AP-MALDI High Resolution MS imaging of skin biopsies

Dana El Assad, Cécile Martias, Gilles Frache
Materials Research and Technology Department, Luxembourg Institute of Science and Technology (LIST),
a MassTech European Application Lab

INTRODUCTION

Latest developments in the field of MALDI imaging has led to a significant impact in the pharmaceutical and cosmetics fields. Sample preparation, high spatial resolution and high selectivity are crucial parameters for reliable identification of molecules of interest on biological samples. MALDI UHR coupled with an LTQ/Orbitrap Elite provided the advantage of accurate mass measurements and high selectivity by tandem MS with a spatial resolution down to 10 µm that allowed the detection and localization of untargeted and targeted molecules in the human skin layers.

WORKFLOW

1) 10 µm-cryosection of human skin

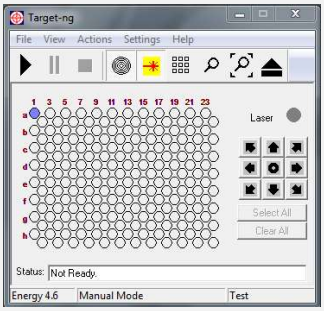
2) Matrix deposition with a TM-Sprayer (HTX Technologies) or equivalent

3) MALDI Source UHR (MassTech)
- Interchangeable with ESI
- Laser spot down to 10 µm

4) LTQ/Orbitrap Elite (Thermo)
- High Mass Resolution (up to 240 000 at m/z 400)
- High Mass Accuracy
- (≤ 1ppm)
- Structural confirmation and selectivity by MS/MS

Data Acquisition:

1) Definition of image parameters (mode, dimension, pixels) in MassTech *Target* (a control software)
→ Parameters are saved as .xml file



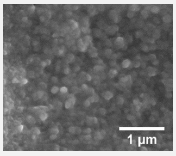
2) Molecular imaging using *AP/MALDI(ng) UHR* ion source (MassTech) with *LTQ/Orbitrap Elite* (Thermo) high resolution MS
→Data saved as .raw file

Data Handling:

1) Data handling with *ImageQuest* (Thermo) using native files (XML and RAW files) OR
2) Conversion of xml and raw files into imzML file using MassTech *imzML Converter*, and Data handling with *MSI reader*, *DataCube Explorer*, *Multimaging* (Imabiotech), *Lipostar* (Molecular Horizon), *SCiLS*, or other MS imaging software.

CASE STUDY:

- A skin biopsie (sample courtesy: Pierre Fabre Dermo-cosmetique) was cryo-sectionned and spray coated with HCCA matrix



HCCA matrix deposition:
- Homogeneous matrix layer
- Crystal size < 300 nm

- MALDI parameters: 1kHz laser frequency, 18% laser energy
- MS parameters: R=15, 30, or 60k, Injection time (fixed)= from 500ms to 1200ms

AP-MALDI Imaging of skin cross section at 10 micron lateral resolution

Many skin lipids from various lipid classes were detected and identified based on exact mass measurement (less than 1 ppm relative error)

Skin Layers	m/z	Tentative assignment with Metlin database
Stratum Corneum *Ceramides are mainly detected*	386.399	[Cer fragment + H]$^+$
	526.519	[Cer(d33:0) + H]$^+$
	554.551	[Cer(35:0) + H]$^+$
	740.712	[Cer(t46:0(2OH)) + H]$^+$
	754.620	[GlcCer(d38:2) + H]$^+$
Epidermis *Different lipids classes Phosphatidylcholine (PC), Sphingomyelin (SM), Phosphoethanolamine (PE)*	703.575	[SM(d34:1)+H]$^+$
	734.569	[PC(32:0)+ H]$^+$
	744.553	[PE(36:2)+H]$^+$
	758.569	[PC(34:2)+H]$^+$
Dermis *Mainly proteins and peptides are located*	582.273	[$C_{20}H_{37}N_{11}O_8$ + H]$^+$
	600.283	[$C_{33}H_{37}N_5O_6$ + H]$^+$

Many skin lipids from various lipid classes were detected and identified based on exact mass measurement (less than 1 ppm relative error)

