Zeiss CRYOSTAGE
USE & SETUP

1. **Use Common Sense.** If in doubt, ask for help.
2. All ICAL Equipment is to be run by qualified ICAL personnel or Users who have received formal training (short course) from a qualified ICAL staff member. No exceptions.
3. **ICAL is a multi-user Facility.** Please keep the work areas clean. Samples left unattended may be lost or damaged and data or files left on computers are subject to loss or erasure. If you need to store your belongings or samples, please make special arrangements to do so.
4. Fill in appropriate entries in the logbooks.
   All MSU Users must provide a Banner grant number and department.
5. No food or drinks in the lab area. Appropriate safety attire is required when handling chemicals, etc.
6. We request that all publications or reports utilizing results obtained in this facility acknowledge the Imaging and Chemical Analysis Laboratory at MSU. For citation information, visit https://nano.montana.edu/mont-acknowledgement-logo/acknowledgementlogos.html

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I. BASIC INFORMATION

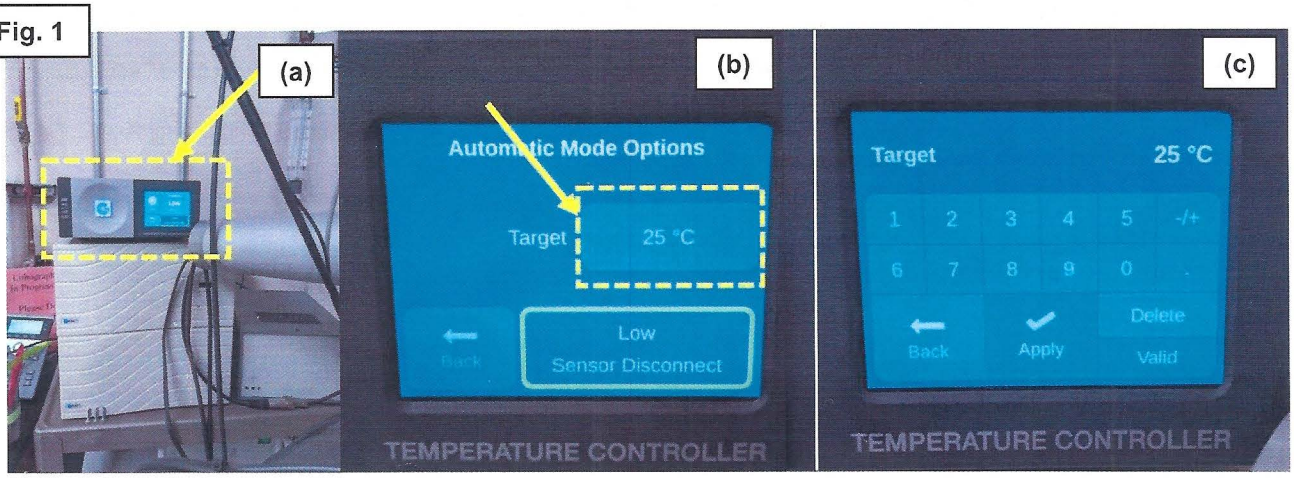
The cold stage should be already set up and ready to use. This manual discusses the protocol for cryostage insertion and analysis in the FEM that are different from standard protocol.
The user will need a supply of dry ice to keep samples/holder cold. Liquid nitrogen will be provided by ICAL.
Wear gloves when handling anything that will go in the microscope chamber – fingerprints are oily and not welcome in any vacuum system
Be careful with items at liquid nitrogen temperature, as they will burn you. For large items, use the temperature resistant gloves to protect your skin.
Use the special cold stage sample holders intended for the cold stage.
Make a note of the location of your samples on the sample mount. In particular, note the orientation of the layers of interest in a cross-section sample. Taking a picture with your phone camera before inserting the holder into the microscope is a good idea.
Make sure there is a sufficient supply of liquid nitrogen for your time on the microscope. It is better to get a fresh supply early before analysis is started than later in the middle of good imaging.
Use the foil-covered wax-coated cup provided to hold the liquid nitrogen intended for sample and holder immersion. Do not immerse the holders and samples into the Dewars or thermos.

II. CRYOSTAGE COMPONENTS (Pre-Assembled and Functioning)

Most times the Cryostage will be set up for you in advance, and the stage will be cooled and ready for sample insertion. In those cases, you will need to know the different elements of the Cryostage and how to run them.

