Technical Report

Compensation of Sample Topography Variability in MALDI-TOF MS using Lock Mass Correction

Simona Salivo[1]

Abstract:

In the typical MALDI process, the sample is mixed with a UV-energy absorbent MALDI matrix to aid ionisation. Depending on the chemical properties of the sample and matrix, there may be some variations in the topography upon drying, leading to ions originating from different heights. This can cause a spread in the time-of-flights ultimately affecting the mass resolution and accuracy. While some sample factors can be compensated by the pulsed extraction, the spatial distribution cannot be corrected at the hardware level. The lock mass feature can effectively correct the mass shifts and improve the mass resolution and accuracy. Here, we show example applications of oligonucleotides, protein profiling and imaging where the topography variability can affect the end results but can be effectively compensated using lock mass correction.

Keywords: MALDI-TOF MS, topography variations, lock mass correction, MALDI Solutions

Introduction

Matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry has emerged as a fundamental tool in many fields of application thanks to its robustness, simple operation and high sensitivity. In a typical MALDI process, the sample containing the molecule(s) of interest is mixed with a UV energy-absorbent organic compound (the matrix). Depending on the chemical properties of the matrix and sample, the dried spots often present variations in height due to surface irregularities. The irradiation by the UV laser generates a plume in which a distribution of ion velocities and positions may arise, ultimately producing a spread in the detected time-of-flights (Figure 1). While the velocity distribution can be effectively corrected by the pulsed extraction, the spatial distribution (i.e., the difference in initial positions) cannot be corrected as this is due to sample topography. The ultimate effect of ion spatial distribution is a decrease in resolution and mass accuracy. One way to correct mass accuracy variations is by applying a lock mass correction, in which a species of known mass (the lock mass) is used by re-aligning the lock mass and the sample species accordingly. In the case of imaging experiments, lock mass correction will also improve the resolution as the alignment is performed in each individual pixel spectra.

Here, we show examples of the benefits of lock mass correction with samples that are affected by topography variations, causing a loss of mass resolution and accuracy. The lock mass correction is a feature available in the *MALDI Solutions™ Data Acquisition, AuraSolution™, QC Reporter™, IonView™* and *IMAGEREVEAL™ MS* software (Shimadzu).

Samples and methods

Examples of oligonucleotides, protein profiling and tissue imaging are provided to demonstrate the benefits of the lock mass correction. The experimental conditions are summarised in Table 1. All data were acquired on Shimadzu MALDI-8000 series benchtop instruments in positive ion mode. Lock mass correction was carried out in the MALDI Solutions Data Acquisition (for the oligonucleotides and protein profiling examples) and IonView (for the imaging example) software.

