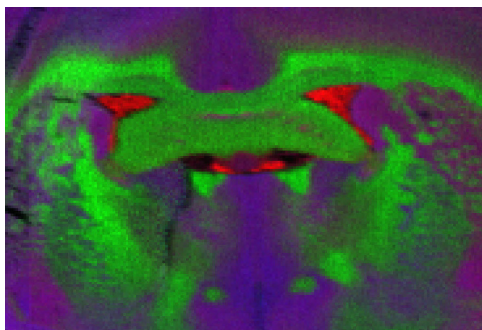Waters

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High Definition Imaging (HDI) 1.4: Lock Mass Recalibration for MS Imaging Data Sets

GOAL

An overview of the recalibration of MALDI-MSI using an external or internal lock mass, and the recalibration of DESI-MSI, using an internal lock mass — both features fully integrated in HDI® Software, v 1.4.

BACKGROUND

High mass accuracy is essential when performing mass spectrometry imaging (MSI) in order to aid subsequent identification. When using an orthogonal-acceleration (oa)Tof instrument, the calibration curve generally remains valid for an extended period of time. However, for additional confidence in the mass accuracy of imaging data, the ability to adjust the calibration curve via a gain function is preferable and available. This feature is especially advantageous for long MS imaging runs where environmental conditions in the laboratory may change during the course of acquisition. With the Waters® matrix-assisted laser desorption/ionization (MALDI) imaging workflow, it is possible to utilize an external calibrant to correct the mass drift. An internal correction method has now been developed for use with the desorption electrospray ionization

External and internal recalibration for improved mass accuracy in MS imaging data sets.
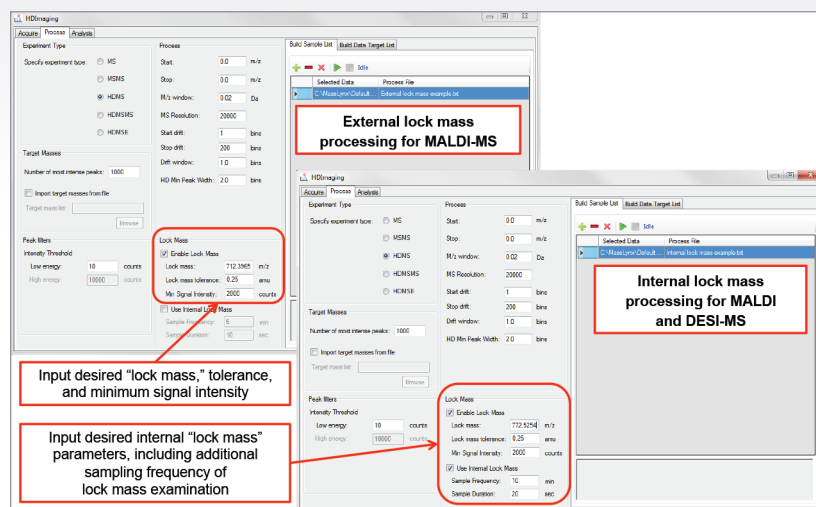

Figure 1. Highlighted parameters for setup of internal and external lock mass recalibration of MS imaging datasets within HDI 1.4.

(DESI) imaging workflow, which can also be utilized for MALDI-MSI experiments. The two methodologies have been assessed and compared for their ability to correct and maintain mass accuracy.

THE SOLUTION

An external lock mass has traditionally been implemented for Waters MALDI imaging experiments. This function is available within the fully-integrated processing page of High Definition Imaging (HDI) Software, version 1.4.

Additionally, a new internal lock mass function has been implemented, allowing for reliable mass correction in both MALDI and DESI experiments. Regardless of the recalibration methods, the lock mass *m/z* is user-defined, along with the lock mass peak tolerance window and minimum signal intensity cut-off. For internal recalibration, a peak of known identity with relatively ubiquitous distribution is selected as the "lock mass." With external lock mass, the sampling frequency and duration are set and fixed as part of the method setup prior to data acquisition. When using an internal lock

mass, the user can input the desired sampling frequency and duration as part of the processing or reprocessing workflow (highlighted in Figure 1). The duration determines the amount of data that is combined prior to lock mass assessment, the frequency determines how often the lock mass is assessed, and the result is a series of gain values over time, which are used for data correction.

Figure 2 shows examples of DESI-MSI after the application of an internal lock mass — in this case, a relatively ubiquitous lipid peak at *m/z* 772.5254. Also included are the mass errors before and after recalibration. The lower mass error is advantageous when attempting to tentatively identify *m/z* species via accurate mass comparisons to established databases.

