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Answering What and Where in complex samples: Advances in Imaging mass spectrometry and the impact of high resolution mass and ion mobility

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Outline

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Part 1:

- Imaging and sample selection
- Sample prep and Ionization

Part 2:

- Technology for analysis

Part 3:

- Setup and data flow
- Considerations for quantitation

MS imaging: an overview

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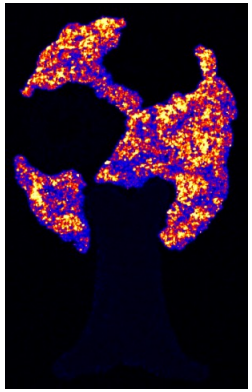

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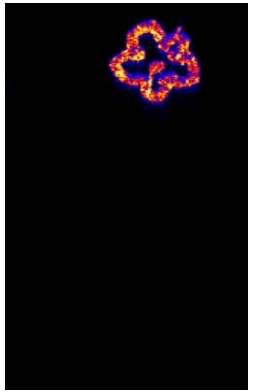

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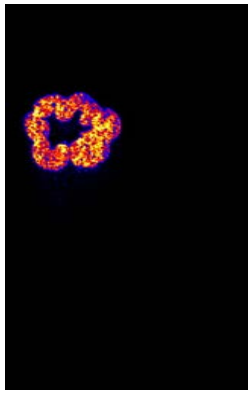

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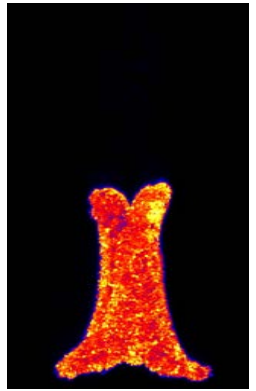

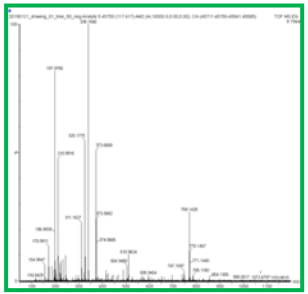

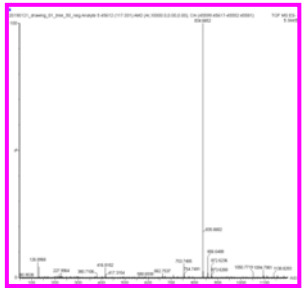

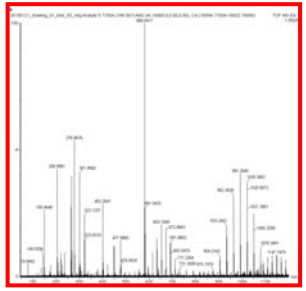

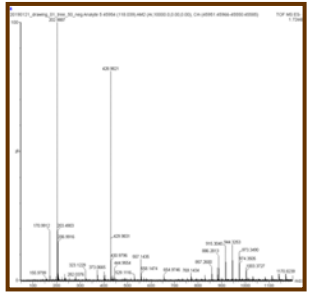

- MS imaging captures **spatially based MS information over a whole surface**

Mass Spectrometry Imaging application areas

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Forensics

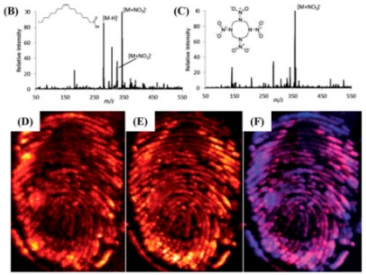
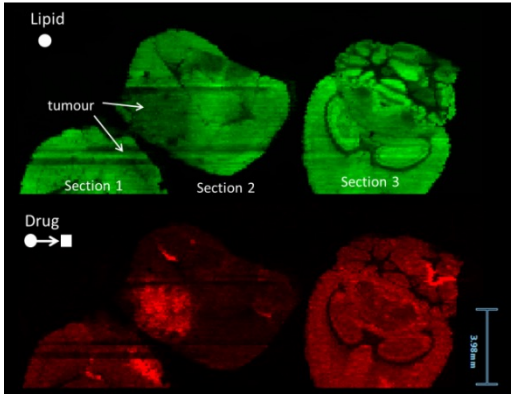
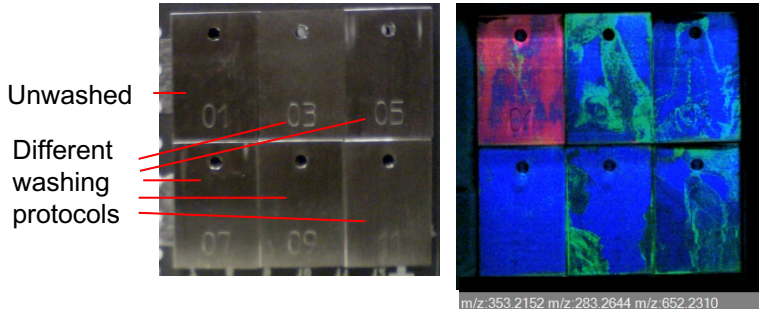


