MALDI Software Users Guide (*simplified version*)

Central Analytical Lab – Ekeley E266

The Acquisition program is called **Voyager Control Panel**. Once you shoot your sample and determine it looks good, you transfer the data to **Data Explorer**, the Analysis software tool.

(I) To Begin Acquisition

i) Starting the Software

- The oscilloscope and video monitor must be ON before opening the software. They are supposed to be ON all the time. (When turning on the scope, wait for a moment until the scope gets to the initial screen.)
- The software is located on the desktop, open **Voyager Control Panel.**

ii) Load a Sample Plate (use a flat plate with no deep wells.)

- Go to "**Hand Icon**" on the tool bar. → Eject → Autosampler will take a few moments to eject the plate holder.
- ***Be very careful when hooking up a sample plate.***
- ***Never put a wet plate or samples in.***
- Go to the Hand Icon again. → Plate ID = 100 wells → Load
- If you're finished for the day, select the **Load No Plate** option to get the empty plate holder back in.
  (Also get the holder back in *when you temporarily leave the instrument* --- Leaving the holder out for an extended period of time is not good for the vacuum system of the instrument.)

iii) Open a Method File (called Instrument Settings)

- Prior to the measurement you must open an appropriate Method File in your Settings folder.
- To open an **Instrument Setting** (*.bic* file), under **File** select **Open Instrument Settings.**
- You can only modify and save changes to *your own* instrument setting files, use disk button (top left) to save.
- *"**Your location**" and Method Files on the computer:*

D:/MALDI data/Your sub-directory/
- Data
- Settings
  - Linear_meth_pos.bic
  - Linear_meth_pos_over10kDa.bic
  - Reflector_meth_pos.bic

iv) Load a Mass Calibration File (see II-v below)

(II) Control Panel Settings

i) File Naming and Saving

- This is located on the upper left hand side of the Control Panel Window.
- Choose your directory in the gray box by selecting the **Browse** button.
- All data should be saved to the Data folder in "***Your location*".**
- Below this is a box for your **Filename** (*use only* **letters, numbers** and **__**.) When the data is saved, the computer will automatically attach 4-digit numbers sequentially, like *Filename-0001.dat, Filename-0002.dat, …..*

ii) Laser Intensity

- In the center of the left hand side is a slide bar for **Laser Intensity.**
- Either slide with mouse or hit the arrow buttons.
- Double arrow = 50 increments, Single arrow = 5 increments
- **Use laser intensities typically between 1300 and 2300. Do not exceed 2500 too much too often, except when you must use high intensities for large proteins and polymers**.
  (Peptide Linear ~1600, Peptide Reflector ~1800, Protein Linear 1800-2500, polymers 1800-2500)

iii) Autosampler Control of Sample Plate

- On the bottom left hand side is an image of the sample plate.
- You can select your spot by clicking the corresponding spot on the grid.
- Or by typing in the spot number or using the drop down menu.

iv) Instrument Settings-Parameters

- On the right side of the Panel are all the parameters your method file has set.
- Do NOT need to change settings in the **Voltages** section.
  (These are settings you should change *only* if you are familiar with them. And if you are in your *own* saved version of the Instrument Setting file.)
- Use **Shots/Spectrum** value of 25 to 100.
- Set the **Mass Range (Da).** Make sure **Low Mass Gate (Da)** is at or below the set low mass.
- After data acquisition over the preset number of laser shots, the laser will automatically stop.

v) Applying Calibration File

- On the bottom right hand side, there is a box to check that reads **Calibration.**
- Once you have created and exported a calibration file (**External File**) to your Data folder, you can load that file here.

  In the box below, **Browse** to open your calibration file *.cal* and apply to your method.
- Otherwise, select **Default.** (This is also used when shooting a standard and making a calibration file; see IV-iii **Creating a Mass Calibration File**).
- If the Default calibration is used, the mass accuracy is typically to within 1 m/z in the low 10^3 mass range. External calibration has better mass accuracy and is recommended for most analysis.

