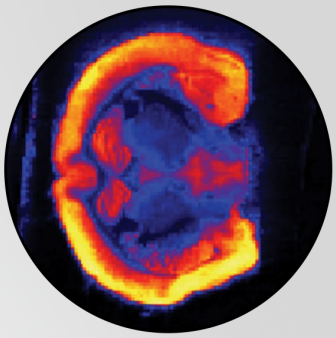
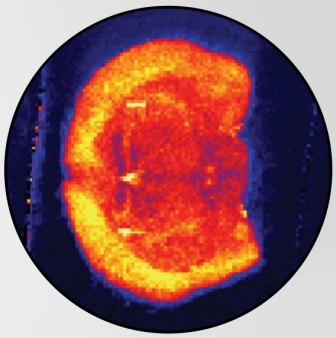
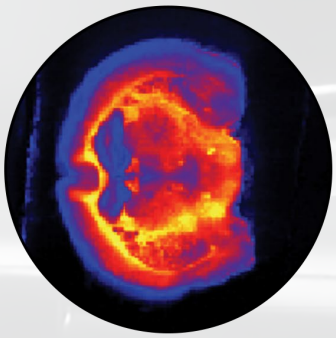
[DESI IMAGING]







DESI Imaging

APPLICATIONS WORKBOOK

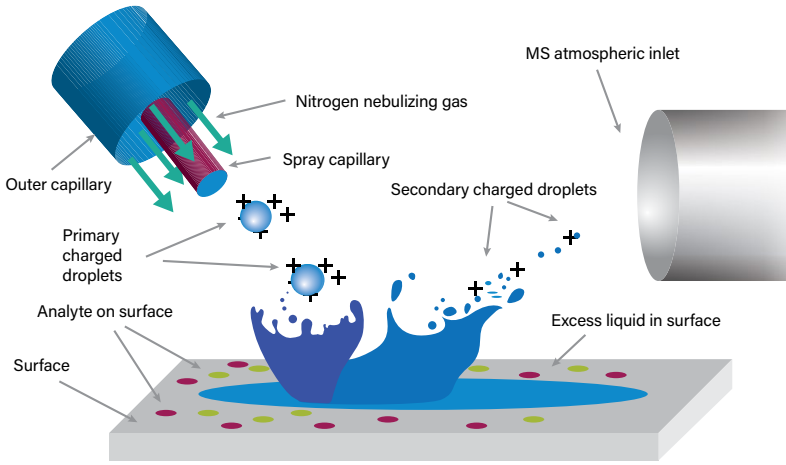
Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Molecular visualization and spatial distribution of compounds provides **mechanistic insight**

The spatial distribution of molecular species in a sample is crucial to gaining insight into biological, chemical, and physiological processes. Mass spectrometry imaging (MSI) with Desorption Electrospray Ionization (DESI) produces label-free, multiplexed, and objective measurement of molecular targets from complex surfaces. This direct-from-sample analytical technique provides researchers with the spatially resolved molecular information needed to quickly and objectively interpret molecular profiles and understand mechanistic insights with confidence.

DESI is a soft ionization technique performed under ambient environmental conditions and requires no sample preparation. This direct tissue analysis provides non-subjective information about biochemical distribution of molecules after just one measurement.



HOW IT WORKS

DESI operates by utilizing a nitrogen carrier gas to focus a jet of solvent at the surface of the sample, causing localized micro-extraction of molecules. The solvent is desorbed from the surface via a droplet pick-up mechanism, which is then deflected into the mass spectrometer for analysis.

