Acquiring MALDI-TOF Mass Spectra
(Using the Applied Biosystems Voyager-DE STR)

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I. Sample Preparation:

Matrix Assisted Laser Desorption Ionization (MALDI) requires that a relatively small amount of sample (1-100 pmol) be mixed with a relatively large amount of matrix compound (ratio of $1:10^3$-10^4 of sample:matrix) on a sample plate.

A. Prepare a matrix solution. [~10 mg/mL]

1. Select an appropriate matrix for your sample: Three specific recommendations are listed. For additional suggestions there is a chart provided in the MALDI reservation notebook (Located on the instrument).

   "**DHB**" – dihydroxybenzoic acid - this is good for a wide range of molecules ranging from small organics to proteins.
   "**α**" – α-cyano-4-hydroxycinnamic acid - this is good for peptides and proteins
   "**DITH**" – dithranol – this is good for some synthetic polymers and organometalic and metallic samples.

2. Prepare a ~10 mg/mL solution of this matrix in a solvent appropriate for your sample & the matrix. Acetonitrile is recommended whenever possible for organic samples. Water/acetonitrile mixtures are recommended for protein samples.

B. Prepare a sample solution. [~10 pmol/μL]

1. To accurately prepare a 10 pmol/μL solution serial dilutions are necessary. However, for organic samples higher analyte to matrix ratios are often tolerated.

2. If possible, dissolve your sample in the same solvent as the matrix solution made above.

C. Prepare a standard solution. [~10 pmol/μL]

1. Prepare a standard solution of a known compound in the same manner as your sample solution.

D. Spot your samples on a MALDI plate

A shared-use MALDI plate is available in the upper left drawer of the main sample bench near the instrument. Two reasonable methods for mixing your matrix and analyte are described below:

1. When using a relatively non-volatile solvent, the matrix and sample can be mixed at the same time as spotting the sample onto the MALDI plate. Use a micropipette to spot 1 μl of the solution containing matrix onto an unused spot on the MALDI plate. Quickly spot 1 μl of the solution containing sample onto the drop of matrix solution. Try not to touch the plate. Draw up the matrix and

sample together into the pipette a couple of times to effect mixing.

2. When using a volatile solvent, premix the sample and matrix solutions prior to spotting. Use an appropriately sized micropipette to place 10 µl of matrix solution into a tube. Then add 10 µl of sample solution to the same tube. Draw up the matrix and sample together into the pipette a couple of times to effect mixing. Use an appropriately sized pipette to spot 1-2 µl of the mixture onto the MALDI plate. Try not to touch the plate.

Remember to write down the plate position numbers of all samples transferred to the MALDI plate. It is a good practice to generate a table as the one shown below:

Spot #	Sample ID	Data File	Molecular Formula	Expected Mass	Result
62	Standard		C18H15PO	278.1	
63	CEH-V-18		C19H23NO3P	344.2	
64	CEH-V-19		C28H41NO3P	470.3	
65	CEH-V-20		C22H37NO3P	394.2	
66	CEH-V-21		C23H39NO3P	408.3	

E. Dry the MALDI plate

For compound loaded in volatile solvents, it is acceptable to dry the droplets in a stream of air or nitrogen. Compressed gas lines are available in the hood towards the back of the room.

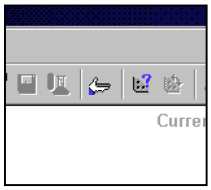
For compounds loaded in water it is necessary to thoroughly dry your MALDI plate in a vacuum desiccator.

After your plate is dried you are ready to acquire data.

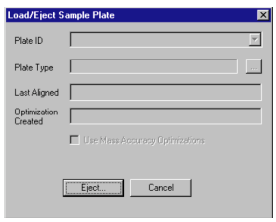
II. Acquiring Data:

Data Acquisition on the Applied Biosystems Voyager-DE STR is done using the ***Voyager Instrument Control Panel.*** If the program is not already open, it can be opened by clicking an icon on the desktop.

