

Mass Spectrometry Core

Operation Protocol Number: MSI001

Version Number: v1

Operation of the Mass Spec Core Automated M3 and M5 MALDI matrix sprayer for Mass Spectrometry Imaging (MSI) samples preparation in Lab E1.27, QMRI

Instrumentations:

M3 HTX TM-Sprayer™ (HTX Technologies, LLC, USA)

M5 HTX TM-Sprayer™ (HTX Technologies, LLC, USA)

Corona N₂ Generator

Consumables and chemicals:

Matrix solution ~6 mL – depends on protocol

MeOH:H₂O 1:1 (500 mL) for solvent flow line

Nitrogen Gas flow at pressure 10 psi

Sample slide for spray

Solvents for washing lines:

10 mL of 5% Aq. Ammonium Hydroxide.

10 mL of 1% Trifluoroacetic Acid (TFA) in ACN

(5% Ammonium Hydroxide in water preparation: 25-30% NH₄OH (aq) solution 1 in 5 dilutions

e. g. 100mL 25% NH₄OH + 400mL H₂O (HPLC Grade) = 500mL 5% NH₄OH

1% TFA in ACN prep: 1 mL in 99mL ACN (HPLC Grade))

1. Set up of Instrument to equilibrate:

- Connect laptop with instrument using a USB comm cable
- Switch on HTX TM-Sprayer™, laptop and Corona N₂ generator at the plug
- Check N₂ generator lights are green (if light is Amber or Red then don't use the sprayer and report to Shazia Khan)
- Turn on fume cupboard air flow and light
- Check the solvent bottle has enough solvent (50% MeOH)
- Check waste bottle is not full (if full, discard waste solvent into appropriate waste container)
- Turn on Nitrogen flow by toggle valve bracket on wall beside the fuming hood - Nitrogen for the system is supplied from the Corona N₂ Generator
- Adjust nitrogen pressure to 10 psi (70kPa) using regulator at back of the system in M3 and at front of M5.
- Turn on TM-Sprayer unit and pump should be already on @ 0.01 mL/min flow in sprayer mode

IMPORTANT: Check that exhaust fan on TM Sprayer is operational. Do not start solvent pump if proper active venting is not functioning.

Before putting sample slide to dry in vacuum desiccator check the stability of the instrument at different flow rates in sprayer mode:

- Set the flow rate 500 uL leave for 10 min to stabilise then observe the back pressure fluctuations and record it in the M3 / M5 sprayer's log book. Pump back pressure should be within range 43-47 MPa (if pressure jump randomly a lot or ±5 and does not stabilise on



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additional 10 min flow then don't use the sprayer and report to Shazia Khan and also note down in the log book)

- k. If pump back pressure is stable at 500ul/min then check further at 200 ul/min (34-37 MPa), 100 ul/min (31-33 MPa) and 10 uL/min (28-29 MPa) and record in log book as below chart.
- l. Also record which matrix you are using with flow rate and back pressure on the log book along with humidity and temp of the fume hood.

Date	User	Solvent flow	M3/M5 Pump back Pressure	Fume hood humidity & Temp
		500ul/min		
		200ul/min		
		100ul/min		
		10ul/min		
		Matrix + flow rate		

- m. Set valve to LOAD position and flush loop with at least 6mL MeOH:H₂O 50:50 using 10 mL syringe.

In LOAD position the flow from pump goes to nozzle and by-passes the loop

In SPRAY position the flow from pump goes to nozzle through the loop

- n. Double click on the TMSP icon to launch the sprayer application.
- Nozzle will initialise positions and temperature display will indicate ambient temperature**
- o. Select the sprayer method if already there and let spray equilibrate at set temperature and flow rate for at least 20-30 min
- p. In case of new method click on add method on the top right-hand side and add spray method details or select a method and then click on copy and add selected method to make a copy and then amend the method. Once method edit click on save methods otherwise method will not save automatically.

2. Matrix application (sample preparation):

- a. Prepare Matrix solution according to protocol – **Make sure matrix is completely dissolved otherwise it will block the flow line which increase back pressure.**
- b. Dry MALDI plate/slide with affixed sample(s) under vacuum desiccator (15-30 min depend on protocol)
- c. TM sprayer control software screen should display:

The screenshot shows the TM sprayer control software interface. On the left, there are controls for Sample Area (X Range: 26-150 mm, Y Range: 26-108 mm), Plate Selection (Calibration Plate), Overspray Margins (Left, Right, Top, Bottom), Current Method (TRYF-01), and Matrix Density (0.000002 mg/mm²). The main area is a grid with a red rectangular spray pattern. On the right, there are buttons for Save Methods, Add Method, Copy And Add Selected Method, and Delete Selected Method. At the bottom, there is a table of method parameters:

