Bruker Microflex MALDI Quick-Start Guide

User Name: administrator

Password: bruker12

For sample preparation guidance, see "MALDI-TOF Sample Preparation" or the "Bruker Guide to MALDI Sample Preparation).

All users should use the **UCSB MS - MALDI** Google Calendar to schedule appointments. This is also used for billing. Include PI and recharge code in title. (**Example**: Bishop, Dezmond - Buratto - ABC123)

Target plate spotting

1. There are two general use MALDI target plates in the MS Facility. Many groups have their own. The general use plates have sheets to track which spots have been used. If there is a fully used plate, please inform staff so that they can clean it.
2. To spot a sample, identify and record an available spot and deposit 0.5-1 µL of sample. Chloroform will spread out significantly which can lead to contamination, so ~0.5 µL is advised. The sample should ideally cover the entire spot, and can be gently spread with the pipette tip if necessary. Avoid touching the tip directly to the target plate as much as possible.
3. Allow samples to fully dry before inserting the target plate into the instrument.
    a. In the sandwich method, commonly used with the matrix sinapic acid, the matrix and analyte solution are spotted and dried independently to create a layer of the analyte between two layers of the matrix.
4. Do not forget to spot an appropriate calibration standard if there isn't a recently spotted one on the target plate (they will last several weeks/months once spotted). See "Calibration Standards" for more information.

Operating the MALDI instrument

1. The green IN/OUT button on the front of the instrument controls the target plate chamber. Press the green button to release the vacuum, which will take a few seconds. The LED display will cycle through the "In Progress" orange light.
2. When the green "Ready" light is lit, open the chamber and remove the blank target plate from the instrument and replace it with your target plate.
3. **GENTLY** close the target plate chamber to prevent damaging the instrument.
4. Press the green IN/OUT button to evacuate the target plate chamber. The LED display will cycle through the "In Progress" orange light. This will take a minute or two. When it is fully under vacuum, the green "Ready" light will be lit. This process can also be monitored from the control software.
5. Following operation, repeat this process to remove your target plate and replace the blank target plate. **The instrument should be kept under vacuum with the blank target plate inside the chamber when not in use.**

Operating the MALDI software

1. The instrument is controlled with the flexControl software. Both the computer and the software use the following login. **User Name: administrator Password: bruker12**

2. Select a method that is appropriate for your sample. Sample names include a two letter code; “L” for linear TOF or “R” for reflectron TOF and “P” for positive ionization or “N” for negative ionization. Following is a description, typically including mass range and/or calibrant used.
    a. Reflectron TOF gives better resolution but worse signal than linear TOF, and has a limited range up to a few kDa.
3. For many uses, methods can be found to use without making changes. If desired, the following parameters can be changed/tuned. **Do not overwrite methods**. You may save your settings as a new method if desired.
    a. AutoXecture: **No Changes**
    b. Sample Carrier: Under advanced, the random walk parameters can be changed if desired. **Do not change anything else.**
    c. Spectrometer: Matrix Suppression can be changed or turned off. This will suppress signal up to a certain mass if the Deflection option is selected. **Do not change anything else.**
    d. Detection: Adjust mass range/mode as necessary. Increase Sample Rate if the mass range allows. Electronic Gains can be increased and Realtime Smoothing can be added as desired. **Do not change Detectro Gains**.
    e. Processing: **No Changes**
    f. Setup: **No Changes**
    g. Calibration: Discussed later.
    h. Status: **No Changes**
4. In the bottom right corner, wait for the software to read “Ready” on a green background (while the vacuum is pumping down and laser is ramping, this will be yellow and read “Preparing”)
5. The target plate grid (middle left side of the screen) moves the instrument by clicking on the circles corresponding to the target plate spots. The number/letter labels match the labels on the target plate.
6. Move to the spot containing the calibration standard(s).
7. Adjust the Laser Power slider (right of camera) to match the matrix.
    a. Start with 20-40% for DTH and CHCA, 60-90% for SA.
    b. Increasing laser power can increase signal. Use as little laser power as is required to get good signal to optimize resolution and prevent dirtying the instrument source.
8. Check the number of Laser “Shots” under the camera.
    a. 200 laser shots per spectrum is standard. This can be increased to increase weak signals.
9. Hit “Start” to collect a spectrum to be used for calibration.
10. Under the “Calibration” tab, make sure that the mass control list matches the calibration standard being used. Change using the dropdown menu if necessary.
11. Identify the most intense mass peak in the spectrum and click on the corresponding entry in the mass control list. This will zoom in on that peak according to the “Zoom Range”. Left click on the peak to assign the peak and hit “Apply” to implement the assignment. (This will shift the spectrum, but not fully calibrate the instrument).
12. “Automatic Assign” will usually assign the remaining peaks. If this fails or if the automatic assignment is off they can also be manually assigned by selecting the entry in the mass control list and left clicking on the peak. Not all peaks/calibrants to be assigned.
13. Check the currently selected “Mode” and change if needed to match the number of calibrants assigned. Change the mode if the number of calibrants or mass range is incompatible.

    a. Cubic Enhanced: Most powerful calibration mode. Extrapolates outside range of calibrants. Requires 4 minimum assigned calibrants.
    b. Quadratic: Reasonable calibration mode but does not extrapolate outside range of calibrants. Required 3 minimum assigned calibrants.
    c. Linear: Avoid using.
    d. Linear Correction: Avoid using.
14. Hit “Apply” a second time to implement the full calibration.
    a. Changing the mass range will invalidate the calibration.
15. Move to your sample position. Adjust laser power and number of shots if needed. Hit “Start” to collect a spectrum.
16. To save the spectrum, press “Save Spectrum As” (**NOT “Save Method”**). All files should be named using the first three letters of the PIs last name, the date, the instrument letter ID “L”, and an index letter. **Example**: BIS05212023LA. Info included in the notes will be included in the printout. Check “Open in the flexAnalysis” to automatically open in the analysis software.
17. Repeat 15-16 for all samples.
18. Remove your target plate from the instrument and replace it with the blank target plate. Evacuate the instrument.
19. Close all open programs on the computer. Do not overwrite shared methods if asked.