Bruker OminiFlex MALDI-TOF Mass Spectrometer Operation Quick Start

Sample preparation for the Bruker OminiFlex MALDI-TOF Mass Spectrometer is of crucial importance. Please take care when preparing your MALDI-TOF samples. The DCIF also has a stock of the MALDI targets. If you need a target please see a DCIF member.

A: Starting the MALDI-TOF

1. Log into the MALDI workstation in the usual fashion by pressing "**ctrl**" "**alt**" and "**delete**" then enter your user ID and password. If you do not have a user id for the instrument you will need to be trained. No exceptions. Please see a DCIF member or email dcif@mit.edu to arrange an appointment.

2. Once you have logged onto the instrument, press the "**Camera**" and "**Light**" buttons on the lower left side of the instrument front panel (Main Control Panel). This will turn on the target camera and target illumination lamp. These should normally be in the "off" position. The Main Control Panel also displays the vacuum pressure and pumping speed.

B: Software and Instrument operation

1. Double click the desktop "**FlexControl**" icon. A Logon Information dialog box will prompt you. Use the default User Name (TOFUser) and click "**OK**". The "FlexControl" software will open (be patient, several computer / instrument initializations need to take place) and you will immediately be asked to "Open FLEXControl Method". Click "**Cancel**".

2. Select "**MethodNew**". Each Group folder includes copies of several methods installed during the instrument installation. Select one of these or a method of your own. You can create a method of your own by selecting an appropriate method, then using the pull-down menu select "**File**" and "**Save Method**", giving the 'new' method a different name. By default method files are assigned a "**.par** " extension. Methods are named for different acquisition modes.

   For example, two standard method files are called:

   REF_P_1230-5700.par_0825011
   LIN_P_5000-16000.par_08252011

   Here:

   REF - Indicates this method configures the instrument using "Reflector Detector", for

mass range 0 – 4000 m/z, the reflector detector has better resolution, but lower sensitivity.

LIN **-** Indicates this method configures the instrument using "Linear Detector", for mass range up to 150,000 m/z, the linear detector has higher sensitivity, but low resolution.

P **-** Indicates a positive ion mode.

N **-** Indicates a negative ion mode.

1000-1600 (or 1230-5700) - Identifies the mass range over this particular method has been optimized.

Important: It is a good idea to start with one of the standard Bruker methods, and then save under a new name before you start any acquisition. If you make any changes to one of the original (or standard) methods, please do not overwrite it as all users in your group will be affected. If you modify a method you must save it under a different name! Doing so will ensure that you always have a baseline to work from if your modification was not as good as you expected.

3. Loading the sample target:

Before you get to this point, you should have read and understood the sample preparation handout. Proper sample preparation is critical for experimental success.

Warning! You must make sure that your target is dry. This includes…

1) That the bottom (or backing) of the target is loose. The center portion of the target must spin freely.

2) The sample positioning "spots" must be free of visible liquids / solvent residue.

3-1. First check the sample status in the green box in the upper left-hand side of the screen and the video view window in the lower left-hand side of screen.

"**IN**" indicates the previous user has left a target inside the ion source.

"**OUT**" indicates no target in the ion source- this means it's okay to load a target.

Importantly, you should also be able to see if the target is truly out by looking at the video view window -- No target spots should appear in this video view window.

Warning! Never load more that a single target into the source region. If you are not sure as to the status, or if a previous target is stuck, please ask a staff member for help. Loading more than one target will severely damage the instrument.

3-2. If a target is **IN** the source region (if not, go to 3-3), you need to unload this target before loading your target by clicking the ▲ button (next to the '**Carrier**' path box) to

unload the target. During the unloading cycle you will see the red probe light (above the camera button) turn on, and then the red pressure light will turn on after automatically opening the loading chamber to the ion source, as this causes some vacuum loss. The red lights will automatically turn off after the loading probe takes the target out of the ion source and the vacuum goes back down to working conditions -- $<1.0 \times 10^{-6}$ torr.

Warning!!! Never open the probe door if any red lights (pressure and/or probe) are on.

3-3. If the ion source is empty or you have just unloaded the target (see 3-2), open the probe door by firmly pulling handle upward (counterclockwise), to make the D cut of the handle base parallel to the blue door box. This will allow the probe door to swing open.

