# Calibrating MALDI-TOF Instruments:

Factors that change calibration:
(1) Method.  Every time the instrument method is changed, a new calibration is needed.
(2) Instrument mass range (detection range).  Every time the range is changed, a new calibration is needed.
(3) Laser power.  Too much laser power leads to wider and squarer-topped peaks.  In general, one needs to use the minimum laser power needed to produce peaks.  Data for calibrants needs to be collected at the same laser power used for the analyte.
(4) Target.  A calibration done on target A may be a bit off when applied to target B.  It is a good idea to put calibrants on the target at the same time one spots analyte.

A two point linear calibration is quick ad useful when the two calibration masses bracket the mass expected for the analyte.  The closer the masses of the calibrants are to the that of the analyte, the better the calibration will turn out.  Also the closer the peak shape is to that of the analyte, the better.  It is a good idea to use proteins to calibrate proteins and peptides to calibrate peptides.  Higher laser powers are often needed for higher masses, so having a low mass calibrant around 500 Da and a high mass calibrant around 66,600 Da is NOT a good idea.

Outline of the external calibration procedure:
(1) Place two calibrants with matrix on one spot on the target.
(2) Place the analyte with matrix on another spot.  [If the two calibrants and the analyte are in the same spot, the calibration is called internal.]
(3) Find conditions that produce a well-defined peak for the analyte.  Collect and save a spectrum.
(4) Use the same conditions to collect and save a spectrum of the two calibrants.
(5) To calibrate in Bruker flexControl program, go to the calibration tab.  Search the calibration list for the calibrants or make a new calibration list,  To generate a new list, you need to know the mass of each calibrant to several decimal places.
(6) Make sure you choose linear and tell the instrument which peak is which.
(7) To calibrate in Bruker flexAnalysis, load the calibrant file and the analyte file.  Open the calibrant file and then choose Internal calibration. [In this case internal calibration means calibrating the open spectrum.] The process is basically the same as for the Bruker flexControl program.  Be sure to save the file once you have it calibrated.

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