

General Plate Maintenance and Prep

1. Wash MALDI plate:
 - Rinse the plate thoroughly with 70% ethanol. Wipe the plate dry using a Kimwipe. Repeat.
 - 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*)

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 50% ACN/0.1% TFA in HPLC water.
 - 10 mg dihydrobenzoic acid (DHB) dissolved in 1 mL of 50% ACN/0.1% TFA in HPLC water.
 - 10 mg α -cyano-4-hydroxycinnamic acid (CHCA) dissolved in 1 mL of 50% ACN/0.1% TFA in HPLC water.
 - 10 mg sinapinic acid (SA) dissolved in 1 mL of 50% ACN/0.1% TFA in distilled water.
 - For binary matrices, 10 mg each matrix material (a total of 20 mg matrix) was dissolved in 1 mL of 50% ACN/0.1% TFA in HPLC water.
 - The tertiary matrices were prepared by dissolving each of 10 mg matrix (a total of 30 mg matrix) in 1 mL of 50% ACN/0.1% TFA in distilled water.
2. Dissolve 10 mg sodium trifluoroacetate 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 μ L of the matrix mixture onto a stainless-steel plate on sampling locations
5. After the spots dry, spot 1 μ L NaTFA solution on top of the spots.
6. Load 2 μ L polymer sample onto each spot.
7. Allow sample plate to dry

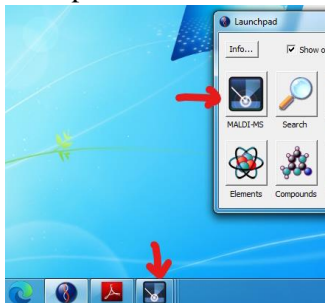
Biological:

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 50/50 ACN/water
 - 35 mg 2',4',6'-Trihydroxyacetophenone (THAP) in 500 microL 50/50 ACN/water
2. Dissolve 25 mg Dibasic ammonium citrate in in 500 microL 50/50 ACN/water
3. To load the sample, spot 1 μ L of the matrix mixture onto a stainless-steel plate on sampling locations
4. After the spots dry, spot 1 μ L Dibasic ammonium citrate solution on top of the spots.
5. Load 2 μ L sample onto each spot.

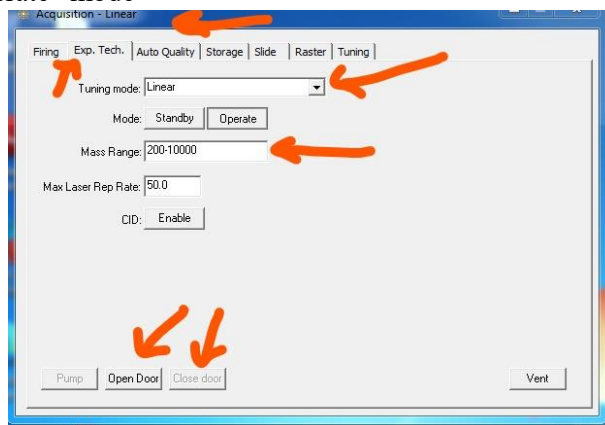
6. Allow sample plate to dry

Sample Analysis Procedure (General)

