MALDI Imaging
Mass Spectrometry

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What is MALDI Imaging?

MALDI:
Matrix Assisted Laser Desorption Ionization

Imaging:
Imaging is a technique in which a sample, often a thin tissue section, is moved in two dimensions while the mass spectrum is recorded.
Things to think about

Goals
• Small molecule/lipids
• Peptide/protein
• Others...

Sample type
• Heart, lung, liver, etc.
• Biofilm
• Others...

Method optimization!
• Appropriate matrix
• Standards

IMS Workflow

Sample Preparation → Matrix Application → Analysis → Processing
Sample Preparation

Fresh Frozen (FF) is best for all IMS experiments.

BUT:
Hundreds and thousands of tissue samples have been “banked” over the years as Formalin Fixed Paraffin Embedded (FFPE).

Lipids and small molecules: FF only.

Intact proteins: FF or FFPE

FFPE samples:
• Tissue sectioning
• Deparaffinization and Rehydration
• Heat induced antigen retrieval
• Histology staining on serial section (i.e. Hematoxylin and eosin)
• Matrix application

FF samples:
• Tissue sectioning
• Washing
• Histology staining on serial section
• Matrix application
Matrix Application

Wet, but not too wet.
- Extraction
- Delocalization

Sufficient matrix application.
- Some
- But not too much

Matrix choice.
- Works well
- Doesn’t work...

And the list goes on...
Automated Matrix Application Methods

Spotted Arrays
- Labcyte Portrait—uses an acoustic pico drop method

Bruker ImagePrep

HTX Technologies Sprayer
TM-Sprayer
Manual Matrix Application Methods

Tissue slice on slide
Wash tissue by pipette or bath
Allow tissue to dry
Apply matrix
MALDI IMS

Common solvents:
- Ethanol
- Acetonitrile

SA, DHA: proteins
HCCA, DHB: peptides

Typical Workflow for peptide and protein imaging

Apply trypsin for digestion
Incubate at 37°C for 2-18 hours
Top-Down or Bottom-Up Analysis?

Top-Down: extract and ID intact proteins in images
- Faster sample prep for imaging
- Fewer steps means less chance for delocalization or other error
- Increase confidence in ID

Bottom-Up: sequence tryptic fragments of larger proteins
- Easier to analyze larger or hydrophobic proteins
- Potential for MALDI MS/MS

Tools for Protein ID post IMS

Top Down Analysis for intact proteins
- Matrix removal
  - Tissue microextraction
    - LC-MS of proteins
      - ETD MS/MS fragmentation
        - De-novo sequencing
          - Match masses of protein ID’s to IMS data

Bottom-Up Analysis for tryptic peptides
- Matrix removal
  - Tissue microextraction
    - LC-MS of peptides
      - CID MS/MS fragmentation
        - Database searching
Typical workflow for lipids and small molecule imaging

Fresh Frozen tissue samples

Wash with 50mM Ammonium formate to reduce salts

Lipids: DHB, DHA, DAN, 9-AA

Apply appropriate matrix

Small molecules: DHB, SA, HCCA, THAP

MALDI IMS

Analysis

Instrumentation in our facility with imaging capability

Bruker ultrafleXtreme MALDI-TOF-TOF

Bruker solariX FTMS - MALDI and ESI
Processing

- A challenge in imaging experiments is the huge amount of generated data. A typical MALDI imaging dataset contains thousands of spectra.

- Software programs allow statistical analysis and overlaying of the histology staining. (SCiLS—Bruker supported.)
H and E staining with tumor indicated

Dataset provided by SCiLS

Total mass spectrum for entire imaging run
Three masses selected as having statistical differences

Wrapping it all up

MALDI IMS is a rapidly growing field within mass spectrometry.

MALDI IMS is providing major contributions to the understanding of diseases, improving diagnostics, and drug delivery.

MALDI IMS is time consuming and involves lots of sample manipulation and data interpretation. (It is not one size fits all.)
For further information, please come see me in BRT 250.

Not actually me...