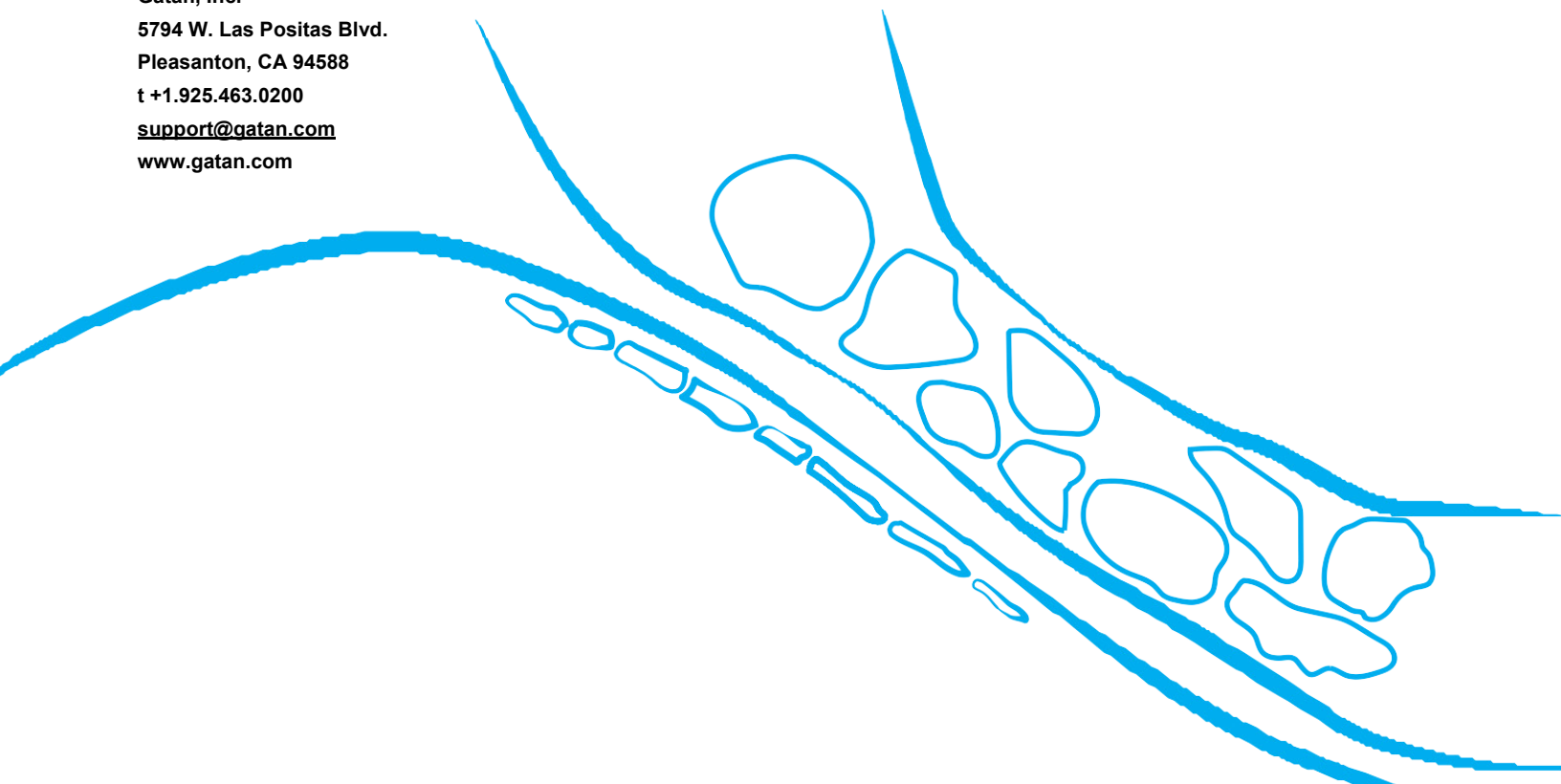




Single Tilt Liquid Nitrogen Cryo Transfer Holder Model 626

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Gatan, Inc.
5794 W. Las Positas Blvd.
Pleasanton, CA 94588
t +1.925.463.0200
support@gatan.com
www.gatan.com



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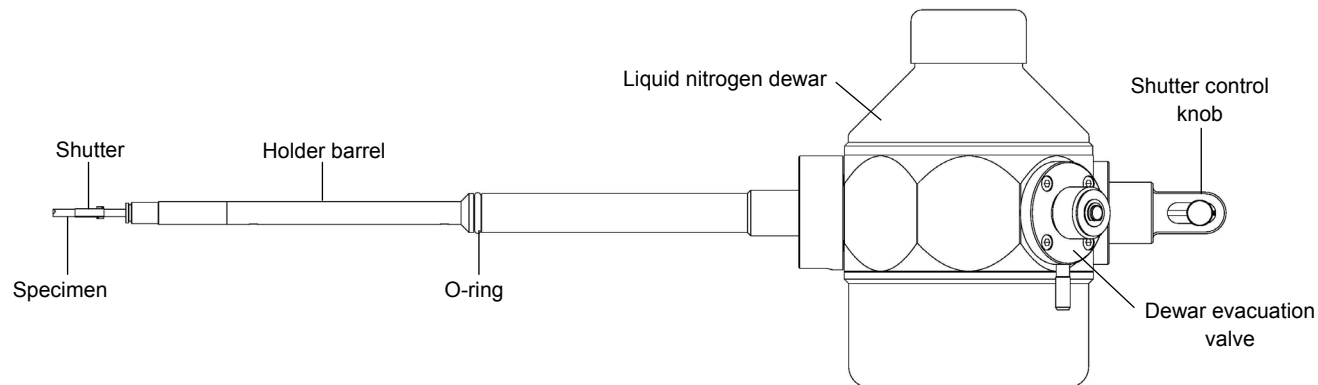
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1 General description

1.1 626 Specimen holder

The 626 Single Tilt Liquid Nitrogen Cryo Transfer Holder is designed for low temperature transfer of frozen hydrated specimens for cryo electron microscopy. There are two versions of the holder: $\pm 60^\circ$ and $\pm 70^\circ$. The $\pm 70^\circ$ version uses a low profile Clipping™ and provides a larger field of view at maximum tilt than the $\pm 60^\circ$ version of the holder. Ultimately, the maximum achievable tilt for either version of the holder is determined by the objective lens pole piece spacing of the electron microscope and any in-lens accessories.

Figure 1: 626 specimen holder



A conductive copper rod located within the holder barrel connects to a liquid nitrogen dewar that maintains the temperature of the specimen holder below -170°C . The distal end of the conductive copper rod has a recess to contain the frozen hydrated specimen. A silicon diode sensor is located near the specimen and the temperature is measured using the Model 1905 Temperature Controller. During use, the complete tip of the holder is cooled. A heater, which is mounted along the conductive copper rod, provides temperature control and rapid warm-up of the specimen to ambient temperature at the end of a cryo session.