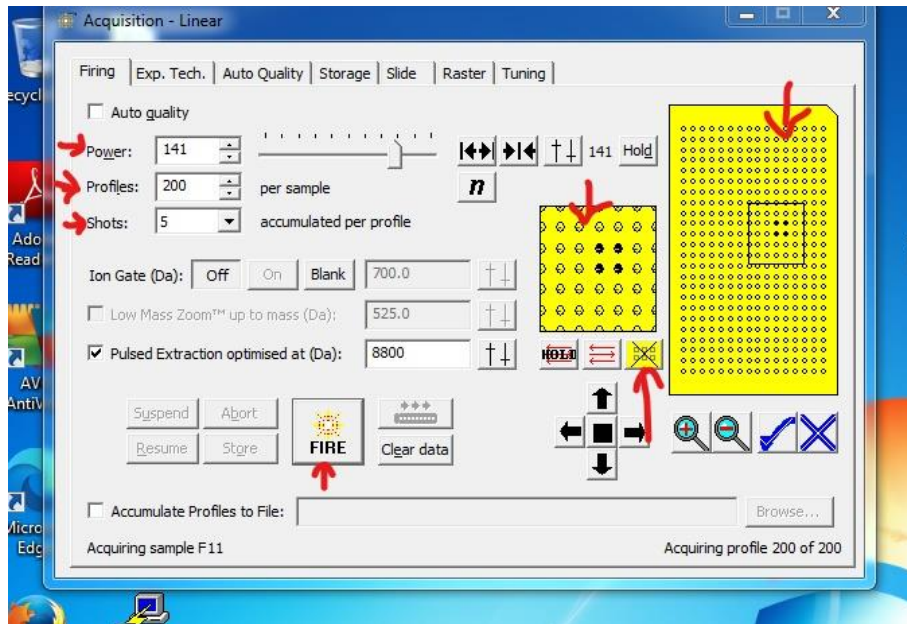
1. Open Biotech Launchpad software and open Maldi -MS icon



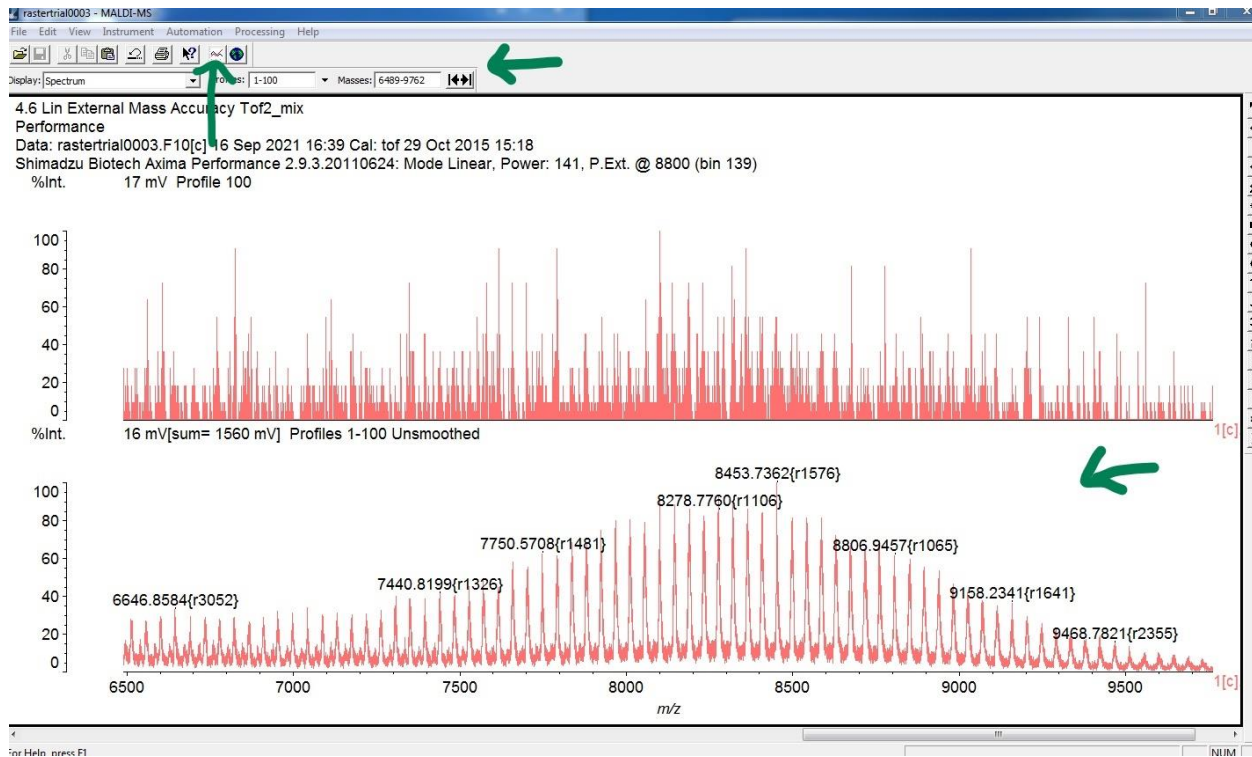
2. In the Acquisition Window go to Exp. Tech. tab and press “**Open Door**” to open Maldi Door
3. Take out previous sample plate if there by holding plates on sides.
4. Insert sample plate by holding plate on the sides and not touching samples.
5. Once placing the plate inside, press “**Close door**” **Allow vacuum pressure to equilibrate before performing 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.

