

IMSERC User Manual for Bruker RapiFlex MALDI-TOF

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IMSERC User Manual for Rapiflex MALDI-TOF

INTRODUCTION

MALDI-TOF Is designed to provide molecular weight and isotope pattern information for ionizable molecules ranging from approximately mass 150 to several hundred thousand daltons. It is a “soft-ionization” technique, ionizing the target molecules with minimal or no fragmentation. MALDI provides an alternative analysis pathway for molecules not suited for other mass spectrometry techniques/molecules not volatile and hence suited for GCMS; Species not ionizable using electrospray ionization (ESI) or easily soluble in solvents suitable for ESI.

MALDI-TOF is suitable for analyzing a wide range of molecules; synthetic small molecules, proteins, oligonucleotides, and polymers; providing molecular weight information, as well as monomer size and polydispersity information for suitable polymers.

Using the IMAGING capability of the instrument, one can also characterize tissue or materials by correlating the presence of specific species to specific local position on the surface of the material or tissue cross-sections, providing information about geographical distribution of different species, in the target sample.

All samples Need to be prepared usually by mixing with an appropriate Matrix to assist in ionization and desorption of the material. Please consult with the staff about Matrices, and appropriate sample preparation.

Use of this instrument is allowed only by qualified users after receiving training by a staff member. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges.

This short set of instructions is meant to serve as a guide for ‘routine’ data collection on the instrument. Please read this standard operating procedure and acquaint yourself with the instrument. If during the course of using the system, something happens that you do not understand, please **stop** and **get help**. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable.

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SAFETY

All users of IMSERC must review the general safety policies at <http://imserc.northwestern.edu/about-policies.html>.

Familiarize yourself with the location of standard safety stations like eye wash and shower stations found in just outside of BG70. Protective eyewear is required in this room, and gloves should be removed when using the computer.

Hazard	Location	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.

To become an independent user of this instrument, you must have the following safety training and certificates which are offered at <https://learn.northwestern.edu>:

- Laboratory Safety
- Personal Protective Equipment

Upon completion of the certificate, it will take an overnight to filter through the different systems and get into the files that NUCore uses.

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DATA MANAGEMENT

Your personal data folder is created during training which must be located under your supervisor's group folder. See a staff member if you do not have a personal folder on this instrument yet. Your personal file folder must contain your Lastname_Firstname. Inside this folder you are able to access your sample table containing your history of samples run, as well as the individual acquisition and processed data files.

It is recommended you save each sample run proceeding with the day's full date, then include your sample information. Over time, files will be archived to a larger file server to save local space on the local drive. If you do not use the designated file structure as outlined in this manual, it may result in a failure to backup your data.

Example: PI name >Lastname-Firstname > YYYY-MM-DD>sample

Specific Example: Einstein > Currie-Marie > 2020-01-01> test-1

Data on this instrument are copied on 'imsercdata.northwestern.edu' under 'MS/MALDI-Rapiflex. Please follow instructions at <http://imserc.northwestern.edu/about-general-faq.html#data> for details about data access. Please remember to use the northwestern VPN when accessing data from a personal computer.

SOFTWARE

Data acquisition can be performed with FlexControl, data reduction and analysis are performed using FLEXAnalysis. Both icons to access each software are located on the instrument in the upper right corner of the computer screen.

For offline analysis after your instrument reservation is complete, please use the following resources:

- Analysis Software is also installed on a communal computer located in the area outside room BG51
- Spectrum data is observable through MNOVA and is available through the online communal server 'imsercterm.northwestern.edu'. You must have the correct patches for Bruker software loaded from MNOVA web site. You must be a registered IMSERC user and have permission to login to the server. Please email IMSERC-ofc@northwestern.edu for details

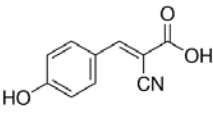
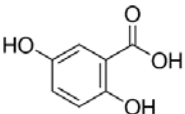
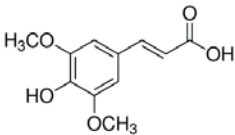
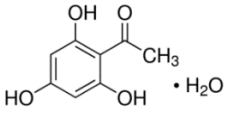
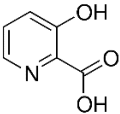
You have the option to use the instrument computer for analyses, but you must reserve instrument time through NUCore.

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SAMPLE PREPARATION

1. Decide if MALDI is the correct technique for your sample. Talk to IMSERC Staff if you have questions.
2. Choose the matrix for your sample (consult with IMSERC staff or utilize the available chart by instrument):

Sample	Matrix
Peptides < 10kDa	α -Cyano-4-hydroxycinnamic Acid (CHCA)
Proteins > 10kDa	Sinapinic Acid Super DHB
Oligonucleotides	3-Hydroxypicolinic Acid (HPA) 2,4,6-Trihydroxyacetophenone Monohydrate (THAP) 2,5-Dihydroxybenzoic Acid (DHB)
Polymers	2,5-Dihydroxybenzoic Acid (DHB)
Carbohydrates (Neutral)	2,5-Dihydroxybenzoic Acid (DHB)
Glycosylated Protein	Super DHB

Matrix Structures				
				
CHCA	DHB	Sinapinic Acid	THAP	HPA

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Matrix Preparation	
Matrix	Preparation
α -Cyano-4-hydroxycinnamic Acid (CHCA) Sinapinic Acid (SA)	50% Acetonitrile, 0.1%TFA in DI water, saturated
DHB	10 mg/ml in water, 50% ACN or other appropriate solvent for analyte
THAP, HPA	50% Acetonitrile, 0.1%TFA in DI water, saturated

- Obtain an appropriate sample plate to spot your sample on.
 - Coin Chips are available from IMSERC staff for \$65.
 - Large plates with 384 spots are available if you need to run much larger sample sets.
- Make a solution of **no more than approximately 10% sample to matrix by mass**. (Disregard solvent for this calculation)
- Apply solution to plates. (**NO MORE THAN 0.5 μ l per spot** at a time)
- Dry the sample before coming to IMSERC (solvent must be evaporated)
- Do not run** any samples that are **not fully dry**, or if they are not crystalline or powdery. **Do not run** samples that are paste like, oily, gooey, etc. This will cause damage to the instrument.

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 and potential damage to instrument will result from wet samples
2	Begin reservation in NUcore Login system	
3	If needed, log into computer	Login Name: flex-user Login Password: youshouldknow
4	Start Flex Control	Log in as tof-user (you don't need to do anything) No password is required
5	Check status lights on instrument	WHITE: Status is OK. RED indicates major error-report to staff. In software: System must be in "Ready" or "Warm-up" mode

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QUICK START ACQUISITION CHECKLIST

1. Begin reservation in NUCore.
2. Introduce sample into instrument through sample docking.
 - a. Place sample plate or Coin chip on carrier plate.
 - b. Place Sample carrier Plate in instrument docking bay.
 - c. Dock the sample.
 - d. Make sure correct plate geometry is chosen.
3. Choose sample spot and location on the spot.
4. Choose method.
5. Make sure Sample carrier movement is set to manual not Auto-Random walk.
6. Verify Mass Range and Mass Suppression. (Low mass cut off)
7. Choose your sample and spot position.
8. Set laser power.
 - a. Start from Laser power loaded in method or a slightly lower setting.
9. Collect the data.
 - a. Raise the laser power slowly during data collection to collect a spectrum with adequate signal.
10. Add any spectra which you want to keep and to build on, into Sum buffer.
11. Toggle peak position (m/z information) and peak resolution information if desired.
12. Save your data (Sum or Signal buffer) and open in FlexAnalysis.
13. Go to Flexanalysis to do data analysis, and to print data.
14. End Reservation in NUCore.

