MALDI-TOF/TOF mass spectrometer system: user information

Mass Spectrometry Research Facility, Department of Chemistry

Instrument description:

Innovative MALDI-TOF and TOF/TOF technology designed and optimized to be easy-to-use; robust systems for both traditional MALDI applications (reliable and detailed protein/peptide characterization, and polymer analysis) as well as cutting edge work (high resolution MALDI Tissue Imaging, glycan analysis. Autoflex MALDI-TOF/TOF instrument works in linear only mode, high resolution reflectron mode and the latest TOF/TOF technology (LIFT™) which enables the use of various MS/MS techniques (e.g. LID, high energy CID, ISD) for fast and sensitive MS/MS experiments.

Instrument location:

Basement MS (00.097)

Typical applications:

Small molecule analysis, Intact mass determination of proteins (up to 150kDa), Peptide Mass Fingerprint (PMF), In Source Decay (ISD) analysis, Oligonucleotide analysis, Polymer characterization

1. Any user of **Bruker Autoflex Speed MALDI-TOF/TOF** instrument has to undertake a training session on it before admitted to its use. Unauthorized use of the instrument is not allowed.
   - If you require MALDI training with the aim of mass/stoichiometry confirmation for small synthetic molecules, metal-organic complexes, polymers, peptides or intact (modified) proteins, you should contact **Victor Mikhailov (victor.mikhailov@chem.ox.ac.uk).**
   - If you require MALDI training for tryptic digests, Top-Down or Bio-tools software analysis you should contact **Elisabete Pires (Elisabete.pires@chem.ox.ac.uk).**
2. Before you run your experiments on Bruker AutoFlex, please make sure that you prepare your samples on a Bruker MALDI plate in your laboratory, as we normally do not provide lab space for sample preparation. Please make also sure that your MALDI spots are thin and well-

dried before you put your MALDI plate in the instrument: **wet MALDI spots prevent the mass spectrometer from reaching the necessary high vacuum condition and can also damage the instrument.** Mind that different solvents take different time to dry, with aqueous spots often taking up to ½ hour. You also need to have in consideration the type of matrix and plate to use according to your sample. Please see **Appendix 1** for MALDI matrices, plates and sample preparation We also recommend that you have several spots with appropriate standards on your plate to mass-calibrate your spectra when required (details can be discussed at your training).

3. Please remember to book a time slot on **Chemistry Instrument Booking System** before you bring your samples in.

4. When at the instrument and before you start with your experiments, please make sure that you have a data file folder under your name for your data files and flexControl method files. **You are only allowed to use and modify method files from your personal folder, not the default ones**. If you have to use a new method file, please copy a default one most closely related to your requirements (see below) from **Bruker\Methods\FlexControl methods\19 July 2019** to your personal folder before downloading and modifying it in flexControl software.

5. Choose the method file based on **a)** ion polarity (positive or negative), **b)** mode of time-of-flight detection (linear or reflectron), **c)** mass range. Linear methods (their names start with 'LP' or 'LN' for positive or negative ions, respectively) provide high mass range, but relatively poor resolution. Reflectron methods (names starting with 'LP' or 'LP'), provide high resolution (monoisotopic for m/z <3500), but lower mass range. Mass range of the default methods can be adjusted after they have been downloaded **from your personal folder**, and then saved under a different name. Please see **Appendix 2** for more information on MALDI process and MS methods.

6. Mass calibration in flexControl is applied to the current method. If you change the method after calibration, you may need to calibrate the new method. Default MALDI methods are checked/ calibrated weekly by MS Facility staff, but you are only allowed to calibrate the methods downloaded from your personal folder.

7. MALDI plate has to be put and fixed on the relevant Bruker plate holder, and the whole assembly should then be put on the movable MALDI plate carrier stage, that goes inside the instrument (Please see **Appendix 3**). **Please make sure that you take your plate off the holder and leave the holder on the desk by the instrument, and also sent the MALDI plate carrier back in the airlock inside the instrument after you finish your work.**

8. Data files are not automatically saved to an external server. **You are required to back up your data files yourself,** e.g. on Mass Spectrometry (Q:) \\ 2020 Data \Group Folder\Personal Folder.

