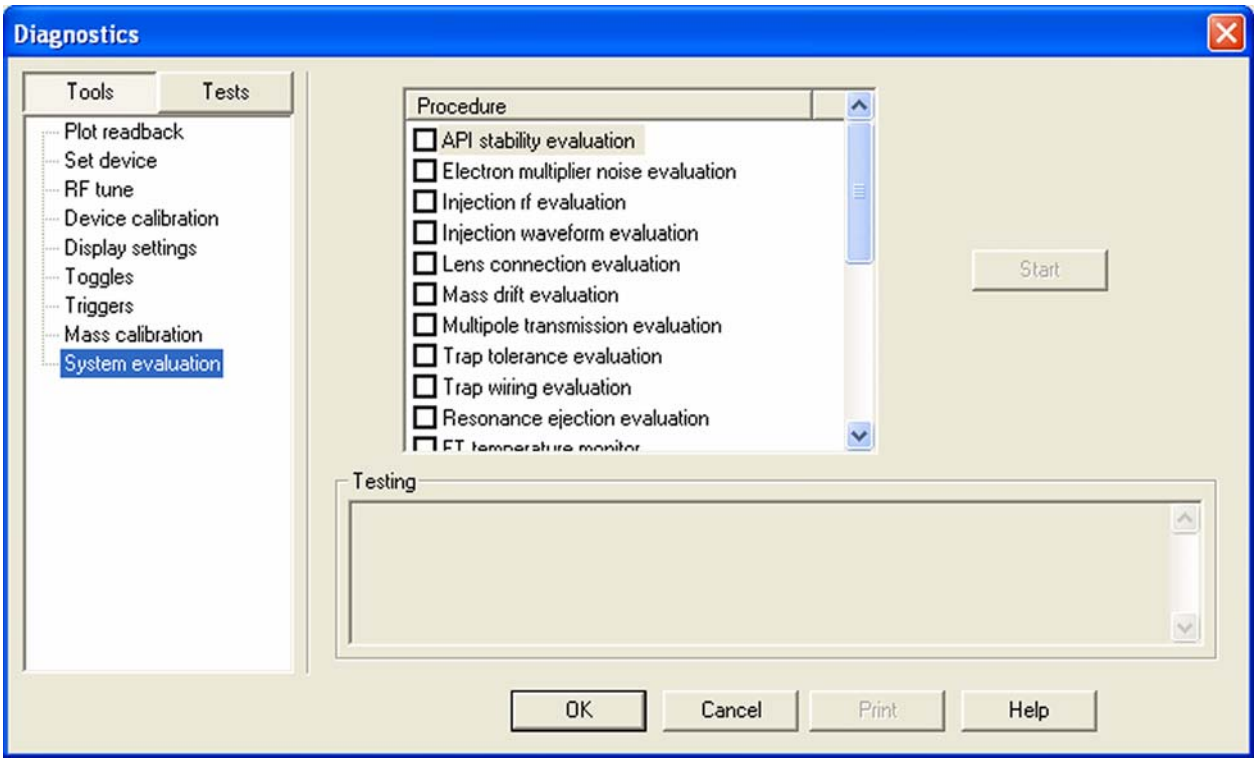


Figure 4-1. System evaluation page of the Diagnostics dialog box

Besides several ion trap relevant system evaluation procedures, you can perform the following FT system evaluation procedures:

- "FT Temperature Monitor" on page 4-3
- "FT Temperature Control Evaluation" on page 4-3
- "FT Preamp Evaluation" on page 4-3
- "FT Sensitivity Test" on page 4-3
- "FT Noise Test" on page 4-4
- "FT Isolation Test" on page 4-4
- "FT Dynamic Range Test" on page 4-4
- "FT Stability Test" on page 4-4
- "FT High Mass Range Target Compensation" on page 4-5

FT Temperature Monitor

External mass accuracy of the orbitrap detector depends on a stable temperature of the analyzer and the electronic components. This evaluation plots a history of the temperature regulation results to the graph view, see "Graph View" on page 2-5.

FT Temperature Control Evaluation

This evaluation procedure allows examining temperature regulation behavior of the instrument by intentionally driving temperatures to extreme values.

Note The evaluation will usually take more than 12 hours where no measurements can be done. After stopping the evaluation, the instrument needs to stabilize temperatures for several hours before high mass accuracy measurements can be started. ▲

FT Preamp Evaluation

This evaluation allows checking the basic FTMS analyzer signal detection path. The instrument needs to run in FTMS analyzer mode. It is recommended to switch to diagnostic transient view, see "FT Include Transients" on page 4-7.

During the evaluation, the preamp input protection switches are activated with a period of 100 ms. This switching can be observed as periodic incidences in the transient if the electronic signal path is operational.

FT Sensitivity Test

The FT sensitivity test is only applicable for an infusion experiment with Reserpine. The test assumes that a Reserpine solution of 5×10^{-9} M (100% methanol, 1% acetic acid) is used. The following test are performed one after another:

1. SIM of m/z 609.28 using the ion trap as analyzer and an AGC target of 2e+03.

2. SIM of m/z 609.28 using the orbitrap detector as analyzer and an AGC target of 5e+03.

3. SIM of m/z 609.28 using the orbitrap detector as analyzer and an AGC target of 5e+04.

4. MS/MS of m/z 609.28 using the orbitrap detector and an AGC target of 5e+04.

The test fails

1. if the inject time which is necessary to reach the selected AGC target value is too high;

2. if the ratio of the reserpine signal to the overall signal inside the SIM window is too low;

3. if the transmission from the ion trap to the orbitrap detector is too low, or

4. if the intensity of the product ions of reserpine is too low.

FT Noise Test

This test determines resistant noise peaks in the selected scan range. In this test ions are "switched off" automatically. At the conclusion of the FT noise test, a list of resistant noise peaks is displayed in the Testing text.

FT Isolation Test

This test is only applicable for an infusion experiment with a solution containing MRFA, for example the standard LTQ calibration solution or a MRFA alone solution (for example 5×10^{-6} M in 100% methanol/water, 1% acetic acid). This test is analogous to the "Check of the ion isolation waveform" on the Check page of the Calibrate dialog box. Here, the isolation of m/z 525.3 is performed at a target of 2000 and analyzed by the ion trap. In contrast to this, the FT isolation test is performed at higher targets and uses the FT analyzer. Thus this test determines the maximum AGC target value that allows performing a unit isolation of m/z 525.3 at the presence of m/z 524.3 and 526.3.

FT Dynamic Range Test

This test is only applicable for an infusion experiment with a solution containing MRFA, for example the standard LTQ calibration solution or a MRFA alone solution (for example 5×10^{-6} M in 100% methanol/water, 1% acetic acid). This test determines the signal-to-noise ratio of an isolated MRFA signal.

FT Stability Test

This test is applicable for an infusion experiment with any sample solution. This test procedure checks the stability of the FT TIC (total ion current) detected in the selected scan range by means of 600 scans. In principle, the test can be performed at any experimental conditions. It is recommended, however, to perform this test in Full scan mode

using one microscan, a resolution setting of 60 000 and a FT Full MS Target of 5e+05 or 1e+06. At the conclusion of the FT stability test, the AGC stability and the corresponding signal variation is displayed.

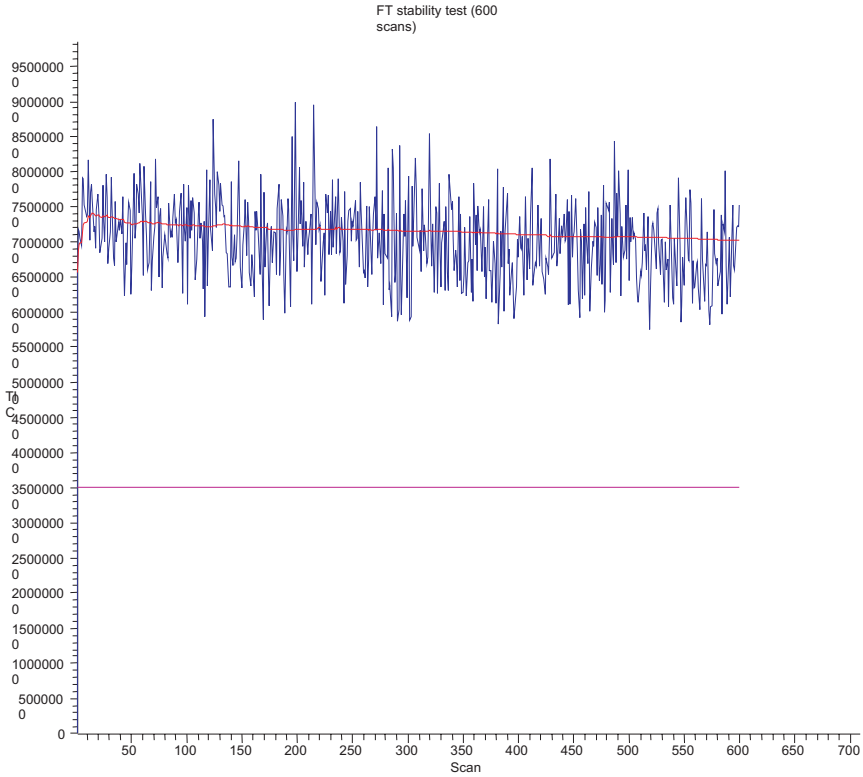


Figure 4-2. Result of the FT stability test displayed in the Graph View

FT High Mass Range Target Compensation

This procedure determines an AGC target compensation factor, which ensures that the FT mass calibration is still valid if the instrument is set into the high mass range mode. The resulting compensation factor will be saved in the calibration file. Usually, it is sufficient to run this procedure once. It is not necessary to repeat this procedure on a regular basis. It is recommended to use the LTQ calibration solution for this test. However, you can also use any other solution that gives reasonable ion signals at 1 000 < m/z < 2 000.

Toggles

The Toggles page in the Diagnostics dialog box allows you to toggle [change] a subsystem from one state to another state. See Figure 4-3.