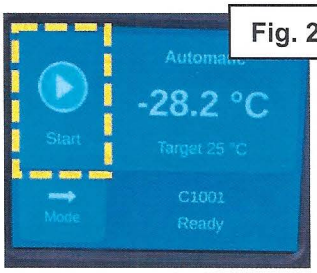
A. Gatan Temperature Gauge

Fig. 1



1. The Gatan Temperature Gauge reads the temperature of the cryostage within the FEM and can control the temperature by heating the stage. The gauge is controlled using the touch screen.
2. Locate the temperature gauge to the left of the FEM instrument, located on the racks (Fig. 1a).
3. To set a target temperature, press the temperature box on the middle right (Fig.1b).
4. In the next screen, choose the target temperature by touching corresponding number pad (Fig. 1c). For negative temperature values, please make sure to choose a negative sign. When your temperature is chosen, press the Apply button.
5. On the screen that comes up, press **Start** to begin working toward the target temperature (Fig. 2).
6. Monitor the temperature of the stage at this Home screen.

Fig. 2



B. Liquid Nitrogen Stage Dewar Cooling System

1. The liquid nitrogen Dewar cooling system comprises tubes containing a flow of dry nitrogen gas that flows from the lab N_2 hookup (Tube 1), through the copper tubing inside the LN2 Stage Dewar, through the FEM door (Tube 2), through the stage, and back out the FEM door to vent (Tube 3).

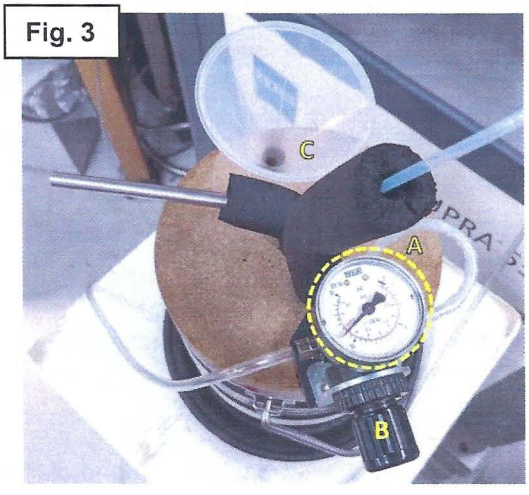
2. The pressure of the cooled N2 gas leaving the Stage Dewar is measured with a gauge at the top of the Stage Dewar (Dashed circle A, Fig. 3). This gauge should read ~6 psi when in normal cryostage operation and ~1 psi when performing the sublimation process.

   a) If the pressure of the vented gas from Tube 3 becomes unstable and begins to pulse and hiss irregularly, slightly reduce the pressure at the gauge using the knob B (Fig. 3).

   b) If the Gatan temperature gauge cannot keep a sufficiently low temperature, increase the N_2 pressure using the knob at the Stage Dewar gauge.

3. The Stage Dewar should have sufficient LN2 to cover the copper tubing inside. Monitor the level of the LN2 to ensure that it does not boil dry and cause heating of the sample inside the microscope. To add LN2, carefully pour it slowly through the small funnel at the top of the Dewar (C in Fig. 3).