Appendix 1. MALDI matrices, plates and sample preparation

Commonly used MALDI matrices

The analyte incorporation with a suitable matrix is the first step of the MALDI process and extremely important to optimize sensitivity and stability for analysis. There is no formula for calculating which matrix is best and a certain amount of trial and error is recommended, particularly for novel analytes. However, certain matrices tend to work well for certain compound classes:

Matrix abbreviation	Matrix Full name	Sample Application	Target type
9-AA	9-aminoacridine	sugars, amino acids, short peptides, small acids, sulphated sugars, metabolites	Polished Steel /Ground Steel
G	graphene	amino acids, polyamines, anticancer drugs, nucleosides, steroids, small molecules, peptides, polymers, lipids	Polished Steel
HCCA	α-cyano-4-hydroxycinnamic acid	pharmaceuticals, agrochemicals, peptides, proteins, lipids, drugs	Polished Steel /Ground Steel /AnchorChip (800µm)
2,5-DHB	2,5-dihydroxybenzoic acid	sugars, lipids, peptides, proteins, drugs	Polished Steel /Ground Steel /AnchorChip (800µm)
SA	hydroxycinnamic acid)	proteins	Polished Steel /Ground Steel
3-HPA	3-hydroxypicolinic acid	peptides, oligonucleotides, proteins	Polished Steel /Ground Steel /AnchorChip small (400µm)
ATT	6-aza-2-thiothymine	oligonucleotides	Polished Steel /Ground Steel /AnchorChip small (400µm)
sDHB	super-DHB	Top-down proteins	Ground Steel /AnchorChip (800µm)
DCTB	trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-prope	polythiophenes and other electroconjugated polymers	Polished Steel /Ground Steel
FU	FULLERENE	Organometalic compounds	Polished Steel /Ground Steel
2,5-DHAP	2',5'-Dihydroxyacetophenone	PROTEINS	Ground Steel

Table 1 - Matrices, sample application and target type information

Note: Additional information about matrices/sample preparation can be found on the MS website: https://massspec.web.ox.ac.uk/files/brukermaldisamplepreparationpdf

Appendix 2. MALDI process and mass spectrometry

1. Time-of-Flight Mass Spectrometry for Matrix Assisted Laser Desorption/Ionisation (MALDI)

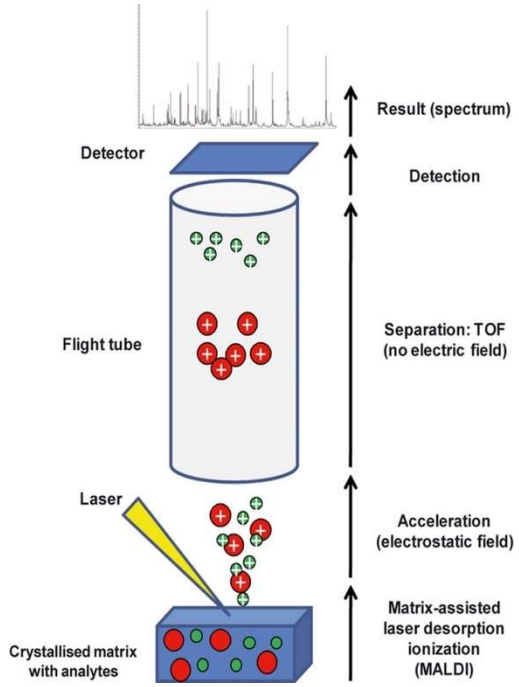


Figure 1 - Schematic of MALDI-TOF MS

- MALDI spot is ablated by the beam of a pulsed UV laser.
- Analyte ions are instantly produced by desorption and proton transfer between matrix and analyte molecules.
- Ions are accelerated by HV electrostatic field. Lighter ions are accelerated to higher velocities than heavier ones.
- Ions then fly in a field-free region and separate in space according to their velocities.
- Lighter ions arrive at the detector earlier than heavier ions thus mass separation is achieved on the time-of-flight scale.