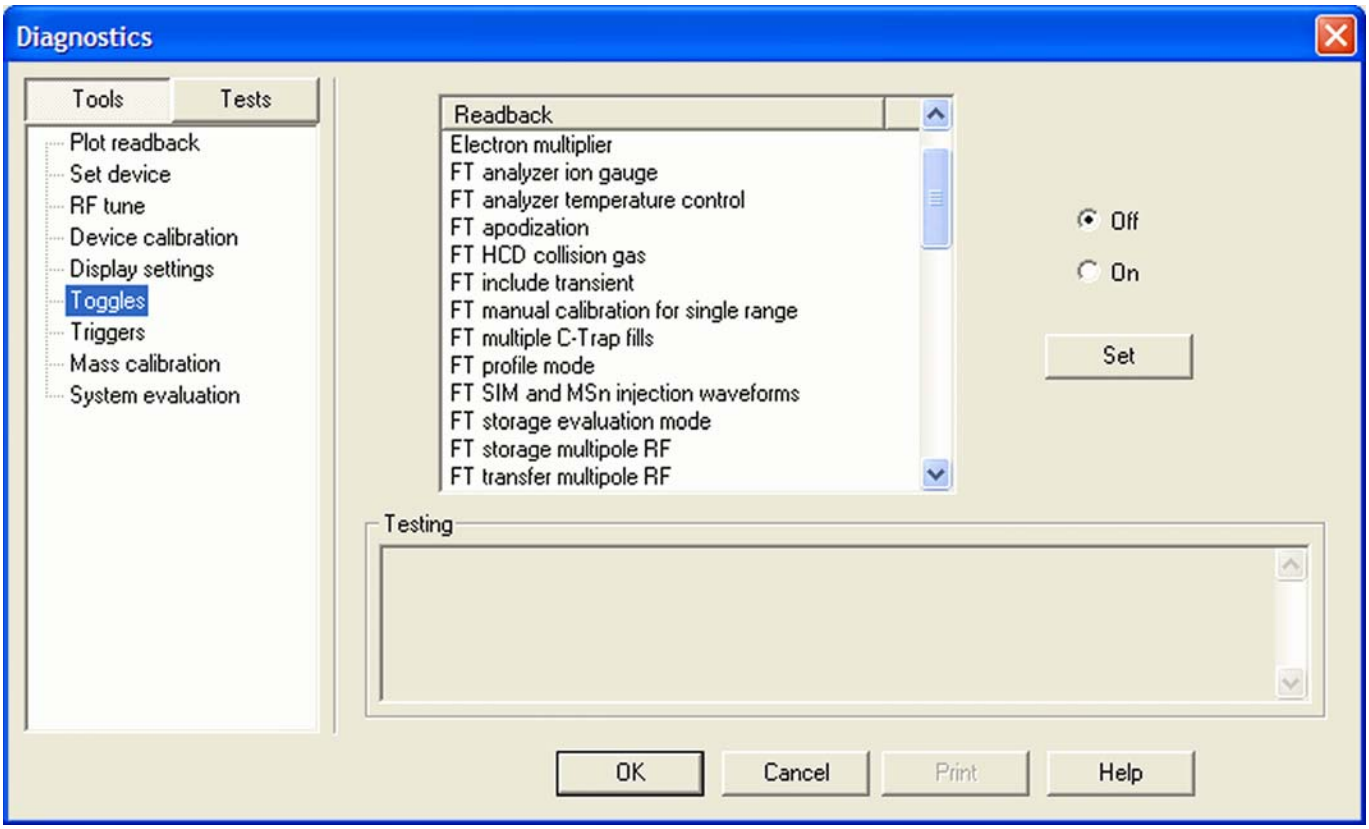


Figure 4-3. Toggles page of the Diagnostics dialog box

To display this page:

From the Tune Plus window, choose Diagnostics > Diagnostics > Tools > **Toggles**.

Caution All toggles should only be used for diagnostic purposes. The functionality of the LTQ Orbitrap XL may be harmed if a toggle is switched to a status that differs from its default value. ▲

If one of the FT toggles is (accidentally) different from its default value during data acquisition, the FT Analyzer Settings of the Scan Header of a raw file contains a reference to this. See Appendix A: "Miscellaneous Information" for further details.

Note The status of a toggle is not saved in the tune method and is set back to its default value after an instrument reset. The toggle state shown by the radio buttons next to the list box does not necessarily correspond to the actual settings. ▲

FT Analyzer Ion Gauge

With this toggle, the ion gauge for the FT analyzer vacuum can be disabled manually for diagnostic purposes. The default setting is enabled.

FT Analyzer Temperature Control

With this toggle, the FT analyzer temperature control regulation electronics can be disabled for diagnostic purposes. The default setting is enabled.

FT Apodization

With this toggle, the apodization can be switched on or off. The default setting is On.

FT HCD Collision Gas

With this toggle, the HCD collision gas can be switched on or off. The default setting is **On**.

FT Include Transients

If this toggle is on, it is possible to display transients in the Spectrum view by choosing **Show FT Transient** in the shortcut menu of the Spectrum view. The menu is displayed when you right-click anywhere on that page. See topic "Spectrum View" on page 2-3 for further details.

Note A transient view is only possible if profile (instead of centroid) is chosen as data format. ▲

During transient display in Spectrum view, the x-coordinate is misleadingly labeled with m/z instead of milliseconds. The default setting is **Off**.

Note It is not possible to acquire transients into an Xcalibur raw file. ▲

FT Storage Multipole RF

This toggle can be used to switch the FT storage multipole RF on or off. The default setting is **On**. If the multipole RF is switched on, the corresponding blue diagnostic LED of the ion optic supply board should be on (indicating that the RF1 generator is switched on). See the *LTQ XL Hardware Manual* for further details.

FT Transfer Multipole RF

This toggle can be used to switch the FT transfer multipole RF on or off. The default setting is On. If the multipole RF is switched on, the corresponding blue diagnostic LED of the ion optic supply board should be on (indicating that the RF2 generator is switched on). See the *LTQ XL Hardware Manual* for further details.

FT Manual Calibration for Single Range

This toggle can be used to influence the behavior of the FT manual calibration procedures, refer to "FT Manual Calibration Page" on page 3-18. In the default behavior, the FT manual calibration procedures calibrate the whole scan mass range for the actual polarity.

In order to be able to use nonstandard calibration substances that cover a limited mass range only, advanced users may enable this toggle. With this toggle enabled, there is no check for mass range coverage of reference mass lists. Instead, the instrument stays in the chosen mass range and calibrates this range only. With this toggle enabled, the user is responsible to cover the whole mass range needed, possibly by calibrating manually in several steps with different substances. If the performed FT manual calibration is not suitable for the scan settings used in an FTMS analyzer data acquisition, the scan header of a raw data file contains a reference to this. See Chapter A: "Miscellaneous Information" for further details.

FT Multiple C-Trap Fills

With this toggle you can allow the C-Trap to accommodate multiple ion injections from the linear trap (and HCD collision cell?). Variation of collision gases and collision energies allow creating mixed ion populations. Subsequently, the ions are injected as a single pulse into the orbitrap. The default setting is **On** (enabled).

Gibt es eine maximale Anzahl an Fills? Wie wird so eine Messung mit mehreren Fuellung aufgesetzt?

FT Profile Mode

With this toggle you can select whether the FT profile mode corresponds to a Full Profile format or to a Reduced Profile format. It is recommended to use the Reduced Profile Mode for data acquisition since the data size of the raw file is significantly decreased by using the Reduced Profile. The default setting is **Reduced**. For further information, see also topic "Data Size of FT Raw Files" on page A-4.

FT SIM and MS^n Injection Waveforms

Usually, for FT SIM and FT MS^n scans, the injection waveforms are automatically enabled. It is not possible to change this setting in the Injection Control dialog box. By means of this toggle, it is possible to disable or enable the injection waveforms manually for diagnostic purposes. The default setting is **On**.

FT View Frequency

If this toggle is switched on, the FT spectrum is shown as a frequency spectrum. If the system is on and the FT is chosen as analyzer, the frequency spectrum is displayed in the Spectrum view. The default setting is **Off**.

Note The x-coordinate is misleadingly labeled with m/z instead of kHz. ▲

This toggle is for diagnostic purposes only. Therefore, it is not possible to acquire frequency spectra.

FT Zero Offset

If this toggle is switched on, an offset is added to the spectrum. This enables to view the full noise band. The default setting is Off, if the Reduced Profile format is used. The setting is *On*, if the Full Profile format is used.

Set Device

The Set device page of the Diagnostics dialog box (Figure 4-4) allows to select devices or experimental parameters from the list and to set the value for that device or parameter. To set a device:

1. Select the device or parameter you would like to set from the Device list box.

2. Enter the device parameter's value in the text box below the Device list box.

3. Click on **Set** to apply the change to the device value or parameter.

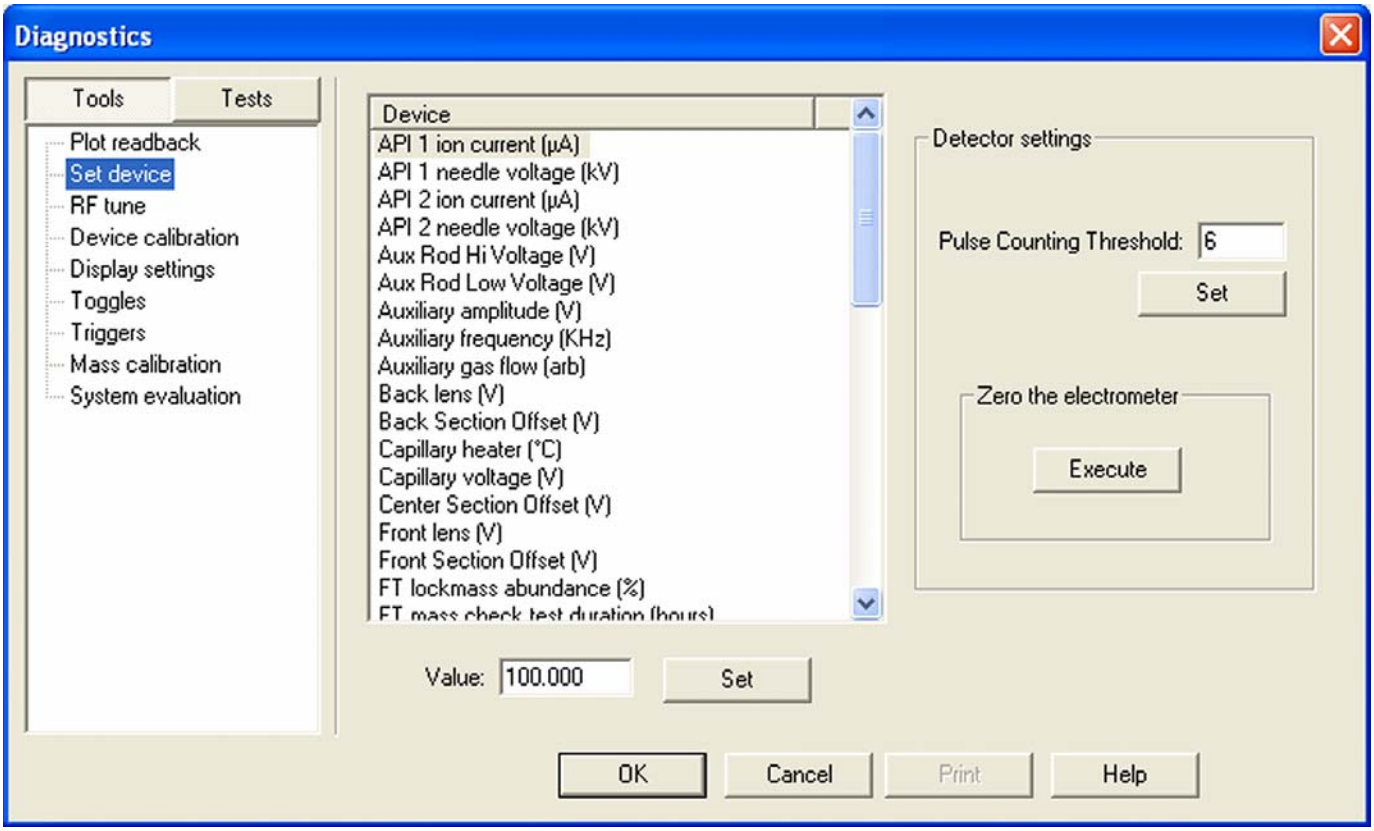


Figure 4-4. Set device page of the Diagnostics dialog box

Note The value in the text box below the Device list box, which is displayed after the call of this page, does not necessarily correspond to the actual value. ▲

There are several FT relevant parameters, which may be changed from this page.

Note After an instrument reset, the manual settings are overwritten with the corresponding calibration parameters. ▲

Caution Changing the instrument settings can harm the functionality of the LTQ Orbitrap XL, especially if followed by saving the calibration parameters (manually or at conclusion of a calibration procedure). Thus, this option should only be used by very advanced users. ▲

FT Lockmass Abundance

This device allows changing the target value of an injected lock mass relative to the actual FT scan target value. The recommended default is 10 percent. Also refer to "Locking" on page 2-11.

FT Mass Check Test Duration

This device allows changing the duration of FT Manual mass calibration checks, see "FT Manual Calibration Page" on page 3-18. By changing this value, a long-term mass stability evaluation can be run. The default behavior of the FT manual mass calibration check is to perform 100 scans checking the mass accuracy. The test duration may be extended to up to 72 hours. The default behavior can be restored by setting the duration to zero. If the duration is set between two and 24 hours, the FT manual mass calibration check will specially control the syringe pump in order to allow running long-term test with a single syringe filling. For durations above 24 hours, it is assumed that an external syringe pump is used.

Setting new FT Transfer Optics Parameters

There are two set device items that can be used to override the FT optics values. These values are originally determined during instrument calibration and set automatically. Overriding calibration values will influence instrument performance and should only be done for diagnostic purposes.

Note If you have changed FT transfer optics settings, it is recommended to perform a full FT instrument calibration afterwards to assure good instrument performance. ▲

Display Settings

The Display Settings page in the Diagnostics dialog box allows you to select a variety of instrument settings for display:

1. Select the instrument settings you want to display,
2. Click on **Start**,
3. The LTQ Orbitrap XL MS detector now displays the requested instrument settings in the Testing text box.

The following FT relevant instrument settings can be displayed:

- FT calibration settings
- FT diagnostics
- FT instrument settings

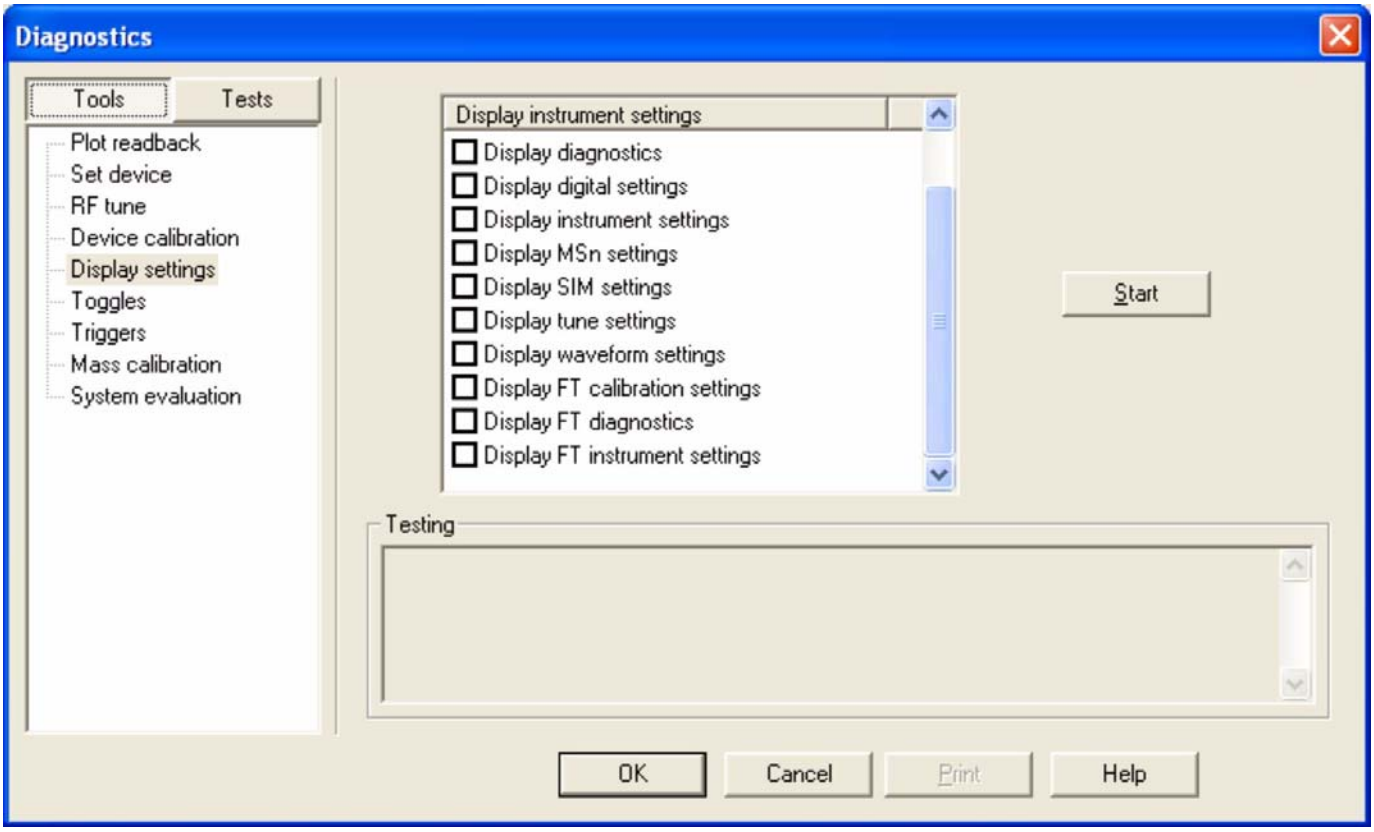


Figure 4-5. Display settings page of the Diagnostics dialog box

Display FT Calibration Settings

Displays all FT relevant calibration parameters in the diagnostics text box.

Display FT Diagnostics

Displays current diagnostic readback values of the FT electronic boards.

Display FT Instrument Settings

Displays the current values of those FT instrument settings that depend on the scan range and ion polarity mode and can be changed manually on the Set Device page of the Diagnostics dialog box.

Chapter 5 Instrument Setup

This chapter takes a look at the “Locking” feature in automated runs and describes the FT relevant topics of the data dependent settings in the Instrument Setup. It contains the following topics:

Using Locking in Automated Runs

In order to use locking in an automated run, use the Instrument Setup program.