Comparison of internal and external recalibration for MALDI-MSI

In Figure 3, it can be observed that the calibration was subject to a degree of drift uniformly across the mass range. The internal and external recalibration methods successfully compensated for the drift. Interestingly, both methods performed comparably for this dataset, showing that for particularly heterogeneous samples where an internal standard is not available, MALDI-MSI data can be successfully calibrated using an externally-deposited standard.

SUMMARY

- The lock mass recalibration has been fully integrated into the processing tab of HDI Software, v 1.4
- Internal recalibration has been successfully applied to DESI-MSI experiments

PC(32:0)K$^+$ m/z 772.5254
Before −4.49 ppm
After −0.66 ppm

PC(34:1)K$^+$ m/z 798.5415
Before −3.25 ppm
After 1.25 ppm

PC(36:1)K$^+$ m/z 826.5728
Before −3.99 ppm
After 0.24 ppm

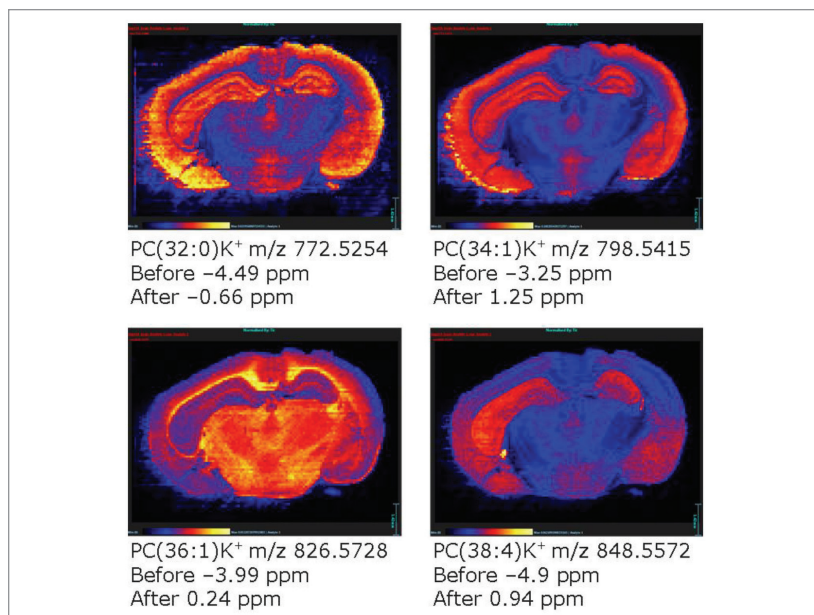PC(38:4)K$^+$ m/z 848.5572
Before −4.9 ppm
After 0.94 ppm

Figure 2. Mass error and DESI-MS images for commonly-occurring lipids in mouse brains. Mass error calculated before and after internal recalibration against m/z 772.5254.

Comparison of recalibration methods

Error (ppm) — m/z

Recalibration
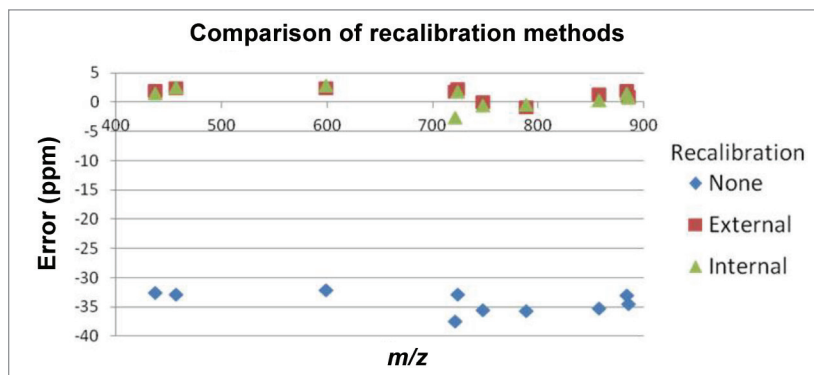- None
- External
- Internal

Figure 3. MALDI-MSI data was acquired on an instrument with offset calibration. A selection of ions was then viewed where no recalibration, internal calibration, or external calibration had been applied. Error in ppm for the selected peaks before and after recalibration is shown along the y-axis.

- In the case of MALDI-MSI experiments, it has been possible to successfully apply a calibrant, both internally and externally
- Both internal and external recalibration show comparable performance when applied to a MALDI-MSI dataset

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