2. Overview of MALDI analyzer -Time of Flight (TOF)

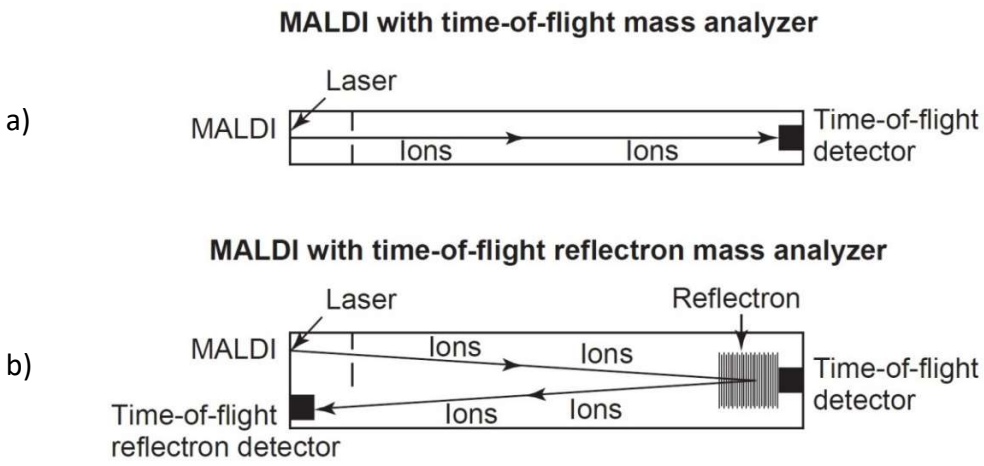


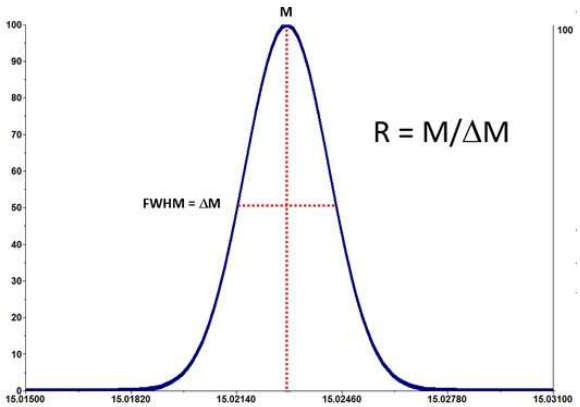
Figure 2 - General schematic for TOF analyzer. (a) Linear TOF analyzer; (b) Reflector TOF analyzer

As shown in Figure 2, the basic principle of TOF is that ions of different m/z are dispersed in time during their flight along a field-free drift path of known length. Provided that all the ions start their journey at the same time or at least within a sufficiently short time interval, the lighter ones will arrive earlier at the detector than the heavier ones.

(a) Linear TOF analyzer vs (b) reflectron TOF analyzer

Theoretically, all the ions are given the same initial kinetic energy, so that after drifting along the field free region, the ions of the same m/z at the detector at the time. However, in practice, the pulse is not felt by all ions to the same intensity and so not all the ions of the same m/z values reach their ideal velocities. To correct this problem, a reflection is often applied to the end of the drift zone. The reflectron consists of a series of ring electrodes with high voltage, which can repulse the ions back along the flight tube usually at a slightly displaced angle. Ions of different kinetic energy penetrate the reflectron to different depths before they get expelled from the reflectron into the opposite direction. Faster ions carrying more kinetic energy will travel a longer path than slower ones, and thus spend more time within the reflectron than slower ions carrying less energetic. In that way, the detector receives ions of the same mass at (about) the same time. Thereby, this design for TOF mass analyzer has increased their resolution significantly. However, reflectron TOF analyzer is not suitable for analytes that are not stable enough to survive the electric field.

