

Data acquisition and handling protocol for AP-MALDI UHR imaging on an Orbitrap instrument

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INTRODUCTION

AP-MALDI UHR module is a flexible add-on for existing Thermo LC/HRMS equipment. This Application Note presents a protocol for AP-MALDI imaging workflows based on a Thermo LTQ/Orbitrap Elite instrument. Brain samples were provided by Professor William J. Griffiths, Swansea University Medical School.

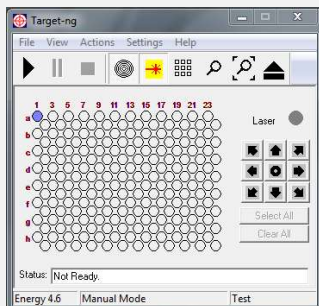
WORKFLOW

Sample Preparation:

- 1) 10-15 μm -cryosection of biological tissue
- 2) Matrix deposition with a TM-Sprayer (HTX Technologies) or equivalent to get homogeneous crystals deposition

Data Acquisition:

- 1) Definition of image parameters (mode, dimension, pixels) in *Target* (Control software)
 - Parameters are saved as .xml file
- 2) Molecular imaging using AP-MALDI UHR Source (MassTech) with LTQ/Orbitrap Elite (Thermo) high resolution MS.
 - Data saved as .raw file



Data Handling:

- 1) Data handling with ImageQuest (Thermo) using native files (xml and raw files)
- 2) Conversion of xml and raw files into imzML file using MassTech imzML Converter
- 3) Data handling with MSI reader, DataCube Explorer, SCILS...



Fig.1: MassTech AP/MALDI UHR add-on

KEY CHARACTERISTICS AND BENEFITS

- MALDI imaging capabilities down to 10 micron lateral resolution
- Switch from the AP-MALDI configuration to LCMS configuration within 5 minutes.
- Provides MALDI analysis and imaging capabilities to high-end LCMS instruments
- Laser focus can be adjusted from 10 to 30 microns

Available modes of operation

- Sequential MALDI analysis of multiple spot analysis using spiral or raster motion (AB Sciex OptiTOF 192, Bruker MTP96) with tunable parameters
- MALDI imaging using Pixel-Map, with tunable in-pixel motion for multiplexed analysis (HRMS, SRM...).
- MALDI imaging using Constant Speed Raster (CSR) modes for increased pixel rate (MS dependent)

CASE STUDY:

- Sagittal mouse brain sections were cryo-sectioned and spray coated with matrix.
- MALDI parameters: 1kHz laser frequency, 18% laser energy
- MS parameters R=15, 30, or 60k, Injection time (fixed)=200ms

Data handling using Thermo ImageQuest:

AP-MALDI UHR imaging has been applied to lipid imaging using a Thermo HRMS equipment. Thermo RAW file can be combined with XML position file (created by MassTech Target® control software) to produce MALDI MS images using Thermo ImageQuest software.

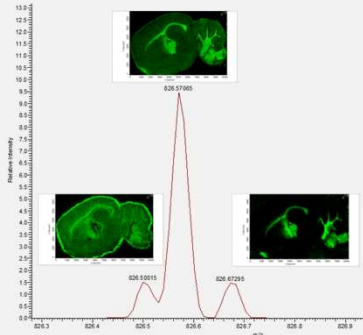


Fig.2: Single pixel mass spectrum showing 3 intact lipids in a 0.3Da mass window

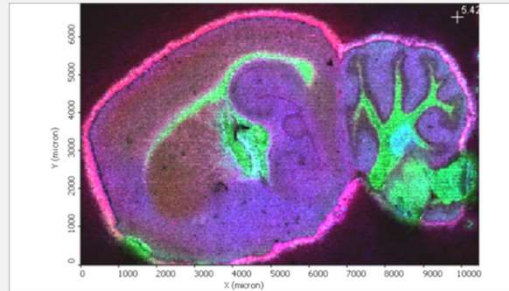
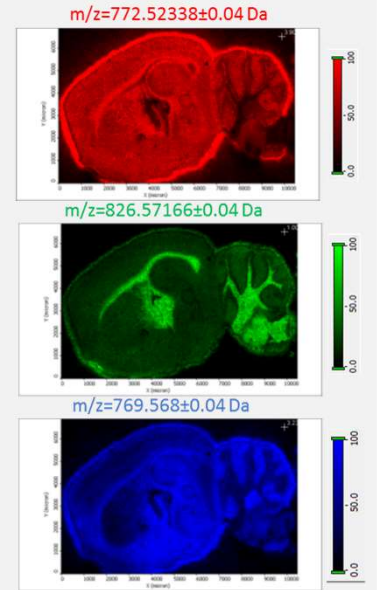


Fig.3: Overlaid AP-MALDI HRMS images of 3 lipids with a mass tolerance of 0.04Da (red: $m/z=772.52338$, green: $m/z=826.57166$, and blue : $m/z=769.568$) obtained using ImageQuest software



Data handling using alternative software:

After conversion of xml and raw files into imzML file using MassTech imzML Converter (or using ImageQuest), alternative software such as **MSI Reader** [1] or **DataCube Explorer** [2] can be used to provide complementary data handling features, such as various normalization possibilities, statistics, overlay with other imaging modalities or spectrum reconstruction from free-shaped Regions of Interest.

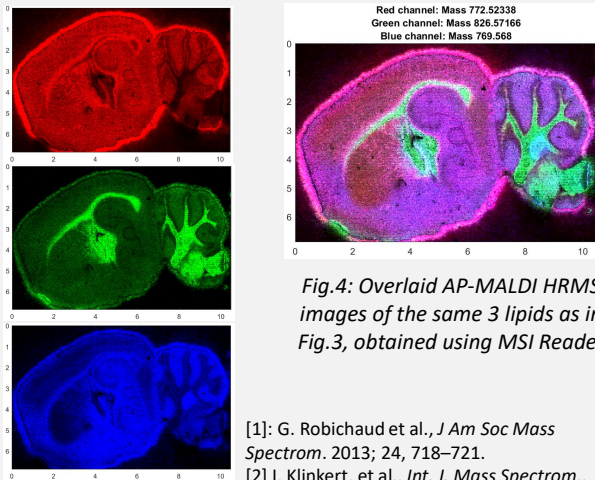


Fig.4: Overlaid AP-MALDI HRMS images of the same 3 lipids as in Fig.3, obtained using MSI Reader

- [1]: G. Robichaud et al., *J Am Soc Mass Spectrom.* 2013; 24, 718–721.
 [2] I. Klinkert, et al., *Int. J. Mass Spectrom.*, 2014. 362, 40-47

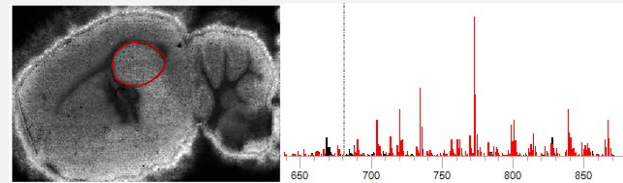


Fig.5: Spectrum reconstruction from free-shaped Regions of Interest using DataCube Explorer

Conclusion:

MassTech AP-MALDI UHR provides imaging capabilities to existing LCMS instruments such as Thermo HRMS equipment. APMALDI/ESI swap is done within minutes. AP-MALDI HRMS images can be handled by Thermo ImageQuest or alternative imzML-based software.

MassTech offers a range of analytical ionization sources, accessories and complete instruments for advanced analytical platforms. The AP-MALDI UHR is available exclusively from MassTech.

To request further information, please contact:

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MassTech selected LIST as European Application Lab. www.list.lu