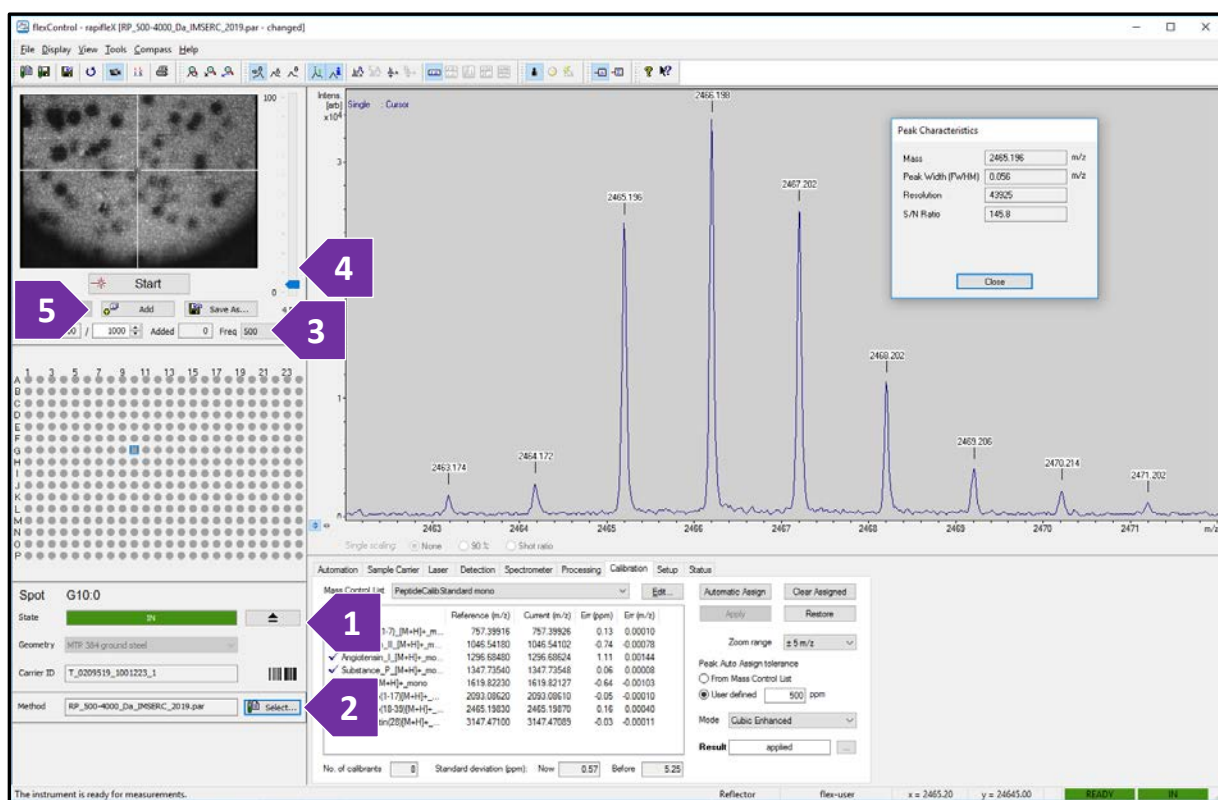
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DATA ACQUISITION – OVERVIEW

1. The computer screen is by default deactivated. You must start your reservation through NUCore to be able to turn on the computer screen. If screen is already on, start your reservation through NUCore.

2. Open FlexControl (if it is not open). You can select the FlexControl icon  located on the top right screen or on the lower tool bar.

Overview of the FlexControl Starting Screen:



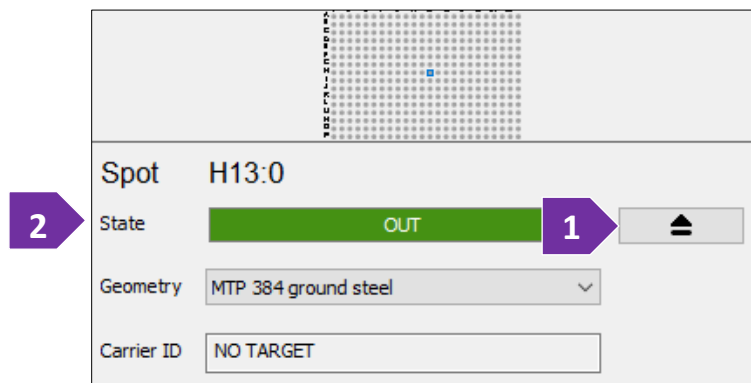
- [1] Plate Docking
- [2] Method Selection
- [3] Number & Frequency of Laser Shots
- [4] Laser Power Control
- [5] Start/Stop Scan

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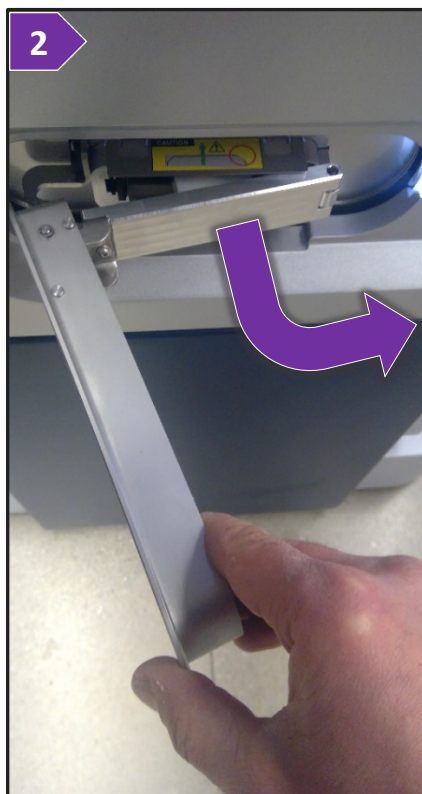
DATA ACQUISITION

1. Loading your Sample:

- Check to see the state of the target: If it is **IN** or **OUT** of the instrument.
- If target is IN, undock the target by clicking the 'State' button (1) and wait until State reads OUT (2).



- When the target state is "**OUT**", open tray door, to access target plate. Open the door from the right hand corner and pull out gently (1). The door will pivot on the left, then you will be able pull the door out and to the right (2).



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2. Load the Sample:

- a. Either the 10 spot 'Coin Chips' or the large '386 Ground Steel' Target plates can be used.



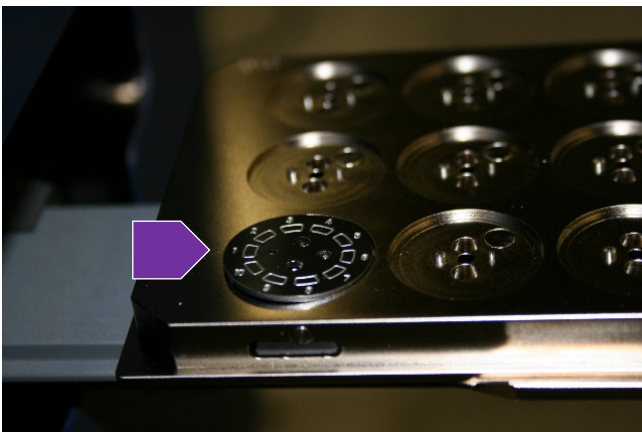
MTP MultiProbe Adaptor



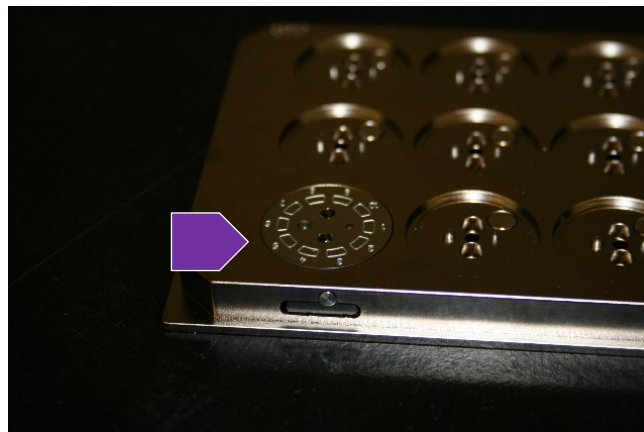
MTP 384 Ground Steel

- b. If using the **"CoinChip"** Multi-probe Adaptor, make sure the Round **"CoinChip"** targets are put onto the carrier correctly:
 - i. Remove Sample Plate.
 - ii. Place **"Coin Chip"** on the plate/carrier.
 1. The plate has cutouts for the coin-chips. They are magnetic. So always use both hands. With one hand hold the multi-probe adaptor onto the surface, with the other hand use the metal forceps to put the coin into place.
 - iii. Sample **"coin chip"** is **keyed to only fit one way**.
 - iv. Ensure **"Coin Chip"** sits **flush** with the top of the carrier and sample plate is flat.

WRONG: Sample may scratch sealing surface and lock!