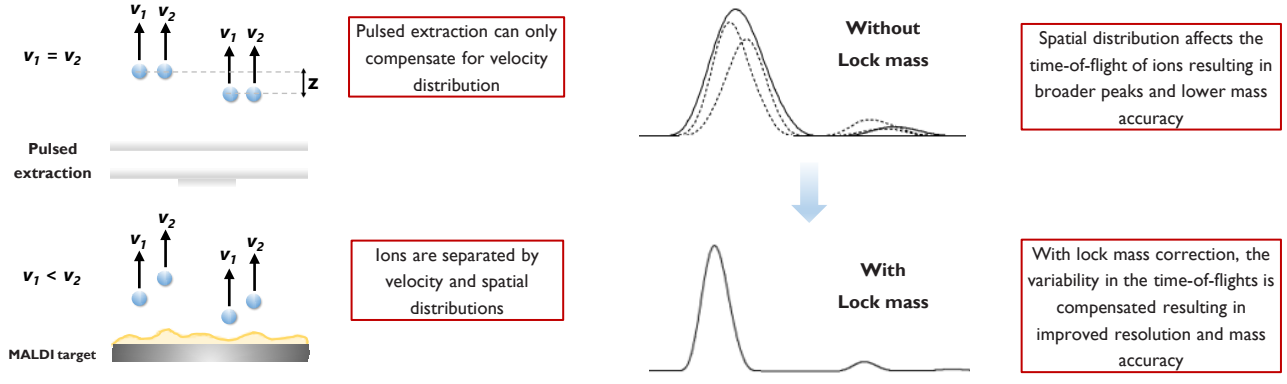

Figure 1. Left: Within the MALDI process, the variations in sample topography can result in ions exhibiting different velocities and spatial distributions leading to a spread in the time-of-flights. While the pulsed extraction can correct the velocity distribution, the spatial distribution is due to topography factors and can contribute to a loss in the mass resolution and accuracy. Right: the lock mass correction can minimise the variability in the time-of-flights leading to improved mass resolution and accuracy.

[1] Shimadzu Manchester, UK

Table 1. Experimental conditions for the example applications described.

Application	Sample	Sample prep	Matrix	Lock mass
Oligonucleotides	12-mer (ATCTTTGGTGTT) + 20-mer I.S. (ACGTACGTACGTACGTACGT)	100 µM in Water. Desalted with cation exchange resin	3-HPA[a] in Ammonium citrate dibasic	20-mer ([M + H]$^+$; *m/z* 6118.05 (Average))
Protein profiling	Haemoglobin Human (Hb)[b]	0.1 mg/mL in 0.1% TFA	Sinapinic acid in 50:50 ACN/0.1% TFA	Hb alpha ([M + H]$^+$; *m/z* 15127.2 (Average))
Imaging	Rat brain	matrix sublimation	2,5-DHB[c]	PC(34:1) ([M + K]$^+$; *m/z* 798.54 (Monoisotopic))

a 3-Hydroxypicolinic acid.
b Merck (P/N H7379).
c 2,5-Dihydroxybenzoic acid.

Results – oligonucleotides

Oligonucleotides prepared with 3-HPA matrix typically produce a unique topography upon drying, with a slightly raised edge and a thin inner layer (Figure 2A). Due to the height difference between the raised edge and centre, the mass resolution and accuracy may be degraded if ions are generated from both regions within the same acquisition. Figure 2B shows the MALDI spectrum of the 12-mer and 20-mer internal standard (I.S.) oligos before and after lock mass correction (top red and bottom blue traces, respectively). The 20-mer oligo was set as the lock mass (*m/z* 6118.05). It can be observed how the mass accuracy improved after the lock mass correction was applied (1.54 Da error (without lock mass) *vs.* 0.06 Da error (with lock mass)).