Note Refer to topic "Locking" on page 2-11 for a basic description on using locking with FTMS analyzer scans. ▲

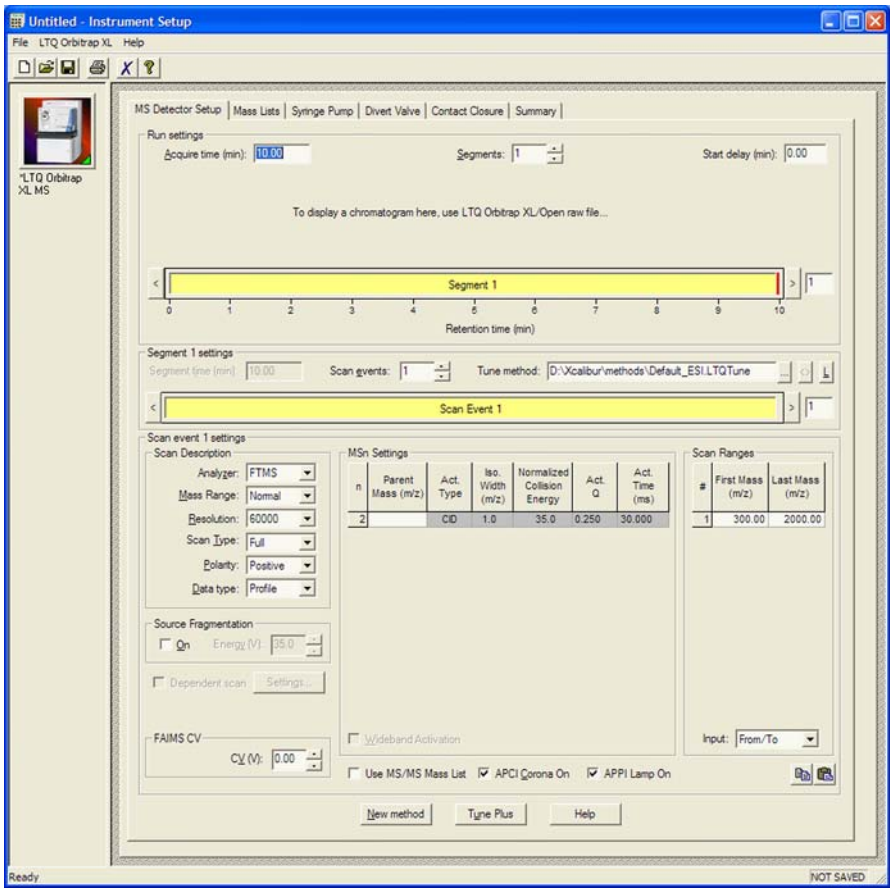


Figure 5-1. MS Detector Setup View - MS Detector Setup Page

The Lock Mass List button in the segment settings group brings up a lock mass list editor dialog. Segment-related lock masses can be entered here. There are separate lists for positive ion and negative ion mode. If the lock mass for a segment is empty, no locking will be applied in the run and the external calibration will be used.

Data Dependent Settings

This section describes the FT relevant topics of the data dependent settings in the Instrument Setup.

Preview Mode

Figure 5-2 shows the Current Segment page of the Data Dependent Settings dialog box.

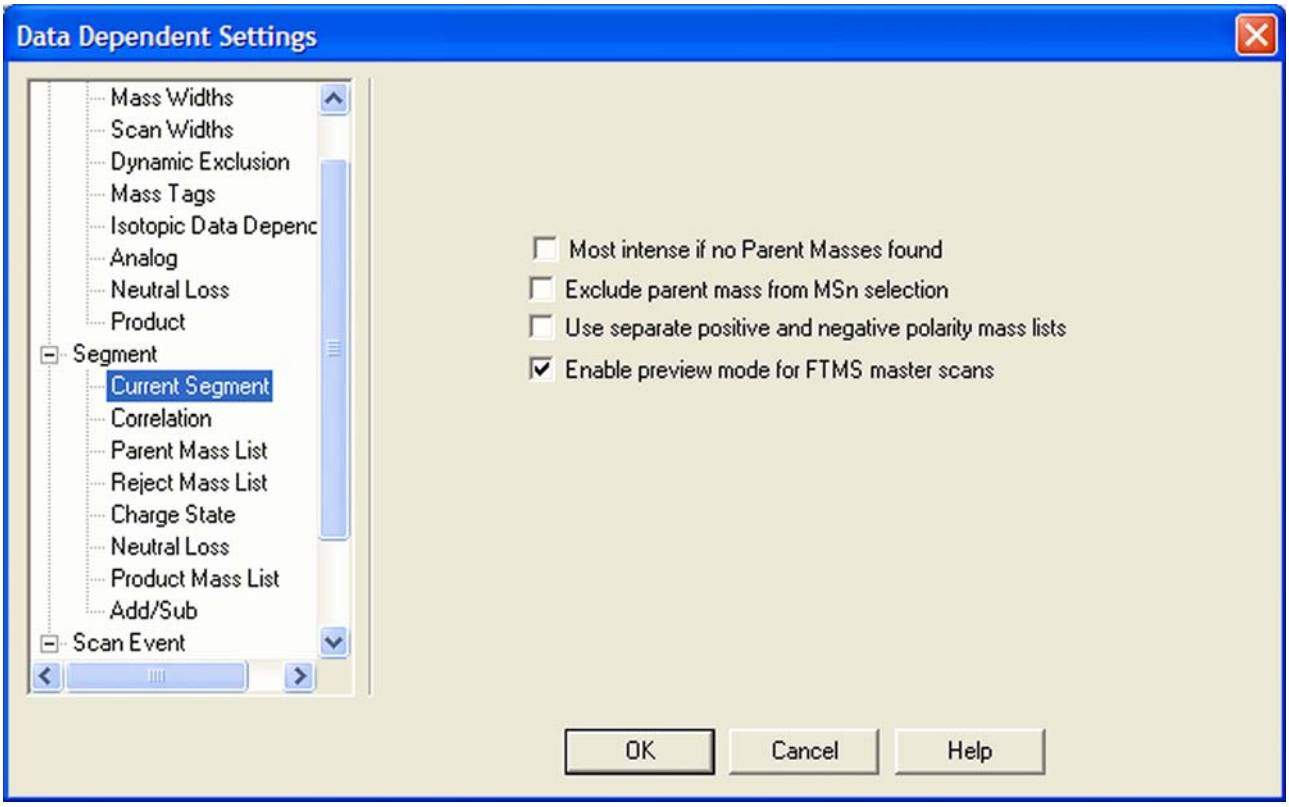


Figure 5-2. Data Dependent Settings dialog box – Current Segment page

If the preview mode for FTMS master scans is enabled on the Current Segment page, the data dependent decision is made on the basis of the FT master scan with lower resolution to increase the duty cycle. The resolution of the FTMS scan itself is not changed. Since the high resolution is usually not required to make the data dependent decision, it is recommended to enable the preview mode.

To prevent making data dependent decisions on basis of lower-resolution preview spectra, disable this option. For example, if there are ions with high charge states to be examined, and the data dependent settings require charge state recognition of precursor ions, this might be a reason to turn off this option. Otherwise, the high charge state clusters may not be resolved and charge states will not recognized in preview mode.

Monoisotopic Precursor Selection

Figure 5-3 shows Charge State page of the Data dependent settings dialog box.

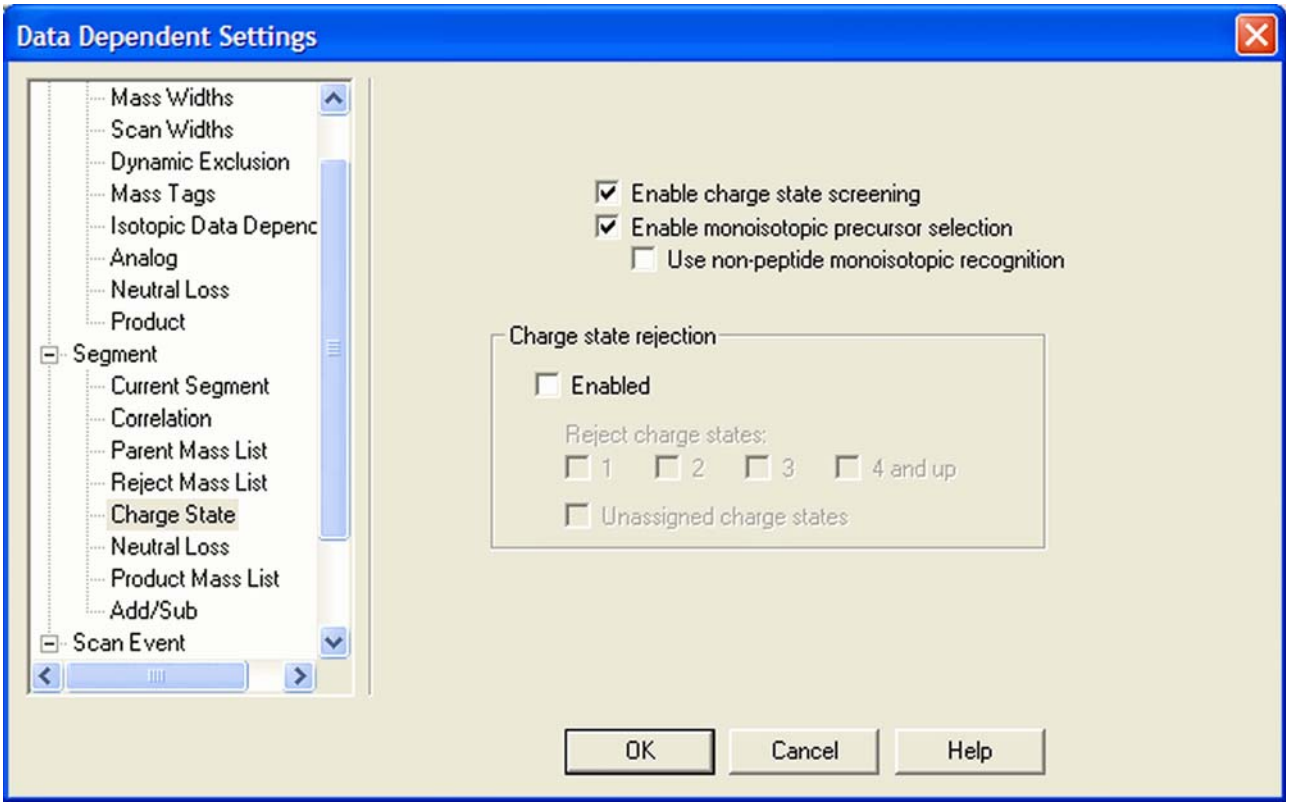


Figure 5-3. Charge State page of the Data dependent settings dialog box with Advanced Features on

If the monoisotopic precursor selection is enabled on the Charge State page, the data dependent scan is only performed for one molecular ion of the corresponding overall ^{13}C isotopic distribution if Dynamic Exclusion is enabled.

This check box is only available on the Charge State page if the Advanced Features are turned on in the LTQ Orbitrap XL menu of the Instrument Setup.

Use Non-Peptide Monoisotopic Recognition

This check box is only available if monoisotopic precursor selection is active. If monoisotopic precursor selection is active and this box is not checked, precursor ions in FT master scans must match peptide-type isotopic distribution in order to identify the monoisotopic peak. If this box is checked, monoisotopic peaks will also be identified for small molecules and precursor ions with non-peptide-type isotopic distributions.