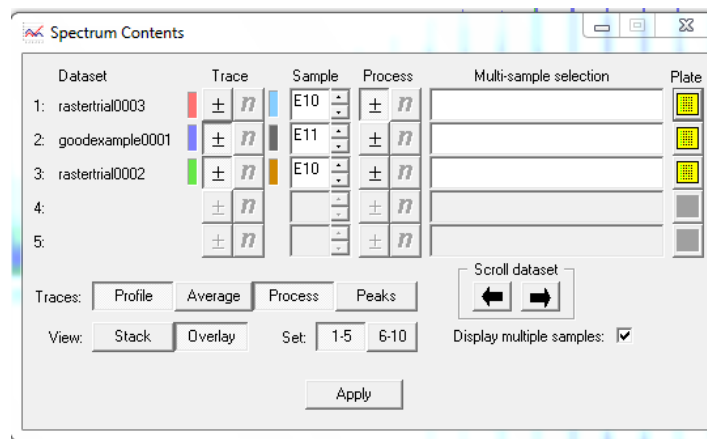
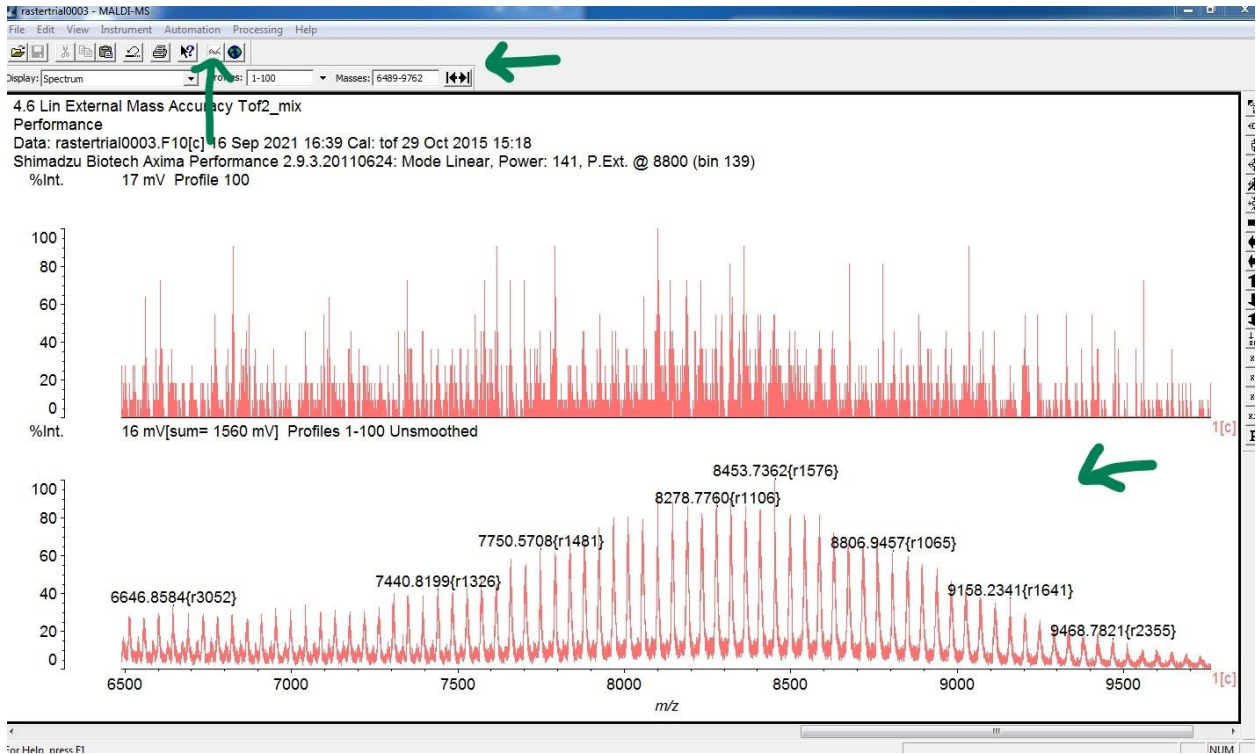