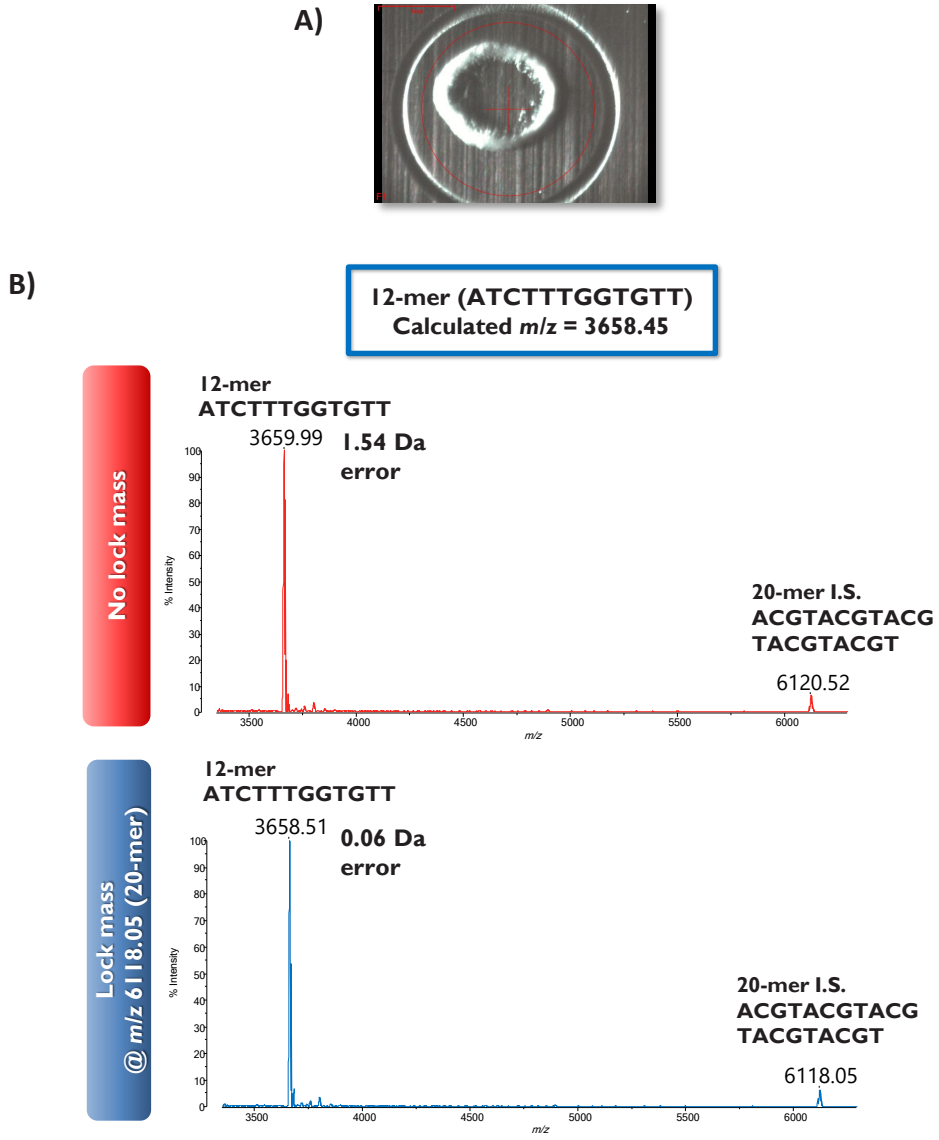

Figure 2. A) Optical image of a dried spot of 12-mer and 20-mer (I.S.) oligos prepared with 3-HPA, showing a raised edge and thin central region. B) MALDI spectra of 12-mer and 20-mer oligos before (top red trace) and after lock mass correction at *m/z* 6118.05 (20-mer) (bottom blue trace). The mass accuracy is improved with the lock mass correction.

Results – imaging

In this example, a rat brain tissue exhibiting topography variations was acquired as an imaging experiment. The tissue was coated with 2,5-Dihydroxybenzoic acid via sublimation using the Shimadzu iMLayer™ sublimation device. The imaging analysis was carried out in positive ion mode at 30 µm pixel size. Figure 3A shows an extract of the MALDI TIC spectrum around the PC(34:1) + Na^+ species (*m/z* 782.568, calculated). It can be seen how the resolution is affected resulting in broader and slightly distorted peaks. Figure 3B-D shows three example pixel spectra extracted from different areas of the tissue (the pixel spectra locations are highlighted in the MALDI TIC ion image in Figure 3E). Here, the peak of the PC species exhibit a shift in mass supposedly due to different tissue heights.

The MALDI ion images corresponding to the observed *m/z* in the individual pixel spectra (Figure 3F-H) clearly show partial tissue features when compared to the TIC ion image (Figure 3E). The lock mass feature in the IonView software allows for correction of the mass drifts at the individual pixel spectrum level. The lock mass was set to *m/z* 798.541 using one of the known detected endogenous phospholipid species (PC(34:1) + K^+). Figure 4 shows the MALDI TIC and pixel spectra (Figure 4A and 4B-D, respectively) which are re-aligned as a result of the lock mass correction. The mass of the PC(34:1) + Na^+ species (*m/z* 782.556) is also more accurate exhibiting a very small error (-0.012 Da). The MALDI TIC ion image of PC(34:1) + Na^+ after lock mass correction is similar to that prior to the correction (Figure 4E).

