

In the Event of an Emergency:
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Gatan Cryo-holder Model 626

Basic Procedures

(Prepared by Aida Razi, Kaustuv Basu and Joaquin Ortega)

1. Initial Set Up

- a. The cryo-holder should be in the dry pumping station under vacuum.
- b. The cryo-holder should be inverted so dust does not accumulate in the dewar and is actively being pumped.
- c. Tubing should be attached from the cryo-holder to the arm of the dry pumping station and the needle valve at each end of the tubing opened as much as possible. When tubing is NOT attached to the cryo-holder dewar, it should be connected to one of the two solid metal posts of the dry pumping station.
- d. Two green lights (MDP STATUS and SYSTEM STATUS) and one yellow light (POWER) on the front panel of the dry pumping station are lighted.



2. Removal of Cryo-holder from the Dry Pumping Station

- a. CLOSE the NEEDLE VALVE on the CRYO-HOLDER and then CLOSE the VALVE on the DRY PUMPING STATION
- b. DETACH the TUBING from the cryo-holder and attach it to a metal post of the dry pumping station
- c. CLOSE the VALVE LABELLED V1 (turn the black lever to the 3 o'clock position).

- d. SWITCH OFF the DRYING PUMPING STATION by pressing the POWER BUTTON at the back of the unit.
- e. Wait approximately five minutes for the lights on front panel to turn off.
- f. Gently REMOVE the CRYO-HOLDER by RETRACTING it from the HORIZONTAL SLEEVE.
- g. After the cryo-holder has been removed, INSERT the PLASTIC PLUG into the empty horizontal sleeve.
- h. Place the CRYO-HOLDER into the WORKSTATION and move it to the transfer table.

3. Transfer of TEM grid into the holder

- a. Position the CRYO-HOLDER and CONTROLLER BOX on the transfer table as shown in the image.
- b. The tweezers and clip ring tool are in the right drawer inside the plastic container. Remove and place them on top of the controller box as seen in the image. Before transferring the TEM grid to the cryo-holder, press the TURBO ON button (changes color from Gray to Orange) on the FEI Tecnai G2 F20 TEM as it takes a few minutes spool up (button turns Yellow when ready).
- c. Transfer the TEM grid onto the holder.
- d. Move the workstation with the cryo-holder to the desk of the FEI Tecnai G2 F20 TEM.
- e. Cover the RIGHT CONTROL PAD and MONTORS with the plastic sheeting. Ensure the BLACK RUBBER MAT is over the viewing window.



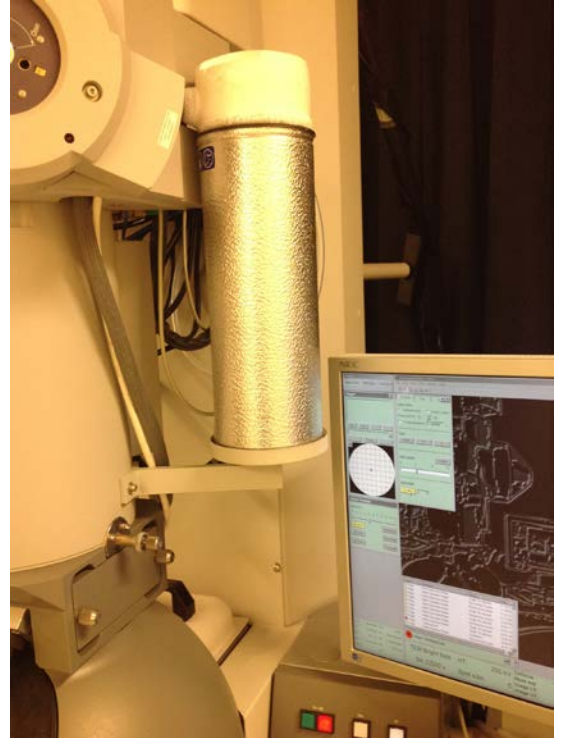


4. Inserting the cryo-holder into the Column of the Microscope

- a. VERIFY on the Vacuum (User) window, the COL. VALVES CLOSED is Yellow.
- b. VERIFY the COMPUSTAGE red LED is OFF. If the red LED is lighted, do NOT attempt to insert cryo-holder. Request assistance of staff.
- c. VERIFY the OBJECTIVE APERTURE is retracted.
- d. Before inserting the cryo-holder into the goniometer, SELECT the SEARCH tab on the Tecnai User Interface (TUI), press the small ARROW button on STAGE window to open the flyout window, SELECT Control tab, and press the HOLDER button (under Reset) to reset all stage translates and tilt to zero.
- e. SET the Alpha Wobbler to -70° and press the ALPHA WOBBLER button (turns Yellow) to rotate the stage.
- f. Click on the PREPUMP AIRLOCK button which will initiate a countdown of a few seconds.
- g. Once the Prepump is completed, INSERT the CRYO-HOLDER into the GONIOMETER making sure it is well-aligned from the beginning according to the images below. (Black dot on the stage with the base of the 'i' in 'Assembled in the USA').