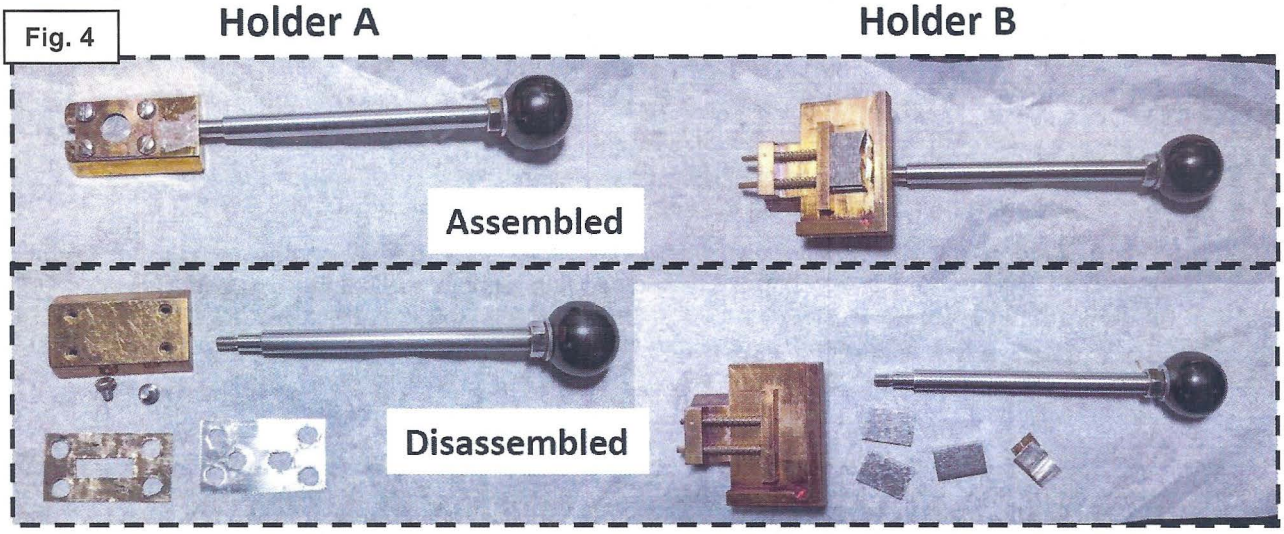
Fig. 3



III. CRYOSTAGE SAMPLE PREP

There are two types of holders for the cryostage that are useful for different kinds of samples. The first (Holder A, Fig. 4) is best for looking at the surface of a flat sample. The second (Holder B, Fig. 4) is best for looking at cross-sections.

Fig. 4



A. Sample Holder A – Flat samples

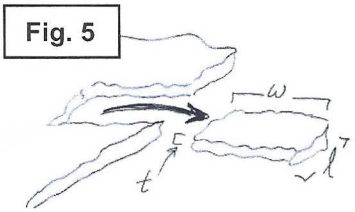
1. For best results, place a section of your flat sample, thawed or frozen, on the brass surface generally where the view holes will be in the aluminum/brass mask you choose.

2. Screw the mask down using two to four of the screws provided.

3. When you are ready to insert the sample into the microscope, attach the handle to the end of Holder A and plunge the entire holder end into liquid nitrogen until the liquid boiling activity settles into a rolling simmer. The sample holder/sample is now at equilibrium with the liquid nitrogen temperature and can be inserted into the microscope.

B. Sample Holder B – Cross-section samples

Fig. 5



1. To create a cross-section of the thickness (*t*) of your wet sample, cut a piece of your sample that is about as long (*l*) as you want it in the sample holder and significantly wider (*w*) than you will need. (See Fig. 5)

2. Cut a piece of thick metal foil about the same width you will want in the sample holder. When the sample is mounted for the cross-section, this will be the height the sample stands in the holder. Cut the metal foil about twice the length (*l*) of your sample.

3. Submerge your cut sample into liquid nitrogen and hold there until the rapid boiling calms.

4. Pull the sample out and quickly snap the frozen sample at the area you want the cross-section using tweezers, razor blades, etc.

5. Mount this snapped sample piece by folding it in a piece of already-prepared thick metal foil (Step 2).

6. After folding your cross-section sample into the foil, pinch the foiled sample into the spring-loaded sample Holder B using a stack of 3-4 metal coupons to create pressure from the spring side. It is best to stick these coupons together with a small amount of double-sided carbon tape to prevent them from falling out of the holder and getting lost inside the microscope during sample insertion/removal.

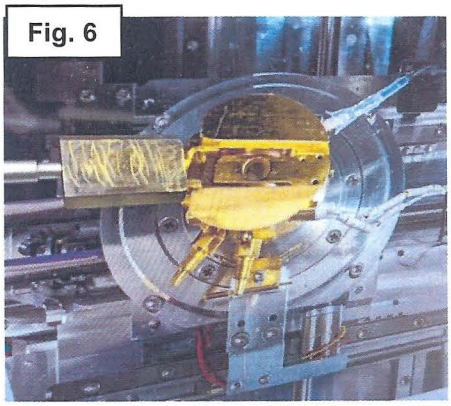
7. When you are done prepping the sample and inserting it into the spring-loaded Holder B, attach the handle to the end of Holder B and carefully plunge the entire holder end into liquid nitrogen until the liquid boiling activity settles into a rolling simmer. The sample holder/sample is now at equilibrium with the liquid nitrogen temperature and can be inserted into the microscope.

