Combine with General Plate Maintenance and Prep

1. Wash MALDI plate:
    - Rinse the plate thoroughly with 100% methanol. Wipe the plate dry with a Kimwipe. Repeat.
    - Rinse the plate thoroughly with 100% acetone. Wipe the plate dry with a Kimwipe. Repeat.
2. Handle plate on the sides with Kimwipe to prevent contamination
3. Cover plate with a plastic lid to protect from dust until use.
4. Prepare a worksheet (A-H/1-12) indicating sample placement on the MALDI plate. For optimum measurement, place sample in 2 wells each, also include 2 standard wells and 2 blanks.

Preparation of Samples (Sample dependent*)

Metallic Ions:

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.
   Options for matrix solutions (depending on specific sample):
    - 10 mg Universal Matrix solution (comprised of DBH/CHCA/SA) dissolved in 1 mL of 50% ACN /50%HPLC water
    - 10 mg dihydrobenzoic acid (DHB) dissolved in 1 mL of 50% ACN /50%HPLC water.
    - 10 mg α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in 1 mL of 50% ACN/50% HPLC water.
    - 10 mg sinapinic acid (SA) dissolved in 1 mL of 50% ACN /50%HPLC water
    - Binary matrices: 10 mg each matrix material (a total of 20 mg matrix) dissolved in 1 mL of 50% ACN /50%HPLC water
    - Tertiary matrices: 10 mg matrix (a total of 30 mg matrix) dissolved in 1 mL of 50% ACN /50%HPLC water
2. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
3. Load 2 µL polymer sample onto each spot.
4. Allow sample plate to dry

Polymers::

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.
   Options for matrix solutions (depending on specific sample):
    - 10 mg Universal Matrix solution (comprised of DBH/CHCA/SA) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
    - 10 mg dihydrobenzoic acid (DHB) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
    - 10 mg α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
    - 10 mg sinapinic acid (SA) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
    - Binary matrices: 10 mg each matrix material (a total of 20 mg matrix) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
    - Tertiary matrices: 10 mg matrix (a total of 30 mg matrix) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water
2. Dissolve 10 mg sodium trifloroacetate in 1 ml of 50% ACN/0.1% TFA in distilled water.

3. Dissolve 10 mg solid sample in 1.0 mL of 0.5% TFA in HPLC water (or solvent that best dissolves material)
4. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
5. After the spots dry, spot 1 µL NaTFA solution on top of the spots.
6. Load 2 µL polymer sample onto each spot.
7. Allow sample plate to dry

Biological:

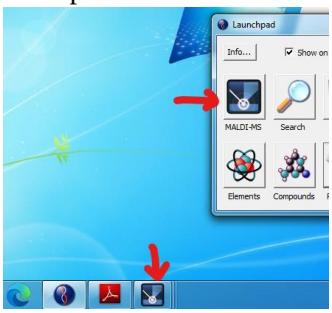
1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.
   Options for matrix solutions (depending on specific sample):
   - 35 mg of 3-hydroxypicolinic acid (3-HPA) in 500 microL 70/30 ACN/water
   - 35 mg 2',4',6'-Trihydroxyacetophenone (THAP) in 500 microL 70/30 ACN/water
2. Dissolve 25 mg Dibasic ammonium citrate in 500 microL 50/50 ACN/water
3. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
4. After the spots dry, spot 1 µL Dibasic ammonium citrate solution on top of the spots.
5. Load 2 µL sample onto each spot.
6. Allow sample plate to dry

Small Molecules:

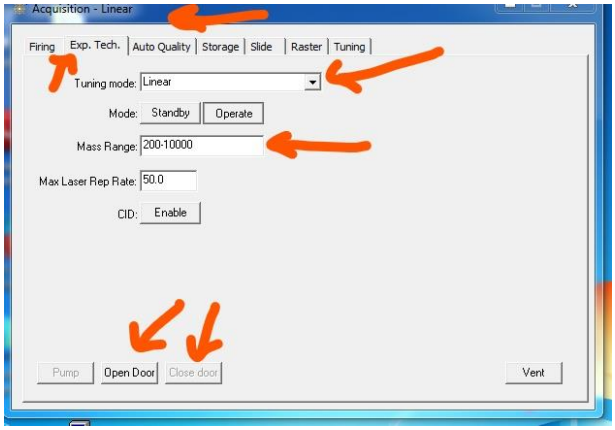
1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.
   Options for matrix solutions (depending on specific sample):
   - 35 mg 2',4',6'-Trihydroxyacetophenone (THAP) in 500 microL 70/30 ACN/water
2. Dissolve 10 mg α-Cyclodextrin in 1 mL water
3. Mix 120 microL of aqueous α-Cyclodextrin with 20 microL of THAP solution.
4. To load the sample, spot 1 µL of the matrix mixture solution (CD+THAP) onto a stainless-steel plate on sampling locations
5. Then load 2 µL sample onto each spot.
6. Allow sample plate to dry