A. Load your MALDI sample plate



1. Locate the sample plate holder button  near the center of the top tool bar. Hovering the mouse over this button will reveal the label: **"Eject"** Click this button.



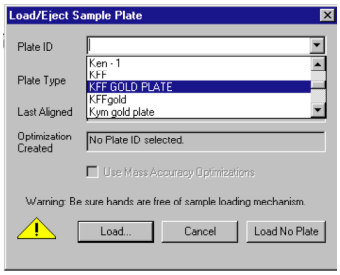
2. In the window that appears, click the "Eject" Button."

3. The arm that holds the plate will take as much as 2 minutes to eject.

DO NOT LEAVE THE ARM OUT FOR MORE THAN 1 MINUTE.

DO NOT LEAVE THE MACHINE UNATTENDED WHILE THE ARM IS IN TRANSIT.

DO NOT LOAD A WET PLATE.



4. After the sample plate holder appears, slide the sample plate into the sample holder until it "clicks" into place. The sample plate only fits into the sample holder in one orientation – the sample should be facing towards the main portion of the instrument.

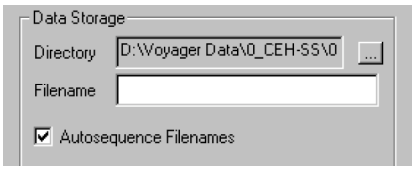
5. Click the sample plate holder button  (the same button as step 1). Hovering the mouse over this button will reveal the label: "**Load**"

6. In the window that appears, select a plate ID by clicking on the pull down menu near the top of the window. For a standard MALDI plate select: "**KFF GOLD PLATE**"

7. Click load. (Bottom Left of the window)

B. Designate a place to save your data

The data storage control panel is usually located in the upper left portion of the screen.



You must specify a directory path and file name for your data:

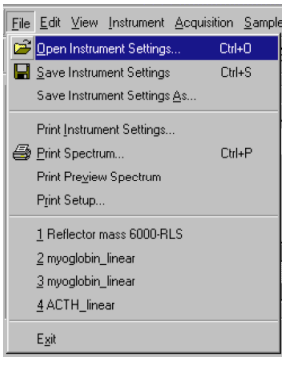
1. Click the ... button  to the right of "Directory"

2. In the window that appears choose the folder where you want to save your data.

STORE ALL DATA IN THE "VOYAGER DATA" FOLDER ON THE d:\\voyager data\
Most people store their data is subdirectories for each research group.

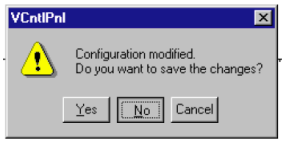
3. Type in a file name, then click the "OPEN" button

C. Load settings



1. Select "Open Instrument Settings" from the "File" menu.

2. A dialogue box will likely appear that states:



Always click "No" to avoid overwriting stored parameters.

3. Select a Instrument Configuration File. All configuration files are stored on the D-Drive in a folder titled "Voyager Methods", i.e. D:\\Voyager Methods\

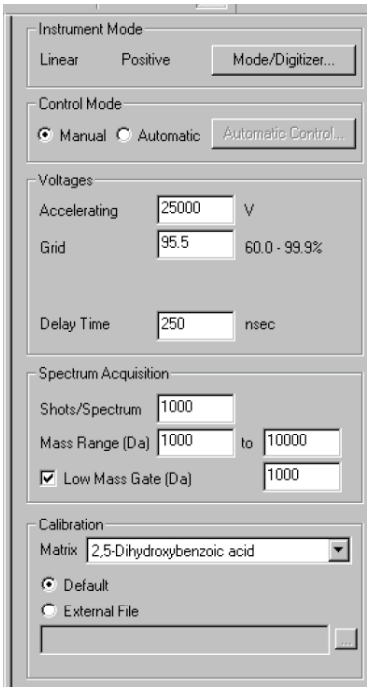
Recommended parameters:

"0_DEFAULT_REFLECTOR" (for compounds with expected mass <10,000 amu)

"0_DEFAULT_LINEAR" (can be used for any compound, but usually used only if reflector mode is not an option)

D. Modifying settings

Several instrument settings will require adjustment from the default settings. A number of settings are listed below, the ones that most commonly need adjustment are underlined. Make sure all of these settings are appropriate for your sample.