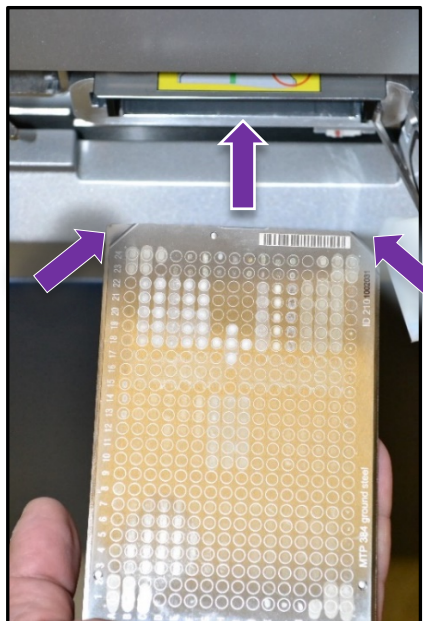
Correct: Sample plate is flat



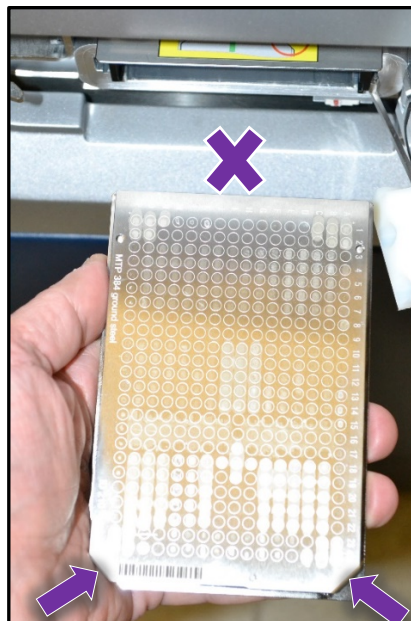
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- c. **Please note:** Direction that the plate is inserted should be correct: The Plate end with **diagonal cutout points** goes into the instrument first.

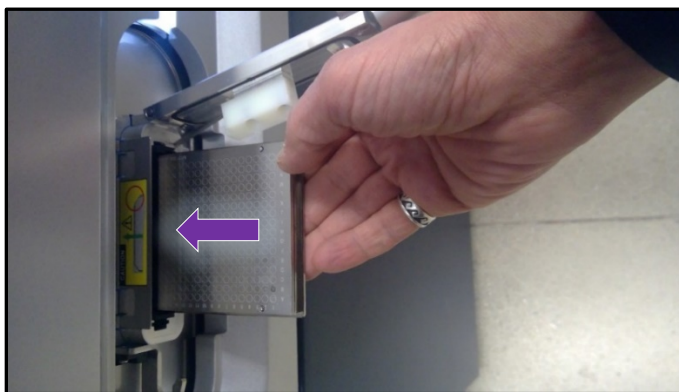
Correct



Incorrect



- d. Slide the Target Plate in:



- e. Close the door. Press the door to ensure it is latched all the way. Otherwise it will show a docking error.

- f. Press the Docking Button  in the starting screen, to load the tray.

- g. The Software will show "Docking" message  until tray is in.

You can abort the Scan Barcode/Height Check  to save time.

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3. Choose the correct **plate geometry**. If the instrument has not automatically chosen the correct plate geometry, Use the drop down menu to do so.

Use: **MTP Multi-Probe Adapter** or
MTP 384 Ground Steel

4. **Method Selection:** Click “Select Method” button from the starting screen. Select the appropriate method for your sample:

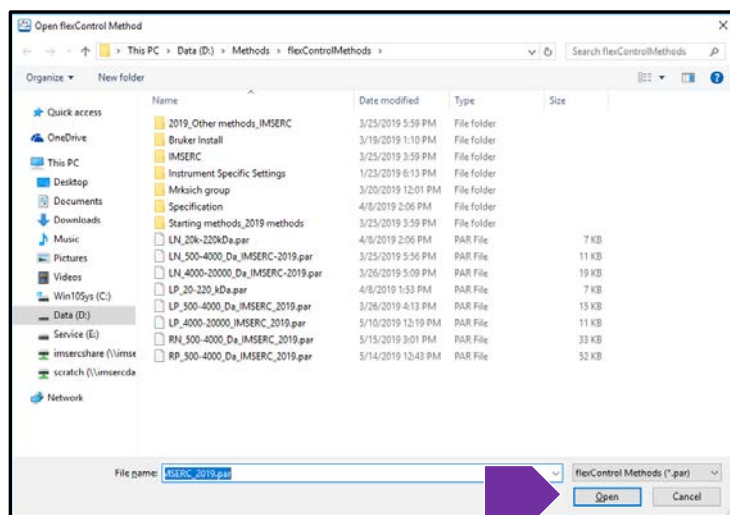
Spot	1:6	
State	IN	
Geometry	MTP Multiprobe Adapter	 
Carrier ID	G_65712ab8-ae2a-4a27-9101-ec0633db67e7	
Method	LP_4000-20000_IMSERC_2019.par	  Select...

The naming convention for basic operation is detailed below. For example: “RP 0.5k-4kDa.par”

First character TOF mode: **L = linear / R = Reflectron**

Second Character Polarity: **P = Positive / N = Negative**

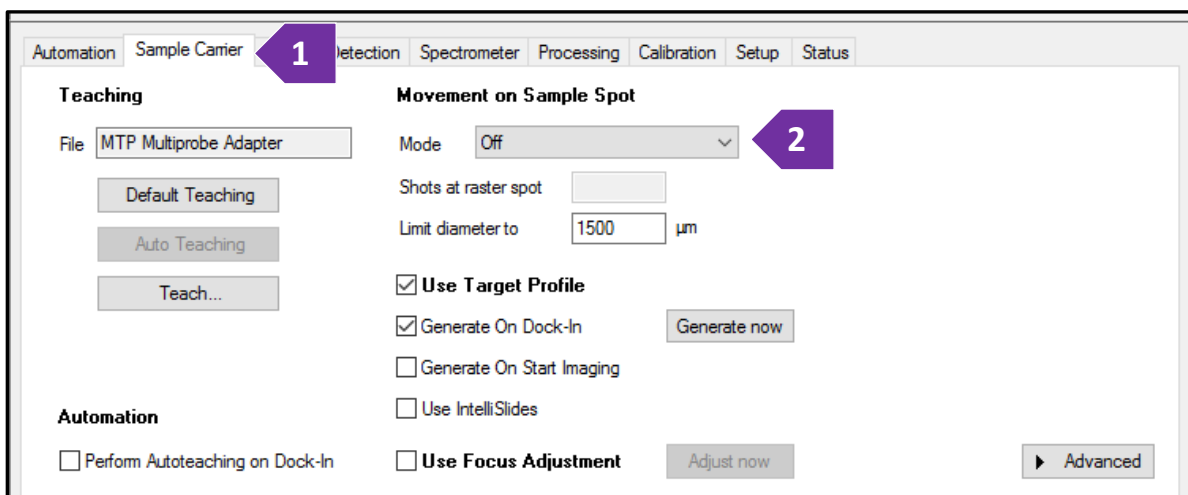
Third phrase Optimized and calibrated **molecular weight range**



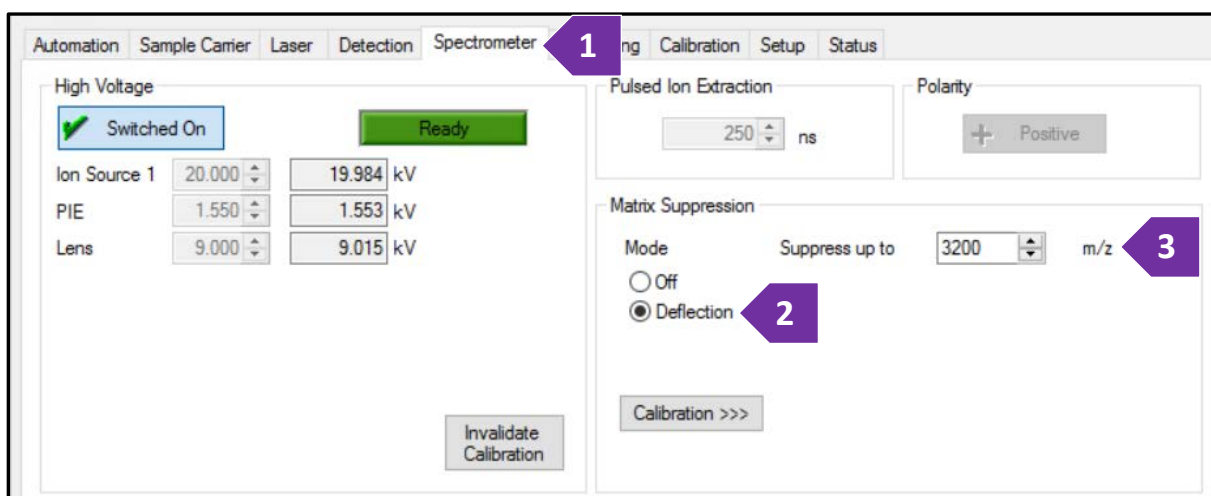
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5. Set **Movement on Sample Spot**: (1) Select the *Sample Carrier Tab* at the bottom of the screen. (2) Ensure that “Movement on Sample spot” mode is set to **Off**.

