Operation of the Mass Spec Core HTX Sublimator for MALDI Matrix Deposition for Imaging Samples Preparation in E1.26, QMRI

Instrumentations:

HTX Sublimator™ (HTX Technologies, LLC, USA)

Huber Water Chiller (Minichiller 600 OLÉ, filled with 20% Ethylene Glycol (CAS 107-21-1) as Coolant) Temperature range: –20 to +40 °C

Vacuum pump

Consumables and chemicals:

Matrix solution ~2 mL – depends on protocol

Water flow at 4-8 ^{0}C – depends on matrix crystal size requirement

Nitrogen Gas flow at pressure 10 psi

Sample slide for matrix deposition (Some regions of slide may not be coated properly; it is recommended to plan tissue mounting on slide accordingly and avoid placing samples in those areas).

Solvents for cleaning:

100% EtOH in spray bottle for cleaning

Acetone for DAN stain cleaning

Important: Check the stability of the instrument first, before taking out sample slide from -80C freezer or dry ice to dry in vacuum desiccator

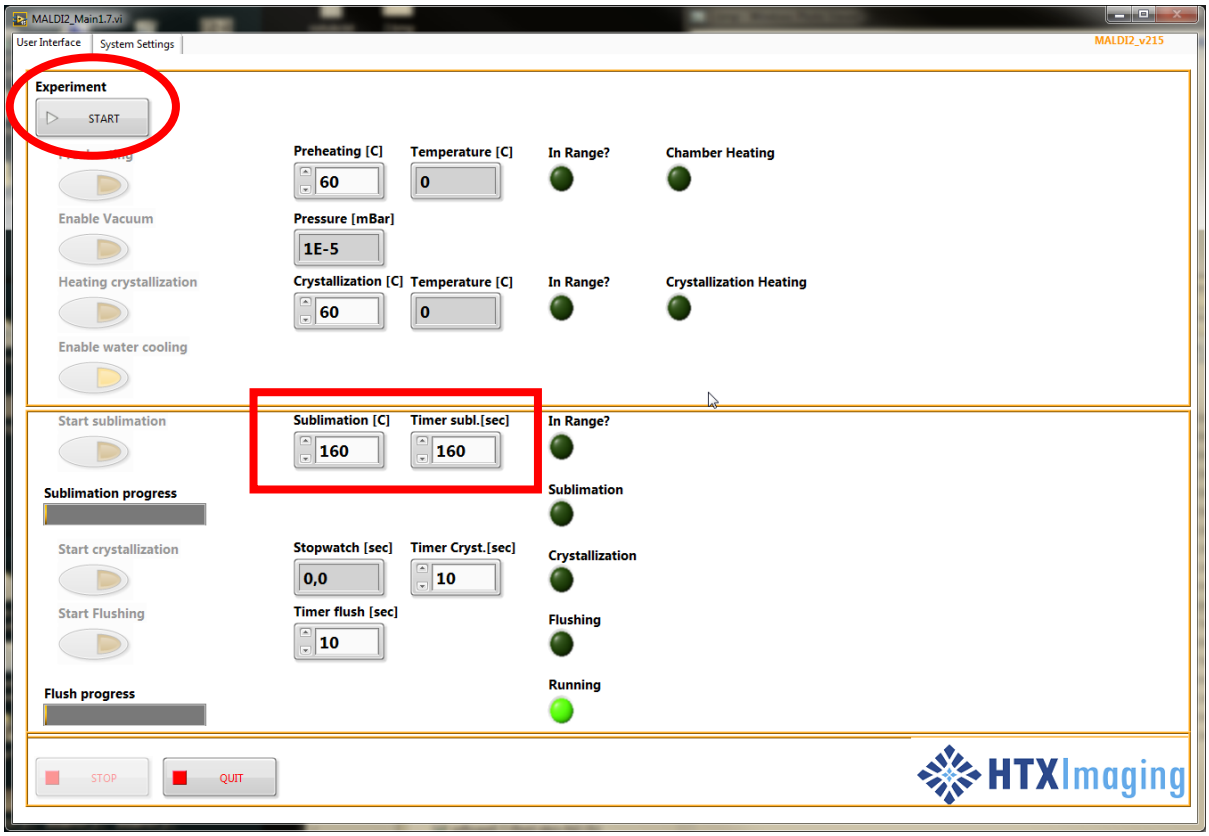
Set up of Instrument:

1. Turn on Fume cupboard light and Fan (airflow).
2. Connect / switch on tablet cable at the plug, leave for 15 min on charge before turn on tablet.
3. Switch on HTX Sublimator™, Vacuum pump and Huber Chiller at the plug
4. Check Huber chiller coolant level. (If level is low then don't use the sublimator and report to Shazia Khan)
5. Set the Chiller required temperature i.e. 4^{0}C and wait (~20 min) until the chiller reaches the setpoint before proceeding.
6. Inspect all tubing / connections for leak.

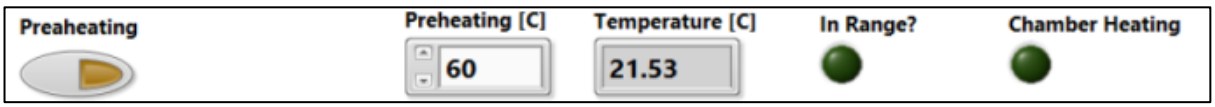
7. Turn on Nitrogen flow fully by toggle valve on the fuming hood - Nitrogen for the system is supplied from the building Generator.
8. Ensure sublimator is cleaned and dried thoroughly to avoid any cross contamination. Special attention must be given to the sealing surfaces of the vacuum chamber and the o-ring surrounding the door.

Matrix Deposition (sample preparation):

1. Prepare Matrix solution according to protocol – Make sure matrix is completely dissolved.
2. Open the software to and press **START** (see image below).

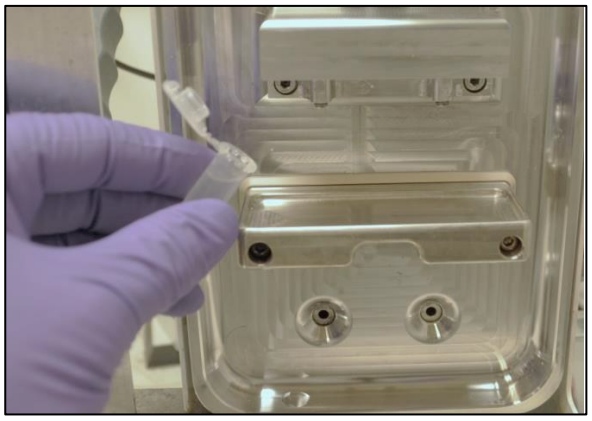


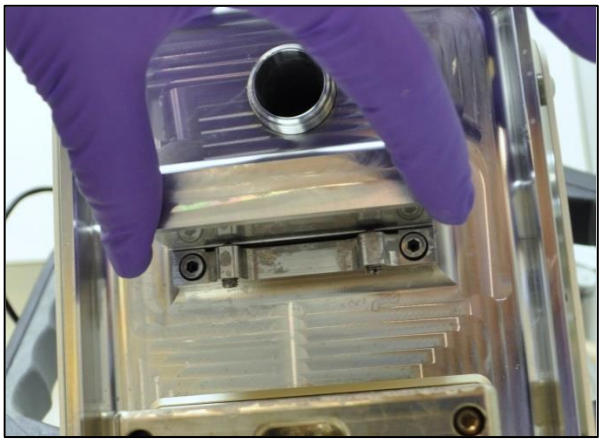
3. The water cooling enables automatically.
4. Set sublimation parameters (temperature and time) in **Sublimation [^{0}C]** and **Timer subl. [sec]** according to user requirements / protocol (highlighted on image up).
5. Set the **preheating temperature [°C]** to the desired value (mostly 60 ^{0}C)
6. Enable the preheating toggle switch (image below).