- First remove the target if one is on probe.
- Load your target onto the sample loading probe, taking care to match the alignment pin with the pinhole size on back of the target. Leave the probe door open.
- Click ▲ load button on computer screen. Wait till red probe light starts **blinking**; this indicates the electronic magnet of the probe has turned on,
- Try (only try, do not pull hard) to gently remove the target from probe, you should feel resistantance from target.
- Now it's ok to gently close the probe door and lock it quickly and tightly, the red probe light should turn into solid red, and the red pressure light will turn on after. When both red lights have turned off, the loading cycle is completed. This may take 2-3 minutes normally. But it will take longer when sample or target is not completely dry.

4.. Check status

- "**Sample**" status – should be "**IN**" in green color. If "**Sample**" status is "**OUT**", go back to 3-2, and followed by 3-3.

- "**System**" status – should be "**Ready**" in green color. If it displays "**Preparing**" in yellow for more than two minutes, go to 5-1. Otherwise, when both Sample and System are green, skip 5-1.

5. Click the "**Spectrometer**" menu tab.

5-1. In the "**High Voltage Options**" box, click the "**On**" button to turn on high voltage. This will change "**System**" status from **Preparing** (yellow) to **Ready** (green).

5-2. "**Matrix Suppression**" is located in the lower right-hand side of "**Spectrometer**" window. Check the box will allow you to suppress the matrix peaks up to the mass showed in the "**Suppress up to** ☐ **Da**" box. This function should be used for scanning mass at least >5000Da.

6. Check mass scanning range and detector gain.

6-1. Click "**Detection**" menu tab; the green colored region in the "Mass Range" window is the scanning range. Move the left slider to set the lower mass and move the right slider to set the upper mass for the scanning range. If the scanning range you have selected shows red color at right side, this indicates that the selected mass range is out of the digitizer's capacity. You need to reduce the mass range (bring up the lower mass) into the green zone. It's good to set the mass range at lest two or three times higher than the target mass (<10,000Da). This will allow you to see if there are any dimmers or trimmers.

6-2. Adjusting detector gain: There are two horizontal sliding bars in the Detector Gain box. One bar for Liner detector and one bar for reflector Detector. Adjust detector gain voltage (by moving the slider on the sliding bar) up to **9.5-10.5 for Linear detector** and **10-11 for Reflector detector**. If you have a weak signal, in addition to adjusting laser power, increasing the detector gain will also enhance the signal intensity.

7. Click "**Sample Carrier**" menu tab.

7-1. Check the target "**Geometry**". From the "**Sample Carrier**" menu, choose "**Scout 49**" for the 49 position target, which is what is you are using.

7-2. Choose the desired position by clicking the left mouse button on the desired spot (i.e. A1, D4 or G5). Once the target spot is selected, wait till the target stops moving. This can easily be seen through the ion source video view window.

- Click the "**Advance>>>**" button to display the "**Manual Fine Control**" options. Left click the "***X***" button in the center of the arrows. This option will activate the key board, it allows you to make fine movement on currently displayed target spot using **(**← → ↑ and ↓) keys.
- To move around on the displayed single spot, you can also use the mouse. To move any desired crystal cluster under the laser pulsing point (the center of the cross in video view window), just click on the crystal cluster. Since the mouse is not as accurate as the keys, you may need repeat this once or twice.

8. Spectrum acquisition. Once the target has stopped moving, click the "**Start**" button to pulse the laser onto the target. Adjust laser power by moving the slider arrow up or down on the vertical bar. Start laser power about 20 ~30%, then increase ~5% each step. Laser saturation can be easily identified by a distorted baseline. Click "**Stop"** to stop laser pulsing when the signal intensity (of the peaks of interest) has increased up to 75 ~95% of the full scale; or let the instrument automatically stop when the preset laser **Shots** number has been reached- this option can be modified using the "**Shots**" input box.

9. To optimize the signal, the target needs to be moved around so the laser lands on a good/big crystal. To make fine adjustments within a single spot of target, follow the steps under 7-2.

10. Fine tuning the ion optics (optional):

Zooming in to the peak that you want to improve, set laser cycle to 500 or more , and then start laser pulsing.

- While the laser is pulsing, select the "**Spectrometer**" menu tab. In the "**High Voltage**" options, the ion optics voltages (i.e. Ion Source 1, Ion Source 2, Lens, and Reflector) may be adjusted. Fine tuning these voltages may be necessary if the laser power adjustment could not achieve the proper peak shape or intensity.
- First try adjusting the "**Lens**" by increasing or decreasing the voltage in ±0.05kV increments.
- Then adjust "**Ion Source 2**" by also increasing or decreasing the voltage in ±0.05kV increments.