Figure 3. (A) Schematics of the DEFFI-MS imaging setup. Spectra in the negative ion mode showing detection of (B) oleic acid and (C) HMX. DEFFI-MS images of an HMX-laden artificial fingerprint deposited onto forensic lift tape for (D) oleic acid and (E) HMX as well as (F) a colocalization map of both oleic acid (blue) and HMX (red). Adapted with permission from ref 47. Copyright 2014 Royal Society of Chemistry.

DMPK



Materials



Synthetic biology

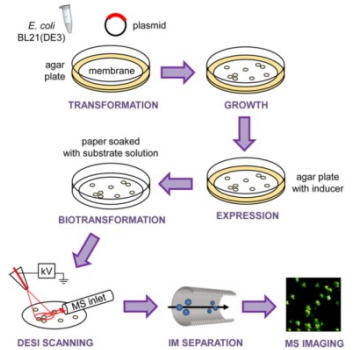
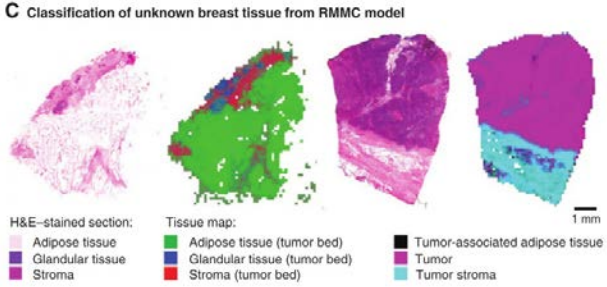
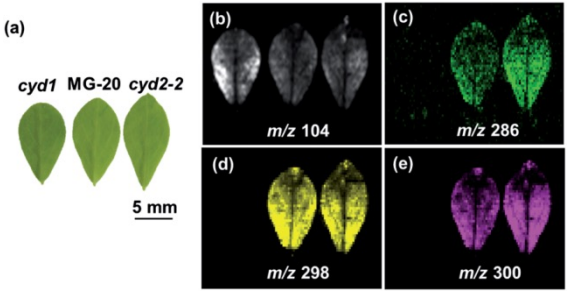


Figure 1. Workflow of DiBT-IMMS imaging of bacterial colonies expressing biocatalysts on agar plates under ambient conditions.

Histopathology



Agriculture



Different MSI techniques/ionisation modes

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MALDI – Matrix assisted laser desorption ionisation
DESI – Desorption electrospray ionisation
Nano DESI – Nanospray desorption electrospray ionisation (also known as liquid micro junction, not DESI and not nano!)
LESA – Liquid extraction surface analysis
SIMS – secondary ion mass spectrometry
LAESI – Laser ablation electrospray mass spectrometry
LDI – Laser Desorption Ionisation

Plus many more – if information can be generated from a discrete position it can be used for imaging

MALDI fundamentals

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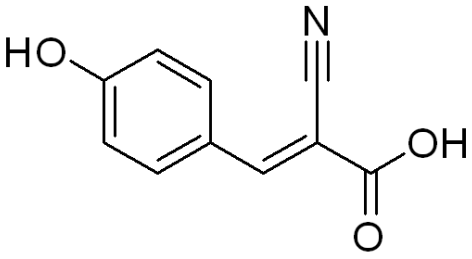
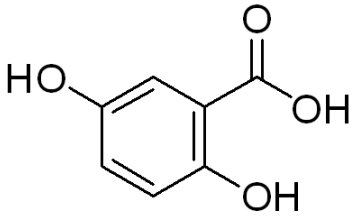
MALDI – Matrix Assisted Laser Desorption Ionisation

- Phrase first used in 1985[1].
- An investigation of laser desorption of amino acids found that pure standard of alanine would not ionise but could be ionised in the presence of tryptophan
- An analyte is co-dissolved with an excess of a “matrix” molecule (typically a small organic acid chromophore such as α-Cyannohydroxycinnamic Acid (CHCA) or 2,5-Dihydroxybenzoic Acid (DHB)
- “soft” ionisation technique



