

# 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 Li Li.**

## **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 Li Li (x 8-9354) or email her ([lili@mit.edu](mailto:lili@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 a "**method**". 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\_800-3000.par  
LIN\_N\_1000-8000.par

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 “Liner Detector”, for mass range up to 150,000 m/z, the liner detector has higher sensitivity, but low resolution.

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

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

**1000-1600 (or 2000-4000)** - 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 over write it as all users in your group will be affected. If you modify a method you must save under different name! Doing so will insure that you always have a bottom line to work with 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 understand 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 sample status in green box in the upper left-hand side of the screen and 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.

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

**Warning!** Never load more than 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 click ▲ button (next to the ‘**Carrier**’ path box) to unload

the target. During the unloading cycle you will see red probe light (above camera button) turns on, and then red pressure light turns on after automatically open loading chamber to ion source which causing some vacuum loss. The red lights will automatically turn off after loading probe take target out of ion source and vacuum back down to working condition --  $<1.0 \times 10^{-6}$  torr.

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

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

- First remove the target if one is on probe.
- Load your target on to sample loading probe, taking care to match the alignment pin with pinhole size on back of the target. And 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, not really do) to gently remove the target from probe, you should feel a resistant to remove the target.
- Now is ok to gently close the probe door and lock it quickly and tightly, the red probe light should turn into solid red, and red pressure light will turn on after. When both red lights 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  $>5000$ Da.

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 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 least 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 **18 ~ 19 for Liner detector** and **16 ~ 17 for Reflector detector**. If you have weak signal, in addition to adjust laser power, increase 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 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 stop 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 also can be done by mouse. To move any desired crystal cluster under laser pulsing point (the center of the cross in video view window), just click on the crystal cluster. Since using mouse is not as accurate as the keys, you may need repeat 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 a distorted baseline. Click “**Stop**” to stop laser pulsing when the signal intensity (of the interests) 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 to allow the laser shot on the good/big crystal. To make fine movement on the 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 when 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  $\pm 0.05\text{kV}$  increments.
- Then adjust "**Ion Source 2**" by also increasing or decreasing the voltage in  $\pm 0.05\text{kV}$  increments.

**Important:** Never adjust "Ion Source 1" and "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 box of Reflector buffer "**Single**" and "**Sum**"

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

- 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  $\pm 10.0\%$  works well.

- Select (highlight) a compound name from the list, and the “**Spectrum Display**” will “zoom in” on the selected mass region ( $\pm 10.0\%$ ).
- 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 liner 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 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 default method, First Go to "File", "Save Method", give 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<sup>th</sup> 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  $\updownarrow$ ). 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  $\updownarrow$  to a +. Now move the cursor to the desired end point and again click 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 (7<sup>th</sup> 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 (7<sup>th</sup> 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 refile 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 peak
- 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<sub>12</sub>H<sub>24</sub>O<sub>4</sub>NBr 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 singly charged negatively ion. Subtract H2 from the molecular formula and change **charge number** to -2 will give you exactmass of doubly charged negative ion.

- To plot a theoretical spectrum type "**isotopicdistrabution**" at the command line, and then enter the molecular formula as same as calculating exactmass. Some times the theoretical spectrum may not plot out due to a software problem; typing "**isotopicdistrabution**" 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 tool bar

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".