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UVPD Module

User Guide

For the Orbitrap Fusion Lumos Tribrid Mass Spectrometer

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DOCUMENTATION SURVEY

ThermoFisher SCIENTIFIC

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Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable North American and European regulations. Your system meets the applicable requirements in the electromagnetic compatibility (EMC) and product safety standards described in this section.

Changes that you make to your system might void compliance with one or more of these regulatory standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with the regulatory standards, order replacement parts and additional components, options, and peripherals directly from Thermo Fisher Scientific or an authorized representative.

Laser product safety

This device complies with the FDA Class 1 requirements in 21 CFR 1040.10. It also complies with the laser safety standards IEC 60825-1: 2017 and ANSI Z316.1-2014.

Low Voltage Directive 2014/35/EU

This device complies with Low Voltage Directive 2014/35/EU and the harmonized safety standard IEC/EN/CSA/UL 61010-1, 3rd Edition.

EMC Directive 2014/30/EU and other EMC test standards

This device was tested by TÜV Rheinland of North America and complies with the following EMC standards:

47 CFR 15, Subpart B, Class A: 2015	VCCI V-3/2015.04	IEC/EN 61000-4-4: 2004 + A1
AS/NZS CISPR 22: 2009	EN 55011: 2009 + A1	IEC/EN 61000-4-5: 2006
BSMI CNS 13438: 2006	IEC/EN 61326-1: 2013	IEC/EN 61000-4-6: 2009
ICES-003: 2014	IEC/EN 61000-3-2: 2006 + A1 + A2	IEC/EN 61000-4-8: 2010
IEC/CISPR 11: 2009 + A1	IEC/EN 61000-3-3: 2008	IEC/EN 61000-4-11: 2004
KN 61000-6-2	IEC/EN 61000-4-2: 2009	
KN 61000-6-4	IEC/EN 61000-4-3: 2006 + A1 + A2	

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FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: This instrument must be used in the manner specified by Thermo Fisher Scientific to ensure protections provided by the instrument are not impaired. Deviations from specified instructions on the proper use of the instrument include changes to the system and part replacement. Accordingly, order replacement parts from Thermo Fisher Scientific or one of its authorized representatives.

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Preface

The *UVPD Module User Guide* describes the primary components for the optional Thermo Scientific™ ultraviolet photodissociation (UVPD) hardware module installed in the Thermo Scientific Orbitrap Fusion Lumos™ (also known as Lumos™) Tribrid™ mass spectrometer (MS). It also provides an example experiment and instructions for calibrating the alignment of the invisible laser beam.

Contents

❖ **To suggest changes to the documentation or to the Help**

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.

Accessing Documentation

The Orbitrap Fusion Lumos MS includes complete documentation. For system requirements, refer to the release notes on the software DVD.

❖ **To view the product manuals**

From the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo Instruments > *model x.x***, and then open the applicable PDF file.

❖ **To view Help**

Do the following as applicable:

- Thermo Tune instrument-control software: Click the **Options** icon,  , and choose **Tune Help**.
- Thermo Xcalibur™ Method Editor: Choose an option from the **Help** menu (or press the F1 key).

❖ **To view user documentation from the Thermo Fisher Scientific website**

1. Go to thermofisher.com.
2. Point to **Services & Support** and click **Manuals** on the left.
3. In the Refine Your Search box, search by the product name.
4. From the results list, click the title to open the document in your web browser, save it, or print it.

   To return to the document list, click the browser **Back** button.

Special Notices, Symbols, and Cautions

Make sure you understand the special notices, symbols, and caution labels in this guide. Most of the special notices and cautions appear in boxes; those pertaining to safety also have corresponding symbols. Some symbols are also marked on the instrument itself and can appear in color or in black and white. For complete definitions, see Table 1.

Table 1. Notices, symbols, labels, and their meanings (Sheet 1 of 2)

Notice, symbol, or label	Meaning
IMPORTANT	Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the product.
Note	Highlights information of general interest.
Tip	Highlights helpful information that can make a task easier.

Table 1. Notices, symbols, labels, and their meanings (Sheet 2 of 2)

Notice, symbol, or label	Meaning
	**Caution:** Read the cautionary information associated with this task.
	**Chemical hazard:** Observe safe laboratory practices and procedures when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	**Heavy object:** The Orbitrap Fusion Series MS, excluding its workbench, weighs over 227 kg (500 lb). Never try to detach and move the instrument from its workbench; you can suffer personal injury or damage the instrument.
	**Hot surface:** Allow any heated components to cool before touching them.
	**Risk of electric shock:** This instrument uses voltages that can cause electric shock and personal injury. Before servicing the instrument, shut it down and disconnect it from line power. While operating the instrument, keep covers on.
	**Risk of eye injury:** Eye injury can occur from splattered chemicals, airborne particles, or sharp objects. Wear safety glasses when handling chemicals or servicing the instrument.
	**Risk of laser radiation:** Failure to understand and comply with laser cautions and operating instructions in this guide can result in property damage or serious injuries to the user.
	**Sharp object:** Avoid handling the tip of the syringe needle.

Contacting Us

Contact	Email	Telephone	QR Code[a]
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
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1

Introduction

The Orbitrap Fusion Lumos Tribrid MS is a member of the Thermo Scientific family of mass spectrometers. The optional UVPD module assembly can come factory-installed with the purchase of the Orbitrap Fusion Lumos MS, or a laser-safety-trained Thermo Fisher Scientific field service engineer can install it at a later date as an upgrade.

Note The UVPD option is not available for the Thermo Scientific Orbitrap Fusion™ (also known as Fusion™) Tribrid MS.

An Orbitrap Fusion Lumos MS with UVPD capability incorporates an assembly consisting of a laser module and a control module, both of which are located inside the instrument. For information about these modules, see Chapter 2, "UVPD Module." For information about the invisible laser beam, see Chapter 3, "UVPD Laser Beam." For laser safety information, see Appendix A, "Laser Safety Information."

For procedures regarding daily operation, maintenance, and system startup and shutdown, refer to the *Orbitrap Fusion Series Hardware Manual*.

CAUTION Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Note The Glossary defines some of the terms used in this manual.

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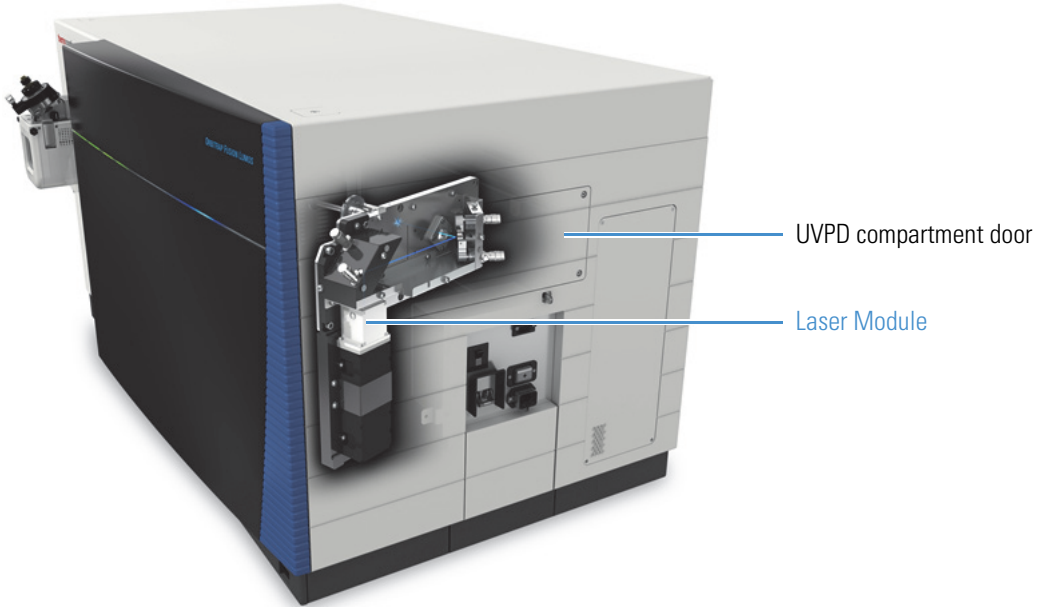
Ultraviolet Photodissociation

The Orbitrap Fusion Lumos MS with UVPD capability incorporates an internal UVPD module, which is located behind the right-side panel that connects to the ion trap chamber manifold. Inside the module, a compact, solid-state laser head (CryLaS GmbH) generates a pulsed 213 nm invisible laser beam that enters the ion trap, irradiating ions and producing fragment ions. While collision-induced dissociation (CID) uses a vibrational excitation mechanism to fragment molecular ions, UVPD uses an electronic excitation mechanism to induce fragmentation.

Figure 1. Approximate location of the UVPD laser module inside the Orbitrap Fusion Lumos MS



As a fragmentation technique, UVPD offers several advantages:

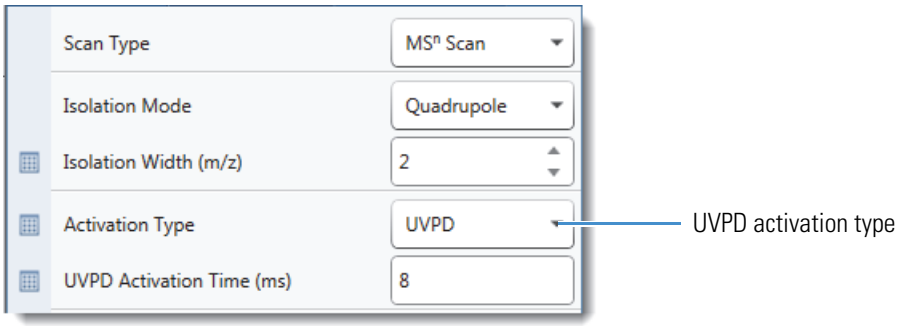
- More informative fragmentation for a wide variety of compounds.
- More complete sequence coverage for intact proteins as well as unique structural information for small molecules.
- Fast and direct cleavage of amide bonds at 5.82 eV per photon.
- Not limited by the precursor charge state, unlike ETD fragmentation.
- A wider array of sequence fragment types (a/x, b/y, and c/z) when activating peptidic precursors.
- Simplicity. Activation time is the only parameter, which the MS sets automatically based on the precursor molecular weight during method runs when the MS can determine the precursor's charge state.

UVPD Scan Parameters

When you select the MS^2 or MS^n scan type in the Thermo Scientific Tune application's Define Scan pane (Figure 2), you can then select UVPD as the Activation Type and set the UVPD activation time (in milliseconds). When you create a method in the Method Editor application, you also have the option of using the molecular-weight-based UVPD activation time that is determined by the Precursor Decay Rate Calibration.

For instructions on how to acquire data by using the Tune application, refer to the *Orbitrap Fusion Series Getting Started Guide*. For descriptions of the parameters in the Define Scan pane, refer to the Orbitrap Fusion Lumos Tune Help.

Figure 2. UVPD scan parameters in the Tune application



UVPD Readback Measurements

In addition to monitoring the overall system readback status in the Tune application, you can monitor individual UVPD readback values on the By Board and By Function pages of the Status pane. Table 2 lists the defaults for the UVPD readback values.

Table 2. Default values for the UVPD readbacks

Readback	Typical value
Laser Status	Ready, Standby, Not Connected
Laser	On, Off
Laser Diode Voltage	1–6 V
Laser Temperature[a]	10–60 °C
Laser Total Hours[a]	(Number of laser power-on hours)
Laser Pulse Count[a]	0–500 Gigashots

[a] By Board page only

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UVPD Module

The UVPD module assembly for the Orbitrap Fusion Lumos MS has two principal components—the laser module and the controller module. Installation of these modules includes connections to three of the instrument printed circuit boards (PCBs):

- Carrier PCB—Provides USB communication between the MS and the laser controller.
- Distribution PCB—Powers the laser module.
- Source PCB—Provides the laser trigger signal during the UVPD scan functions.

