MALDI Imaging – Looking beyond Classical Histology

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Schedule of the Seminar

1. 25-min presentation to introduce MALDI imaging

2. Software demo on real imaging data
   • Mouse kidney by FTICR
   • Rat testis by MALDI TOF
What is Imaging?

Imaging involves the visualisation of sample information in different ways.

- H&E
- IHC
- Nuclear Magnetic Resonance Imaging (MRI/MRS)
- Nuclear computed tomography (CT)
- Positron Emission tomography (PET)
Imaging Mass Spectrometry

Exploiting Mass Spectrometry for the analysis of multiple analytes, from small molecules and metabolites to lipids, peptides and proteins

**MALDI** (Matrix-Assisted Laser Desorption Ionization)
**SIMS** (Secondary Ion Mass Spectrometry)
**DESI** (desorption electrospray ionization)
MALDI stands for...
Matrix-Assisted Laser Desorption/Ionization

- invented by Tanaka (Nobel prize award in 2002), Hillenkamp and Karas in the mid 80s
MALDI-Imaging Mass Spectrometry (IMS)

The Principles
The Principles
MALDI Imaging Workflow

1. Tissue
2. Cryosection
3. Matrix coating
4. MALDI Mass Spectrometry

Steps include:
- H&E staining
- Image analysis
- Mass spectrometry
Co-registered Image & Virtual Microscopy

- H&E staining after MALDI measurement on the same sample: Unambiguous correlation of optical and molecular imaging modalities
- High-resolution virtual slide provide full access to molecular and histological information
## MALDI Imaging Applications

<table>
<thead>
<tr>
<th>Discovery “Profiling”</th>
<th>Small molecules (&lt;2-4 kDa)</th>
<th>Large molecules (&gt;2-4 kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipidomics, Metabolomics and Peptide Profiling</td>
<td><strong>FTICR</strong> 😊 <strong>TOF</strong></td>
<td>Proteomic Biomarker Discovery</td>
</tr>
<tr>
<td>Drug &amp; Metabolite Detection</td>
<td><strong>FTICR</strong> 😊 <strong>TOF</strong></td>
<td>Protein Detection</td>
</tr>
<tr>
<td>Targeted Drug &amp; Metabolite Detection</td>
<td><strong>FTICR</strong> 😊 <strong>TOF</strong></td>
<td><strong>TOF</strong></td>
</tr>
<tr>
<td>Targeted Protein Detection</td>
<td><strong>TOF</strong></td>
<td><strong>TOF</strong></td>
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</tbody>
</table>
Effect of MS resolving power on phospholipid analyses

TOF spectrum

FTICR spectrum
Isotopic Fine Structure
Read chemical formula directly from mass spectrum

**SFXR Results**

- **C_{27}H_{30}O_{16}**: ✔️
- **C_{24}H_{29}O_{18}N_{2}**: ✗
- **C_{25}H_{25}O_{14}N_{6}**: ✗
- **C_{22}H_{17}O_{8}N_{16}**: ✗

**Experimental**

- N = 0
- O = 16
- N ≠ 2
- O ≠ 18
- N ≠ 6
- O ≠ 14
- N ≠ 16
- O ≠ 8

**Mass Spectrum**

- 633.5
- 634.0
- 634.5
- 635.0
Lateral Resolution is Important to Understand Tissue Morphology

Rat testis sample: Charles Pineau, Univ. Rennes, France
Lateral resolution is Important to Understand Tissue Morphology

- Cover whole tissue
- Hot spots
- Good Sensitivity
- Similar to QWBA

≥ 100 µm

100 <-> 50 µm

- Large portion tissue
- Some gross tissue features
- Compare Distributions
- Medium Sensitivity

< 50 µm

- Small portion of tissue
- Histology overlays
- Explore mechanisms
- Compare Distributions
- Low Sensitivity

Target Monitoring and Therapeutic Compound Distribution
MALDI imaging in DMPK – how it started

- MALDI imaging as a tool to complement whole body autoradiography (WBA)

<table>
<thead>
<tr>
<th>Feature</th>
<th>MALDI imaging</th>
<th>WBA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sensitivity</strong></td>
<td>50-200 ng/g, drug dependent can be lower</td>
<td>high</td>
</tr>
<tr>
<td><strong>cost</strong></td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td><strong>quantitation</strong></td>
<td>requires extra effort</td>
<td>yes</td>
</tr>
<tr>
<td><strong>unique advantages</strong></td>
<td>No radioactivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can differentiate drug and metabolites</td>
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</tbody>
</table>
MALDI imaging requires **no radioactive label** for drug imaging and can differentiate drug and different metabolites.
Rapid Assessment of Drug and Metabolite Distribution in Whole-Body

- Male Sprague-Dawley rats, single 10 mg/kg PO dose olanzapine. Euthanized at 1, 3, 6, 12 and 24 hours post-dose and flash frozen.
- Tissue section: 20 mm; mounted on steel MALDI plates
- Matrix: spray coated with DHB (40 mg/mL)
- Lateral resolution: 500 µm
- Number of pixels per image: 20,000 – 25,000 pixels
- Instrument: 7T FTMS