File Name	Author	Matrix	Solvent	Temp (°C)	# Passes (Count)	Conc. (mg/ml)	Flow Rate (ml/min)	Velocity (mm/min)	Track Spacing (mm)	Pattern	Pressure (psi)	Gas Flow Rate (l/min)	Drying Time (s)	Nozzle Ht. (mm)	Dab. Coat
TRYF-01	Lisa/ David	Trypsin at 0.05	Aeolic	30	8	0.05	0.0075	750	2	HH	12	3	0	40	8/10
TRYF-02	Michael/ Jane	Trypsin at 0.05	Aeolic	30	8	0.05	0.03	750	2	HH	12	3	0	40	10/1
DHB-00	User Training	DHB	70% Methanol	75	8	40	0.1	1200	3	CC	10	3	10	40	3/4
DHB-01	Johan	DHB	50% Methanol	85	8	10	0.075	800	3	CC	10	3	0	40	8/10

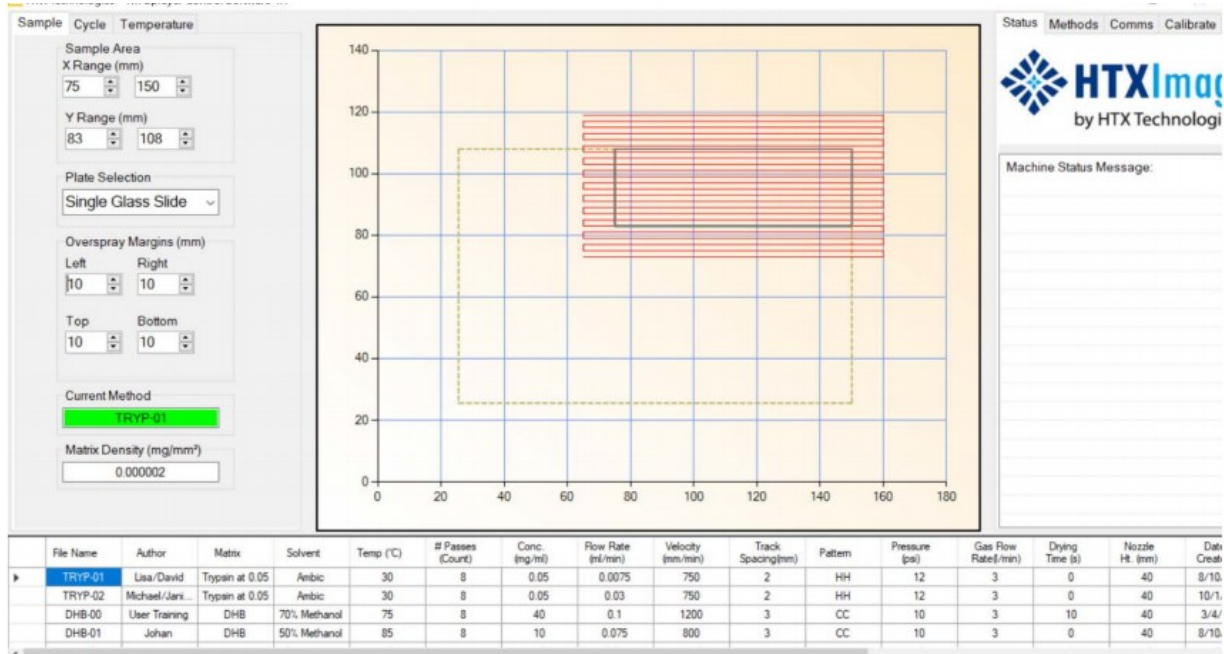
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- d. The red striped area in the centre represents the available spray area.
- e. Begin on the Sample Tab at the far left of the screen select the type of plate from drop down menu. Once selected, the red striped area will also automatically update.

Note: The Sample Area is defined by the X Range and Y Range of the sample.