Note You cannot service the PCBs. If you need assistance, contact Thermo Fisher Scientific Technical Support.

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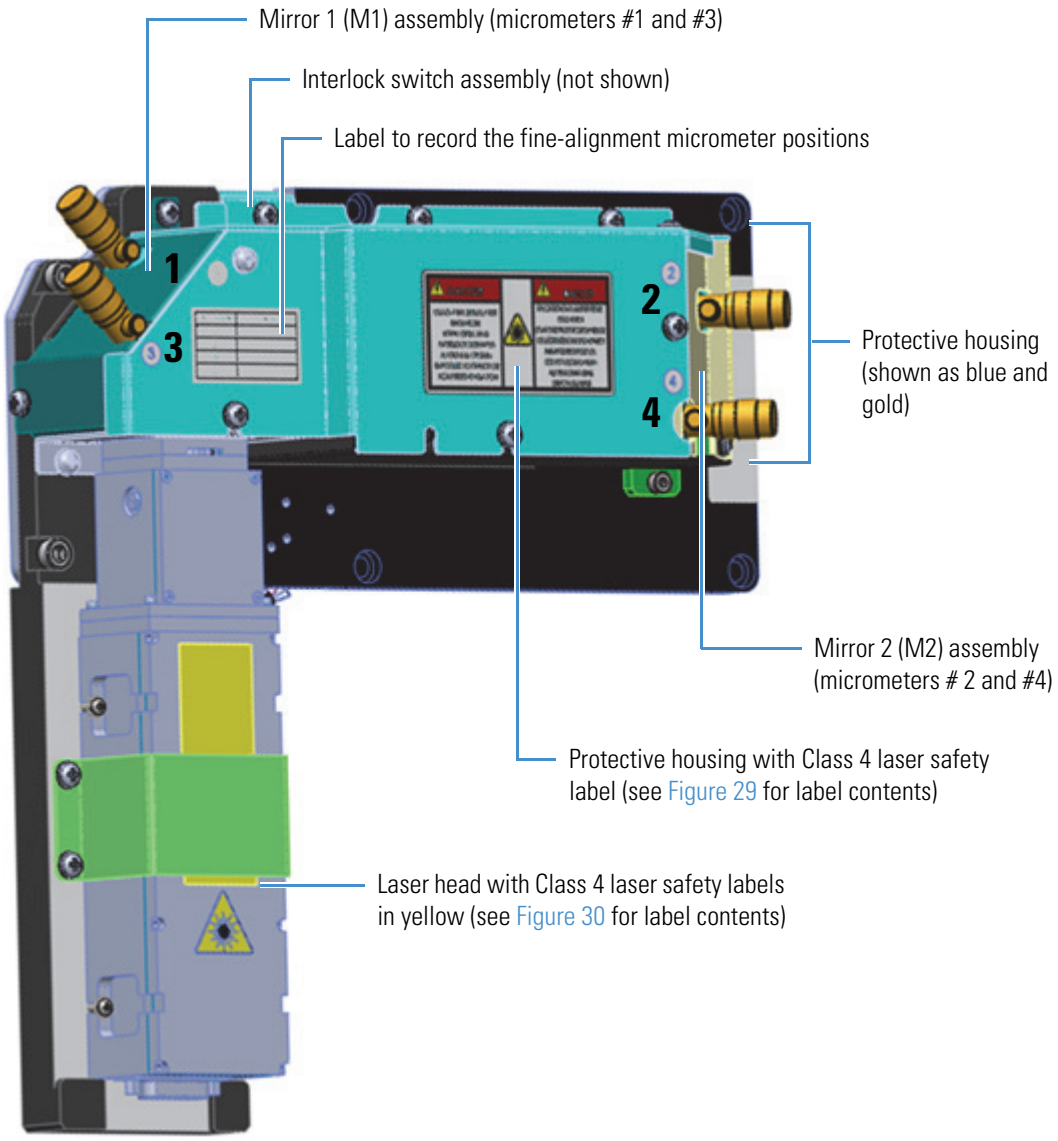
Laser Module

The laser module (Figure 3) is located inside the MS on the right side. The user-accessible laser compartment is behind the removable side door. The laser module assembly includes a 213 nm laser head, two adjustable mirror assemblies (each with two micrometers), and an interlock switch assembly.

CAUTION Do not remove the laser beam protective housing. The protective housing and safety interlock system prevent access to the invisible Class 4 laser beam. Exposure to the beam can cause serious skin and eye injuries, including blindness.

Figure 3. Laser module



Adjacent to each micrometer is a numbered label (1 to 4). Figure 4 shows the numbering and the assigned "complementary pairs" of micrometers, which are 1-2 (top micrometers) and 3-4 (bottom micrometers). Complementary pairs work in the same plane of motion—that is, movement that is vertical (pair 1-2) or horizontal (pair 3-4).

Figure 4. Complementary pairs of micrometers (front view)

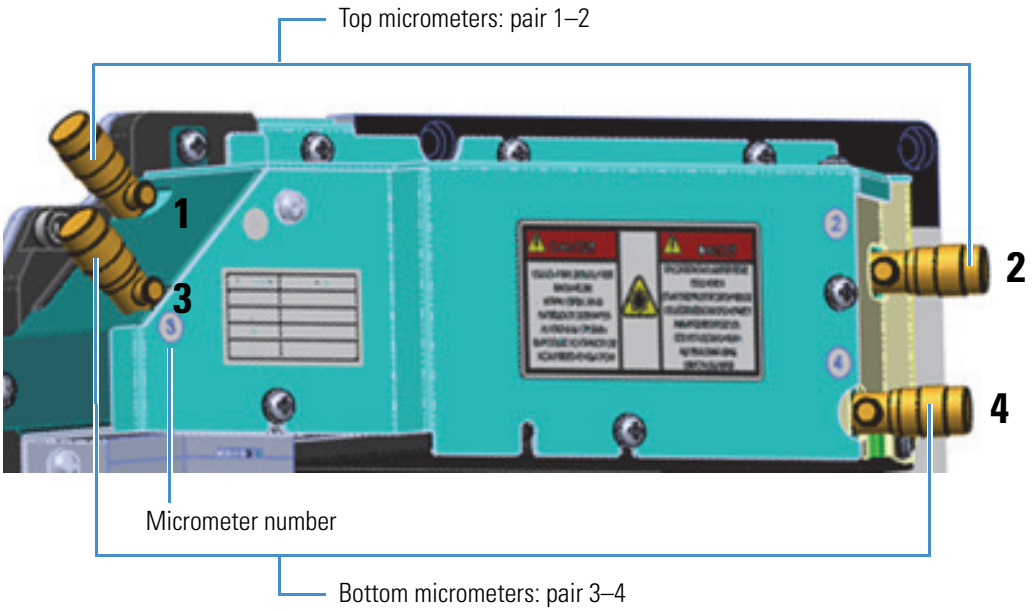


Figure 5 shows the mirror assemblies behind the protective housing. Each mirror assembly has two micrometers that control the position of its mirror.

Figure 5. Mirror assemblies behind the laser beam protective housing

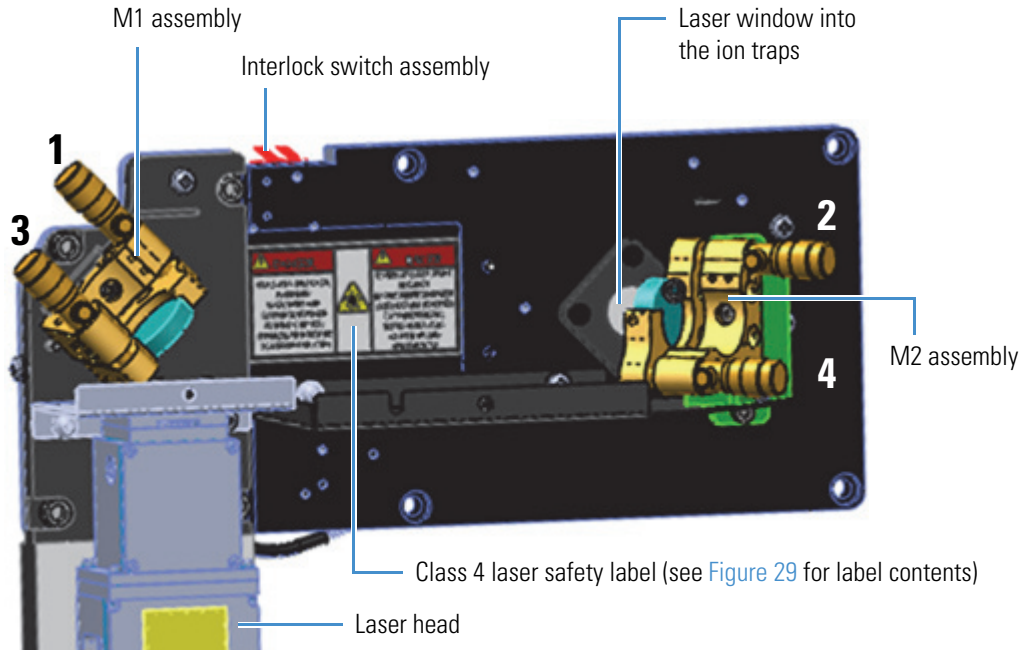
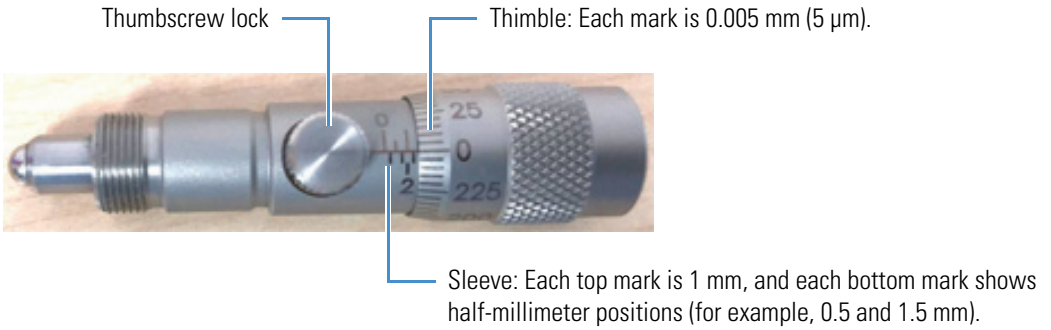


Figure 6 shows the micrometer and defines the graduation markings on its sleeve and thimble.