16. Set mass Range of interest. Or also use the mouse to close up on range of interest.
17. To stop the run early hit Abort. Or hit Suspend and then Resume to pause the run.

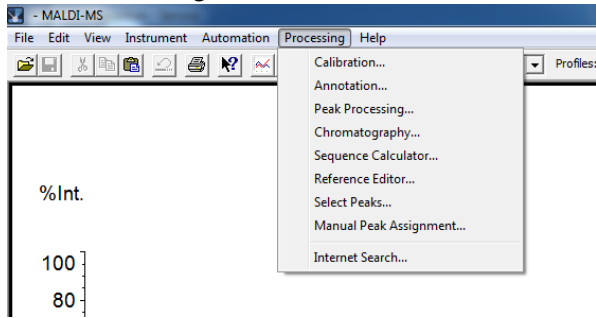


Data Analysis Procedure

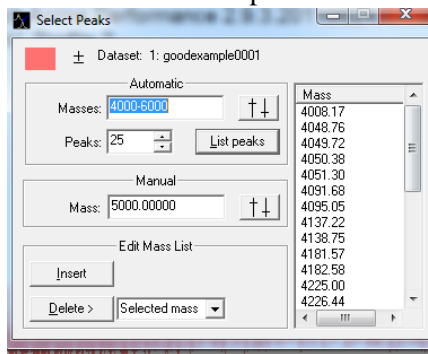
1. Press Spectrum Contents button in Data window
2. Select which data to look at in Spectrum Contents window.
3. Scroll through data in Spectrum Contents window with black arrows.
4. In the Spectrum Contents window, choose to Profile or Average data/ Process or mark Peaks/ view Stack or Overlay



