

## Non-Linear Correction (NLC) Functionality to Improve Mass Accuracy in MALDI-TOF Analyses

Reflectron time of flight (TOF) mass analyzers, coupled with matrix assisted laser desorption ionization (MALDI) sources utilizing delayed extraction, are capable of high resolution ( $M/\Delta M > 20,000$ ) and high mass accuracy ( $<5$  ppm) measurements. MALDI TOF analyzers operate based on the principle that ions are accelerated into a field free region, and the final velocity of the ions is dependent on their mass. It is the flight times of the ions, i.e. the time from application of the acceleration voltages to detection of the ions, that are actually measured. The flight times are subsequently converted to mass-to-charge ( $m/z$ ) via a calibration which is generated by measuring the flight times of standards whose masses are accurately known. The flight time ( $t$ ) of an ion of mass  $m$  is proportional to  $\sqrt{(m/z)}$ , where  $z$  is the number of charges on the ion.

However, the observed relationship between  $t$  and  $\sqrt{(m/z)}$  in MALDI-TOF analyses is not strictly linear. Therefore, to improve the mass accuracy, we have developed a new calibration procedure that utilizes a non-linearity correction (NLC) functionality to correct the observed deviations from linearity during a multipoint ( $n \geq 3$ ) calibration. The procedure works by automatically generating a least squares fit to the observed deviations and applying an offset such that the residual errors are 'randomized' around zero.

This is demonstrated in Figures 1 and 2. Figure 1 shows a MALDI-TOF MS spectrum obtained for a mixture of peptide standards. This spectrum was internally calibrated using the peaks detected for all seven peptides. Figure 2 shows the residual errors (calibrated  $m/z$  minus theoretical  $m/z$ ) for each peptide mass following internal calibration. In this figure, the red line represents the residual errors obtained following calibration without the NLC functionality. As can be seen, the residual errors range from +80 mDa to -40 mDa and also have a systematic distribution. Internally recalibrating the same spectrum using the new NLC functionality results in significantly reduced residual errors ( $\pm 4$  mDa; black dashed line) which are centered around zero with a random distribution.

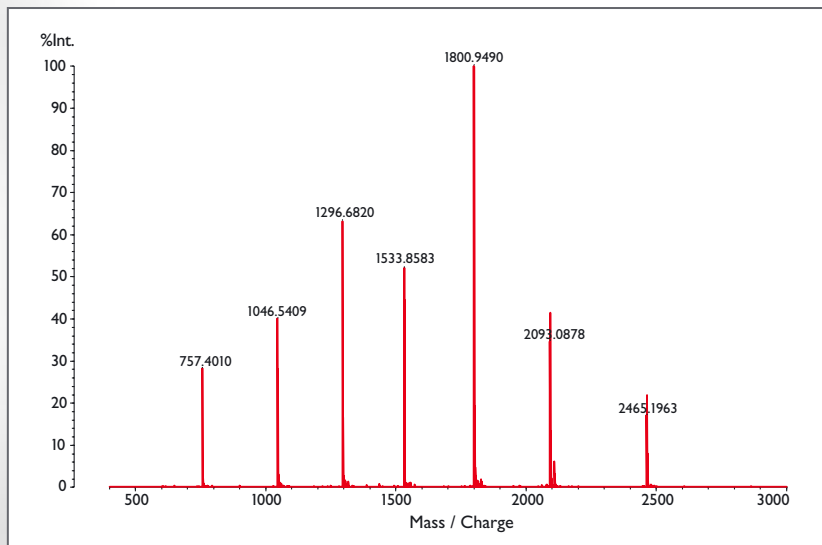


Figure 1: Internally calibrated mass spectrum obtained for a seven peptide mixture.