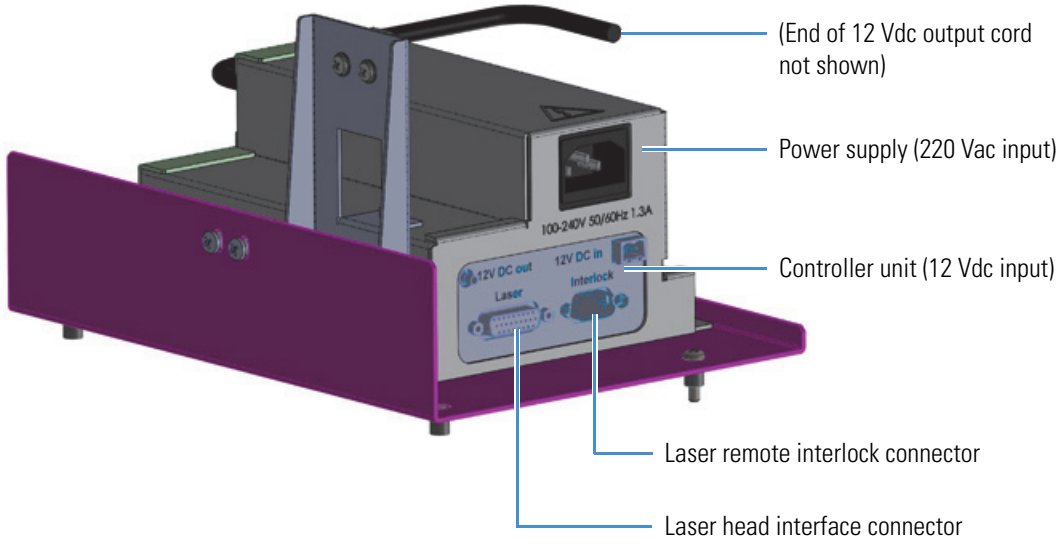
Figure 6. Micrometer locking thumbscrew and graduation markings



Controller Module

The controller module (Figure 7) is located inside the MS behind the back panel near the distribution PCB. Enclosed within the controller module assembly is a power supply and a controller unit, which controls the laser head.

Figure 7. Controller module



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UVPD Laser Beam

Before your first use of the Orbitrap Fusion Lumos MS with UVPD capability, the Thermo Fisher Scientific factory or service engineer aligns the invisible laser beam. However, you might have to periodically fine align the beam to overlap with the ion clouds within the ion trap assembly. The goal of the laser alignment is to both center the laser beam in the ion trap cells and maximize fragmentation efficiency.

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Laser Alignment Quality Score

The mass spectrometer's dual-cell ion-trap configuration allows for a new and unique laser alignment procedure that guarantees optimum laser beam alignment while minimizing the chance for laser-generated noise occurring from the beam's impact with metal surfaces within the instrument. During the UVPD Laser Alignment calibration, the Tune application displays the precursor fragmentation percentage and the laser beam's alignment quality score (AQS), which is calculated as follows:

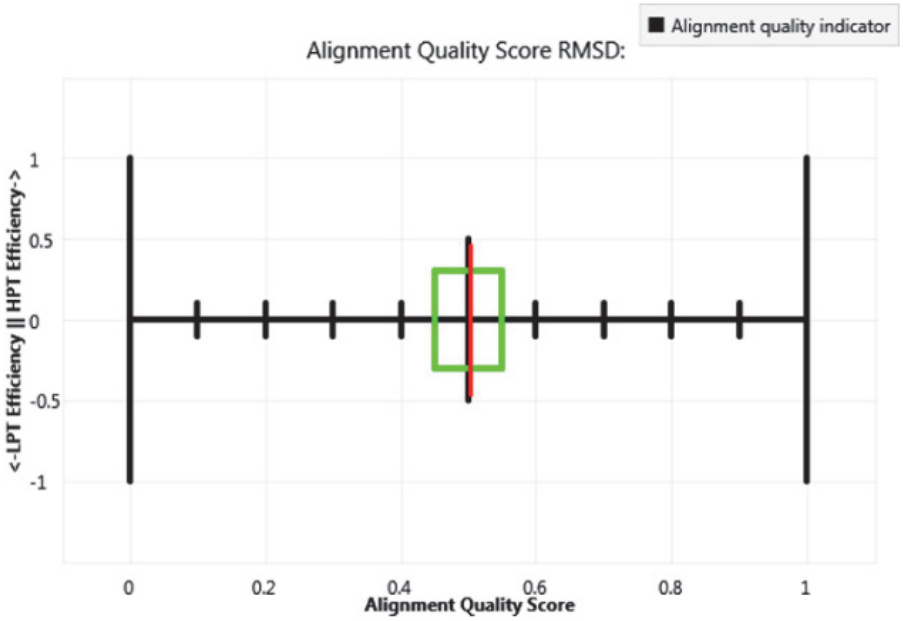
$$AQS = \frac{I_{HPC}}{I_{HPC} + I_{LPC}}$$

In this formula, I_{HPC} is the amount of precursor remaining in the ion trap's high-pressure cell (HPC) and I_{LPC} is the signal remaining in the low-pressure cell (LPC) after a fixed amount of irradiation time. The AQS ranges from 0 (no irradiation in the HPC) to 1 (no irradiation in the LPC), with 0.5 being the target value that represents the perfect balance between the two ion trap cells.

In the following figure, the green box represents the minimum required combination of UVPD efficiency and acceptable AQS. The red bar's top and bottom ends indicate the absolute values of the UVPD efficiency (*y* axis) in the LPC and HPC. The AQS is the red bar's *x*-axis value, which must be 0.5 ±0.05 where the tolerance values are indicated by the

green box's side borders. When the laser beam is properly aligned (AQS is 0.5), the red bar is in the box on the *x* axis and outside the box on the *y* axis (Figure 8). Or, if the red bar is as long as and overlaps with the *x* axis's center black mark, the laser beam is aligned for optimal UVPD efficiency.

Figure 8. Example of optimal UVPD efficiency and AQS



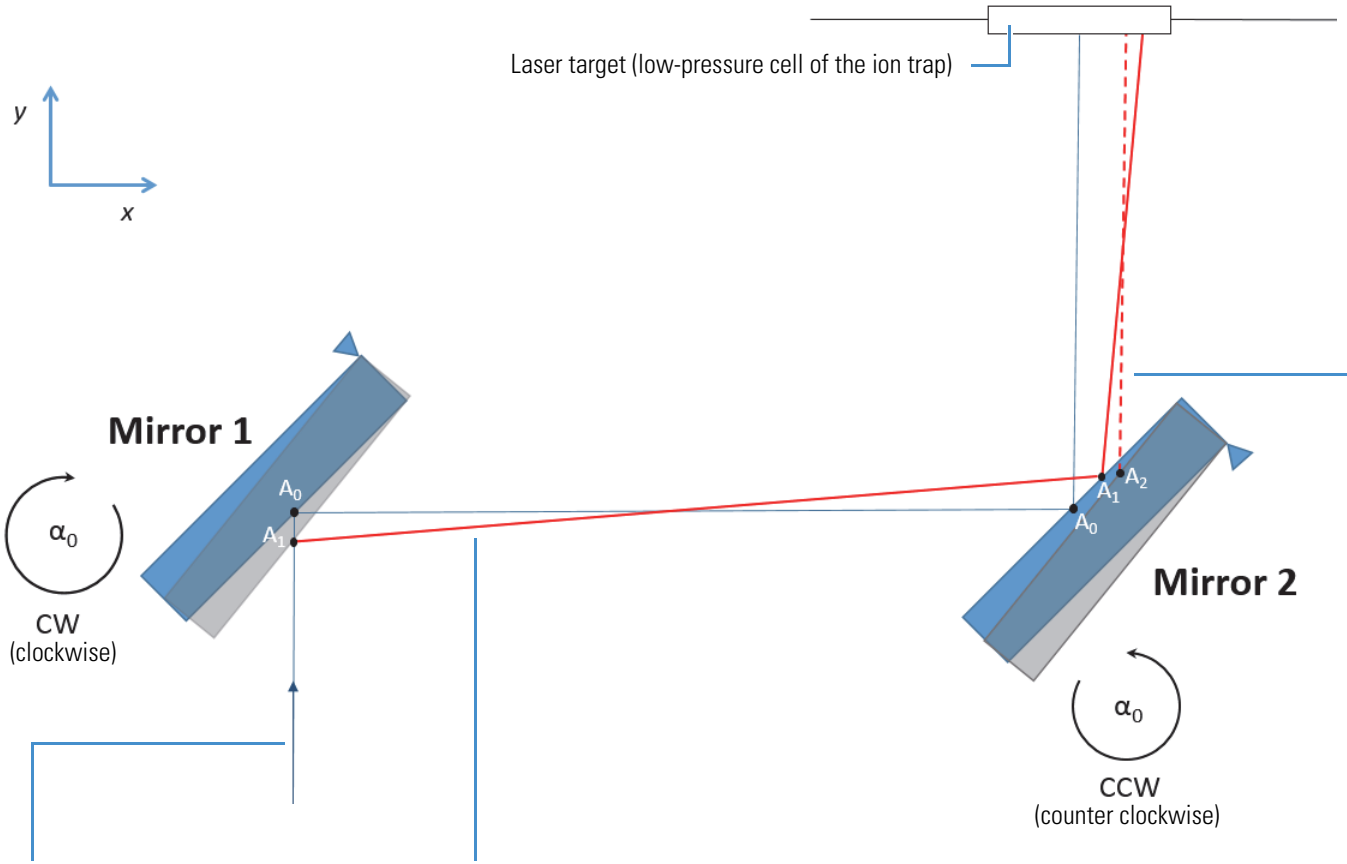
Laser Beam Movement

As shown in Figure 9, the three-dimensional orientation (plane) of the first mirror (M1) in relation to the second mirror (M2) determines the angle of the transmitted laser beam into the linear ion trap chamber. If the mirror positioning is correct, the laser beam enters the chamber and optimally overlaps with the ion clouds inside the chamber.

Two types of movement result from adjusting the mirrors' orientation:

- Uncompensated movement—Any movement/adjustment to the position of a single micrometer at M1 displaces the laser beam at M2, resulting in an angular change as the beam travels the space between M2 and the window into the ion trap chamber. For example, with this type of movement, a beam that is parallel to the ion trap cells then becomes off-centered and at a wider angle (Figure 12).
- Compensated movement—A compensated movement corrects the uncompensated (first) movement by making an equal and opposite movement of the second micrometer in that complementary pair. The beam retains the same angular dependence that it had before the first compensated movement, but it is now displaced by some amount of distance. For example, a beam that is parallel and centered within the ion trap remains parallel but now becomes off-centered; in turn, this reduces the overlap with the ion cloud in both cells in the ion trap, which then reduces the UVPD efficiency (Figure 11).

Figure 9. Laser beam paths after adjusting one or more micrometers



Laser Beam Overlap with the Ion Clouds in the Ion Trap Chamber

As shown in Figure 10, the transmitted laser beam (blue circle) is typically only slightly wider than each ion cloud (the green circles represent the ion cloud that is located in either cell of the dual-cell ion trap. Therefore, the position and angle of the laser beam determine the amount of overlap of the beam with the ion cloud, which affects the overall UVPD efficiency. Examples 1 and 3 show poor UVPD efficiency, while the overlap in example 2 is ideal.

Figure 10. Laser beam (blue) overlap with an ion cloud (green) in the ion trap

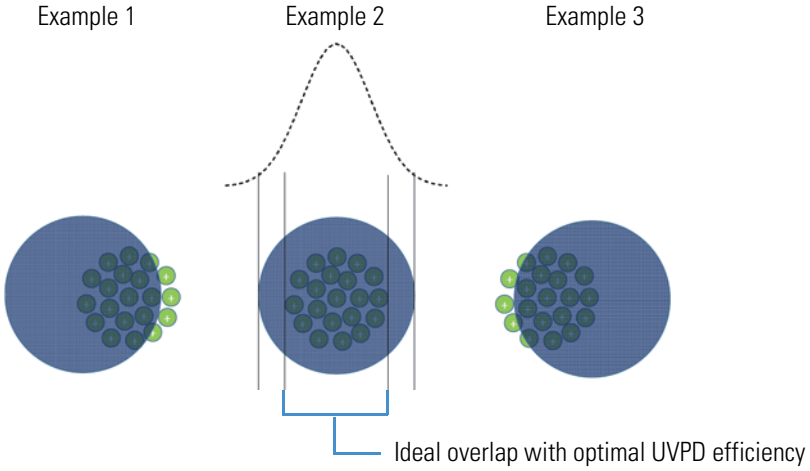


Figure 11 shows that the laser beam is parallel to the ion clouds as it passes through the ion trap's low- and high-pressure cells. However, a beam that is not centered (as in image B) has less overlap with both ion clouds; this reduces the overall UVPD efficiency, even with the AQS at the 0.5 target value.