A shutter mechanism surrounds the specimen area and prevents frost from forming on the specimen during transfer of the holder from the workstation to the microscope. The shutter can be opened or closed using the shutter control knob located at the back of the dewar. A molecular sieve within the vacuum space of the dewar acts as a sorption pump and prevents rapid boil-off of the liquid nitrogen within the dewar. Small quantities of water vapor adsorb onto the shutter actuator rod each time the shutter is actuated. Eventually the molecular sieve becomes saturated, which leads to degradation of the vacuum space within the dewar.

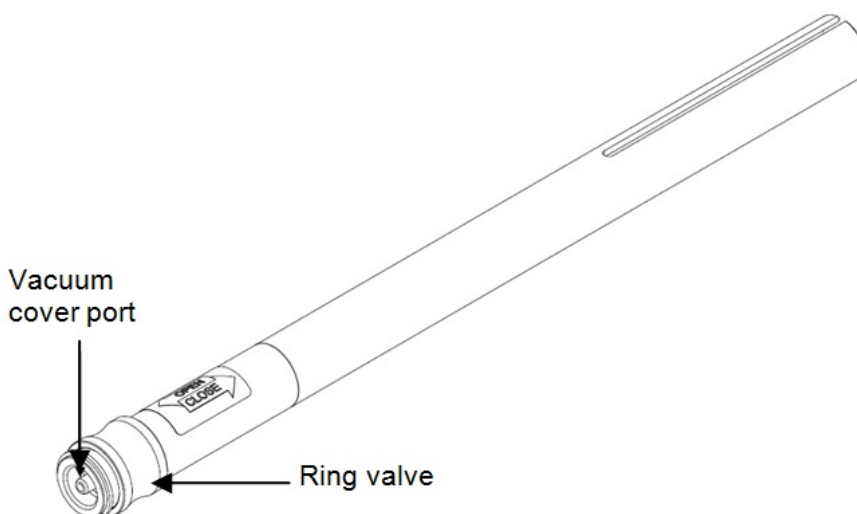
Single Tilt Liquid Nitrogen Cryo Transfer Holder
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The molecular sieve is regenerated by adjusting the temperature of the holder's built-in heater with the Model 1905 Temperature Controller while the vacuum space of the dewar is evacuated using the Model 655 Turbo Pumping Station.

1.2 Vacuum cover assembly

The holder is shipped with a protective vacuum cover assembly. This vacuum cover can be used to safely store the barrel of the holder under vacuum when the holder is not being used.

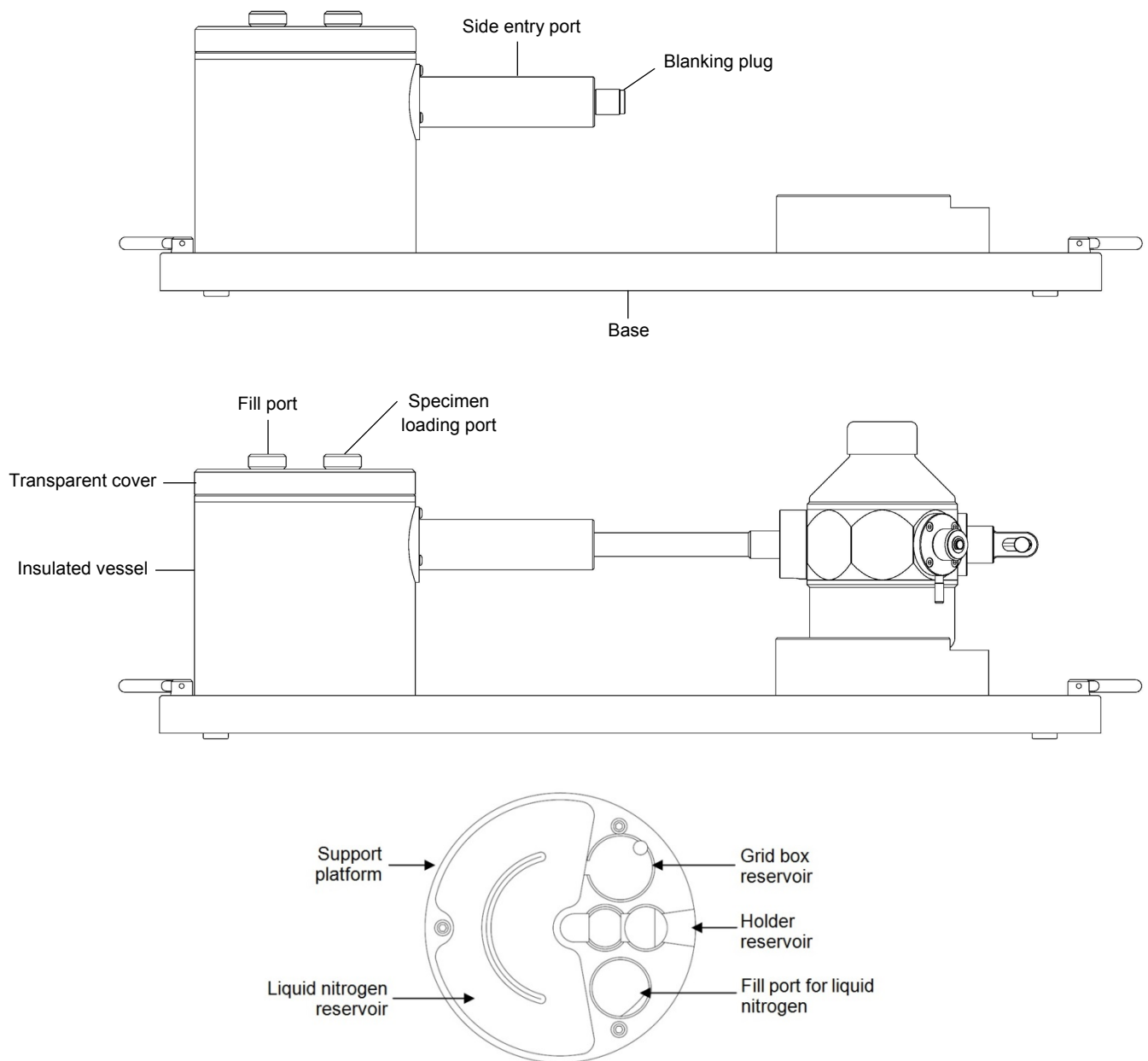
Figure 2: *Vacuum cover assembly*



1.3 Workstation

The Workstation consists of a Styrofoam insulated vessel with a side entry port, and a stand which aligns the specimen holder to the support platform located within the insulated vessel. The support platform supports the specimen tip during specimen loading and also includes two reservoirs; one to hold liquid nitrogen for precooling the specimen loading tools and one to contain the specimen grid box. During use, the insulated vessel is partially filled with liquid nitrogen to cool the specimen tip and surround the specimen with a protective atmosphere of cool, dry nitrogen gas.