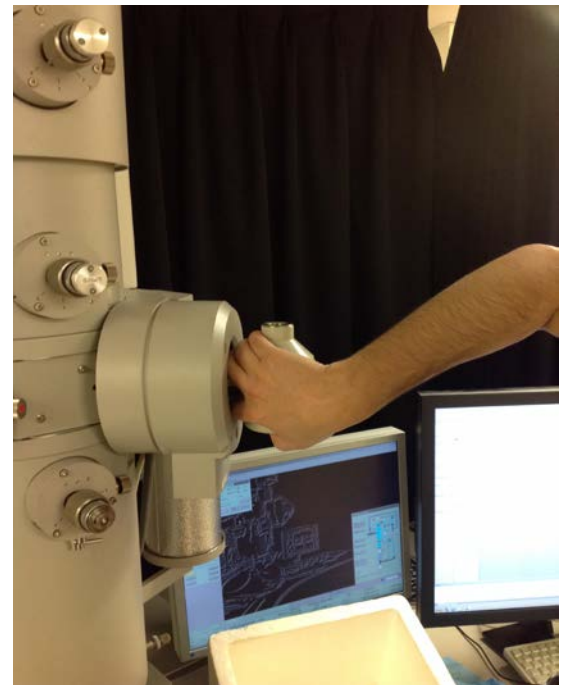


- h. In the message window, select CRYO-HOLDER in the PULL-DOWN MENU.
- i. Wait until the countdown reaches zero, ensuring the red LED is OFF.
- j. PRESS the SEARCH tab again, and under the CONTROL window, press the ALPHA WOBBLER button (Yellow at this point). Place your left hand lightly on the stage. Immediately with your right hand, GRIP the cryo-holder dewar and start ROTATING the CRYO-HOLDER as the STAGE ROTATES. Once the stage reaches zero degrees, ROTATE the HOLDER and INSERT.
- k. Once the cryo-holder has been inserted into the column, PRESS the TURBO ON button again to stop the turbo molecular pump (changes color from Yellow to Gray).
- l. When IGP1 is lower than 18 Log, PRESS the COL. VALVES CLOSED button to open the column valves.



5. Removing the Cryo-Holder from the Column

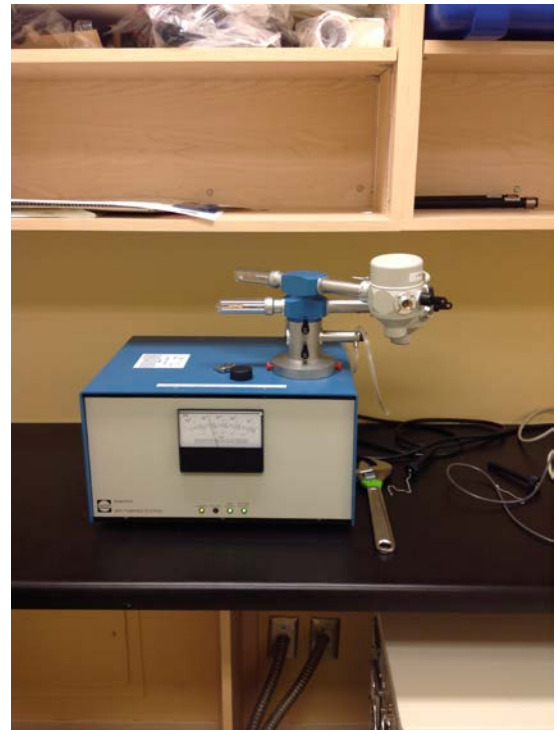
- a. PRESS the COL. VALVES CLOSED button (turns Yellow)
- b. PRESS the TURBO ON button and wait until the button turns Yellow.
- c. REMOVE the OBJECTIVE APERTURE from the beam path; it is ESSENTIAL to remove the objective aperture in case the cryo-holder touches it during removal.
- d. To retract the cryo-holder, grip it with your RIGHT HAND as shown in the image and with your LEFT HAND lightly pressing the purple plate of the goniometer.
- e. Slowly and smoothly RETRACT the CRYO-HOLDER until in STOPS. ROTATE the CRYO-HOLDER CLOCKWISE 150° until it STOPS. With your thumb and index finger of your LEFT HAND, carefully pull the CRYO-HOLDER completely out.