Figure 11. Examples of optimal and poor UVPD efficiency (level laser beam through the trap)

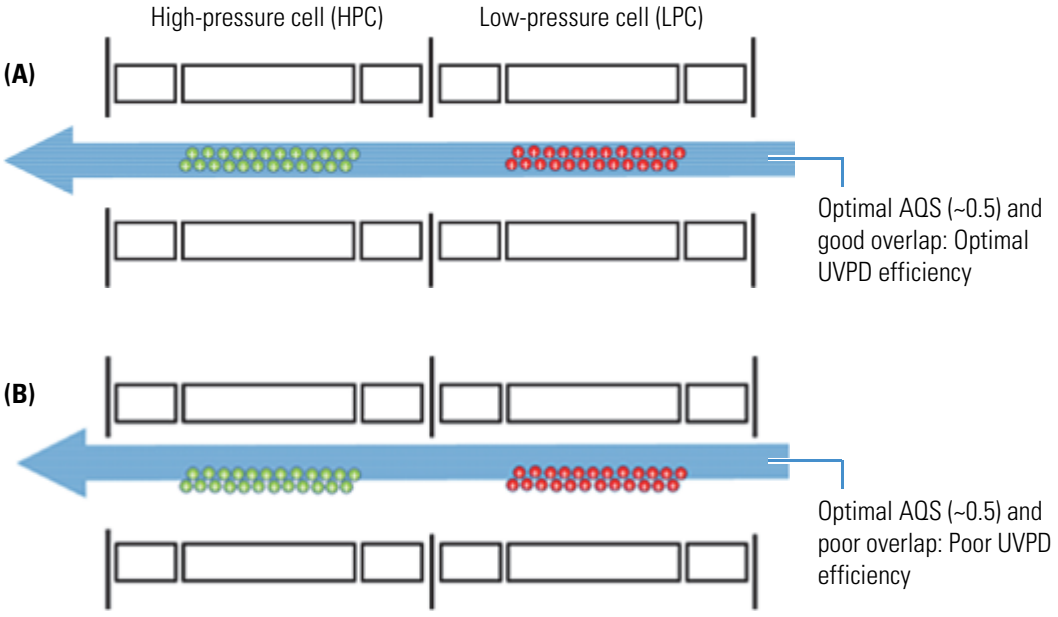
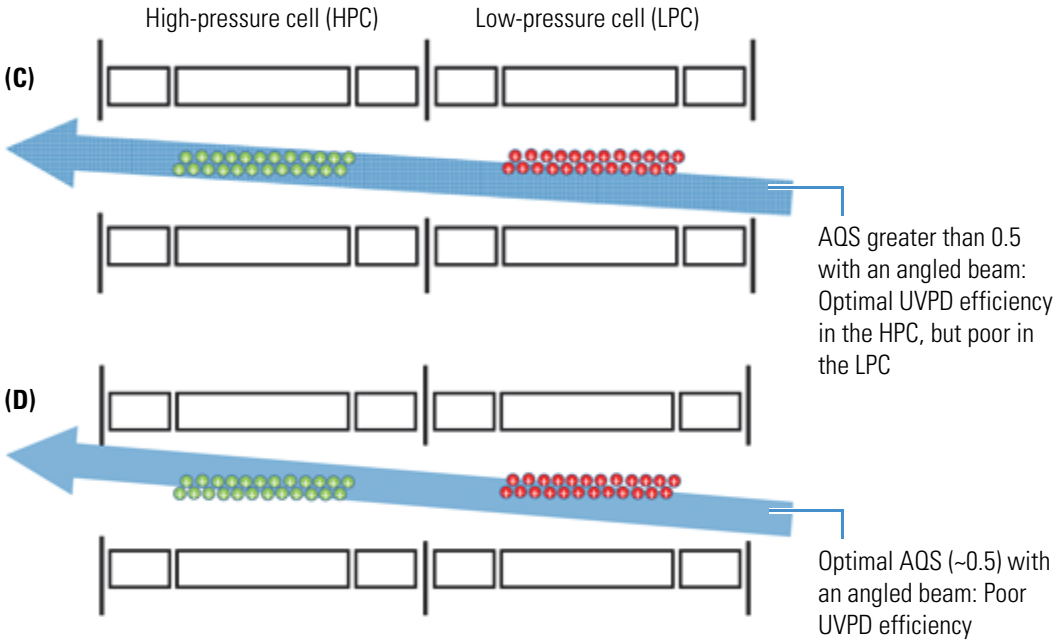


Figure 12 shows the laser beam transmitted through the ion trap at an angle. Depending on the angle, there might be full overlap with an ion cloud (as in the HPC in image C), or the amount of ion cloud overlap in both the HPC and LPC can be reduced (as in image D). The overall UVPD efficiency depends on the AQS value, the amount of overlap, and in which ion trap cell the overlap occurs.

Figure 12. Examples of optimal and poor UVPD efficiency (angled laser beam through the trap)



3 UVPD Laser Beam

Laser Beam Overlap with the Ion Clouds in the Ion Trap Chamber

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UVPD Calibration

Before operating the Orbitrap Fusion Lumos MS with UVPD capability, run the standard calibrations in H-ESI mode for positive and negative ion polarity modes with passing results, and then run both of the UVPD calibration procedures in positive ion polarity mode. For the H-ESI mode calibration instructions, refer to Chapters 5–7 in the *Orbitrap Fusion Series Getting Started Guide*.

Note

- Generally, you must run the UVPD Precursor Decay Rate Calibration every month and the Laser Alignment Calibration every 3 months for optimum performance.
- If you want to generate calibration reports, click the **Options** icon,  , choose **Preferences**, and then select the **Show Report Generation Options Dialog Box** option.
- For descriptions of the UVPD calibration options, refer to the Orbitrap Fusion Lumos Tune Help.

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Laser Alignment Calibration

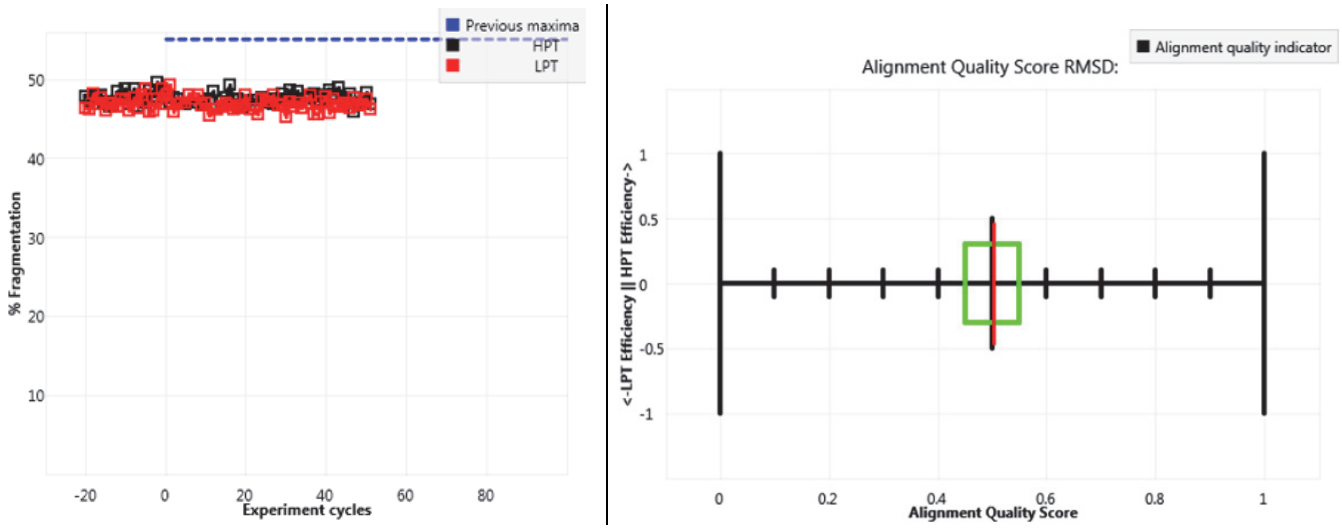
The Laser Alignment calibration and calibration check measure the laser's fine alignment quality and UVPD fragmentation efficiency. This calibration procedure maximizes the overlap of the laser beam with the ion clouds in the low- and high-pressure cells of the ion trap (LPC and HPC, respectively) to obtain the highest possible fragmentation efficiency. For calibration instructions, see Running the UVPD Calibration Procedures.

Note The Laser Alignment calibration and calibration check pass if the measured AQS is 0.5 ±0.05 with a fragmentation efficiency of 30 percent or higher in both ion traps.

Figure 13 shows the real-time plots that appear in the Tune application during this calibration procedure and their ideal behavior. The left-side graph shows overlapping HPC and LPC precursor depletion signals, each with an acceptable result above 30 percent. The right-side graph shows an AQS of approximately 0.5, which is indicated by the red bar's *x*-axis value. For additional information about the AQS plot, see Laser Alignment Quality Score.

A calibration failure indicates that the laser beam is out of alignment with respect to at least one of the ion trap cells, requiring you to use the internal micrometers to manually adjust the beam position.

Figure 13. Laser Alignment calibration displays (percentage of precursor fragmentation and AQS)



Precursor Decay Rate Calibration

The Precursor Decay Rate calibration measures the caffeine precursor ion's decay rate in the LPC so that the Tune application can predict the UVPD activation time and automatically set it during method-controlled sample analysis. Figure 14 and Figure 15 show the real-time plots that appear in the Tune application during this calibration procedure. For calibration instructions, see Running the UVPD Calibration Procedures.

