ART CONSERVATION WITH DESI MS IMAGING: DIRECT MAPPING OF COMPOUND LOCALIZATION ON WOOD SAMPLES

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INTRODUCTION

Analyzing historic wooden artifact samples offers unique challenges in understanding their composition. Sampling compounds directly from wood without any preparation provides a promising approach to noninvasive detection and identification of an object's endogenous molecules. Desorption electrospray ionization (DESI) is an ambient ionization, direct sample analysis technique that is often used with Mass Spectrometry Imaging to map molecular spatial distributions from a sample surface.

Frequently, DESI Mass Spectrometry imaging (MSI) is used with tissue samples to map biomolecule distributions. However, DESI is minimally destructive and requires no sample preparation, making it attractive for use with precious art and archaeology samples in heritage science. Consequently, DESI MS Imaging mapped the molecular distribution of endogenous wood metabolites on historic 19th century French furniture wood samples without perturbing the wood.

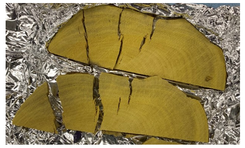
SAMPLES

Reference and calibration standards:

Leucine-Enkephalin lock mass (Waters™ P/N 186006013)
- 200 pg/µL in DESI solvent

MS Calibrants:
- Sodium formate (50 mM in 50:50 ACN:H_2O) for ESI/DESI

French barberry wood (*Berberis vulgaris*), 19th century:



Cuban mahogany (*Swietenia mahagoni*), 19th century:

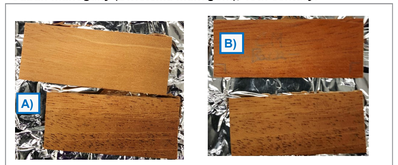


Figure 1. A) Uncoated mahogany surfaces; B) Shellac-coated rear surface of mahgony (top)

METHODS

Ion mobility-mass spectrometry (HDMS) MS Imaging

Source: Waters DESI XS source

Mass Spectrometer: SYNAPT™ XS ion mobility QToF (Figure 2).

DESI conditions:
- 98:2 methanol:water with 0.01%(v) formic acid at 2 µL/min
- Nebulizing gas pressure of 0.9 bar N_2 w/ 0.7 kV sprayer voltage
- Waters DESI XS high-performance sprayer
- Cone Voltage: 40 V; Source Temperature: 120ºC

Polarity: Positive and Negative

Mass range: 50 -1,200 m/z; 0.3 to 0.5 s per MS scan

MS Imaging Pixel size: 50 µm

Data management

MSI data were acquired using MassLynx™ 4.2. Experimental parameters were defined, raw files processed, and imaging data visualized using High Definition Imaging (HDI™) 1.6 software.

Compound Identification

Peak annotations based on accurate mass match to Human Metabolome Database (HMDB)[1] with 10 ppm search window for H^+, Na^+, and K^+ for positive mode; H^- and Cl^- for negative mode. Proposed ID cross referenced with additional search using Refs. 2 and 3.

MASS SPECTROMETRY IMAGING

Ion mobility-mass spectrometry instrumentation for MS Imaging

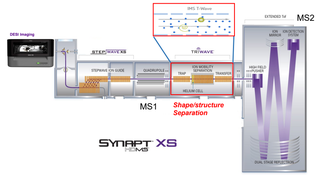


Figure 2. Schematic of the DESI XS SYNAPT XS mass spectrometer with ion mobility shape/structure separation prior to ToF MS

Mass Spectrometry Imaging (MSI) basic concept

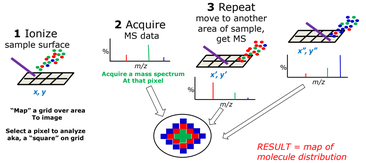


Figure 3. Illustration of how to do Mass Spectrometry Imaging (MSI).