Sample Analysis Procedure (General)

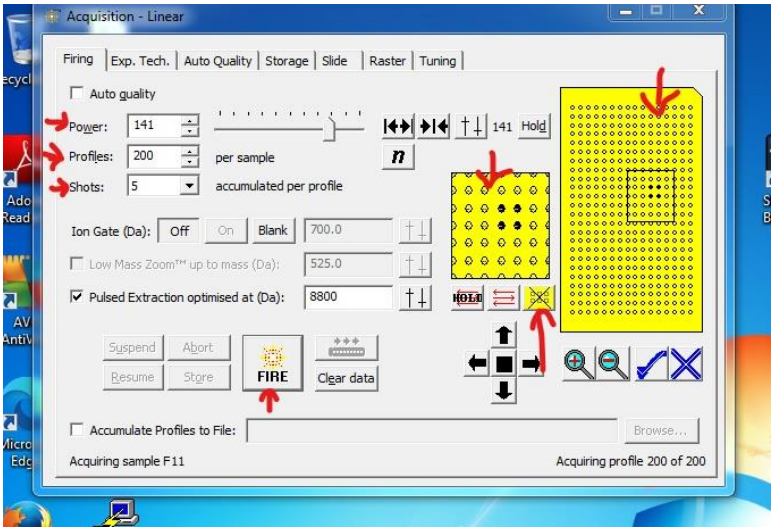
1. Open Biotech Launchpad software and open Maldi -MS icon



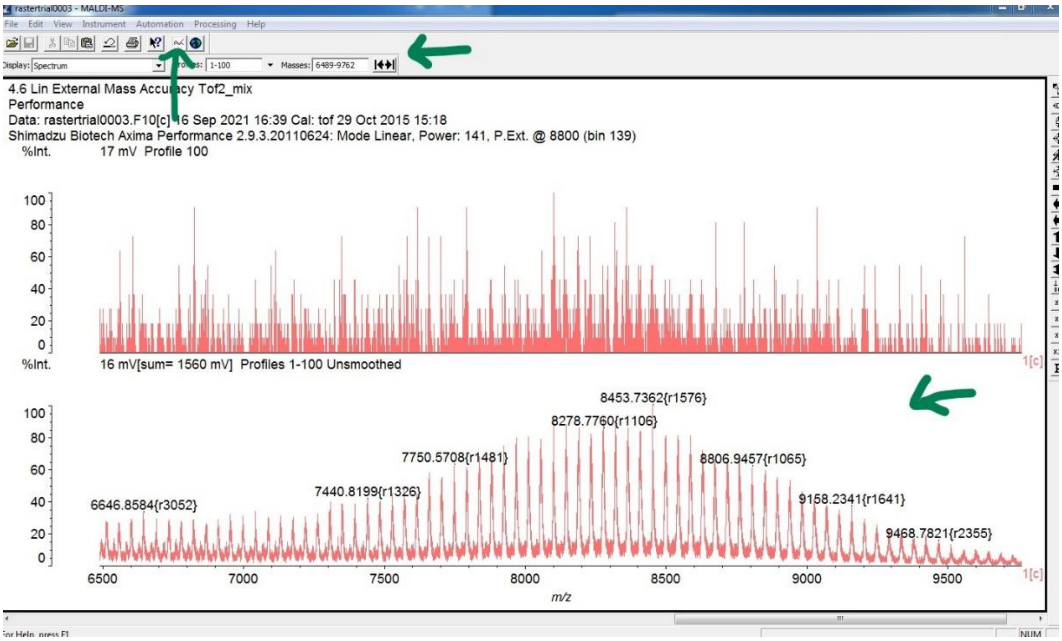
2. In the Acquisition Window go to Exp. Tech. tab and press "**Open Door**" to open Maldi Door
3. Take out previous sample plate if there by holding plates on sides.
4. Insert sample plate by holding plate on the sides and not touching samples.
5. Once placing the plate inside, press "**Close door**" **Allow vacuum pressure to equilibrate before preforming experiments.**
6. Set tuning mode to Linear, Linear_neg, Reflectron, Reflectron_neg, Reflectron_HiRes, or Reflectron_HiRes_neg. (Depending on what mode works best for your specific sample)
7. Set the mass range of interest.
8. Set instrument in "Operate" mode