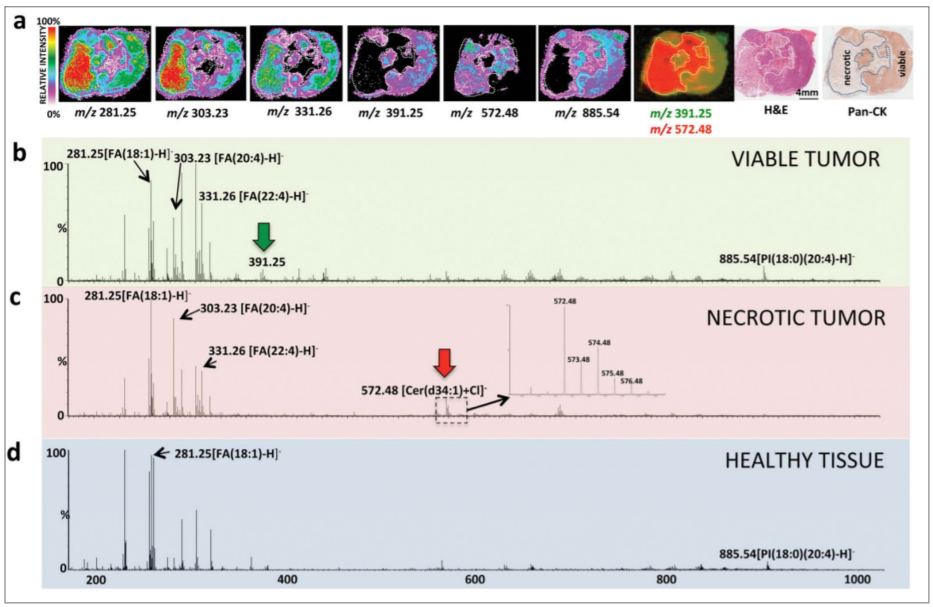
KEY FEATURES

- Ambient analysis technique to visualize the spatial localization and distribution of molecules
- No sample preparation – collect molecular information directly from tissue samples
- Compatible with current histopathological workflows such as H&E staining for morphological analysis
- Measure small molecule drugs, lipids, and endogenous metabolites
- Routinely capable of 20–25 µm pixel sizes; when expertly optimized, 2–5 µm pixel sizes can be achieved
- Rapid and highly sensitive technique for high throughput experiments with images in the order of minutes (depending on pixel size)
- High-performance sprayer and heated transfer line technologies offer huge leaps in ease-of-use while delivering increased information depth and vastly improved spatial resolution
- Seamless workflow with High Definition Imaging (HDI) software which allows multiple DESI imaging experiments to be queued, maximizing data collection and improving sample throughput
- The DESI XS source is the only commercially available DESI source and has been designed with the user in mind, delivering both reliability and simplicity for MS imaging

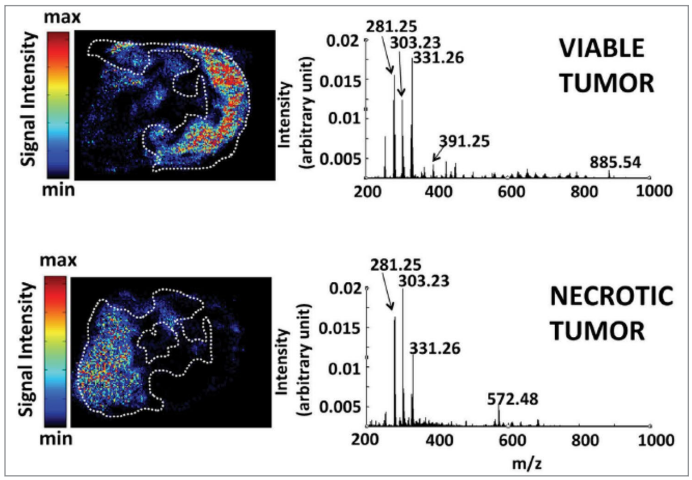
DESI MS IMAGING APPLICATIONS IN

Breast Cancer Research

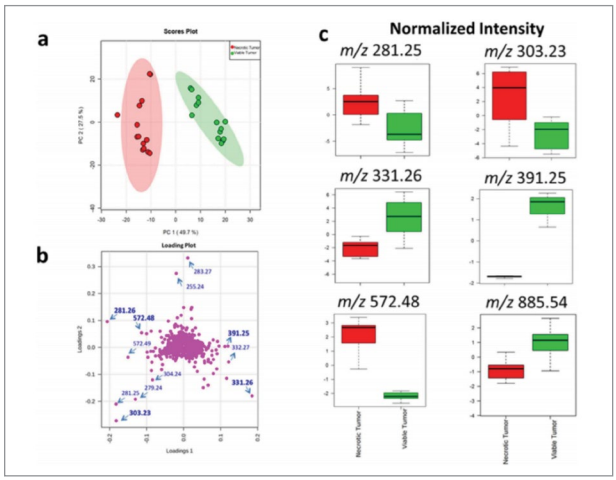
- MS images can be used to understand spatially resolved differences in the metabolites of biological samples.
- Metabolite distribution images can be used to differentiate pathological regions of human tumors.



DESI MS imaging of necrotic breast cancer tumor. (a) DESI MS ion images of the markers alongside H&E and Pan-CK immunostained images of the tumor. (b) The average DESI-MS spectrum of the viable cancer tissue. (c) The average DESI MS spectrum of the necrotic region (d) DESI MS spectrum of healthy mammary fat pad breast tissue.



Non-Negative Matrix Factorization (NMF) analysis of a DESI-MS imaging dataset and their characteristic MS profile. The NMF analysis suggests two regions containing highly correlated MS spectra. The borders marked with white dashed lines delineate areas of viable (a) and necrotic (b) cancer tissue from independent pathology.



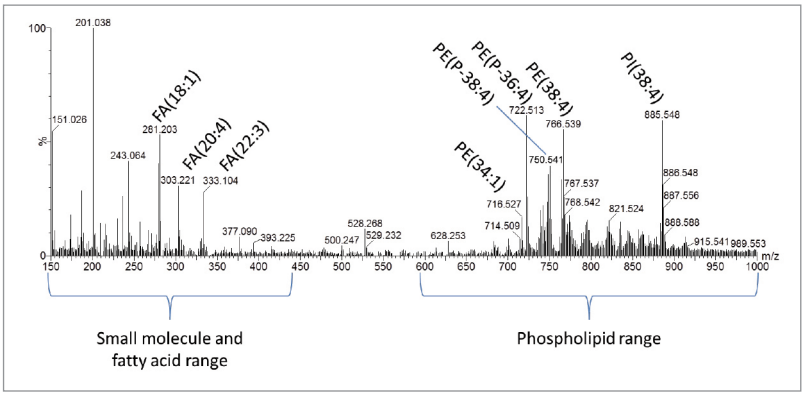
Principal Component Analysis (PCA) of the necrotic breast cancer. (a) PCA scores plot shows the statistical discrimination between MS profile of the necrotic and viable cancer tissue within the same tumor. (b) PCA loading plot illustrating the ions that contribute strongly to statistical separation between the viable and necrotic cancer profiles. (c) The box plots indicating changes in ion intensity (normalized to TIC) of biomarker ions predicted by NMF analysis to contribute most significantly to the statistical discrimination between necrotic and viable tissue.