Figure 3: *Workstation*



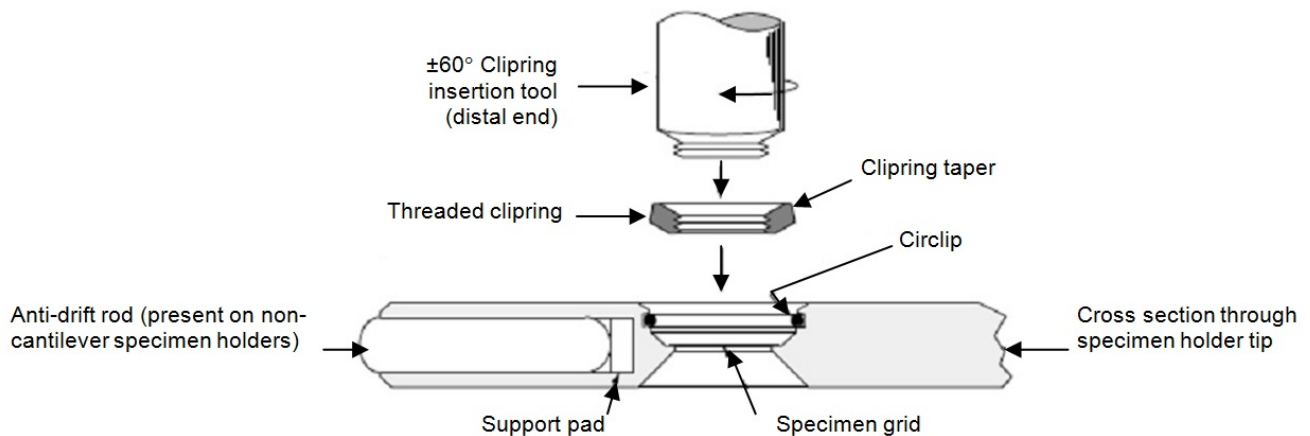
1.4 ± 60° Clipping specimen clamping system

The ±60° Clipping is a tapered metal ring that screws onto the distal end of the Clipping insertion tool. The Clipping insertion tool consists of a rod with a threaded extension. To secure the specimen grid, the ring is gently pressed into a circlip set within the tip of the specimen holder. The circlip squeezes the taper of the Clipping to create a gentle downward force that clamps the specimen grid in place. The Clipping is released by rotating the insertion tool counter-clockwise. Pressing the precooled proximal end of the insertion tool vertically downward on the upper surface of the Clipping ensures that it is properly seated within the specimen grid recess.

Figure 4: ±60° Clipping specimen clamping system

Important: It is helpful to practice inserting and removing the Clipping at room temperature to develop the skills required to handle these small tools.

Important: Always precool the Clipping and the Clipping insertion tool in liquid nitrogen prior to securing the frozen hydrated specimen grid.



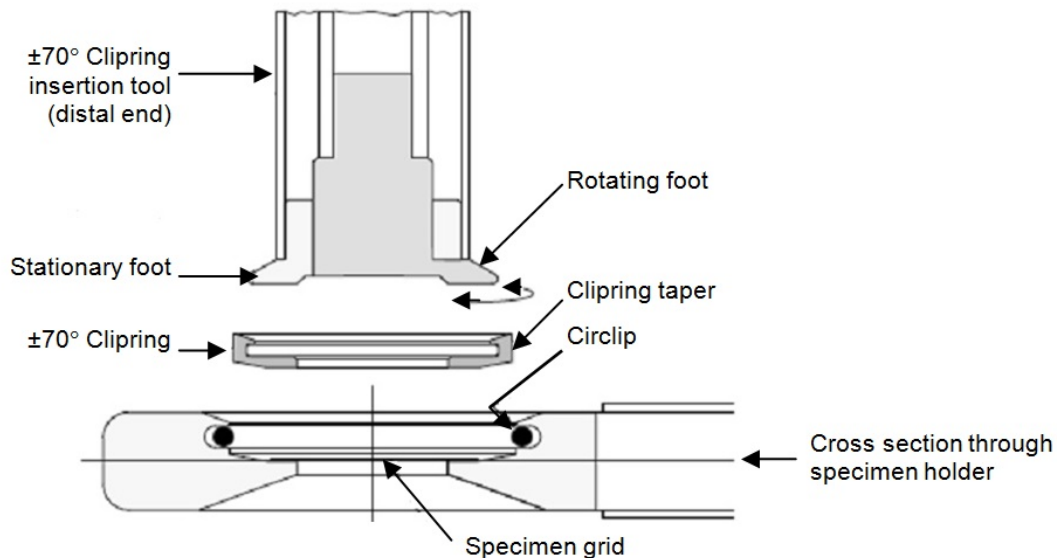
1.5 ± 70° Clipping specimen clamping system

The ±70° Clipping is a tapered metal ring that connects to the distal end of the Clipping insertion tool. The Clipping insertion tool consists of rod with a stationary and rotating foot at the distal end, and a rotating knurled knob at the proximal end. To secure the specimen grid, the ring is gently pressed into a circlip set within the tip of the specimen holder. The circlip squeezes the taper of the Clipping to create a gentle downward force that clamps the specimen grid in place. Rotating the knurled knob of the insertion tool releases the Clipping. Pressing the precooled proximal end of the insertion tool vertically downward on the upper surface of the Clipping ensures that it is properly seated within the specimen grid recess.

Figure 5: ±70° Clipping specimen clamping system

Important: It is helpful to practice inserting and removing the Clipping at room temperature to develop the skills required to handle these small tools.

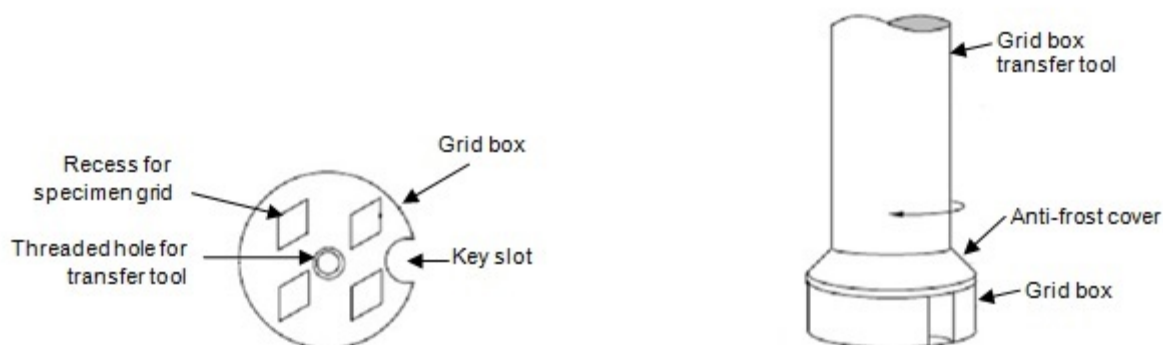
Important: Always precool the Clipping and the Clipping insertion tool in liquid nitrogen prior to securing the frozen hydrated specimen grid.