DESORPTION ELECTROSPRAY IONIZATION

- A shower of charged ESI solvent droplets focuses into a beam by a high pressure gas flow (N_2)
- The beam washes the surface to desorb analytes on the sample.
- Desorbed analytes were then ionized and carried into an inlet capillary that transferred the ions to the MS for analysis.
- DESI is minimally destructive and allows multiple imaging analyses off of the same sample with adjustable height for thicker samples

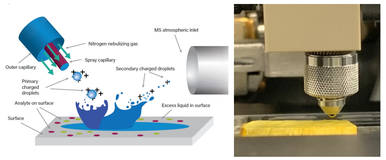


Figure 4. DESI XS high-performance sprayer analyzing a French barberry wood sample in transverse plane cross section (Fig. 6)

ION MOBILITY SPECTROMETRY

Shape/structure separation with ion mobility before MS

- Ion mobility separation is based on an ion's structural size and shape so it is a complementary separation method to MS.
- Traveling Wave IMS (TWIMS) in the SYNAPT uses a DC pulse moving down a series of elements like a wave as seen in Figure 4, guiding the ions against a counter flow of neutral gas (nitrogen here).
- The larger, bulkier molecular structures (red) will not move as easily through the gas flow as smaller, more compact ones (yellow). Therefore, the smaller structures arrive earlier.

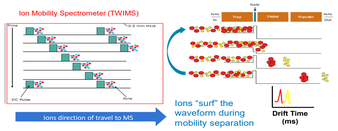


Figure 5. Ion mobility separation using traveling wave IMS (TWIMS)[4]

RESULTS

- French barberry wood sampled at transverse plane cross section, where anatomical wood features are highlighted (Fig. 6).
- Berberine distribution imaged with DESI followed the tree ring structure of the wood labeled in Fig. 7, visualized by overlaying a photo of barberry wood with the DESI MS image of berberine (Fig.8)
- In Fig. 9, the berberine DESI MS image in upper section of barberry wood sample matches the false ring structure vs. phosphatidic acid lipid PA(28:4) that followed the tree rings illustrated by the red lines.
- DESI creates 2+ adducts via ESI ionization, detecting LysoPI(16:0) lipid isolated in bark (Fig. 10, top). Bottom overlay clearly shows distinct spatial localization of metabolites along wood structures

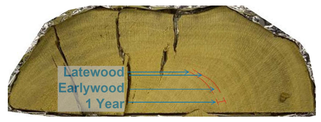


Figure 6. Transverse cross section of French barberry wood

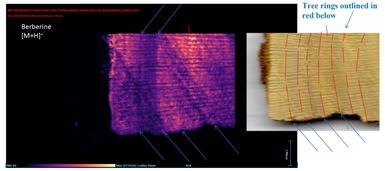


Figure 7. 50 µm pixel DESI image of natural berberine distribution mapping the tree ring structures (positive ion mode)

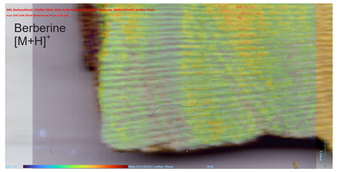


Figure 8. DESI image of natural berberine distribution overlayed with the wood image where intensity follows ring contours and toolmarks

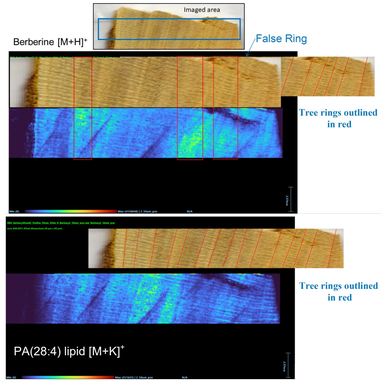
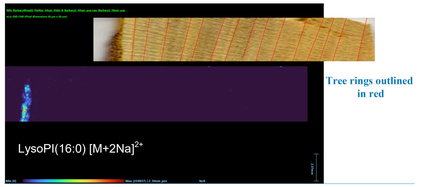


Figure 9. DESI image of berberine followed the false rings (top) while the PA lipid was distributed along the real tree ring structure



