# ultrafleXtreme Series User Manual


Revision C1 (May 2021)

# Legal and Regulatory Notices



## Document History

ultrafleXtreme Series User Manual Revision C1 (May 2021)

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# Warnings, Cautions and Notes

Warnings and cautions appear in this manual. These warnings and cautions will detail the specific hazard, describe how to avoid it, and specify the possible consequences of not heeding the warning or caution. Read all warnings and cautions carefully and observe them at all times. Their format is:

**WARNING**

A ‘Warning’ message appears in the manual when failure to observe instructions or precautions could result in death or injury. Symbols depicting the nature of the specific hazard may also be placed alongside warnings.

**CAUTION** A ‘Caution’ message is used when failure to observe instructions could result in damage to equipment.

**Note** A ‘Note’ is used to give advice or additional information.

# Table of Changes

| Revision | Date | Changes | Remarks |
|---|---|---|---|
| 1 | 2009-07-01 | First Edition | |
| 2 | 2012-10-18 | Update laser information | |
| 3 | 2013-08-03 | Text and structural changes, remove reference to Twister and transponders | |

# Table of Contents

**Legal and Regulatory Notices** ... **2**
**Contact** ... **4**
**Warnings, Cautions and Notes** ... **5**
**Table of Changes** ... **5**
**Table of Contents** ... **6**
**1 ultrafleXtreme Mass Spectrometer** ... **9**
1.1 Purpose of the ultrafleXtreme ... 9
1.2 Weight and Dimensions of the ultrafleXtreme ... 9
1.3 Site Preparation Specification ... 10
1.4 Safety ... 10
1.4.1 General Safety Instructions ... 10
1.4.2 Safety Symbols ... 11
1.4.3 Operating Precautions ... 12
1.4.4 Electrical Safety ... 13
**2 Installation and Setup** ... **15**
2.1 Facility and Electrical Requirements ... 15
2.2 Setup ... 16
**3 Instrument Layout** ... **19**
3.1 System Components ... 19
3.2 Schematic of the Mass Spectrometer ... 19
3.2.1 Instrument Controller ... 20
3.2.2 Rear Panel ... 22
3.2.3 Vacuum System ... 25
3.2.4 Scout MTP Ion Source ... 25
3.2.4.1 "Panoramic" PAN™ Mass Resolution ... 26
3.2.5 TOF/TOF Analyzer ... 28
3.2.5.1 Precursor Ion Selector (PCIS) ... 28
3.2.5.2 Understanding TOF MS/MS ... 29
3.2.5.3 How Does LIFT Work? ... 31
3.2.5.4 Reflector ... 34
3.2.5.5 Detector ... 35

3.2.5.6 Digitizer ... 37
3.2.5.7 Bruker smartbeam-II™ Laser System ... 37
3.2.5.8 Video CCD Camera ... 38
3.3 Target Plates ... 39
3.3.1 SCOUT MALDI MTP Target ... 39
3.3.2 AnchorChip Target ... 39
3.4 PC Configuration ... 40
**4 Remote Service Capability ... 41**
**5 Operation ... 43**
5.1 Turning the Instrument On and Off ... 43
5.2 Checking the Instrument for Operational Readiness ... 43
5.3 Controlling the Instrument During Analytical Operations ... 43
5.4 Moving Targets In and Out with the Manual Cartridge ... 43
**6 Instrument Maintenance ... 49**
6.1 Maintenance Intervals ... 49
6.2 Ventilation Air Filter ... 49
6.3 Roughing Pump ... 50
6.4 Source Cleaning ... 50
**7 Index ... 51**

# 1 ultrafleXtreme Mass Spectrometer

## 1.1 Purpose of the ultrafleXtreme

The ultrafleXtreme is a MALDI (Matrix Assisted Laser Desorption/Ionization) tandem mass spectrometer specially designed for automated MS and MS/MS high-throughput identification of proteins and peptides. Tandem Mass Spectrometry is a technique that utilizes more than one mass selective stage in a mass spectrometer.

The most common form in practice is a two-stage arrangement to record MS/ MS or $(MS)^2$ spectra.

The incorporated LIFT device allows full fragment ion spectra to be acquired within a single scan and replaces traditional measurements of segmented spectra with stepwise-reduced reflector potentials.

## 1.2 Weight and Dimensions of the ultrafleXtreme

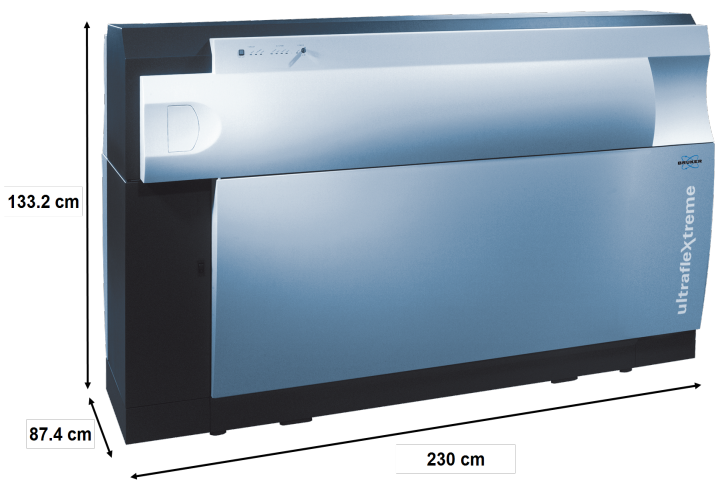


**Figure 1-1 Dimensions of an ultrafleXtreme instrument (L x W x H = 230 cm x 133.2 cm x 87.4 cm; Weight 550 kg)**

## 1.3 Site Preparation Specification

Before starting the installation of the instrument the site must be properly prepared. Please refer to the Site Preparation Specification document that is sent to all customers before the instrument is shipped.

It contains information regarding the device requirements, such as operating environment, gas supply, power, exhaust, venting, grounding, etc.

This document must be verified and returned with your signature to Bruker before a service representative can start the installation.

## 1.4 Safety

Safety considerations for the ultrafleXtreme spectrometer include:

- General safety instructions (see section 1.4.1)
- ultrafleXtreme safety symbols (see section 1.4.2)
- Operating precautions (see section 1.4.3)
- Electrical safety (see section 1.4.4)

### 1.4.1 General Safety Instructions

Disconnect mains electrical supply before removing any covers from the instrument as defined in accordance with the DIN EN 61010-1 Safety Standard. This instrument poses no electric shock hazard during normal use. All service and maintenance of this equipment must only be conducted by personnel who have undergone training and certification by Bruker Daltonics GmbH & Co. KG..

The instrument casing and panels protect the operator and others from hazards including high voltages and laser radiation. If the panels are to be removed for any purpose, the main electrical supply cable to the instrument must first be fully disconnected from its socket.

Bruker engineers are trained in the specific procedures necessary to operate the equipment safely with the covers removed.

Before starting the installation, pay attention to these important safety instructions!

## 1.4.2 Safety Symbols

The following symbols may be found on or near various components of the mass spectrometer (see Table 1-1):

**Table 1-1 Safety Symbols**

| Symbol | Description |
|---|---|
|  | Indicates the danger of electric shock due to the presence of high voltage, if precautions are not followed. |
|  | Indicates that laser light may be present.<br><br>The ultrafleXtreme is a Class I Laser product with the safety cover closed.<br><br>Only Bruker-trained and -certified personnel are permitted to service or maintain the instrument with the covers removed |
|  | Indicates that there is a risk of danger. This can be any type of hazard. When this symbol is observed, refer to the safety pages in the manual for further information. |
|  | Indicates that a terminal either receives or delivers alternating current or voltage. |
|  | Indicates that a protective grounding terminal must be connected to earth ground before any other electrical connections are made to the instrument. |
|  | Indicates the OFF position of the main power switch. |
|  | Indicates the ON position of the main power switch. |

## 1.4.3 Operating Precautions

To protect yourself from harm and prevent system malfunction, observe the following guidelines:

- Before using the instrument, read all safety instructions contained in this manual.
- Wear appropriate protective clothing, including safety glasses and gloves, when preparing samples and solutions for use with this instrument.
- Follow the correct safety procedure and the manufacturer's recommendations when using solvents.
- Clean the exterior surfaces of the instrument with a soft cloth dampened with a mild detergent and water solution. Do not use abrasive cleaners or solvents.
- The ultrafleXtreme mass spectrometer weighs 550 kg / 1212 lb. Exercise caution, wear appropriate clothing, and use appropriate equipment when moving the instrument.

**CAUTION** When shipping or moving the instrument, it is critical that the target is in the OUT position. Otherwise, the instrument will be damaged.

**CAUTION** Do not restrict ventilation air intake at the rear of the instrument or the exhaust at the back of the instrument.

To ensure proper operation, check the ventilation air filter at the rear of the instrument every three months and replace if necessary (see section 6).