This will allow you to choose the spot manually rather than instrument changing and moving the laser spot automatically.

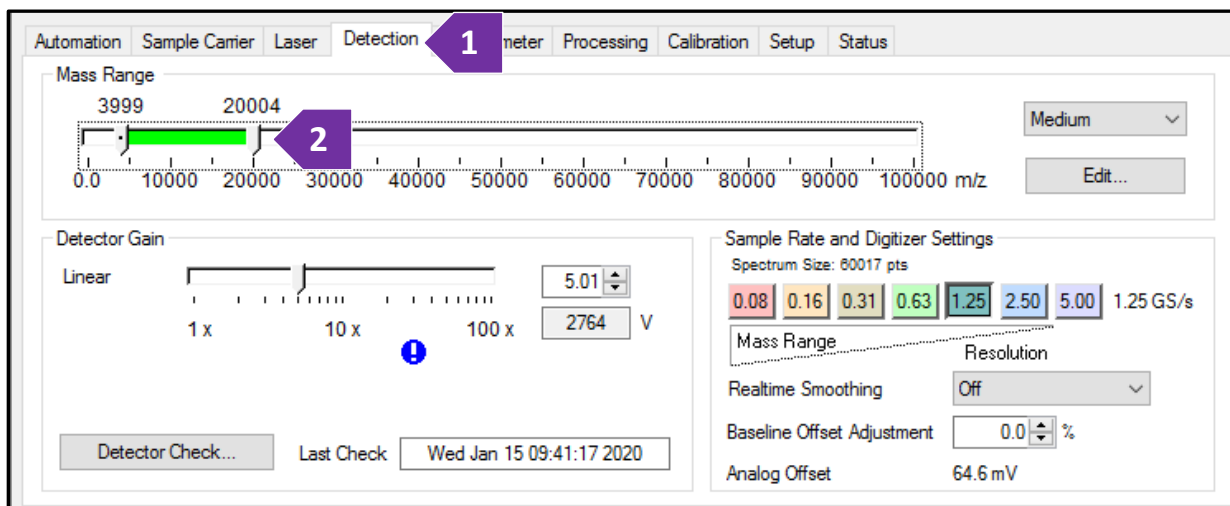


Matrix Suppression: This value sets the limit to which ions are deflected and not seen suppressing the high matrix ions at the low end. Select the *Spectrometer Tab* (1). Ensure that under Matrix Suppression “Deflection” is checked (2) and that the Suppress up box is a suitable value for your sample (3).

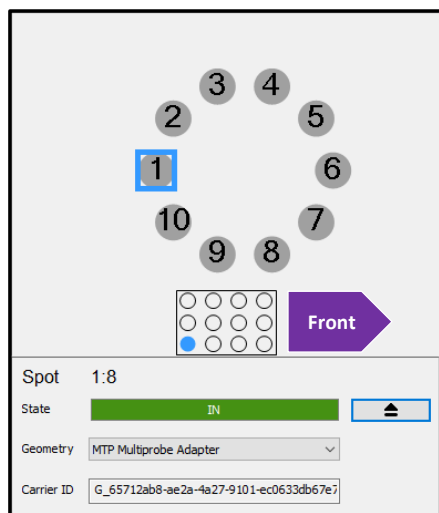


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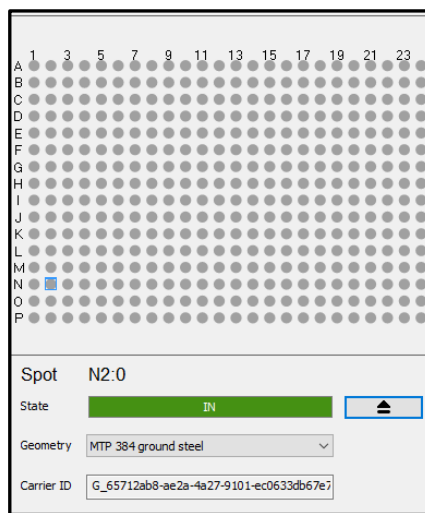
6. Set the **Mass Range**: Choose the Detection Tab (1) then choose the mass range to look at (2). The 'Deflection' setting in the spectrometer tab still controls suppression of the ions at the low end of the Mass Range. It will suppress ions no matter what "viewing" mass range is set on the 'Detection' tab.



7. **Sample Position**: 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)



MTP MultiProbe Adaptor

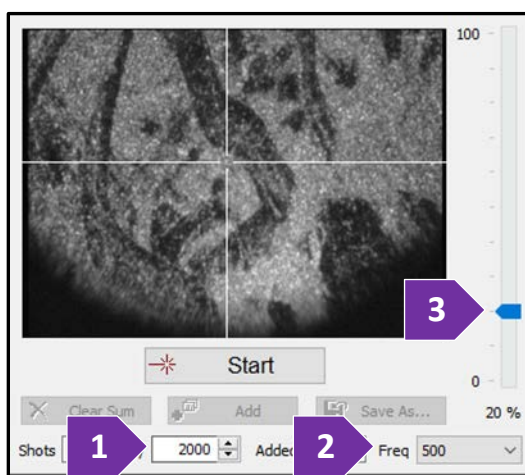


MTP 384 Ground Steel

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8. Set the **Laser Power**, number of **Shots** and laser shot **Frequency** located above where you have previously selected your sample position. Select an appropriate number of shots (1) and frequency of shots (2) for your sample. Also, set the laser offset to approximately 10 - 15% (3). Note: this does NOT turn off the laser.

ALWAYS start at a low power and then increase it if needed!



Typical Settings:

Parameter	Allowable Values	Description / Warning
Laser Power	0-80%	Remember that higher laser power leads to broad and unresolved peaks
Shots	Any (500-1000 Best)	Sum shots to build signal with lower power (multiple spectra can be added)
Frequency	200-2000 (1000 Best)	Use low frequencies to move around and see changes
Sample Carrier (Movement on spot)	Mode (on / partial / off)	Mode: OFF
Spectrometer	Do not change	Settings are optimized for selected mass ranges- Can override Ion Suppression
Detection	Use default Window	Zoom in later

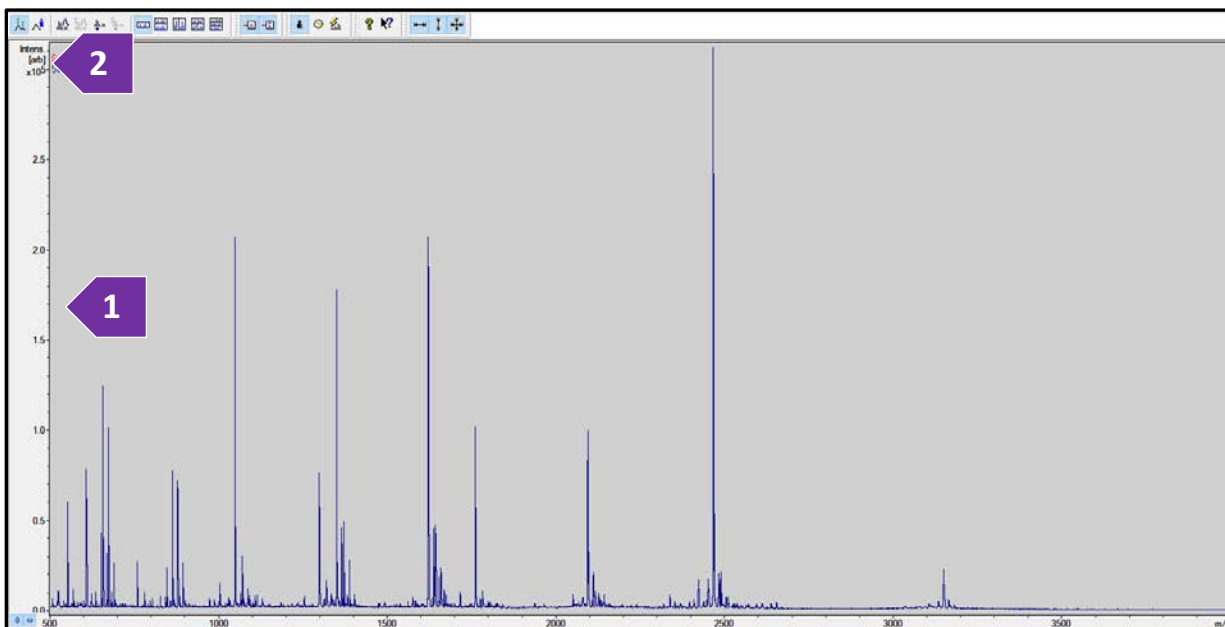
IMPORTANT: Before any parameter is set outside the limits in this table, approval must be obtained from IMSERC-MS staff members. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees or removal of IMSERC privileges

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9. **Data Collection:** Find a good spot with good density of sample to start with. Press  **Start**. You can move the crosshairs around the sample (left click with mouse) to find other good spots. You can also change laser power using the slide tab which can be controlled with the wheel on the mouse.
- Watch the Intensity on Y-axis (1).** The intensity of the peaks should build up progressively and smoothly.
 - If it rises **very rapidly** and in an uncontrolled manner, you are likely using **too much power**.
 - Max signal should be **in the low 10^4 (2)**. Anything over that is TOO MUCH. You are likely using too much laser power.
 - If you are not getting any peak, but horizontal plateaus, either you do not have enough power, or sample is not flying. It is possible the sample or matrix is unsuitable or is not prepared well.

