MS-MALDI User's Manual

Update 11/14/2012

Table of Contents

Safety

1. General Lab Safety Requirements:
   All users of the IMSERC facility must review the generic IMSERC guidelines before starting training. The guideline will be posted by the sign on computer and at http://pyrite.chem.northwestern.edu/analyticalserviceslab/ASL%20Final%20Guidelines.htm

2. Do not run instrument without approval from Saman, Jaekuk or lab TA. Failure to do so may cause injury, damage the instrument, produce invalid data, and result in additional fees or removal of IMSERC privileges.

3. Instrument Specific Safety

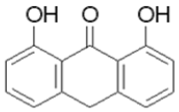
Hazards	Location on equipment	PPE required / Hazard Mitigation
Sample to be run		Wear gloves and safety goggles
Pinch Hazard	Sample Inlet	Keep hands clear of sample inlet once you press insert/eject button. This is especially true if you notice the sample holder is misplaced.

Sample Preparation

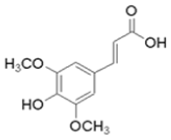
1. Decide if MALDI is the correct technique for your sample:

2. Choose the matrix for your sample:

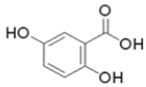
Sample	Matrix
Peptides < 10kDa	α-Cyano-4-hydroxycinnamic acid (CHCA)
Protein > 10kDa	a. Sinapinic acid b. Super DHB*
Oligonucleotides	a. 2,5-Dihydrozybenzoic (DHB) b. 3-Hydroxypicolinic acid c. 2,4, 6-trihydroxyacetophenone monohydride (THAP)
polymer	a. α-Cyano-4-hydroxycinnamic acid (CHCA) b. 2,5-Dihydrozybenzoic (DHB) c. Dithranol
Glycosylated protein	Super DHB*
Neutral Carbohydrates	2,5-Dihydrozybenzoic (DHB)



Dithranol



Sinapinic Acid



DHB

•**Alpha-cyano**: 50% acetonitrile, 0.1% TFA in deionized water
•**Sinapinic acid**: 30-50% acetonitrile, 0.1% TFA in deionized water
•**DHB**: 10 mg/ml in water, 50% CAN or other appropriate solvent for analyte
•**3-HPA**: prepare saturated solution of 3-HPA in Milli-Q water (~50g/L) and 50g/L solution of diammonium citrate. Combine 3-HPA:NH_4citrate 9:1
•**Super DHB**: DHB+ 10% 5-methoxysalicylic acid, Preparation: Solution A= 10 g/L DHB in 20% CAN, Solution B=10g/L 5-methoxysalicylic acid in 50% CAN, Combine A:B (9:1)

3. Obtain an appropriate sample plate (Coin Chip) to spot your sample on. Coin Chips are available from IMSERC staff for $35. Plates with pre-applied matrix with calibrant spots (AnchorChip) and matrix-free plates (NALDI) are available (cost is ~ $100/plate or $1/site).
4. Make a solution of approximately 10% sample to matrix by mass. (disregard solvent for this calculation)
5. Apply solution to plates. (~ 1ul per spot)
6. Dry the sample before coming to IMSERC (solvent must be evaporated).