C. Sample Insertion

1. Vent the system by clicking on Vac Fine Vac: ✓ Gun: ✓ EHT: ✗ and selecting vent (If the EHT is still on Vac: ✓ Gun: ✓ EHT: ✓, click on EHT and choose EHT Off). Under stage navigation, check that the rotation and tilt are set to zero. (Note: The stage should not be tilted or rotated in cryo-mode.) Wait about 1 minute for the system to vent sufficiently to allow you to *gently* slide open the door. Be careful to move the LN2 Stage Dewar table away with the microscope door to ensure the cryostage gas lines are slack and not in the way.

2. Pull your holder/sample out of the liquid nitrogen or from atop the dry ice and quickly insert it onto the cryostage. The insertion will be done from the *left side* as is shown in Fig. 6 (this is different from the room-temperature stage).

Fig. 6



3. Unscrew the holder handle from the holder and gently close the microscope door. Quickly click on Vac and pump the system. You may have to move the plastic nitrogen tubing out of the chamber door to close it.

NOTE: Steps 2 and 3 should be done as quickly as safely possible to mitigate the accumulation of frost on the sample holder and cryostage.

4. Wait 2–5 min for the system to pump. The system is ready when the check mark next to Vac turns green. Fine Vac: ✓ Gun: ✓ EHT: ✗ (i.e., ~ 10^{-5} Torr)

5. Set up the computer workspace. Open/toggle the following if necessary:

   a) SEM Control window (Ctrl-G, or Tools_Control Panel).
   b) Data Zone (View_Data Zone_Toggle Data Zone)
   c) Hard Front Panel (View_Toolbars)
   d) Stage Navigation (Stage_Navigation)
   e) Soft Joystick

D. Sample Sublimation

1. Once the sample is in the microscope chamber and the chamber is pumped, check the sample using the TV camera. If there is a lot of visible accumulated frost, you will need to sublimate the sample and sample holder.

2. The ice is sublimated by reducing the nitrogen flow at the Sample Dewar and raising the target temperature at the Gatan Temperature Gauge

   a) Decrease the nitrogen flow to **~1 psi** using the pressure gauge at the Stage Dewar.

   b) Increase the target temperature to ~ **−76°C** at the Gatan Temperature Gauge. Push START

3. After several minutes you should see a color change in the TV camera view of the sample holder as the white ice sublimates off the brass holder. It will progress something like the images Fig. 7a to Fig. 7b. If you don't see any color change after several minutes, increase the target temperature a few degrees.

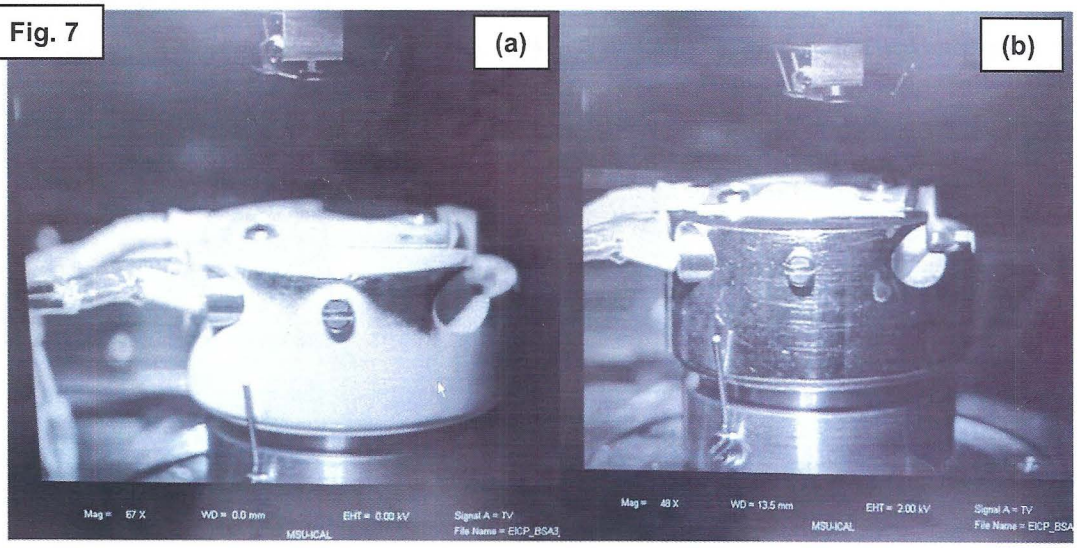
4. Wait until the color of the entire visible sample holder is changed (Fig. 7b) before restoring the settings:

   a) Decrease the target temperature to **−180°C** at the Gatan Temperature Gauge. Push START

   b) Increase the nitrogen flow **to 6 psi** at the pressure gauge at the Stage Dewar.

