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 We use this protocol and it's working

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## 🌐 Deposition of matrix using an M5 TM sprayer for high resolution MALDI analysis V.2

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### ABSTRACT

This protocol describes the use of an HTX M5 TM sprayer to deposit small molecule matrices onto tissue sections for high resolution MALDI imaging mass spectrometry (IMS). Spraying conditions will vary depending on matrix, and this protocol focuses on matrices for lipid IMS such as DMACA and DHB.

### SAFETY WARNINGS

⚠ All steps involving organic solvents should be performed in a fume hood.

## Autofluorescence Scan

- 1 Remove slides from freezer and place in desiccator for 00:30:00 30m
- 2 Collect autofluorescence scan using a Zeiss AxioScan Z1: [Pre-IMS Autofluorescence Microscopy](#)

## TM Sprayer Setup

- 3 Set nitrogen flow to 8 psi. turn on TM sprayer.

- 4 Open HTX software
- 5 Set nozzle temperature to 60C, and heated stage temperature (if applicable) to 45C.
- 6 Clean sample loop with 10mL of methanol with sprayer set to "load". Switch to "spray" and allow to spray for 2 minutes.
- 7 Make matrix solution of DMACA or DHB at a concentration of 12mg/mL in a solution of 60% THF and 40% acetone containing 5uL/mL DMF. Sonicate solution for 10 minutes.
- 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 ~30 seconds. 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:
  - 1350 mm/min nozzle velocity
  - 1.5 mm track spacing
  - 0.05 mL/min flow rate
  - CC pattern
  - 4 passes
  - 40 mm nozzle height
  - No 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.

## Cleanup

- 14 Set TM sprayer nozzle and stage temperatures to 30C
- 15 With sprayer set to "load", load loop with 10 mL each of acetonitrile followed by methanol. Set sprayer to "spray" and allow to flow for ~5 minutes.
- 16 Spray nozzle tip with methanol, taking care not to touch the tip.
- 17 Clean the stage with methanol. Do not spray solvents directly onto the stage.
- 18 Replace the bench liner in front of the instruments
- 19 Dispose of syringes in biohazard container
- 20 Shut down HTX software and close the computer. Turn off the TM sprayer.
- 21 Shut off the nitrogen flow.