Data quality control and mass recalibration standardize MALDI Imaging and enhance interpretability across large datasets

Bram Heijs[1], Andra Pascale-Henke[2], Kai Zoellner[2], Harrison Taylor[3], Joe Ippolito[4], Christopher Clark[5], Tobias Boskamp[2], Richard R. Drake[3], Peggi Angel[3]
[1]Bruker Nederland B.V., Leiderdorp, The Netherlands; [2]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [3]Medical University of South Carolina, Charleston, SC, USA; [4]Washington University in St. Louis, St. Louis, MO, USA ; [5]Bruker Scientific LLC, Billerica, MA, USA.

Introduction

MALDI Imaging delivers highly detailed and spatially resolved molecular data, making it a valuable addition to the spatial biology toolkit. Monitoring and ensuring mass precision and accuracy are key to confident insights, as m/z shifts lead to peak misalignment, incorrect molecular annotations, and decreased comparability between pixels within and across data sets. In this context, we introduce the new SCiLS™ QC View, Mass Variation Statistics, and Mass Recalibration/Alignment workflow (**Fig. 1**), which provide a new perspective on data quality, as well as tools to uphold reproducibility and maintain analytical integrity in MALDI Imaging.

Methodology

Human samples were prepared for on-tissue bottom-up proteomics analysis by MALDI Imaging using published methods. Data were acquired using a timsTOF fleX instrument in oTOF mode over m/z 500-2,500 and at pixel sizes of 50x50 µm² and 60×60 µm². Data were imported into a preview version of SCiLS Lab 2027a. Data quality metrics were studied using the new QC View. Mass precision was quantified using the Mass Variation Statistics tool, and mass spectral recalibration or alignment were performed using the Mass Recalibration/Alignment workflow.

Results

MALDI Imaging using internal calibrants

The addition of internal reference standards enables standardized quality monitoring and assurance between experiments and across laboratories. Here, a multi-peptide internal reference standard was applied to a human hepatocellular carcinoma tissue section prepared for bottom-up proteomics analysis by MALDI Imaging. The recalibration results, generated in SCiLS Lab, (**Fig. 2**) show that the procedure slightly improved the overall calibration error of the data set.

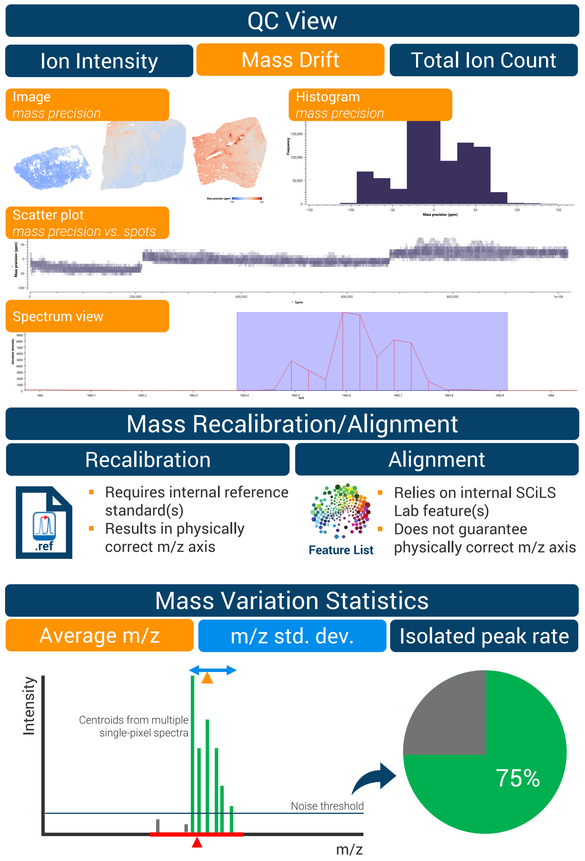

Figure 1: Overview of the four panels in QC View, offers insights in the ion intensity and mass drift for the active ion, or the total ion count *(top)*, Mass Recalibration/ Alignment workflow *(middle)*, and Mass Variation Statistics tool *(bottom)* introduced in SCiLS Lab 2027a.

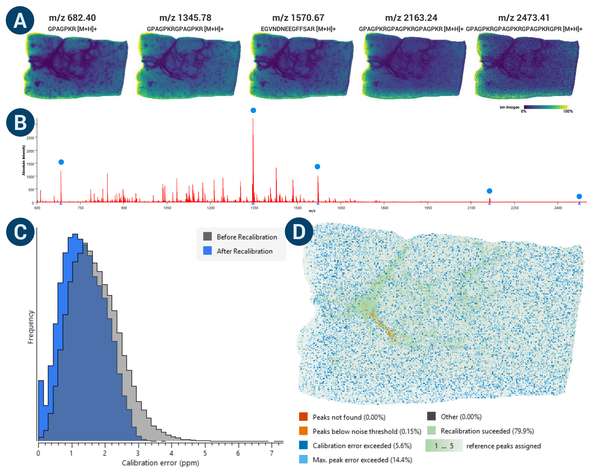

Figure 2: (A) Reference peptide images and **(B)** their corresponding peaks in the tissue mean spectrum. **(C)** Calibration error histogram and **(D)** calibration results image obtained from the mass recalibration workflow.