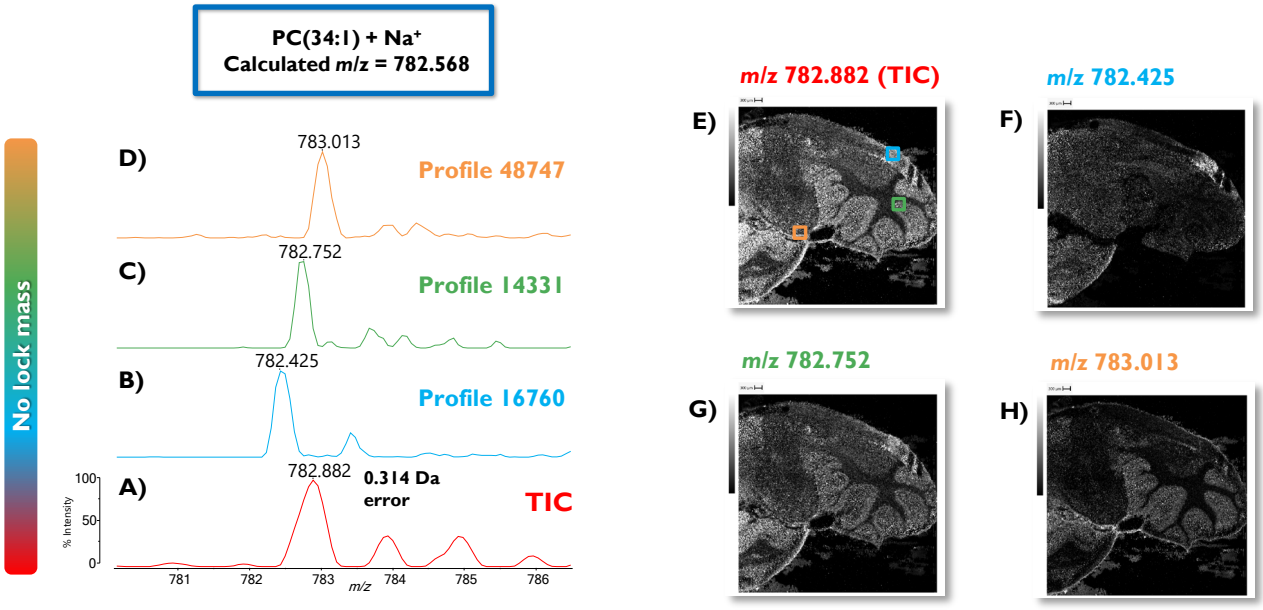

Figure 3. MALDI spectra and ion images of PC(34:1) + Na^+ (*m/z* 782.568, calculated) before lock mass correction. A) MALDI TIC spectrum showing a broader and slightly distorted peak of the PC(34:1) + Na^+ species (±0.4 Da window), as a result of the topography variations. The mass accuracy is also affected, showing a large error (0.314 Da). B-D) Pixel spectra of the PC species showing the mass shifts possibly due to different heights in the tissue. E) MALDI TIC ion image of the PC(34:1) + Na^+ species before lock mass. The coloured boxes highlight the approximate locations from where the pixel spectra in B-C) were extracted. F-H) MALDI ion images corresponding to the observed *m/z* in the individual pixel spectra in B-C showing partial tissue features.