CHCA



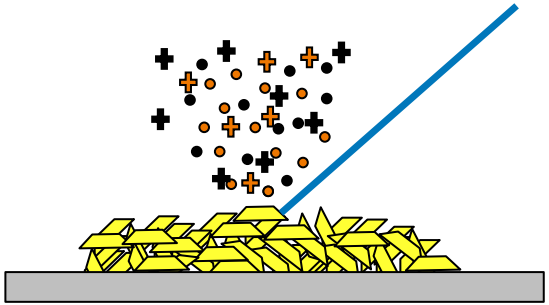
DHB

1. Karas *et al*, *Analytical Chemistry* **1985**, 57, 2935-2939

MALDI fundamentals

- Matrix absorbs UV light / energy protecting the analyte – triggers rapid gas phase transition

Co-crystalised analyte / matrix
Neutral analyte molecule
Neutral matrix molecule
Charged analyte molecule
Charged matrix molecule



Energy pooling through singlet singlet annihilation[2]

Primary ionisation

$$M + h\upsilon \rightarrow M^*$$

$$M^* + M^* \rightarrow M^{**} + M$$

Secondary ionisation

$$M^{\bullet+} + A \rightarrow [M\text{-}H]^{\bullet} + [A+H]^+$$

2. Ludemann *et al*, *Rapid Communications in Mass Spectrometry* **2002**, 16, 1287-1294

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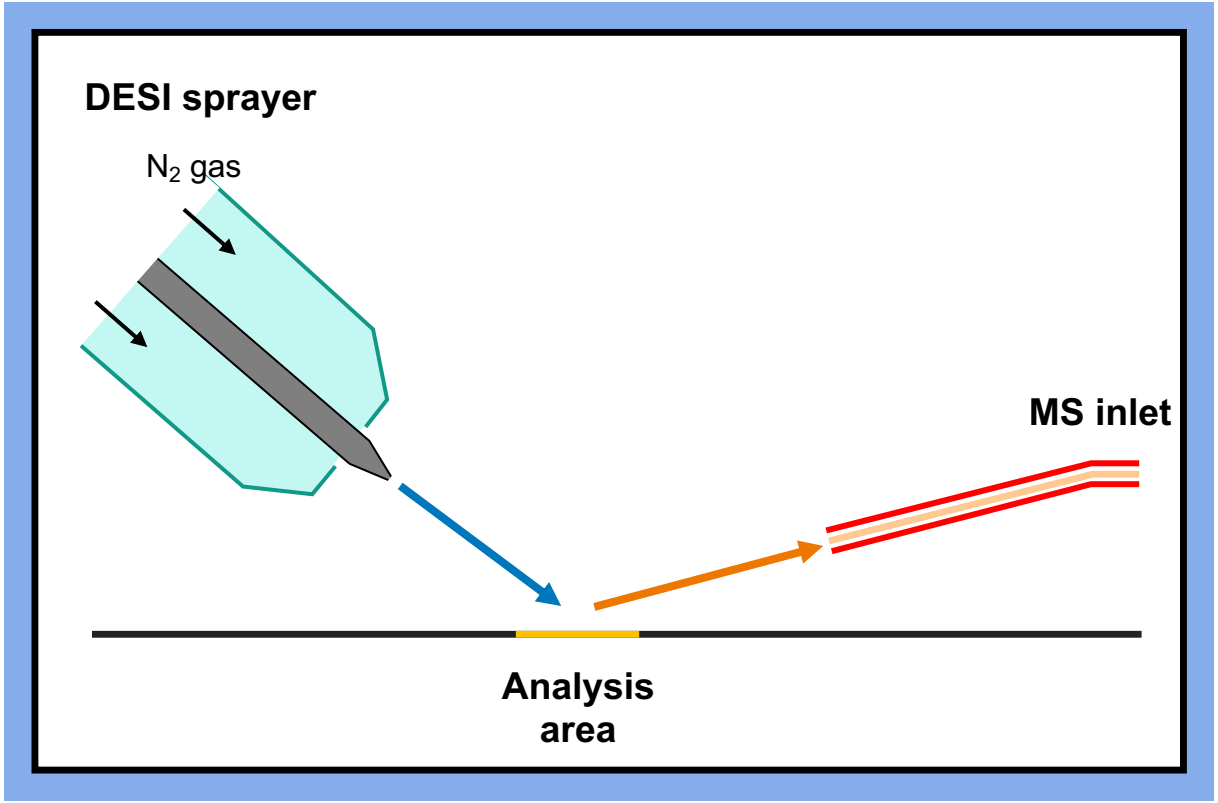
Making DESI More Accessible