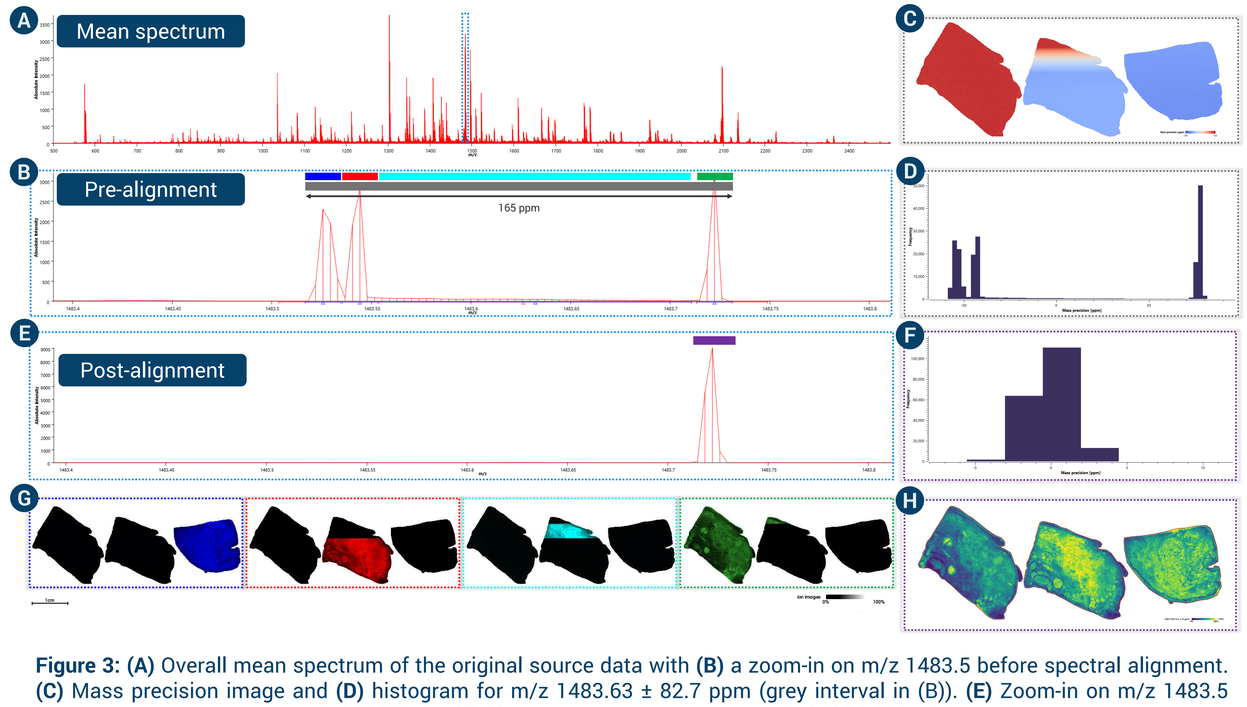

Figure 3: (A) Overall mean spectrum of the original source data with **(B)** a zoom-in on m/z 1483.5 before spectral alignment. **(C)** Mass precision image and **(D)** histogram for m/z 1483.63 ± 82.7 ppm (grey interval in (B)). **(E)** Zoom-in on m/z 1483.5 after spectral alignment on m/z 1302.6545. **(F)** Mass precision histogram of m/z 1483.7222 ± 10 ppm. Ion images for the m/z intervals **(G)** color-coded in (B), and **(H)** color-coded in (E).

Trouble-shooting MALDI Imaging data sets

In the timsTOF fleX, the flight tube is water-cooled to prevent thermal expansion that would cause temperature-dependent variations in drift path length. The drift path length is assumed constant in the conversion from time-of-flight to m/z; thus, variations herein lead to a loss of mass precision and accuracy. In this example involving valuable human prostate cancer patient samples, a malfunction in the water cooler caused significant mass drifts both within and between data sets (**Fig. 3**), evident in the mean spectrum, mass precision image, histogram, and ion images. Unfortunately, recalibration using the [Glu]-Fibrinopeptide B internal reference standard was not possible due to a low isolated peak rate of 1.4%. The isolated peak rate measures the proportion of pixels in a region where a given m/z interval contains only one signal above the noise threshold. Instead, another feature at m/z 1302.6545 (isolated peak rate: 92.7% over a 165-ppm wide m/z interval) was used to perform spectral alignment and fully corrected the mass precision artifact. After alignment, the [Glu]-Fibrinopeptide B internal reference standard achieved a mass accuracy of 1.8 ppm.

Overall, the new SCiLS QC View, Mass Variation Statistics, and Mass Recalibration/Alignment workflow have proven to be crucial tools for evaluating data quality and ensuring reproducibility in MALDI imaging data.

Conclusions

- **Assessing data quality and ensuring reproducibility leads to confidence in molecular annotations and biological findings in MALDI Imaging data sets.**
- **SCiLS Lab QC View, Mass Variation Statistics and Mass Recalibration/ Alignment are essential tools to maintain analytical integrity in MALDI Imaging data sets.**

Imaging MS: Computational Methods, Software & Analysis

Conflict of interest statement

B.H., A.P.-H., K.Z., C.C. and T.B. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.