1.6 Specimen grid holder and grid box

The specimen grid box transfer tool is used to move the grid box from a liquid nitrogen storage dewar to the workstation. The transfer tool has a threaded flange at one end to secure the grid box. The anti-frost cover of the transfer tool provides an airtight seal to prevent frost from forming on the frozen hydrated specimen during transfers. A small key slot on the base of the grid box connects to a grid box reservoir within the support platform of the workstation and prevents rotation of the grid box when the transfer tool is engaged or disengaged.

Figure 6: Specimen grid holder and grid box transfer tool

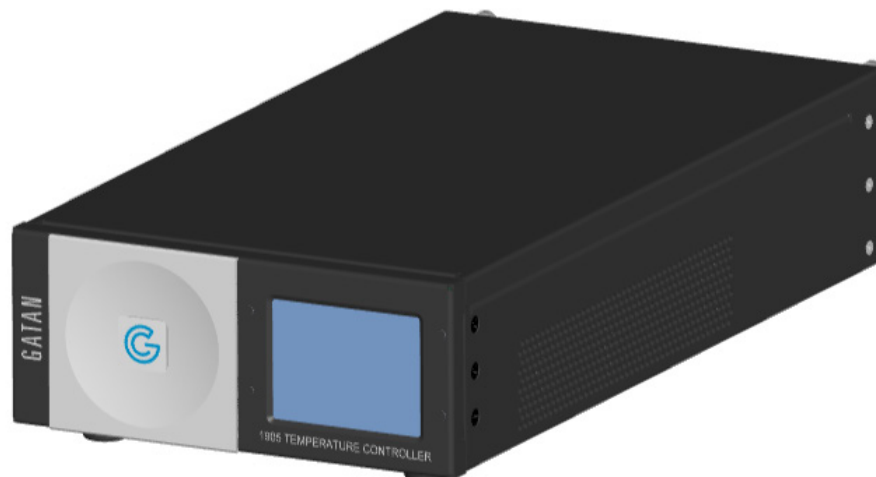


1.7 Model 1905 Temperature Controller

The Model 1905 Temperature Controller monitors specimen temperature, and displays all menu functions for Gatan heating, cooling and cryo transfer holders. The controller has multiple-functions and all are controlled by using the GUI on the front panel. These functions are also accessible through the Ethernet port on the rear panel.

Figure 7: Model 1905 Temperature Controller

Important: Refer to the 1905 Temperature Controller User Manual for full operating instructions.



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1.8 Faraday cup (optional)

To facilitate quantitative X-ray analysis, the holder can be ordered with a small Faraday cup with an entrance hole located between the specimen and the distal end of the holder tip. The tip of the holder is electrically isolated from the microscope and is connected to a miniature BNC connector mounted on the outside of the specimen rod. The beam current entering the Faraday cup is measured by plugging a picoammeter into the BNC connector. A grounding plug is provided to ground the specimen tip to the microscope when the Faraday current is not being measured.

Important: The grounding plug must be installed for the holder heating functions to operate normally. The grounding plug should only be removed when checking beam current.

1.9 Faraday cup adjustment bracket

In order to locate the electron beam into the Faraday cup, the specimen must first be centered (geometric center of the microscope goniometer). This may be facilitated by working at a low magnification for the initial set up for locating the Faraday cup. The next series of steps change depending upon what make of TEM is being used.

Hitachi or FEI TEM

The specimen holder should be withdrawn and the Faraday bracket adjustment tool placed in the space between the specimen holder dewar flange and the face of the goniometer. The IN/OUT adjustment knob should then be rotated clockwise until the measured current reaches its peak.

JEOL, Zeiss or Topcon TEM with a left hand stage drive

The Faraday bracket adjustment tool can be omitted and the stage drive can be used to align the electron probe into the faraday cage until the measured current reaches its peak.

JEOL TEM without a left hand stage drive

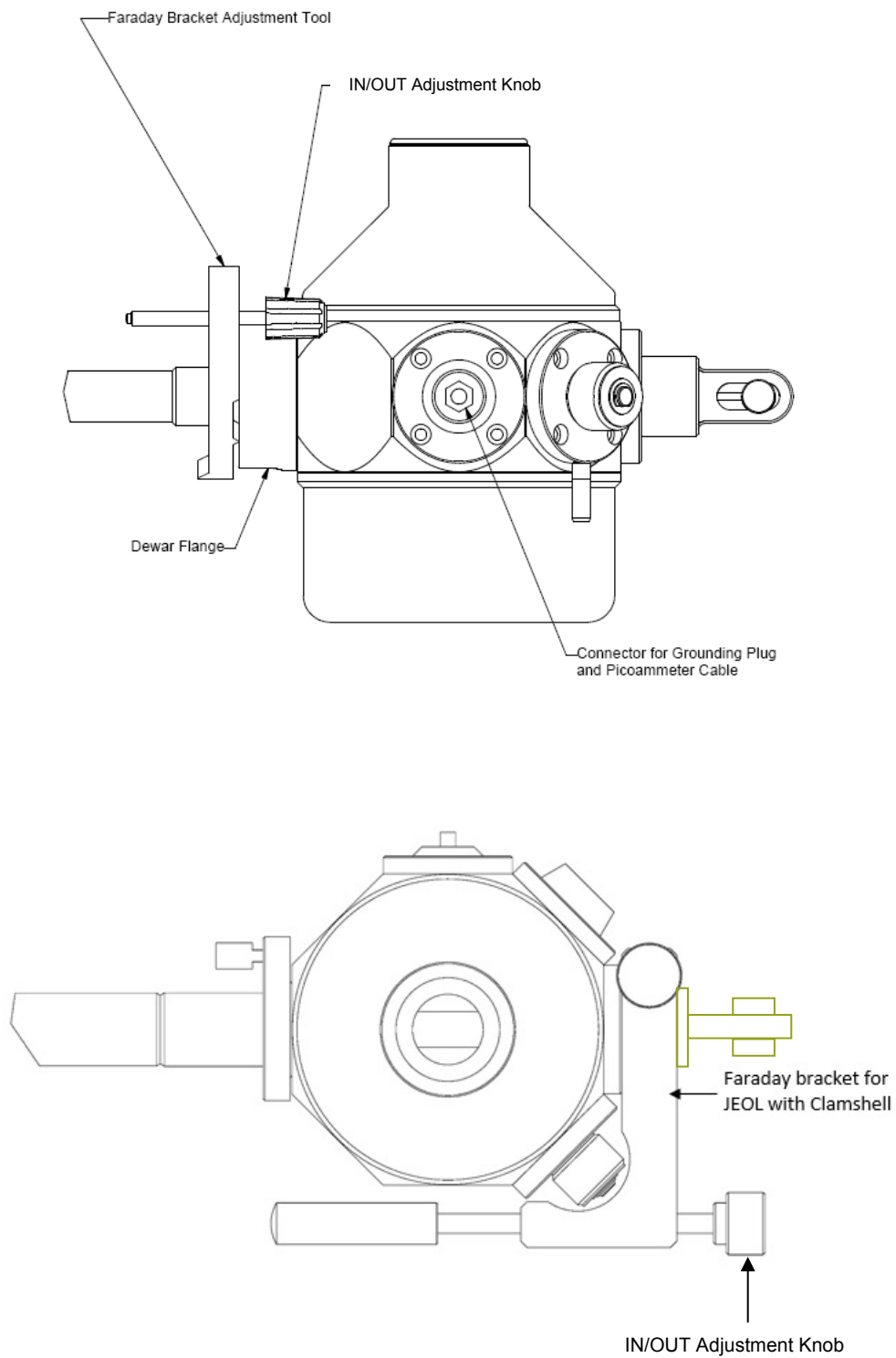
The specimen holder should be withdrawn and the Faraday bracket adjustment tool placed in the space between the specimen holder dewar flange and the face of the goniometer (as shown in figure 8). The adjustment tool should then be operated until the probe is observed to pass through a 250 μ m locating hole next to the Faraday cup. At this point the in-out adjustment knob should be rotated about 1/2 turn clockwise to move the beam into the Faraday cup.