Tata, A.; Woolman, M.; Ventura, M.; Bernards, N.; Ganguly, M.; Gribble, A.; Shrestha, B.; Bluemke, E.; Ginsberg, H. J.; Vitkin, A.; Zheng, J.; Zarrine-Afsar, A. Rapid Detection of Necrosis in Breast Cancer with Desorption Electrospray Ionization Mass Spectrometry. Scientific Reports ***2016, 6, 35374****.*

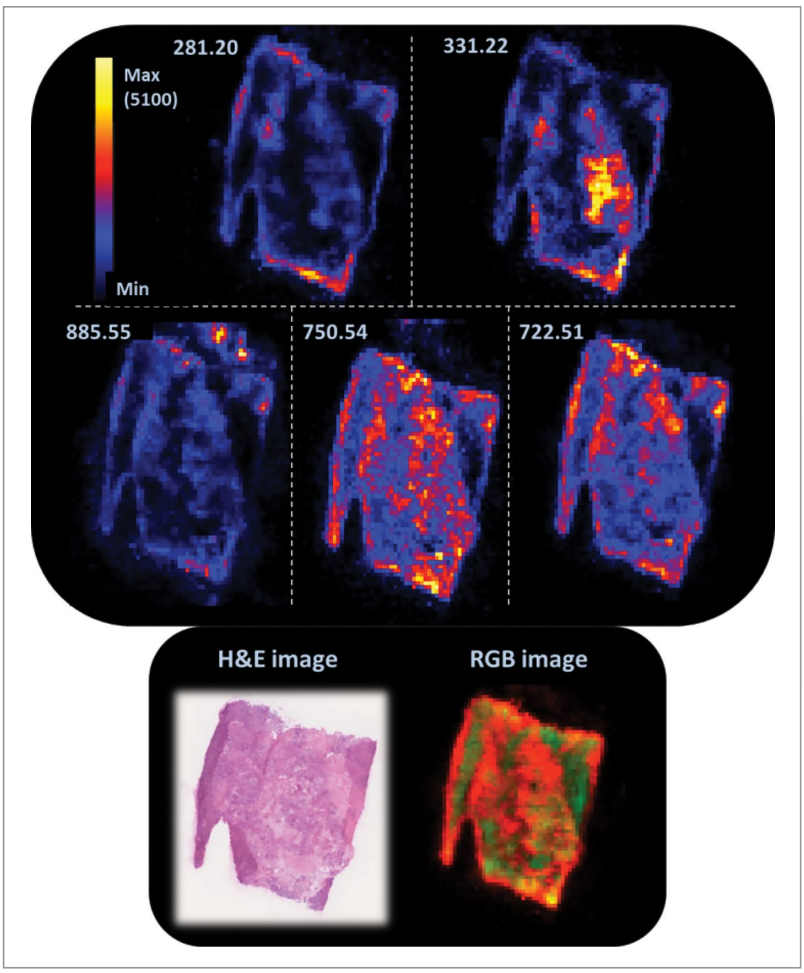
DESI MS IMAGING APPLICATIONS IN

Ovarian Cancer Research

- The DESI MS imaging workflow is compatible with traditional hematoxylin and eosin (H&E) tissue staining to give an extra dimension of molecular information on the sample.
- The molecular information from DESI MS imaging allows higher confidence in determining specific differences, such as tumor regions in an ovarian cancer sample.



An averaged DESI MS imaging spectrum, acquired in negative mode, from tumor tissue. Fatty acids (FA), phosphatidylethanolamines (PE), phosphatidylinositol (PI) can be observed in this spectrum.

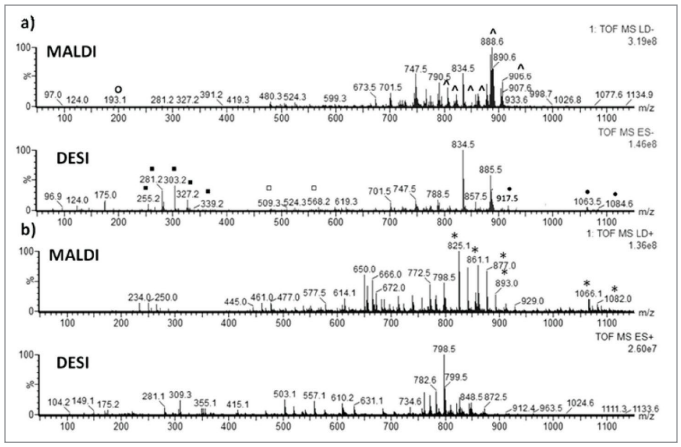


DESI MS images of specific fatty acids and phosolipids from an ovarian serous ovarian carcinoma sample together with an optical H&E image and RGB image.