Important: Never adjust "Ion Source 1" or "Detector"!

- Select the "**Detection**" menu tab. Here the operator can adjust the instrument sensitivity and mass range (using the displayed slider bar). Only adjust the "**Detector Gain**" when the desired results were not obtained by adjusting the laser power and the ion optics. A "**Sample rate**" of 1 and an "**Electronic Gain**" of 1x should normally be used.

4. Saving your spectrum:

- Click "**Save as**" to save your spectrum in the proper directory. All data is to be saved on the D: drive in accordance with your group and user id. For example, if you are John Doe working in the Swager research group and you wish to save a file called my_nobel_prize the path would be as follows:

  D:\data\swager\SWjdoe\ my_nobel_prize

- Check both the "**Single**" and "**Sum**" boxes for Reflector buffer

Important: Do not check "UseAutoXecute" box or "AddID" box.

- Click "**Save**" to save current spectrum to current file.
- Click "**Add**" to add the current spectrum to last spectrum (sum buffer), the added spectrum could be from same, or different, sample (one from a standard and one from a sample).

5. Select the "**Calibration**" menu tab after data acquisition. This will allow for the creation or updating of the calibration file.

- From the "**Reference list**" pull down menu, select a proper mass list. For example, selecting the file called "postrefma" produces a mass list with peaks commonly used for positive ions.

- You may also wish to add the "**Zooming**" option. A value of ±10.0% works well.
- Select (highlight) a compound name from the list, and the "**Spectrum Display**" will "zoom in" on the selected mass region (±10.0%).
- Move the cursor to the spectrum then click on the left side of the corresponding peak.
- Repeat for the next compound
- Select a calibration "**Fit**" option. A linear fit requires two or more points and is best when your mass is "outside" the range of the calibration end points. The quadratic fit also requires a minimum of two (and preferably more) points. Use this option if your mass is within the limits (extremes) of the calibration points.
- Once a calibration is selected, left click the "**Accept**" button.
- Click the "**Update Method**" button to update the calibration curve in the current method. This calibration curve will be used for the following samples as an external standard.

Important: Only "Update Method" to your own method file. If you are using a default method, First Go to "File", "Save Method", give it a new name, then "Update Method".

- An internal calibration can be done the same way as external standard for each data file or through the data processing software xTOF (see the data processing section below).

C: Data processing

1. Double click on XMASS OmniFlex desktop icon

2. Open an existing data file (or load a spectrum) for processing.

   - Using the pull down menus, select "**File**", then select "**open spectrum**". An 'Open Dialog' window will open.
     - In the '**DataDir**' box highlight your "group" directory.
     - Select your user id in the '**Sample**' box.
     - Highlight the desired data set in the '**Experiment (EXPNO)**' box.
     - Highlight the process number in the '**Process (PROCNO)**' box.
     - The data set along with the full path name will be displayed in the '**Selection**' box.
     - Next press the "**Apply**" button then press the "**OK**" button. The selected data set is now inserted into xTOF and ready for processing.

3. Peak labeling:

   - Click peak icon with numbers on the top bar (5^{th} icon from the left)
   - Click the "**Label region**" button on the right side of screen. By default, the entire spectrum is selected. Left mouse click inside of the region to label all peaks or,

using the cursor, point to the corner of the region box, hold down left mouse button and resize the box to redefine the label region.

4. Selectively delete peak labels:

   - Click on the "**Del peak**" button on the right-hand side of the program. Use the left mouse button to "frame" the unwanted peaks. Once selected, move the cursor to inside the framed region and click the left mouse button.

5. Zooming in and out and general manipulation:

   - Move the cursor over the spectrum baseline (it will change from an arrow to a ↕). Using the middle mouse button click on the baseline to define the starting point of region to be expanded. A vertical line will appear and the cursor will change from a ↕ to a +. Now move the cursor to the desired end point and again click the middle mouse. Presto!
   - Left click on the " > < " button under the spectrum to zoom back a little at a time.
   - Left click on the "**Full**" or "|<->|" buttons to display full spectrum (these both do the same thing!).
   - Left click on the "***2**" (times two) or "***8**" (times eight) buttons to increase peak height (scale) 2 or 8 times
   - Left click on the "**/2**" (divide by 2) or "**/8**" (divide by 8) buttons to decrease the peak height by 2 or 8 times