You may also need to change the aforementioned settings.

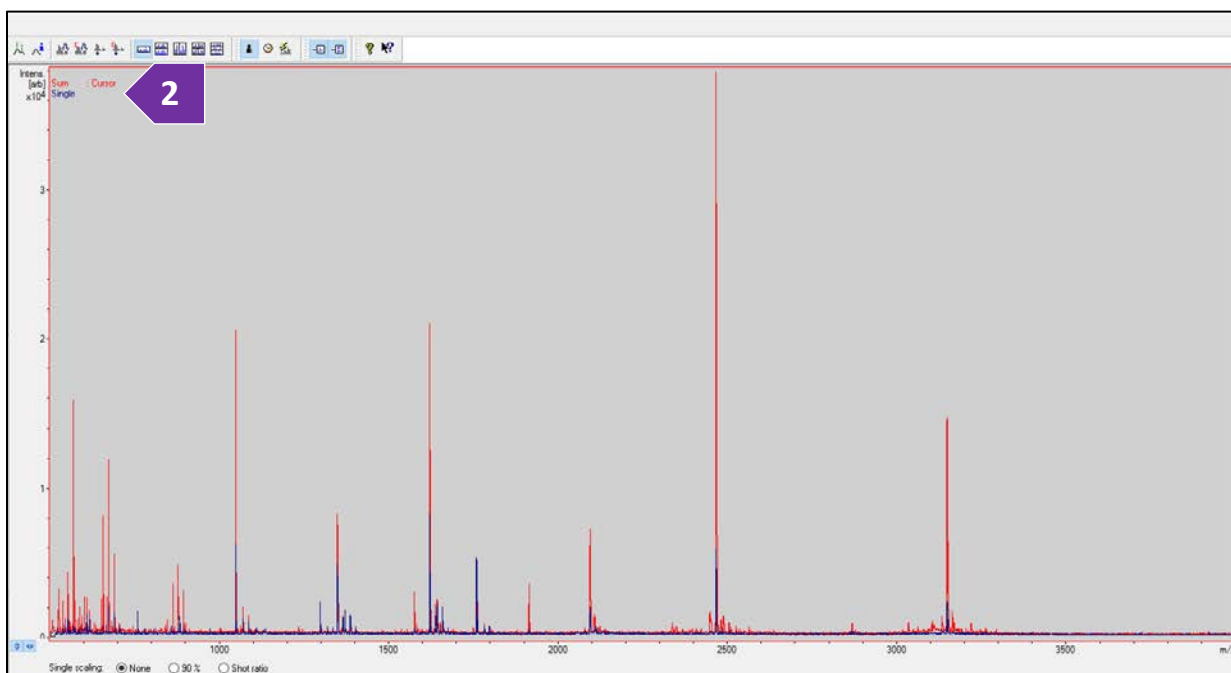
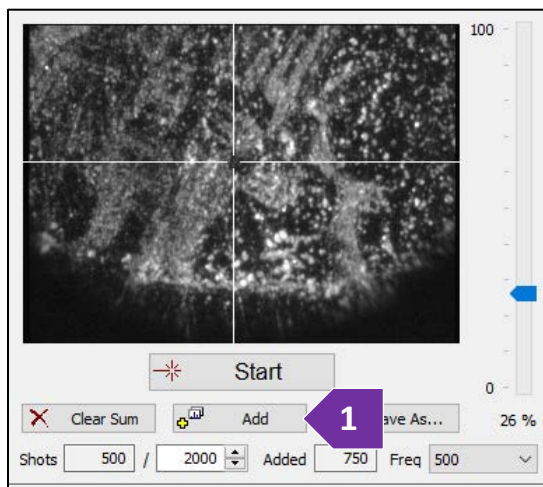
*Note: if you are getting a "hump" near the left of the spectrum, you are likely using **too much laser power**.*



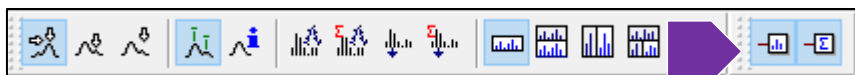
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10. **Adding spectra to Sum Buffer:** After obtaining a satisfactory spectrum, press **Add**  **Add** (1)

to move spectrum to **SUM** buffer . You can add multiple spectra together in the SUM buffer to build a higher cumulative intensity. Once the first spectrum is added, visible as RED, to the SUM buffer (2) you can collect more spectra and continue to add them if they are satisfactory. You can skip adding any spectra from the SINGLE buffer, visible as blue, which is deemed not high quality.



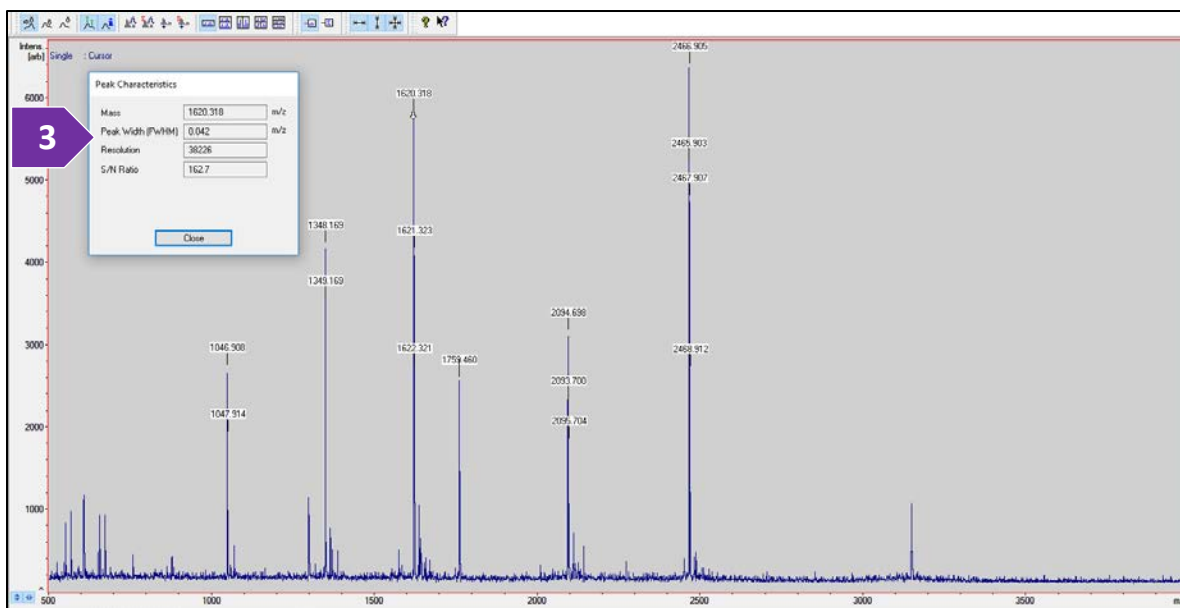
SINGLE  and SUM  Buffer spectra displays can be toggled On or Off using the buttons in the upper tool bar.



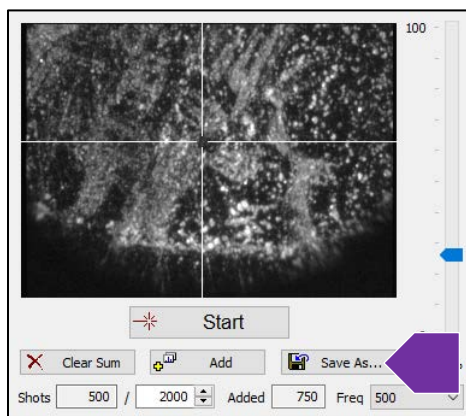
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11. **Peak position and Peak Information:** Maximum cursor Left  button (1) is used to **tag any peak**.