## 1.4.4 Electrical Safety

The following electrical safety considerations should be taken into account:

- General Safety: Before installing or operating the ultrafleXtreme mass spectrometer, read the following information concerning hazards and potential hazards. Ensure that anyone involved with installation and operation of the instrument is knowledgeable in both general safety practices for the laboratory and safety practices for the ultrafleXtreme mass spectrometer. Seek advice from your safety engineer, industrial hygienist, environmental engineer, or safety manager before installing and using the instrument.
- Position the ultrafleXtreme mass spectrometer in a clean area that is free of dust, smoke, vibration, and corrosive fumes, out of direct sunlight, and away from heating units, cooling units, and ducts.
- Verify that there is an adequate and stable power source for all system components.
- Verify that the power cord is the correct one for your laboratory and that it is a nationally approved power cord set.

**WARNING**

**Connecting an instrument to a power source that is not equipped with a protective earth contact creates a shock hazard for the operator and can damage the instrument. Likewise, interrupting the protective conductor inside or outside the instrument or disconnecting the protective earth terminal creates a shock hazard for the operator and can damage the instrument.**

**WARNING**

**The instrument must be disconnected from its power source before any cover is removed or it is opened.**

**WARNING**

**All connections of the instrument must be used as intended. The instrument should only be used with the wires and cables delivered with the system or otherwise provided by the manufacturer.**

**WARNING**

**DO NOT attempt to make adjustments, replacements or repairs to this instrument.**

**Only Bruker Daltonics Service Representatives or similarly trained and authorized personnel are permitted to service the instrument.**

**WARNING**

**When it is likely that the electrical protection of the ultrafleXtreme mass spectrometer has been impaired:**

1. **Switch off the ultrafleXtreme mass spectrometer.**
2. **Disconnect the line cord from the electrical outlet.**
3. **Secure the instrument against any unauthorized operation.**

**WARNING**

**The ultrafleXtreme mass spectrometer and MALDI- TOF analyses use very high voltages. Under normal operation, the instrument requires NO user access to the inner components of the instrument.**

**NEVER OPERATE the ultrafleXtreme mass spectrometer with the protective cover removed as this exposes the user to risk of severe electrical shock and harmful, invisible, ultraviolet and infrared laser radiation.**

# 2 Installation and Setup

**Note** Please refer to the Bruker Site Preparation Specification document for your mass spectrometer.

Installation and setup consists of:

- Facility and Electrical Requirements (see section 2.1).
- Setup (see section 2.2).

## 2.1 Facility and Electrical Requirements

The facility must provide:

- 208 to 230 V, 50/60 Hz, 2000 VA. The instrument back panel is fitted with an IEC60320–C14 mains inlet (see Figure 3-3). The instrument is supplied with a 3 m long IEC60320 line cord and a mains plug, suitable for use in your country.
- The ultrafleXtreme mass spectrometer requires approximately 2 $m^2$ of space on a surface that can safely support the full 550 kg weight of the instrument.
- To ensure proper ventilation and convenient access to the connections and main switch, maintain at least 80 cm of clearance behind the instrument.

**WARNING**

The main electrical supply must provide adequate grounding.

The system has an exhaust port to allow venting. This port is located on the rear of the instrument. Individual facilities may have safety guidelines that specify ventilation requirements. It is the responsibility of the user to adhere to the requirements of their respective facility.

## 2.2 Setup

**►► To set up the instrument**

1. At the rear of the instrument, plug the following cables into the corresponding sockets (see Figure 3-3).
   - AC line cord — **Mains** inlet
   - Remote control cable — **Control > Remote Control** socket
   - Reflector detector— **Acquisition > Det Ref** socket
   - Linear detector — **Acquisition > Det Lin** socket
   - Trigger — **Acquisition > Trigger** socket
   - Digitizer:
     - Plug the digitizer synchronization cable into the **Acquisition > Digitizer Sync** socket (digitizer card in PC)
     - Plug the supplied 3 m connecting cable into the **USB** port (digitizer in instrument, see Figure 2-1)

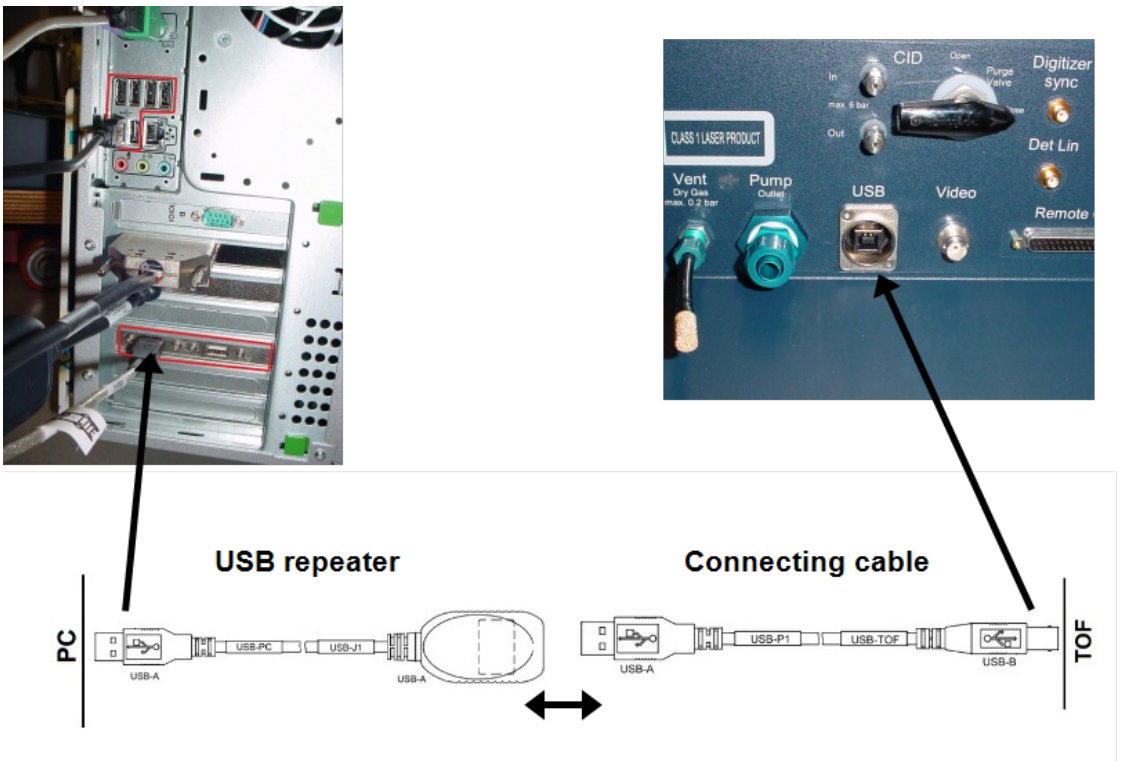


**Figure 2-1 Connecting the internal digitizer to the PC using USB cables**

2. Insert the plug of the AC line cord into a suitable AC outlet.

**CAUTION** Read and follow all electrical and safety precautions (see section 1.4)

3. Plug the data system end of the signal cables into the appropriate socket on the rear of the PC (see Figure 2-2).

   **Note** If the internal digitizer is installed in your system, connect the cable from the USB port of the instrument to a free USB port at the rear of the PC using the supplied 5 m USB repeater cable (see Figure 2-1).

4. Plug the data system end of the remote control cable into the COM-1 port on the PC.

5. Switch on the instrument using the AC mains switch at the rear of the instrument.

6. Make sure that the **POWER > MAINS** LED is lit (see Figure 3-2).

After switching on the instrument for the first time, it may take as long as 12 hours before the instrument is ready for operation. The **SYSTEM > READY** LED is lit when the instrument is ready for operation (see Figure 3-2).

**Note** If any faults occur within the system, the **SYSTEM > ERROR** LED (see Figure 3- 2) is lit and the system enters standby mode. Contact your authorized service personnel for help.


**Figure 2-2 Connections at the rear of the PC**
**1 Digitizer Sync (Handshake), MCX connector**
**2 Input 2 – Reflector, SMA connector**
**3 Input 1 – Linear, SMA connector**
**4 Trigger, MCX connector**

# 3 Instrument Layout

## 3.1 System Components

Bruker flex systems, such as the ultrafleXtreme, have two main components:

1. Mass spectrometer (see section 3.2)
2. Data system (PC) (see section 3.2.1).

## 3.2 Schematic of the Mass Spectrometer

Figure 3-1 shows the working principle of the ultrafleXtreme mass spectrometer.

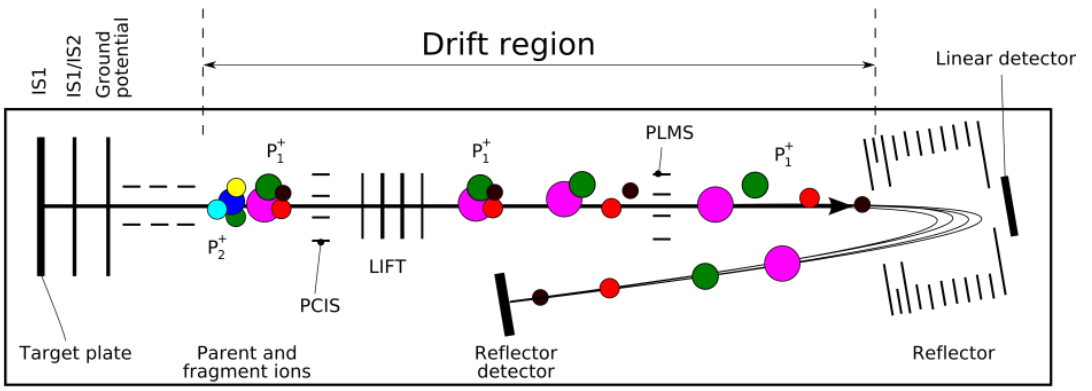


**Figure 3-1 Schematic of the instrument**

## 3.2.1 Instrument Controller

When the instrument is ready for operation, the three green LEDs **MAINS**, **READY** and **ACCESS** are lit (see Figure 3-2).