9. In the Acquisition window go to Firing tab
10. "**Profiles**" should always be set to 200
11. Set "**Power**" to at least 90. Adjust Power accordingly Higher power may increase quality of data.
12. Set "**Shots**" accordingly. Less shots take less time but more shots with result in better quality data.
13. Check "**Pulsed Extraction optimized at (Da):**" and enter in Mass m/z value predicted.
14. To select all wells hit blue check mark. To unselect all wells hit blue x.
    For automatic or manual mode:
    a. For automatic sampling choose which well(s) to analyze by clicking circles in yellow box and click the red arrows below. Make sure yellow button with x across is not selected.
    b. For manual sampling click the small yellow box with the x and move location using black arrows and dragging window. Or right click yellow box and enter in well location of choice.
15. When ready to analyze samples hit "FIRE". Name file.

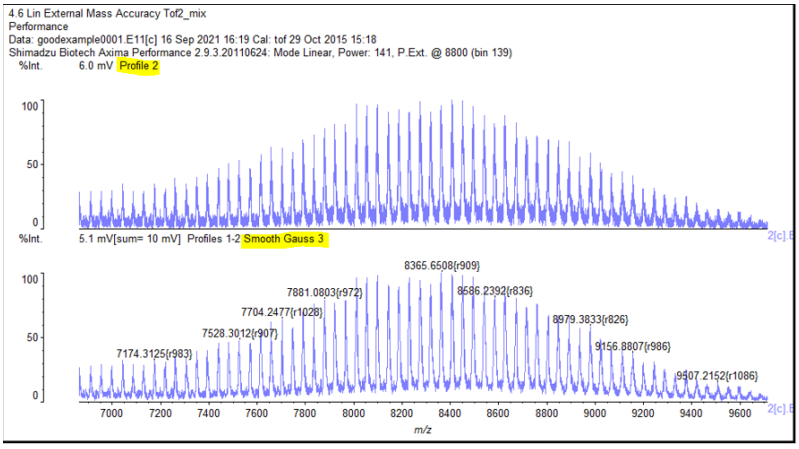


16. Set mass Range of interest. Or also use the mouse to close up on range of interest.
17. To stop the run early hit Abort. Or hit Suspend and then Resume to pause the run.

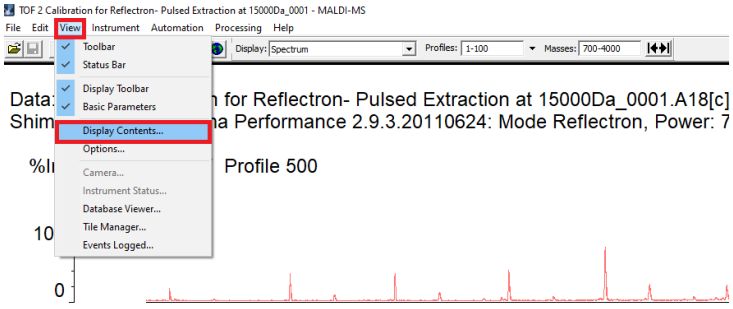


The spectra are for the same sample the difference is the type of spectra. The difference between the two spectra is that the one on top is of a profile In this case below profile number 2 is the last profile captured. The spectra at the bottom is the process spectra after processing parameters have been applied.

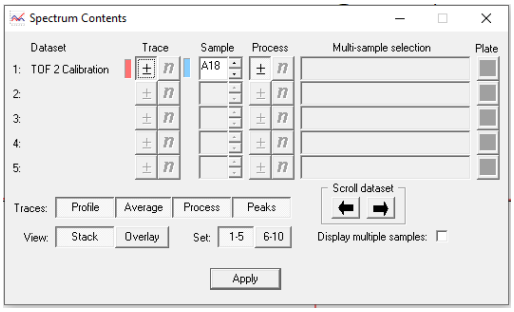
The spectra on the top is states a specific profile, “Profile 2” highlighted in yellow. The spectra on the bottom it states” Smooth Gauss 3” highlighted in yellow which means the smoothing method is Gaussian and a smoothing filter width of 3 is applied.



If you want change which type of spectrum you see you can do this under View < Display Options.