6. Edit Reference List. If your standard compound is not on the list, you may add additional compounds to the list.

   - Click the icon of the spectrum with "**?**" mark (7th icon from the left)
   - Left click the "**reflist**" radio button on the upper right-hand corner of the spectrum graphics window.
   - A pop-up window will open titled "**Mass-Reference Table**". Using the pull-down menu select "**File > New**" to create a new reference list or select "**File > Load**" to open an existing list for editing.
   - From the pull-down menu select "**Edit > Add**", and then enter the mass and structure information.
   - Repeat for additional compounds
   - When finished editing, go to "**File > Save**" and give the modified reference list a new name.

   Important: Any new reference list created in xTof program can not be used in FlexControl program.

7. Calibration for an internal standard (standard and sample mixed on same spot)

   - Click the icon of the spectrum with "**?**" mark (7th icon from the left)
   - Left click the "**caliblist**" radio button (directly under the "**reflist**" radio button)

- A pop-up window titled "**Calibration Table**" will open. Using the pull-down menus select "**File > Load**" and choose a proper reflist from the pop-up window listing.
- Under compounds list left click on "**auto select**"
- Left click on each compound name that the corresponding peak appears in the spectrum. If found a "+" will mark the top of each peak.

  Note: If any one is not marked correctly:
  (a) Left click "**Manually select peak**"
  (b) Zoom in the mis-marked peak
  (c) Highlight the compound name from the list
  (d) Click the top spot of the peak
  (e) Repeat (c) and (d) for additional peaks

- When finished selecting peaks, click the "**caldt**" button from right hand side tool bar to calibrate (click the Return button when finished).
- To print a calibration report, left click the "**Calibms**" button and type "**calstat**" at the command line.

8. Calibration for an external standard (standard and sample on different spot)

   - Follow the steps under 7.
   - Click "**StoCal**" after click "**caldt**" . This will store the new calibration curve in to the memory.
   - Go to **File → Open spectrum,** choose a data file that you need to apply calibration to it.
   - Label peaks by same steps as 3.
   - Click calibration button on top tool bar, and then click "**RecCal**" on the right side of spectrum.

   Important: "**StoCal**" and "**RecCal**" should be used only when both standard data and sample data acquired by same method file in FlexControl.

9. Some additional useful command:

   - To edit (or create) a title on your spectrum type "**ti**" at the command line. A pop-up window will allow you to input text.
   - To calculate the molecular weight type "**exactmass**" at the command line. Enter the molecular formula by symbol, space, number, space, next symbol, space, next number,…. etc.,

     For example: $C_{12}H_{24}O_4NBr$ would be entered as C 12 H 24 O 4 N 1 Br 1 H 1

     Important:
     - Add H1 at the end will include the element that brings a single charge to the component into the calculation. H1 could be Na1, K1 or Ag1

instead. To calculate a doubly charged positive ion, the H1 or Na1…. should be to H2 or Na2....., and **charge number** in the next box should be 2.

- Subtract H1 from the molecular formula and change the **charge number** to "-1" will give you the exactmass of a singly charged negative ion. Subtract H2 from the molecular formula and change **charge number** to -2 will give you exactmass of a doubly charged negative ion.

- To plot a theoretical spectrum type "**isotopicdistribution**" at the command line, and then enter the molecular formula, in the same way as for calculating exactmass. Sometimes the theoretical spectrum may not plot out due to a software problem; typing "**isotopicdistribution**" one more time will do the job.

10. Click the right mouse button to get a pop-up menu

- Click on "**check resolution**" then move cursor to a peak, the resolution of this peak will be displayed.
- Click on "**mass diff**", then click on first peak, again click on second peak, the mass different between these two peaks will be displayed.

11. Print spectrum

- Using the pull-down menus select "**File > Plot**"
- You may also click on the printer icon on the top toolbar

12. When finished analysis

- From the pull-down menus select "**File > Exit**" (xTof)
- Click the ▲ button to unload the target from FlexControl
- Once the cycle is complete, open the probe door, and remove the target from the probe
- Close the target door
- From the pull-down select "**File > Exit**" (this will exit you from the FlexControl software)

12. Turn off "**Camera**" and "**Light**" by pressing both buttons.

13. Log out from the workstation. Go to "**Start > Shut Down…**", and choose "close all programs and log on as different user".