DESI imaging: principles



- Solvent directed at surface
- N2 gas focuses solvent
- Surface impact of solvent conducts **micro-extraction**
- **Ambient** method
- Little to **no sample preparation**
- ***In-situ*** examination
- **Rapid** analysis

New technologies for DESI XS

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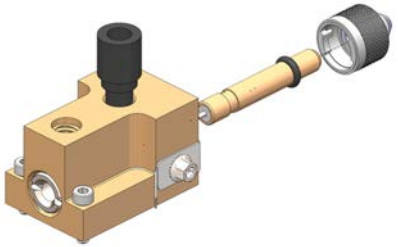
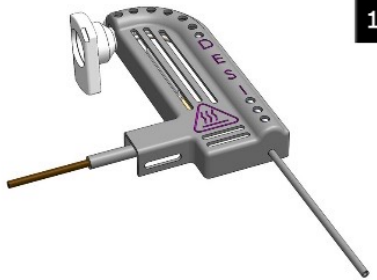
High- Performance Sprayer

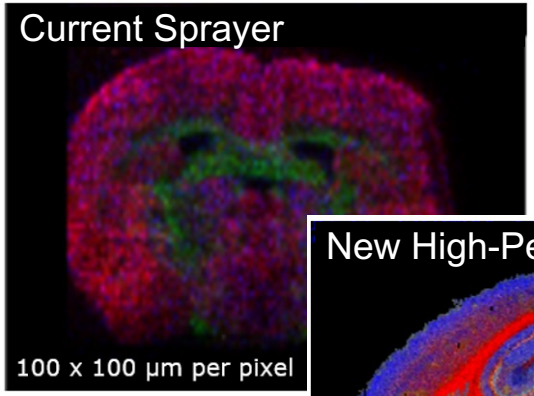
- Improved spray focus delivering greater sensitivity and spatial resolution (25µm)
- Consumable cartridge with fixed emitter gives ‘plug and play’ ease of use

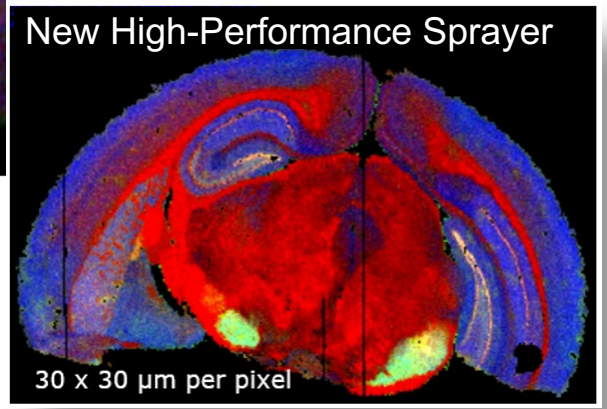


Heated Transfer Line

- Enhances the sensitivity performance
- Enhances the molecular range of the technique







Both improvements together can give >100x sensitivity increase
Enable rapid DESI imaging I Improved usability I Improved robustness