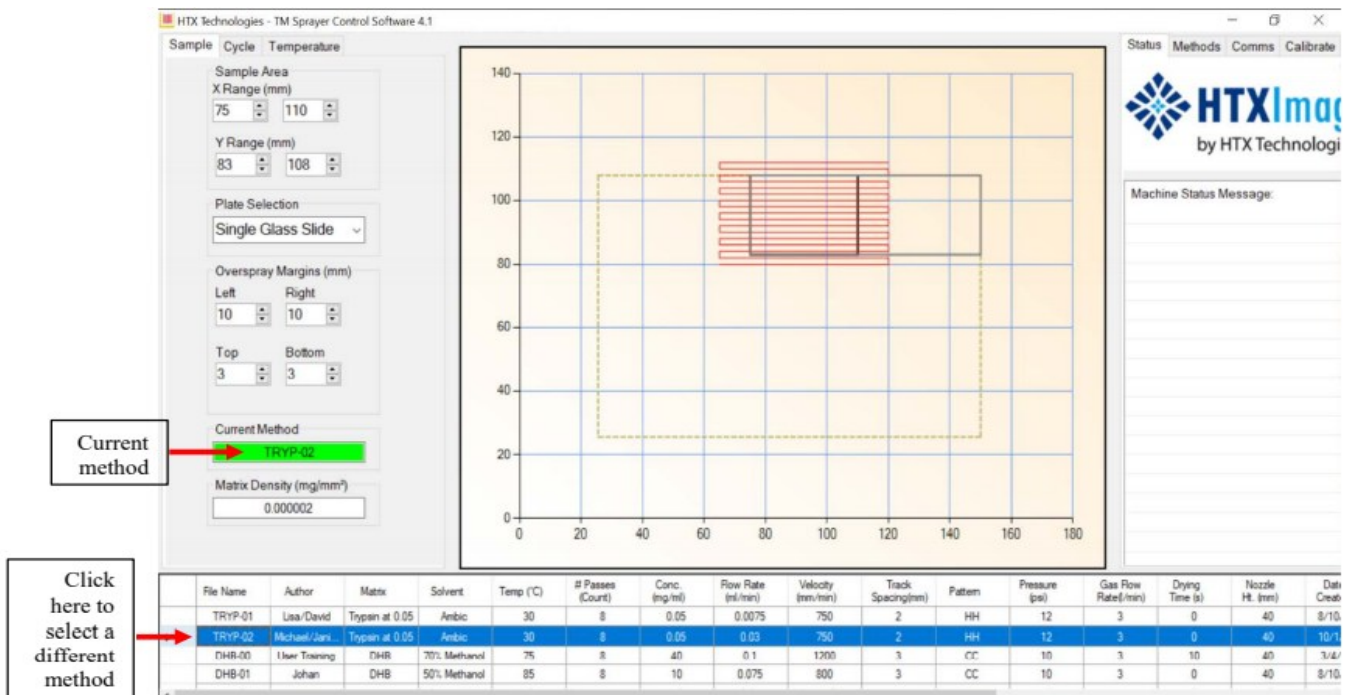
- f. If sample is significantly differently sized than manually set the lower and upper value of the X and Y ranges to match the coordinates of the edges of the sample.



The screenshot shows the HTXImag software interface. On the left, the 'Sample' tab is active, displaying configuration options for 'Sample Area' (X Range: 75-150 mm, Y Range: 83-108 mm), 'Plate Selection' (Single Glass Slide), 'Overspray Margins' (Left: 10, Right: 10, Top: 10, Bottom: 10 mm), 'Current Method' (TRYP-01), and 'Matrix Density' (0.000002 mg/mm³). The central area is a 2D grid showing a red striped spray area within a yellow dashed sample boundary. On the right, there are tabs for 'Status', 'Methods', 'Comms', and 'Calibrate', and a 'Machine Status Message' field. At the bottom, a table lists various methods:

File Name	Author	Matrix	Solvent	Temp (°C)	# Passes (Count)	Conc. (mg/ml)	Flow Rate (ml/min)	Velocity (mm/min)	Track Spacing(mm)	Pattern	Pressure (psi)	Gas Flow Rate(l/min)	Drying Time (s)	Nozzle Ht. (mm)	Date Creat
TRYP-01	Lisa/David	Typsin at 0.05	Ambic	30	8	0.05	0.0075	750	2	HH	12	3	0	40	8/10
TRYP-02	Michael/Jani...	Typsin at 0.05	Ambic	30	8	0.05	0.03	750	2	HH	12	3	0	40	10/1
DHB-00	User Training	DHB	70% Methanol	75	8	40	0.1	1200	3	CC	10	3	10	40	3/4/
DHB-01	Johan	DHB	50% Methanol	85	8	10	0.075	800	3	CC	10	3	0	40	8/10

- g. Adjust the overspray margins from 0 to 10 mm, a default 10 mm overspray is applied in all four directions
- h. Select a saved method or create a new method (right side tabs) include all the parameters related to spray conditions, author, date created, and user comments



This screenshot is similar to the previous one but with annotations. A red arrow points to the 'Current Method' dropdown menu, which is labeled 'Current method'. Another red arrow points to the 'TRYP-02' row in the table below, which is labeled 'Click here to select a different method'. The 'Current Method' field now displays 'TRYP-02' in a green box, and the 'TRYP-02' row in the table is highlighted in blue.

- i. The selected method is always displayed in the **green box** under the Sample Tab.

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- j. To select a different method, click in the far most left column in the methods menu at the bottom of the screen as shown above.

Note: If you click anywhere other than the first column on a different method, it **WILL NOT change the current method.**

- k. The Cycle Tab on left of the screen provides matrix usage data, timers and reading of the actual temperature of the spray nozzle as well as starts, pauses, aborts and confirms completion of a Cycle.
- l. To spray matrix switch valve to **LOAD** position manually on TM Sprayer unit.
- m. Load matrix in loop with syringe. Adjust Matrix Volume in Cycle tab if different than the full loop volume value.

