Sample preparation

Preparation of matrix solution

ACCA (α-cyano-4-hydroxycinnamic acid) for peptides/proteins:
10 mg ACCA is dissolved in 1 mL of MeCN–H2O–TFA 500:475:25.

Sample preparation

Prepare a solution (i.e. analyte) of the compound to be analyzed in MeCN-H2O (≥50% water – to ensure that the sample will give a well-defined droplet). The concentration should be approx. within the range 0.01-0.1 mg/mL (typically a fraction from preparative HPLC will be suitable also).

Target (plate; Geometry: MSP96)
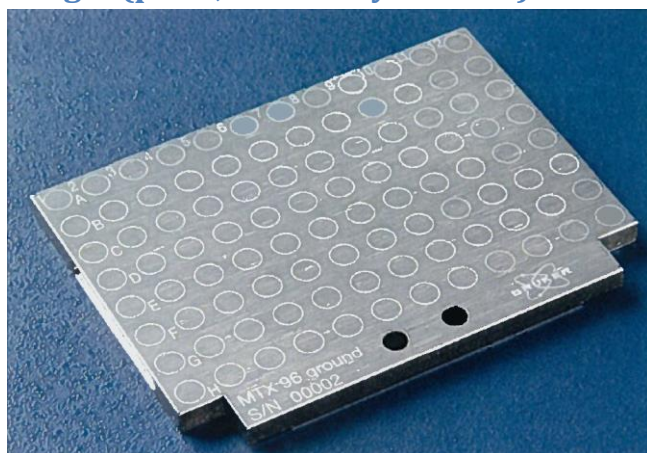
## Preparation of co-crystallization spots on target

Using a micropipette (should preferably go below 1.0 µL) a droplet of 1.0 µL of each analyte is applied to a position (please insert this in the sample sheet next to the apparatus) on the target.

When all samples have been applied and allowed to dry completely (approx. 5 min) a droplet of 1.0 µL of matrix solution is added on top of each sample spot. Again the spots should be completely dry (> 5 min) before proceeding.
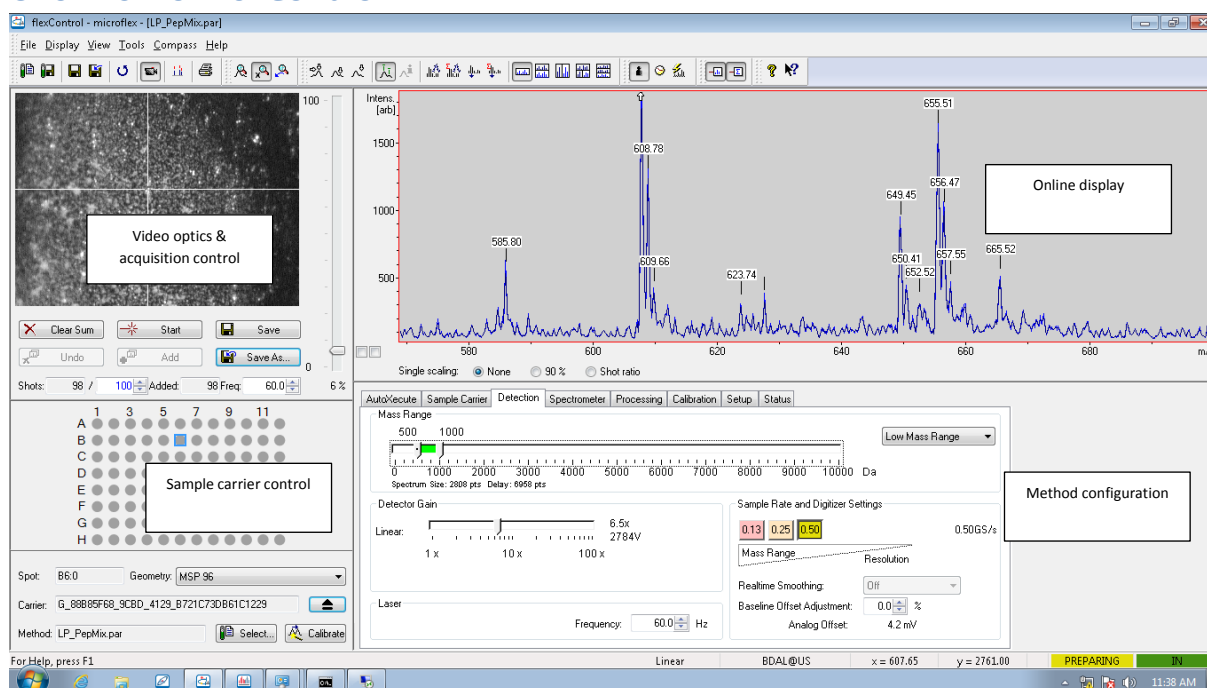
Acquisition