Figure 14. Plot of the precursor decay rate in the ion trap's low-pressure cell

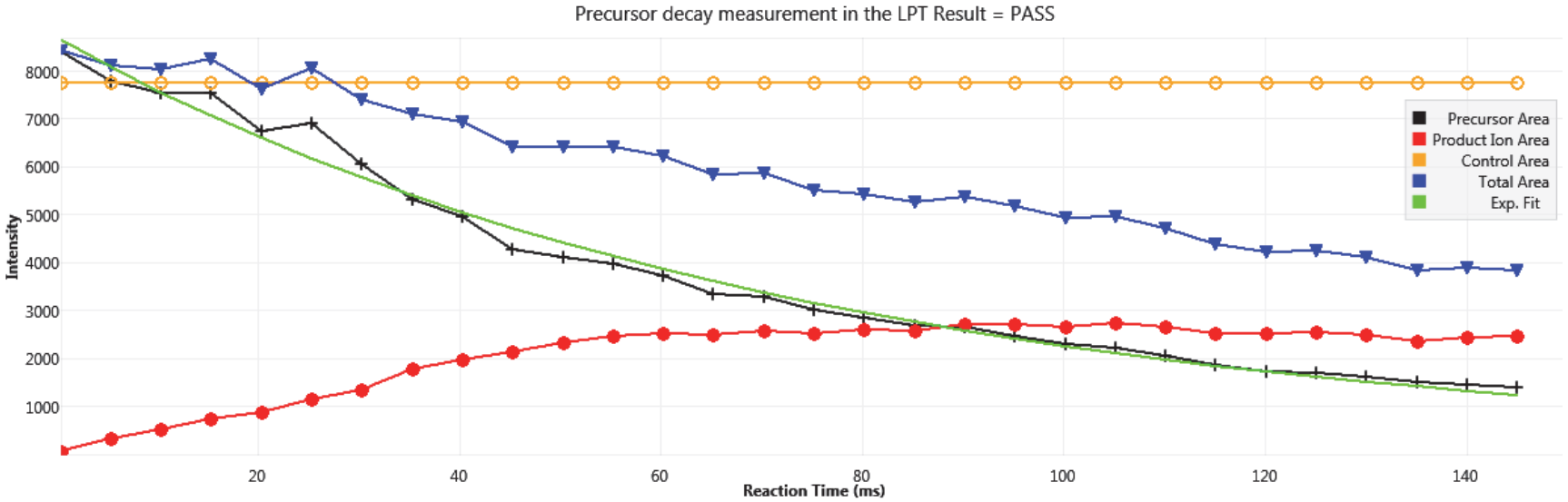
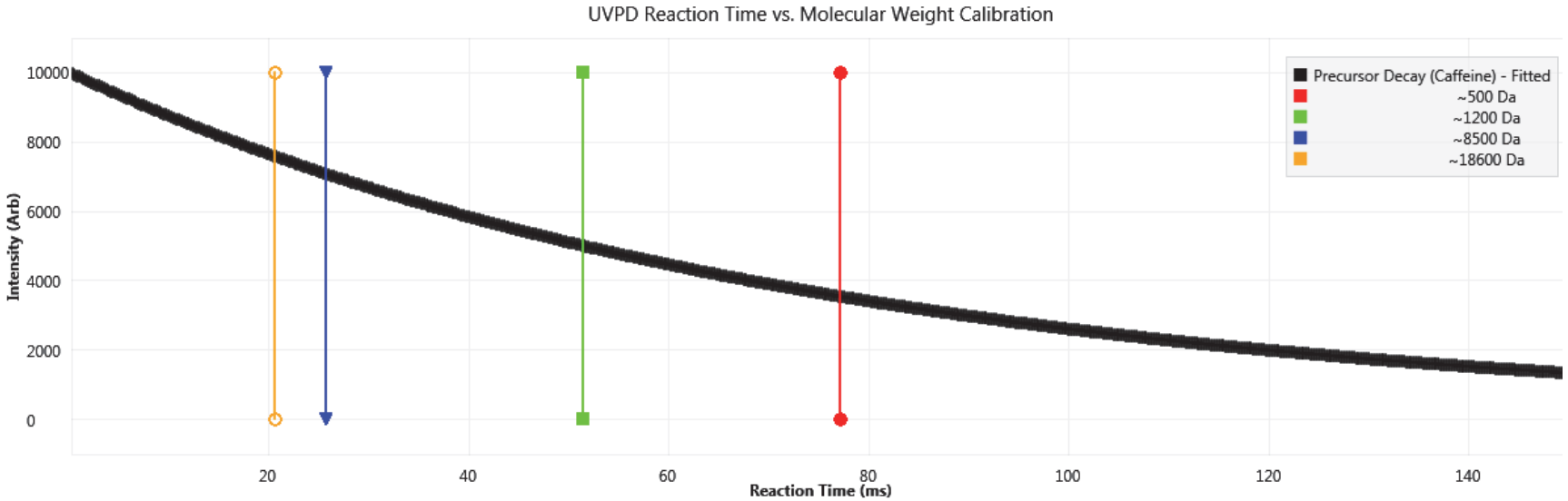


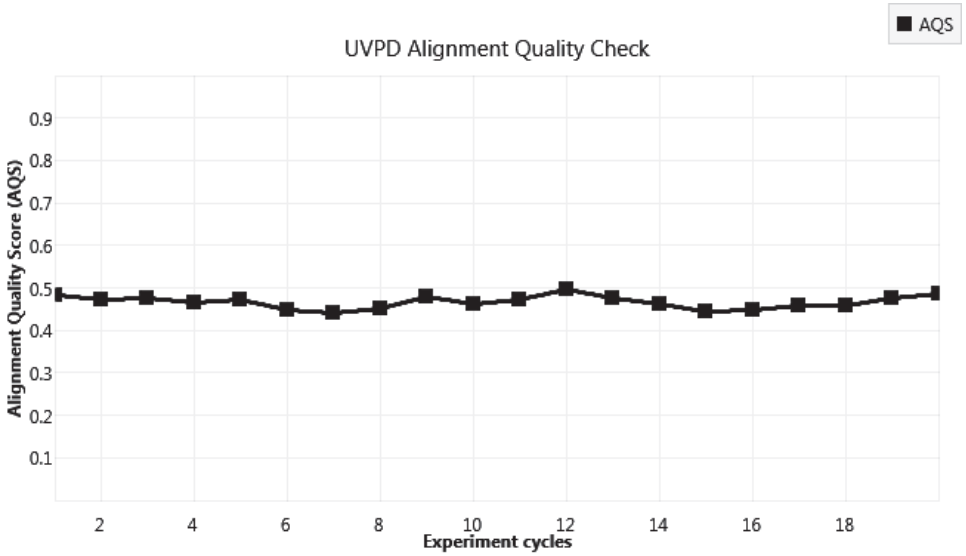
Figure 15. Plot of the UVPD reaction time for the caffeine precursor



Laser Alignment Check

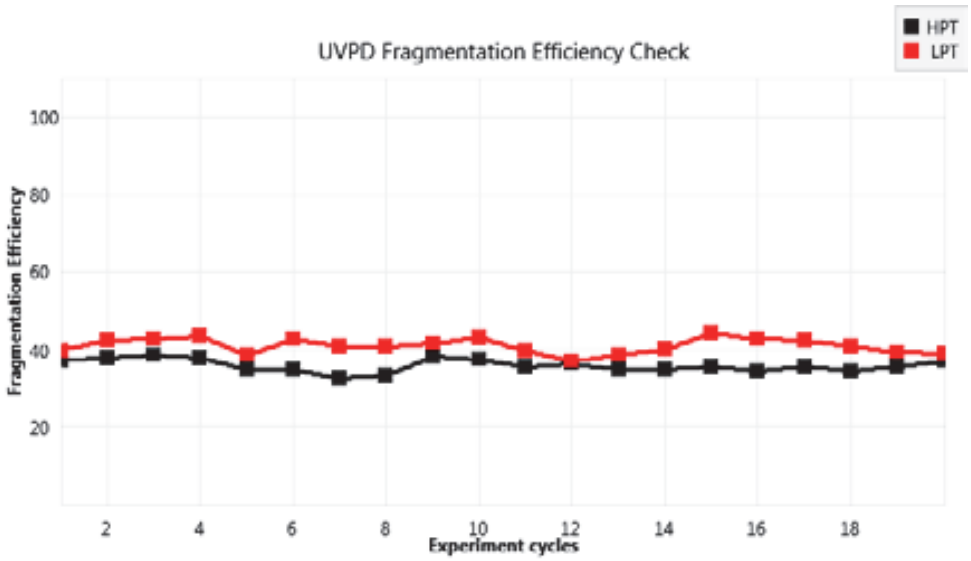
When you select the Check Only check box (Figure 18) with the Laser Alignment calibration, the Tune application performs two checks of the current UVPD calibration, starting with the AQS. Figure 16 shows the plot of the AQS check, where the target value is 0.5 ±0.05.

Figure 16. Plot of the UVPD alignment quality check



Next, the application checks the fragmentation efficiency in both the HPC and LPC by using the molecular ion of caffeine (Figure 17). A valid value is 30 percent or higher.

Figure 17. Plot of the UVPD fragmentation efficiency check



Running the UVPD Calibration Procedures

In the Tune application, the Calibration pane lists the dates when you last ran any of the calibration procedures and when to repeat those tests to maintain instrument performance. However, if you suspect that your data might not be optimized, you can run the "check" version of the calibration procedure to determine if the MS is out of calibration.

Note After the calibration (or check calibration), you see either a green check mark (✔) adjacent to the calibration name to indicate a successful calibration or a red X mark (✖) to indicate a failed calibration.

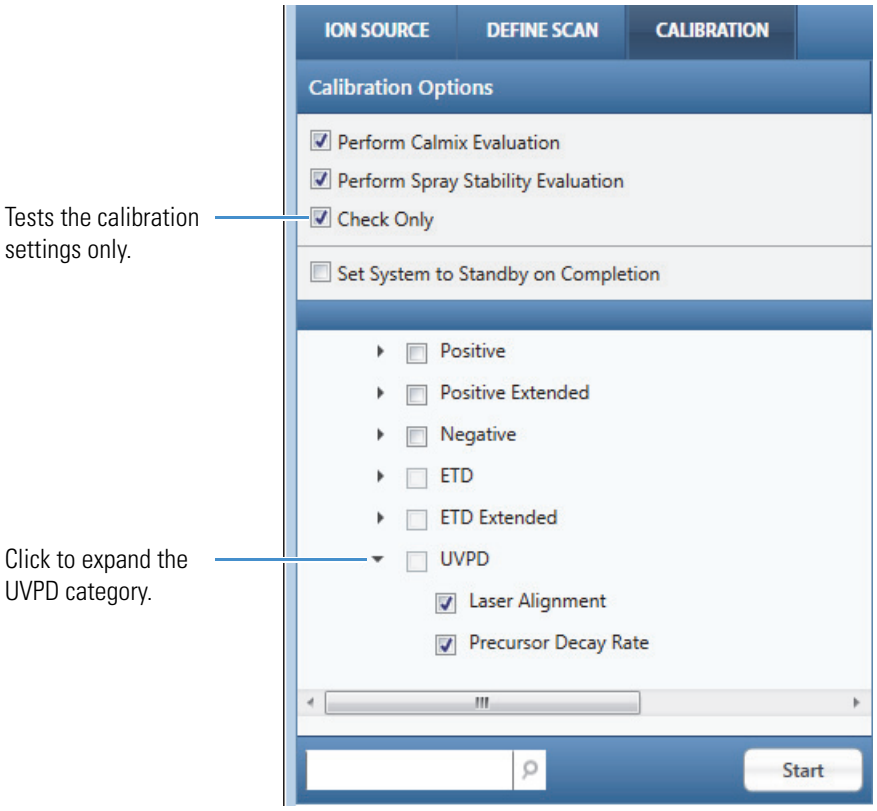
❖ To check the UVPD calibration settings

1. Prepare the system for positive mode calibration (refer to Chapters 6 and 7 in the *Orbitrap Fusion Series Getting Started Guide*).
2. In the Tune window, in the Calibration pane, select the check boxes as shown in Figure 18, and then click **Start**.

   Note You must select the **Check Only** check box to run the calibration check.

   After clicking Start, the button label changes to "Stop," the calibration check process begins, the status area appears in the Calibration pane, and the real-time plots appear.