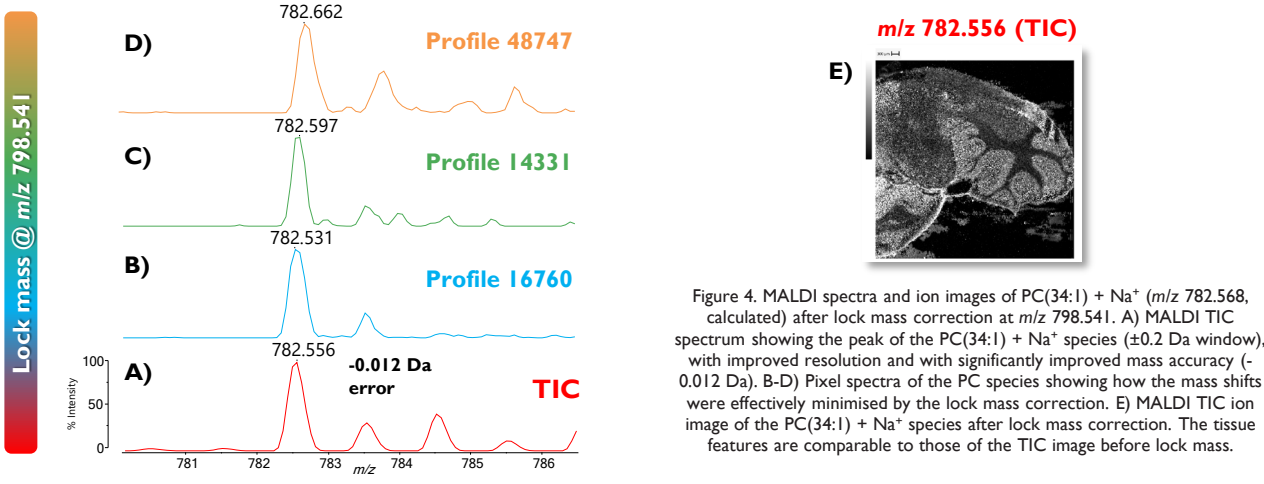

Figure 4. MALDI spectra and ion images of PC(34:1) + Na^+ (*m/z* 782.568, calculated) after lock mass correction at *m/z* 798.541. A) MALDI TIC spectrum showing the peak of the PC(34:1) + Na^+ species (±0.2 Da window), with improved resolution and with significantly improved mass accuracy (-0.012 Da). B-D) Pixel spectra of the PC species showing how the mass shifts were effectively minimised by the lock mass correction. E) MALDI TIC ion image of the PC(34:1) + Na^+ species after lock mass correction. The tissue features are comparable to those of the TIC image before lock mass.

Results – protein profiling

In this example of protein profiling, Human Haemoglobin (Hb) was used given its involvement in several genetic blood disorders such as sickle cell syndromes [1]. Given the existence of several variants of the beta chain, it is important that the analytical method is accurate in ensuring the correct variant is detected. Here, we demonstrated how the lock mass correction can increase the reliability of the Hb variant detection. To demonstrate the benefits of the lock mass correction, two scenarios were explored: 1) Hb was spotted on the whole MALDI target, mass calibration performed on each designated external calibration position and Hb acquired in three groups of 16 spots (NB. this is the recommended scenario as a local calibration would give the best mass accuracy performance); 2) Hb was spotted on the whole slide and mass calibration performed on the central external calibration position (NB. This scenario is not typically recommended as the variation in the mass accuracy across the whole MALDI plate would be inevitably larger than performing local calibrations). The Hb alpha peak was set as the lock mass (*m/z* 15127.21). Figure 5A shows the MALDI spectra of the Hb standard before and after lock mass correction (blue and orange traces, respectively). The Hb beta peak is only partially resolved, making accurate detection even more challenging. The box plots in Figure 5B-C show the mass errors (Da) of the Hb beta peak (*m/z* 15868.05, calculated) before (blue box) and after lock mass correction (orange box). The box plot in Figure 5B, corresponding to scenario no. 2 described above (i.e. a single calibration at the central external calibration position), shows a large variability in the mass errors spanning ~ ±15 Da. Despite the (non-optimal) single calibration strategy used, the lock mass feature was able to correct the mass drifts and significantly reduce the errors to <1 Da. The box plot in Figure 5C, corresponding to scenario no. 1 (i.e. local designated external calibration and acquisition of the surrounding 16x spot group), shows how the mass errors are much smaller than the single middle point calibration, however there is still a spread in the masses due to sample variations. Again, the lock mass correction was able to reduce the mass errors to <1 Da.