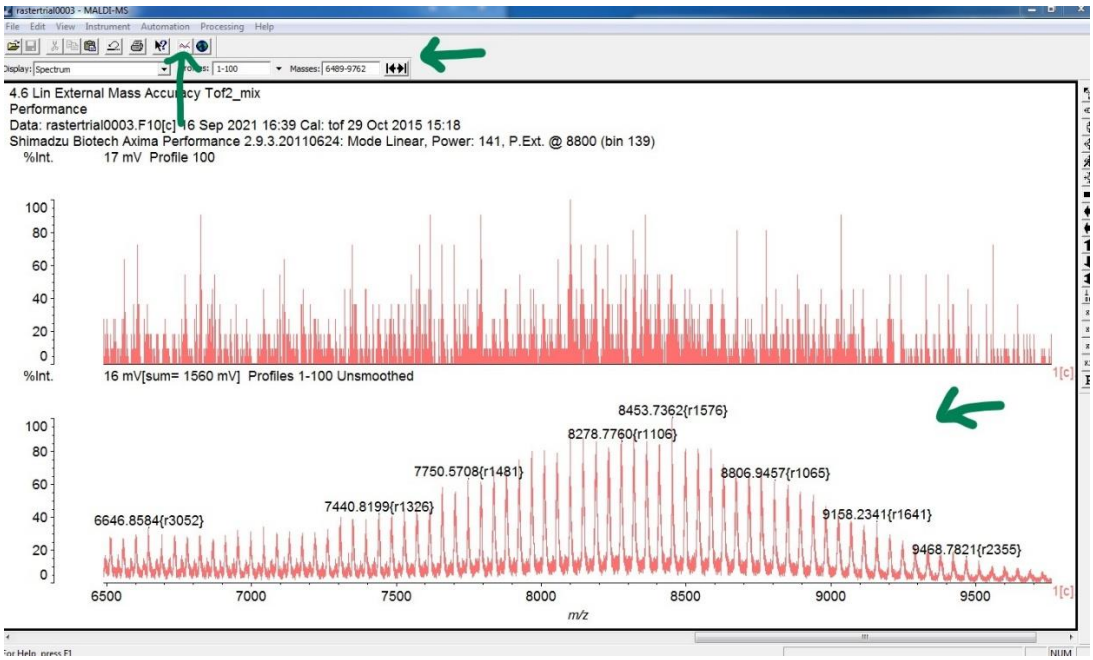


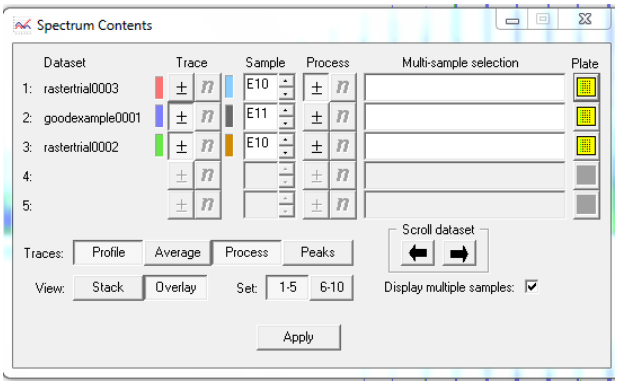
Then click on the type of spectra (profile, average, process, and/or peaks) she wants to see or hide and hit apply for the changes to take effect.



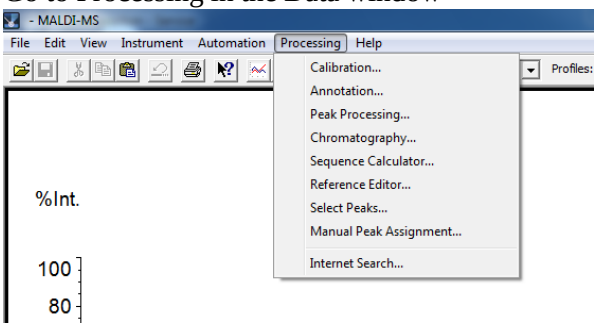
Data Analysis Procedure

1. Press Spectrum Contents button in Data window
2. Select which data to look at in Spectrum Contents window.
3. Scroll through data in Spectrum Contents window with black arrows.
4. In the Spectrum Contents window, choose to Profile or Average data/ Process or mark Peaks/ view Stack or Overlay