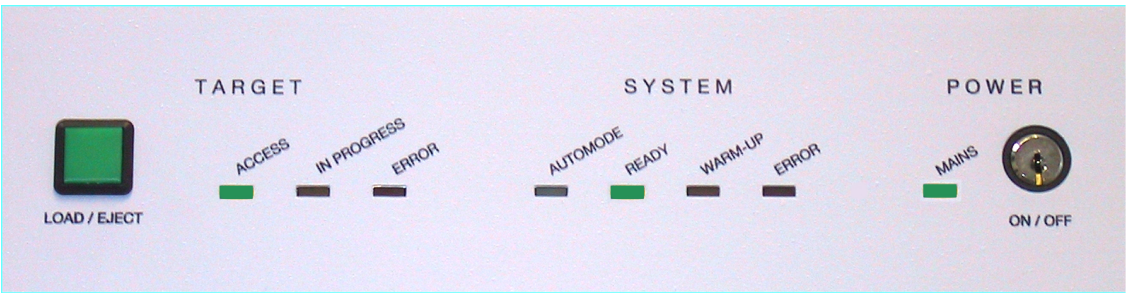


**Figure 3-2 Control panel at the front of the instrument**

1. **LOAD / EJECT**: Moves the target in or out.
2. **TARGET** LEDs
   a. **ACCESS**
      - The **TARGET > ACCESS** LED is lit when the tray is either inside or outside the instrument in the end position.
      - The **TARGET > ACCESS** LED is switched off while the target is moving in or out the source.
   b. **IN PROGRESS**: Lit while the docking procedure is in progress (**TARGET LOAD/EJECT**).
   c. **ERROR**:
      - The **TARGET > ERROR** LED is lit when there is malfunction that requires intervention of the user or a Bruker service technician. Error messages are displayed in flexControl software.
      - The **TARGET > ERROR** LED flashes (together with **SYSTEM > ERROR**) during Service mode or a firmware update.

3. **SYSTEM** LEDs

   a. **AUTOMODE**: Lit while an AutoXecute run is in progress. During an automatic run, the LOAD/EJECT button cannot be used to eject the target.

   b. **READY**:

   - When the **READY** LED is lit, the instrument is ready to acquire spectra; that is, all interlocks are closed, $N_2$ pressure is correct, there is a sufficient vacuum, HV is present, and so on.
   - The **READY** LED is switched off during the docking procedure.

   c. **WARM-UP**:

   - The **WARM-UP** LED is lit during the warm-up phase of the integrated laser device and when the system is not ready to acquire spectra; that is, the docking procedure is still in progress, HV is off, and so on. The readiness of the system to acquire spectra is displayed in the flexControl software.
   - The **WARM-UP** LED flashes when a non-critical malfunction has occurred that can be resolved by the user. Such situations include low $N_2$ pressure, an open interlock, and so on.

   d. **ERROR**:

   - The **SYSTEM > ERROR** LED is lit when a malfunction that requires the intervention of the user or a service technician has occurred. ToolTips in flexControl will display information about the malfunction.
   - The **SYSTEM > ERROR** LED flashes (together with **TARGET > ERROR**) during Service mode or a firmware update.

4. **POWER**

   a. **MAINS**: The **POWER > MAINS** LED is lit when the instrument is switched on using the AC mains switch at the rear of the instrument.

   b. **ON / OFF**: Turning the key switch to the **OFF** position vents the instrument.

### 3.2.2 Rear Panel

The following interfaces are at the rear of the instrument (see Figure 3-3):

**CID**

- **Purge Valve > Open/Close**: Switch for flushing the CID (Collision Induced Dissociation) cell after changing the collision gas.
- **In** — Inlet for collision gas (argon or helium; maximum pressure 6 bar) and flushing the collision cell.
- **Out** — Outlet for collision gas and flushing the collision cell.

**Gas Supply**

- **Slide Valve Compressed Air** — Compressed air connection for operating the slide valve. Alternatively, this port can be connected to an external $N_2$ supply.
- **Vent Dry Gas** — Gas inlet for venting the instrument.

**Acquisition**

- **Digitizer Sync** — MCX connector for digitizer synchronization cable.
- **Trigger** — MCX connector for trigger cable.
- **Det Lin** — SMA connector for linear detector cable.
- **Det Ref** — SMA connector for reflector detector cable.

**Control**

- **Video** — BNC connector to carry the video signal of the sample spot to a video capture card mounted on the PC.
- **Aux** — Serial port to connect to auxiliary equipment (not used).
- **Remote Control** — Serial port to connect the PC (HOST).
- **USB** — USB port to connect internal digitizer (where applicable).

### Pump

- **Outlet** — Vacuum system exhaust 13 mm (1/2 inch) Ø plastic tubing (Tygon), with a filter mounted inside. Individual facilities may have safety guidelines that specify ventilation requirements. It is the responsibility of the user to adhere to the requirements of their respective facility.

### Service Only

- Key switch for service and maintenance. Only to be used by personnel trained and certified by Bruker.

### Mains

- The instrument is fitted with an IEC60320-C14 mains inlet. Instrument power is controlled using the mains switch:
- 0 = OFF (vents the instrument)
- I = ON

There is an air intake with a filter behind the cover at the rear of the instrument. Replace the air filter every three months. Keep the fan area free of obstructions to ensure a free flow of cooling air.

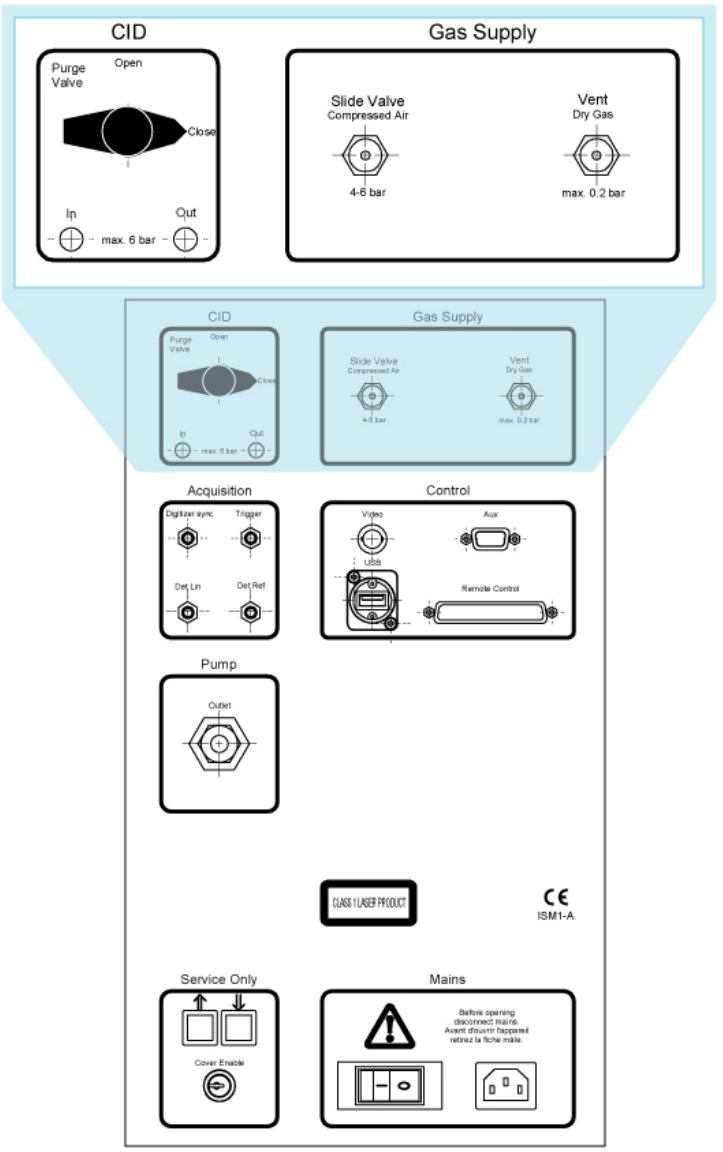


**Figure 3-3 The interface panel at the rear of the instrument**

### 3.2.3 Vacuum System

The vacuum system of the ultrafleXtreme consists of two high-vacuum and three rough-vacuum regions. The vacuum lock of the SCOUT MTP ion source and the exhaust of both turbomolecular pumps are coupled and guided via valves to the vacuum side of the roughing pump.