Data Dependent FT SIM Scans

A data dependent FT SIM scan is performed around the center mass determined in a previous reference scan event if the check box "Same MS order as referenced scan event" is selected on the Current Scan Event page of the Data Dependent Settings dialog box as shown in Figure 5-4.

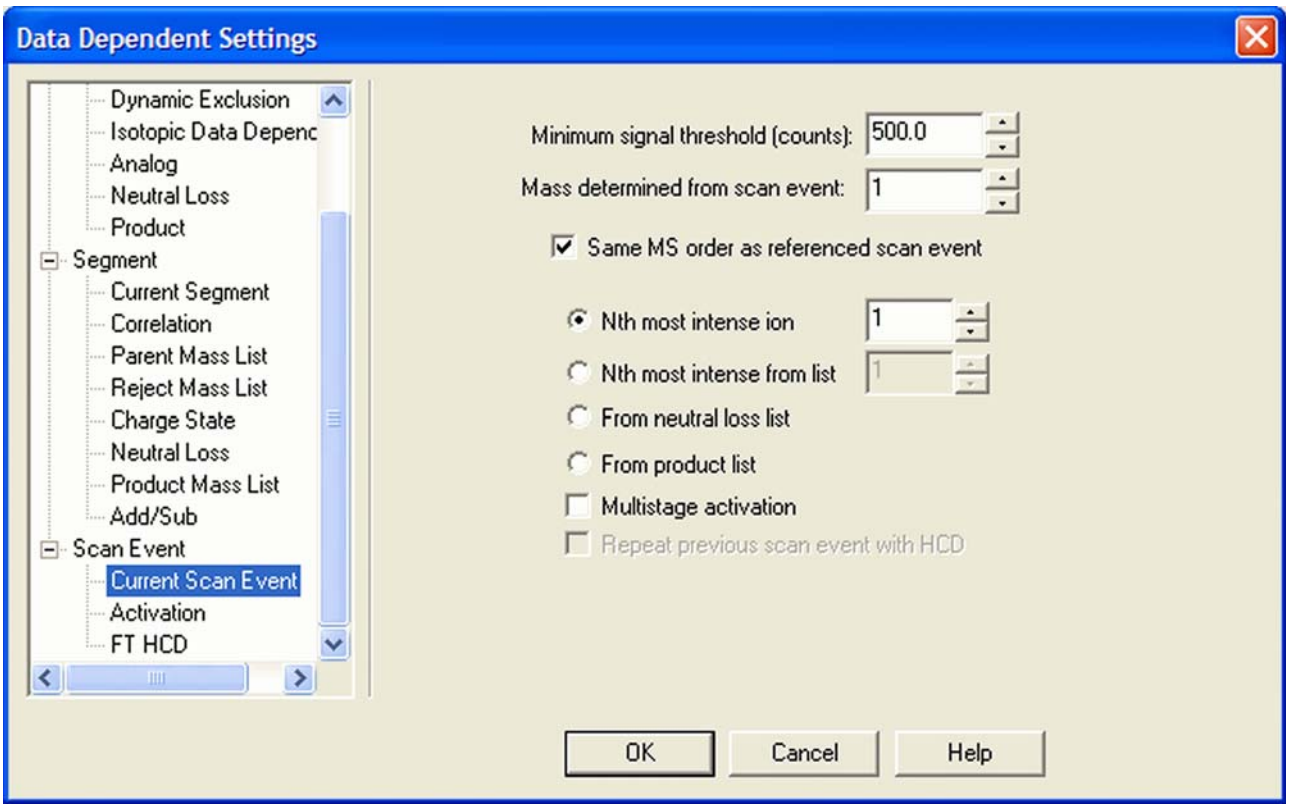


Figure 5-4. Data Dependent Settings dialog box – Current Scan Event page

Repeat previous Scan Event with HCD

The Current Scan Event page allows repeating the previous scan event by using HCD. See Figure 5-5. The respective check box becomes available when the precursor mass is determined from the neutral loss list or from the product list. To repeat the previous scan event with HCD, select the corresponding check box.

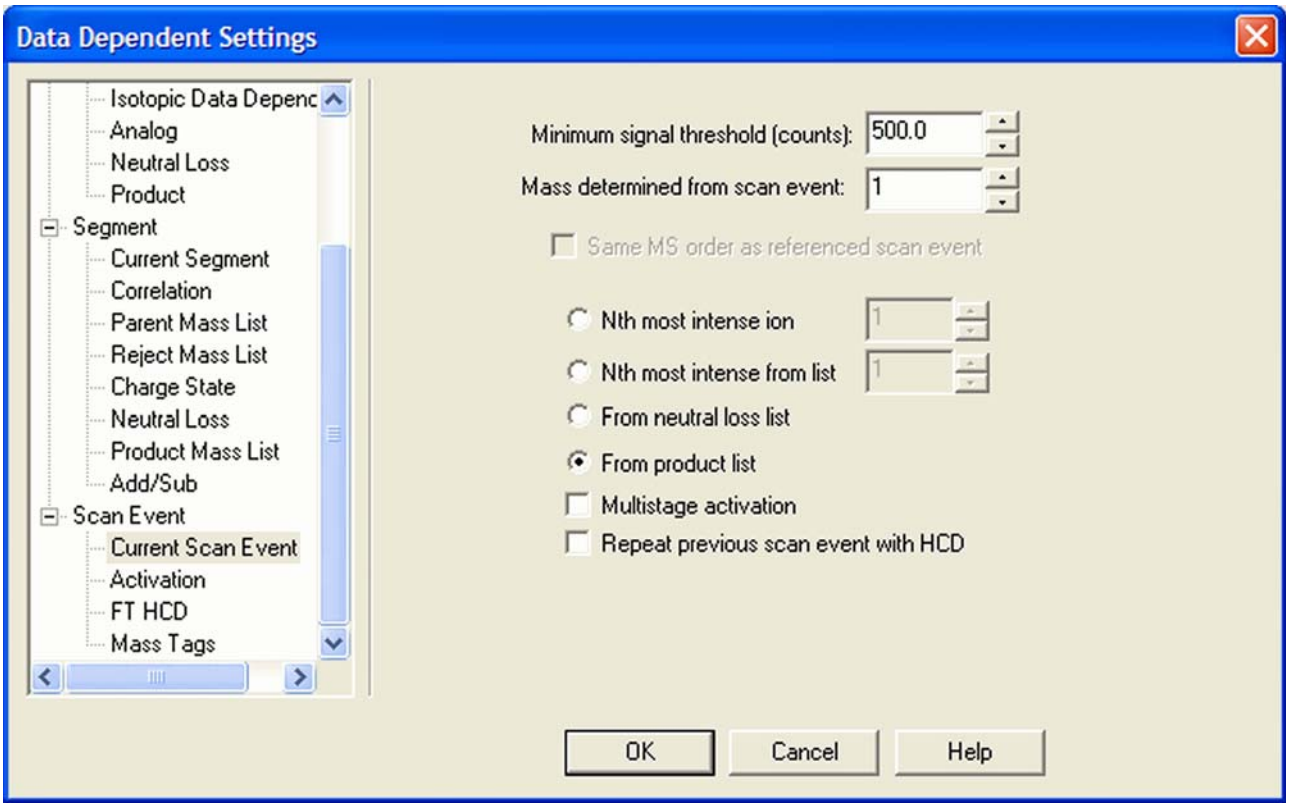


Figure 5-5. Current Scan Event page - Repeat previous scan event with HCD

Scan Width The scan width of the data dependent FT SIM scan can be selected on the Scan widths page of the Data Dependent Settings dialog box. See Figure 5-6.

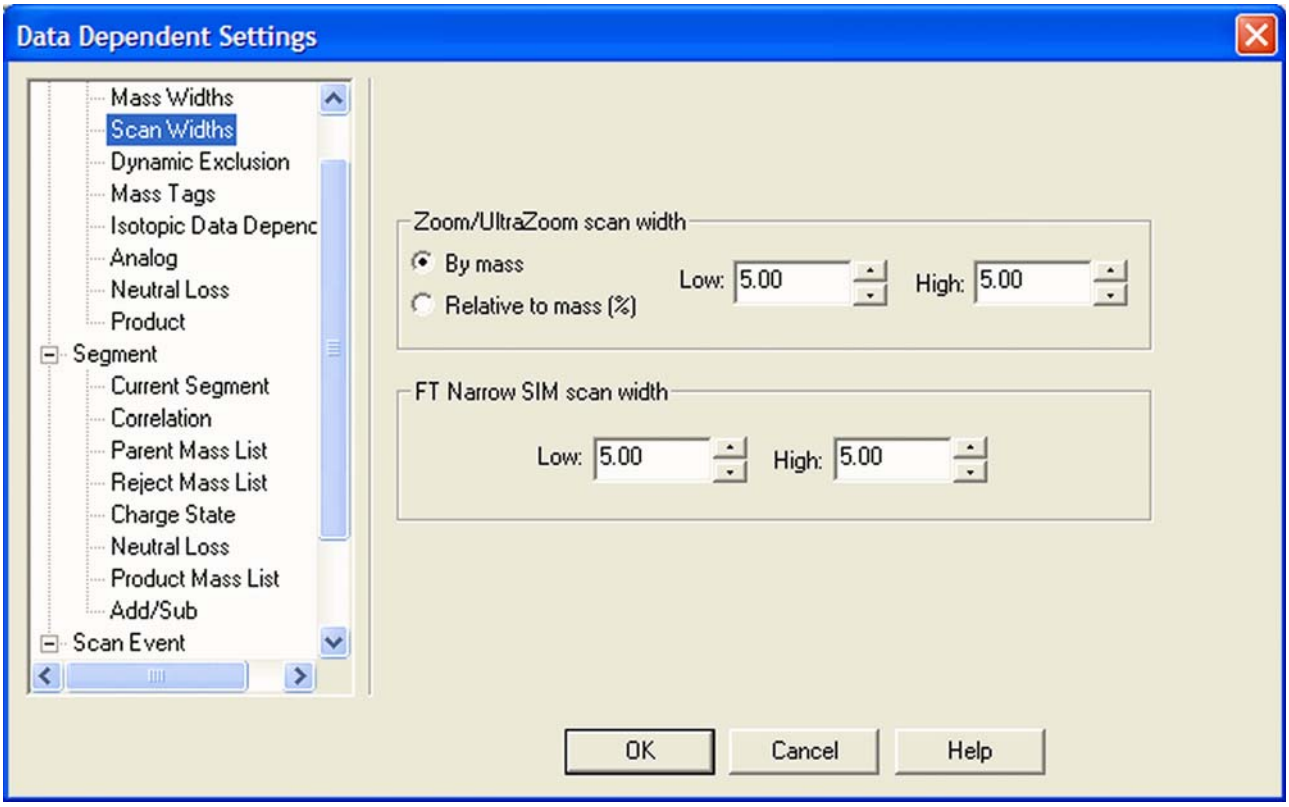


Figure 5-6. Data Dependent Settings dialog box – Scan Widths page

Activation Type

On the Activation page of the Data Dependent Settings dialog box, the Activation type list box allows you to specify how the ion is activated for fragmentation during a data dependent experiment. See Figure 5-7. It has the following options:

- CID (Collision-induced dissociation)
- PQD (Pulsed-Q dissociation)
- HCD (high-energy CID)

If you select HCD, the Activation Q spin box becomes unavailable.