(III) Shooting Spectra

- When all Parameters have been set, you are ready to shoot spectra.
- Hit the **A button** on the Joystick and drive for a good location on your sample.
- Aim for dark clusters of dark crystals, they tend to be the "hot spots" (The opposite may also be true; it is quite empirical what spots give the best result.)
- The software will automatically turn off after it reaches the set number of shots/spectrum.
  (The T button on the joystick box is unnecessary; the spectrum is automatically transferred to the computer.)
- **Maximum peak intensity is 6.4E4**. Approaching this saturation value we lose both mass resolution and accuracy, so best avoid peak saturation.
- Reducing the laser intensity can help avoid peak saturation.
- On the oscilloscope, the flashing 1 or 2 is the mark for ½ saturation. Use this as a guide while shooting sample.
- When you get a spectrum you like, give it a **Filename** (if not given earlier).
- Then **Save** the file by clicking the ***Burning* Disk Icon** in the tool bar to save.

(IV) Data Analysis

i) Data Analysis Program

- Once you have named and saved spectra, always go under the drop down menu **Application.**
- Select **Launch Data Explorer.**
- The program should begin and your last saved spectrum will be displayed on a larger screen.

ii) Spectral Analysis (on Data Explorer)

- Mouse control is very similar to those in the Voyager Control Panel.
- The **Left Mouse Button,** if dragged across the peak, will zoom in. **Right** click will zoom out.
- There are a variety of tools available for analysis, for example,

  Tools → S/N Calculator → Method = Auto and enter Peak Position

  Tools → Resolution Calculator → Peak Height = 50% and enter Peak Position

  To give an unlabeled peak the mass number, **Peaks → Insert Peaks** → right-drag across the peak → Calculate

  To predict peak positions and isotope distributions, **Applications → Isotope Calculator** → enter Elemental Formula → Calculate
- When you are done with manipulating the spectrum, print by selecting a **Print** option under **File.**

iii) Creating a Mass Calibration File

--- For calibration with standard peptides, ask someone in the Mass Spec Facility ---

- You can do this after a standard is shot, saved and transferred to the **Data Explorer** program.
- **Zoom** in on peaks to visualize isotopic resolution using the **Left Mouse Button.**
- In the reflector mode, you should see a 3-4 peak pattern 1.0 amu apart with the most intense peak first.
- If this is not the case, re-shoot a spectrum for better resolution.
- Under the drop down menu **Process**, select **Mass Calibration**, then **Manual Calibration.**
- A **Calibration Window** will appear.
- Use a three-point calibration when possible, right click and drag the mouse across the first peak of interest.
- The **Calibration Window** will display peak mass and find that mass in the reference list.
- Note if the mass from the reference list is **Resolved** or **Average.**
- For peptides in the reflector mode, a ***Resolved*** mass should be picked. The ***Resolved*** mass should also be picked in the linear mode when you observe a unit mass resolution.
- The Calibration window will want to know if you agree with the mass it selected, hit **Okay** and move to the next peak to calibrate.
- Repeat the right mouse drag and examine the mass that is selected from the reference list. We typically do a three-point calibration
- Once all peaks have been selected, hit **Plot** then **Apply Calibration**, then **Export** (to your Data folder) and finally **Close.** Your calibration file has been saved as a *.cal* file.
- Be sure to save the *.cal* file in the same folder as the folder containing its source data file.

- Refer to II-v, Applying Calibration File to use the calibration file for new acquisitions.

Apppendix

How to create an electronic file (*.txt*):

(Excel and other graphing applications should be able to read it.)

1) Open a data file in Data Explorer.

2) File → Export → ASCII spectrum….

3) Use your e-mail to send the .txt file to yourself.

****** To save the disc space, please delete .txt files after e-mailing them!! ******

How to clean a MALDI plate:

"Avoid scratching the surface. A scratched area cannot be used for MALDI."

1) Wear rubber gloves.

2) Soap water / Rub the surface with gloves.

* For sticky spots, use acids (e.g., formic acid)

3) Tap water / (ditto)

4) Clean water / (ditto)

5) Methanol / (ditto)

6) **Completely dry the entire body of the sample plate. Very Important!!** (Solvents may be hiding behind.)