Mass Spectrometry Imaging of Protein and Lipid Distribution in *Ex Vivo* Human Skin

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MALDI-MSI Microprobe Mode

**What do MALDI Images Represent?**

- Each image pixel correlates to the corresponding region of the original sample.
- Images are produced for intensity of a selected ion or ions.
- Ion intensity is shown as a change in "brightness/hue" for each pixel.
- Images of different ion distributions can be overlaid for more complex analysis.
Sections

• Some Comments on Xenobiotic Imaging by MALDI-MSI
• Lipid Imaging in Skin
• Protein Imaging in Skin
• Other Projects

Positive Ion MALDI Mass Spectrum of Imipramine RMM 280 Showing An Intense \([\text{M+H}]^+\) ion at \(m/z\) 281
For compounds that do not "fly" by MALDI-MS derivatisation can be used. Here a carbonyl compound has been derivatised with DMNTH (4-dimethylamino-6-(4-methoxy-1-naphthyl)-1,3,4-triazine-2-hydrazine) (Buttarro et al 2007) to yield a derivative RMM 424 with good MALDI properties.

......and sometimes the mass spectrometry is difficult

MALDI-MS Images of the Distribution of A Carbonyl Compound as its DMNTH Derivative in ex-vivo Human Skin. The 30 µm MALDI-MSI data is shown overlaid on H&E stained sections and indicates that the compound does not penetrate into the dermis.

......and when it does all work quantification may be possible......
Human Skin Analysis

- Intact Protein Analysis
  - Sections washed to remove salts/lipids coated with sinapinic acid matrix for direct MALDI analysis
- On-tissue digestion for identification of proteins
  - Sample Preparation
    - Overnight on tissue tryptic digestion (sample was sprayed with trypsin) for peptide analysis
    - Ethanol washes, 70% and 90% followed by a brief chloroform wash
    - α-CHCA matrix application using SunCollect autosprayer (KR Analytical)
  - Instrumentation
    - Applied Biosystems Voyager-DE STR (modified with Nd:YAG laser)
    - Applied Biosystems, MALDI-Q TOF “Q-Star Pulsar -”(modified with Nd:YAG laser)
    - Waters, MALDI-HIDMS Synapt G2

Bottom-up “Shotgun” Proteomics

- Lyse cells
- Mixture of 1000’s of peptides
- Trypsin
- 2-D LC-MS/MS
- RPLC-MS/MS
- LC-IMS-MS/MS
- Database searching - matching MS/MS data with peptide sequence

On-Tissue Bottom-up “Shotgun” Proteomics

- Tissue Section Sprayed or Printed with Trypsin
- Mixture of 1000’s of peptides
- MALDI-PMF
- Database searching - matching MS/MS data with peptide sequence
From skin all peptide MS/MS spectra we acquire without ion-mobility show the presence of lipid peaks – multiple species.
Incorporate IMS Separation on Synapt-G2

Mobilogram shows at least 3 species at this m/z
Acquire MS/MS Data with mobility separation no evidence of lipid peaks

Localisation of Peptide Signals

Proteins Identified

- Collagen
- Decorin
- Keratin
- Haemoglobin
- Serum Albumin
- Lumican

In reality a very small list: suggests complementary techniques (conventional proteomics) needed for identification and only use MALDI-MSI for imaging.
Analysis of Treated Human Skin in Multiple Sample Experiments

MALDI Imaging of Multiple Samples: Upregulation in Treated Skin

A MALDI image of a peptide species present at m/z (A) and m/z (B), both of which are thought to belong to a single protein. The image shows difference in levels of expression between: (i) human skin that was treated with the acetone:olive oil vehicle, (ii) sodium lauryl sulphate, (iii) untreated, (iv) treated with glycerol, (v) DNCB and (vi) sulfamethoxazole.

Experimental Setup

- MALDI-MS image, acquired at a spatial resolution of 150 µm x 150 µm, from untreated human skin.
- Data displayed using Waters HD imaging software.

MALDI-MS imaging of lipids in ex vivo human skin

- Philippe A. Haro
- Emmanuel Francone
- Emmanuel Claude
- St. Luke Woodcock
- Malcolm A. Church
High mass resolution, positive ion MALDI Mass Spectrum of normal human skin, using a-CHCA/ANI as a matrix, with an enlarged inset showing the peak resolution achieved (35,000-40,000 FWHM) (Hart et al., 2011).

MALDI mass spectra taken from regions of treated skin sections.

Principal Component Analysis Scores and Loading s Plots for a Series of MALDI Mass Spectra Taken from Different Layers of Ex-Vivo Human Skin.

IMS Separation of Lipid Species from Human Skin - Synapt G2.
Instrument is also a 40,000 FWHM Resolution Instrument Capable of 1ppm Accurate Mass Measurement

<table>
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<tr>
<th>Instrument Description</th>
<th>Spectrometer</th>
<th>Mass Range (amu)</th>
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MALDI-MS Images of the Distribution of Selected Lipid Species in Human Skin (150 µm)

(A) Positive Ion MALDI Product Ion Mass Spectrum of the m/z species 703, identified to be SM(18:1/16:0) [M+H]+. (B) Positive Ion MALDI Product Ion Mass Spectrum of the lithium adduct of SM(18:1/16:0) ([M+Li]+) m/z 709.5, displaying the corresponding molecular structure (Hart et al., 2011).
Images at 30 µm Spatial Resolution

MALDI images of a species at m/z 417. The image shows differences in levels of expression between:

(A) human skin treated with hydroquinone, (B) sulfamethoxazole, (C) SLS, (D) the acetone:olive oil vehicle, (E) DNCB, (F) cinnamaldehyde and (G) human skin left, untreated.

Other Projects

Response to Treatment in LSE

Oilatum 24 hours  Oilatum 4 hours  Physiogel 6 hours  Physiogel 24 hours
Compound formulations studied

Physiogel A.I Cream
Active ingredient: Palmitoylethanolamide (PEA) [299.2824 m/z]
Composed of purified water, olive europaea, glycerol 92.0473 m/z, pentylene glycol, palm glycerides, cika, hydrogenated lecithin, equol, olea 410.3913 m/z, betaine 117.0790 m/z, palmitoylethanolamine (0.3% active ingredient), stearic acid 287 m/z, octamide MEA, hydroxyethylcellulose, sodium carboxylic, carbomer and Xanthan Gum.

Oilatum Junior Cream (control)
Oilatum is composed of Active ingredients; light paraffin 6.0% and soft paraffin 15%. Other ingredients include; Macrogol 1000, monobutyrate, cetostearyl alcohol, glycerol, potassium sorbate, benzyl alcohol, citric acid, povidone and purified water.

Image of LSE sections (across 3 different treatment groups). The samples were incubated for 24 hours after the treatment, (Control group untreated). The image was acquired at a 25 um x 25 um resolution; normalised to the total ion count (Data produced 15th/4/13).
Conclusions

- The use of IMS with MALDI images aids specificity.
- Statistical analysis of the large data sets obtained is essential.
- Using complementary techniques provides a means to identify targets which can be related to the MALDI imaging data set.

Current/Future Work

- MS/MS and TLC/MS/MS using SYNAPT G2 of LSE for identification of species detected.
- Knock down LSE models which mimic disease state.
- HDMS® simultaneous MS – MS/MS of each peak during a MALDI-MS acquisition using preset ramping collision energies.
- Statistical analysis using Matlab (refining of methodology)

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