Figure 18. UVPD check calibration procedures



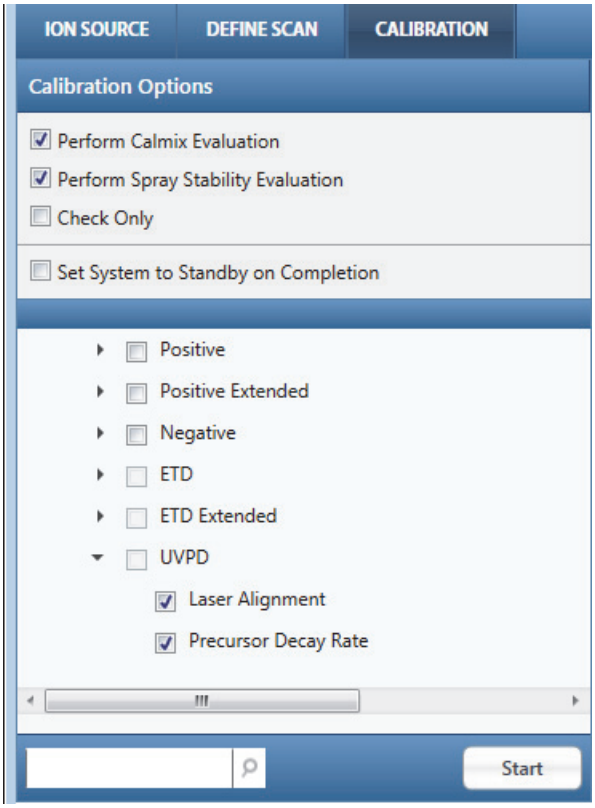
3. If the check calibration fails, follow the procedure To calibrate the UVPD laser beam.

❖ To calibrate the UVPD laser beam

1. Prepare the system for positive mode calibration (refer to Chapters 6 and 7 in the *Orbitrap Fusion Series Getting Started Guide*).
2. In the Tune window, in the Calibration pane, select the check boxes as shown in Figure 19, and then click **Start**.

IMPORTANT The Laser Alignment calibration requires user interaction—do not walk away from the system during this test. When the alignment is optimal, you must manually stop the calibration procedure by clicking Stop to avoid wasting the sample.

Figure 19. UVPD calibration procedures



3. If the AQS is outside the range of 0.45–0.55 or the UVPD efficiency is below 30 percent, follow the procedure in Manually Aligning the Laser Beam to recalibrate the MS.
4. After you manually adjust the AQS and UVPD efficiency to acceptable values, click **Stop** in the Calibration pane.

   After completing the calibration, the Tune application adds a change record to the History pane under History Logs. A date appears in the Last Calibrated column for each successful calibration procedure. A new date does not appear for failed calibrations.

This completes the UVPD calibration process.

Manually Aligning the Laser Beam

If the UVPD Laser Alignment calibration fails, the laser beam is out of alignment with the center of the ion traps. This means you must manually adjust the UVPD mirrors to reposition the beam. This alignment process requires eye-hand coordination and can take 10–60 minutes to complete.

CAUTION Risk of laser radiation. Failure to understand and comply with laser cautions and operating instructions in this guide can result in property damage or serious injuries to the user.

CAUTION Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Note Before you continue, read and understand Chapter 3, "UVPD Laser Beam."

❖ To prepare the MS for the UVPD fine-alignment procedure

1. Ensure that the Laser Power key, which is located below the UVPD compartment, is in the **On** position.
2. Using a 4 mm hex key, remove the UVPD compartment door (Figure 20).

Figure 20. Laser module compartment with door removed

3. Unlock the micrometers by slowly rotating each locking thumbscrew (Figure 6) counterclockwise (CCW) 15–30 degrees.
4. Position the data system monitor with the Tune application in view while standing near the open UVPD compartment.

To maximize the overall fragmentation efficiency in both ion traps (AQS close to 0.5), use the micrometers to iteratively align the mirrors vertically (10–15 µm adjustments) and horizontally (15–50 µm adjustments) so that they converge to form the best fine alignment of the laser beam.

Note Read and understand the following procedure before you begin.

❖ To manually align the laser beam

1. In the Tune window, in the Calibration pane, choose **UVPD > Laser Alignment**, and click **Start**.

   Tip During this procedure, watch the left graph in the Tune application (Figure 13) to see how the fragmentation efficiency percentage responds to each step. The Tune application takes a couple of seconds to respond to adjustments.

2. Align the mirrors vertically by using micrometers #1 and #2 as follows:

   a. If the HPC and LPC signals overlap at the start, go to step 2c. If they do not overlap, use **micrometer #1** to overlay the signals.

      Slowly rotate **micrometer #1** in both the counterclockwise (CCW) and clockwise (CW) directions, and select the direction that yields the higher fragmentation efficiency in the graph for both traps. The overall ion signal might decrease with this step, which is okay.

   b. Record the fragmentation efficiency and the settings for the vertical micrometer #1 (Figure 6).

   c. With the signals overlaid, rotate **micrometer #2** CW and then CCW to see how the fragmentation efficiency responds in both traps for both directions. Depending on the response, do one of the following:

      - If the fragmentation efficiency increases in both traps, continue rotating **micrometer #2** in the same direction until the fragmentation efficiency decreases in one of the traps—at this point, stop and go to step 3 (horizontal alignment).
      - If the fragmentation efficiency increases in one cell and decreases in the other, continue rotating **micrometer #2** to increase the fragmentation efficiency 2–10 percent, and then go to the next step.

        Note Observe which ion trap cell had the increased efficiency.

   d. Repeat step 2a and step 2b to evaluate whether the overall fragmentation efficiency in both traps has improved compared to the starting percentage that you recorded.

e. Do one of the following:

- If the overall fragmentation efficiency has improved, iteratively repeat step 2a through step 2c until you maximize the overlaid signals.
- If the overall fragmentation efficiency has not improved, repeat step 2c by rotating **micrometer #2** in the opposite direction.

–or–

- If the overall fragmentation efficiency did not improve with either micrometer direction, return **micrometer #1** to its settings after step 2a.

3. Align the mirrors horizontally by using micrometers #3 and #4 as follows:
   a. Use **micrometer #3** to overlay the HPC and LPC signals.

      Slowly rotate the micrometer in both the CCW and CW directions and select the direction that yields the higher fragmentation efficiency in the graph for both traps.

   b. Record the fragmentation efficiency and the settings for the horizontal micrometer #3.

   c. With the signals overlaid, use **micrometer #4** to increase the fragmentation efficiency in one of the traps. Consider the following scenarios:

      - If the fragmentation efficiency increases in both traps, continue rotating **micrometer #4** in the same direction until the fragmentation efficiency decreases in one of the traps—at this point, stop and return to step 2 (vertical alignment).
      - If the fragmentation efficiency increases in one cell and decreases in the other, continue rotating **micrometer #4** to increase the fragmentation efficiency 2–10 percent, and then go to the next step.

   d. Record which ion trap cell had the increased efficiency.

4. Repeat step 3a and step 3b to evaluate whether the overall fragmentation efficiency is better or worse. If it is worse, repeat step 3c, rotating **micrometer #4** in the opposite direction. After you have maximized the overall signal by iteratively performing the vertical and horizontal alignment, click **Stop** in the Calibration pane.
5. Repeat the Laser Alignment calibration until the procedure passes.
6. When the results are acceptable, do the following:
   a. Lock the micrometer positions by slowly rotating each locking thumbscrew CW until they are fingertight.

   b. Record the final settings for the micrometers.

   c. Reattach the UVPD compartment door.

Note If you are unable to complete the laser alignment process, contact your local Thermo Fisher Scientific service engineer.

5

UVPD Experiment

This chapter demonstrates the use of UVPD on the Orbitrap Fusion Lumos MS. This experiment uses a direct infusion of the enfuvirtide high-mass range calibration solution, which has molecular ion charge states at *m/z* 1123 (4+ charge state) and *m/z* 1498 (3+ charge state). For instructions on how to prepare the calibration solution, refer to Appendix D in the *Orbitrap Fusion Series Getting Started Guide*.

IMPORTANT To minimize the possibility of cross-contamination, use a different syringe and length of PEEK tubing for each type of solution.

Contents

Setting Up the MS/UVPD System

Follow these procedures:

1. To set up the inlet for direct infusion
2. To configure the syringe pump
3. To set the MS parameters

❖ To set up the inlet for direct infusion

1. Turn on the syringe pump's power switch (on the back of the device).
2. Load a clean, 500 µL syringe with the enfuvirtide solution.
3. Set up the syringe pump and plumb the inlet for direct infusion.

   For instructions, refer to Chapter 3 in the *Orbitrap Fusion Series Getting Started Guide*.

❖ To configure the syringe pump

1. In the Tune window, place the MS in **On** mode,  .
2. Set the syringe pump parameters as follows:
   a. Click **Syringe Off** to turn on the syringe pump.

      The button name changes to Syringe On.

   b. Click the arrow next to the Syringe On/Off button to open the syringe pump settings box (Figure 21), and then enter the following:

      Flow Rate (µL/min): **3**

      Volume (µL): **500**

      Figure 21. Syringe pump settings box

   c. Click **Apply**.
3. Verify that the inlet plumbing connections do not leak.
4. Open the syringe pump settings box, and press and hold **Prime** to prime the syringe at 100 µL/min.

❖ To set the MS parameters

At the top of the Tune window, set the MS parameters as listed in Table 3.

Table 3. Instrument parameter settings

Parameter	Setting
Data type	Profile
Ion polarity mode	Positive
Spectrum scan averaging	Off
Pressure mode	Std. Pressure Mode

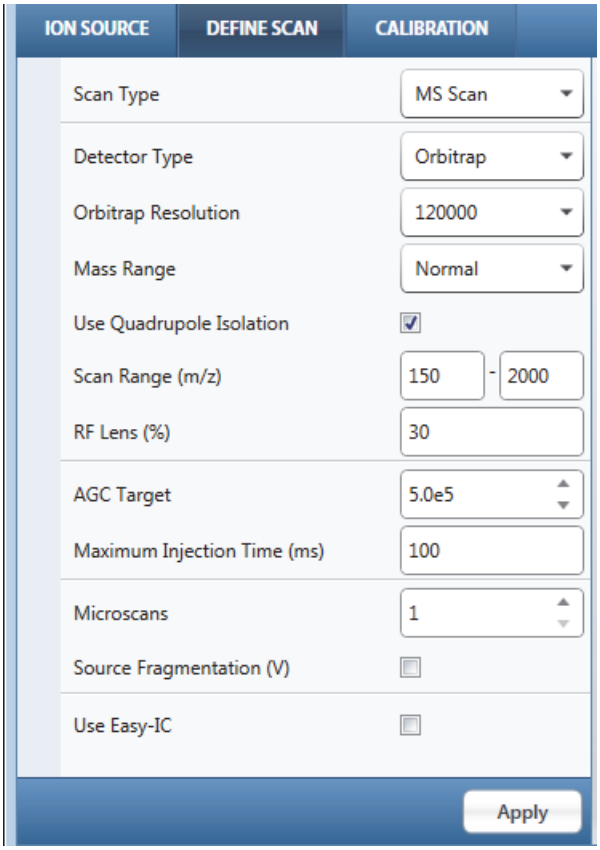
This completes the instrument setup. Go to the next section.

Optimizing the API Source Parameters

❖ **To optimize the API source parameters**

1. In the Define Scan pane, set the MS Scan type parameters as shown in Figure 22, and then click **Apply**.

   Figure 22. Define Scan pane showing the scan parameters for optimizing the ion source



2. In the Ion Source – Optimization page, select the **Pos Ion Spray Voltage (V)** option, enter the settings for the ion spray voltage as listed in Table 4, and then click **Optimize**.

   Table 4. Conditions to optimize the ion source

	Pos Ion Spray Voltage (V)	Sheath Gas (Arb)	Aux Gas (Arb)
Start Value	2000	0	0
Stop Value	4000	50	25
Step Size	100	5	2
Signal Type	TIC	TIC	TIC

3. After the optimization is complete, click **Accept**.
4. Repeat step 2 and 3 for the sheath and auxiliary gases.

Figure 23 shows a real-time graph of the relative standard deviation (RSD) of the total ion current (TIC) for the ion source optimization scan. In this example, the signal stability rating is "Good" and the RSD value is below the maximum 15 percent threshold (black line) for at least 100 scans.

