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Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometric Imaging (MALDI-MSI)_V2.0 V.2

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We use this protocol and it's working

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Abstract

Mass spectrometry imaging is a cutting-edge molecular technology that enables simultaneous analysis of multiple molecular components directly from single cells, tissues, and organs. In combination with histological methods, this technique provides information about the spatial distribution of molecules in various biological tissues. Particularly, MALDI-MS imaging increases the coverage of metabolites by using different matrices and ion modes. We have recently developed and optimized spatial metabolomics and lipidomics approaches to image small molecules and lipids in human kidneys and biopsy material. By capitalizing on our joint expertise in spatial metabolomics at UTHSA, PNNL and EMBL, we have established methods for identifying metabolites in human kidneys, employed ultra-high mass resolution MS imaging for tissue analysis, and developed a bioinformatics resource (METASPACE) to annotate metabolites for anatomical localization and 3-D reconstruction.

MALDI-FTICR-MS offers the highest attainable mass spectrometer performance, with mass resolution >120,000 and mass measurement accuracy <1 ppm being standard in the typical lipid mass region (i.e., 700-1000 m/z). The ultrahigh resolving power provides enhanced confidence in molecular formula assignment for untargeted MSI analysis and for advanced metabolite annotation using METASPACE. While numerous lower magnetic field MALDI-FTICR-MSI instruments exist elsewhere, PNNL houses one of the only few 15 Tesla (T) MALDI-FTICR-MSI in the world. Because all key measures of FTICR-MS performance improve linearly (e.g., resolution, acquisition rate) or quadratically (e.g., broadband mass measurement accuracy, dynamic range) with increased magnetic field strength, the 15T FTICR-MSI platform provides enhanced spectral acquisition rate and attainable sensitivity, thus enabling high-throughput measurements at higher spatial resolution than at lower magnetic field strengths. Similarly, ultrahigh resolution will enable the determination of the fine isotopic structure for a wide range of metabolites, thus providing unequivocal molecular formula and additional chemical and structural information.

The Thermo Scientific Q Exactive HF-X hybrid quadrupole-Orbitrap mass spectrometer is the latest and most advanced MS instrument in the Q Exactive MS product portfolio. Ultra-high field Orbitrap mass analyzer providing highest resolution > 100,000 FWHM at m/z 200 with mass accuracy < 1 ppm. In combination with a novel elevated pressure MALDI/ESI interface (Spectroglyph, LLC), the UTHSA Q Exactive HF-X MS imaging platform has increased sensitivity characterized to a limit of detection of ~400 zmol, when using a laser repetition rate of 2 kHz, a laser wavelength of 355 nm, and a laser energy of 2 µJ in a 5 ns pulse. The use of the combined MALDI/ESI interface with Q Exactive Orbitrap mass spectrometer offers high sensitivity, high mass and spatial resolution, and high mass accuracy and represents a robust and reproducible approach for MSI of complex biological tissues. UTHSA MALDI-Q Exactive HF-X MSI is complementary with PNNL 15T MALDI-FTICR-MSI to increase the coverage of metabolites, validate data obtained from different MS imaging platforms, and increase the spatial resolution.

The METASPACE cloud platform is used by us to find molecules in the generated big MALDI-MSI data, annotate small molecules and lipids with confidence, exchange the information between the involved partners, share and provide results to involved experimental and computational scientists, store metadata, and overlay MALDI-MSI data with microscopy images. METASPACE also used for dissemination of the MALDI-MSI results obtrained in the KPMP project within the project as well as within the broader scientific community.

Significance of the data/analysis generated:
1. Generated high quality MSI data at high spatial resolution (e.g., 20 µm and 5 µm).

2. Identified new lipid marker (e.g., sphingomyelin d34:1) for localization of glomeruli.
3. Increased the coverage of metabolites.
4. Developed protocols for detection and mapping of compounds (e.g., ATP, ADP, and AMP) indicative of tissue state.
5. Integration of spatial metabolomics and snDrop-seq data identified new glomerulus-specific gene markers.

Guidelines

QA/QC for MALDI -15 T FTICR MS

1. Test CalMix (Agilent) to make sure instrument is tuned properly, and to see if instrument is performing at optimal conditions.
2. Ablate laser paper (Zappit) to measure laser spot size.
3. Adjust laser parameters to make sure the TIC is at 1E9.

