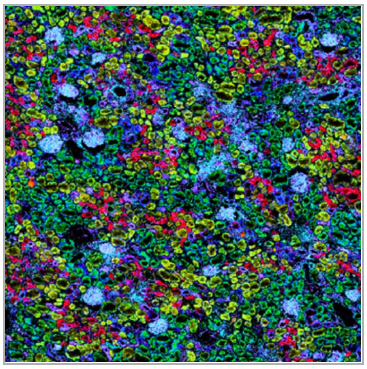

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High Spatial Resolution MALDI Imaging Mass Spectrometry Data Acquisition

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ABSTRACT

This protocol provides the workflow and instrument parameters for setting up a MALDI imaging mass spectrometry experiment on a Bruker timsTOF Flex instrument. This protocol is intended for 10µm spatial resolution imaging of lipids (*m/z* 400 - 2000) in positive and negative ionization mode data acquisition in qTOF mode of operation.

MATERIALS

Agilent ESI-L Tune Mix (Agilent Technologies, Santa Clara, CA)

Keywords: MALDI IMS, Lipid Imaging

1 Place slides in a MTP 2-slide holder. Scan slides using flatbed scanner, allowing for sufficient contrast to visualize the tissue boundary. Ensure the slides have fiducials.

Note

Fiducials are markings on the slide that can be used as reference points for teaching the slides (Step 4).

2 Load the 2-slide holder in the instrument. Open timsControl software and select appropriate MALDI IMS data acquisition method. Generate target profile by ensuring "Use Target Profile" is enabled. Check the height at a few different locations on the slide and ensure the difference is <10 µm.

Note

Example Positive/Negative Ionization Mode Parameters for 10µm MALDI IMS:
m/z 400 - 2000
Laser Power: 35 - 45%, Frequency: 10 000 Hz
Shots: 100 - 150 per pixel
Spot size: 10 µm with Beam scanning function disabled
Source temperature: ~50 ^{o}C

3 Calibrate the TOF with an external calibration using red phosphorus. Proceed if all calibration points are <1ppm standard deviation error and the calibration score is >99%.

Note

Red phosphorus is commonly used to perform calibration, however Agilent ESI-L Tune Mix can also be used for electrospray ionization.

List of Red Phosphorus Mass Calibration Peaks

	A	B
	Positive mode	**Negative mode**
	278.7633	278.7644
	402.6583	402.6594
	464.6058	464.6069
	526.5534	526.5545
	650.4484	650.4495
	774.3435	774.3445
	898.2385	898.2396
	960.1860	960.1870
	1022.1335	1022.1346
	1146.0286	1146.0297
	1393.8187	1393.8198
	1517.7137	1517.7148
	1641.6088	1641.6099
	1765.5038	1765.5049
	1889.3989	1889.4000

4 Use the FlexImaging software to load the optical image of the slides. Teach the target position with three teaching points. Check the accuracy of the teaching by moving the stage to a fourth location.

5 Select desired tissue measurement region. Start data acquisition in the FlexImaging software.