Imaging MS: Molecular Histology
(Everybody Loves a Pretty Picture)

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Imaging Mass Spectrometry

- Map distribution of known and unknown analytes in solid samples
- Traditionally done on harvested tissue sections

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• Map distribution of known and unknown analytes in solid samples
• Traditionally done on harvested tissue sections

Wide Range of Analytes
Compatible with Many Sample Types
Can be Targeted or Discovery
Multiple Ionization and Mass Analyzers

Imaging Mass Spectrometry

• Use mass spec to generate a spatially accurate map of the biomolecular species present in sample
• Raster laser across a solid sample and acquire mass spectra
• Appealing technique (people like pretty pictures)

First Imaging Paper

**Molecular Imaging of Biological Samples: Localization of Peptides and Proteins Using MALDI-TOF MS**

Richard M. Caprioli, * Terry B. Farmer, and Jocelyn Gile

- Cited 1,097 times (as of 7/9/18)
- Describes IMS concept
- Demonstrates imaging from:
  - Coomassie on target, C-18 resin blot, buccal cells and tissues

**Figure 1** Conceptual representation of the MALDI MS imaging procedure on a tissue slice.

**Figure 2** Copyright symbol on MALDI target using Coomassie dye

**First Imaging Paper**

**Figure 3.** Aggregate of human buccal mucosa (cheek) cells: A) under UV light (B) Selected ion image of m/z 7605 (IB-1 protein fragment) C) Representative MALDI MS spectrum obtained from cheek cells

**Figure 7** Representative MALDI MS spectrum of the C-18 blotted target for a section of rat pituitary to identify proteins and peptides

Nomenclature

- **IMS** can be Imaging Mass Spectrometry or Ion Mobility Spectrometry
- Many members of the imaging community have switched to **MSI** (Mass Spectrometry Imaging) to avoid confusion.
- Some retain the use of IMS.

MALDI versus DESI Imaging

- **Matrix-Assisted Laser Desorption/Ionization (MALDI):**
  - Traditionally been more versatile—proteins, peptides, lipids, metabolites, etc.
  - More common instrumentation.
- **Desorption Electrospray Ionization (DESI):**
  - Lipids, recent demonstration of protein ionization.
  - No matrix interference.
  - Better spatial resolution.
  - Fewer DESI instruments.
Palmer, A., Trede, D. & Alexandrov, T.
“Where imaging mass spectrometry stands: here are the numbers”
*Metabolomics* 12, 107 (2016).

Data collected in 2015; Published in 2016

### Important Details

<table>
<thead>
<tr>
<th>Assay</th>
<th>Measures</th>
<th>Channels</th>
<th>Spatial Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALDI Imaging</td>
<td>Protein/Peptide/Lipid/Metabolite</td>
<td>200-300 analytes</td>
<td>20 μm*</td>
</tr>
<tr>
<td>nanoDESI Imaging</td>
<td>Lipid/Metabolite</td>
<td>200-300 analytes</td>
<td>8 μm</td>
</tr>
<tr>
<td>Microscopy</td>
<td>~20-30 analytes</td>
<td>&lt;1 μm</td>
<td></td>
</tr>
</tbody>
</table>

*MALDI imaging can be performed at 10 μm spatial resolution, with reduced laser power.

Average mammalian cell is 10μm diameter

*Compared to spectroscopic methods, IMS provides greater chemical information at the expense of spatial resolution*
Imaging Mass Spec of 3D Cell Cultures

Sample Preparation

- Embed in gelatin
- Freeze
- Section with cryostat (~10-20 μm thick)
- Thaw-mount onto ITO slides
- Wash to remove lipids
- Dry in desiccator
- Application of MALDI matrix
More on Sample Prep

• If protein/peptide imaging, series of alcohol (Ethanol or Isopropanol) washes to:
  – Reduce salts and lipids
  – Gentle fixation protocol
  – Improves intensity of protein signals

