

Common Autoloader Standard Operating Procedure

version 1.0

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1. Purpose

- 1.1. To transfer clipped autogrids into a TFS autoloader cassette and use a NanoCab to transfer them into an autoloader system
- 1.2. To care for and maintain autoloader tools (autoloader cassettes, transfer stations, and NanoCabs)

2. Definitions:

- 2.1. An Autoloader system is a robotic system for loading cryo-EM grids into the microscope, in which the user inserts autogrids into a cassette that is loaded into the microscope using a retractable arm.
- 2.2. An Autogrid is a Cryo-EM grid secured into a C-clip using clip rings (and prepared by the user through “grid clipping”).
- 2.3. NanoCab is specially made vacuum insulated capsule that holds a cassette and docks with Autoloader.
- 2.4. Liquid Nitrogen (LN₂) is a cryogenic liquid stored under pressure.
- 2.5. Definition of terms for tools/equipment can be found in Figure 1.

3. Supplies & Equipment

- PPE (BSL-1)
 - Laboratory Coat
 - Nitrile Gloves
 - Goggles / Safety Glasses
 - Cryogenic Gloves
 - Face Mask
- Chemicals/Reagents
 - Liquid Nitrogen
- Table-top Transfer Station assembled with Covers, Metal Block, and Cassette Gripper Handle
- NanoCab and Lid
- Autogrid Cassette
- Autogrid Tweezers
- Cassette Tweezers
- Standard Fine-tip Tweezers
- Autogrid box(es)
 - Containing vitrified samples on clipped grids
- Autogrid Box Opening Tool
- Large Forceps
- Hair dryer or heat block (if needed to dry tools)

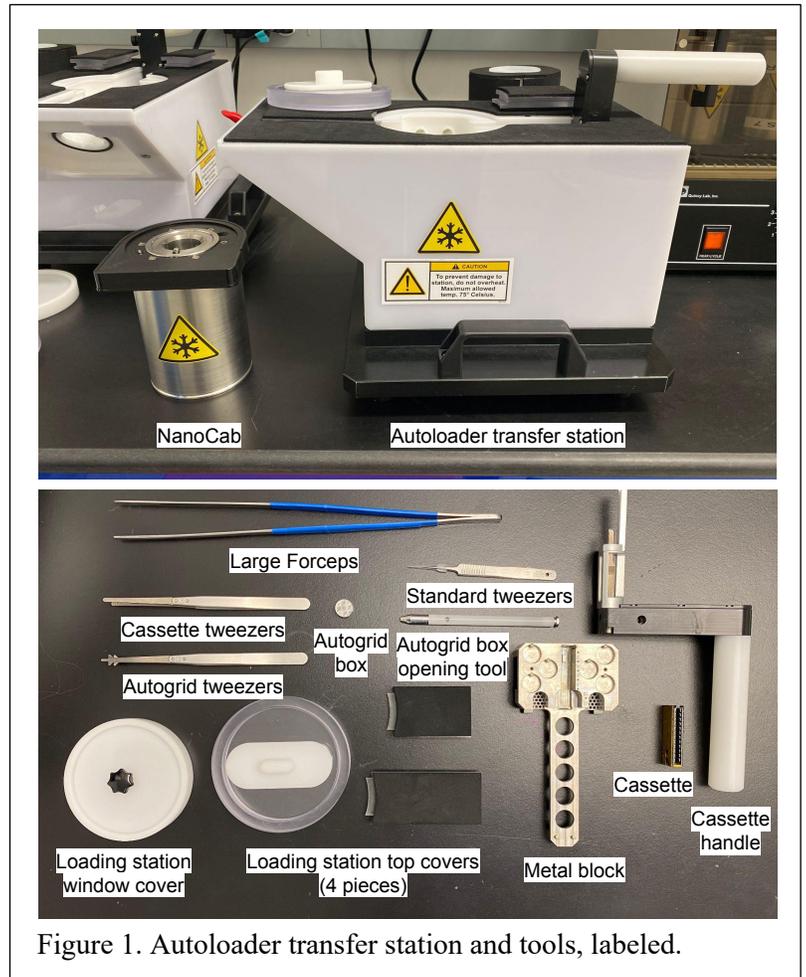


Figure 1. Autoloader transfer station and tools, labeled.

4. Procedure:

4.1. Prepare the Transfer Station

- 4.1.1. Gather the autoloader transfer station and tools (fig 1).
- 4.1.2. Assemble the transfer station with the loading station window cover, metal block and handle, and the four top covers. Make sure the station does not have any hairs, fibers, or dust.
- 4.1.3. Remove the two chamber plugs and white center cover (fig 2). Cool the transfer station with LN₂ without overfilling (fig 3).
 - 4.1.3.1. Initial fill should be performed from the top, through the opening in the clear cover.
 - 4.1.3.2. Remove and replace white cover with each pour.
 - 4.1.3.3. Do not fill higher than the top of the metal block (fig 3).
- 4.1.4. Once the LN₂ has finished bubbling, the temperature has stabilized.
- 4.1.5. Reattach the chamber plugs. The third outlet always remains unobstructed.