COMPLEMENTARY MS IMAGING INFORMATION GAINED WITH

MALDI and DESI with Ion Mobility

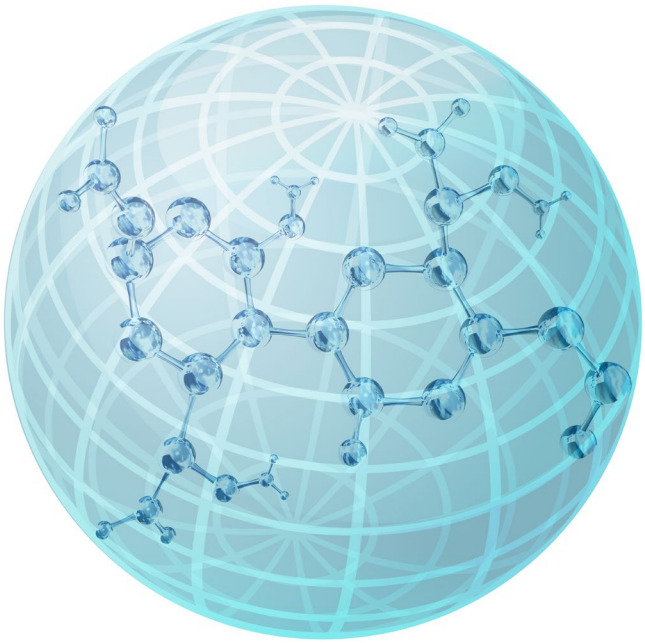
- DESI MS imaging offers complementary technique to MALDI imaging with no matrix addition.
- The mechanism of using charged droplets for DESI MS imaging allows a diverse coverage of compounds similar to electrospray ionization.
- Ion mobility separation with DESI MS imaging can help differentiate isobaric species and allow the removal of matrix/background signals.

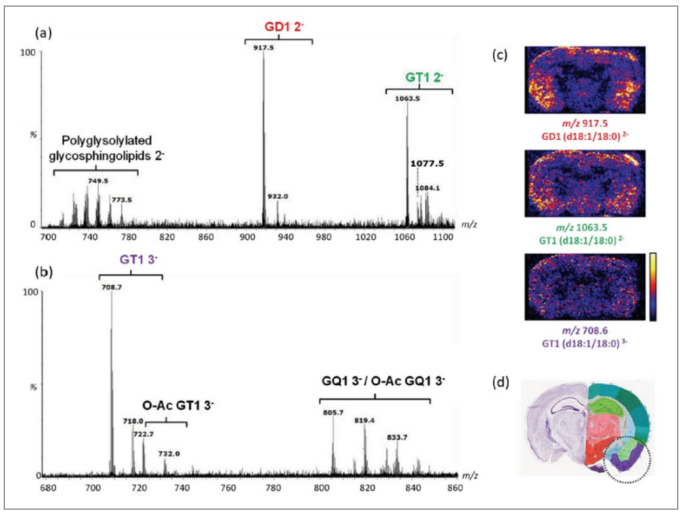


Averaged a) negative and b) positive ion mass spectra of MALDI and DESI MSI experiments performed on a mouse brain without ion mobility separation.

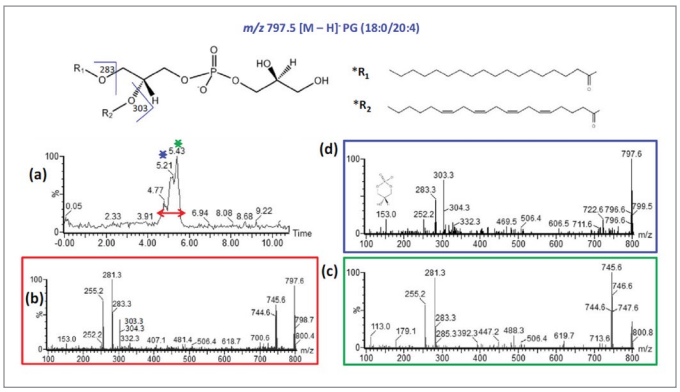
MALDI and DESI both show complementary information with MALDI showing higher ionization of sulphatides and DESI for fatty acids. MALDI mass spectrum suffers from the presence of matrix clusters which make the lipid region unclear. DESI greatly alleviates this problem as no matrix is required for ionization.

*Legend: o matrix 9-AA, ^ sulphatides, ▪ fatty acids, ▫ lysolipids, ● multiply charged ions, * matrix clusters (CHCA).*





DESI MSI detects gangliosides as doubly and triply deprotonated ions. The olfactory area is shown in a violet color within the dotted circle.



DESI MS Imaging with ion mobility allows separation of isobaric lipids.

a) Drift time plot showing three peaks

b) MS/MS spectrum showing fragments detected from all ions without ion mobility separation.

c) Fragments of the slowest ion. Molecular species remained unidentified.

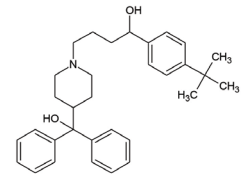
d) Fragments of the slower ion identified as phosphoglycerol with stearic (18:0) and arachidonic (20:4) acid.