Instrument overview
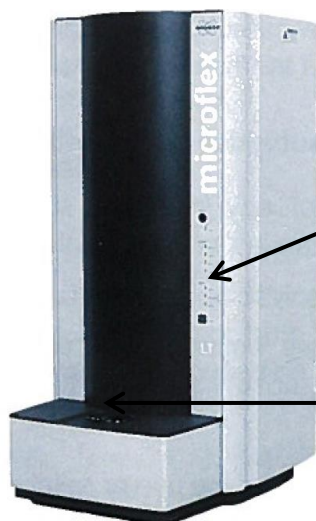
MALDI control panel

Load port

Overview of flexControl

Insertion of target

In the sample carrier control window in the lower left corner of the **flexControl** user interface you can see whether the carrier is docked in the measuring chamber (see below). **NOTICE** that a green light in "Access" on the control panel does **not** mean that the lid of load port can be opened!!

If "Carrier" displays a long name it is in the "docked position", then click on the open/close button (⬆ above "Calibrate" button): on the MALDI control panel (under TARGET) a yellow light for "in progress" is turned on while the carrier is being ejected (ca. 1-2 min). Green light in "Access" is turned on, and now the lid of the load port may easily be opened.

The target is inserted by holding it on the sides and lowering it carefully into the "pocket".

If a target has been left in the carrier it is taken out by carefully squeezing down on both sides next to the target.

If the carrier is not docked: "Carrier" displays "NO_TARGET" (and the green light for "Access" is turned on) and the lid of the load port may be opened and a target inserted as above.

In any case the lid is subsequently closed followed by clicking on ⬆. After docking (1-2 min) a long name is displayed in "Carrier".

Choice of method

"**LP_PepMix par**" can be recommended for peptides/proteins.

Getting a spectrum

a) Select the desired spot by clicking at it in the sample control window displaying the entire target (plate).

b) In the lower right window click on "Detection" and choose the desired mass range (low mass range: 0-10 kD or medium mass range: 0-100 kD). Fine tune range by moving the highlighted bar or by dragging at the ends of the bar to reduce/enlarge the range.

c) In the upper left window (acquisition control): First click on "Clear sum", and then adjust the laser power (typically to 3-15% of the max. level) on the scrolling bar to the right:
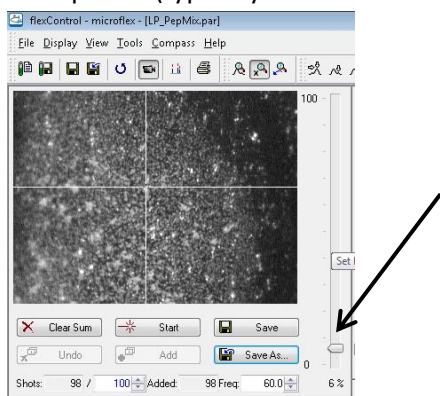

d) In the upper left corner (a microscope video camera picture shows the selected target position): click on a bright part of the spot (i.e. at crystals) and then click on "Start" (the shots are briefly visible).

e) In the toolbar the "Display single buffer" button should be activated:
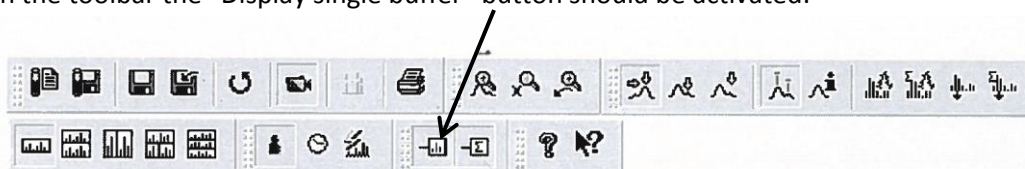
After a while (ca. 30 sec) a spectrum appears in the upper right corner (i.e. in the online display). If a satisfactory signal (largest peak should still be sharp) is obtained, and the noise level is sufficiently low the spectrum may be saved by clicking "Save as":

More spectra may be added via the sum buffer (e.g. use scaling to 90% for each spectrum by selecting this below the spectrum in the online display): click "add", and now it is possible to

