Optimizing the mass accuracy for automated analysis of MALDI images

Sophie Rappe[1], Mathieu Tiquet[1], Kune Christopher[1], Andrea MCCann[1], Raphael La Rocca[1], Johann Far[1], Loic Quinton[1], Eppe Gauthier[1], Edwin De Pauw[1]

[1]*Mass Spectrometry Laboratory, MolSYS Research Unit , **University of Liège, Liège, Belgium***

LIÈGE université
Mass Spectrometry Laboratory
Contact : e.depauw@uliege.be

Overview

MALDI Mass Spectrometry Imaging is now a mature method presenting a large panel of applications. Data acquisition has been largely improved and data analysis is now the bottleneck in the experimental workflow. Several software solutions exist usually based on spectral patterns defining regions of interest. Here, we present a careful analysis of the experimental conditions ensuring a homogeneous distribution of mass accuracy across the whole image in order to improve the image analysis using automated classification and identification of homologous analytes.

Method TOF

Sample:

- Sample: 1µL **Lactosyl C12 ceramide** 2.5µM in EtOH/$CHCl_3$ 50/50 (v/v) (sodium adduct : m/z = 828.5444)
- Brain tissue sections from mouse
- Support: Indium tin oxide coated glass slide (**ITO**)
- Matrix: 2,5-Dihydroxybenzoic acid (**2,5-DHB**) saturated in acetonitrile or **ethyl acetate** + 10% hexan and 1% formic acid

Acquisition method:

- Calibration: red phosphorus
- Laser power: **60%**
- Number of shots: 1000
- Freq: 10000 Hz
- Raster width: 25 µm
- Scan range: 25µm x 25µm
- Mass range: 100 – 1200 amu
- Mode: reflectron

Method FTICR

Mouse brain → CryoStar NX70 → SunCollect II, HCCA → SolariX XR 9.4T

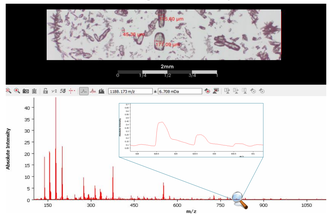
Data processing

SCiLS lab 2016b

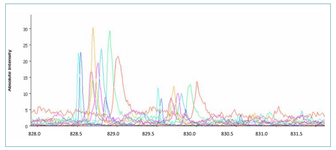
Python version 2.7

MALDI TOF MS

Full average mass spectrum

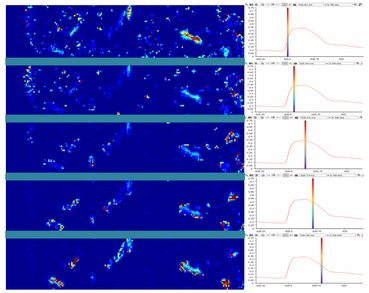


Single pixels spectra overlay



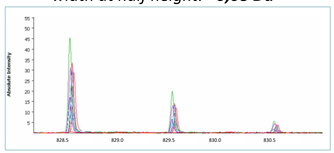
Dirty drop method

Image reconstruction as a function of the crystal height



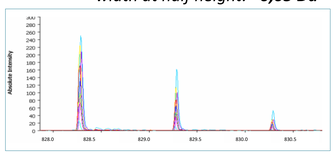
Sprayer

*width at half height: **~0,08 Da***



Sublimation

*width at half height: **~0,05 Da***

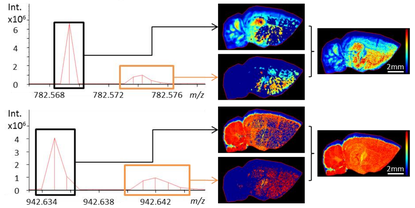


Isotopic distribution of Lactosyl C12 ceramide, sodium adduct (m/z = 828.5444).
Individual spectra from random selection of pixels

MALDI FT-ICR MS

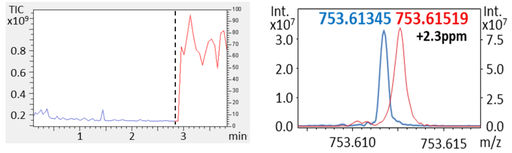
Problems

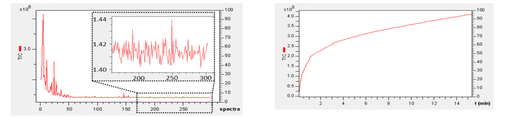
Poor mass accuracy and occasionally peaks multiplication incomplete images.



Origin

Fluctuation of the Total Ion Current (TIC) during the acquisition, dramatically affecting the mass accuracy.

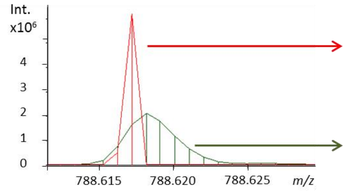




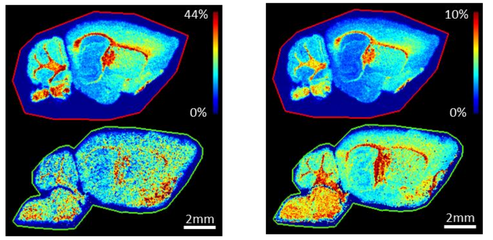
Total ions count evolution

Evolution of the TIC shot by shot (left) and over time (right) for an acquisition summing 400 laser shots.

Controlled TIC **Non-Controlled TIC**



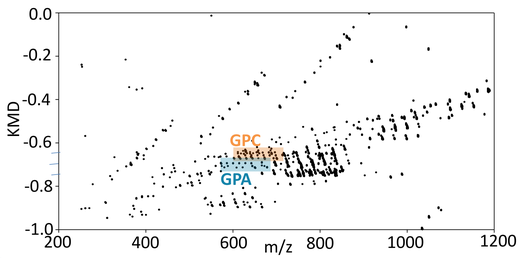
No normalization **TIC normalization**

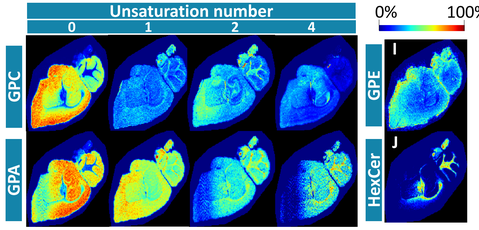


TIC control

No TIC control

Kendrick Mass Defect map for rapid identification and localization of families of lipids by MALDI MS





KMD plot enables a semi-targeted approach for lipids families visualization, based on their -CH_2- repeat unit.

This method can also be automatized to compare images or to target a specific lipid family, knowing the its KMD.

Conclusions

Optimizing experimental conditions providing constant sub-ppm mass accuracy on the whole image allowed efficient KMD filtering of MSI data. KMD axis was used instead of the usual m/z axis from mean. Reconstruction of regions of interest (ROI) based on non-targeted, semi-targeted (specific family of homologous) or localisation of selected chemical can be easily performed with KMD. The Python software will be soon released.

Acknowledgments This work is funded by the Interreg Euregio Meuse-Rhine, the H2020 European infrastructure project EU_FT-ICR_MS, The University of Liege Research Council and the FNRS-FWO Rhizoclip project. Thanks to Ellis Shane (Maastricht University, Netherlands) for the sprayer and sublimator