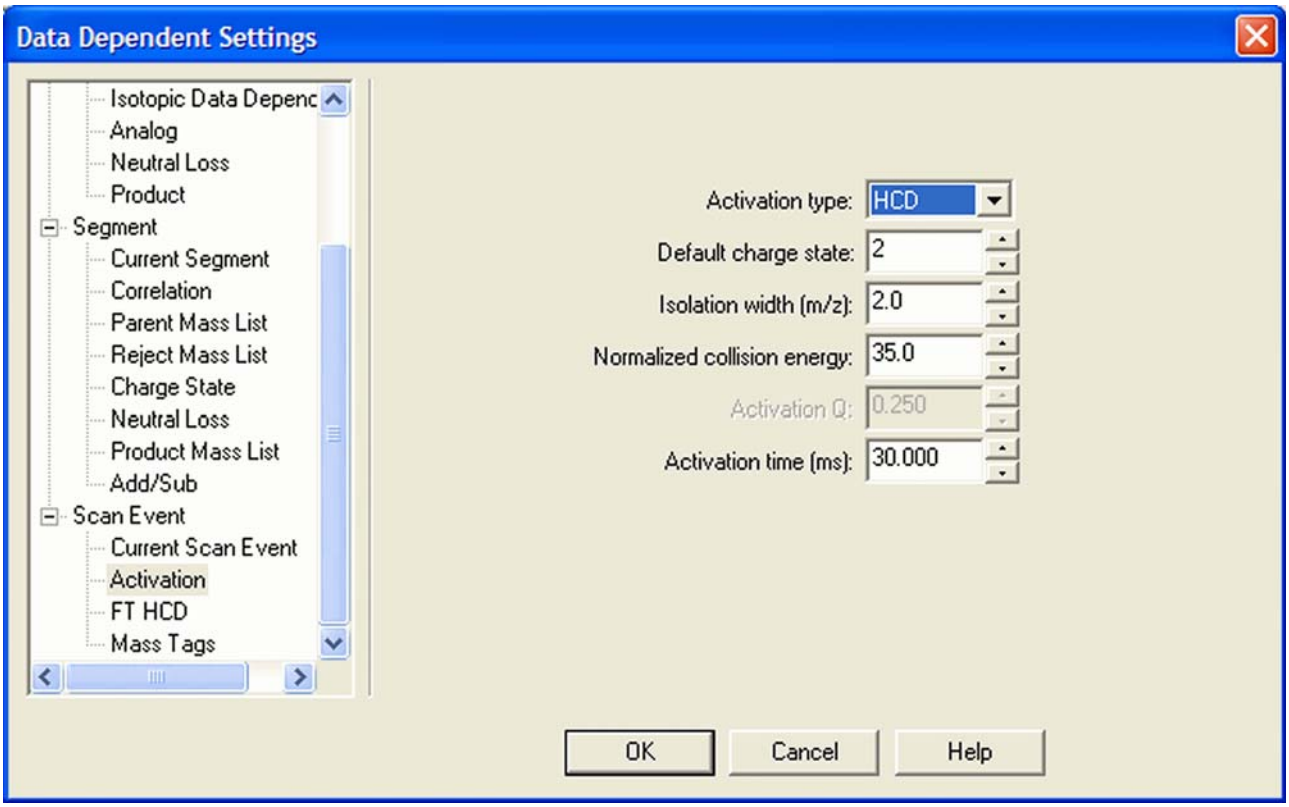


Figure 5-7. Data Dependent Settings dialog box – Activation page

FT HCD

The FT HCD page of the Data Dependent Settings dialog box offers you two modes for choosing the first mass:

- a mass with a fixed m/z value

  To change the m/z value, click on the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set m/z to any value from 50 to 4000; default is 100. Alternatively, you can enter a value in the spin box text field.

- a mass with an m/z value that is relative to the precursor mass.

  To change the percentage, click on the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set the percentage to any value from 0 to 4; default is 0.25. Alternatively, you can enter a value in the spin box text field.

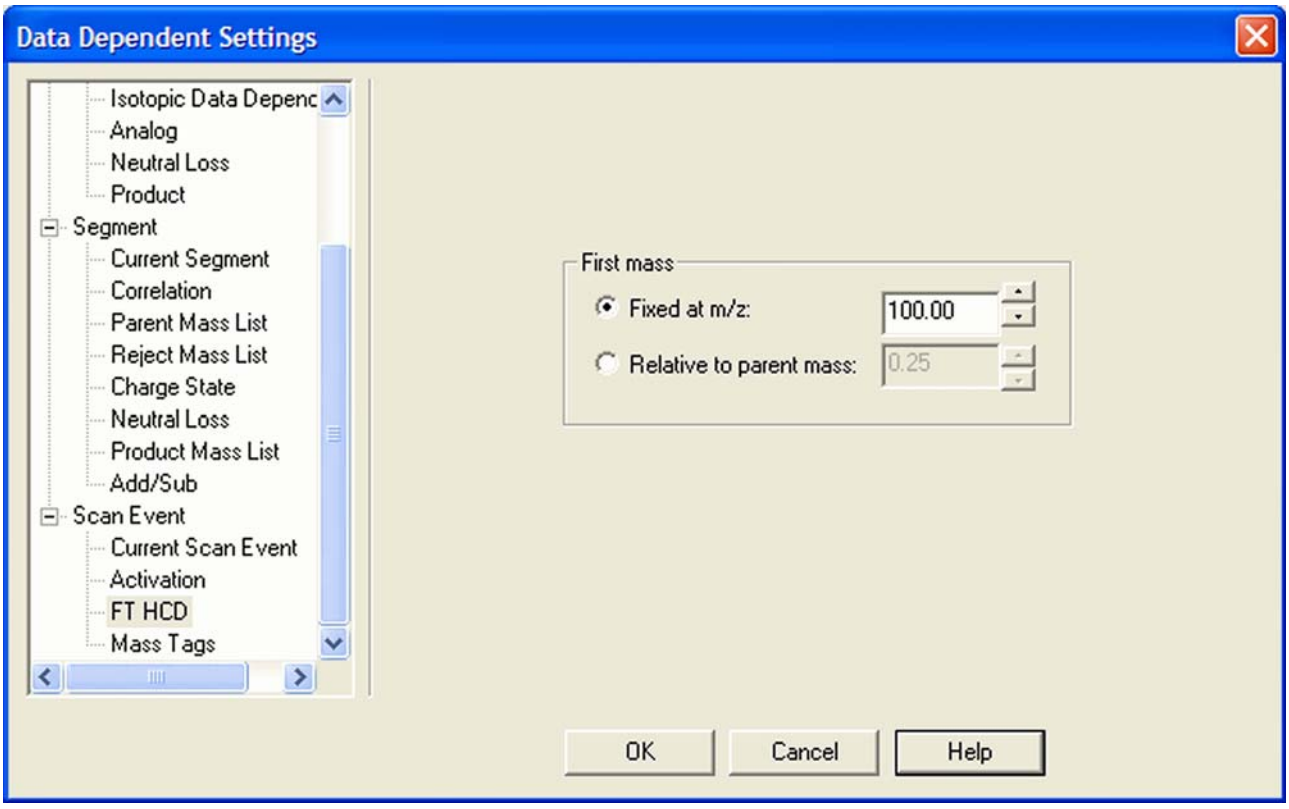


Figure 5-8. Data Dependent Settings dialog box – FT HCD page

MSn Settings for HCD Experiments

Similar to the Define Scan dialog box, the Instrument Setup allows selecting the Activation type (CID/PQG/HCD). If HCD is selected as activation type, the HCD charge state input box becomes available and the Activation Q input box is disabled.