Application of the new technologies

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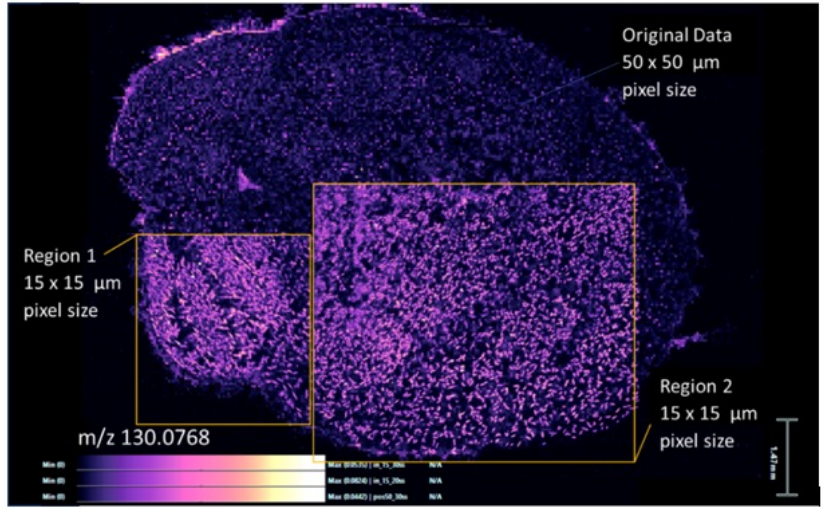
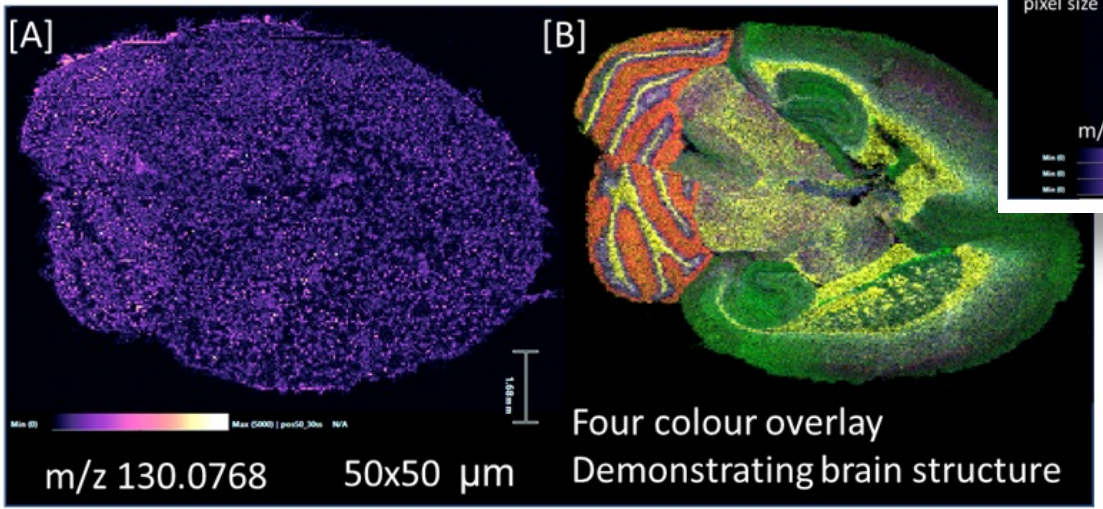
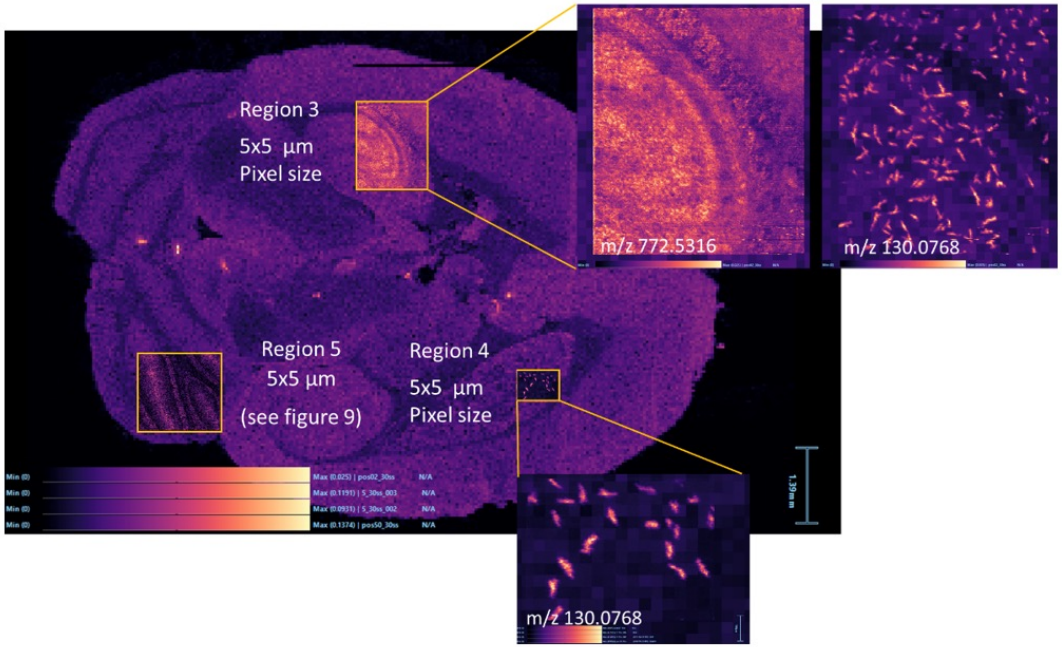
The ability to re-analyse the same sample at different spatial resolutions allows the DESI user to obtain the required information without large time burdens.