5. When the stage has reached a temperature less than −160°C, you are ready to obtain images.

Fig. 7



IV. CRYOSTAGE MICROSCOPE OPERATION

A. Readying and Imaging the Sample in FEM

1. Locate the Gatan Temperature Gauge. Set the target temperature on the gauge to −180°C and confirm that the stage is less than −160°C.

2. Insert your sample following the instructions in **Section III:C**.

3. If necessary, sublimate your sample following the instructions in **Section III:D**.

4. Operate the FEM for imaging following the instructions in the FEM User Guide. Typically, lower beam voltages (1–2 kV EHT) are best to avoid damaging or altering cold samples.

B. Shutting Down the System

1. Turn off the accelerating voltage (EHT) by clicking on EHT 

and choosing EHT Off (Do NOT select Shutdown Gun, as this turns off power to the filament).

2. Un-tilt your sample if necessary, and confirm the sample rotate (R) is zero. Wind out the EDX detector if it was used.

3. Vent the system by clicking on Vac 

and selecting vent. The stage will automatically reset to the lowest position. Wait about 1 minute for the system to vent sufficiently to allow you to *gently* slide open the door. Carefully move the Stage Dewar table to move it out of the way of the chamber door and ensure the cooling tubes are slack.

4. Remove the sample holder by screwing the cold stage holder into the screw hole in the left of the cryo-stage and sliding the stage off the holder.

5. Gently close the chamber door, moving the Stage Dewar table with the door so the cooling lines remain slack. Pump the system by clicking on Vac and choosing Pump System. Leave the system under vacuum with the EHT Off when you are done 

.

6. Inform ICAL staff that you are finished with the Cryo-FEM so that the FEM can be reconfigured for standard FEM imaging and use.

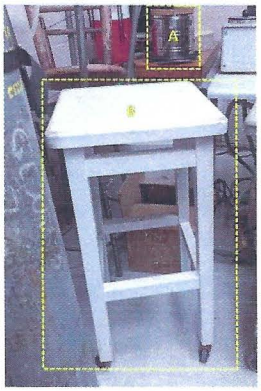
7. Be sure to enter your time and Banner # in the logbook. Please clean the sample prep area workspace and take your samples with you. Clean the sample holders you used and leave them assembled on the table for ICAL staff to store.

V. CRYOSTAGE SETUP

Unless you have been trained to install the cryostage, please contact qualified ICAL personnel to perform this for you. This should never be done without ICAL supervision or express approval.

A. Installing the Cryostage

Fig. 8



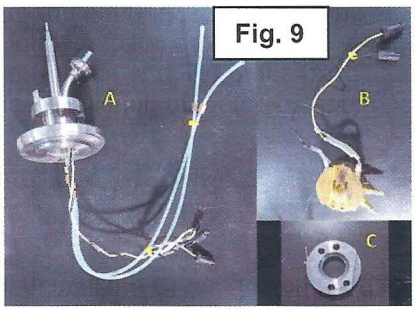
1. Locate the cardboard box containing the cryo stage and accessories (cryobox). It is typically on the wooden shelf running along the wall, set on the south wall (behind the LN2 Dewar cart).

2. Locate the Stage Dewar (on the table behind the LN2 Dewar cart, C in Fig. 8).

3. Locate the small rolling table used for the Stage Dewar (B in Fig. 8)

4. From the cardboard box, locate the Port Connection (A in Fig. 9), Cryo Stage (B in Fig. 9), and Cryo Stage Chuck (C in Fig. 9).

Fig. 9



5. Vent the FEM and remove the standard sample stage installed in the instrument. You will do this using a **4 metric (5/32 imperial) allen wrench**, removing the four bolts holding the mount to the stage (Fig. 10a). Also carefully remove the brass ground pin (Fig. 10b). Wrap the standard stage mount in foil and place in the cryobox, and store the ground pin and two of the bolts in a safe place for de-installation of the cryostage.