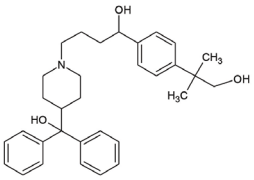
DRUG METABOLISM AND PHARMACOKINETIC

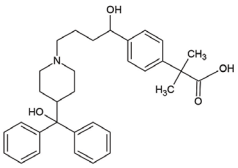
DMPK Studies with DESI MS Imaging

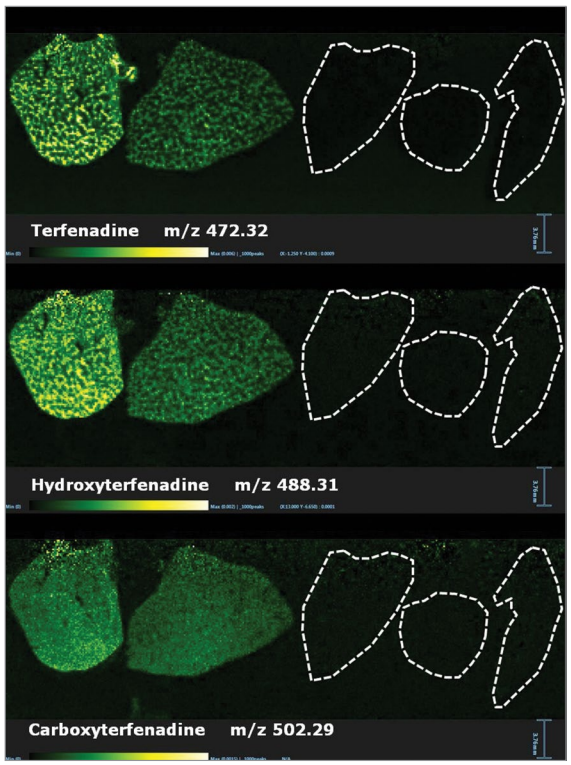
- The traditional approach in understanding the metabolism of a drug in a biological sample is mostly based on whole tissue analysis.
- DESI MS imaging offers a unique approach in visualizing the drug localization including its metabolites in the biological sample.
- DESI MS imaging has been used to analyze tissue sections from cassette dosed drug metabolism and pharmacokinetic (DMPK) models.

Drugs Dosed

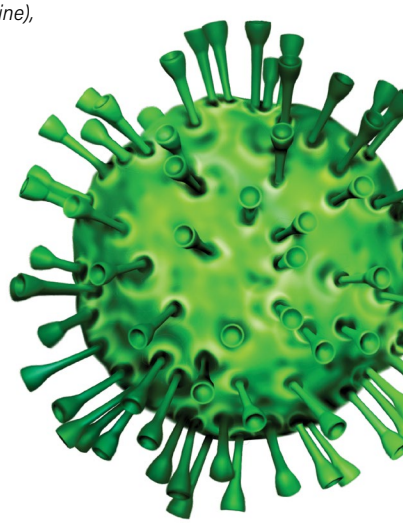


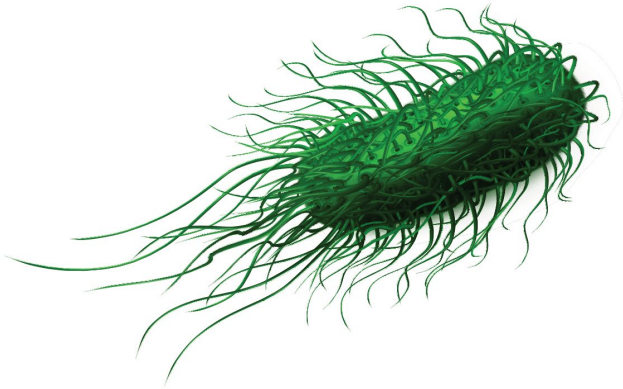




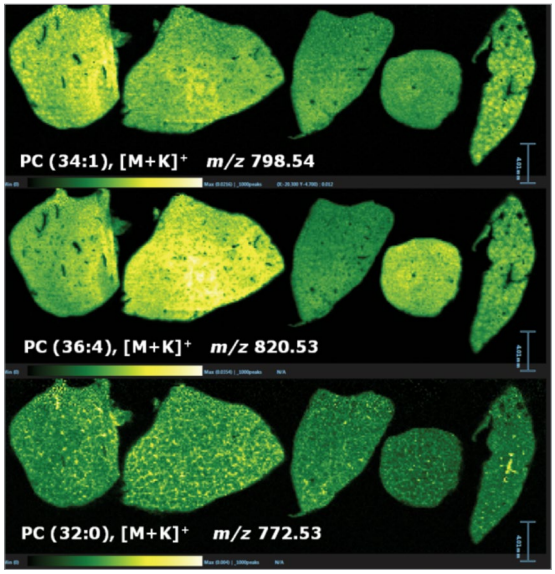


DESI MS images of terfenadine and two of its metabolites (hydroxyterfenadine and carboxyterfenadine) in the liver tissue sections from: 2 and 6 hour post-cassette dosed models, 2 and 6 hour discrete dosed models (no terfenadine), and untreated models.