Challenge: Identification

• Identification of protein species
• Complement the imaging with nLC-ESI-MS/MS
• Sampling by laser capture microdissection or other means
• Improved fragmentation leads to increased number of protein IDs
Matrix Selection

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Mass (Da)</th>
<th>Laser λ (nm)</th>
<th>Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,5-Dihydroxybenzoic acid (DHB)</td>
<td>154</td>
<td>337</td>
<td>Lipids, small peptides, carbohydrates and nucleotides</td>
</tr>
<tr>
<td>α-cyano-4-hydroxycinnamic acid (HCCA)</td>
<td>189</td>
<td>266, 337</td>
<td>Peptides, small proteins, lipids, glycoproteins and nucleotides</td>
</tr>
<tr>
<td>3,5-dimethoxy-4-hydroxycinnamic acid (SA, sinapinic acid)</td>
<td>224</td>
<td>266, 337</td>
<td>Large proteins, glycoproteins and hydrophobic proteins</td>
</tr>
<tr>
<td>4,6-Trihydroxyacetophenone (THAP)</td>
<td>186</td>
<td>NA</td>
<td>Oligonucleotides</td>
</tr>
<tr>
<td>3-Hydroxypicolinic acid (3-HPA)</td>
<td>139</td>
<td>337, 355</td>
<td>Oligonucleotides</td>
</tr>
<tr>
<td>Picolinic acid</td>
<td>123</td>
<td>266</td>
<td>Oligonucleotides</td>
</tr>
<tr>
<td>2,6-Dihydroxyacetophenone (DHAP)</td>
<td>152</td>
<td>337, 355</td>
<td>Phospholipids and large proteins</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>123</td>
<td>266, 337, 355</td>
<td>Proteins and oligonucleotides</td>
</tr>
</tbody>
</table>

Sunscreen--Selected to Absorb UV Light

Most Popular Matrices

- **Protein (>5kDa):** Sinapinic acid in 50-60% ACN
- **Peptide:** CHCA or DHB in 50% ACN
- **Metabolites:** 9-Amino acridine (9-AA)
- **Lipids:** DHB & 2,6-dihydroxyacetophenone (DHA) in 60-70% ethanol
- Sinapinic acid, CHCA, DHB best in + mode
- DHA good in + or – ion mode
- 9-AA best in negative mode
Matrix Application

- Critical to obtain high quality spectra map
- Need to be very careful so as to not add too much liquid, as molecules will migrate

**Goal:** Homogenous coating of the smallest possible matrix crystals that are co-crystallized with the analyte of interest; Spatial resolution is limited to crystal size

Matrix Application 1: Pipet

- **Manual application 1: With Pipet**
  - Simple & quick
  - Get a pretty big spot (1-2 mm)
  - Poor reproducibility of matrix coverage
  - Variable MS signal
  - Reduces spatial resolution

www.pipettes.com
Matrix Application 2: Printers

- **Robotic spotter (Inkjet Printer)**
  - Good control over location of matrix deposition
  - Ordered array of pL spots deposited at discrete locations
  - Helpful if correlating with histopathological samples
  - More reproducible than pipetting
  - Smaller spot size (80-200 μm)
  - Resolution limited by size and density of spots


LabCyte Image: https://www.selectscience.net/products/portrait-630-reagent-multi-spotter

Matrix Application 3: Airbrush

- Spray matrix solution
- Homogeneous field of 1-20μm crystals formed
- Lateral resolution is determined by size of laser spot
- Don’t overwet sample by spraying for too long
- 12 applications in 1 hr (5 min intervals)

Image: https://www.blickart.com/products/airbrushes
Matrix Application 4

- **Spraying**: Nebulizer
  - Spray matrix solution
  - Homogeneous field of 1-20μm crystals formed
  - Lateral resolution is determined by the size of the laser spot
  - Don’t overwet sample by spraying for too long
  - 12 applications in 1 hr (5 min intervals)
  - Can also *spray Trypsin to digest proteins into peptides*

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Tryptic Digestion to Detect Peptides

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Matrix Application 5: Sublimation