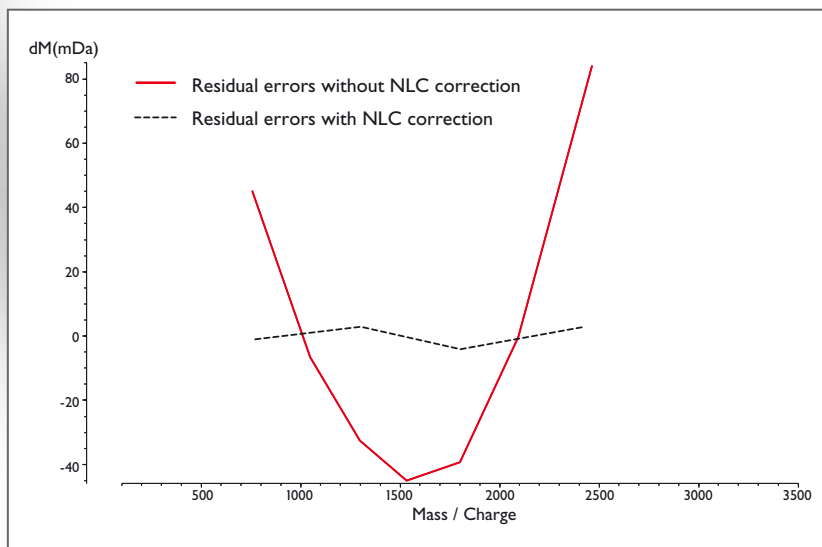


Figure 2: Residual mass errors (in mDa) following internal calibration of the spectrum shown in figure 1, using the standard calibration function i.e. without NLC (red line) and using the new NLC calibration functionality (black dashed line).

Figure 3 shows a zoom image around the (Pro)14Arg (P14R) peak (1450-1615  $m/z$ ) for five internally calibrated spectra of the same peptide mixture, calibrated using the NLC functionality. Table 1 shows the observed  $m/z$  and calculated mass errors (in ppm) for the five spectra, with and without the NLC. The RMS error for the five P14R peaks is **2.3 ppm**. This can be seen to be a significant improvement when compared with the result without using the NLC which is calculated as 32 ppm. While the NLC functionality was developed primarily for use with internally calibrated data, the functionality can also improve mass accuracy, to a lesser extent, using an external calibration procedure. The NLC functionality effectively increases mass accuracy in cases where the data itself is intrinsically accurate (i.e. of sufficiently high quality) and the systematic errors can be quantified and minimized. In order for the NLC functionality to have any effect on externally calibrated data, care must be taken to minimize the introduction of additional systematic errors which may limit the effect of the NLC functionality on mass accuracy.

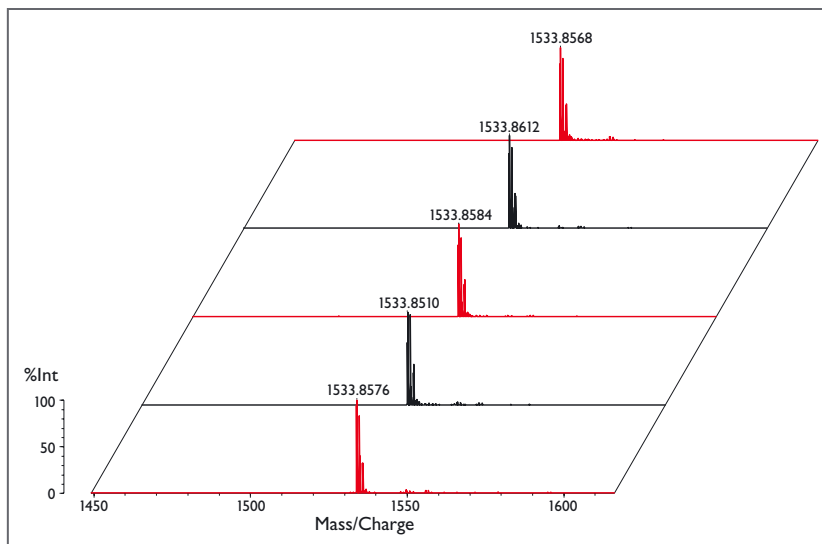


Figure 3: Zoom region (1450-1615  $m/z$ ) around the P14R peak for five internally calibrated mass spectra obtained for the same seven peptide mixture. Spectra were calibrated using peaks detected at 757, 1046, 1296, 1801, 2093 and 2465  $m/z$  using the NLC functionality.

## Conclusions

- A new calibration procedure has been developed to improve mass accuracy during multipoint ( $n \geq 3$ ) internal calibration
- The procedure works by automatically minimizing the residual errors, resulting in a random distribution of errors around zero
- By automatically performing the calculation for each calibration, the procedure even corrects for variations from sample to sample.

Spectrum #	Observed $m/z$		Error (ppm)	
	With NLC	Without NLC	With NLC	Without NLC
#1	1533.8576	1533.9057	-0.4	31.0
#2	1533.8510	1533.9016	-4.7	28.3
#3	1533.8584	1533.9120	0.1	35.1
#4	1533.8612	1533.9105	2.0	34.1
#5	1533.8568	1533.9056	-0.9	30.9
Average	1533.8570	1533.9071	2.3 (RMS)	32.0 (RMS)

Table 1: Observed  $m/z$  and calculated mass errors (in ppm) for the five P14R peaks shown in figure 3, calibrated with and without the NLC functionality.