Figure 23. RSD results for spray stability when optimizing the ion source parameters

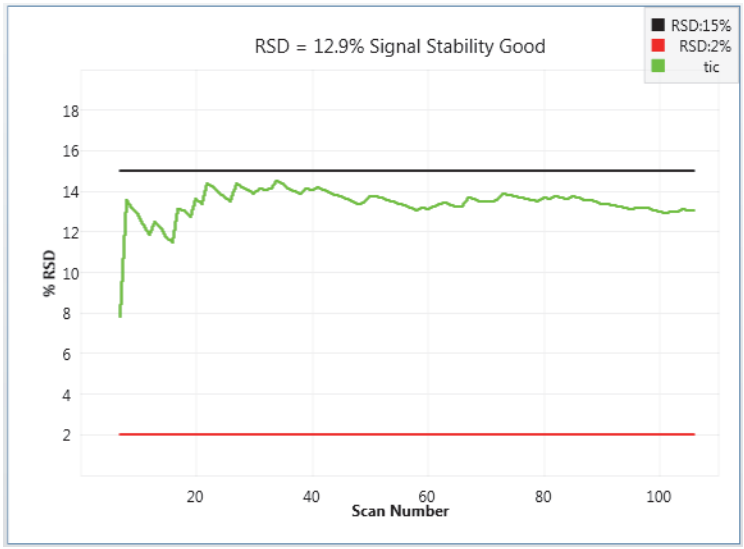
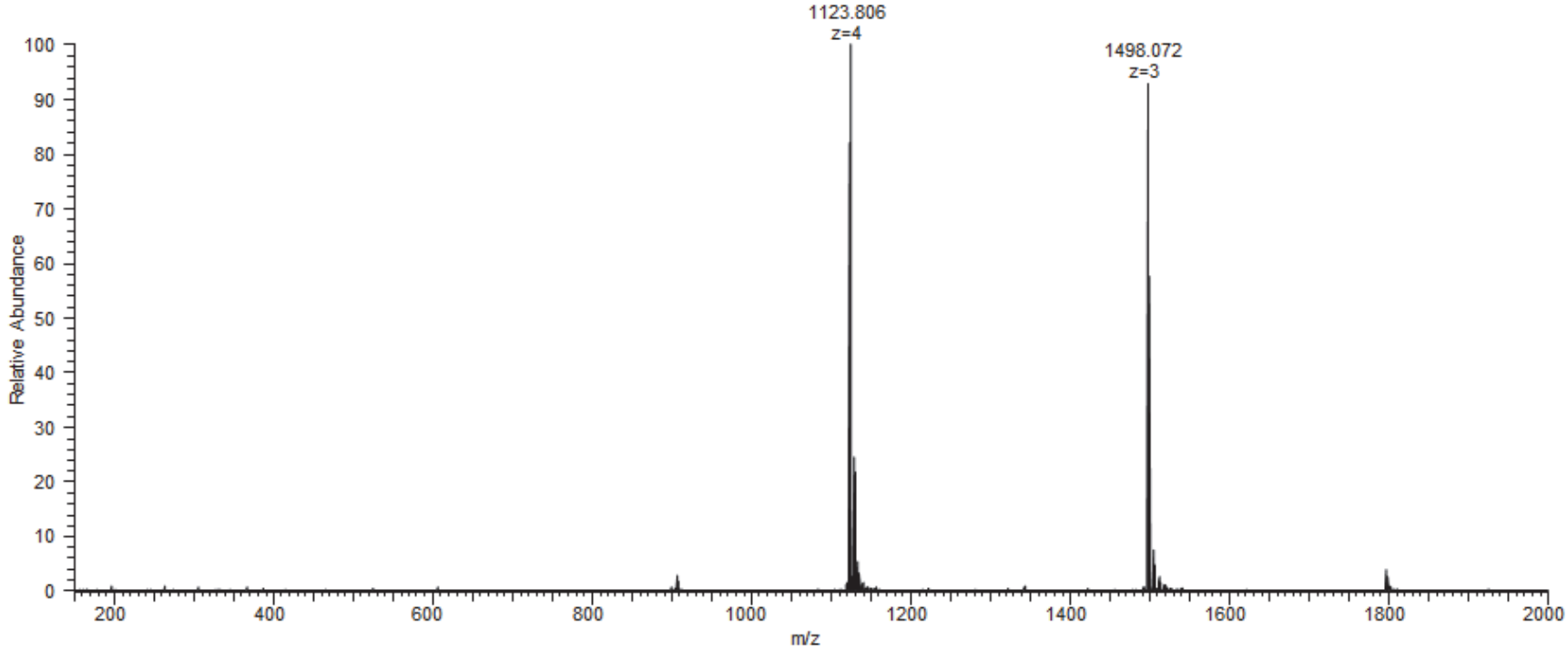


Figure 24 shows the full-scan spectrum of the enfuvirtide solution and its charge states.

Figure 24. Full MS scan spectrum of the enfuvirtide solution (profile mode)



This completes the optimization of the ion source parameters. Go to the next section.

Defining the Scan Parameters for the Experiment

❖ **To define the scan parameters for the experiment**

In the Define Scan pane, set the **MS^n Scan** type parameters as shown in Figure 25, and then click **Apply**.

Figure 25. Define Scan pane showing the scan parameters for the UVPD experiment

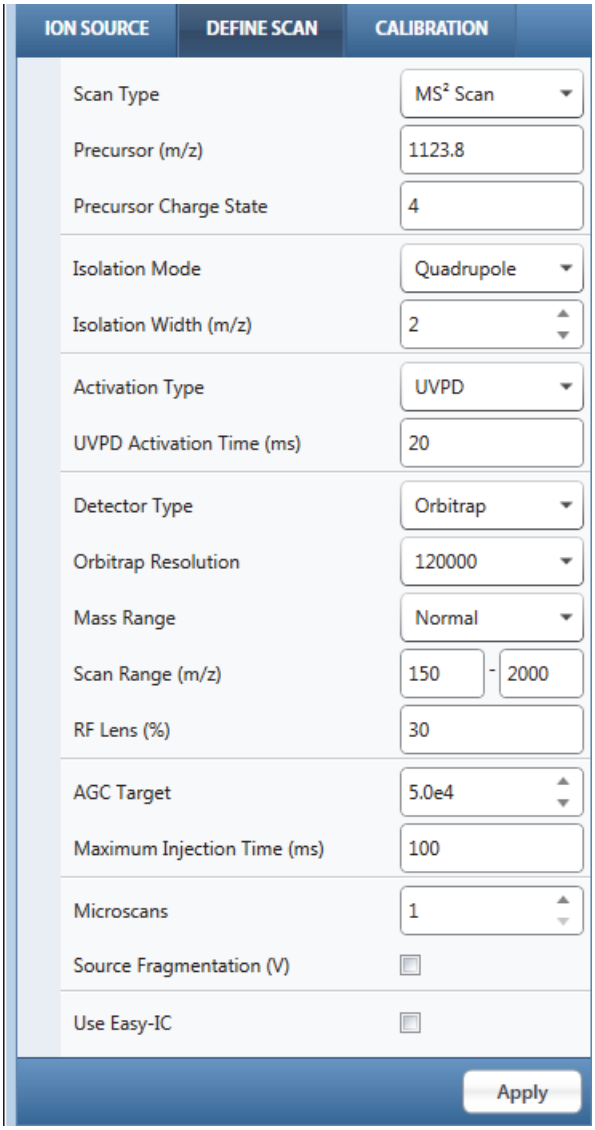
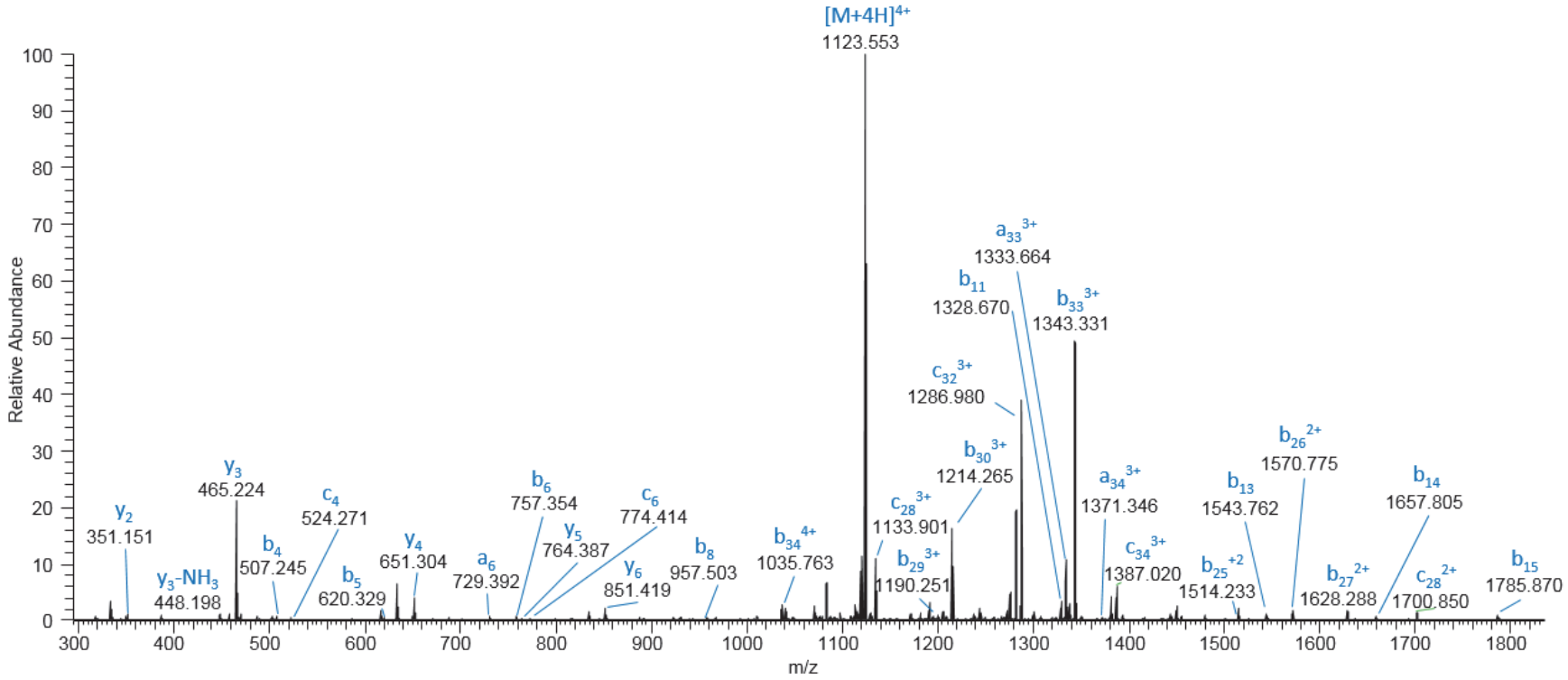


Figure 26 shows the spectrum of the UVPD fragmentation of the $[M+4H]^{4+}$ enfuvirtide sample.

Figure 26. MS^2 scan spectrum of the enfuvirtide solution (profile mode)



Acquiring Sample Data

After you optimize the API source and scan parameters for the analyte, use the Tune application or the Method Editor application to acquire sample data.

For instructions on how to set the external start instrument and use the Tune application to acquire sample data, refer to Chapter 8 in the *Orbitrap Fusion Series Getting Started Guide*.