toggle the display between the "Display Single buffer" and the "Display Sum buffer".
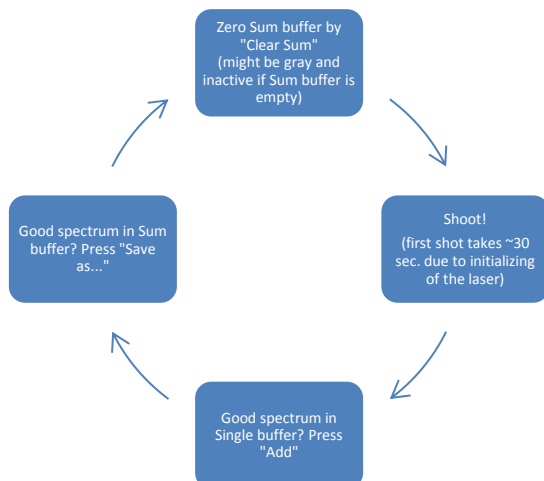
f) In order to save your spectra correctly choose the appropriate subdirectory (group or personal) click "Browse": a list of folders appears – select your group/initials. The entry "Sample Name" should be a folder name indicating user and date (example nn.140221). No spectra files are overwritten so one name is ok for a series of measurements. All spectra are saved in subfolders indicating position – if parameters are changed (m/z-range or power) the spectra are saved as different datasets.
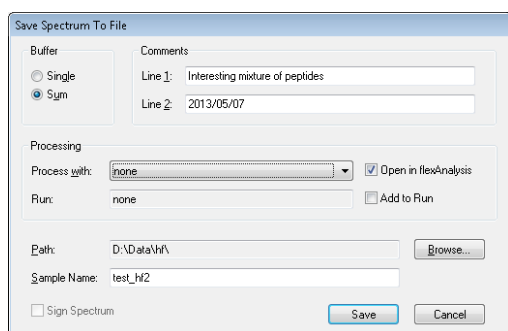

g) In the **flexControl** interface it is possible to use the "Zoom" commands (e.g. "zoom X axis only") as well as the **"Label Peaks"** (should always be turned on) and "Smooth Spectrum" commands present on the toolbar.

Cleaning of target (plate)

The target is sonicated in MeOH–H_2O 1:1 (v/v) for 15 min, followed by wash with MeOH and H_2O. The target is dried with a flow of N_2.

Processing and printing in flexAnalysis
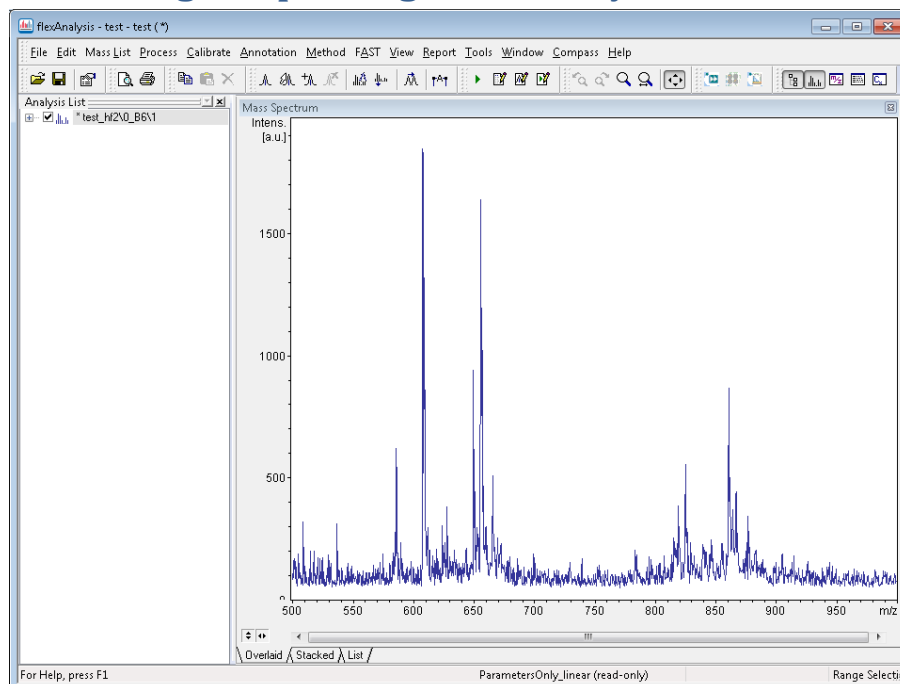
Open data file

a) In the "File" drop-down list: first choose "Close all" to remove any open spectra from the analysis window (remember to select "no" in the following dialogue box).

b) Then click "Open" and "Browse" to select the appropriate subdirectory (GROUP/initial), and then select the desired spectrum folder. Then in "Spectrum Browser" window select the box (or click the "Select all" button) and then click "Open".
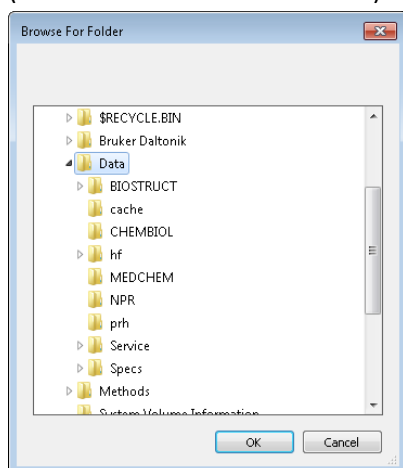
Mark peaks

a) Press F5 for automatic peak detection, or zoom in and select peaks by using the mouse while pressing [ctrl]+[alt].

Print

a) Under "View" (drop-down menu) choose "Set Display Range" (auto scaling off) and insert desired spectral range.

    b)   Use "Print" button on the toolbar to print the spectrum or save spectrum as pdf using the "Report"-menu.