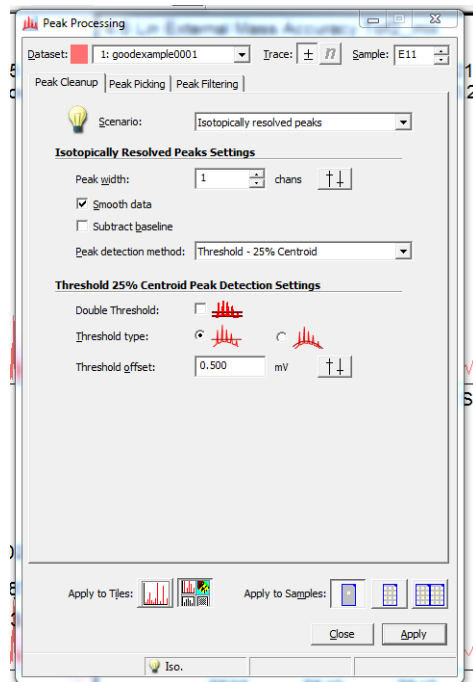
5. Go to Processing in the Data window



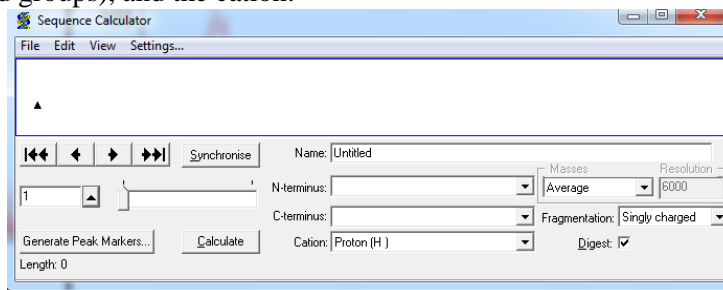
6. In Processing tab, go to Select Peaks for full list of peak in selected peak range



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



- 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

- In Biotech Launchpad click on the compound database



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

Cat	Compound	Formula	Mass (av.)	Mass (n.a.)	Mass (mon)
Gener	ACTH_fragmenti_xviii:ACTH_xvi	C95 H145 N29 O23 S	2093.4484	2093.0817	2092.0789
Gener	ACTH_fragmentxviii_xxxix:ACT	C112 H165 N27 O36	2465.7087	2465.1937	2464.1911
Prot	Acetanidomethyl:Acn	C3 H6 N O	72.0870	72.0449	72.0449
Prot	Acetyl:Ac	C2 H3 O	43.0453	43.0184	43.0184
Prot	Adamentyloxy:Adao	C10 H13 O	149.2134	149.0966	149.0966
Amino	Alanine:Ala:A	C3 H5 N O	71.0790	71.0371	71.0371
Gener	Aldolase:Ald	C1733 H2773 N489 O525 S11	39211.8752	39210.9227	39187.2250
C-	Anide	H2 N	16.0227	16.0187	16.0187
Gener	Angiotensin_i:Angi	C62 H89 N17 O14	1296.4987	1295.6769	1295.6775
Gener	Angiotensin_ii:Angii	C50 H71 N13 O12	1046.1972	1045.5340	1045.5345
Gener	Angiotensin_iii:Angiii	C46 H66 N12 O9	931.1085	930.5071	930.5076
Gener	Anthranilic_acid:Anth	C7 H7 N O2	137.1384	137.0476	137.0477
Amino	Arginine:Arg:R	C6 H12 N4 O	156.1881	156.1010	156.1011
Amino	Asparagine:Asn:N	C4 H6 N2 O2	114.1041	114.0429	114.0429
Amino	Aspartic_Acid:Asp:D	C4 H5 N O3	115.0887	115.0269	115.0269
Gener	Azothiothymine:Att	C6 H5 N3 O S	167.1900	167.0153	167.0153
Prot	Benzoyl:Bz	C7 H5 O	105.1164	105.0340	105.0340
Prot	Benzyl:Bzl	C7 H7	91.1331	91.0547	91.0548
Prot	Benzylxy:Bz10	C7 H7 O	107.1324	107.0496	107.0497
Prot	Benzylxycarbonyl:BcZ	C9 H7 O2	135.1427	135.0445	135.0446
Prot	Benzylxymethyl:Bom	C9 H9 O	121.1594	121.0653	121.0653
Gener	Bovine_insulin:Ins	C254 H377 N65 O75 S6	5733.5815	5732.6062	5729.6009
Gener	Bovine_serum_albumin:Bsa	C2935 H4582 N780 O899 S39	66430.0694	66427.4319	66386.5910
Gener	Bradykinin:Brad	C50 H73 N15 O11	1060.2273	1059.5609	1059.5614
Gener	Bradykinin_fragmenti_iii:Br	C35 H52 N10 O9	756.8620	756.3915	756.3919

- To add Cations or another category, choose "Cation" category in the dropdown menu and add "New"

Compound	Formula	Mass (av.)	Mass (n.a.)	Mass (mon)
Proton	H	1.0080	1.0078	1.0078
Sodium	Na	22.9898	22.9898	22.9898

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