It will automatically lock on and pick any peak to its RIGHT as long as it is higher than the cursor position. 'Peak Position'  and 'Peak Information'  buttons (2) can be used to tag peaks and provide position, peak width, resolution, and S/N information for any tagged peak. Peaks must be tagged to get the information (3)



12. **Saving Spectra:** You can save spectra from either SUM or SINGLE buffer. Click the  Save As... button.



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You will have options to:

- Save either spectrum from either SUM or SINGLE buffer (1).
- Open the saved spectrum in Flex Analysis automatically or later manually (2).
- Chose the file Path (3) and Sample Name (4).

The screenshot shows the 'Save Spectrum' dialog box. It has several sections: 'Buffer' with radio buttons for 'Single' and 'Sum' (callout 1 points to 'Sum'); 'Comments' with two text input fields 'Line 1' and 'Line 2'; 'Processing' with a 'Process with' dropdown set to 'none', a checkbox for 'Open in flexAnalysis' (callout 2 points to this checkbox), and a 'Run' field; 'Path' with a text input field showing 'D:\Data\IMSERC' and a 'Browse' button (callout 3 points to 'Browse'); and 'Sample Name' with a text input field showing 'Cali_RP_CHCA' (callout 4 points to this field). At the bottom are 'Save' and 'Cancel' buttons.

13. When finished:

- Press the docking button to eject the plate.
- Remove your sample and close the tray door.
- Take your sample back to your lab and dispose of it there.
 - Be mindful of your chip location and DO NOT take the IMSERC calibration chip.

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DATA ANALYSIS

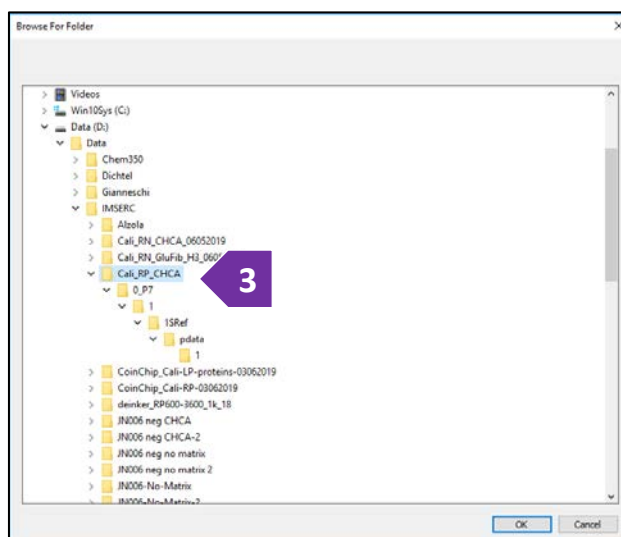
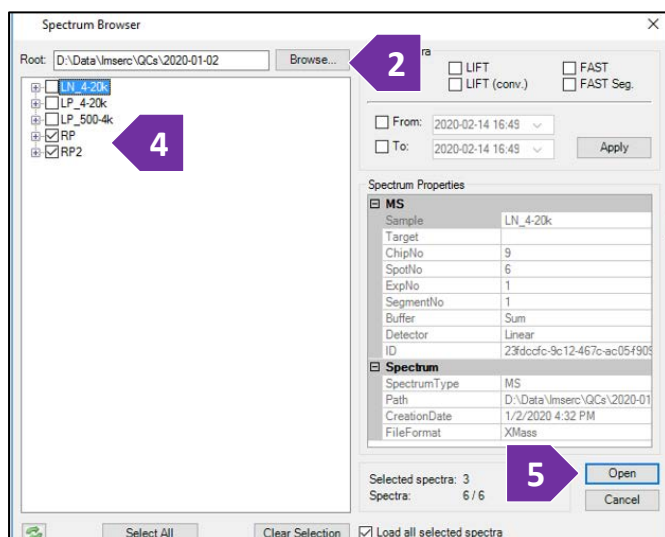
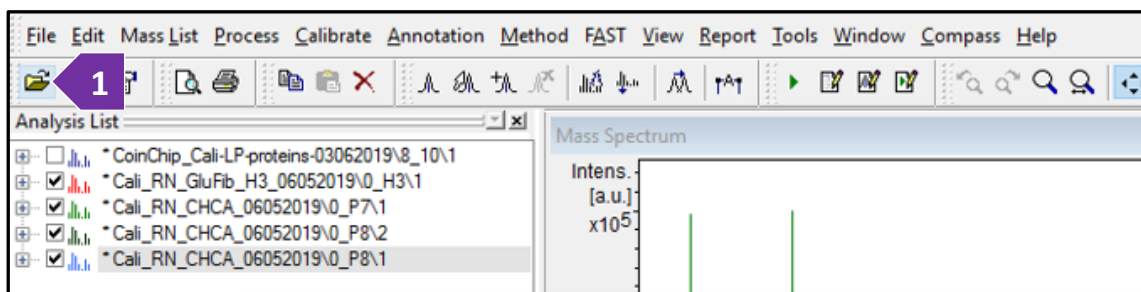


Use FlexAnalysis software for Data Analysis. Open it from the Desktop if not already open.

1. **Opening Files:** If you chose to automatically open the data files in FlexAnalysis when saving them, they will be open as soon as you click okay. If you did not, or if you need to re-open them, then choose “Open

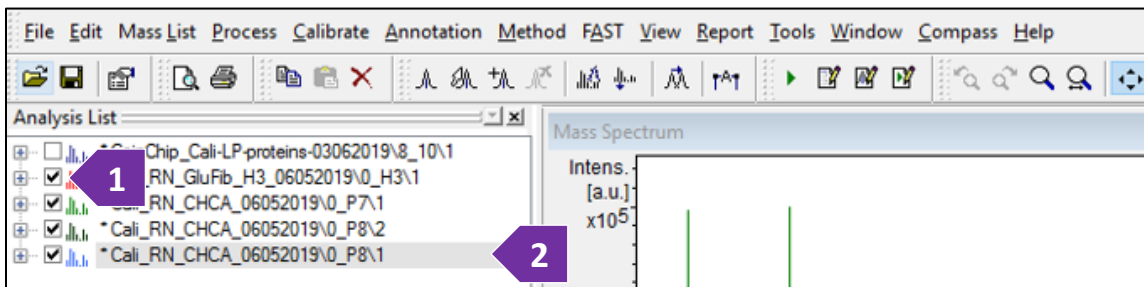
data file”  (1). To navigate to your file:

- a. In the dialog box use browse to go to your folder. Note that you can also search by date (2).
- b. In your folder, choose the data file you want to open. **Do not go into sub-folders** (3).
- c. In the dialog box click the file(s) you want to open (4) and Click “OPEN” (5).

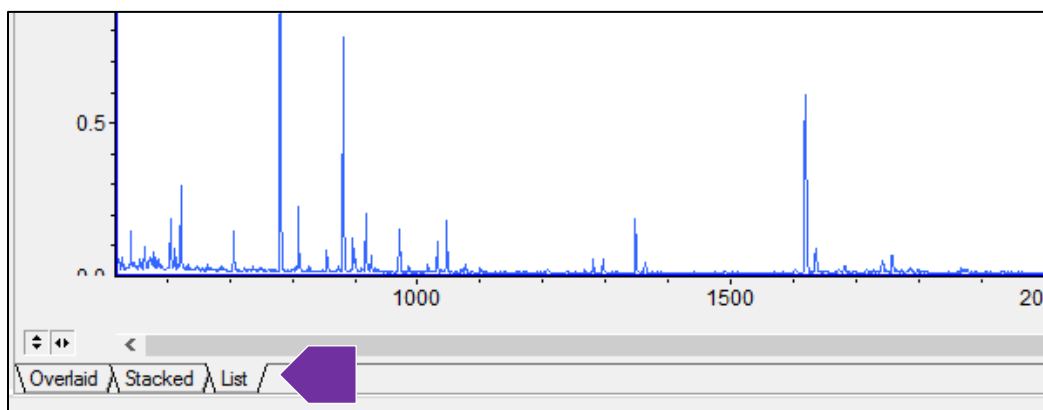


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2. **Displaying Files / Choosing files:** You may have multiple files open. The spectra can be displayed as overlaid or separately.
- Files can be toggled On and Off on the right side by Checking ☒ their individual boxes (1).
 - The “Highlighted” file is what is considered the “Active” file (2). All operations and processing will be done to the “active” file, even though others may be displayed concurrently.

