MALDI STANDARD OPERATION PROTOCOL

This is a guide to using the MALDI-TOF for those who have received training. If you have any questions or problems whilst using this instrument please contact a member of the Mass Spectrometry Facility Staff.

Figure 1-1 MALDI micro MX External View

Note: all users must obtain training from a member of the mass spectrometry staff before using the MALDI mass spectrometer

Booking time on the MALDI:

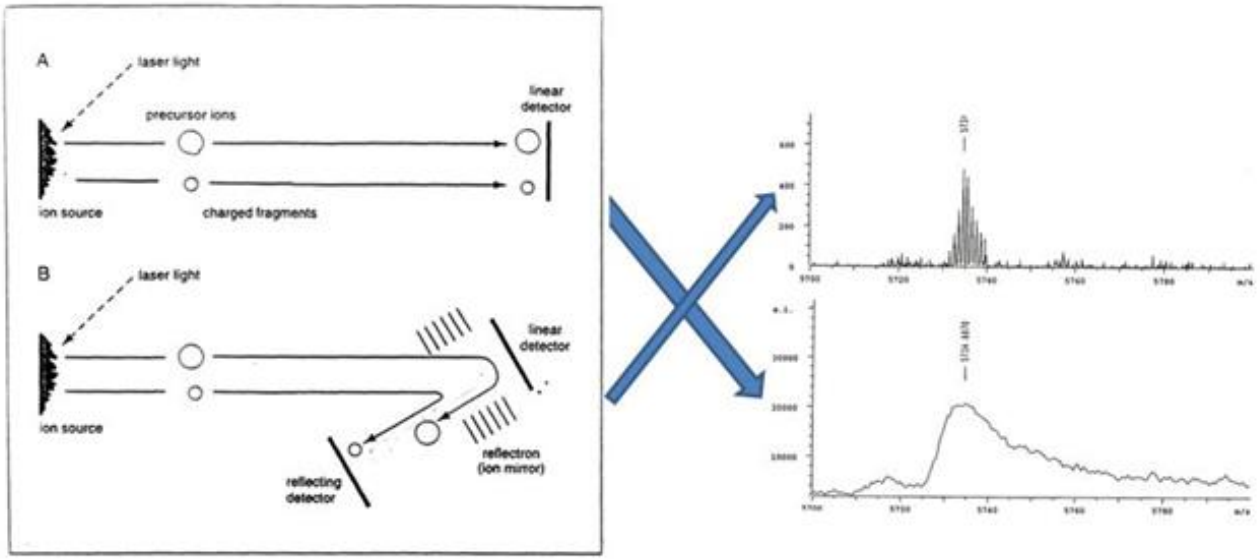
The MALDI is used much less frequently so there is no booking procedure. Just turn up and use. Please fill in in the log book: date - name - supervisor - mode used - number of samples. It is in a blue folder beside the MALDI.

Introduction

The Waters MALDI micro MX can be operated in Linear or Reflectrons modes. Linear covers the whole mass range of the instrument (0-500 kDa) but has a low resolution (approximately 1,000) whereas

Reflectron gives much higher resolution (approximately (12,000) but the mass range is restricted (0 -15 kDa). Whilst both positive and negative ionisation can be used most sample types are analysed in positive mode. Note: the use of matrices in sample preparation usually raises the effective starting mass range from 500 Da.

(a) Linear versus (b) Reflectron mode



Sample preparation:

The method used to prepare the sample, and the specific matrix to use will depend upon the sample characteristics. Chapter 6 (pp83-102) in the Operators Manual which is kept by the instrument, gives a description of the common matrices, their preparation, the type of compounds suitable for each matrix and preparation of standards. Typically the sample should be at a concentration of 1- 100 pmol/µL and the matrix at 1 mg/mL. Ideally both the sample and matrix should be prepared in the same solvent which should evaporate easily at room temperature. The sample spots must be completely dry before the plate is put into the instrument to reduce the vacuum pump down time and the risk of acing which the high voltage is applied to the extraction grid. Make sure the well on the MALDI plate where you are going to put your sample is clean.

