

Gatan Pumping Station Operation

Starting cryo-session (check list):

1. Make sure that vacuum valves (V2, V3) on the pumping station and valve on holder are open and that the soft transparent tube is connected to the metal outlet beneath vacuum valve of holder dewar. The holder dewar must be pumped for at least 30 mins (vacuum reading should be $\leq 1 \times 10^{-5}$ Torr) at room temperature. **Note: make sure you know how to fully open and close V1, V2, V3 and valve on holder and on how to connect/disconnect the soft transparent tube correctly. Please ask facility staffs and do not assume that you know it when you are not confident.**
2. Wait for Step 1 to finish, check the controller to make sure that no heating cycle is on and the temperature of the holder is at room temperature.
3. Following the order to close valves: valve on holder dewar, V3 (close V1 after V3 if V1 is open), V2. **Note: V3 and valve on the holder are multi-turn valves, when closing such valves, you only need fingertip force to turn the black knob to fully close them, there is no need to increase force to further tighten them.**
4. Disconnect the soft transparent tube from the metal outlet of holder dewar slowly and connect it to the blunt metal rod on the back. **Common mistake: the transparent tube was disconnected from holder dewar but did not connect to the blunt metal rod.**
5. Use both hands to pull holder from the pumping port of the station slowly until vacuum breaks. Then, slowly remove holder from the station and be carefully not to let the guide pin to scratch the inner surface of the pumping tube.
6. Fully insert the black stopper into the pumping port of the station where the holder was removed. **Common mistake: this stopper was not inserted fully into the pumping port.**
7. Insert holder into cryo transfer station horizontally. Pour LN₂ into the holder dewar and station reservoir to cool down the system to about -196°C before loading specimen grid to the holder.
8. Remember to close holder shutter and disconnect controller cable after loading grid to holder.

Finishing cryo-session (check list):

1. Remove holder from TEM and empty LN₂ in holder dewar.
2. Make sure the holder pumping station is on and all valves (V1, V2, V3) on the station are closed. **Note: make sure you know how to fully open and close all valves and on how to connect/disconnect the soft transparent tube correctly. Please ask facility staffs and do not assume that you know when you are not confident.**
3. Remove black stopper from pumping port, insert holder slowly into the pumping port fully and connect controller cable.
4. Rotate the holder inside pumping port to let holder dewar upside down (make sure the dewar cap is place near the pump). Do not connect the soft transparent tube now.
5. Open V2, and then open V1. **Note: make sure you know how to fully open and close V1 and V2. Please ask and do not assume that you know it if you are not confident.**
6. For old model controller (Controller for Holder-1), turn on controller (power switch is on the back) and start "Warm Up Cycle".
 - a. Press the large black knob once and turn it to select "Warm Up Cycle".
 - b. Press the same black knob one more time to show options, press button below "Yes" to start the warm up cycle.

- c. Warm up cycle takes about 20 mins to warm up holder to 40°C and the heating will stop automatically when the temperature reaches 40°C.

For New model controller (Controller for Holder-2)

- a. Turn on controller (press and hold the Gatan logo on the front) and confirm controller cable connection on touch screen. Program enters main screen.
 - b. Current program is shown above the temperature at the upper right corner of main screen. Press "Mode" and select "Automatic" if not in Automatic mode.
 - c. Press "PLAY" to start the automatic program. The holder tip will be heated to 30°C and be maintained there until the program is turned off manually.
 - d. Alternative, Press "Mode" to select "Warm Up" and "Play" to start the warm up program. The holder tip will be heated to ambient temperature and be held there for the duration of user-defined time. The default of 2 hours is the total program running time, including the heating step.
7. After the tip is warmed up to room temperature, rotate the holder to make holder dewar upright, put the cap back to the holder dewar opening (Extra step for new controller: stop "Automatic" mode by pressing "STOP" button).
 8. Make sure V3 is still closed, disconnect one of the soft transparent tubes from blunt metal rod, connect it to the metal outlet on holder dewar, slowly open V3 and wait for the vacuum meter to stabilize, and then open valve on the holder.