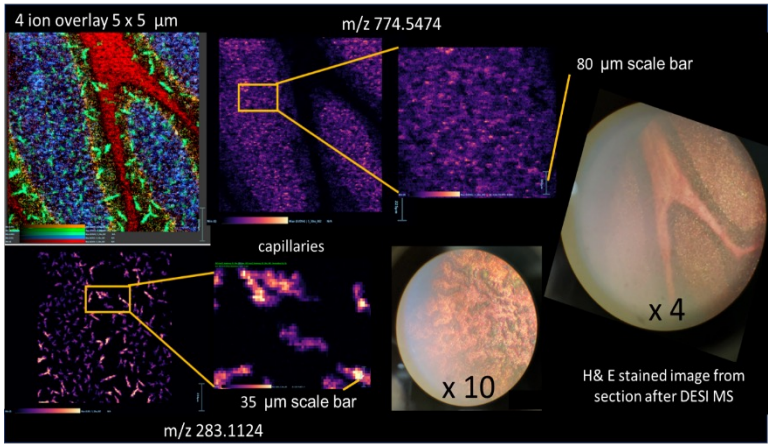


Application of the new technologies



Two areas of the tissue analysed at **5x5 µm**

A staining procedure can enable accurate co-registration between the known structures of the tissue and MS data



Showing features which are consistent in size to **single cells within the tissue**

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Sample Preparation

Imaging MS Workflow

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1 SECTIONING

Cut tissue into sections (10-15µm thick) using a cryostat

2 SAMPLE PREPARATION

MALDI: Matrix application

DESI: No Sample Prep

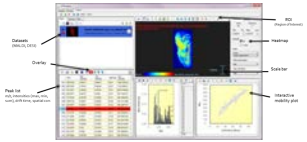
3 EXPERIMENT SET-UP

Co-registration &definition of acquisition parameters

4 MS IMAGING ACQUISITION

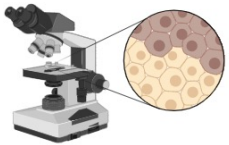
Data acquisition

5 DATA PROCESSING & VISUALIZATION



Data processing and visualization in HDI

6 TISSUE STAINING



Optional staining

Sample Preparation Considerations

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Sample and source dependant

- non-imaging applications can be as simple as a 1 µL spot of sample
- E.g. surface analysis

Some samples for DESI do not require preparation but can be analyzed directly in their native state.

- E.g. Pharmaceutical tablets, Tape used for skin analysis or plant surfaces.

Some samples may require cleaning or treatment prior to analysis

- E.g. Leaves may require their waxy layer removed using solvent
- E.g. Tissue will require digestion if looking for peptides

Many samples will benefit from cryosectioning prior to analysis

- To flatten the surface for optimal imaging
- To cut through a samples and allow imaging of the inside cross-section.

MALDI requires a flat surface for good results and will require the application of a r the surface of the sample.

Setting Up

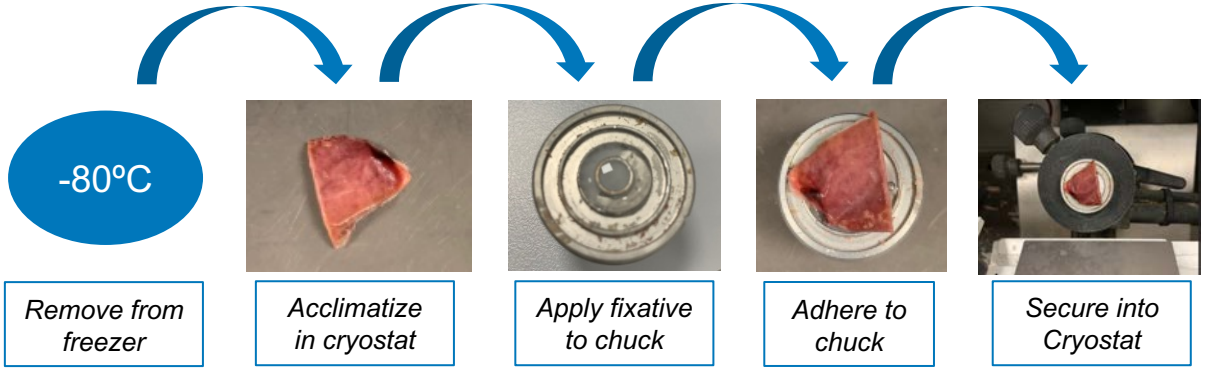
Depending upon sample type, steps for sectioning will vary

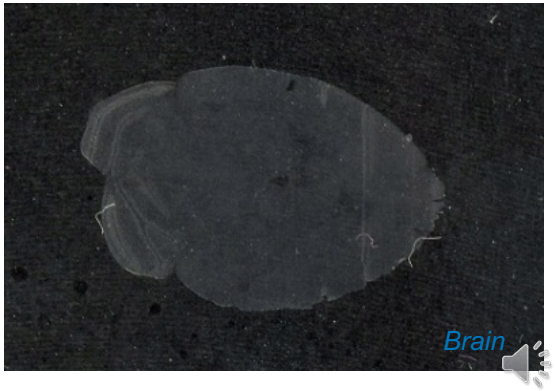
Typically, applications will be tissue based

Tissue must be frozen prior to sectioning

Remove the tissue from the freezer and adhere in a suitable orientation to the chuck.