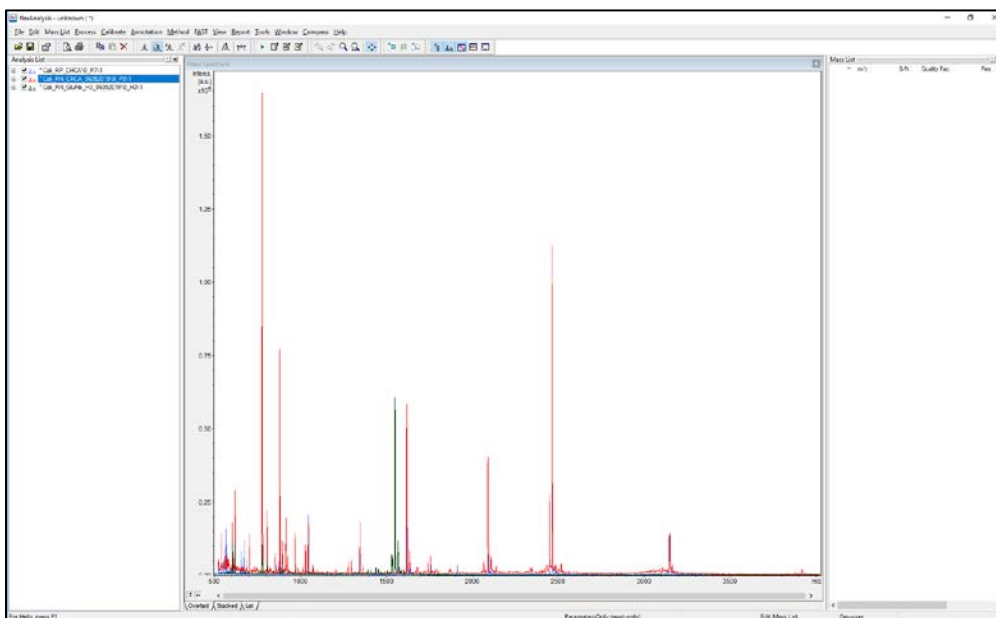


- File can be displayed in Overlaid or List mode (Stacked mode is less useful). The mode is chosen at the lower left corner of the spectrum display.

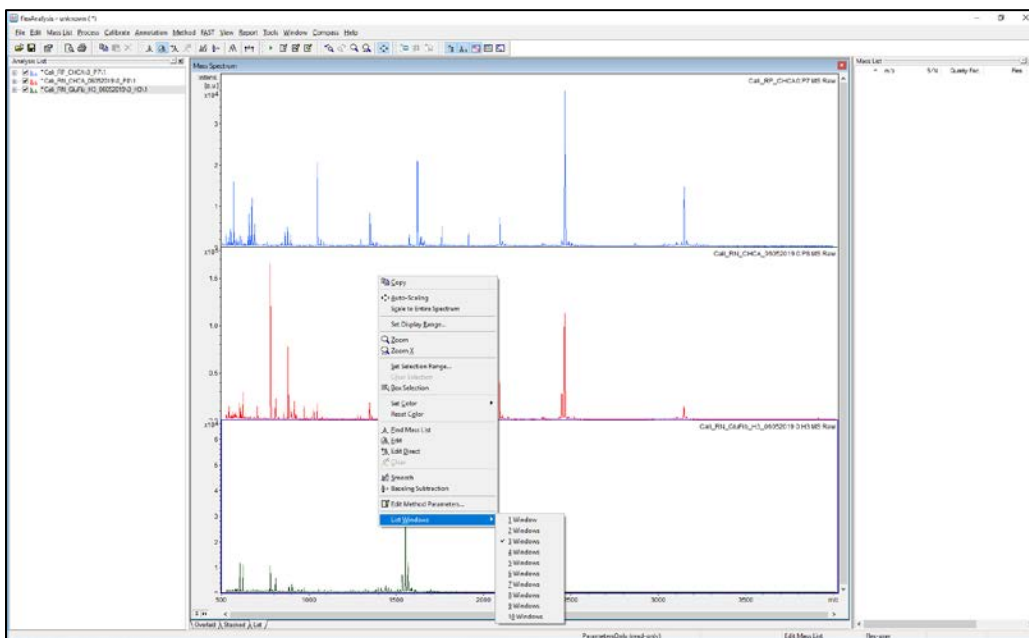


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- i. **Overlaid Mode:** Multiple files are overlaid. All files share and are scaled to a common Axis. Their intensities relative to each other are highlighted.



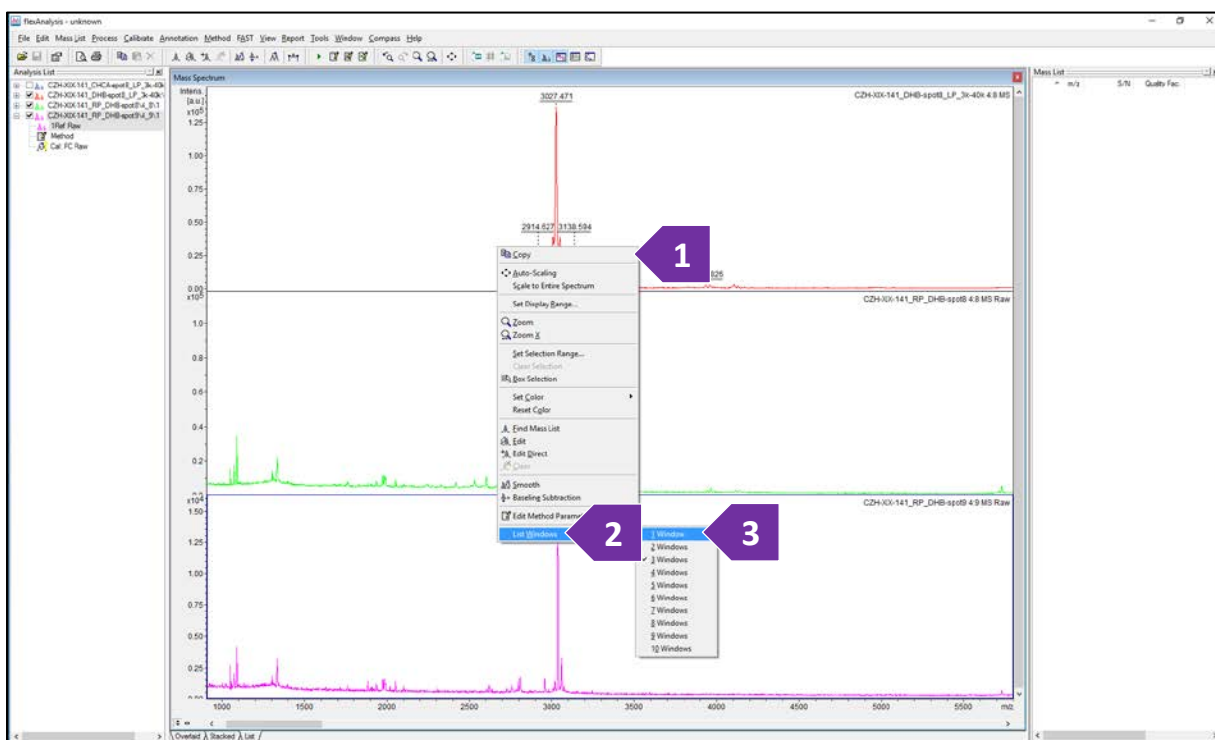
- ii. **List Mode:** Files are scaled independently and use independent axes. Keeping the y-axis scaled independently, qualitative differences in peaks seen is highlighted- presence or absence of; or increase or decrease of the intensity of certain peaks). Moreover, number of windows can be changed to match the desired number of spectra, using List windows option.



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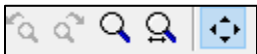
When using 'List' mode the number of windows displayed (with a different spectrum in each window) can be changed by:

- Right clicking in the spectrum (1).
- Choosing 'List Windows' (2).
- Choosing the number of windows to display, 1 to 10 (3).



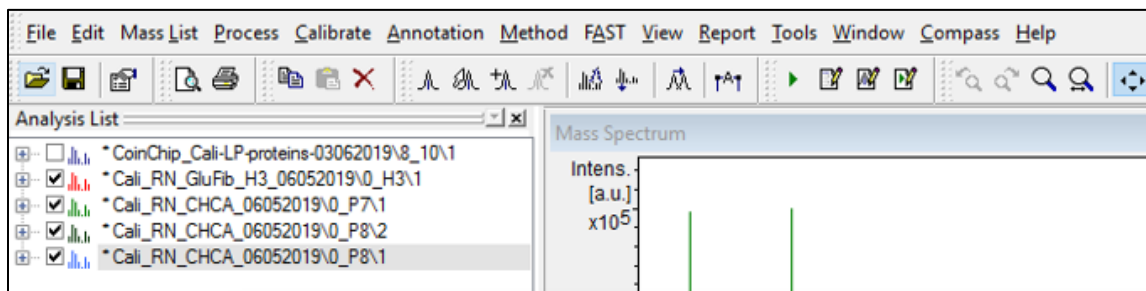
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3. **Zooming and Unzooming** can be done using the **zoom**  **unzoom**  and **auto-scale**  buttons.