Depending on the system status — for example, when changing a target plate — each of the rough vacuum lines can be closed separately by computer-controlled valves. The input of the turbomolecular pumps is attached directly to the high-vacuum regions of the ion source housing and the ion flight tube, respectively.

The instrument operates at a high-vacuum of about $1 \times 10^{-6}$ mbar or better. The rough-vacuum pressure ranges from atmospheric pressure to around 3 mbar.

### 3.2.4 Scout MTP Ion Source

The Scout MTP ion source (see Figure 3-4) is the part of the mass spectrometer where ions are formed using the MALDI technique. The source consists of three main components.

1. The x-y-table accommodates the target plate, transports it into the ion source, and moves the target along x-y-coordinates, according to the selected shot position.

2. The vacuum lock transfers the target from atmospheric pressure to the high vacuum region.

3. The ion optic consists of the positively or negatively charged MTP target plate (P1); a second voltage plate (P2) for time-lag focusing; and a grounded acceleration electrode.

   When the laser hits the analyte/matrix mixture the ions formed are accelerated by the delayed application of an electrical field and focused by a lens system before they leave the source.

CID is an acronym for Collision Induced Dissociation. This term stands for the procedure when molecules decay by collisions during a passage through a particular cell that is filled with gas, for example, argon. CID has proved to be useful to enhance intensities of fragments in the low fragment mass range.

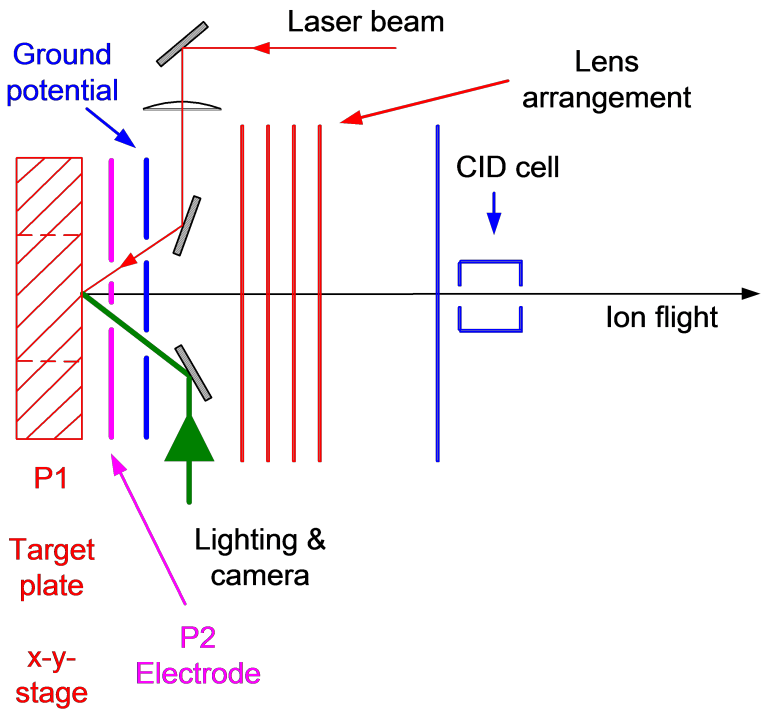


**Figure 3-4 The flex series time-lag focusing ion source**

### 3.2.4.1 "Panoramic" PAN™ Mass Resolution

An important factor in the resolution of a MALDI experiment is the energy distribution of the ions created during the ionization process. This distribution arises because not all analytes are desorbed and ionized at exactly the same time and the same location. Repulsive electrical forces also contribute to the initial energy distribution of the ions.

These differences in energy mean that ions of the same mass leave the source with a range of kinetic energies, leading to them being detected at slightly different time points. This leads to a broadening of peak widths and a reduction in resolving power.

PAN is a technique for enhancing both resolution and sensitivity of a TOF mass spectrometer.

After desorption, ions are submitted to delayed extraction (phase 2 and 3 discussed below). This method benefits from:

- A softer acceleration process, because ions do not pass the dense plume of matrix molecules
- A time-lag focusing effect (provided that the correct parameters are applied).

Three components of the ion source are involved in the PAN process:

1. The target plate (P1), on which the analyte is deposited.
2. The second voltage plate (P2), an electrode mounted opposite to and some millimeters apart from the target plate.
3. The subsequent grounded acceleration electrode (see Figure 3-1 and Figure 3-4).

PAN can be broken down into three phases.

**Phase 1**

Both the target plate (P1) carrying the analyte and the second voltage plate (P2) are held at a potential IS/1. This means that the analyte is not exposed to any external effects until the laser shot, which represents the transition into the second phase.

**Phase 2**

In this phase, molecules and ions are set in motion by laser ionization/desorption. The analyte is ionized and ions move with a typical velocity of 700 m/s from P1 towards P2.

At this point, the ions are still not exposed to any external effects: their kinetic energy derives from the MALDI process. During the next few hundred nanoseconds the analyte moves further towards P2.

However, not all ions start with the initial velocity of 700 m/s. Some ions with identical masses fly faster than others, and in a given time, travel further. This velocity distribution decreases the resolution of a TOF mass spectrometer in linear mode.

**Phase 3**

In this phase, potential P2 is pulsed down from IS/1 to IS/2, generating an electrical field whose strength forces all charged particles to move towards P2.

This means that the fast, more energetic ions that were able to fly further towards the P2 plate in phase 2 are exposed to a lower electrical potential than the slower ions that are closer to P1.

This results in the slower ions receiving a bigger "boost" than the faster ions, and consequently they will fly faster in the field-free region, allowing them to "catch up" with the faster ions at the detector (if the correct potential gradient is applied between P1 and P2).

## 3.2.5 TOF/TOF Analyzer

A special feature of the ultrafleXtreme analyzer is the LIFT device located in the flight tube, which allows acquisition of a full fragment ion spectrum in a single scan.

### 3.2.5.1 Precursor Ion Selector (PCIS)

The **Precursor Ion Selector** (PCIS) shown in Figure 3-5 works like a mass filter to separate a particular precursor ion and related fragments from all other ions for MS/MS analysis.

The PCIS consists of vertical layers of deflector plates. Consecutive electrodes are coupled to a high-voltage supply with alternating polarity, according to the Bradbury-Nielsen principle. The potential difference between the plates generates an electrostatic field perpendicular to the direction of ion flight, which deflects all the ions entering this electrode arrangement.

Just at the moment when the selected ions enter the deflecting field, the field is switched off. The potential between the deflector plates is kept at zero until the ions leave the deflector.

After the selected ions have left the deflector, the deflecting field is switched on again, with a reversed polarity. This mode compensates for the partial deflection that occurs in the stray field areas at the front of the electrodes. This technique enables extraordinarily short selection times to be applied, which contributes to improved resolving power.

The ultrafleXtreme is equipped with a second deflection unit: the **PLMS** (**P**ost **L**ift **M**etastable **S**uppressor) located between the LIFT device and the reflector. This assembly can be used to remove fragment ions that are formed after the LIFT procedure.

As a result, only the fragments formed between the source and the LIFT continue their journey to the reflector, where they are separated by mass.

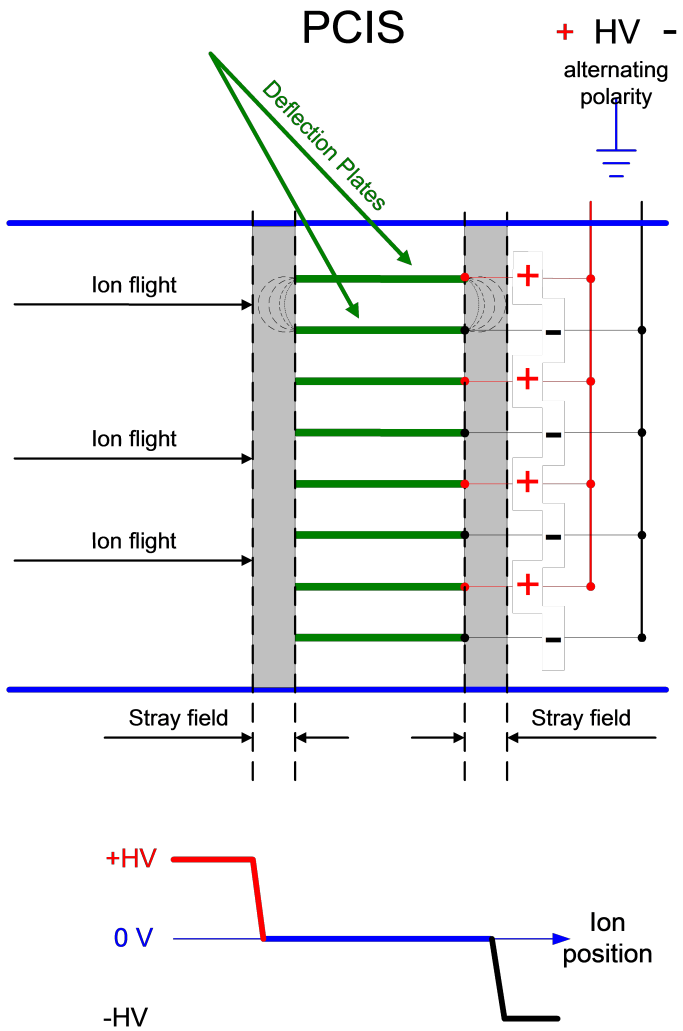


**Figure 3-5 Operation principle of the precursor ion selector**

### 3.2.5.2 Understanding TOF MS/MS

Because the kinetic energy release is only a few eV, molecules fragment after acceleration in the source. This means that fragments have a lower kinetic energy than precursors
($E_{kin} \propto$ m).