Fig. 10

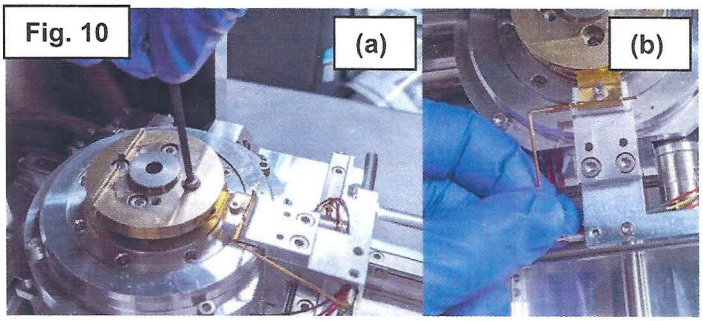
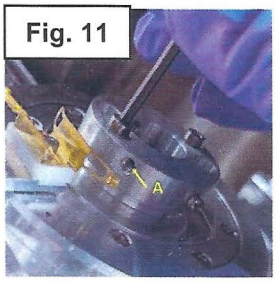


Fig. 11



6. Install the Cryostage chuck using two of the bolts from the standard stage chuck (Fig 11) and the **4 metric allen wrench**. Keep in mind the accessibility of the set screws on the sides of the chuck (A on Fig. 11) as you install, so that you will be able to tighten and loosen the set screws without difficulty.

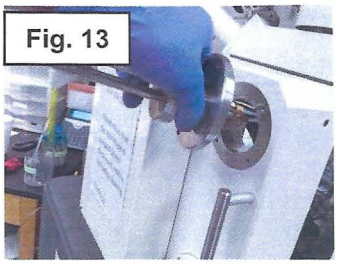
7. Insert the Cryostage into the center of the chuck, tightening the set screws with a **1.5 metric allen wrench**. Make sure you orient the Cryostage so that the wires for the heater and thermocouple are on the right side of the chamber and the gas line connectors are to the left.

8. Locate the grounding pin on the side of the Cryostage Chuck. Rotate the pin and tighten it against the Cryostage using a **flat-bladed screwdriver** so there is good contact between the chuck and the stage (Fig. 12). Confirm the good contact using a **multimeter**, by measuring the resistance between the stage and ground.

Fig. 12

9. Remove the top left port cover from the door of the FEM using a **5 metric allen wrench**. Carefully thread the wires and gas lines of the Port Connection through the opening left after the cover removal (Fig. 13) and bolt the Port Connection to the door using the bolts removed from the standard port cover. Wrap the standard port cover in foil and store it in the cryobox.

Fig. 13



10. Connect the thermocouple wires (yellow and white) and stage heater wires (black) from the Port Connection to the Cryostage by clipping the connections together (Fig. 14).

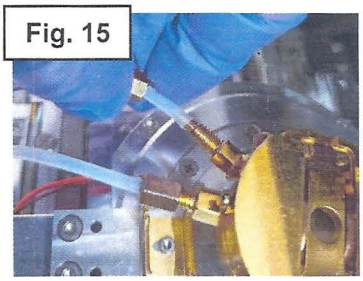
Fig. 14

11. Connect the gas lines from the Port Connection to the Cryostage by pushing each line to one of the brass nipple connectors on the stage and screwing the nut tightly to seal the connection (Fig. 15). Use a ¼ **crescent wrench** to do the final tightening. Do NOT wrench the connectors too tightly.

12. Close the FEM door and confirm that the gas lines and the wires do not interfere with the seal of the door.

13. Pump the chamber of the FEM and confirm that the chamber can be pumped to the point that the green check appears.

Fig. 15



B. Cooling the Cryostage

To begin cooling the stage, you must establish a supply of chilled dry nitrogen through the Cryostage. The dry nitrogen originates from the valve at the right side of the sample prep table, through the input of the Stage Dewar and out to the input of the Port Connection. The chilled dry nitrogen will exit the unused gas tube of the Port Connection. To achieve a good supply of chilled air, follow the steps below.

1. Place the Stage Dewar on the rolling table and arrange them close to the front of the FEM chamber door.

2. Remove the flexible 7 mm gas tubing from the lab-gas output on the sample prep table and attach the long flexible 7 mm gas tubing from the Stage Dewar to this output.

3. Open the gas valve to allow dry nitrogen to flush through the Stage Dewar tubing. This will ensure removal of any humidity present that may freeze upon reduction to liquid nitrogen (LN2) temperature.