Pre-Run Checklist

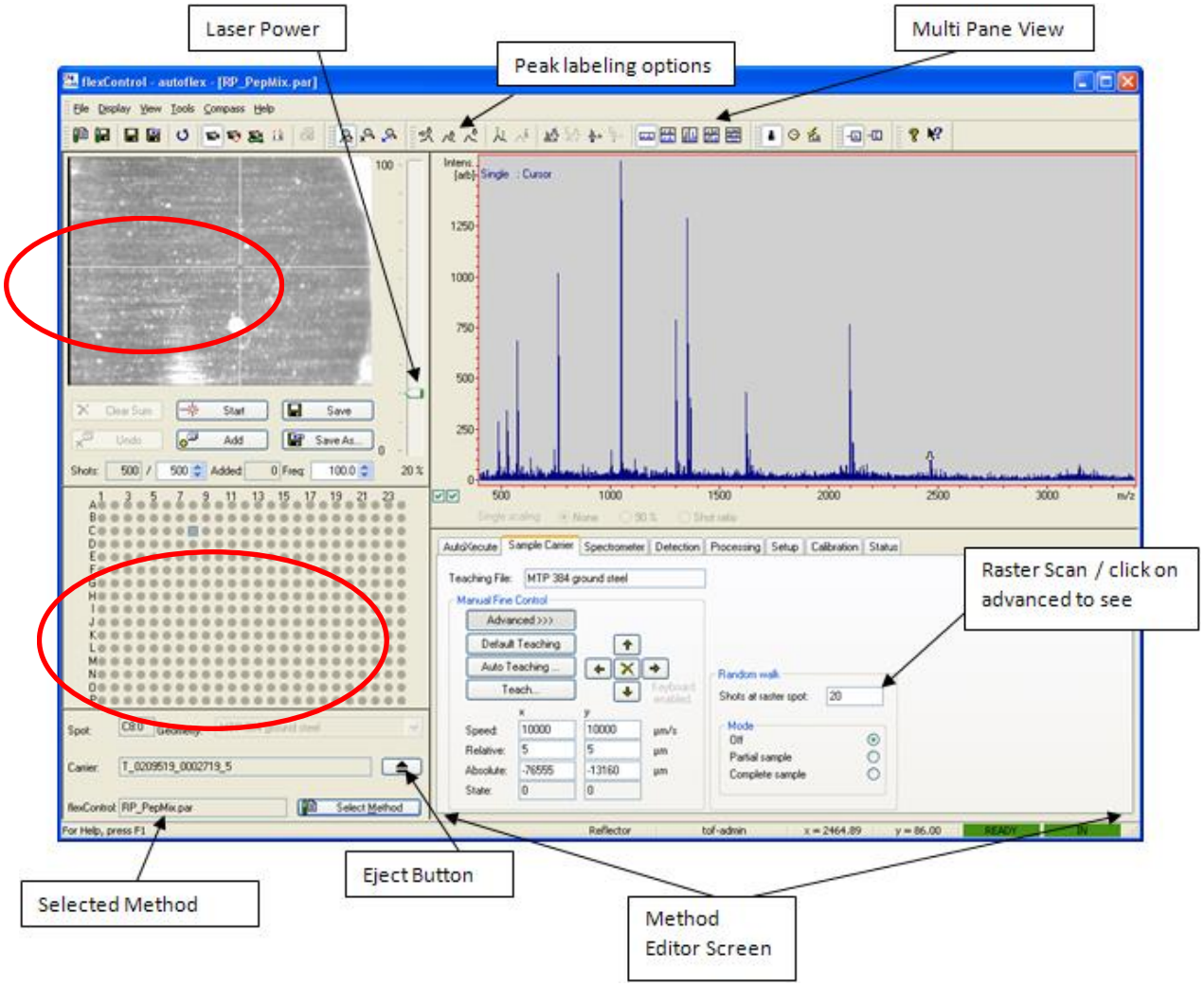
Step	Instruction	Comments
1	Verify sample is completely dry on the sample plate	Poor vacuum (poor signal and resolution) as well as long transfer times will result from wet samples
2	Log on to instrument in Login system.	
3	If needed, log into computer	Login Name: tof-user Login Password: youshouldknow
3	Start Flex Control	Log in as tof-user (you don't need to do anything) No password is required
4	Check status lights on instrument panel	Mains: Green System: Ready or Warm-up Target: Access

Bruker Autoflex III Operating Procedures

Northwestern University Analytical Services Laboratory

Last Updated: 8/8/2008 by AWO

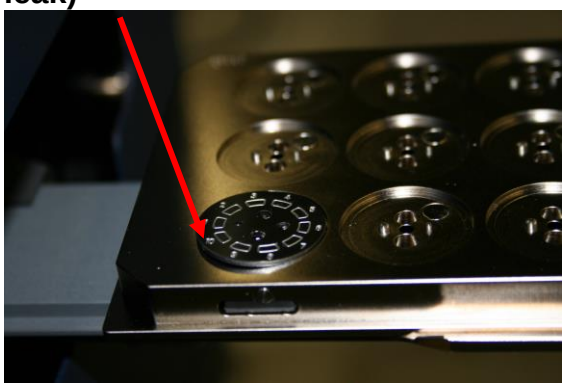
Starting Screen:

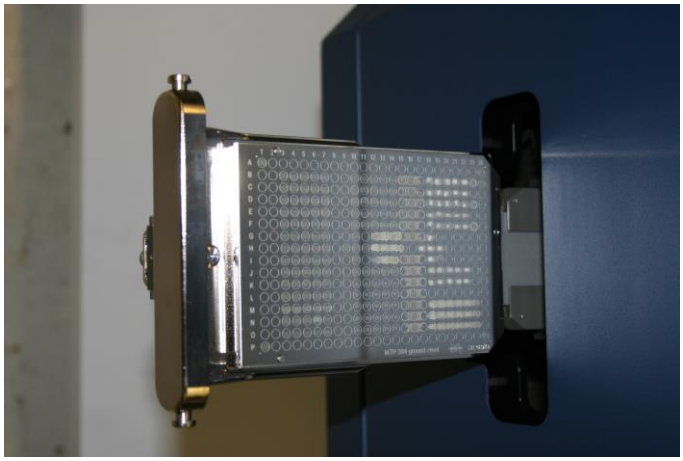
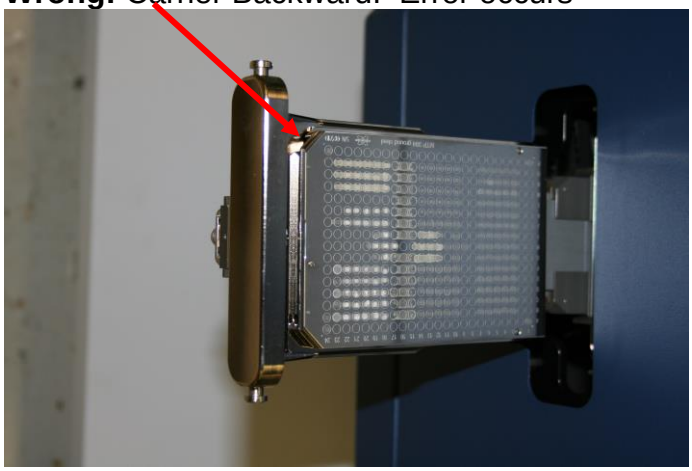


Step #1: Press the Eject Button in the starting screen (or the large green button on the side of the MS-MALDI. The Tray will open slowly – Make sure the tray path is not obstructed.

Step #2: Load the Sample:

Step	Instruction	Comments
1	Remove sample plate	Always store sample carrier in instrument to ensure that the next person can find it

2	Place plate on carrier	Sample “coin chip” is keyed to only fit one way. Ensure plate sits flush with the top of the carrier and sample plate is flat. Wrong: **(Sample may scratch sealing surface and cause a leak)**  Right: 
3	Load Plate into Loading Dock	Right: Carrier flush against back of transfer system 

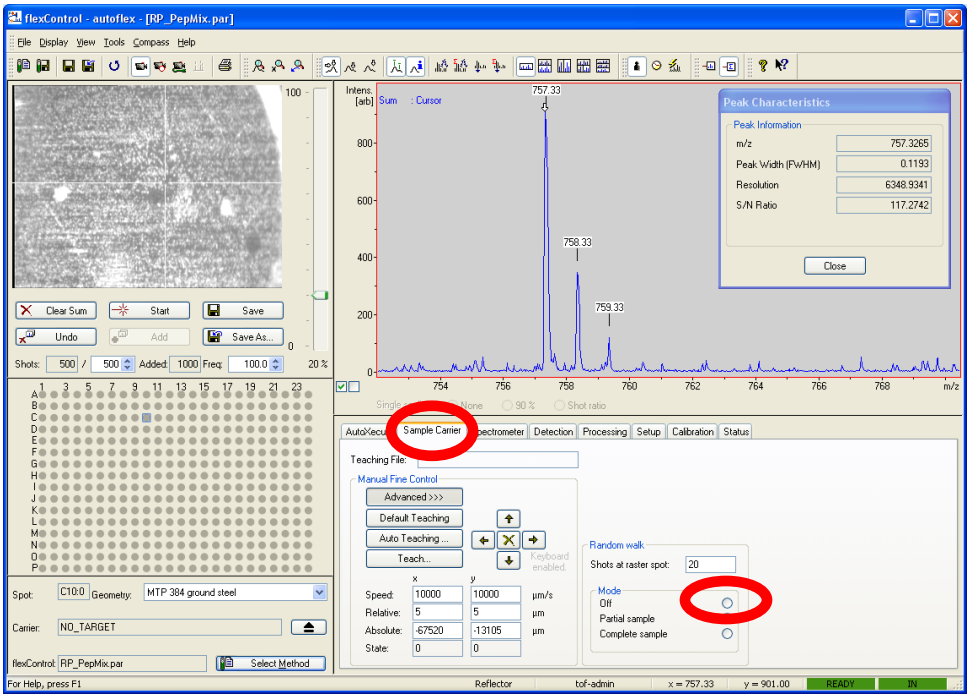
		Wrong: Carrier two far forward. Sealing surface can be scratched  **Wrong:** Carrier Backward. Error occurs 