QA/QC for MALDI – Q Exactive HF-X

1. Thermo FlexMix in ESI direct infusion mode. Injection times (1-2 ms) are similar to those of the S lens configuration that uses a larger ID inlet capillary.
2. Signal intensities (NL) were in the range of 1E6 at ion injection times of 50-100 ms. Spot size is in the range 20-25 um (estimate).

Materials

STEP MATERIALS

MTP Slide-Adapter II **Bruker Catalog #**8235380

Protocol materials

MTP Slide-Adapter II **Bruker Catalog #**8235380 Step 2

MALDI-15T-FTICR MS (Bruker Solarix) with SmartBeam II laser source– Spatial Lipidomics

1 External calibration of instrument is performed using TuneMix via electrospray ionization (ESI), resulting in mass measurement accuracy within 1 ppm across the entire m/z range..

Equipment

MALDI-15T-FTICR-MSI	NAME
Imaging mass spectrometer	TYPE
Bruker Daltonics	BRAND
n/a	SKU

Equipment

TuneMix	NAME
Agilent	BRAND
G1969-85010	SKU

Note

Tuning permits us to test the sensitivity of the instrument day-today and ensures that the correct Fourier transform calibration constants are used for processing the raw data to mass spectral data.

2 Slide(s) is (are) removed from the HTX sprayer, remounted in the MTP slide Adapter II and loaded into MS instrument.

MTP Slide-Adapter II **Bruker Catalog #**8235380

3 The laser is stepped across the sample in 35 µm, 50µm or 75 µm increments. For each spatial resolution, the sample is analyzed in triplicate.

Note

Image data is acquired using FlexImaging (v4.1, Bruker Daltonics). Peak picking is performed during the run so that all ions with intensity higher than 1e5 and a signal-to-noise ratio higher than 1 are collected.

4 **For positive-ion mode:** Analyzer parameters are optimized for range of analysis m/z 200-1500 in resolution of 1M (correspond to 140,000 at 400 m/z). Laser focusing is set to “minimal”, 19% of laser power is used and 200 shots with frequency of 2,000 Hz are used to ablate material from one pixel position.

5 **For negative-ion mode:** Analyzer parameters are optimized for range of analysis m/z 200-1500 in resolution of 1M (correspond to app 140,000 at 400 m/z). Laser focusing is set to “minimal”, 36% of laser power is used and 100 shots with frequency of 2,000 Hz are used to ablate material from one pixel position.

6 Imaging data are opened via SCILS software, where they are “Exported to METASPACE” in form of .imzML and .ibd files.

7 .imzML and .ibd files are uploaded to METASPACE at **https://metaspace2020.eu/**, the metadata is entered according to the METASPACE metadata template and requirements.

MALDI-Q Exactive HF-X (Thermo) MS– Spatial Metabolomics

8 The slide is removed from the HTX sprayer and loaded into the slide holder in the MALDI injector (SpectroGlyph).

9 Turn on the vaccum pump for MALDI source.

10 Open the ‘Gate Wall’ between MALDI and Q Exactive HF-X. Adjust the HPF pressure (MALDI) at 7 TORR and QE fore vacuum pressure at 1.5 mbar.

11 Enable the MALDI system. External calibration of instrument is performed using FlexMix (Thermo), resulting in mass measurement accuracy within 1 ppm within the m/z range 50-700. Image data is acquired using 'MALDI Injector Software'.

12 The laser is stepped across the sample in 20 µm (routinely) or 5 µm (regions of interest) increments. The sample is analyzed in four replicated sections. Peak picking is performed during the run so that all ions with intensity higher than 1e5 and a signal-to-noise ratio higher than 4 are collected.

13 **For Negative-ion mode (MALDI-Q Exactive):** Analyzer parameters are optimized for range of analysis m/z 70-700 in resolution of 1M (correspond to 120,000 at 200 m/z). Laser current is set at 1.95 Amps, laser repetition rate is set at 1000 Hz.

14 Imaging data are opened via ImageInsight software (MALDI-Q Exactive). The data are converted into the form of .imzML and .ibd files in the centroid mode.

15 .imzML and .ibd files are uploaded to METASPACE at **https://metaspace2020.eu/**, the metadata is entered according to the METASPACE metadata template and requirements.

16 The slide is removed from the HTX sprayer and loaded into the slide holder in the MALDI injector (SpectroGlyph).