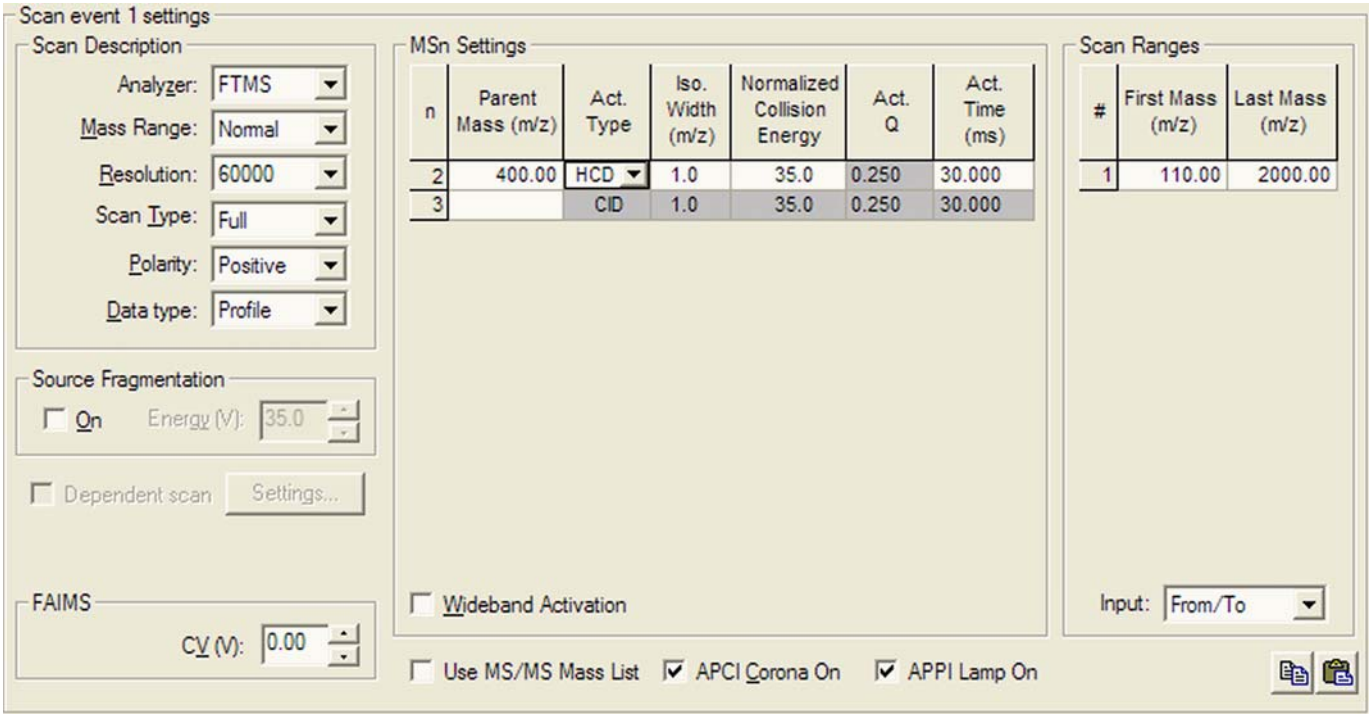


Figure 5-9. MS Detector Setup Page - Scan event settings with HCD experiment

Chapter 6 Instrument Configuration

This chapter gives instructions on how to configure your instrument. It contains the following topics:

Starting Instrument Configuration

From the Instrument Configuration dialog box, click on the LTQ Orbitrap XL MS button in the Configured Devices group box. See Figure 6-1. Then, click on the **Configure** button to open the LTQ Orbitrap XL Configuration dialog box.

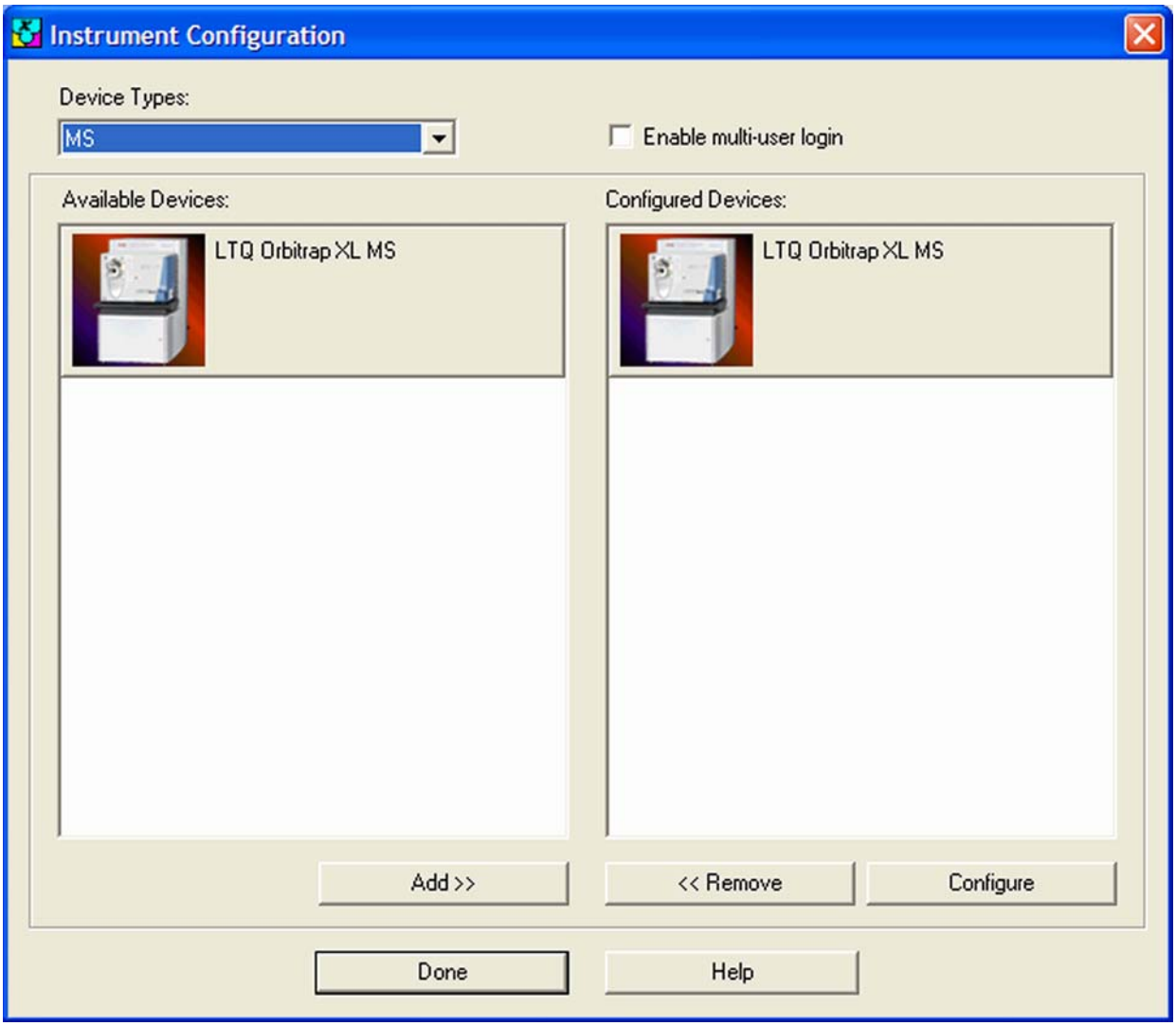


Figure 6-1. Instrument Configuration dialog box

The LTQ Orbitrap XL Configuration dialog box allows you to enter LTQ Orbitrap XL configuration information by using several pages, including the FT Settings page and the FT Manual Calibration page.

The elements of the pages are described in the following topics.

FT Settings Page

The FT Settings page of the LTQ Orbitrap XL Configuration dialog box allows you to turn on Active Noise Reduction and to enable the HCD collision gas.

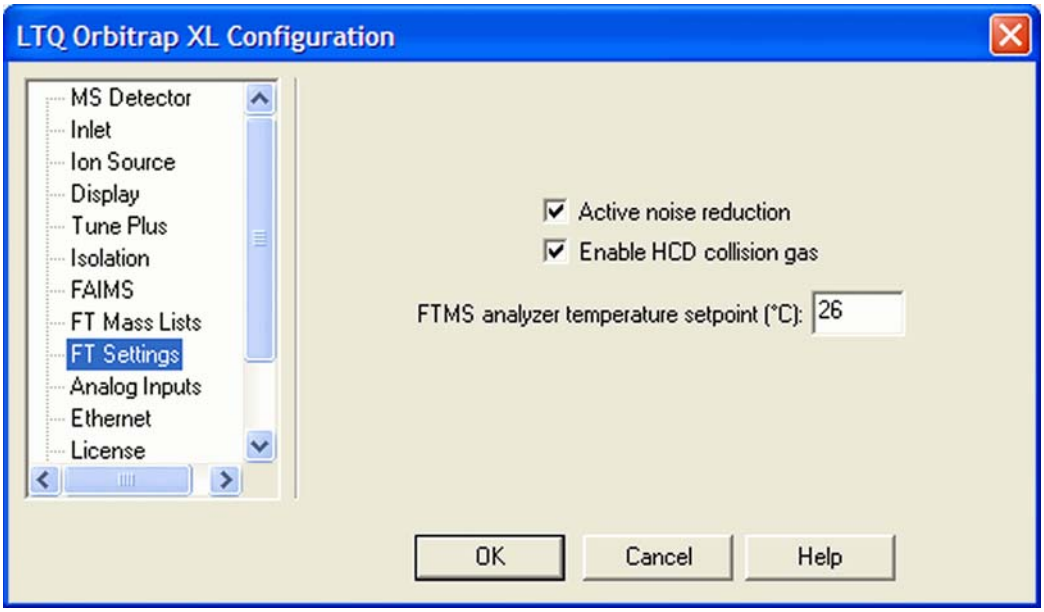


Figure 6-2. LTQ Orbitrap XL Configuration dialog box – FT Settings page

Using the FT Settings page of the LTQ Configuration dialog box, you can select the following parameters:

Active Noise Reduction Select this check box to turn on Active Noise Reduction. If the Active Noise Reduction is turned on, an advanced algorithm is used to decrease the statistic noise in an FT spectrum without increasing the noise threshold. If the Active Noise Reduction is turned on the data size of acquired raw files is decreased.

For further information, see also topic "Data Size of FT Raw Files" on page A-4.

Enable HCD collision gas Select this check box to enable the HCD collision gas.

FTMS analyzer temperature setpoint (°C) Enter the desired temperature for the orbitrap analyzer chamber. The default value is 26.

Note Configuration changes will become effective when you reboot your instrument. ▲

FT Mass Lists Page

The FT Mass Lists page of the LTQ Orbitrap XL Configuration dialog box allows you to manipulate the mass lists that are displayed in the FT Manual Calibration page of the Calibration dialog box in Tune Plus. See Figure 6-3. You can also import or export a mass list as a text file.