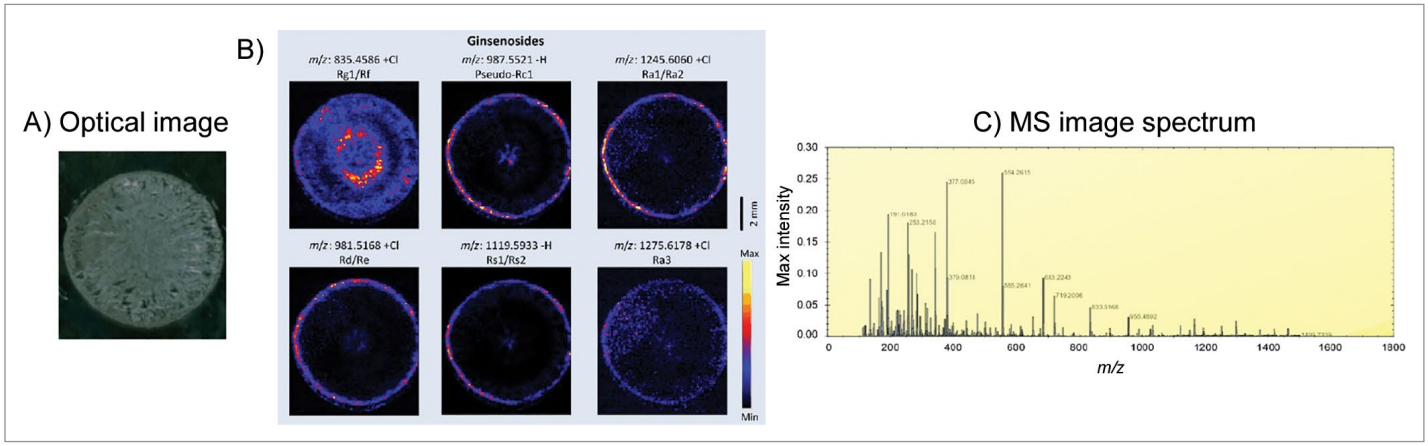
DESI MS imaging of endogenous phosphatidylcholines in liver tissues from the: 2 and 6 hour post-cassette dosed models, 2 and 6 hour discrete dosed models, and untreated models.



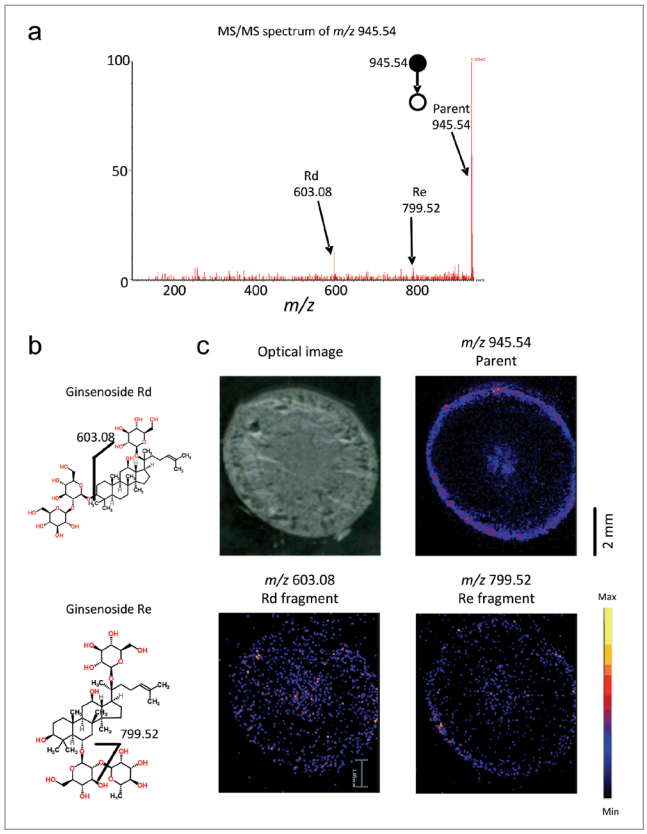
DESI MS IMAGING OF

Natural Products and Traditional Medicine

- DESI MS imaging can be used to visualize the metabolite distribution in natural products such as the traditional medicine, *Panax ginseng*.
- Understanding the localization of key metabolites in a natural products allow a higher mechanistic understanding of its activity and function.



The optical image of the ginseng root (Figure a) and the DESI MS imaging of the distribution of the ginsenosides in P.ginseng root (Figure b). For each of the pixel of the MS image, a comprehensive MS spectrum can be acquired (Figure c).



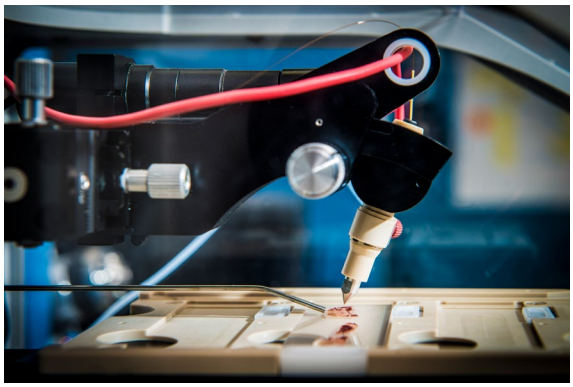
The DESI-MS/MS allowed the detection of ginsenoside fragments which aids in higher confidence for identification. Figure a and b shows the identification of two ginsenosides, Rd and Re, through their fragmentation spectra and their distribution in the MS image.