The gridless reflector in the ultrafleXtreme can focus ions whose kinetic energies differ by around 30%; for example, from the precursor to fragments with around 70% of the precursor's mass. Less energetic ions do not hit the detector.

This means that to obtain a full MS/MS spectrum, the reflector voltage must be stepped down, as is this case in segmented PSD or FAST (see Figure 3-6).

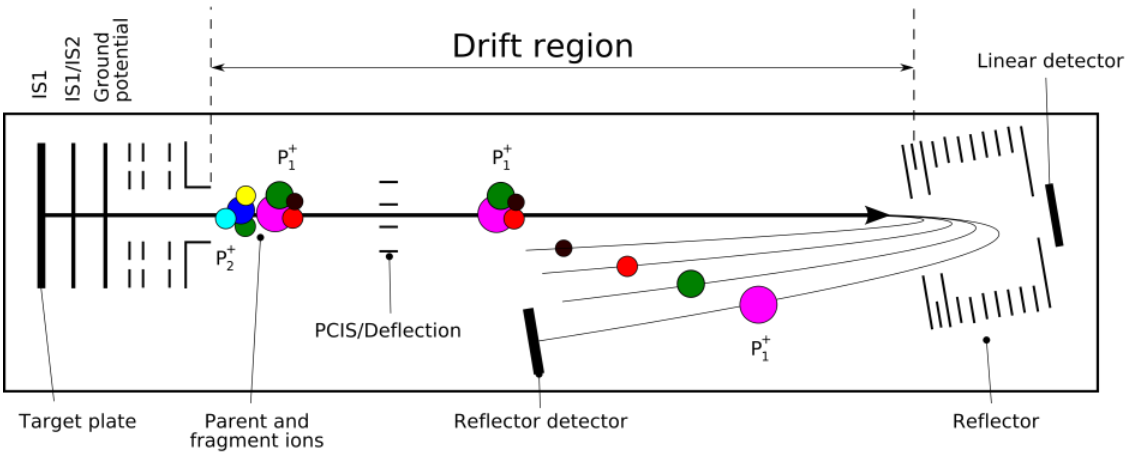


**Figure 3-6 Principle of a conventional PSD measurement**

### 3.2.5.2.1 Bruker LIFT Technology

The basic idea of LIFT is to raise the kinetic energies of precursor ($P_s^+$) and fragment ions ($F^+$) to a level where the energy difference between the precursor and smallest fragment does not exceed 30% (see Figure 3-7). In such cases, all fragment ions can be simultaneously detected with the precursor ion.

The **Precursor Ion Selector** (PCIS) (see section 3.2.5.1) is able to pick a precursor with its associated fragments out of a mixture, such as a digest. Because they have the same velocity, fragments that are produced before arriving at the PCIS assembly pass the unit together with the precursor.

If switched on, the **Post Lift Metastable Suppressor** (PLMS) deflects the precursor together with those metastable fragments that are formed after LIFT (see Figure 3-1 and Figure 3-7). Switching off the PLMS enables detection of the precursor, for example, for calibration purposes.

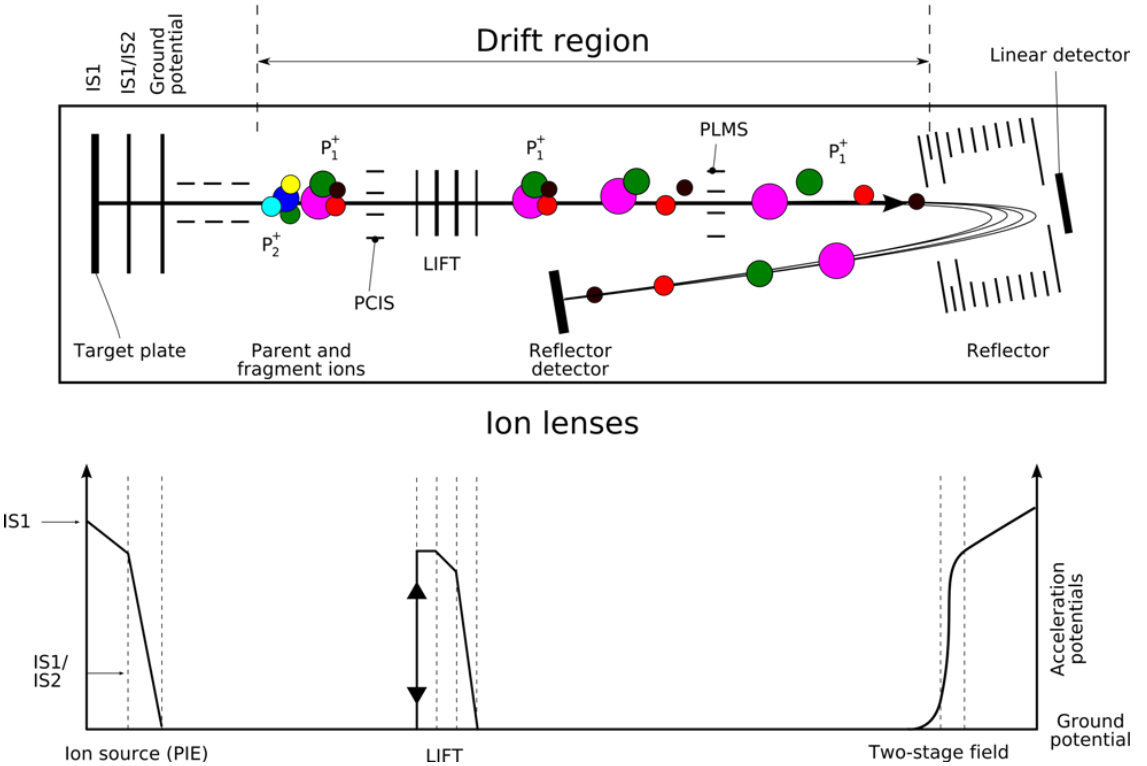


**Figure 3-7 Working principle of Bruker LIFT technology**

### 3.2.5.3 How Does LIFT Work?

The LIFT assembly, which is located just behind the PCIS, consists of an arrangement of four electrodes forming three chambers (see Figure 3-8):

- LIFT chamber
- Pulsed Acceleration Chamber
- Acceleration Stage

Electrodes 1 and 2 are always connected in parallel, forming a cell that shields ions from all external effects. Therefore, this cell can be regarded as a Faraday cage.

Ions leave the ion source with an energy of around 8 keV. When the precursor and related fragments enter the LIFT chamber, the four electrodes have the following potentials:

1. Electrode 1 and 2 are connected to ground.
2. Electrode 3 is connected to 19 kV.
3. Electrode 4 is always connected to ground.

While the ions move inside the first cell, the electrical potential of electrodes 1 and 2 will be raised from ground to around 19 kV. Because there is no voltage difference and no field between the two electrodes, the ions' motion is not influenced in any way.

The ions continue moving as before, but at this increased electrical potential. This jump in potential is necessary for phase 4 to take effect.

During phase 3, ions move from chamber 1 to chamber 2 at the raised potential.

Phase 4 begins by pulsing down the electrical potential of electrode 3 by 2–3 kV while precursor and related fragments are still inside the pulsed acceleration stage. This stage acts as a first (delayed) acceleration stage.

When ions enter the acceleration stage between electrodes 3 and 4, they are further accelerated by the remaining voltage difference. This pulsed extraction resembles PAN (see section 3.2.4.1) and also makes use of **SVCF** (**S**pace-**V**elocity **C**orrelation **F**ocusing).

This two-stage acceleration by a potential difference of 19 kV increases the kinetic energies of precursor and fragments to the extent that they are efficiently reflected and directed onto the reflector detector.

***Example*** After acceleration by LIFT, the energy of the precursor ion is raised to 27 keV (8 keV + 19 keV). A fragment with a molecular weight $^{1}/_{20}$ of the precursor ion would have an energy of 19.4 keV ($^{8keV}/_{20}$ + 19 keV). This is around 28% less energy than the precursor, allowing this fragment ion to be focused together with the precursor ion onto the detector.

In this way, post-acceleration by LIFT may be considered a segmented MALDI FAST measurement using only one voltage segment to record a full fragment spectrum.