In collaboration with Genentech
Olanzapine distribution over time

Olanzapine, m/z 313.1481

1 hour post-dose

3 hours post-dose

6 hours post-dose

12 hours post-dose

24 hours post-dose

In collaboration with Genentech

Quiason, et. al. ASMS 2012
Olanzapine Metabolites Distribution

Oxidized olanzapine, m/z 329.1431

N-desmethyl olanzapine, m/z 299.1325

1 hour

3 hours

6 hours

12 hours

24 hours

In collaboration with Genentech

Quiason, et. al. ASMS 2012
Automated Tissue State Assignment for High Resolution MALDI-FTMS Imaging Data

- Fully automated tissue assignment
- Hierarchical clustering following smoothing and normalization of first 7 PCs
  - 21 clusters shown, generated from the mass range 400 – 900 m/z
Biomarker Discovery
Typical proteomics approach to find biomarkers for tumor

- Non-tumor
- Tumor

Typical approach:
1. Non-tumor tissue
2. Tumor tissue
3. Blending
4. Scissors
5. LC-MS/MS
Pathologists’ understanding of tumor

- Invasive tumor cells
- Carinoma in situ (early stage tumor cells)
- Neoplastic cells (pre-tumor cells)
- Epithelial cells
- Lymphocyte infiltration
- Inflammation
- Connective Tissue

Tumor cells are not homogenous:
- Tumor stem cells (*)

- Different stages
- Different clones

The surrounding of the tumor (stroma) influences the tumor:
- Tumor microenvirement
- Molecular differentiation
Pathologists’ understanding of tumor

- The tissue is a complex mixture of different cell types in different ratios
- If the tissue is homogenized for a shotgun proteomics approach, all the spatial information is lost
- It is not even possible to differentiate tumor and inflammation markers
- The spatial organization of the protein expression is crucial for understanding the sample
HER2: Therapy prediction mammacarcinoma

**Immunohistochemistry**

- Score 0 and 1+
  - Negative
- Score 2+
  - Equivocal
- Score 3+ (see table 1)
  - Positive

**Fluorescence in situ hybridization**

- HER2 ratio < 2.00
  - Negative
- HER2 ratio ≥ 2.00
  - Amplified
  - Positive

**No Therapy**
- Trastuzumab (Herceptin®)

**Therapy**
- Trastuzumab (Herceptin®)
MALDI imaging to discover HER2 markers in breast cancer

48 breast cancer tissue samples pre-characterized with IHC and FISH:

<table>
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<tr>
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<tbody>
<tr>
<td>HER2 pos.</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>HER2 neg.</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

MALDI imaging spectra from tumour cell populations

Rauser, S. et al, J. Proteome Res. 2010, 9, 1854–1863
Direct correlation of 8.4 kDa protein as HER2+ surrogate maker

Markers Discovery by Statistical Analysis

P value for each < 0.05

AUC = 0.84

AUC = 0.93

J Proteome Res 2010;9(4):1854-63
Direct correlation of 8.4 kDa protein as Human Epidermal Growth Factor Receptor 2 (HER2)+ surrogate maker.
Direct correlation of 8.4 kDa protein as HER2+ surrogate maker
Identification of 8.4 kDa Protein

J Proteome Res 2010;9(4):1854-63
Direct correlation of 8.4 kDa protein as HER2+ surrogate maker

8.4 kDa Protein identified as CRIP1_HUMAN

J Proteome Res 2010;9(4):1854-63
Validation: Tissue microarrays (n = 75) of breast cancer tissue using anti-HER2 and anti-CRIP1 antibodies

HER2+ case

HER2- case

α-HER2

α-CRIP1
Comparison of IHC & MALDI-IMS

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<thead>
<tr>
<th></th>
<th>Immunohistochemistry</th>
<th>MALDI Imaging mass spectrometry</th>
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<tbody>
<tr>
<td>Spatial resolution</td>
<td>++ +</td>
<td>++ (+)</td>
</tr>
<tr>
<td>Multiplexing</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Label-free analysis</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Identification</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Quantification</td>
<td>+/-</td>
<td>+ +</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>High throughput</td>
<td>+++</td>
<td>+ +</td>
</tr>
<tr>
<td>Low sample amount</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Modified from Laboratory Investigation (2015), 1-10
MALDI imaging enables simultaneous mapping of multiple analytes directly from tissue without radioisotope or fluorescence labeling.

Unambiguous identification of small molecule based on high resolution and high mass accuracy.

Statistical analysis enables automatic tissue state assignment.

MALDI imaging is a powerful tool for biomarker discovery.

- Prediction of HER2 status in breast cancer based on 7 proteins in the 4-8 kDa range.
Software Demo
Targeted Drug Monitoring - Olanzapine

- Tissue:
  - Mouse kidney
  - Single 100 mg/kg PO olanzapine
  - Sacrificed after 2 hours after dosage
- Matrix:
  - DHB (30 g/L in 50% MeOH, 0.2% TFA)
  - Sprayed by ImagePrep
- Spatial resolution: 200 um
- No. of laser shot: 300/pixel
- No. of pixel: 1031
- Instrument: 7T solariX XR FTICR

Data from: Professor Masaya Ikegawa, Doshisha University
Protein Analysis

- Tissue:
  - Rat Testis
- Matrix:
  - HCCA (7 g/L in 50% ACN, 0.2% TFA)
- Spatial resolution: 20 um
- No. of pixel: ~11000
- Instrument: MALDI-TOF ultrafleXtreme