For full loop 20% overfill is recommended. E.g.: Use 6 mL syringe to fill 5 mL loop

- n. Check that flow rate, back pressure and temperature are correct and stable
- o. Review sample position and define spraying area in TM-Sprayer software
- p. Select method and press START in the Cycle tab. Option changes to CONTINUE.
- q. This will communicate the method temperature to the unit, and activate the Status tab dialog prompts. The Spray Nozzle Temp will be displayed in red until it reaches the temperature specified by the selected method when it will turn green.
- r. When prompted, switch the valve to **SPRAY** and click CONTINUE.
- s. Unit will delay start to allow full purge of the delay volume between valve and nozzle tip. The cycle will proceed without further input.
- t. At the end of the Cycle switch valve to LOAD. This action stops the matrix usage preserving the unused matrix inside the loop for next sample.

Note: The interface (Cycle Tab) keeps track of matrix usage and volume left in the loop. If the volume remaining is insufficient for the next Cycle, and error prompt will be displayed.

- u. **Before start actual tissue sample slide, visually check for matrix spray quality using dummy plate.**
- v. After spray end, change the nozzle temperature to 25C and keep solvent and gas flow on and wash the lines/loop.
- w. Set valve to LOAD position and flush loop with at least 6mL MeOH:H₂O 50:50 then toggle the valve to the "SPRAY" position and flush loop with remaining 4mL MeOH:H₂O 50:50.

IMPORTANT: Keep gas and pump flow on so until nozzle temp reach to 25C so that clean solvent flows to nozzle and prevents matrix residues from crystallizing and clogging the capillary and spray nozzle

3. Flushing procedure before shut down sprayer:

1. Set the pump flow rate to 0.500mL/min
2. Nitrogen does not have to be on during the wash procedure if nozzle temperature is below 30C.
3. Check the nature of your matrix before start the rinsing sequence.

Important: Most matrices are acidic (DHB, CHCA, SA, etc...) so start with the Ammonium Hydroxide (basic solution). A few matrices (9-AA, DAN, etc...) are basic, for those switch the rinsing sequence and start with the acetic acid rinse, then ammonium hydroxide solution. Always end with neutral.

- a. Switch the valve back to the "LOAD" position and inject 10 mL of 5% ammonium hydroxide solution into the 5 mL loop. This process rinses the loop entirely and loads the loop with a remaining 5 mL of ammonium hydroxide. Switch the 6-port valve to the "SPRAY" position. Allow to flow for 10 minutes.

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- b. Switch the 6-port valve back to the "LOAD" position and Inject 10 mL of 1% TFA into the loop then switch the valve to the "SPRAY" position and allow to flow for 10 minutes.
 - c. Switch the valve back to the "LOAD" position and rinse the lines with normal push solvent (50% Methanol) 10 mL then switch the valve to the "SPRAY" position and allow to flow for 2-3 minutes at 0.500mL/min.
 - d. Fill the syringe with 50% MeOH and leave the pump flow on @ 10uL/min at SPRAY position.
4. **Shut Down:**
- a. Exit out of TM-Sprayer software
 - b. Turn TM-Sprayer off.
 - c. Turn PC off and Nitrogen supply off if not done before.
 - d. Make sure pump flow set at 10uL/min and valve at SPRAY position.
 - e. Clean sample holder with solvent and water.
 - f. **Empty waste bottle if it is full or top up flow solvent if going to finish soon**
 - g. Clean all glass wares and dispose vials and gloves appropriately.

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Replacing solvent bottle and prime pump:

Around 1000ml of 50% MeOH:H₂O solution needed for solvent line last up to a month depends on frequency of sprayer use.

After replacing solvent, set pump at 0.500 mL/min and prime pump to remove air bubble as follow.

With pump on, and flow on, prime pump by opening pump valve completely (counter clockwise) and let solvent flow out then pulling on syringe, solvent should flow.

Optimization of matrix deposition:

With the TM-Sprayer™ you can control deposition quality and quantity using five fully adjustable parameters:

- Number of passes (NP)
- Matrix solution concentration (Cm)
- Matrix solution flow rate (FRm)
- Relative speed of the nozzle to the sample surface or Velocity (V)
- Track Spacing (TS)

Because these parameters can be adjusted independently from one another they give you the ability to change the matrix deposition quality (wetter spray vs. dryer spray) without changing the amount deposited, or change the amount deposited (less vs. more) without changing the deposition quality.

Calculating the amount of matrix deposited on a sample:

The amount or weight of matrix deposited per linear distance (W) is given by the formula:

$$W = (NP \times Cm \times FRm) / (V \times TS)$$