The tissue must then be left in the cryostat for a minimum of 30 minutes to acclimatize to the same temperature as sectioning will occur





Thickness

Tissue section thickness will depend upon the imaging technique;

MALDI section thickness **is critical**.
MALDI you **must** section at 12 µm or less.

- if sections are thicker than this: salts within the samples will interfere
  with data quality.
- The salts from the tissue are preferentially drawn into the matrix and can
  negatively affect crystalisation and ionisation.

DESI section thickness is not critical
Typically for DESI in house we section at 16 to 20 µm

- this generates robust easily handled sections.
- these sections give good signal strength by DESI
- this thickness allows for multiple passes without loss of tissue regions.

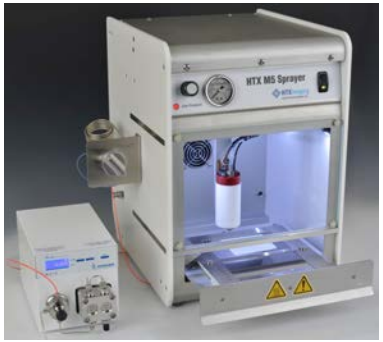
Video

Matrix applicators

Labcyte Portrait spotter

Sunchrom Suncollect sprayer

HTX M3+ Sprayer



HTX M5 Sprayer

HTX sublimator

Options Pros & Cons

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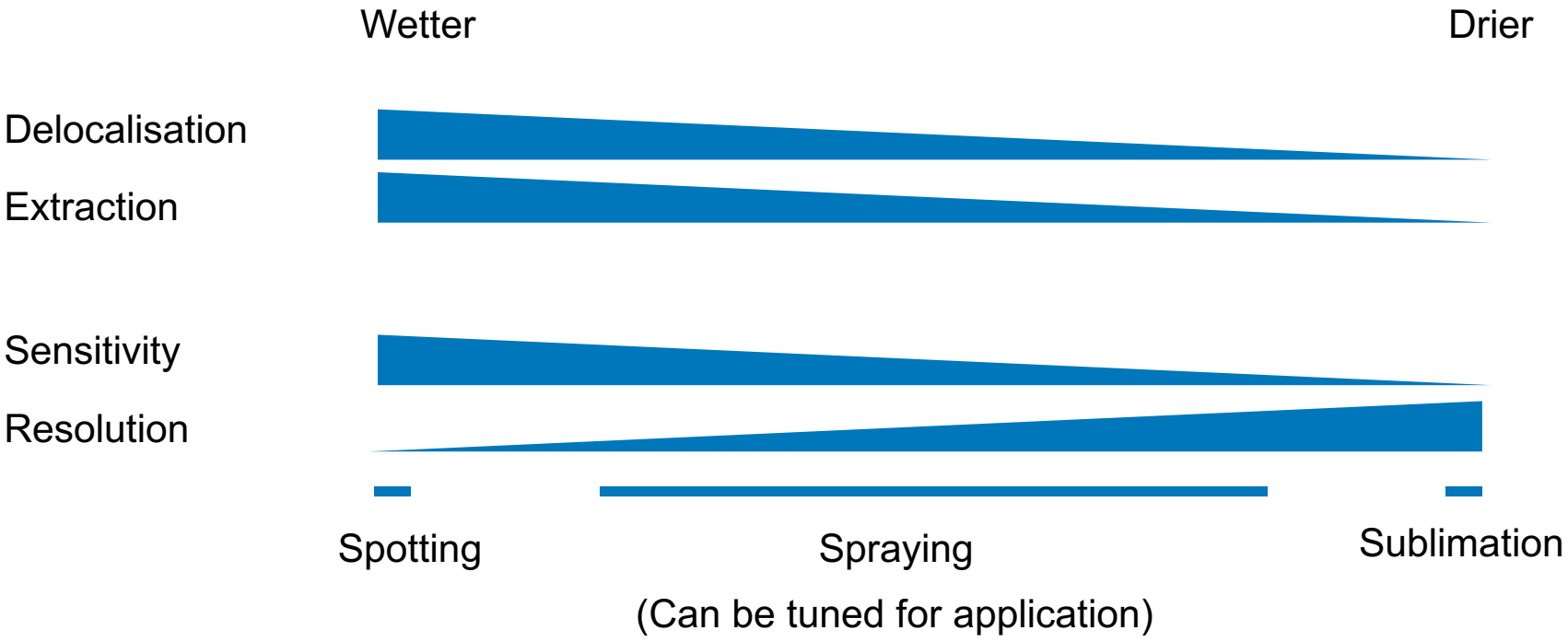
Spotted matrix
- Highest sensitivity but very limited resolution
- Typically, spots are 100 µm+
- Can be used for enzyme application