- f. PLACE the CRYO-HOLDER back onto the workstation.

6. Insertion and removal of Cryo-holder in Dry Pumping Station

- a. REMOVE the plug from the HORIZONTAL SLEEVE of the DRY PUMPING STATION.
- b. INSERT the CRYO-HOLDER in the horizontal sleeve of the dry pumping station with dewar TILTED UPSIDE DOWN.
- c. SWITCH ON the DRYING PUMPING STATION by pressing the POWER BUTTON at the back of the unit.
- d. ENSURE VALVES V1 and V2 are in 12 o'clock position.
- e. CONNECT the CABLE from the CONTROLLER BOX and START the WARM UP CYCLE.



7. Removing the CRYO-HOLDER from the DRY PUMPING STATION

- a. TURN VALVE V1 to the 3 o'clock position.
- b. CLOSE the NEEDLE VALVE on the CRYO-HOLDER and then CLOSE the VALVE on the DRY PUMPING STATION
- c. DETACH the TUBING from the cryo-holder and attach it to a metal post of the dry pumping station
- d. CLOSE the VALVE LABELLED V1 (turn the black lever to the 3 o'clock position).
- e. SWITCH OFF the DRYING PUMPING STATION by pressing the POWER BUTTON at the back of the unit.
- f. Wait approximately five minutes for the lights on front panel to turn off.
- g. Gently REMOVE the CRYO HOLDER by RETRACTING it from the HORIZONTAL SLEEVE.
- h. After the cryo-holder has been removed, INSERT the PLASTIC PLUG into the empty horizontal sleeve.

- i. Place the CRYO-HOLDER into the WORKSTATION and move it to the transfer table (do NOT walk around the room with the cryo-holder in your hand).

8. Pumping the Dewar of the Cryo-holder

- a. PLACE the CRYO-HOLDER in the PUMPING HORIZONTAL SLEEVE with the dewar in the UPRIGHT POSITION.
- b. SWITCH OFF the UNIT using power switch at the back of the unit. VERIFY VALVE V2 is at 12 o'clock POSITION and VALVE V1 is at 3 o'clock POSITION.
- c. CONNECT the TIGON TUBE to the DEWAR from the SLEEVE to the right of VALVE V2.
- d. SWITCH ON the UNIT using power switch at the back of the DRY PUMPING STATION and wait for five minutes to achieve a good vacuum.
- e. Open valve indicated by finger in the image. VALVE V1 should be at the 3 o'clock position.
- f. OPEN the VALVE of the DEWAR.
- g. CONNECT the CABLE from the CONTROLLER BOX and START the ZEOLITE CYCLE for two hours (no longer).
- h. To remove a cryo-holder that is pumping the dewar, reverse the operation. First close valve of the dewar and close valve indicated by finger in the image. VALVE V1 should be at the 3 o'clock position. Turn off the POWER SWITCH at the back of the unit and WAIT five minutes for the turbo to slow down. RETRACT the CRYO-HOLDER holder from the HORIZONTAL SLEEVE and replace with the PLUG.



Notes: Potential Issues when Inserting the Cryo-holder

1. Misalignment of the holder during insertion

- a. If the alignment of the cryo-holder at entry is not correct, the turbo molecular pump (TMP) on the TEM will make a very loud noise. If this occurs, retract the

cryo-holder as quickly as possible. Re-insert the cryo-holder into the workstation and refill with liquid nitrogen. Press Pre-Pump Airlock on the TEM again, the red LED will be lit and a countdown of about six minutes will begin. Wait until the countdown reaches zero and the red LED goes off before attempting to re-insert the cryo-holder. Keep adding liquid nitrogen to the cryo workstation to save your grid.

2. Dry Pumping Station

- a. The turbo pump will run continuously except when air is dumped into the system while operating at full speed. The red FAILURE light will indicate when this has happened.
- b. The butterfly valves (V1 and V2) can get dirty and when this occurs they will often not close completely. If this situation occurs, a user will do everything the correct way (as outlined above), but the vacuum will still crash and the red FAILURE light will light up. When this occurs, please contact the staff as soon as possible.
- c. The vacuum gauge occasionally gets dirty and fails to read properly - the needle is pinned to the right (indicating no vacuum, even though it is clear there is some vacuum in the horizontal sleeves and the red FAILURE light is not lit). When this happens, please stop using the dry pumping station and contact the staff.

3. If the HT crashes, get assistant from staff.