MALDI MASS SPECTROMETRY IMAGING APPLIED TO SMALL MOLECULES LOCALIZATION

Guitton, Y.[a], Mondeguer, F.[c], Nicolau E.[c], , Royer F[c], Hilbert, J.L.[a], Fournier, I.[b], Wisztorski, M.[b]

[a] INRA USTL UMR SADV 1281, Stress Abiotiques et Différenciation des Végétaux Cultivés, Université Lille Nord de France, Lille 1, SN2, F-59655 Villeneuve d'Ascq, France
[b] MALDI Imaging Team, Laboratoire de Spectrométrie de masse Biologique, Fondamentale, Appliquée, EA-4550, Université Lille Nord de France, Lille 1, SN3, F-59655 Villeneuve d'Ascq, France
[c] Laboratoire Phycotoxines, IFREMER Rue de l'ile d'Yeu, BP 21105 44311 Nantes, France

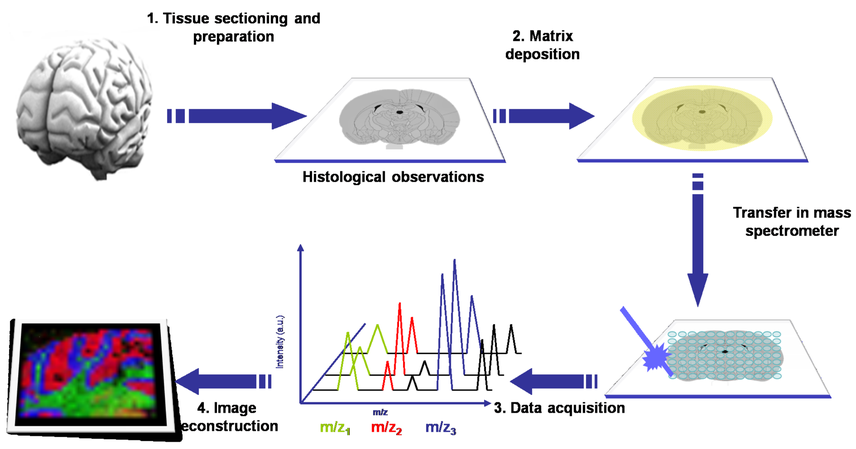
Overview

Purpose :
We present here the MALDI mass spectrometry imaging technique applied to plant and animal metabolomic

Introduction

MALDI Mass Spectrometry Imaging (MALDI-MSI) is a two-dimensional MALDI mass spectrometric technique used to visualize the spatial distribution of a large variety of biological molecules (from small metabolites to large proteins) without extraction, purification, separation or labelling of biological samples[1]. Since its introduction by Caprioli *et al* in 1997[2], it has become one of the most important molecular histology methods for biomarker hunting and for understanding the spatial distribution of biomolecules in various tissues. MALDI-MSI is thus ideal for complementing the expanding field of metabolomics[3]. Despite the fact that MALDI-MSI have been very successfully applied in divers studies (even in clinical applications) its use remains far from routine, and there is still a need to adapt protocols to suit specific tissues[4] or metabolites. Here we describe the methodology of MALDI-MSI with examples taken from applications in plant (*Cichorium intybus*) and in seashell (*Mytilus edulis*).

MALDI Imaging: Molecular localization directly on tissue section

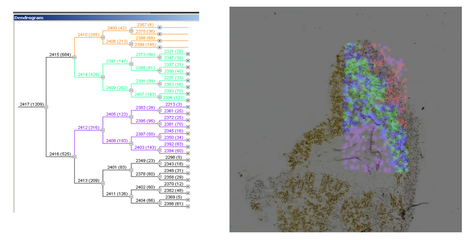


Schematics workflow for MALDI-MSI experiment

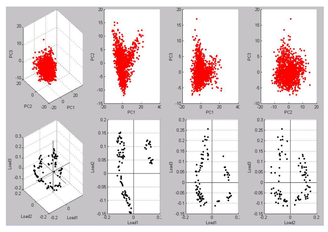
Image Analysis softwares
- ImageQuest (Thermo)
- FlexImaging (Bruker)
- Other software see www.maldi-msi.org

Statistical analysis
- ClinProTools (Bruker)
- Other software see www.maldi-msi.org