Step #3: Press the Eject Button in the starting screen (or the large green button on the side of the MS-MALDI to insert your sample.

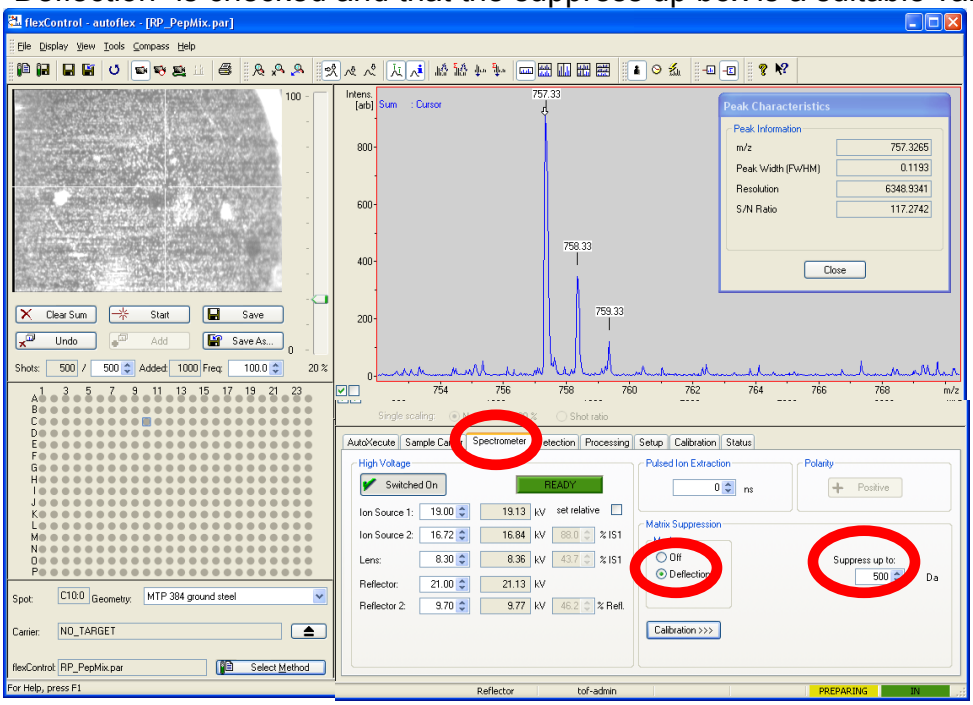
Step #4: While sample is loading, click “Select Method” button from the starting screen.
Select the method appropriate for your sample:
The naming convention is for basic operation “RP_(0.5k-4kDa).par”
First character = TOF mode: L = linear / R = Reflectron
Second Character = polarity: P = positive / N = Negative
Third phrase = optimized and calibrated molecular weight range

Step #5: Press “Open” to choose your method.

Step #6: Select the Sample Carrier Tab at the bottom of the screen. Ensure that “Off” is checked for Mode.