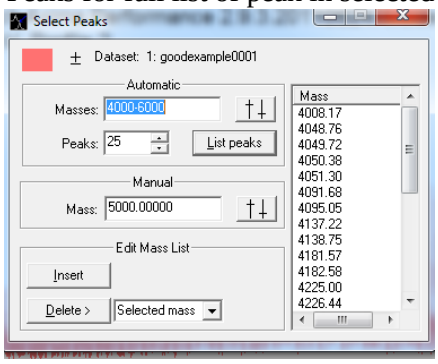




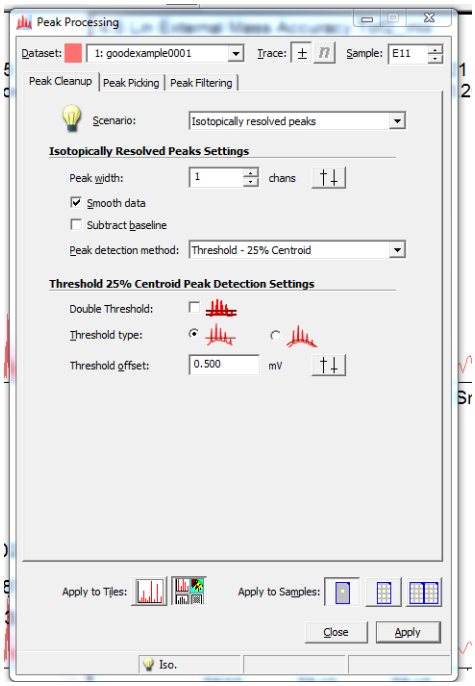
5. Go to Processing in the Data window



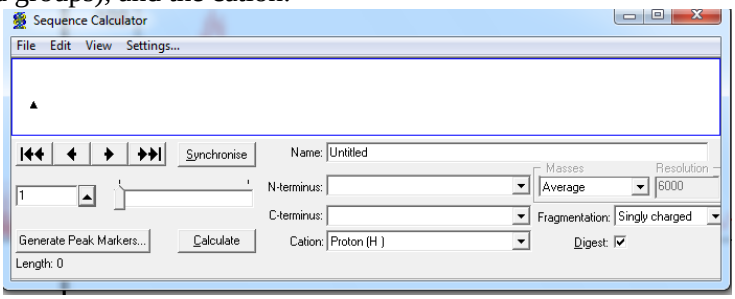
6. In Processing tab, go to Select Peaks for full list of peak in selected peak range



7. In the processing tab, go to peak processing more accurately identify peaks qualities

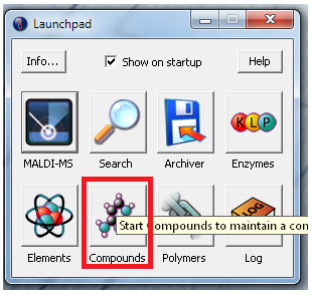


8. To understand sequence, use sequence calculator in Processing tab. Enter in N-Terminus and C-Terminus (the end groups), and the cation.

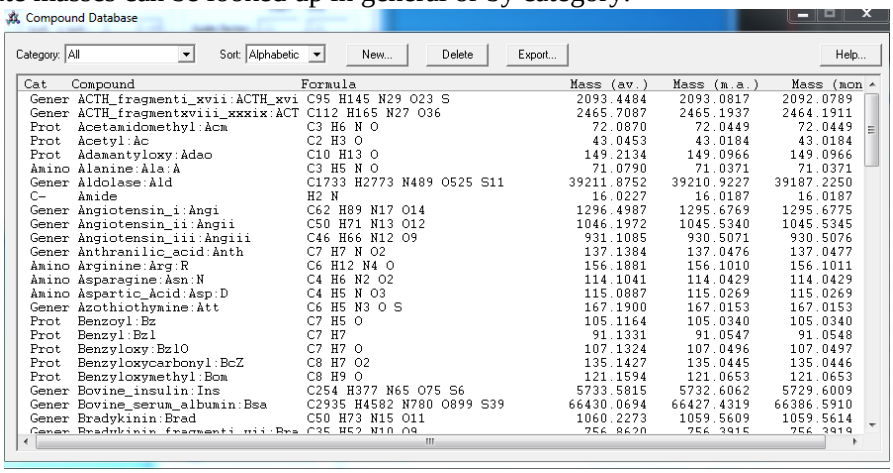


Database Look Up

1. In Biotech Launchpad click on the compound database



2. Approximate masses can be looked up in general or by category.



3. To add Cations or another category,
choose “Cation” category in the dropdown menu and add “New”

5. Type out the name and formula of the cation to add and click okay