- Many matrices sublimate readily (which is why they are ideal for MALDI)
- Put them and slide in chamber and reduce pressure
- Matrix sublimes onto slide
- Results in smaller crystals
- More work, but worth it
- Chambers need to be specially constructed in glass shops

Website: “Applying Your MALDI Matrix Like a Boss”
https://bitesizebio.com/36681/applying-maldi-matrix/

Dried Droplet Versus Sublimation

Matrix crystals resulting from dried droplet preparations on stainless steel plate (left) & from sublimed preparations on hydrophobically coated aluminum oxide surfaces (right) shown in 500 (large pictures) and 20,000 magnifications (small pictures).
3D Imaging

- Lingjun Li, Wisconsin
- 3D distributions of neuropeptides in *C. borealis* brain
- Individual optical images of each layer of section are shown in the top panel
- Tissue sections were prepared using matrix spraying method
- Six neuropeptides
- 7 slices collected, with 132 μM between each slice

Chen et al. JASMS, 2009, 20(6), 1068-1077.

Whole Animal Imaging

- Detection of drug and metabolite distribution
- Whole rat sagittal tissue section dosed with Olanzapine

A. Optical image of a 2 h post OLZ dosed; Organs outlined in red. Scale Bar, 1 cm
B. MS/MS ion image of Olanzapine (*m/z* 256)
C. MS/MS ion image of *N*-desmethyl metabolite (*m/z* 256)
D. MS/MS ion image of 2-hydroxymethyl metabolite (*m/z* 272)

Correlating Histology & IMS

• Use in tandem with other imaging approaches
• H&E Staining
• Hematoxylin stains negatively charged species (nucleic acids, etc) blue
• Eosin stains proteins bright pink
• Red blood cells stain intense red


Correlation of IMS & Histology

• Two options for histology-directed IMS
  1. **Slice two close sections**: IMS for one, histo the other
     • Advantage: Can optimize each slice for specific method
     • Disadvantage: Different slices
  2. **One slice, IMS first, then stain**: 
     • Advantage: Same slice
     • Disadvantage: IMS can deplete the sample, making histological detection less reliable
Imaging from FFPE Tissues

- Ron Heeren (Univ. Maastricht, IMS) and Peter Verhaert (Insect Neuropeptides)
- Formalin-fixed paraffin-embedded (FFPE) popular approach to preserve tissues, especially in clinical settings
- Neuroendocrine tissue from cockroaches (*P. americana*)
- Embedded in paraffin for **30 years**
- Analyzed by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)

Paine et al. *Anal. Chem.*, Just Accepted Manuscript. DOI: 10.1021/acs.analchem.8b01838

- Localized more than 20 peptide ions, including 14 known neuropeptides
- Protocol: Brief deparaffinization steps involving xylene and ethanol
- Neuropeptides are amenable to this type of analysis; no digestion needed

Paine et al. *Anal. Chem.*, Just Accepted Manuscript. DOI: 10.1021/acs.analchem.8b01838
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DESI and MALDI

- DESI developed by Graham Cooks (Purdue)
- Prosolia--DESI sources that are compatible with multiple mass spec vendors

A-C) DESI-MS ion images of mouse brain coronal section
D-F) After DESI-MS imaging, same tissue section subjected to MALDI-MS imaging
G-I) Optical image of same tissue section after removal of MALDI matrix and H&E staining

DESI Excels at Lipid and Metabolite Imaging

Desorption Electrospray Ionization Mass Spectrometry Imaging of Proteins Directly from Biological Tissue Sections
Kyana Y. Garza, Clara L. Feider, Dustin R. Klein, Jake A. Rosenberg, Jennifer S. Brodbelt,* and Livia S. Eberlin†

Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, United States

Supporting Information

ABSTRACT: Analysis of large biomolecules including proteins has proven challenging using ambient ionization mass spectrometry imaging techniques. Here, we have successfully optimized desorption electrospray ionization mass spectrometry (DESI-MS) to detect intact proteins directly from tissue sections and further integrated DESI-MS to a high field asymmetric waveform ion mobility (FAIMS) device for protein imaging. Optimized DESI-FAIMS-MS parameters were used to image mouse kidney, mouse brain, and human ovary and breast tissue samples, allowing detection of 11, 16, 14, and 16 proteoforms, respectively. Identification of protein species detected by DESI-MS was performed on-tissue by top-down ultraviolet photodissociation (UVPD) and collision induced dissociation (CID) as well as using tissue extracts by bottom-up CID and top-down UVPD. Our results demonstrate that DESI-MS imaging is suitable for the analysis of the distribution of proteins within biological tissue sections.

DESI Protein Imaging from Tissue

Washed mouse brain tissue section

Human normal mammary and breast cancer tissue

Quantification

• Often performed with deuterated samples
• MALDI quantification
  – Spray standard layer before matrix layer
  – Standard MUST co-crystallize completely with matrix
• DESI quantification
  – Deuterated standard added with DESI solvent
  – No need to worry about crystallization

A couple cool applications

Molecular Cartography & Handheld Ionization Sources

Bouslimani et al. PNAS 2015;112:17:E2120-E2129
3D topographical maps of molecules and microbes distributed on human skin

- **Pieter Dorrestein** (UCSD)—Imaging MS & **Theodore Alexandrov** (EMBL)—Data visualization
- Study chemical makeup of human skin surface and correlate to microbes that live in skin
- Correlations between molecules and microbes give insights into the chemical milieu in which these different microbial communities live

Bouslimani et al. PNAS 2015;112:17:E2120-E2129

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Examining skin chemistry and microbes

- Swab sampling of a female and male human
- Swabs analyzed by MALDI-TOF-MS for proteins/peptides and UPLC-QTOF-MS/MS for small molecules
- Microbes determined by 16S RNA

Bouslimani et al. PNAS 2015;112:17:E2120-E2129
Correlated Topographical Maps

Correlate chemicals on the skin with microbial locations

Bouslimani et al. PNAS 2015;112:17:E2120–E2129

Mass Spectrometry-Based Visualization of Molecules Associated with Human Habitats

- Examine chemicals in the cars we drive, the homes we live in, the restaurants we visit, and the laboratories and offices we work in
- Provide a snapshot of the various molecules to which humans are exposed on a daily basis in five habitats
- Examined frequently (door handles) and less frequently touched surfaces (bike frame)
- Most MS/MS spectra from:
  - Personal care products
  - Plasticizers
  - Cleaning supplies
  - Food
  - Food additives
  - Medications

Molecular 3D cartography of human buildings

a) Chemical features detected in a public party room during a social gathering event

b) Two-bedroom apartment

c) Public drinking fountain as well as persons interacting with these environments

- Relative ion intensities are shown in log scale as 3D heat maps through color gradient from red (highest) to blue (lowest).


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**DESI Examining Tumors Margins**

- Livia Eberlin; UT Austin

- Developing DESI imaging approaches to characterize metabolic profiles of ovarian tumors

- Identified predictive markers of cancer aggressiveness, including various free fatty acids, metabolites, and complex lipids such as ceramides, glycerophosphoglycerols, cardiolipins, and glycerophosphocholines

- Built classification models from DESI images of 78 different tissue samples

- Enabled diagnosis and prediction of cancer and normal tissues, with overall agreements to histology of 96.4% and 96.2%

**Cancer Res. 2017;77(11):2903-2913.**
Handheld Ambient Ionization in the Clinic

**iKnife:**
- REIMS ionization
- Developed by Zoltan Takats, Imperial College
- Commercialized by Waters

**MassSpec Pen:**
- DESI ionization
- Developed by Livia Eberlin, UT Austin)

*Scalpel is replaced by ionization source*


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**Takeaway Messages**

- Enables spatial localization of proteins, peptides, lipids and metabolites from human tissues, animal models, cell cultures and organoids
- Rich chemical information, though poorer spatial resolution compared to spectroscopic approaches