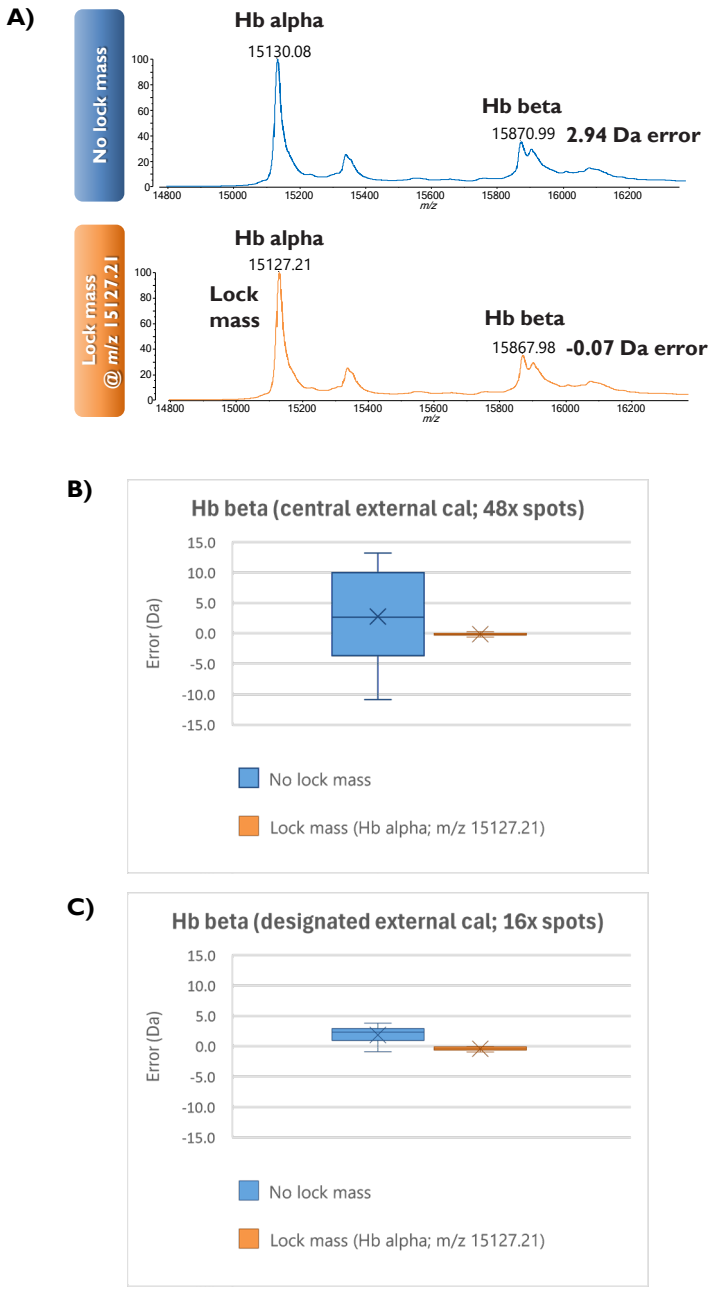

Figure 5. A) MALDI spectra of the human Hb standard before and after lock mass correction (blue and orange traces, respectively). The lock mass was set to the Hb alpha peak (m/z 15127.21). B) Box plot showing the mass errors of the Hb beta peak from scenario no. 2 (central external calibration position): the lock mass correction was able to correct the significant error spreads due to a non-ideal starting calibration. C) Box plot showing the mass errors of the Hb beta peak from scenario no. 1 (designated external calibration positions): although the mass errors are improved with the local calibrations, there is still a spread in the masses which was effectively compensated by the lock mass correction.

Conclusion

In MALDI-TOF mass spectrometry, it is widely accepted that sample topography variability can negatively affect the mass resolution and accuracy. Unlike some sample factors, the spatial distribution cannot be compensated at the hardware level. Lock mass correction can effectively correct mass drifts due to ions originating from different sample heights. Here, we have shown examples of oligonucleotide, protein profiling and imaging applications where the sample topography has negatively affected the mass resolution and accuracy. With the lock mass correction, we demonstrated that the mass resolution and accuracy can significantly improve.

References

[1] Titus H.J. Huisman, Marianne F.H. Carver, and Georgi D. Efremov. *A Syllabus of Human Hemoglobin Variants (1996).* The Sickle Cell Anemia Foundation, Augusta, GA, USA.

First Edition: April 2025

Shimadzu Corporation

www.shimadzu.com/an/