DESI MS IMAGING

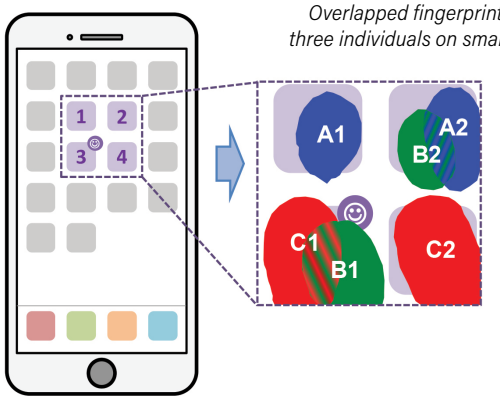
Forensic Applications

- DESI MS imaging can be used to separate overlapping fingerprints, potentially generating important information from previously inaccessible sources.
- The molecular signatures acquired from DESI MS imaging can be modeled to identify the fingerprints of multiple individuals.

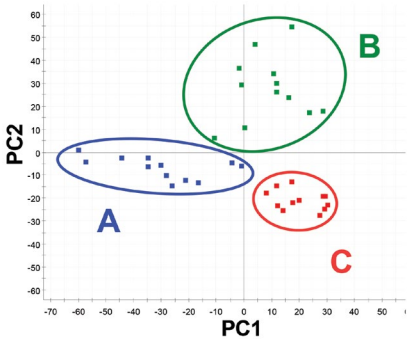
Overlapped fingerprints from three individuals on smart phone.



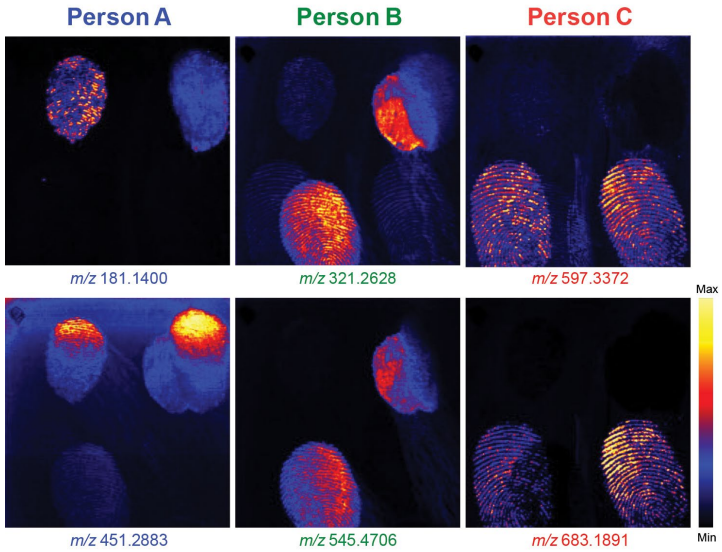
Principal Component Analysis in separating the three individual's fingerprints.

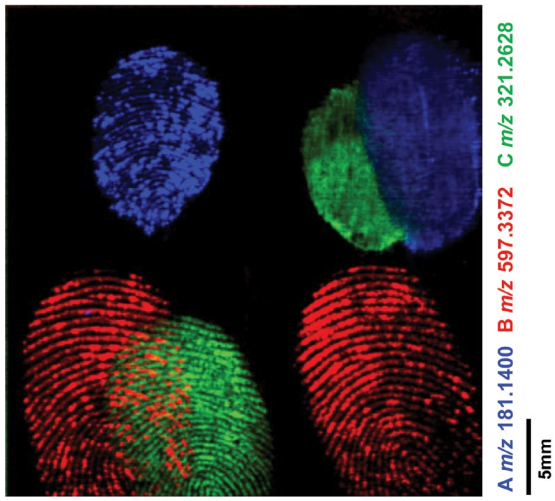


Distinct DESI MS images of the three individual's fingerprints.



MS Image of three distinct molecular masses at 181, 597, and 321 m/z.