3. Mass-resolution



Resolution R defines, how well two peaks are separated from each other:

$$R=m/\Delta m$$

Δm is usually derived from a mass peak´s full width at half maximum (FWHM), as shown in Figure 5.

Figure 3 - Calculation of resolution

4. MALDI process: role of the matrix

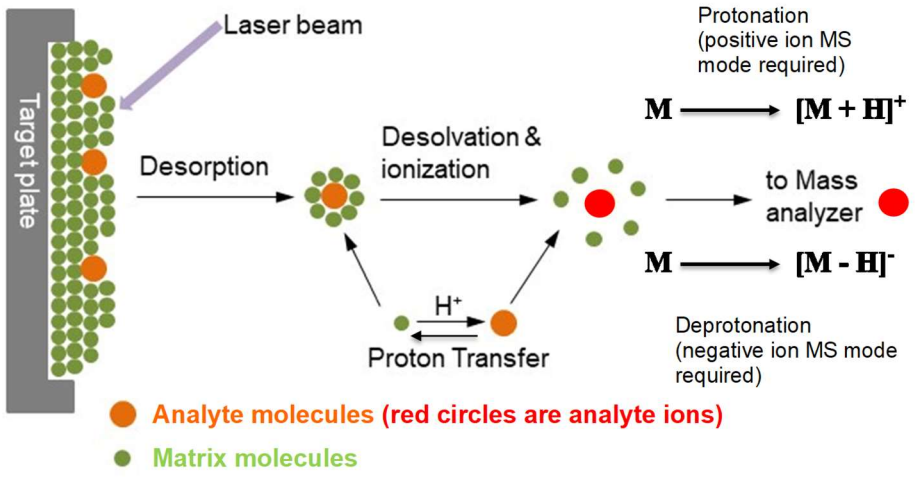


Figure 4 - MALDI process: desorption and proton transfer.

- Matrix molecules are in far excess of analyte molecules in the dried spot. Matrix shield analyte molecules from the UV radiation, so analyte molecules stay intact, without much dissociation happening.
- Matrix molecules desorb from the surface under laser ablation. Thermal energy transfer drives desorption of the analyte molecules embedded in the matrix.
- Proton transfer reactions take place between the matrix and analyte molecules producing even-electron ions of the analyte: $M+H^+$ or $[M-H]^-$ ions. (Note: for matrices involving metal cations, *e.g*. Na^+, $M+Na^+$ ions can also be observed. Metal-organic complex may also produce radical M^+ ions upon MALDI).

Appendix 3. Bruker plate holder and MALDI plate carrier stage

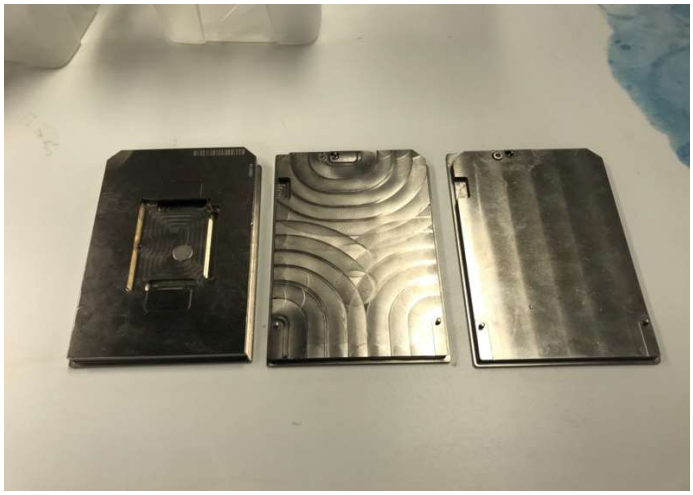


Figure 5 - Bruker plate holder

Figure 6 - MALDI plate carrier stage