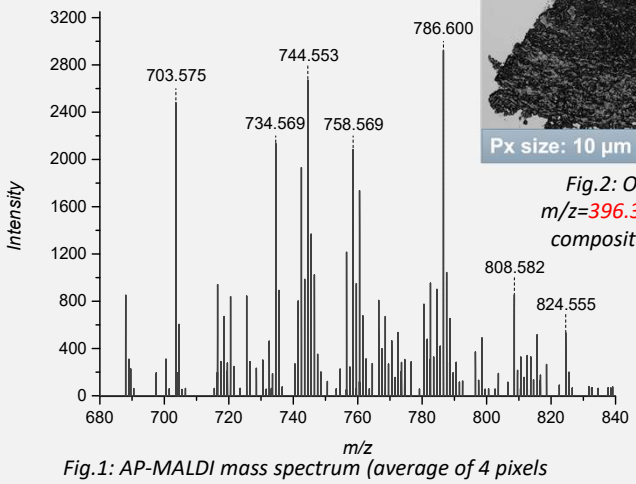


Fig.1: AP-MALDI mass spectrum (average of 4 pixels in the epidermis)

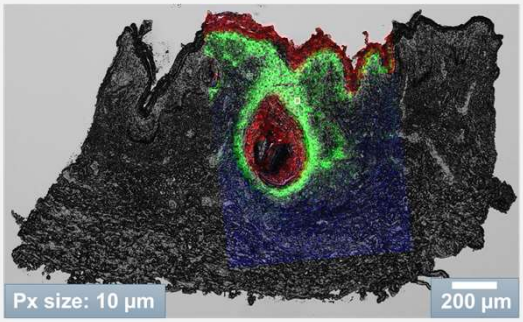


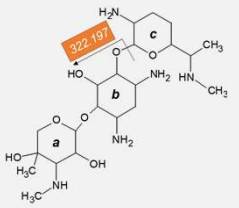
Fig.2: Overlaid optical and RGB image of m/z=396.399, 744.553, 582.273, showing lipid composition in skin layers near a hair follicle

AP-MALDI HRMS imaging

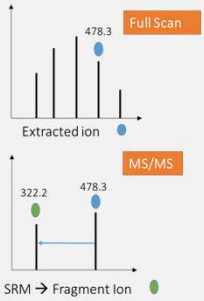
- Detection of endogenous molecules in the different layers of the skin
- Identification based i

Simultaneous Full Scan/SRM by AP-MALDI-HRMS Imaging

A signal at m/z 478.324, tentatively assigned as Gentamicin C1 (migrating from the cell culture medium used during the tissue manipulations) was observed in the dermis.

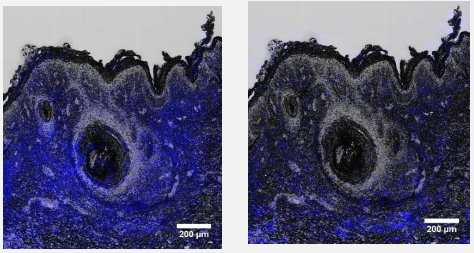


Antibiotic Gentamicin C_1
$[M+H]^+$; *m/z 478.324*



Specific localization of the antibiotic by SRM

Gentamicin C1 was observed by Simultaneous Full Scan/SRM by MALDI-Imaging.



Full Scan m/z 300-1000 + SRM (m/z 478.3 → 322.2)

Orbitrap — ***In each pixel*** — *Ion Trap*

SIMULTANEOUS FULL SCAN/SRM

- High selectivity
- Targeted localization of an antibiotic in the skin
- Applications in pharmaceutics and cosmetics field

- *Sample preparation with HCCA Matrix enabled to obtain MALDI Imaging on skin sections with a spatial resolution down to 10 µm while keeping a good sensitivity for the detection of endogenous molecules in the different skin layers.*
- *A method providing simultaneous Full Scan/SRM was developed to specifically localize targeted molecules such as active substances in the skin.*

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

To request further information, please contact:
sales@apmaldi.com

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

6992 Columbia Gateway Drive, Suite 160
Columbia, MD 21046, USA
Tel.: +1-443-539-1758
Email: msms@apmaldi.com
Web: www.apmaldi.com