But it is best done using the **Mouse buttons**:

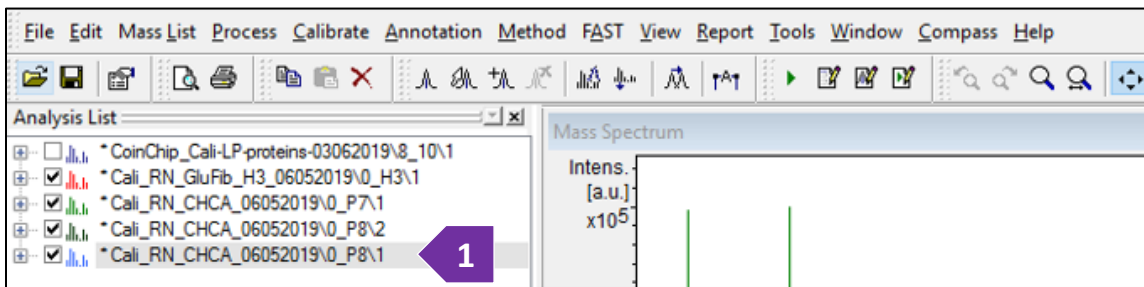
- Move the Cursor to just below the X-Axis to get 
- Use the **LEFT** mouse button to **MOVE** the spectra. (Hold down left mouse button with cursor under the axis)
- Use the **RIGHT** mouse button to **EXPAND** or **CONTRACT** the spectra. (Hold down left mouse button with cursor under the axis)
- Use the **WHEEL** to zoom in on the spot you have clicked on.
- Double click the Axis** to Auto scale back to **full** scale.
- Alternatively, you can use the Zoom tools    to zoom or unzoom, but this may require going back and forth between the spectrum and the tools.



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4. **Labeling peaks and Creating Mass Lists:** *Please Remember* all processing is done on the “ACTIVE” file (highlighted file), whether it is visible or not (1).

Please Note that you can have a file invisible (box is unchecked) and as long as it is still highlighted in the file list, all operations are still going to be done on that file- not any of the VISIBLE files.



a. **Mass list generation:**

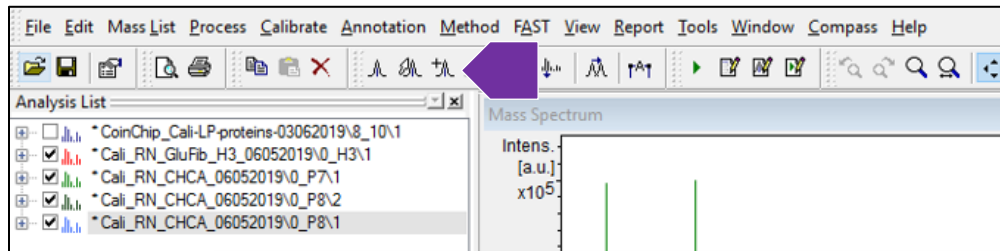
- ii. **Automatic** - A list of all the peaks can automatically be generated by clicking the Mass

List icon .

- iii. **Manual** - Individual peaks can be labeled and added to the mass List by choosing Edit

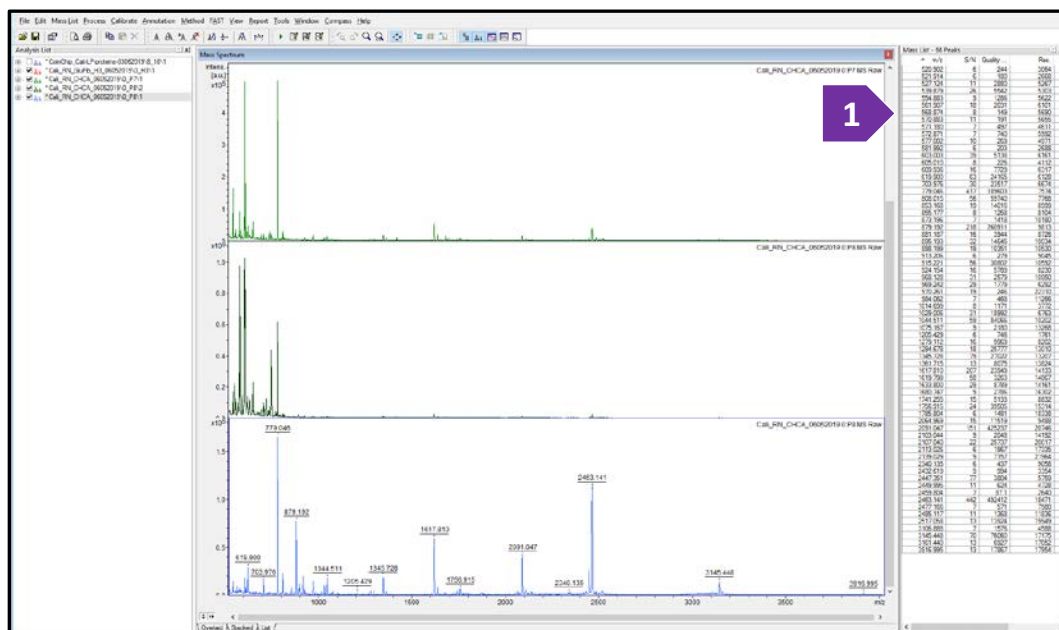
Mass List icon . Peaks are chosen by moving the cursor to the left of any peak, and then dropping it to below the top of the peak. As soon as the cursor moves lower than the top of any peak to its right, it will automatically “grab” that peak, and one can save it to the mass list.

- iv. You can **Delete** the Mass List and start over at any time using the delete icon .



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The Mass list will be displayed on the left side of the spectrum for the “**ACTIVE**” file. It can be printed with the spectrum (1).



5. **Printing: A hardcopy or pdf** can be generated. Pdf's can be saved in your folders or other folders/drives such as the scratch drive. Printouts (whether harcopy or pdf) will include the scan you are seeing, the acquisition parameter information, and the Mass List for the “ACTIVE” file.

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PUBLICATION

EXPERIMENTAL SECTION

Mass Spectrum data was collected on Bruker Rapiflex MALDI-TOF, using FlexControl data acquisition software and processed using FlexAnalysis software for data analysis.

Sample Preparation: Users need to include pertinent information regarding Sample preparation- Matrix used, Solvents, and Ratio of Matrix to sample.

For Example: Sample was dissolved at concentration of [i.e.50ug/mL] in 50%Water/50% acetonitrile solution with 0.1 % TFA. [20ul] of sample was mixed with [20ul] of saturated CHCA matrix in Acetonitrile/Water solution. Please consult IMSERC staff with sample preparation questions, and how to report them,

ACKNOWLEDGEMENT

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“This work made use of the IMSERC at Northwestern University, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the State of Illinois, and the International Institute for Nanotechnology (IIN).”

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TROUBLESHOOTING

NUCORE / RESERVATION

1. The computer Screen will not Turn On?
 - a. Begin Your reservation in NUCore to initiate access to the instrument
1. There is an error with my reservation?
 - a. If you have already started your reservation using NUCore, please logoff by selecting the error reporting option and a brief description about the issue.
 - b. If you have not started your reservation using NUCore, please report problems with the instrument at <http://imserc.northwestern.edu/contact-issue.html> add place the 'Stop' sign near the instrument computer. 'Stop' signs are located at XXXX and online at the link above.
 - c. Email or talk to a staff member.

INSTRUMENT

1. The instrument shows an error - The lower right corner has red error label, and/or the instrument front lights turn red:
 - a. Fill in Bug Report.
 - b. CONTACT IMSERC STAFF:

Saman Shafaie sepehr@northwestern.edu

Arsen Gaisin arsen.gaisin@northwestern.edu

Gabby Allison gabrielle.allison@northwestern.edu

Ben Owen benjamin.owen@northwestern.edu
 - c. Put "Stop Sign" on instrument keyboard if staff is unavailable.

APPENDICES

REVISIONS

V1.0 2020/05/31	• Release of original version of USER MANUAL for Maldi-Rapiflex
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