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Deposition of NEDC Matrix using TM Sprayer for High Resolution MALDI Analysis of Metabolites

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We use this protocol to map primary small molecules in negative mode at 10 µm spatial resolution

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Abstract

MALDI imaging mass spectrometry (IMS) encounters difficulties in effectively analyzing low molecular weight ions due to challenges with sensitivity and spatial resolution, limiting the ability to perform a detailed characterization. This protocol describes an automated robotic sprayer (HTX M5 TM sprayer) to deposit N-(1-naphthyl) ethylenediamine hydrochloride (NEDC) onto tissue sections for negative ion mode analyses or high resolution MALDI imaging mass spectrometry (IMS). We have optimized a method for matrix application to maximize analyte extraction from tissue and minimize delocalization for the best lateral resolution of metabolites.

Autofluorescence Scan

1 Remove slides from freezer (-80C) and place in a desiccator for 30 min.

2 Collect autofluorescence scan using a Zeiss AxioScan Z1: (https://www.protocols.io/edit/autofluorescence-microscopy-data-acquisition-b39kqr4w)

TM Sprayer Setup

3 Set nitrogen flow to 10 psi. turn on TM sprayer.

4 Open HTX software.

5 Set nozzle temperature to 55 ^{O}C, and heated stage temperature to 30 ^{O}C.

6 Clean sample loop with 3mL of methanol with sprayer set to "load". Switch to "spray" and allow to spray for 2 minutes.

7 Make the matrix solution at a concentration of 14 mg/mL in a solution of 90% methanol. Sonicate solution for 2 min.

8 Switch sprayer to "load" and load sample loop with 2 mL of matrix solution using a syringe.

9 Switch sprayer to "spray" and allow to spray for ~1 min. Check that matrix is spraying by placing a glass slide under the nozzle.

10 Tape slide onto heated stage, and adjust the spray area in the HTX software.

11 Set the method in the HTX software:

11.1 1350 mm/min nozzle velocity

11.2 2.0 mm track spacing

11.3 0.05 mL/min flow rate

11.4 CC pattern

11.5 14 passes

11.6 40 mm nozzle height

11.7 2 seconds drying time

12 Ensure that the method is highlighted. Press start under the Cycle tab. Click Continue. Wait for purge delay and confirm that the nozzle is moving.

13 When matrix deposition is finished, remove the slides and store them in a slide box. Press "valve load confirm" in the software.

Celanup

14 Set both TM sprayer nozzle and stage temperatures to 30 ^{O}C.

15 With sprayer set to "load", load loop with 3 mL of methanol methanol. Set sprayer to "spray" and allow to flow for ~5 min.

16 Spray the nozzle tip with methanol and acetonitrile, not touching the tip.

17 Clean the stage with Kimwipes tissue that is sprayed with methanol.

18 Shut down HTX software and close the computer. Turn off the TM sprayer.

19 Shut off the nitrogen flow.

20 Dispose of syringes in biohazard container.