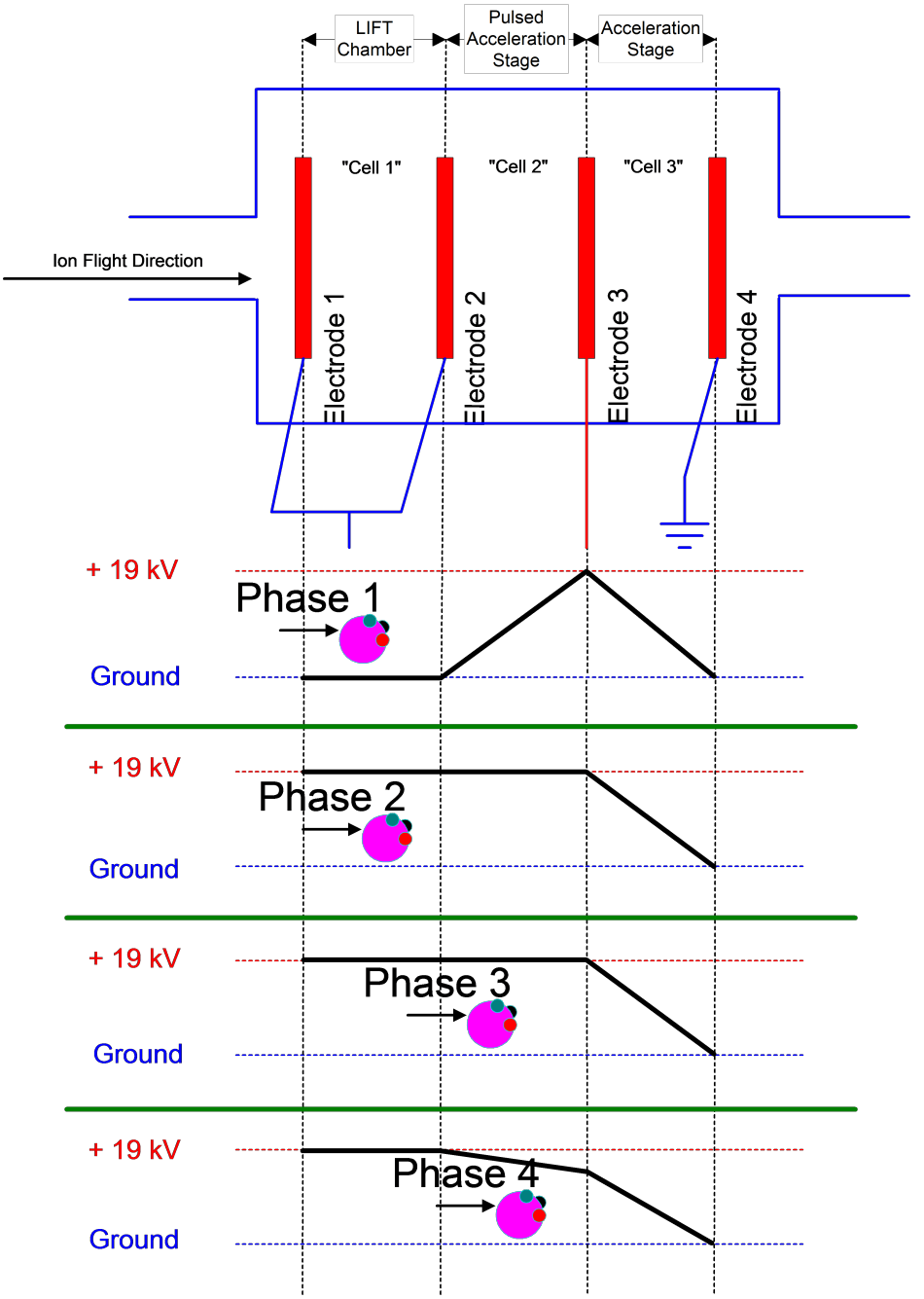


**Figure 3-8 Potentials during the LIFT process on the respective electrodes**

### 3.2.5.4 Reflector

In a MALDI TOF/TOF instrument, the main task of a reflector is to compensate flight times of ions with different energies.

Metastable decay of ions is caused by an energy excess occurring during the complex MALDI process. Because precursors and fragments continue traveling with the same velocity, they hit the linear detector simultaneously, producing a single peak rather than a fragment spectrum (see Figure 3-10).

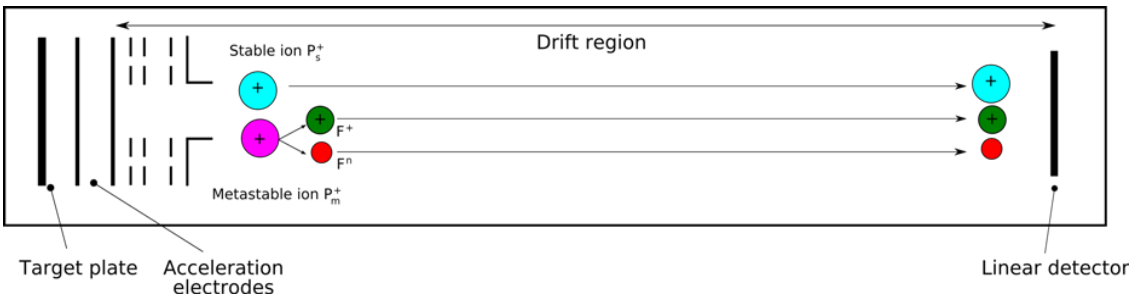


**Figure 3-9 Precursors and fragment ions formed by metastable decay reach the linear detector simultaneously**

For fragment mass separation, a reflector can be incorporated into the mass spectrometer to separate metastable ions. Ions of different masses have different kinetic energies and penetrate to different depths into the electrical field before they are reflected to strike the reflector detector at different times.

To obtain good mass spectra with a reasonable signal-to-noise ratio, the geometry of a reflector must fulfil specific electrical and size requirements. These requirements are mainly concerned with the dimensions of the flight tube, and the type and size of the reflector detector.

The ultrafleXtreme and other Bruker “flex”-series TOF mass spectrometers use a gridless reflector with ion lenses (see Figure 3-10).

Fragments that form inside the reflector instead of during their flight in the drift region represent an undesirable high chemical background. To minimize residence time of ions inside the reflector, a double stage design is used, with the first stage generating a stronger field.

As a result, the reflector is shorter than a single-stage TOF reflector design and the free drift field length is longer. This construction also deflects all the smaller fragment ions

that would otherwise contribute to the background noise. In addition, the gridless entrance lens of the reflector creates a **space focusing** effect, which increases the sensitivity.

The reflector operates at a potential that exceeds the acceleration voltage of the ion source by about two kV.

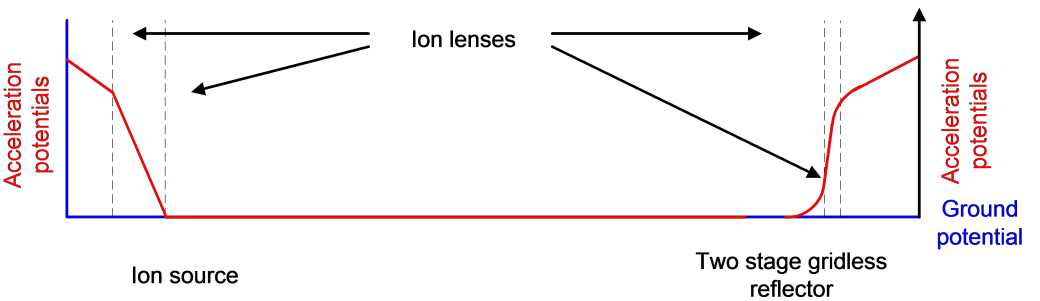


**Figure 3-10 Potential distribution in a two-stage gridless reflector**

### 3.2.5.5 Detector

The detector within a mass spectrometer converts an ion current into an electrical current, which is then digitized and delivered to the PC.

The ultrafleXtreme is equipped with the latest detector technology, which creates sub-nanosecond electrical pulses per ion. The ions arrive at an extremely flat surface and produce secondary electrons, meaning that several electrons are emitted from the metal impact surface per impact.

These electrons are guided and accelerated towards another surface by the superposition of electrical and magnetic fields. Here again, several secondary ions are generated per impacting electron.

These events are repeated many times downstream of a channeltron-like amplification curve that ends in a collector anode. This anode guides the accumulated charge towards the digitizer, where it creates a voltage across a 50 Ohm termination resistor. This voltage is converted to digital values at a rate of up to 4 billion times per second (4 GS/s) and these values are stored on the hard drive. The detector principle is illustrated in Figure 3-11.

In normal operation, a bias of 2000 - 4500 is applied to the detector typically starting with 2300 V and increasing throughout the ageing of the unit.

A further performance parameter is the time response of the detector, which is important for the prevention of deterioration of the peak resolution. Although microchannel plate detectors deliver an output voltage with a rise time of less than 1 ns, the uncertainty in the impact surface, that is, the penetration depth into the channels, widens the average peak at the exit of the detector. The flat and perfectly oriented impact surface of the FlashDetector eliminates this uncertainty, resulting in the same narrow pulse for both individual events as well as for additive signals.

The linear detector uses the same principle but its parameters are optimized to minimize detector saturation. This leads to a reduced time response, but provides the excellent sensitivity that is required for large molecules like proteins, polymers and complex ions.