4. Attach the shorter rigid 4 mm gas tubing from the Stage Dewar to either of the Port Connector gas input/output nipples. On the second Port Connection nipple attach the flexible 7 mm gas tubing that was removed from the lab-gas output (Fig. 16).

Fig. 16

5. Allow the dry nitrogen to circulate through the tubing and stage for a few minutes while you prep the Stage Dewar.

6. Ensure that the ICAL LN2 Dewar is full. (You and the user may need a lot of LN2 during the running of the Cryostage and you don't want to have to run down to refill the Dewar if you can avoid it.)

7. Carefully remove the Stage Dewar lid and copper tubing assembly from the Dewar bucket and balance it safely on the table. Fill the bucket three-quarters full of LN2 (Fig. 17a).

8. When the liquid nitrogen in the Stage Dewar bucket is no longer vigorously boiling, slowly insert the copper tubing into the LN2 until the lid is resting on the top rim of the bucket (Fig. 17b).

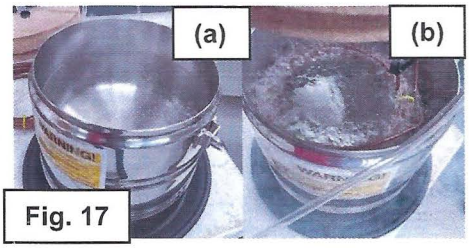


Fig. 17

9. Wait several minutes for the stage and tubing system to cool. You can monitor the process by observing the amount of gas venting from the Port Connection.

a) Initially, the warm dry nitrogen will be venting at a stable flow rate

b) As the dry nitrogen begins to be cooled by the LN2, you should notice that the flow slows and actually stops

c) With further cooling, the flow will increase and will pulse.

10. At this point, plug in the Gatan Temperature Control into the Port Connection. The plug will be typically found coiled on top of the control box.

a) Plug the 10-pin plug into the correct port, noting the unique orientation necessary for insertion (Fig. 18a).

b) Slide the ground wire onto the metal tab provided on the Stage Connector (Fig. 18b).

c) The connection should now look like the image in Fig. 18c.

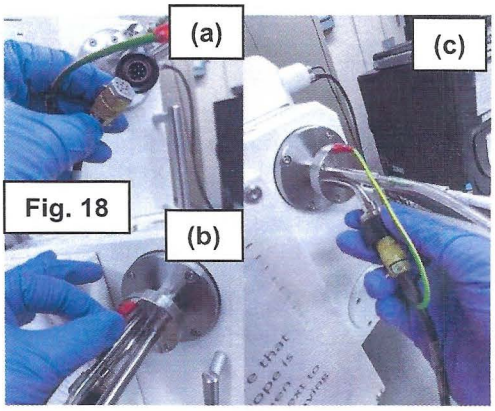


Fig. 18

11. Turn on the Gatan Temperature Control by pressing and holding the  button.

12. Wait for the controller to turn on and tap the  notification on the touchscreen. This should connect the Control to the cryostage thermocouple and heater.

13. At the next screen, tap the yellow dashed section of the screen in Fig. 19a to set the target temperature.

14. Enter the desired Target temperature using the touchscreen keypad. Be sure to enter a negative in front of your temperature value (typically −180 °C). When you have entered the Target temperature value, tap the  button on the touchscreen.

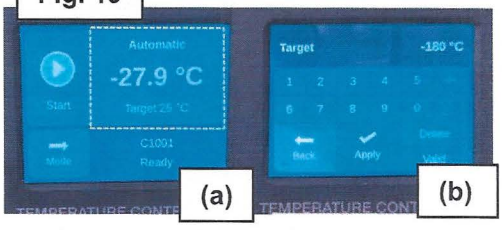


Fig. 19

15. The screen will go back to the one shown in Fig. 19a. Tap the  button on the touchscreen to begin the monitored cooling of the stage.

16. For initial cooling, you will want the flow rate of cooled nitrogen to be fairly high; around **6 psi** at the pressure gauge at the Stage Dewar.

17. Once the temperature of the stage reaches the Target temperature, you can reduce the nitrogen flow to around **5 psi** at the pressure gauge at the Stage Dewar. Continue to monitor the temperature and adjust the nitrogen flow to help keep the stage temperature stable.