How to wash the MALDI plate:

- Wash in hot water/light detergent using Q-Tip to remove previous spots
- Sonicate in beaker of Methanol for about 10 minutes
- Sonicate in beaker of Acetone for about 10 minutes
- Air Dry or Bake o/n at 60-100˚C to prevent spots spreading on plate

Alternative sample preparation:-

- Dissolve your sample (preferably in methanol, ethanol, isopropanol, chloroform, dichloromethane, water) - although for this you can use any solvent (bearing in mind it needs to evaporate off on the plate). I would go at least 0.2-0.5 mg/mL - you only need 1 to 5 µL.
- Into an Eppendorf, add 1- 5 µL of your sample and 5 µL of matrix
- Vortex thoroughly to mix well.
- Take 5 µL of the mixed sample and very carefully drop a spot into the centre of a well on the

plate (making a note of where you have put it). The wells are in bunches of fives - your sample needs to go in one of the outer four wells

- If you are running a calibration sample as well, again mix the calibrant (usually ACTH) with matrix and place in the centre well of the five where your sample is.
- Allow solvent to evaporate - preferably air-dry (on top of an oven) or in the oven if you are confident the sample will not degrade.
- Once dry, the plate is ready to go.

Instrument Operation

1. On the panel at the front of the MALDI itself, press unload (orange light should flash). This will unload the plate inside and takes a minute.

2. Once the orange light stops flashing (and the plate inside is completely visible through the window), press hard on the open button in the centre. This will flip the lid. Remove the plate inside.

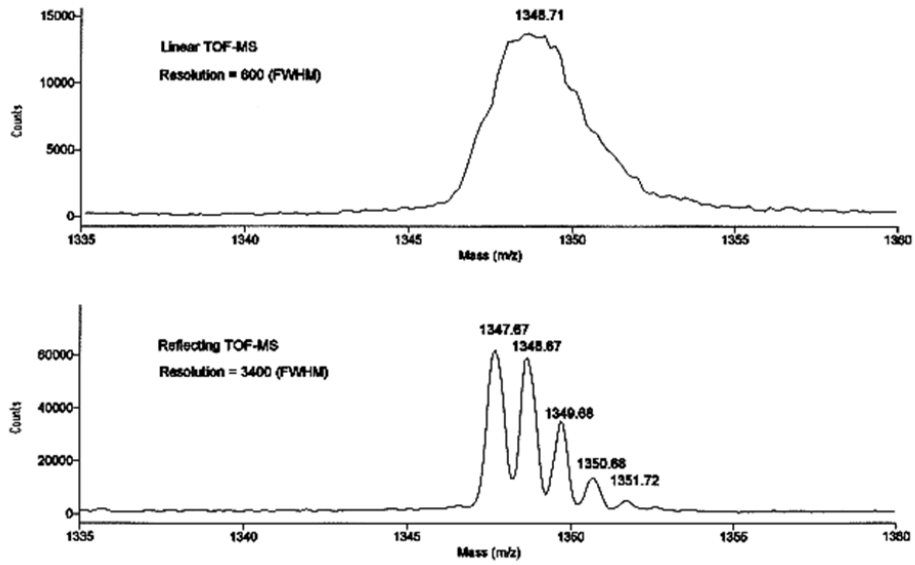
3. Carefully place your MALDI plate onto the holder. In the bottom left hand corner of the plate is a notch removed, this corner goes at the bottom right of the holder as you look down at the holder. The plate should fit snugly in position with only very slight movement.

4. Shut the lid and press load.

5. On the PC, click 'Instrument Settings', 'Camera control', 'On' (the camera may already be minimized on the bottom bar). This allows you to see the plate inside the MALDI.

Choosing which mode to use on the MALDI:

This is an example of running the same sample in linear (top) and reflectron (bottom) modes. The reflectron mode focusses ions of the same *m/z* giving higher resolution but need more sample. Try your sample in linear mode first and then changing to reflectron.



6. The MALDI should always be left in linear mode. To change to reflectron mode, wait for the pressure to drop inside the MALDI (the three pressure gauges: analyser/backing/inlet, should all be in the green - this will take a minute or two after you load the plate.), then press the 'stop' button next to Operate (box will turn red)

7. Next, click 'Mode' on the toolbar, 'mode' and then 'refelctron'

8. Then 'File', 'Open', REFL_POS.ipr

9. Hit the 'On' button to operate (box will turn green).

10. To switch back to linear, repeat steps 6-9, changing mode to 'linear' and then 'File', 'Open', Lin_Pos.ipr

11. **<u>The MALDI should ALWAYS be left ON in linear mode when you finish.</u>**

12. In the box next to 'Sample' (towards the bottom left hand corner) you will see A1. Change this to wherever your sample is and hit return. You will see the plate move on the camera to this position. If the plate has not been put in correctly, the well will not line up.

13. To run the sample, 'Acquire' on toolbar, 'Acquire'. Choose a folder to save the data to and write the filename. You can leave the text box blank if you want.

14. Change the mass range to include your sample eg. if your compound has a mass of 4500, you would choose something like 2000-7500. Again, the MALDI gets a bit dodgy (particularly in linear mode) below masses of 500. **If using a lock-mass calibbrant make sure the range includes the mass of this.**

15. You can change the laser parameters if you want but shouldn't need to. Laser firing rate should be 10Hz, shots per spectrum should be 5.