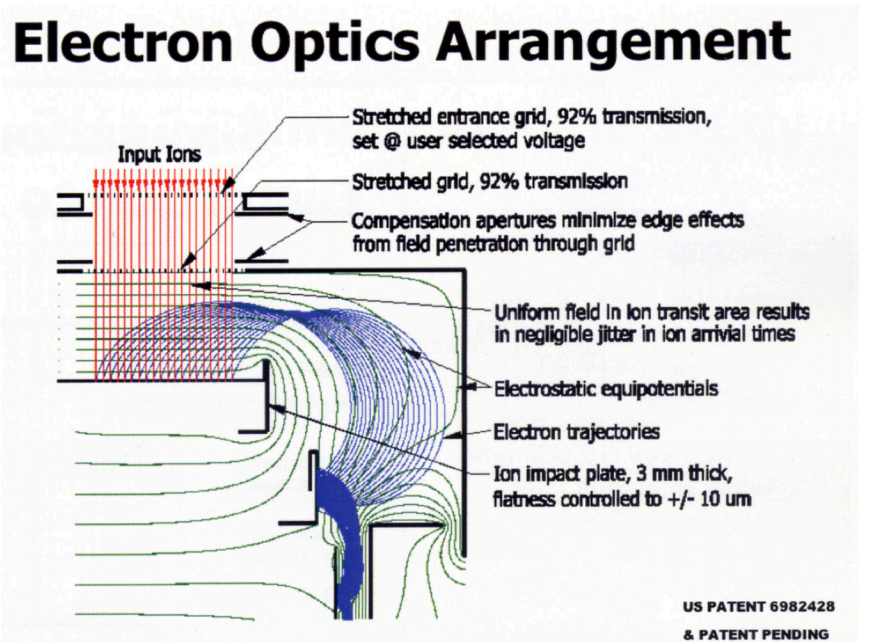


**Figure 3-11 Principle of an electron multiplier detector** [1]

[1] *Reference: SGE Analytical Science, Ringwood (Victoria), Australia*

### 3.2.5.6 Digitizer

Following the laser shot, the digitizer records the incoming analog signals from the detectors and converts them into digital information. The digitizer card can achieve a sample rate of up to 4 GS/sec.

The digitizer assembly is installed in the PC or the instrument. Instrument settings to control the digitizer are found on the **Detector** page in flexControl.

### 3.2.5.7 Bruker smartbeam-II™ Laser System

The ultrafleXtreme laser system consists of a pulsed smartbeam-II UV laser with an attenuator that allows fine adjustment of the laser fluence, a lens system to focus the laser beam and a mirror system to direct the beam into the ion source and onto the target plate.

The laser light can be pulsed at a frequency of (1000 x 1/*n*) Hz (where *n* is any integer) onto a small spot on the target. The laser settings are defined in flexControl.

The smartbeam-II laser features Bruker proprietary spatial beam modulation, simplifying the operation of the laser. There are five predefined shot patterns that provide optimized results for different applications. The "minimum" pattern allows MALDI imaging at a resolution in the range of 10 µm.

**WARNING**

**This instrument contains a Laser class 4 product option. The enclosure surrounding the instrument is designed to protect the user from indirect exposure to the invisible radiation. Operating the instrument with opened covers can expose the user to harmful laser radiation, which may result in blindness.**

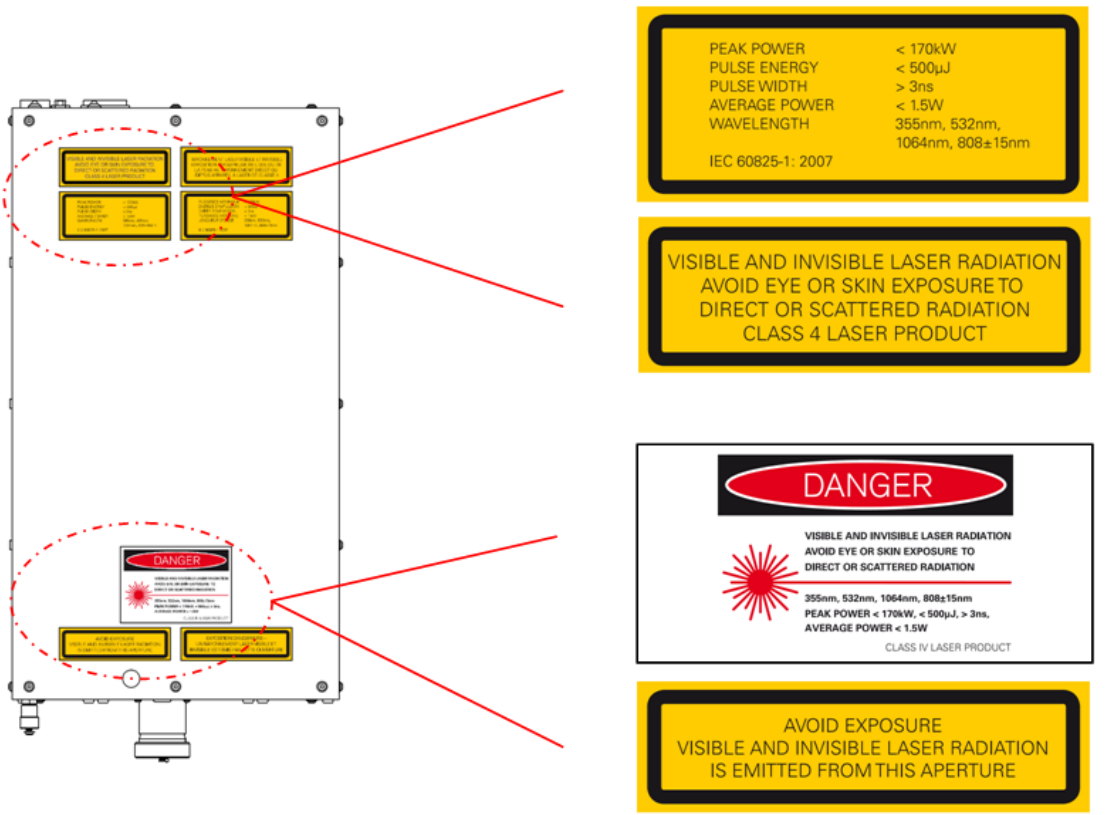


**Figure 3-12 Labels on the smartbeam-II laser inside the instrument**

For further information about the smartbeam-II laser system, please refer to Holle, A; Haase, A; Kayser, M; & Höhndorf, J. *Optimizing UV laser focus profiles for improved MALDI performance* , Journal of Mass Spectrometry, Vol **41** , Issue 6. doi:10.1002/jms.1041

### 3.2.5.8 Video CCD Camera

The camera delivers an image of the sample spot to the Target Manipulation Segment of the Graphical User Interface.

# 3.3 Target Plates

## 3.3.1 SCOUT MALDI MTP Target

Bruker SCOUT MALDI MTP targets (see Figure 3-13) meet the "quasi standard" of 384-well plates in regard to shape and spot localization and fit any liquid- or plate-handler.

A video CCD camera mounted inside the instrument and a video capture card installed in the PC provide the operator with high-resolution images of the sample.

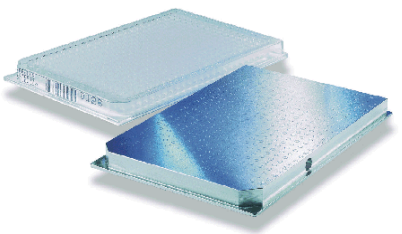

**Figure 3-13 A microwell plate and a SCOUT MALDI MTP target**

## 3.3.2 AnchorChip Target

AnchorChip targets are SCOUT MALDI MTP targets with a special surface coating that localizes and improves the homogeneity of the crystallization process of MALDI samples. This technique benefits from:

- 10 – 100 fold improvement in sensitivity due to increased analyte concentration.
- improved automation that minimizes searches for "sweet spots".

For more information, see the AnchorChip Instructions for Use documents.

## 3.4 PC Configuration

The PC controls the mass spectrometer and acquires and stores data. The following components make up the data system:

- LCD display, min. resolution 1280 × 1024, True Color
- Microsoft® Windows® operating system
- Compass for flex Series software package
- Laser printer

# 4 Remote Service Capability

To maximize operating time, the ultrafleXtreme has a remote service capability (see Figure 4-1). This feature allows troubleshooting over the Internet with the customer PC being fully controlled by the Bruker Daltonics Service Hotline. Software and firmware updates can also be performed using this feature.

In case of instrument malfunction, the overall service process is accelerated by enabling the service engineer to arrive on site with the appropriate spare parts after remote diagnosis.