JEOL TEM with no left hand stage drive and clamshell

A TEM with clamshell has a flexible rubber gasket around the holder opening so there is no solid material to contact with the standard adjustment bracket. The bracket supplied with these holders clamps over the shutter actuator at the rear of the dewar and provides a longer foot to contact against the solid face of the clamshell.

In all TEMs, small adjustments of the IN/OUT knob may be required to maximize or minimize the measured current. Note that the measured current may be larger when the electron beam does not enter the Faraday cup. This is because secondary electrons leaving the specimen tip may be scattered back onto the tip from surrounding surfaces.

Figure 8: *Faraday cup adjustment bracket*



2 Operating instructions for cryo electron microscopy

2.1 Equipment needed:

- Model 626 Single Tilt Liquid Nitrogen Cryo Transfer Holder
- Model 1905 Temperature Controller
- Model 655 Turbo pumping station

IMPORTANT: To ensure ultimate performance, the holder must be evacuated and heated with the bakeout cycle for a minimum of two hours (preferably overnight) before use. A two hour bakeout cycle is a conservative estimate for regenerating the molecular sieve within the vacuum space of the dewar. In the event that the molecular sieve becomes heavily contaminated, the duration of the bakeout cycle may need to be extended to twenty four hours or longer.

IMPORTANT: The dewar evacuation valve should only be opened when the valve is connected to the Model 655 Turbo Pumping Station.

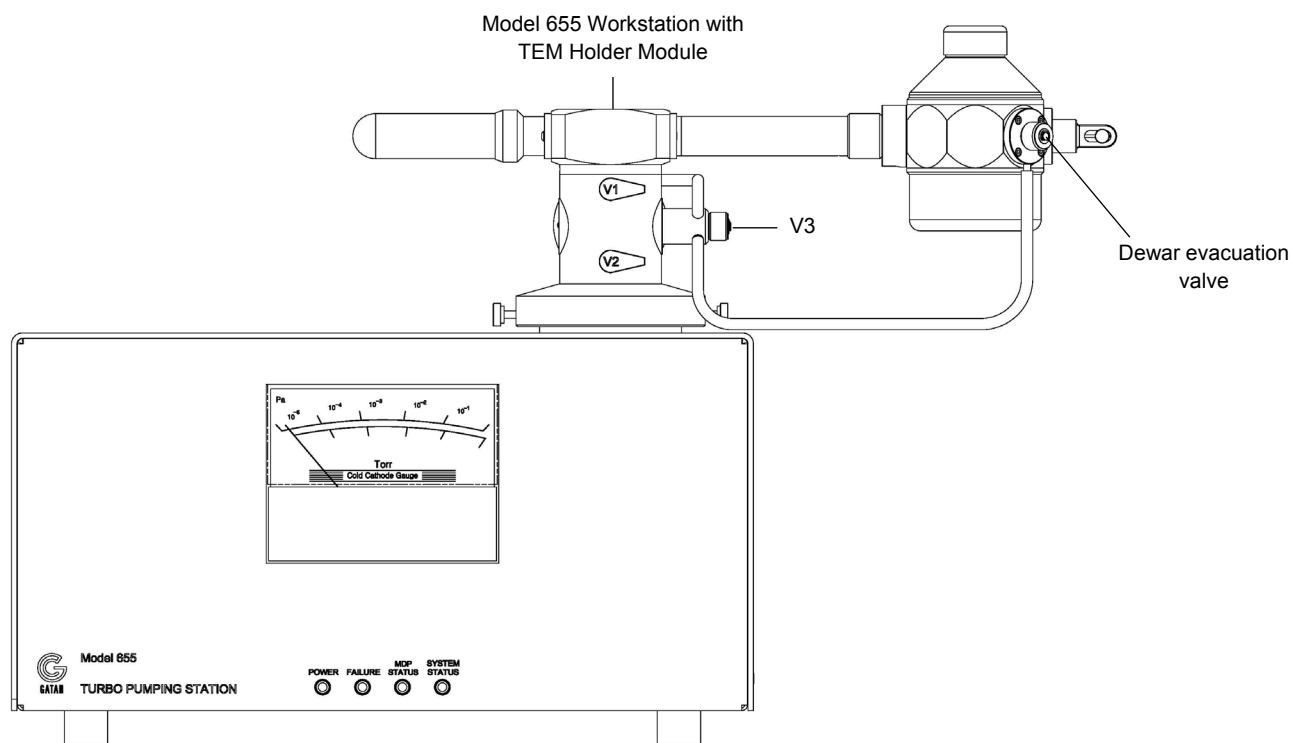
IMPORTANT: Use the correct cable to connect the holder to the Model 1905 Temperature Controller. Ensure that the correct Product is selected on the front panel GUI. Any holder that has a 5-pin connector is a type 6 or 626.6. Any holder that has a 7-pin connector is a type 5 or 626.5.

IMPORTANT: The Model 655 Turbo Pumping Station is specifically designed for the purpose of maintaining and protecting Gatan TEM specimen holders. Although other oil-free pumping systems may be available, it is recommended that the Model 655 Turbo Pumping Station is used for the following procedure to ensure the expected result.