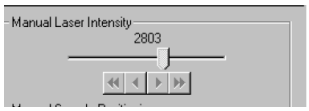
Control Mode: Most users will leave this set to "Manual".

Voltages:
Accelerating: 25000 is a normal value
Grid: 70 is a good value for reflector mode. In linear mode this value will be considerably higher and also needs to be adjusted in response to the mass of your sample.

Delay Time: This value is adjusted empirically in reflector mode, 400 is a good starting value. You may need to change it to improve your signal. In linear mode, delay this is a more crucial parameter and needs to be adjusted with Grid is response to the mass of your sample.

Spectrum Acquisition:
Shots/Spectrum: The laser will stop firing after this number of shots. (The default value is 1000, usually the acquisition will be stopped by the user before it reaches this value.)
Mass Range (Da): Adjust the mass range appropriately for your sample.
[Box] Low Mass Gate [Da]: **Set this equal to the lower limit of your mass range**

Calibration: For your initial acquisition leave calibration set on default.
Matrix: (ignore this setting – it does nothing)
o Default (check default unless you have already created a
o External File calibration file, see section IV of this write-up)

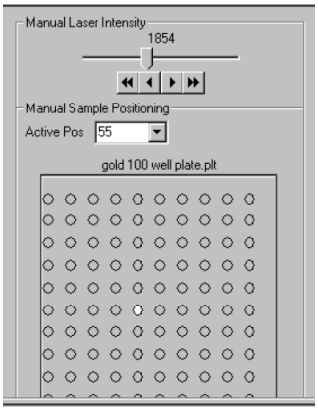


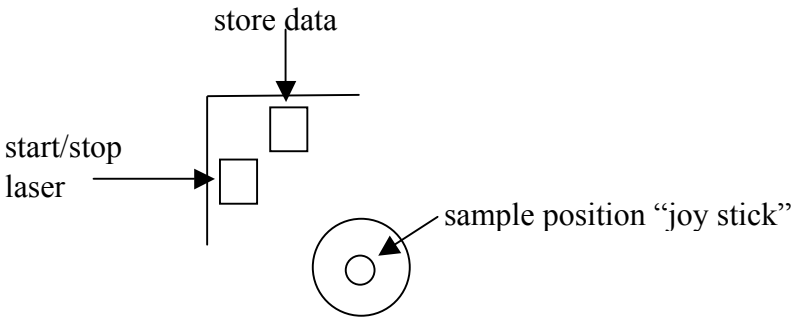
Manual Laser Intensity:
Slide bar
The single arrows increase or decrease by 5 units
The double arrows change the value by 50 units

E. Sample Positioning

There are three ways to position the plate:

1. Joystick
2. Pull down menu – type in the spot number or select a spot from the menu
3. Diagram of plate - Click on your spot number in the plate diagram.





F. Acquiring Data

1. To fire the laser, press the **start/stop laser button** once. (The first time you fire the laser and anytime the voltage settings are adjusted it can take up to 10 seconds to start pulsing, otherwise it should start firing immediately)
2. While the laser is firing move the laser around the sample spot.
3. Also, while the laser is firing gradually increase the laser intensity until a strong signal is seen. (The total ion count is recorded above the spectra to the right. A good number for this value is anywhere between 2,000 and 20,000 counts)
4. Adjust Laser Intensity, delay time (and grid in linear mode) and reacquire the data as required to optimize your spectra.
5. When you are satisfied with your spectra, stop the laser by pressing the **start/stop laser button** again.
5. If you want to save your data, press the **store data** button on the joystick to save the data displayed on the computer screen.