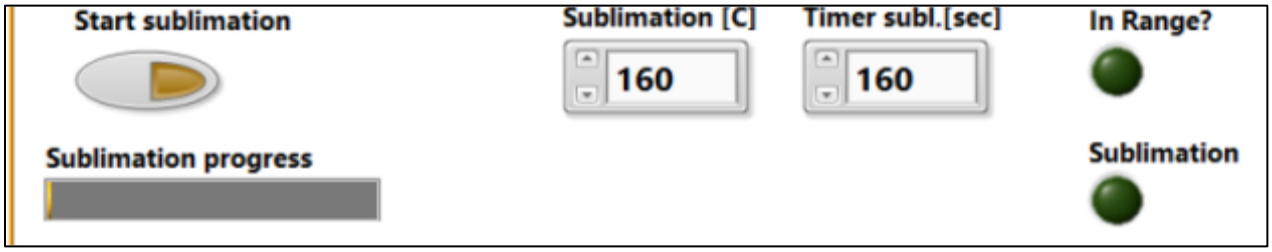
7. The current temperature is displayed in the **temperature [°C]** indicator, when in range, the **In Range?** indicator light **lights up**.
8. Make sure the sample holder is cooled (if not then check Huber water chiller temperature and flow).

9. Load the dissolved matrix solution onto the sublimator's tray evenly (see image). Use pipette or dropper for better control to spread matrix evenly. Handle with care, this surface is hot!.
10. Wait for the matrix substance to dry completely.
11. Insert the sample glass slide into the slide holder, sample facing downward. Ensure the front edge of the slide is in-line with the front face of the slide holder (see image).
12. Close the chamber and make sure the surrounding O-ring is in place.
13. Enable the **Enable Vacuum** toggle switch (see image) and allow for the vacuum to reach the desired value (~0.005-0.006 bar / 5-6 mbar) which can be read on either the digital pressure gauge on top of the chamber or on the **Pressure [mBar]** readout in the software.



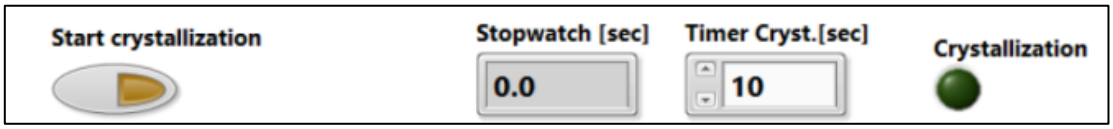


14. Set / check the sublimation parameters (Sublimation [°C] and Timer subl. [sec])

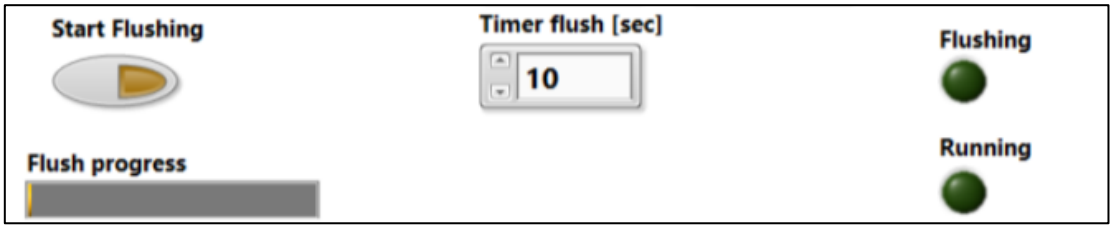


15. When the desired vacuum (~0.005-0.006 bar / 5-6 mbar) has been reached, then **Start sublimation** toggle switch (image above)
16. Sublimation temperature start to rise from **preheating temperature** (60 ^{0}C) to the set temperature gradually within the set time as sublimation progress.
17. Indicator lights show when the temperature is in range and if the sublimation process is enabled.
18. The matrix will sublime and deposit onto the sample slide surface as temperature rises.
19. Once set time reached temperature start to drop gradually.
20. The condensation of matrix on the inside of the viewpoint can be used as in interpretation if the sublimation process has been taken place (see image below).
21. Wait until the sublimation process completed and temperature reach back to 100 ^{0}C in order to aid the condensation of the matrix onto the sample slide.