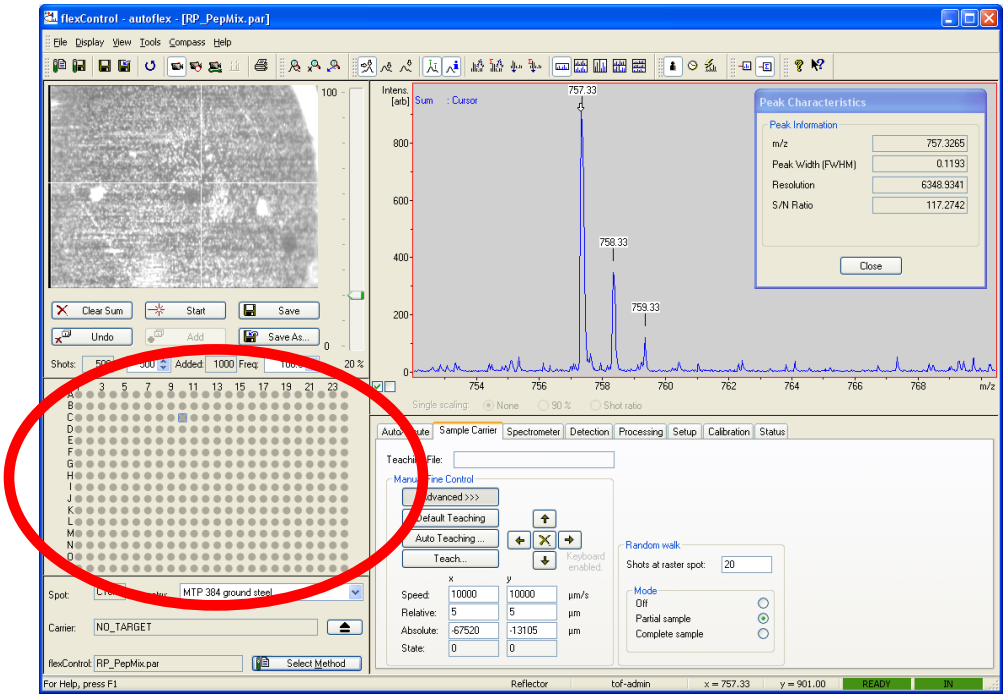
Step #7: Select the Spectrometer Tab at the bottom of the screen. Ensure that “Deflection” is checked and that the suppress up box is a suitable value for your sample.



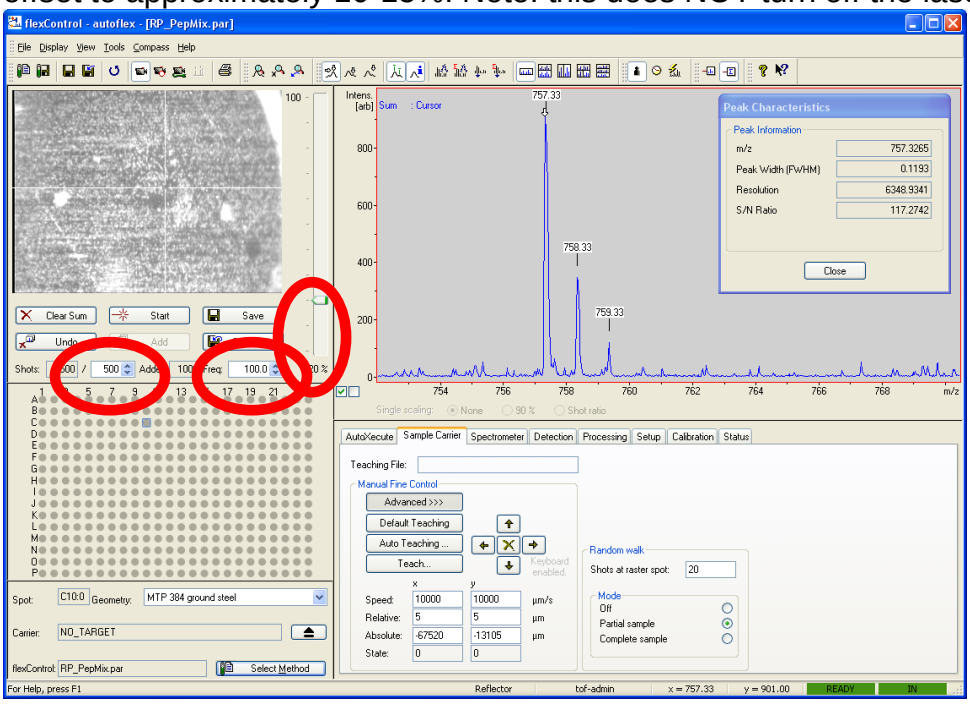
Step #8: Select the Processing Tab at the bottom of the screen. Ensure that the file name at the bottom is an IMSERC file. If it is not, then contact the appropriate MS personnel.