G. Data Processing

At this time you may want to process your data – please see that section

H. Finishing up: How you should leave the instrument

1. Eject your sample plate by, clicking the **Eject Button** as before
2. Click Eject from the menu that appears.
3. Wait for the sample plate to eject (1–2 minutes)
4. Remove the sample plate from the loading arm.
5. Click the **"Load No Plate"** Button to return the sample arm to the instrument.
6. Wait for the arm to return to the instrument.

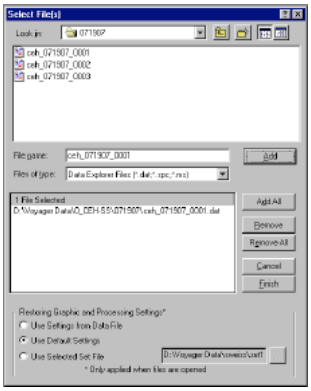
7. a. Clean up the sample preparation area
   b. If you used the MIC's MALDI plate RETURN IT TO ITS DRAWER.

III. Processing Data:

A. Opening Data Explorer/Opening Data

1. Data processing on the Applied Biosystems Voyager-DE STR is done using the program ***Data Explorer.*** If the program is not already open, it can be opened by clicking an icon on the desktop.

2. From inside Data Explorer your data can be opened as follows:
   a. Under the "File" menu choice Open
   b. A window will appear titled "Open File(s)"
   c. In the top portion of this window select the files that you want to open. With the chosen files selected click the "Add" button.
   d. Select any undesired files that are listed in the bottom portion of the window. With the undesired files selected click the "Remove" button.
   e. Click the "Finish" button to open all of the files listed in the bottom portion of the window.



3. It is often convenient to open your data in Data Explorer immediately after you acquire it. For this purpose a shortcut has been included in the software. Inside the Voyager Control Panel Program click the  button (located in the upper toolbar) to open the most recently saved data file.

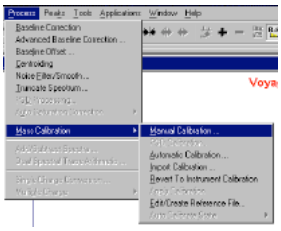
B. Basic Commands

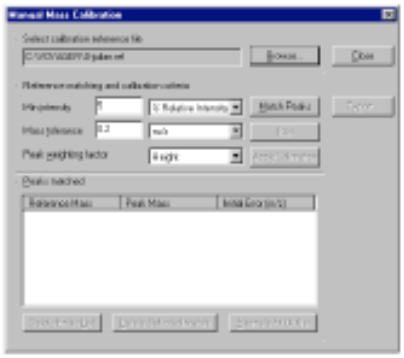
1. Zooming/Unzooming is accomplished with mouse controls. **LEFT click and drag** a box to zoom in around the boxed region. This can be done as many times as you like. To unzoom, **right click** and select "Single Unzoom" from the menu that appears to go to the previous magnification or **right click** and select "Full Unzoom" to display the full spectrum.

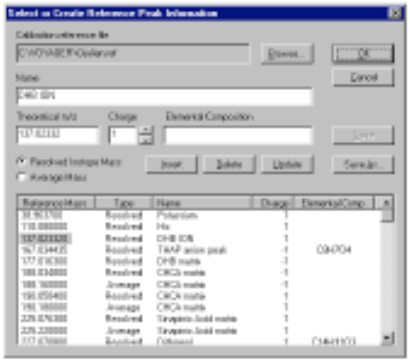
2. a. To specify an exact mass range to display, under the "Display" menu choose "Range" and then "X Range" from the pop out menu that appears.
   b. A window will appear, in which you can specify a precise mass range to display.