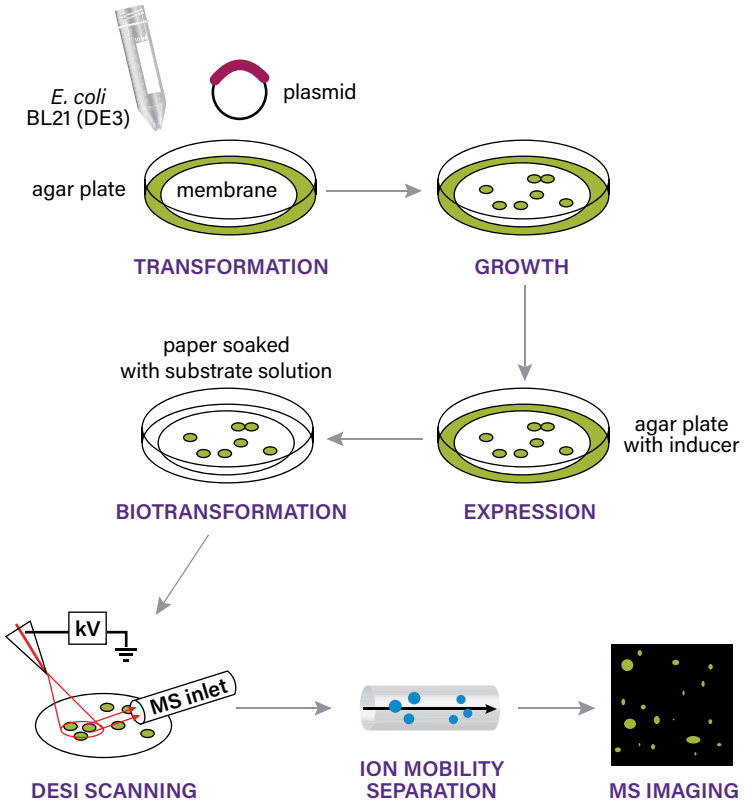


REAL-TIME SCREENING OF METABOLITES USING DESI MS IMAGING IN

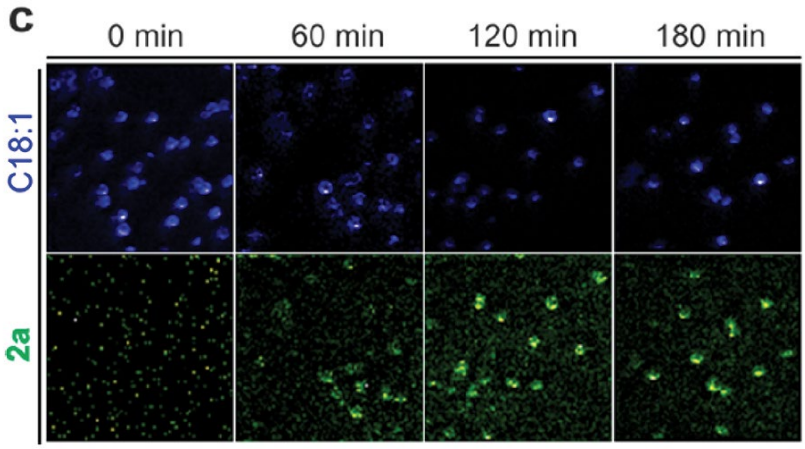
Bacterial Colonies

- DESI MS imaging provides a unique perspective on the metabolites produced from microbial colonies real-time.
- This novel approach allows the investigation in the biotransformation of microbial colonies after gene modifications.

DESI MS IMAGING WORKFLOW OF BACTERIAL COLONIES ON AGAR PLATES IN AMBIENT CONDITIONS



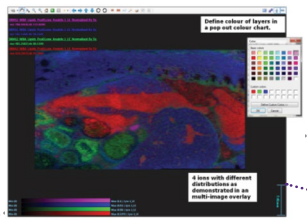
DESI MS IMAGING OF BACTERIAL COLONIES AT DIFFERENT TIMES WITH COLONIES EXPRESSING BIOTRANSFORMATION (2A)



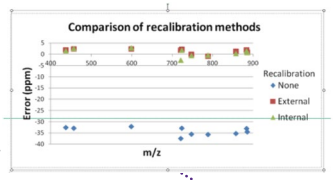
Yan, C.; Parmeggiani, F.; Jones, E. A.; Claude, E.; Hussain, S. A.; Turner, N. J.; Flitsch, S. L.; Barran, P. E. Real-Time Screening of Biocatalysts in Live Bacterial Colonies. Journal of the American Chemical Society ***2017, 139 (4), 1408-1411****.*

FLEXIBLE
DESI Imaging

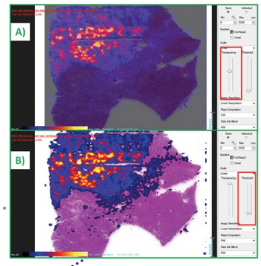
DISTINCTIVE VISUALIZATION MAKES USE OF A WIDE PALETTE OF GRADIENTS AND SOLID COLORS



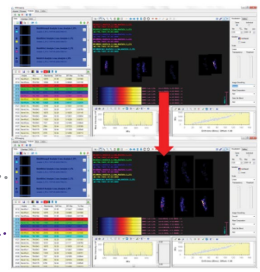
HIGH MASS ACCURACY MS IMAGING



MANAGE MULTIPLE ION IMAGE LAYERS



INDIVIDUAL MULTIMODE IMAGE MANIPULATION



Fully integrated and complementary technologies delivering flexible DESI Imaging

DESI ■ MALDI ■ Ion Mobility TOF ■ MS/MS ■ MS^E ■ SONAR

- DESI™ XS is available on the Xevo™ G2-XS QTof, SYNAPT™ XS, SELECT SERIES™ Cyclic™ IMS, and SELECT SERIES MRT platforms.
- DESI and MALDI (Matrix Assisted Laser Desorption Ionization) are uniquely integrated on the SYNAPT XS and SELECT SERIES MRT platforms for Full Spectrum Molecular Imaging with or without ion mobility, respectively.
- DESI can be coupled with Ion Mobility on the SYNAPT XS and SELECT SERIES Cyclic IMS for additional compound separation, especially with isobaric species.
- High Definition Imaging (HDI™) software is easy and intuitive allowing for the set up of multiple imaging experiments with comprehensive data analysis.

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