Step #9: Select the Setup Tab at the bottom of the screen. Set the Laser Power and the Offset to suitable values for your sample. ALWAYS start at a low power and then increase it if needed.

Step #10: Above where you had previously selected your method, select the correct position of your sample. Remember: the part of the plate that goes into the MALDI first is on the right of the screen. (Image is rotated clockwise 90 degrees)



Step #11: Above where you had previously selected your sample position, select an appropriate number of shots and frequency of shots for your sample. Also, set the laser offset to approximately 10-15%. Note: this does NOT turn off the laser.

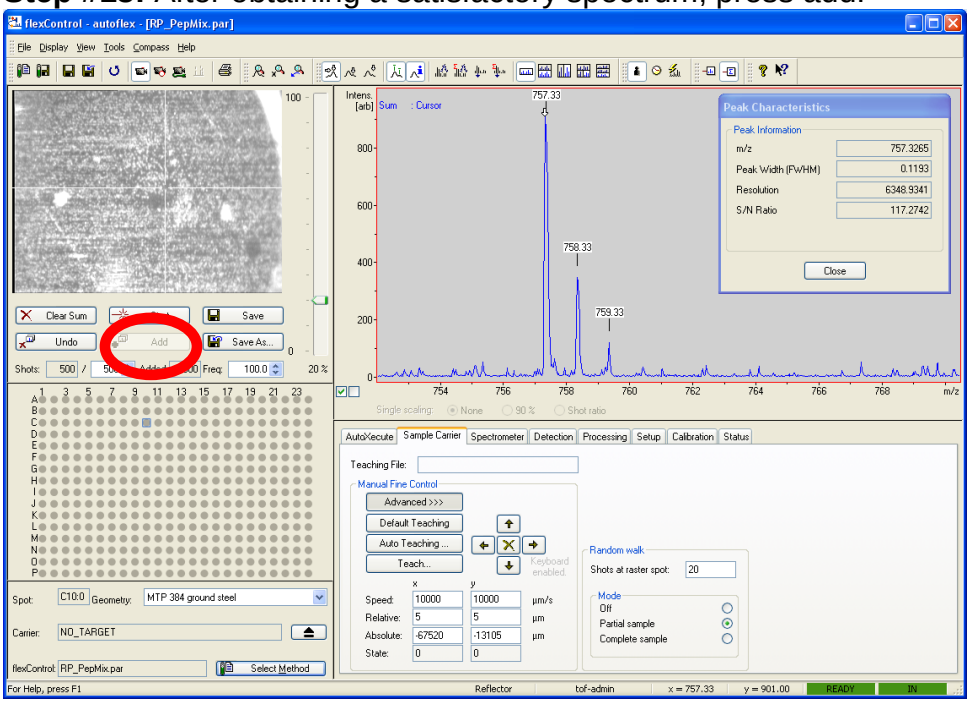


Parameter	Allowable Values	Description / Warning
Laser Power	0-50%	Remember that higher laser power leads to broad and unresolved peaks
Shots	Any	Sum shots to build signal with lower power (multiple spectra can be added)
Frequency	0-200	Use low frequencies to move around and see changes
Sample Carrier (Random walk)	Shots / spot Mode (on / partial / off)	If sample is being depleted, use random walk to automatically move to a new spot
Spectrometer	Do not change	Settings are optimized for selected mass ranges
Detection	Use default Window	Zoom in later

Before any parameter is set outside the limits in this table, approval must be obtained from IMSERC staff members. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees or removal of IMSERC privileges

Step #12: Press start. Move the crosshairs around the sample (left click with mouse) to find a good spot. You may also need to change the aforementioned settings. Note: if you are getting a “hump” near the left of the spectrum, you are likely using too much laser power.

Step #13: After obtaining a satisfactory spectrum, press add.



Step #14: You may choose to run additional scans, pressing add each time to sum them. Otherwise, go to File → Save As, and save your data. **IMPORTANT: DO NOT just press Save.** <u>This overwrites the prior spectrum, even if it is not yours</u>.