22. **Please note: it is not possible to perform a recrystallisation step by using the Start crystallization toggle switch due to high vacuum.**



23. To finish the cycle and open the vacuum chamber, enable the Start Flushing toggle switch. Chamber will be thoroughly flushed with nitrogen to remove possible remaining gasses.
24. The **timer flush [sec]** box indicates the set flush time. This value can be changed based on user requirements.



25. After the set time, the main vacuum valve is closed and N2 will remain flushing in order to open the vacuum chamber door.
26. The water cooling can be disabled by checking the water-cooling toggle switch.
27. A popup window will be displayed, indicating the completion of the cycle.
28. Open the door and carefully remove the sample slide by holding it by the edges and sliding it out of the sample holder.

Important: Some regions of slide that are covered by the sample holder may not be coated sufficiently; it is recommended not to place samples in this area.

Cleaning and maintenance:

It is important to clean the device properly after use. Cleaning not only helps the desired vacuum to be achieved faster and more efficient, but also prevents matrix contamination within the sample.

1. All metal components are preferably cleaned with either 100% ethanol or isopropanol. This includes the inside and outside surfaces of the vacuum chamber, sample holder, sublimation tray, the base plate and the metal frame of the device.
2. Special attention must be given to the sealing surfaces of the vacuum chamber rear and the vacuum chamber door.
3. Special care must also be given to the O-ring that forms the seal between these surfaces.
4. Outside viewport of the vacuum chamber door and the protective cover around the recrystallization bottle are made out of polycarbonate, these are best cleaned with a mild detergent only.

Waste Disposal:

- **DAN Waste:** Must be collected / disposed of **inside fume hood** to prevent exposure.
- **Cleaning Waste:** Dispose of all cleaning residues according to the **chemical/matrix SDS**.
- **Solvent Waste:** Solvent waste to be disposed to appropriate solvent wasted bottles (Halogenated or Non-halogenated)

Shutdown Procedure:

- Exit the Sublimator software and **shut down the tablet completely**.
- Turn **off the Sublimator**, **Huber chiller**, and **vacuum pump**.
- Turn off the **nitrogen supply** (if not already done).
- Clean the **fume hood** to remove any spills.
- Replace the **waste container** if it is full.
- Clean all **glassware** and dispose of **vials and gloves** according to lab safety guidelines.

Recommended Sublimation Protocols (can be use as starting point)

Matrix	Amount (mg)	Solvent (2mL)	Preheat temp	Temp in C	Time (sec)	Comments
DHB	50	Acetone	60	160	160	
Norharmane	50	MeOH	60	140	180	
CHCA	50	ACN:H2O (7:3)	70	180	300	
DHA	50	IPA	60	160	300	
NEDC	40	MeOH	60	160	120	
DAN	20	MeOH	50	120	60	Very thin layer & matrix left in container
DAN	50	ACN (DAN is not soluble in MeOH @50mg)	60	160	300	Optimised protocol

Recrystallisation	**Vacuum valve must be closed during recrystallization process**		
	Temperature	**Time**	**Comments / Observations**
IPA	35	15 secs	Not worked
IPA	60	1.5 mins	Not worked
This instrument does not support automated recrystallisation; users should perform recrystallisation manually.			

Optimization of matrix deposition:

You can control deposition quality and quantity using below adjustable parameters:

- Amount of matrix (matrix quantity effect density on slide)
- Sublimation time (longer more deposition)
- Temperature of matrix deposition (temp to fully sublime the matrix)
- Additional manual recrystallisation step (solvent, temp and time)