For data treatment several software are available and other are on the way. **A common data exchange format (imzML**) have been developed. Specific algorithm are developed (Intensity correlation analysis cf Franceschi *et al* 2012 J Exp Bot 63-3, p 1123-33). See also Trede *et al* 2012 J integr Bioinform 9-1 p 189



Hierarchical correlation analysis a tool for Histochemical tissu caracterisation

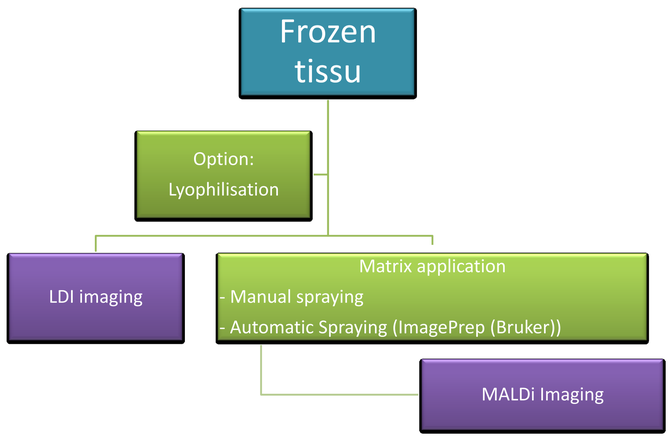


PCA a tool for Biomarker hunting

MALDI-imaging mass spectrometry enables the visualization of the distribution of a range of biomolecules that have varied structures in the cells and tissue sections. This emerging imaging technique was initially developed as a tool for protein imaging, but recently it is increasingly being used for the imaging of small organic molecules. For reviews see Kaspar *et al* 2011 Proteomics 11-9, p1840-50

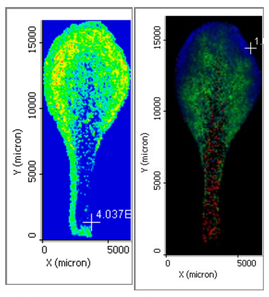
MALDI – MSI Images from two ongoing studies

MALDI – MSI Images from *Cichorium intybus*



Workflow used for those two studies for MALDI-MSI experiment

LDI imaging

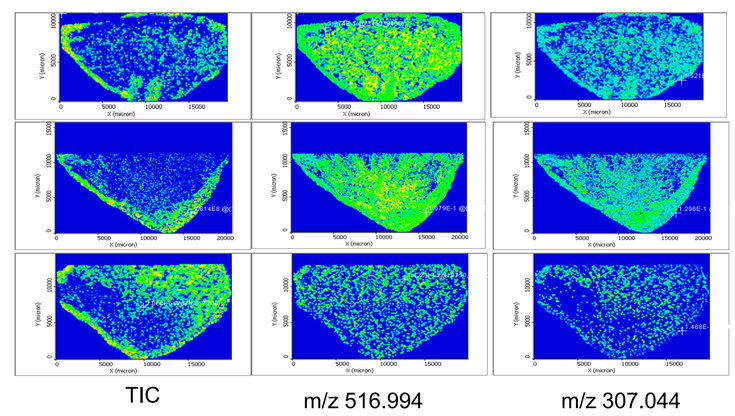


TIC

Red: 189.025
Green: 253.972
Blue: 245.947

LDI-MSI of a *Cichorium intybus* 7 days old cotyledon Cultivated *in vitro* on solid MS medium. Left : Intensity of all m/z for each pixel. Right: combo image for 3 discriminante m/z