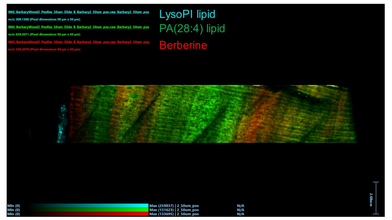
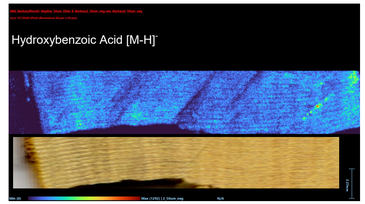


Figure 10. LysoPI doubly-charged Na^+ adduct only detected in bark (top); three color overlay of metabolites with barberry wood image demonstrating the spatial localization with wood topology (bottom)



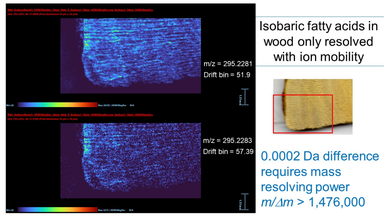


Figure 11. Negative ion mode DESI image of hydroxybenzoic acid wood metabolite (top); ion mobility pre-separation resolves 2 isobaric free fatty acids not detectable without extreme mass resolving power (bottom)

- Negative ion DESI MS Imaging easily detected organic acids (Fig, 11, top), with additional isobaric acid structures resolved only when using ion mobility shape/structure separation prior to Tof MS (Fig. 11, bottom) showing two free fatty acids with only 2 mDa difference.

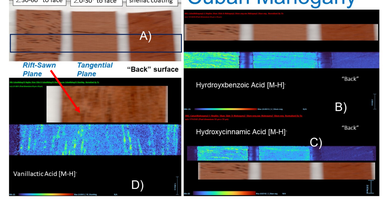


Figure 12. Cuban mahogany sample orientation (A); Neg. mode 50 µm pixel DESI images of 3 surfaces: B) hydroxybenzoic acid; C) hydroxycinnamic acid; D) rift-sawn surface image of vanillactic acid

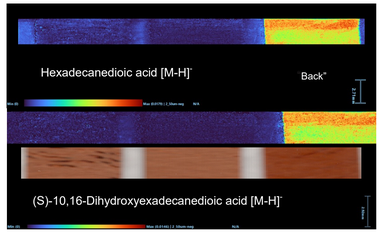


Figure 13. Compounds localized to rear shellac coated surface.

- Negative ion DESI MS Imaging of Cuban mahogany mapped compounds localized to inner uncoated vs. rear coated surface (Figs. 12 and 13)

CONCLUSIONS

- **DESI MS Imaging directly mapped the spatial localization of endogenous compounds in historic wood samples without any sample preparation**
- **DESI sampling produced essentially no damage to the wood samples, allowing additional analyses and conserving precious artifacts**
- **Wood metabolites were shown to localize to specific anatomical wood features when compared to photographic images of wood samples**
- **Adding ion mobility shape/structure complementary pre-separation before Tof MS analysis resolved isobaric peaks not detectable with MS only**

References

1) Wishart DS, Guo AC, Oler E, et al., *HMDB 5.0: the Human Metabolome Database for 2022.* Nucleic Acids Res. 2022. Jan 7; 50(D1):D622–31. doi: 10.1093/nar/gkab1062.

2) Yang X, et al., *Comparative metabolomics analysis reveals the color variation between heartwood and sapwood of Chinese fir (Cunninghamia lanceolata (Lamb.) Hook.* Ind. Crop Prod. 2021. May 26; 169:113656. https://doi.org/10.1016/j.indcrop.2021.113656.

3) Abreu IN, et al., *A metabolite roadmap of the wood-forming tissue in Populus tremula.* New Phytol. 2020. June 26; 228:1559-1572. doi: 10.1111/nph.16799.

4) Waters Pub. #720004176EN, April 2012.

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