Step #15: Press the Eject Button in the starting screen (or the large green button on the side of the MS-MALDI.

Step #16: Remove your sample, then insert the plate back into the MS-MALDI. Make sure for the correct plate loading orientation!!!

Step #17: Press the Eject Button in the starting screen (or the large green button on the side of the MS-MALDI. (Plate should be inside the MALDI at the end of your session)

Step #18: Take your sample back to your lab and dispose of it there.

Method Information Table

Linear Method Constants

Approximate Mass	IS1	IS2	Lens	Delay
<1600	20	18.55	9.0	0ns
6000	20	18.20	9.0	130ns
9000	20	18.10	9.0	170ns
12K	20	17.90	9.0	190ns
17K	20	17.80	9.0	220ns
25K	20	17.80	9.0	220ns
66K	20	17.50	9.0	240ns

Reflector Method Constants

Approximate Mass	IS1	IS2	Lens	Delay
<1000	19	16.90	9.0	0ns
2000	19	16.85	9.0	80ns
3000	19	16.75	9.0	210ns
6000	19	16.60	9.0	230ns
12K	19	16.35	9.0	360ns
17K	19	16.15	9.0	420ns

Initial Starting Method Constants

Method File Name	IS1	IS2	Lens	Refl.	Refl 2	Delay
RP_(0-1kDa)	19	16.72	8.3	21	9.7	0
RP_(0.5k-4kDa)	19	16.53	8.49	21	9.7	0
RP_(3k-6kDa)	19	16.72	8.55	21	9.7	150
LP_(0.5k-4kDa)	20	18.6	7.0	N/A	N/A	0
LP_(2k-20kDa)	20	18.5	8.5	N/A	N/A	150
LP_(10k-150kDa)	20	17.85	9	N/A	N/A	300
LP_(30k-300kDa)	20	17.86	9	N/A	N/A	500
RN_(0-1kDa)	19	16.75	7.5	21	9.7	0
RN_(0.5k-4kDa)	19	16.76	8.3	21	9.7	0
RN_(2k-10kDa)	19	17.1	8.55	21	8.4	250
LN_(0.5k-20kDa)	20	18.1	8.5	N/A	N/A	150

New Methods vs Old Methods

New Methods	Old Methods
RP_(0-1kDa).par	
RP_(0.5k-4kDa).par	RP_PepMix.par
RP_(0.5k-4kDa)_AnchorChip.par	RP_PepMix.par
RP_(0.5k-4kDa)_NALDI.par	RP_NALDI.par
RP_(3k-6kDa).par	RP_ProtMix.par
RP_(3k-6kDa)_AnchorChip.par	
LP_(0.5k-4kDa).par	LP_PepMix.par

LP_(2k-20kDa).par	LP_ProtMix.par
LP_(10k-150kDa).par	LP_66kDa.par
LP_(30k-300kDa).par	
RN_(0-1kDa).par	
RN_(0.5k-4kDa).par	RN_PepMix.par
RN_(2k-10kDa).par	
LN_(0.5k-20kDa).par	LN_ClinprotMix.par
	LN_PepMix.par
	LN_ProtMix.par

Tips for MALDI analysis

- If you observe poor resolution with a linear mode of operation (only), you must check whether "Turbo" checkbox is checked or not. You can find the option under Detection Tap>Detector Gain>Turbo. Disable the Turbo checkbox for better resolution. This option is only good for MW over 50 kDa, enhancing sensitivity of linear detector by compromising resolution. We found this option remains enabled regardless of loading a new method if a previous user had enabled. This will decrease mass resolution significantly for MW below 10 kDa if enabled.
- For users who analyze proteins above 100 kDa, you may have better sensitivity by increasing "High Mass Accelerator" up to 8.0 kV under Detection Tap>Detector Gain>. But mass resolution and S/N ratio will be significantly decreased as the HMA voltage increases.
- If the baseline increases too high along with increased laser energy, change the electronic gain to “Regular” under Detection>Electronic Gain>. The default value is “Enhanced”. You may have better signal sensitivity with “Highest” setting for MW above 10 kDa by sacrificing signal-to-noise ratio.

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