Sprayed matrix
- Multiple passes to build layers.
- Possible delocalisation if tissue gets too wet.
- Highly tuneable with latest sprayer technology (heated spray heads and sample trays).
- Can be sued for enzyme application

Sublimation
- Minimal delocalisation due to matrix being in gas phase
- Low sensitivity due to no liquid extraction

Non-Imaging applications matrix can be mixed 1:1 directly with liquid samples prior to spotting onto target plates.

Application Techniques



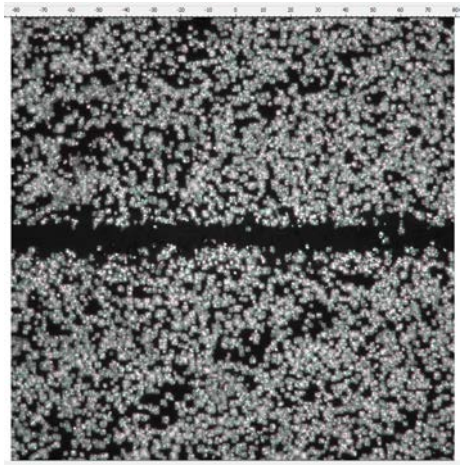
Laser focus

Crylas 2.5KHz Nd: YAG laser

User software control of laser focus via z-motor

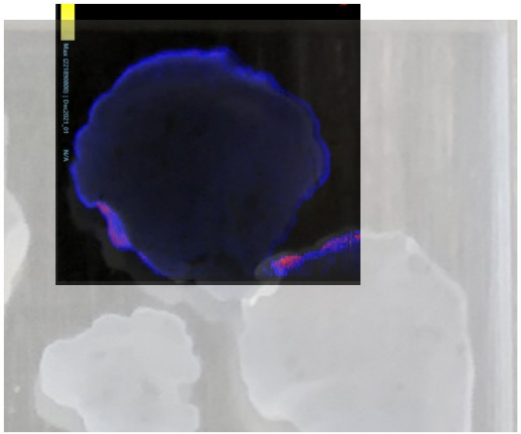
User software control of ND filter control (fine/ coarse)

<10µm to >100um (defocused laser)



5µm laser focus

Thin film prep



20µm laser focus

Mass spectrometry – analysis consideration

- Targeted or untargeted
- Ion mobility
- Mass resolution/mass accuracy
- Analysis time (cost$$)

Benefits of ion mobility

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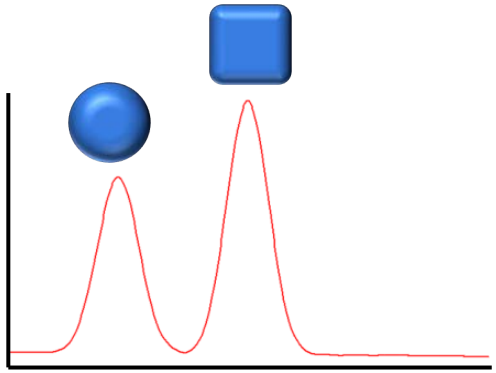
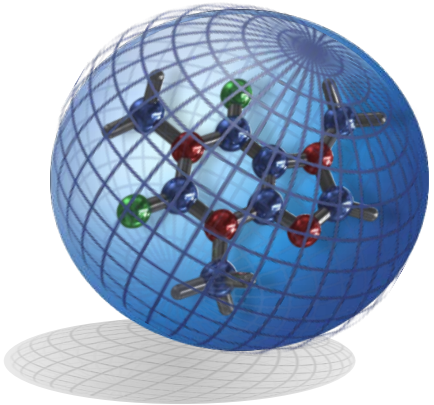


IMS



Orthogonal Separation

- Improved Peak Capacity
  - The ability to see more or increase speed of analysis whilst maintaining resolution
- Improved Selectivity
  - Resolution of isomers
  - Cleaner spectra

Collision Cross Section

- IMS drift time ⇒ CCS
- Robust measurement reflecting, m/z, charge, conformation and shape
- Matrix independent & System independent