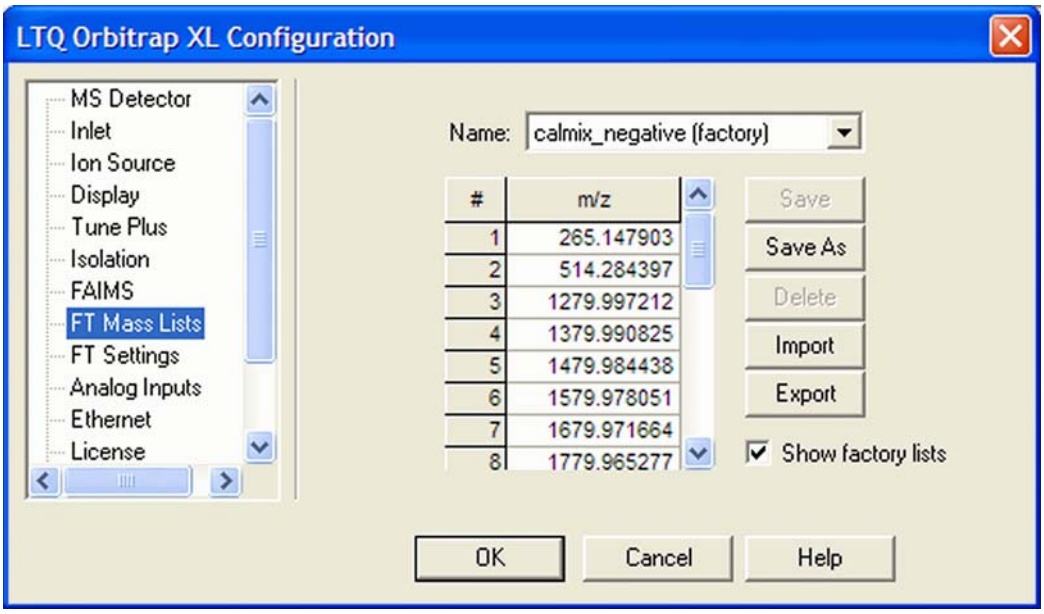


Figure 6-3. LTQ Orbitrap XL Configuration dialog box – FT Mass Lists page

Using the FT Mass Lists page of the LTQ Orbitrap XL Configuration dialog box, you can select the following parameters:

Name This list box lists the names of the factory supplied and user created mass lists.

Mass List This table lists the mass-to-charge ratios of the ions that you are using to calibrate the orbitrap mass analyzer. You can select an existing mass list in the Name list box, or you can create or modify a mass list by clicking on and editing the entries in the Mass List table.

Note Ensure that you use calibration masses of sufficient accuracy (sub ppm). ▲

Save Click on **Save** to save the mass list with the name that is selected in the Name list box.

Save As Click on **Save As** a to save the mass list with a new name.

Delete Click on **Delete** to delete the mass list that is selected in the Name list box.

Import	Click on **Import** to import a mass list that is a text file.
Export	Click on **Export** to export a mass list to a text file.
Show Factory Lists	Select this check box if you want to show the factory calibration mass lists in Tune Plus.

Appendix A Miscellaneous Information

This appendix contains supplemental information for the previous chapters. It contains the following topics:

FT Analyzer Information in Scan Header

The Qual Browser window allows you to open a raw file and to display scan header information for a selected scan in any of the cells. Choose View > **Scan Header** to display the Scan Header of the current scan in the active cell.

FT Analyzer Settings

The scan header information of an FT scan includes information about the FT Analyzer Settings, which is not available in the usual Reports (Tune method, Instrument method, Status log, or Error log):

T=1e5	AGC Target for this scan (here: 1e+05)
PsIT=0.65	Prescan Inject Time (here: 0.65)
Tog=(…)	Manual diagnostic toggles are set different from their default values. See Table A-1 below for detailed information.
iWf	Inject waveform on for this scan.
PvR=3e4	Preview analysis active for this scan
DiagManualSettings	Calibration parameters were manually changed under Diagnostics.

Table A-1. Actual settings of manual toggles

Tog = (…)	Relevant Toggle	Current setting	Default setting
ApoOff	FT apodization	Off	On
TrExp	FT include transient	On	Off
FulIP	FT profile mode	Full	Reduced
IWFoff	FT SIM and MS^n injection waveforms	Off	On
Freq	FT view frequency	On	Off
Offset	FT zero offset	On	Off

FT Analyzer Messages

The scan header of an FT scan includes also so-called FT Analyzer Messages:

Ufill=0.45	Maximum ion time reached. Here: the real number of ions is only ~45% of the target value.
MCal=4d	Last mass calibration for this scan range is several days old (here: 4)
Est=0x24	Machine-readable result message for post-processing tools
DAC=0.98	FT transient measurement near saturation, this might result in spectral harmonics (typically target value too high)
TCal=[195..]	This is a hint that the current scan range settings for the FT analyzer are outside the calibrated storage/transfer mass range. Transfer parameters are extrapolated.
Lock=(inj524.3,1/1,+3ppm)	Information about lock mass settings, extra SIM injection of lock mass ions, number of identified lock masses in the spectrum, and deviation of corrected (locked) masses compared to the external calibration.
Stable=15min	Shows the elapsed stabilization time of the FTMS analyzer high voltage electronics after last off state or polarity switch. For best external mass accuracies, it is required to let the FTMS analyzer high voltage electronics stabilize before performing an acquisition or mass calibration.

TempDiff=1	There is a temperature difference in the FTMS analyzer temperature between mass calibration time and current state. This may be caused by setting a different analyzer temperature setpoint in instrument configuration, by rapid significant changes in the ambient temperature, or by not waiting for temperature stabilization after instrument (temperature regulation) was off.
PkOvf	Internal Peak detection overflow in the FT spectrum analysis

Note The actual FT Analyzer Messages can also be displayed in the Tune Spectrum view, see "Spectrum View" on page 2-3. ▲

Data Size of FT Raw Files

The data size of a raw file with FT data depends on many parameters, for example on the number of scans, the resolution setting, the data format and on the status of the Active Noise Reduction.

Table A-2 below displays typical data sizes (per scan) of an FT spectrum (standard LTQ Orbitrap XL calibration solution, scan range 120–1200, AGC target 5E5, 1 microscan, resolution setting 60000) at different FT data formats and with Active Noise Reduction off.

Table A-2. Typical data sizes (per scan) of an FT spectrum

FT Data Format	Active Noise Reduction	Typical data size / scan
Centroid	On	ca. 10 kB
Reduced Profile	On	ca. 20 kB
Centroid	Off	ca. 35 kB
Reduced Profile	Off	ca. 80 kB
Full Profile	On/Off	ca. 2800 kB

Glossary

The following abbreviations and terms are used in this manual. This glossary also includes acronyms, metric prefixes, symbols, and abbreviations.

A ampere

ac alternating current

ADC analog-to-digital converter

APCI atmospheric pressure chemical ionization

API atmospheric pressure ionization

APPI atmospheric pressure photo ionization

ASCII American Standard Code for Information Interchange

b bit

B byte (8 b)

baud rate data transmission speed in events per second

°C degrees Celsius

cfm cubic feet per minute

CI chemical ionization

CID collision-induced dissociation

cm centimeter

cm^3 cubic centimeter

CPU central processing unit (of a computer)

CRC cyclic redundancy check

CRM consecutive reaction monitoring

<Ctrl> control key on the terminal keyboard

d depth

Da dalton

DAC digital-to-analog converter

dc direct current

DS data system

DSP digital signal processor

EI electron ionization

EMBL European Molecular Biology Laboratory

<Enter> Enter key on the terminal keyboard

ESD electrostatic discharge

ESI electrospray ionization

eV electron volt

f femto (10^{-15})

°F degrees Fahrenheit

***.fasta* file** extension of a SEQUEST search database file

ft foot

FFT Fast Fourier Transformation

FT Fourier Transformation

FTMS Fourier Transformation Mass Spectroscopy

FTP file transfer protocol

FWHM full width at half maximum

g gram

G Gauss; giga (10^9)

GC gas chromatograph; gas chromatography

GC/MS gas chromatograph / mass spectrometer

GUI graphical user interface

h hour

h height

HCD Higher Energy Collision Induced Dissociation

HPLC high-performance liquid chromatograph

HV high voltage

Hz hertz (cycles per second)

ICIS™ Interactive Chemical Information System

ICL™ Instrument Control Language™

ID inside diameter

IEC International Electrotechnical Commission

IEEE Institute of Electrical and Electronics Engineers

in inch

I/O input/output

k kilo (10^3, 1000)

K kilo (2^{10}, 1024)

KEGG Kyoto Encyclopedia of Genes and Genomes

kg kilogram

l length

L liter

lb pound

LC liquid chromatograph; liquid chromatography

LC/MS liquid chromatograph / mass spectrometer

LED light-emitting diode

µ micro (10^{-6})

m meter

m milli (10^{-3})

M mega (10^6)

M^+ molecular ion

MALDI matrix-assisted laser desorption/ ionization

MB Megabyte (1 048 576 bytes)

MH^+ protonated molecular ion

min minute

mL milliliter

mm millimeter

MRFA A peptide with the amino acid sequence methionine–arginine–phenylalanine–alanine.

MS mass spectrometer; mass spectrometry

MS MS^n power: where n = 1

MSDS Material Safety Data Sheet

MS/MS MS^n power: where n = 2

MS^n MS^n power: where n = 1 through 10

m/z mass-to-charge ratio

n nano (10^{-9})

NCBI National Center for Biotechnology Information (USA)

NIST National Institute of Standards and Technology (USA)

OD outside diameter

OT orbitrap

Ω ohm

p pico (10^{-12})

Pa pascal

PCB printed circuit board

P/N part number

ppm parts per million

RAM random access memory

RF radio frequency

RMS root mean square

ROM read-only memory

s second

SIM selected ion monitoring

SRM selected reaction monitoring

TCP/IP transmission control protocol / Internet protocol

TIC total ion current

Torr torr

TWA time weighted average

u atomic mass unit

V volt

V ac volts alternating current

V dc volts direct current

vol volume

w width

W watt

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