**Note** The ultrafleXtreme control PC must have Internet access to use the remote service capability.

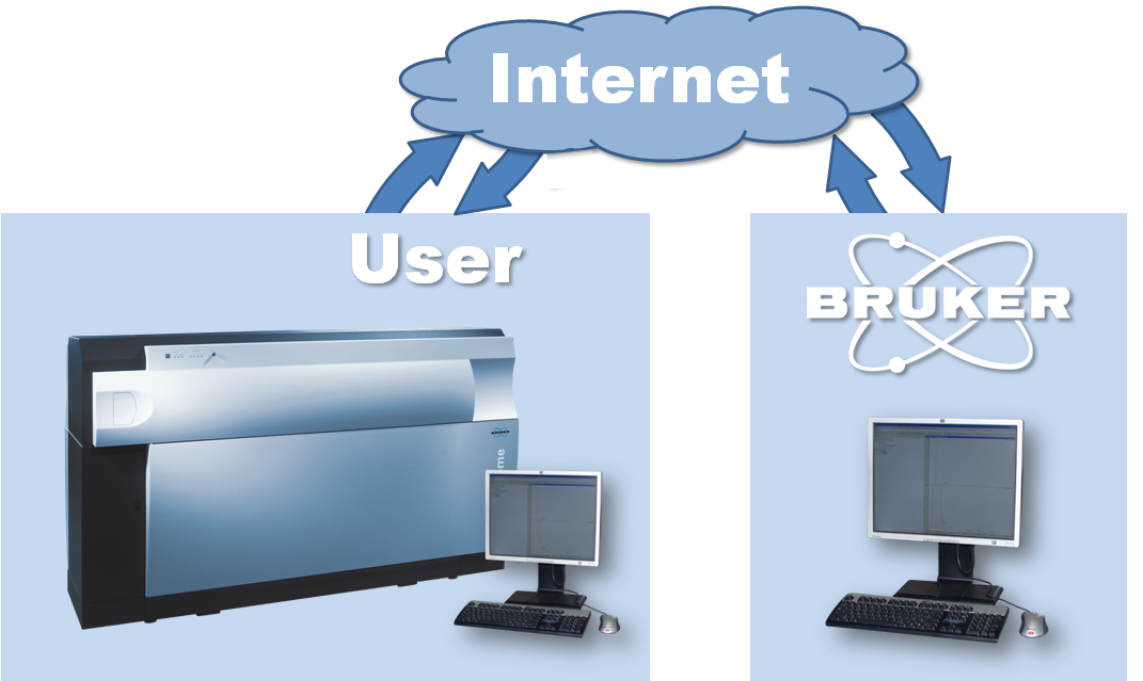


**Figure 4-1 Operating principle of the remote service**

**►► To request a remote service session**

- Send an e-mail to maldi.support@bdal.de providing the following information:
  - Your name and location.
  - The serial number of your instrument.
  - Version numbers of software used to run the instrument and process MALDI data. The version number can be found by opening the software and selecting **Help > About**.

Bruker will contact you as soon as possible by e-mail and provide instructions and a Web link for a remote service session.

**Note** The connection between Bruker and the customer's PC is automatically severed when Internet Explorer is closed.

# 5 Operation

## 5.1 Turning the Instrument On and Off

Instrument power is controlled using the mains switch (see Figure 3-3), where:

- 0 = Off.
- I = On.

The front panel **POWER > MAINS** LED (see Figure 3-2) is lit when the instrument is connected to the power supply and ready for operation.

## 5.2 Checking the Instrument for Operational Readiness

The front panel **SYSTEM > READY** LED (see Figure 3-2) is lit when the instrument is ready for acquisition.

If the **SYSTEM > READY** LED is not lit, either the target is in the OUT position or the vacuum inside the source is not sufficient for proper operation.

## 5.3 Controlling the Instrument During Analytical Operations

Controlling the mass spectrometer during analytical operations is described in the *flexControl* and *flexAnalysis User Manuals*.

## 5.4 Moving Targets In and Out with the Manual Cartridge

**►► To change a target in the manual cartridge**

1. Make sure that the **TARGET > ACCESS** LED is lit on the instrument front panel.
2. Press the **PUSH** button on the cover lid (see Figure 5-1).


**Figure 5-1 Closed cover lid of the manual cartridge with PUSH button**

2. Wait until the cover lid has reached the lower position (see Figure 5-2).

   Keep fingers well away from the pinch point.

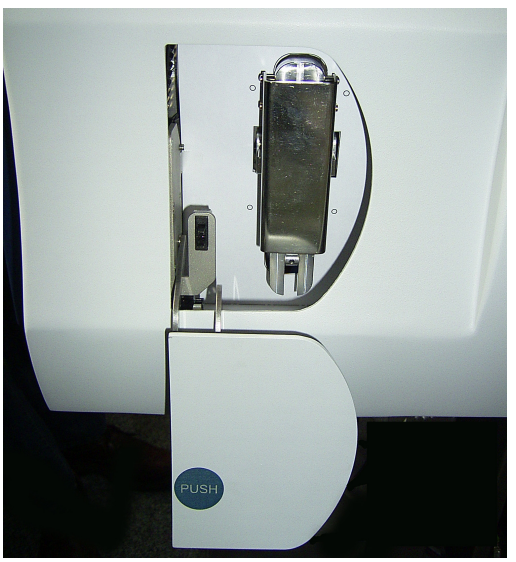


**Figure 5-2 Open cover lid giving free access to the manual cartridge**

3. Open the manual cartridge by pulling the lever (see Figure 5-3) upward and outward with a finger.

**Figure 5-3 Opening the cover plate of the manual cartridge**

4. Pull the lever forward until it reaches the horizontal position (see Figure 5-4 and Figure 5-5).

**Figure 5-4 Manual cartridge during opening**

**Figure 5-5 Manual cartridge completely opened**

5. Pull out the target out from the target slide.

**CAUTION** Take care not to drop anything into the load port. The presence of debris in or around the load port can adversely affect instrument operation.

6. Place the new target onto the target slide and push it into the load port until it reaches the end position.

   **IMPORTANT** Make sure that the target is inserted in the correct orientation, with the sample surface facing right and the cut off corner at the bottom of the front edge.

7. Push the lever up until the cartridge cover closes.
8. Push the lever down until it locks into its end position.
8. Lift the cover lid and push it closed.
9. Press the **LOAD/EJECT** button to move the target into the measurement position inside the vacuum system.
10. Wait for the **READY** LED to illuminate before beginning acquisition.

    The time between pressing **LOAD/EJECT** and the **READY** LED being lit should not exceed five minutes and is typically less than two and a half minutes.

# 6 Instrument Maintenance

**Note** Maintenance and service procedures that require removal of the safety covers on ultrafleXtreme instruments must be performed by Bruker-trained and - certified personnel. Contact your local Bruker office for advice on regular maintenance.

## 6.1 Maintenance Intervals

| Component | Maintenance frequency | | | |
|---|---|---|---|---|
| | Monthly | 3-monthly | Yearly | 2-yearly |
| Check intake filter, fan grids | | ● | | |
| Exchange intake filter, fan grids | | | ● | |
| Exchange diaphragm of optional diaphragm pump | | | | ● |
| Check ion source (P2 plate) | ● | | | |

## 6.2 Ventilation Air Filter

Inspect the ventilation air filter every three months. If the filter is visibly clogged with dust, it must be replaced to ensure proper instrument function.

Replace the filter by lifting the old filter up and out of the holder and sliding in a new one.

**Note** Replace only with a genuine Bruker filter, # 8073121.

For service, please see section "Contact" on page 4.

## 6.3 Roughing Pump

Maintenance is required if there is a dramatic change in performance of the pump.

**►► Dismounting the ultrafleXtreme membrane pump for maintenance**

1. Switch off the instrument using the **POWER** key switch.

   Turning the key switch to the **OFF** position vents the instrument.

2. Wait 20 minutes to ensure that the vacuum system is completely vented.

3. Activate the cover switches using the **Cover Enable** key switch at the lower left of the interface panel at the rear of the instrument.

4. Raise the cover using the ⇧ cover switch.

5. Switch off the instrument and disconnect the mains plug from the mains electrical supply.

6. Using a spanner, release the 2 latches on the end door of the instrument.

7. Undo the 4 finger nuts on the rough-pump housing.

8. Remove tubing and fixing bolts.

9. Remove membrane pump and perform maintenance in accordance with the manufacturer's instructions.

**►► Remounting the ultrafleXtreme membrane pump**

- After maintenance, reverse the dismounting procedure to remount the membrane pump.

## 6.4 Source Cleaning

The ultrafleXtreme Source Cleaning Kit (# 8705193) is available for cleaning the instrument source.

# 7 Index


## A

Analytical operations 43

Analyzer 28

AnchorChip 39

## B

Backpanel of the PC 17

## C

Camera 38

Checking for instrument operational readiness 43

CID 25

Copyright 2

## D

Deflector voltage/matrix suppression 36

Detectors 35

Digitizer 37

## E

Electrical safety 13

## F

Facility and electrical requirements 15

## G

Gas supply 10

Grounding 10

## I

Installation and setup 15

Instrument controller 20

Instrument layout 19

Instrument maintenance 49

## L

Laser device 37

Laser system 37

LIFT 31

## M

MALDI 9

Moving targets in 43

## O

Operating environment 10

Operating precautions 12

## P

PCIS 28

PLMS 28

Power 10

Purpose of the instrument 9

## S

Safety instructions 10

Safety symbols 11

Scheme of the ion source 25

Setup 16

Site preparation specification 10

## T

Table of changes 5

Tandem mass spectrometry 9

Target plates 39

Targets 39

Turning the instrument ON or OFF 43

## V

Vacuum system 25

Ventilation air filter 49

Venting 10