For instructions on how to use the Method Editor application to acquire sample data, refer to the Instrument Setup and Sequence Setup topics in the Xcalibur Help.

A

Laser Safety Information

The Orbitrap Fusion Lumos MS with UVPD capability is a Class 1 laser system that complies with the 21 CFR 1040.10 requirements from the FDA. The instrument contains one 213 nm Class 4 laser head.

Contents

Laser Safety Cautions

CAUTION Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

CAUTION Risk of laser radiation. Failure to understand and comply with laser cautions and operating instructions in this guide can result in property damage or serious injuries to the user.

CAUTION Laser hazard. Only laser-safety-trained Thermo Fisher Scientific service engineers can perform laser maintenance or diagnostic tasks.

CAUTION Do not remove the laser beam protective housing. The protective housing and safety interlock system prevent access to the invisible Class 4 laser beam. Exposure to the beam can cause serious skin and eye injuries, including blindness.

Laser Safety Labels

The Orbitrap Fusion Lumos MS with the factory-installed UVPD option has the following laser safety labels affixed to the instrument in the locations indicated in Figure 27. When the UVPD option is an upgrade to the Lumos MS, your Thermo Fisher Scientific service engineer affixes the label shown in Figure 28, which is in the UVPD Upgrade Kit (P/N UVPD1-20000).

Figure 27. Location of the laser safety label on the Orbitrap Fusion Lumos MS with the UVPD option

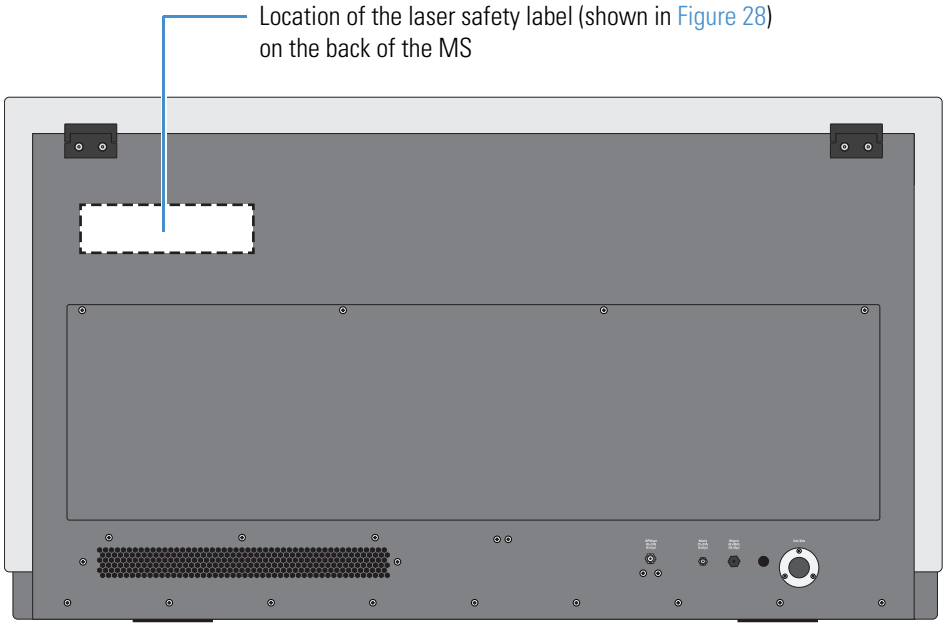


Figure 28. Laser safety label for the Orbitrap Fusion Lumos MS with the UVPD option

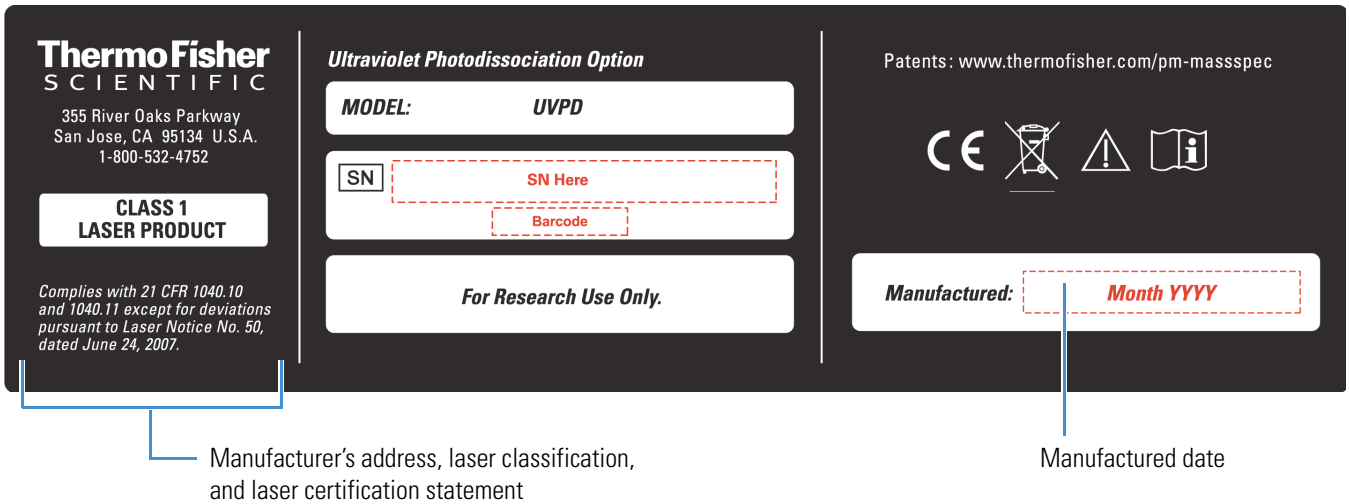
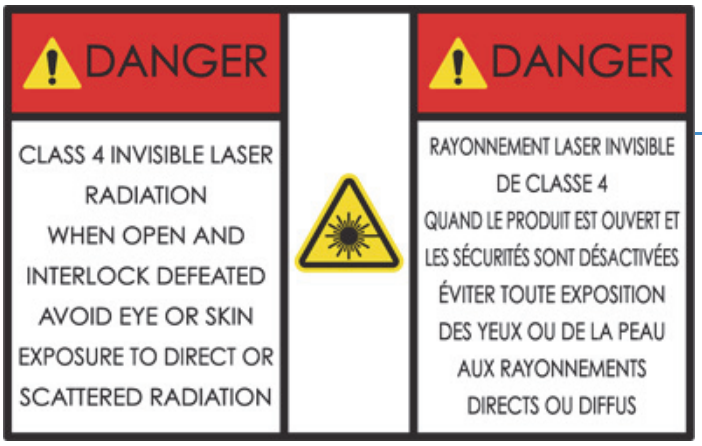


Figure 29. Internal laser safety label (on and behind the laser beam protective housing)



For label locations, see Figure 3 and Figure 5.

Figure 30. Laser head's Class 4 laser safety label

For label location, see Figure 3.

Laser Head Specifications

These are the specifications for the CryLaS™ Class 4 laser head.

Parameter	Specification
Wavelength (nm)	213
Duration (pulse width FWHM) (ns)	Less than or equal to 1.0
Maximum radiant energy (µJ/pulse)	3.0 (at 2.5 kHz)
Maximum radiant (peak) power (kW)	1.5 (at 2.5 kHz)
Laser class	4

Note The laser head is a consumable component that requires periodic service. For details, contact Thermo Fisher Scientific Technical Support.

Glossary

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

A

a ions Ions that result from the cleavage of the C-C bonds of a peptide backbone, with the N-terminal fragments retaining the charge. The three types of a ions are a, a*, and a°. The * refers to a ions that have lost an ammonia molecule (NH_3). The ° refers to a ions that have lost a water molecule (H_2O).

B

b ions Ions that result from the cleavage of the C-N bonds of a peptide backbone with the N-terminal fragments retaining the charge. The three types of b ions are b, b*, and b°. The * refers to b ions that have lost an ammonia molecule (NH_3). The ° refers to b ions that have lost a water molecule (H_2O).

C

c ions Ions that result from the cleavage of the N-C bonds of a peptide backbone with the N-terminal fragments retaining the charge.

collision-induced dissociation (CID) A method of fragmentation where molecular ions are accelerated to high kinetic energy and then allowed to collide with neutral gas molecules such as helium, nitrogen, or argon. The collisions break the bonds and fragment the ions into smaller pieces.

E

electron transfer dissociation (ETD) A method of fragmenting peptides and proteins. In ETD, singly charged reagent anions transfer an electron to multiply protonated peptides within the ion trap mass analyzer. This leads to a rich ladder of sequence ions derived from cleavage at the amide groups along the peptide backbone. Amino acid side chains and important modifications such as phosphorylation are left intact.

F

flow rate, syringe pump status The syringe pump injection flow rate in milliliters per minute (mL/min) or microliters per minute (µL/min) for the current sample, as defined in the current experiment method.

fragment ion A charged dissociation product of an ionic fragmentation. Such an ion can dissociate further to form other charged molecular or atomic species of successively lower formula weights.

H

higher energy collision-induced dissociation (HCD) Collision-induced dissociation that occurs in the ion routing multipole (IRM). The IRM consists of a straight multipole mounted inside a collision gas-filled tube. A voltage offset between the C-trap and IRM accelerates parent ions into the collision gas

inside the IRM, which causes the ions to fragment into product ions. The product ions are then sent to the ion trap or the Orbitrap™ mass analyzer for mass analysis. HCD produces triple quadrupole-like product ion mass spectra.

M

molecular ion An ion formed by the removal (positive ion) or addition (negative ion) of one or more electrons to/from a molecule without fragmentation of the molecular structure.

P

precursor ion An electrically charged molecular species that can dissociate to form fragments. The fragments can be electrically charged or neutral species. A precursor ion can be a molecular ion or an electrically charged fragment of a molecular ion.

precursor mass The mass-to-charge ratio of a precursor ion. The location of the center of a target precursor-ion peak in mass-to-charge ratio (*m/z*) units.

product ion An electrically charged fragment of an isolated precursor ion.

Q

qualitative analysis Chemical analysis designed to determine the identity of the components of a substance.

quantitative analysis Chemical analysis designed to determine the quantity or concentration of a specific substance in a sample.

R

relative standard deviation (RSD) A measure of the dispersion of a group of measurements relative to the mean of the group. Relative standard deviation is expressed as a percentage of the average value. The percent relative standard deviation is calculated as:

$$\%\text{RSD} = 100 \times (S/\bar{X})$$

where S is the standard deviation and $\bar{X}$ is the sample mean.

T

total ion current (TIC) The sum of the ion current intensities across the scan range in a mass spectrum.

U

ultraviolet photodissociation (UVPD) A method of fragmentation based on photon absorption by a precursor molecule. Photon absorption causes the molecule to undergo an electronic transition to an excited state followed by subsequent dissociation to fragment species. UVPD produces abundant fragments and is applicable to peptides, proteins, and many other compound classes.

UVPD activation time The time in milliseconds that the pulsed laser beam used for fragmentation is applied in an ion trap. In general, shorter activation time results in less fragmentation and a longer activation time results in more fragmentation.

X

x ions Ions that result from the cleavage of the C-C bonds of a peptide backbone with the C-terminal fragments retaining the charge.

Y

y ions Ions that result from the cleavage of the C-N bonds of a peptide backbone with the C-terminal fragments retaining the charge. The * refers to y ions that have lost an ammonia molecule (NH_3). The ° refers to y ions that has lost a water molecule (H_2O).

Z

z ions Ions that result from the cleavage of the N-C bonds of a peptide backbone with the C-terminal fragments retaining the charge. The * refers to z ions that have lost an ammonia molecule, or NH_3.

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