16. Click on 'Start' - this starts the laser going.

17. To get a good quality MALDI spectrum you then have to establish optimum parameters:
    - **A)** The laser power - this is a sliding bar towards the bottom left of the screen going from 0-500. Drag with mouse, you could start low and gradually increase (its fine to go up to 500 if needs be).
    - **B)** Where the laser fire. This is done by dragging the crosshair in the target in the bottom left hand corner. You may need to adjust this parameter as the sample layers on the MALDI plate heterogeneously. Therefore, some areas may generate spectrum some not.

18. Once you are acquired good quality spectra (or want to change modes), there are two options:
    - **A)** If you just want a representative spectrum to give you an idea your product is in there, click 'Acquire', 'Stop' and 'Yes' - go to **Viewing Chromatograms** section.
    - **B)** If you have spotted a calibrant in the centre well to accurately calibrate your sample check the 'Lock Mass' box.

19. Having clicked the 'Lock Mass' box, the laser will shift to the centre well and acquire data. The spectrum for the calibrant should be of good quality. Again you may have to adjust parameters of the laser power and where the laser is hitting. Once you have a decent peak (it only needs a couple of scans), click 'Acquire', 'Stop' and 'Yes' 'Acquire', 'Stop' and 'Yes'.

20. <u>Viewing chromatograms:</u>

21. To view the chromatogram, open the MassLynx window, minimised on the bottom toolbar.

22. Click on 'Chromatogram' near the top and then in the chromatogram window click on the 'stopwatch' icon. This brings up the last sectrum run. Alternatively, if you want to open an older file, just 'File', 'Open' etc.

23. Right-click and drag over the part of the spectrum where you saw your peak, this should correlate to a large % abundance peak on the chromatogram. Remember, if you 'locked mass' the final few scans will be of the calibrant.

24. If you need to change number of decimal places - go 'Display', 'Peak annotation', change 'decimal places' and click 'OK'

25. Print

26. **To calibrate the spectrum**, right click and drag over the part of the spectrum corresponding to the lock mass calibrant (the last few scans). On the mass spectrum, left click and drag over the correct calibrant mass (it should be strong and very clear) so it is the largest peak in the spectrum.

27. Click 'Process', 'Center', and 'OK' (don't change any of the parameters here)

28. Click 'Tools', 'Lock mass' and enter the mass - I would advise putting the M+H here. Window should be 100 Da. Ticking the 'Use Monoisotopic Peak' box will automatically make the calibrant M+H (you can choose whether to use this or not). Click 'OK', 'Yes'.

29. The mass of the calibrant peak should change accordingly. If you need to change d.p. - see step 23.

30. Now, go back to the chromatogram and right click and drag over the part of the spectrum corresponding to your sample. This will now give you a calibrated/accurate mass for the sample.

31. Print.

32. <u>Finishing:</u>

33. 31. Unload your MALDI plate.

34. 32. Insert plate you took out to start with and load.

35. 33. **Make sure the MALDI is left in <u>linear mode</u>, with a plate loaded, and operate 'on'.**

List of MALDI matrices

α-cyano-4-hydroxycinnamic acid (CHCA): Good for peptides smaller than 5000 daltons. The solution must be saturated to be useful (dissolve in 50% MeCN/H_2O + 0.1% formic acid).

Sinapinic acid (SA): Good for peptides and proteins larger than 5000 daltons.The solution must be saturated to be useful (dissolve in 50% MeCN/H_2O + 0.1% formic acid).

2,5-Dihydroxybenzoic acid (DHB): Good for small molecules and peptides or proteins that do not ionize well with CHCA or SA.Place 10-15 mgs of dihydroxybenzoic acid into 1 mL of methanol. If DHB is old, desalt by washing with 500 µL of water twice, then adding methanol. Unlike SA or α-cyano, DHB generates a complete solution rather than a saturated solution.

Trihydroxyacetophenone (THAP): Good for oligonucleotides and phosphorylated peptides. Mix 12 mg of 2,4,6-Trihydroxy acetophenone and 7 mg Ammonium citrate, dibasic. Dissolve with 1 mL of 50% acetonitrile in water.

You can also try using silver salts added to the matrix - there will be protocols on the web.

We have all the matrices down in the mass spec lab - have a look in the fridge - they are around somewhere.

List of standards

A quick Google search:

http://www.sigmaaldrich.com/life-science/proteomics/proteomics-products.html?TablePage=9627473

One of the most common is ACTH fragment 18-39 with a mass of 2464.1989.