2.2 Perform bakeout cycle for the holder

1. To perform the bakeout cycle for the molecular sieve, refer to the Model 655 Turbo Pumping Station and the Model 1905 Temperature Controller manuals

Figure 9: Model 655 Turbo Pumping Station with holder



2.3 Prepare the electron microscope

1. Follow the microscope manufacturer's instructions for alignment of the optical system for low electron dose imaging
2. Cool the liquid nitrogen anti-contaminator on the microscope and monitor the vacuum until the system reaches operating vacuum

2.4 Remove the vacuum cover assembly from the holder

1. Hold the holder in one hand and the vacuum cover in the other
2. Carefully grasp the vacuum cover ring valve and slide it to the 'open' position to vent the vacuum inside the cover
3. Gently and carefully remove the vacuum cover
 - a. It is important to keep the vacuum cover parallel with the holder barrel until the specimen tip is visible to prevent damage to the holder

2.5 Insert the holder into the workstation

Important: The holder may also be precooled in the microscope or the Model 655 Turbo Pumping Station and then transferred to the precooled workstation.

Important: The outside surface of the dewar will be cold when first filled with liquid nitrogen, but it will warm to room temperature once the sorption pump (molecular sieve) activates. Turn the knurled knob anti-clockwise and rotate it so that the holder barrel clip is visible

1. Remove the blanking plug from the side entry port and carefully insert the holder into the workstation
2. Slowly add liquid nitrogen to the dewar of the holder
 - a. There will be a rapid boil off of liquid nitrogen because the dewar is at ambient temperature
 - b. Wait for the initial boiling to subside and then fill the dewar to the half fill point
3. Monitor the temperature of the holder with the Model 1905 Temperature Controller

2.6 Transfer the specimen grid box into the workstation reservoir

Important: Failure to precool all tools (tweezers, transfer tools, etc.) that contact the grid or the grid box can result in devitrification of the frozen hydrated specimen. Precooling is accomplished by placing the working end of the tool in a small bath of liquid nitrogen for approximately one minute. The reservoir within the support platform can be used for this purpose.

1. Transfer the grid box containing the frozen hydrated specimen grids into the workstation
 - a. Remove the plastic caps on the transparent cover
 - b. Precool the grid box transfer tool
 - c. Affix the specimen grid box to the end of the precooled transfer tool under liquid nitrogen to protect the frozen hydrated specimen grids
 - d. Quickly transfer the specimen grid box into the workstation and locate it in the keyed grid box reservoir or within the liquid nitrogen reservoir of the support platform
 - i. Quickly replace the transparent cover if it was removed to perform this step

2.7 Load the frozen hydrated specimen grid into the holder

Important: The holder should be at operating temperature before proceeding.

Important: To protect the frozen hydrated specimen grid during transfer from the grid box to the specimen holder, it is helpful to work through the access ports of the transparent cover.

1. There are two common methods for loading the frozen hydrated specimen grid into the holder
 - a. Loading the specimen grid in cold nitrogen gas (produced by the evaporation of liquid nitrogen in the workstation)
 - i. Maintain the liquid nitrogen level in the workstation just below the side entry port
 - ii. Maintain the reservoir of liquid nitrogen within the support platform of the workstation
 - iii. Maintain the level of liquid nitrogen in the dewar at the half fill point
 - iv. The transparent cover is easily rotated such that the holes in the cover can be positioned to allow access to all areas of the support platform while loading the specimen grid
 - v. Precool the Clipping insertion tool, with Clipping attached, in liquid nitrogen
 - vi. Open the holder shutter to expose the specimen grid recess

- vii. Place the frozen hydrated specimen grid within the grid recess using precooled tweezers
 1. Work very close to the metal surface of the workstation platform (approximately no higher than one centimeter) when moving the grid from the grid box to the tip of the holder as this area is cold and the grid will be protected from devitrification
 2. The specimen grid should be centered within the specimen recess to maximize the available viewing area
- viii. Holding the Clipping insertion tool perpendicular to the holder tip, insert the Clipping and gently snap into place
- ix. Release the Clipping tool
- x. Close the holder shutter
- xi. Precool the proximal (rounded) end of the Clipping tool in liquid nitrogen for approximately 1 minute
- xii. Open the shutter and gently press the end of the tool vertically on the Clipping
 1. Visually inspect the Clipping to ensure that it is uniformly seated within the specimen grid recess
- xiii. Close the shutter
- xiv. Add a small quantity of liquid nitrogen to the workstation and to the holder dewar to maintain temperature
- xv. Check the temperature of the holder using the 1905 Temperature Controller
- xvi. Switch the controller off and disconnect the cable from the holder
- xvii. The transparent cover should be in position to protect the workstation environment
- xviii. Protect the remaining frozen hydrated grids by placing them into a liquid nitrogen storage vessel

b. Loading the specimen grid under liquid nitrogen

Important: Loading a frozen hydrated grid under liquid nitrogen is challenging. To help prevent liquid nitrogen from entering the side entry port of the workstation, place an object under the proximal end of the workstation to raise it approximately 1 inch in height. Work with the minimum amount of liquid nitrogen to just cover the grid area at the tip of the holder. Do not breathe into this area during loading as this will cause the frozen hydrated grid to devitrify.

- i. Maintain the liquid nitrogen level in the workstation just below the side entry port while keeping the tip of the holder immersed in liquid nitrogen
- ii. Maintain the reservoir of liquid nitrogen within the support platform of the workstation
- iii. Maintain the level of liquid nitrogen in the dewar at the half fill point
- iv. The transparent cover is easily rotated such that the holes in the cover can be positioned to allow access to all areas of the support platform while loading the specimen grid
- v. Precool the Clipping insertion tool, with Clipping attached, in liquid nitrogen
- vi. Open the holder shutter to expose the specimen grid recess
- vii. Place the frozen hydrated specimen grid within the recess using precooled tweezers
 1. Work very close to the metal surface of the workstation platform (approximately no higher than 1 cm) when moving the grid from the grid box to the tip of the holder as this area is cold and the grid will be protected from devitrification
 2. The specimen grid should be centered within the specimen recess to maximize the available viewing area
- viii. Insert the Clipping and gently snap into place
 1. Be sure to align the Clipping insertion tool taking into account the slight angle of the workstation and holder tip
- ix. Release the Clipping tool
- x. Close the holder shutter

- xii. Precool the proximal (rounded) end of the Clipping tool in liquid nitrogen for approximately 1 minute
- xiii. Open the shutter and gently press the end of the tool vertically on the Clipping
 1. Visually inspect the Clipping to ensure that it is uniformly seated within the specimen grid recess
- xiv. Close the shutter
- xv. Add a small quantity of liquid nitrogen to the workstation and to the holder dewar to maintain cryo temperature
- xvi. Check the temperature of the holder using the 1905 Temperature Controller and then switch the controller off and disconnect the cable from the holder
- xvii. The transparent cover should be in position to protect the workstation environment
- xviii. Protect the remaining frozen hydrated grids by placing them into a liquid nitrogen storage vessel

2.8 Insert the holder into the electron microscope

Important: Liquid nitrogen will spill out of the dewar when the holder is inserted into the specimen holder airlock of the microscope. Protect the microscope column and viewing port, and prepare a receptacle to catch this residual liquid nitrogen, whenever the holder is inserted or removed or whenever liquid nitrogen is added to the dewar.