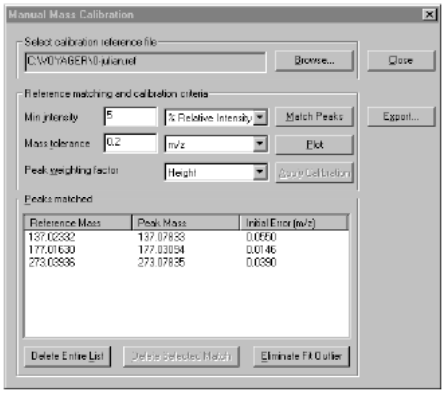
3. a. Sometimes you may be interested in a peak that is relatively small compared to the largest peaks in the spectra and thus not labeled in the default peak detection settings. To adjust these settings choose "Peak Detection" from the "Peaks" menu.'
   b. This will bring up the "Spectrum Peaks Detection Setup" window. The threshold for peak labeling can be changed by modifying the "% of Max Peak Area" located under "Global Thresholds."

C. Calibrating Spectra









1. To calibrate a spectrum, you must know unambiguously the assignment of a particular peak shown in the spectra. For example DHB ionizes with several characteristic peaks, they are: 137.02, 177.02 and 273.04. If you used DHB as a matrix and your compound of interest has a low molecular weight (<600) you can use these peaks as calibration peaks.

2. From the "Process" menu, choose "Mass Calibration" and then "Manual Calibration" from the pop out menu that appears.

3. A window will appear titled: "Manual Mass Calibration." The next thing you must do is to select a calibration file. To do this, click the "**Browse**" button and select the file "**0-julian**" in the window that appears.

4. With a mass calibration file loaded, **Click and drag using the right mouse button** around the peak belonging to your calibrant.

5. When you release the mouse button a window will appear titled: **"Select or Create Reference Peak Information"** This will contain a list of peaks, the one corresponding to your reference peak should be selected in grey – if it is correctly identified click the "OK" button.

6. Repeat steps 4 and 5 for every standard peak in your spectra.

7. Each peak you selected should be displayed in the list at the bottom of the **"Manual Mass Calibration"** window. If everything is correct, press the **"Plot"** button followed by the **"Apply Calibration"** button.

8. Your spectra is now calibrated. To return to it, press the **"Close"** button.

B. Exporting Data

1. **Printing:** You can print your spectra in several formats:
   a. Select: File:Print:Print File with Instrument Settings to print the displayed spectra and a tabulation of the settings used to acquire the data.
   b. Select: File:Print:Print View to print only the displayed spectra.

In either case the select: "**HP LaserJet 4000 Series PCL6**" as the printer in the Print window to printer to a printer in the mass spec lab.

2. **Printing to PDF:** To print to a pdf file follow the steps above but choose "Acrobat PDF Writer" as the printer In the Print window when it appears. After you click "Print" you will be asked to name your PDF file and choose a location to save it. **Please save your PDF files in the same folder that you saved your data!**

3. **ASCII Spectrum:** Some users prefer to export their data in ASCII format. To do this under the "**File**" Menu Select "**Export**" and then "**ASCII Spectrum**"

IV. Acquiring Data with External Calibration:

(Note: *this section assumes you have read and practiced many of the procedures in the sections on: Acquiring Data & Processing Data*)

A. Acquire a spectra of your reference peak

(Follow the steps in ***Section II***)

B. Calibrate your reference spectrum

1. Open your reference spectra in **Data Explorer**
2. Calibrate your reference spectrum following steps 1–7 of **Section III C.**
3. After the spectra is calibrated, but before you close the "**Manual Mass Calibration**" window click the "**Export**" button
4. In the window that appears click "**Save.**" This will create a calibration file with the same name as your data file, but with the extension .cal instead of .dat
5. In "**Voyager Control Panel**" under **Calibration** choose "**External**" and select the calibration file that you saved in step 4.
6. Acquire spectra of your samples.