18. At this point, the Cryostage should be ready for the user to insert their sample. Good job!

VI. CRYOSTAGE BREAKDOWN

The Cryostage breakdown instructions will commence at the point the user has removed their sample and the chamber has been pumped to vacuum.

A. Warming the Cryostage

1. At the Gatan Temperature Control, set the Target temperature to **20 °C** using the method described in **Section V:B 13–15**.

2. Reduce the nitrogen flow to **~1 psi** at the Stage Dewar gauge.

3. Allow the stage temperature to slowly increase, causing any frost to sublimate. This is the safest way to warm the stage so that liquid water will not be present at the stage or in the chamber.

4. Once the stage is at room temperature, turn off the Gatan Temperature Control box and disconnect the cable and ground from the Port Connector.

5. Disconnect the nitrogen gas lines from the Port Connector.

6. Remove the **long flexible 7 mm gas tubing** from the gas outlet so that the Stage Dewar is completely disconnected from everything.

7. Reconnect the length of **flexible 7 mm gas tubing** used at the vent on the Port Connector back to the gas outlet for sample prep use.

8. Move the table with the Stage Dewar away from the FEM chamber door and pour off the remaining LN2 from the Stage Dewar bucket into an open thermos container to boil away. Do NOT pour the remaining LN2 back into the ICAL LN2 Dewar as any contaminants or water ice present will then contaminate and accumulate inside the Dewar.

9. Restore the lid on the Stage Dewar and put the assembled Dewar back on the shelf where it is stored.

B. Disassembling the Cryostage

1. Vent the FEM chamber.

2. Remove the gas lines from the cryostage by loosening the nuts with a ¼" **crescent wrench**.

3. Disconnect the thermocouple and stage heater wires between the cryostage and Port Connector. This can be difficult to do, so be careful not to yank on the wires as you pull the snap connector apart.

4. Remove the Port Connection using a **5 metric allen wrench**, holding the bolts ready for the next step.

5. Locate the blank port flange stored in foil in the cryobox and reseal the open port hole in the chamber door using the four bolts you just removed from the Port Connection.

6. Wrap the Port Connection gently in foil and place into the cryobox.

7. Loosen the grounding pin on the cryostage at the chuck using a **flat-bladed screwdriver**. Only turn the screw a part of a complete revolution so the grounding pin rotates freely but does not completely separate from the chuck.

8. Remove the cryostage using the **1.5 metric allen wrench** to loosen the two set screws.

9. Wrap the Cryostage carefully in foil and store in the cryobox.

10. Remove the Cryostage chuck from the stage by loosening the two bolts with a **4 metric allen wrench** (see Sec. V:A 6), holding the two bolts ready.

11. Wrap the Cryostage chuck carefully in foil and store it in the cryobox.

12. Locate the standard stage that you had wrapped in foil and stored in the cryobox. Mount the standard stage into the microscope using all four bolts and the **4 metric allen wrench** (see Sec. V:A 5).

13. Insert the brass grounding pin (Fig. 10b). Confirm that there is good contact between the stage and ground using a **multimeter** by measuring the resistance between the stage and ground.

14. Gently close the FEM chamber door and pump the chamber.

15. Organize and place in appropriate bags all of the cryo sample holders and tools, and neatly place everything related to the Cryostage back into the cryobox. Place the cryobox in its space on the wooden shelf.

16. Confirm that a sufficiently low vacuum in the chamber has been reached.

ICAL USERS GUIDELINES

1. ICAL is a multi-user facility. Personal belongings (including samples and data) left unattended are subject to loss, disposal, or erasure.

2. **All equipment is to be run by qualified ICAL personnel or Users who have received formal training** (short course) from qualified ICAL personnel. *(Users may not train other users. Students may not train other students.)*

3. **All MSU Users must provide a department and Banner number** for every visit to ICAL. This information is to be entered in the appropriate instrument log book. For other billing arrangements please talk to ICAL personnel.

4. ICAL is a busy place and the equipment can be in great demand. If you sign up for time and are more than 15 minutes late, you may lose your time slot. **Please call or let us know if you are going to be late or cancel an appointment.**

5. Please treat the equipment and tools with respect. Do not use our nice delicate tweezers as scraping tools or pliers. Put things back where you found them.

6. Drinks and food should be consumed in the office area only.

7. Appropriate safety attire is required when handling chemicals, etc.

Questions or comments should be directed to ICAL Personnel
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406-994-4199, 406-994-4190
ical@sympa.montana.edu