1. Prepare the microscope specimen holder airlock to receive the specimen holder
 - a. If desired, follow the microscope manufacturer's instructions for pre-tilting the goniometer to minimize spillage of liquid nitrogen from the dewar during the insertion process
2. Pre-pump the specimen airlock on the microscope and wait for all pre-pumping indicators on the microscope to complete before proceeding
3. Check that the holder shutter is closed
4. Switch the 1905 Temperature Controller off and disconnect the cable from the holder
5. Minimize the transfer distance of the holder with respect to the microscope airlock by placing the workstation on the microscope console
6. Carefully and quickly insert the holder into the microscope specimen holder airlock
7. Wait for the microscope pre-pumping indicators to indicate that it is safe to proceed before inserting the holder fully into the microscope column
8. Fill the holder dewar with liquid nitrogen (do not overfill)
9. Insert the debubbler tool to stabilize the liquid nitrogen within the dewar
 - a. The debubbler tool will cause the liquid nitrogen to become still (i.e. no bubbling)
 - b. **Important:** Be sure to aim the exit port of the debubbler tool away from people or sensitive equipment
 - c. View the surface of the liquid nitrogen in the dewar
 - i. A small LED flashlight is useful to detect bubbling within the dewar
10. Place the cap on the dewar of the holder
11. Refill the microscope anti-contamination device with liquid nitrogen
12. Check the temperature of the holder using the 1905 Temperature Controller and then switch the controller off and disconnect the cable from the holder while collecting data on the microscope
 - a. Disconnecting the cable prevents transmission of vibration to the holder, which would impede imaging at high resolution
 - b. Secure the cable to the microscope column to minimize vibrations to the holder whenever it is desired to constantly monitor the temperature or add a small quantity of heat to the holder tip

13. Wait for the microscope vacuum to recover to its required operating range before operating the electron gun
14. Refill the holder dewar as needed while working
 - a. A full charge of liquid nitrogen should last approximately 4 hours once the dewar is at minimum operating temperature

2.9 Remove the holder from the electron microscope to replace the specimen grid

Important: Portions of the following instructions are abridged. Refer to previous sections of the operating instructions for detailed explanation.

1. Precool the workstation and insert the specimen grid box
2. If the microscope is in a pumping cycle, wait for all pre-pumping indicators to complete before proceeding
3. Protect the electron gun
4. Close the shutter
5. Remove the holder from the specimen holder airlock following the microscope manufacturer's instructions
 - a. If desired, follow the microscope manufacturer's instructions for pre-tilting the goniometer to minimize spillage of liquid nitrogen from the dewar during the insertion process
6. Insert the holder into the precooled workstation
7. Open the shutter
8. Remove the Clipping
 - a. Be sure to align the Clipping insertion tool perpendicular to the long axis of the holder barrel
 - b. Take care that the grid being removed from the holder is safely set aside so that it will not be accidentally carried back into the electron microscope
9. Insert a new frozen hydrated grid
10. Insert the Clipping to secure the new grid
11. Close the shutter
12. Insert the holder into the microscope holder airlock following the manufacturer's instructions

2.10 Remove the holder from the electron microscope at the end of a cryo session

Important: Portions of the following instructions are abridged. Refer to previous sections of the operating instructions for detailed explanation.

1. If the microscope is in a pumping cycle, wait for all pre-pumping indicators to complete before proceeding
2. Protect the electron gun
3. Remove the holder from the specimen holder airlock following the microscope manufacturer's instructions
4. Empty any residual liquid nitrogen from the holder dewar
5. Remove the specimen
6. Insert the holder into the appropriate specimen holder module on the 655 Turbo Pumping Station
7. Follow instructions to evacuate the specimen holder module on the 655 Turbo Pumping Station
8. Connect the 1905 Temperature Controller and activate the warm-up cycle

9. Perform a bakeout cycle on the holder when the holder reaches ambient temperature
 - a. Refer to instruction manuals for the Model 1905 Temperature Controller and the Model 655 Turbo Pumping Station

2.11 Storing the holder after use

Important: When the holder is not being used on the microscope, it should be stored under vacuum using the vacuum cover or the appropriate port on the Model 655 Turbo Pumping Station.

1. Protecting the holder using the vacuum cover
 - a. To protect the holder when it is not being used, insert the holder barrel into the vacuum cover
 - i. Keep the cover parallel with the holder barrel to avoid damage
 - ii. A slight resistance may be felt as the vacuum cover slides over the holder barrel O-ring
 - b. To evacuate the space inside the vacuum cover
 - i. Connect the vacuum cover port to valve 'V3' on the Model 655 Turbo Pumping Station using the tubing supplied
 1. Refer to the Model 655 Turbo Pumping Station instruction manual
 - ii. Check that the ring valve on the vacuum cover is in the 'open' position
 - iii. With the Model 655 Turbo Pumping Station operating at working vacuum, open valve V3 for a few minutes and then slide the ring valve to the 'close' position
 - iv. Close valve V3 and disconnect the tubing from the vacuum cover port
2. Protecting the holder using the Model 655 Turbo Pumping Station
 - a. Store the holder on the appropriate port of the Model 655 Turbo Pumping Station
 - b. Refer to the Model 655 Turbo Pumping Station instruction manual

3 Helpful techniques to obtain best results

3.1 Maintain the molecular sieve

- a. Indicators of an unsuitable dewar vacuum will result when liquid nitrogen is added to the dewar and the follow occurs
 - i. The outside of the dewar becomes coated with frost
 - ii. The temperature of the holder cannot reach the operating temperature of less than -170 °C
- b. Perform a bakeout cycle for at least two hours (preferably overnight)

3.2 Manage drift

Important: Always verify that the drift performance of the room temperature single tilt holder provided with the microscope meets the manufacturer's specifications

- a. Drift can occur if the holder O-ring is not properly lubricated
 - i. Apply a light coating of vacuum grease to the O-ring as prescribed by the microscope manufacturer
- b. Seat the holder within the goniometer of the microscope
 - ii. Gently tapping on the back end of the dewar (towards the microscope column) once the temperature has stabilized will help seat contacting surfaces of the holder and microscope airlock components to help minimize drift
- c. Thermal instabilities in the microscope room
 - iii. Protect the holder from thermal instabilities and drafts