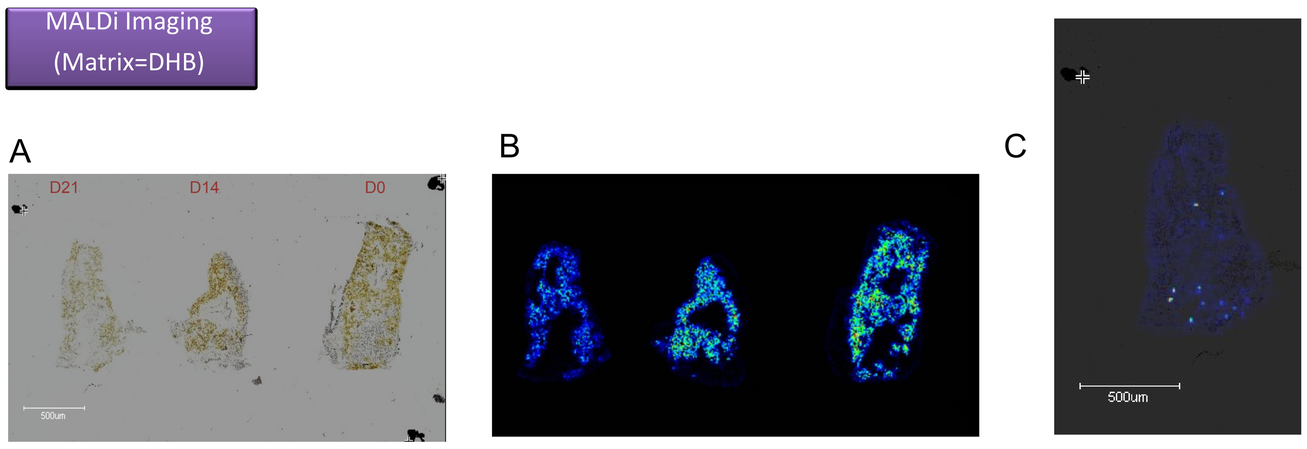
MALDi Imaging (Matrix=DHB)



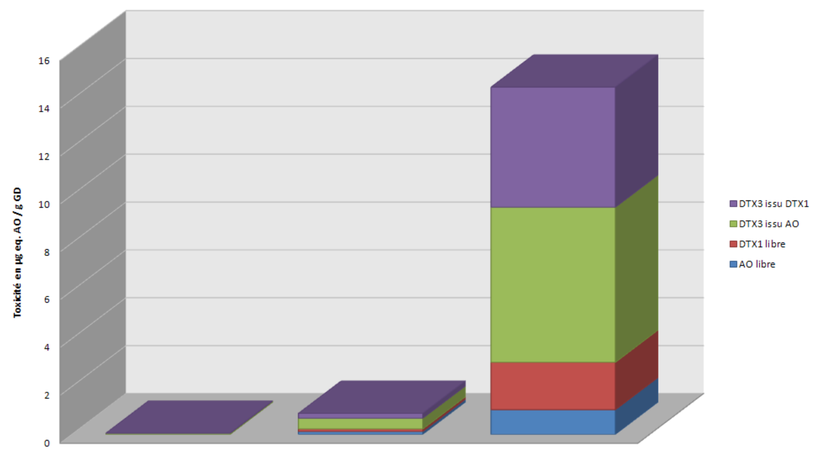
MALDI-MSI of a *Cichorium intybus tuberised* root sections. DHB Matrix deposition was made with ImagePrep (Bruker Daltonics). A Root was harvested from field and stored in a pot (4 C, dark) until cut. T0= cut and immediatelly put in liquid N2, T10: and T60 cut and put in liquid N2 after 10 min and 60 minute respectively.

MALDI – MSI Images from *Mytilus edulis*

MALDi Imaging (Matrix=DHB)



A: Thin 12 µm tissue sections were obtained from digestive glands using a cryostat (Leica Microsystems) and applied onto an indium-tin oxide (ITO)-coated conductive glass slides (Bruker Daltonics). The matrix solution was then deposited using an automatic vibration vaporization system (ImagePrep, Bruker Daltonics) to cover the whole surface of the tissue section. Data were acquired at different positions on the tissue section using an UltraFlex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) equipped with a Smartbeam ™ laser. Direct profiling data were performed in positive reflectron mode, for a mass range from m/z 600 to 2000. Spatial resolution was set to 90 90 µm2. Statistical analyses were performed using ClinProTools 2.2 software (Bruker Daltonics,). After loading data of each condition, mass spectra were internally recalibrated and normalized. **B:** Localization of m/z 641.3854 over time **C:** localization of m/z 872.354 after 11 days



Comparison of toxins levels in digestive galndes of *Mytilus edulis* after feeding with *Prorocentrulm Lima* during (0, 4 or 11 Days, from left)

Conclusions

- **MALDI-MSI combine the advantages of mass spectrometry (MS) and microscopy in a single experiment and allow detection of hundred molecules in one analysis.**
- **Analysis can be achieved in a relatively short time scale (hours)**
- **Possibility to detect various compounds**

Acknowledgments

Supported by grants from the Nord Pas de Calais region

More informations about MALDI Imaging team Laboratory: isabelle.fournier@univ-lille1.fr http://www.maldi-imaging.com

Corresponding author: yann.guitton@univ-lille1.fr