3.3 Manage vibration

- a. Vibration of the specimen can be caused by inadequate specimen clamping, which can result in ice crystallization on the specimen during data collection
 - iv. Test to ensure that the Clipping is properly securing the grid
 - 1. Place a TEM grid into the tip of the holder at ambient temperature
 - 2. Insert the Clipping and try to move the grid using the tips of a tweezer
 - a. The grid should not move but it may tear from the force of the tweezer trying to move the grid
 - b. If the grid moves freely, then the Clipping is not properly seated within the specimen grid recess of the holder
 - i. Replace the Clipping as it may be worn
 - ii. Contact Gatan customer service
- b. The cable for the 1905 Temperature Controller is connected to the holder
 - v. Disconnect the cable to minimize vibration to the holder when collecting data
- c. Liquid nitrogen within the dewar is bubbling
 - vi. Use the debubbling tool provided
 - vii. Replace existing liquid nitrogen with fresh liquid nitrogen
 - viii. Check for ice crystals in the dewar
 - 1. Ice crystals that form within the holder dewar serve as nucleation sites, which can cause the liquid nitrogen to bubble
 - a. If consistent bubbling is observed in the dewar, take a cotton swab and gently rub the spot generating the bubbles to dislodge any ice crystals that may be present, then use the debubbler tool to eject the crystals from the dewar

3.4 Maintain the level of liquid nitrogen

- a. Insert the proximal end of the Clipping tool into the dewar for a few seconds and then remove the tool
- b. Observe the condensation that forms on the tool to determine the liquid level within the dewar
- c. Add liquid nitrogen as needed to maintain operating temperature

3.5 Improve the microscope vacuum performance

- a. The TAC100 Anti-contaminator is designed to minimize contamination in the vicinity of the specimen within the microscope

4 Holder maintenance

4.1 Regenerating the Sorption Pump

Once the molecular sieve becomes fully saturated, the surface of the dewar will be cold to the touch; frost will form on the outside of the dewar during use and the holder will no longer maintain operating temperature. The molecular sieve is regenerated by running a bakeout cycle for two hours (preferably overnight) with the Gatan 1905 Temperature Controller while the dewar is evacuated using the Gatan 655 Turbo Pumping Station.

4.2 Replacing the anti-drift rod for non-cantilever holders

A broken anti-drift rod can be replaced by following instructions supplied with the replacement part.

4.3 Applying vacuum grease to the holder o-ring

A dry O-ring can cause specimen drift and a vacuum leak at the microscope holder airlock. Clean the O-ring and apply a light coating of high vacuum grease as prescribed by the microscope manufacturer.

4.4 Cleaning the specimen rod

Remove the specimen holder O-rings using a wooden toothpick or other non-sharp object.

Important: Care must be taken to avoid scratching the sealing groove surfaces while removing the O-ring. Place a graduated cylinder in an ultrasonic cleaning bath and insert the holder barrel into the graduated cylinder.

Important: The cylinder needs to be longer than the holder barrel to keep the specimen tip from contacting the bottom. Fill the graduated cylinder with enough 100% ethyl alcohol to just cover the region of the uppermost O-ring groove of the holder. Sonicate for about one minute. Air-dry the holder barrel and insert new O-rings. Apply a light coating of high vacuum grease to the O-rings as prescribed by the microscope manufacturer prior to using the holder.

5 Warnings

1. A detailed knowledge of the interior construction of the specimen holder and workstation is required for servicing
 - a. Contact Gatan customer service regarding specific operating issues with the holder or workstation
 - b. Do not perform any service operation on the holder or workstation as special tools are required to avoid damage
2. Do not tilt the holder beyond the limits allowed by the microscope manufacturer
 - a. Use caution when inserting the holder into a microscope with a narrow pole piece gap
 - i. Consult Gatan customer service if you are concerned about the specimen holder tilt limits in your microscope
3. Do not over-tighten the circumferential seal of the dewar evacuation valve
 - a. This valve does not require high compression to activate
4. Never vent the dewar to atmosphere
 - a. This dewar evacuation valve should **only** be opened when it is connected to a vacuum source
5. Never use a rotary pump to evacuate the dewar
 - a. Rotary pump oil will contaminate the molecular sieve and destroy its sorption capability leading to excessive boiling of liquid nitrogen in the dewar, increased vibration of the holder at low temperature and subsequent loss of resolution
 - i. Oil vapor traps do not effectively block the passage of oil to the dewar
 1. It is difficult to regenerate oil contaminated molecular sieve by heating since the oil simply migrates from hotter to cooler surfaces inside the dewar
 2. Oil contaminated molecular sieve will need to be replaced
 - a. Contact Gatan customer service
6. Do not put an extra heater or direct a heat gun into the holder dewar or the workstation to speed up the drying or heating process as there is a great danger that certain parts in the holder or workstation will become overheated causing damage
7. Do not use coolants other than liquid nitrogen
8. Do not subject the dewar to large mechanical shocks
9. Do not subject the holder barrel to excessive force
10. Do not operate the shutter knob any more than necessary
 - a. Each time the shutter is actuated, small quantities of water vapor adsorb onto the shutter actuator rod, eventually saturating the molecular sieve, which leads to degradation of the vacuum space within the dewar
11. Do not heat the dewar above 110°C
 - a. Use the 1905 Temperature Controller as specifically designed for the holder
 - b. Do not try to use the older style 613-0500 cold stage power supply with the 626 specimen holder since this controller can deliver heater currents in excess of 750mA

6 Optional accessories

626.07000 Cryostorage System (100 specimens)

655 Turbo Pumping Station

TAC100 TAC100 Series Anti-contaminator

7 Spares

626.03141	Specimen grid holder x5 (lid not included)
626.03151	Specimen grid holder transfer tool x5
626.04046	Clipring (x5) - aluminum
626.04047	Clipring (x5) - phosphor bronze
626.04051	Clipring, $\pm 60^\circ$ 200-300 micron SiN grid
626.04052	Clipring, $\pm 70^\circ$ 300 micron SiN grid
626.04053	Clipring, $\pm 70^\circ$ 100-200 micron SiN grid
626.04054	Clipring, $\pm 70^\circ$ 50 micron SiN grid
626.04190	Clipring insertion tool
626.07040	Specimen grid holder top cover x5
626.07101	Cryo-storage bottle with dewar hook x5
626.13361	$\pm 70^\circ$ Clipring
626.53204	$\pm 70^\circ$ Clipring tool
626.54047	Carbon pack
1905.CBLC100	Temperature cable (ref. 1905.50670, Type 6)

8 Warranty

Gatan specimen holders are warranted per the terms and conditions outlined in the product warranty. Contact Gatan customer service at <http://www.gatan.com/support>.

1. Examples of items not covered under warranty include
 - a. Damage caused by operating the holder beyond the tilt limit imposed by the microscope pole pieces
 - b. Replacement of a damaged anti-drift rod (non-cantilever holders)
 - c. Repair of a damaged specimen tip
 - d. Replacement of a lost Clipping