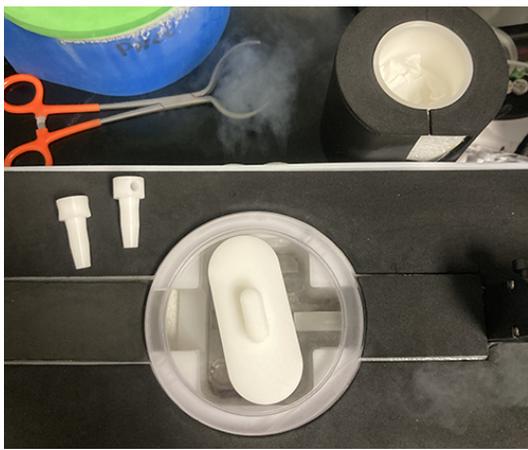


Figure 2. Top down view of a covered loading station. Two chamber plugs are removed for initial cooling. Funnel has paper towel or wipe to reduce ice contamination.



Figure 3. Red line shows the highest level to fill LN₂ in the loading station.

4.2. Cool the NanoCab

- 4.2.1. Retrieve the dry NanoCab, with lid, for the microscope you'd like to load (fig 4).
- 4.2.2. Cool the NanoCab with LN₂.
 - 4.2.2.1. Replace the lid with each pour to mitigate frost buildup.
 - 4.2.2.2. Be mindful not to overfill above the bottom of silver ring (fig 4).
- 4.2.3. When the LN₂ has stopped bubbling, the NanoCab is cooled.

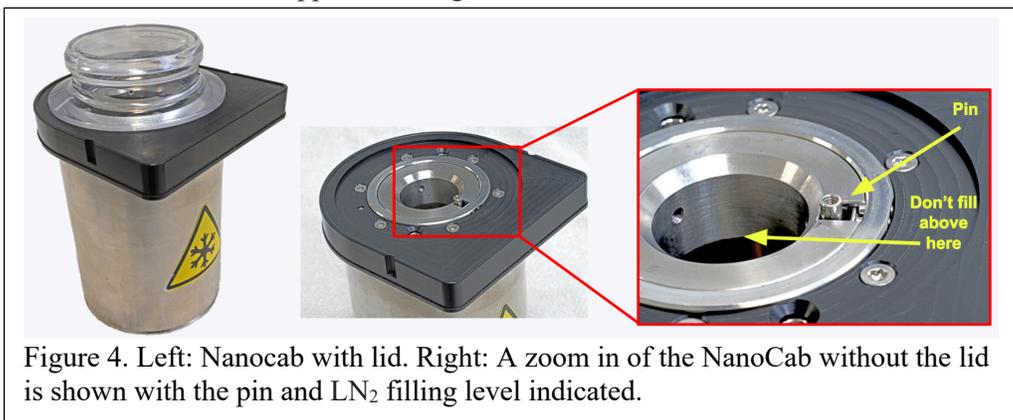


Figure 4. Left: Nanocab with lid. Right: A zoom in of the NanoCab without the lid is shown with the pin and LN₂ filling level indicated.

- 4.2.4. Verify the cassette lock pin (fig 4) is not stuck by pulling upward on the pin (it is spring loaded).
- 4.2.4.1. The pin's operation is essential for docking and undocking cassettes from the microscope. If the pin is stuck, notify center staff to get a different NanoCab until the defective one is repaired.

4.3. If Cassette is in the Microscope: Retrieve Cassette

- 4.3.1. Before docking the cold NanoCab to the Autoloader, check:
- 4.3.1.1. The microscope is not doing any operations.
 - 4.3.1.2. Column valves are closed.
 - 4.3.1.3. Temperature of all components is cryogenic.
 - 4.3.1.4. Position 1 (cross-grating) is loaded onto the stage or no grid is loaded.

4.3.2. Open the enclosure door and locate the autoloader docking stage (fig 5).

4.3.3. With the NanoCab label facing you, remove the cap and slide the NanoCab into Autoloader dock.

- 4.3.3.1.1. Krios: Push green dock Button (fig 6A).
- 4.3.3.1.2. Arctica/Glacios: Push touchscreen "Dock" button.
- 4.3.3.1.3. Any autoloader: Click "Load" on the microscope computer autoloader interface (fig 6B).

4.3.4. If docking button is not available, remove the NanoCab and check cassette lock pin (fig 4).

- 4.3.5. Wait for autoloader to complete the docking procedure. When complete you will see:
- 4.3.5.1. Krios: "NanoCab locked" light off, "cassette in loader" light on (fig 6A).
 - 4.3.5.2. Arctica/Glacios: "NanoCab can be removed" displayed on the screen (fig 6B).

4.3.5.3. Any autoloader:
Interface says "You can now remove the NanoCab" (fig 6C).

- 4.3.6. Remove NanoCab from Autoloader dock by sliding directly back off of the stage.
- 4.3.7. Visually confirm the cassette is in the NanoCab and replace cap.
- 4.3.8. Close enclosure.
- 4.3.9. Proceed to step 4.5.

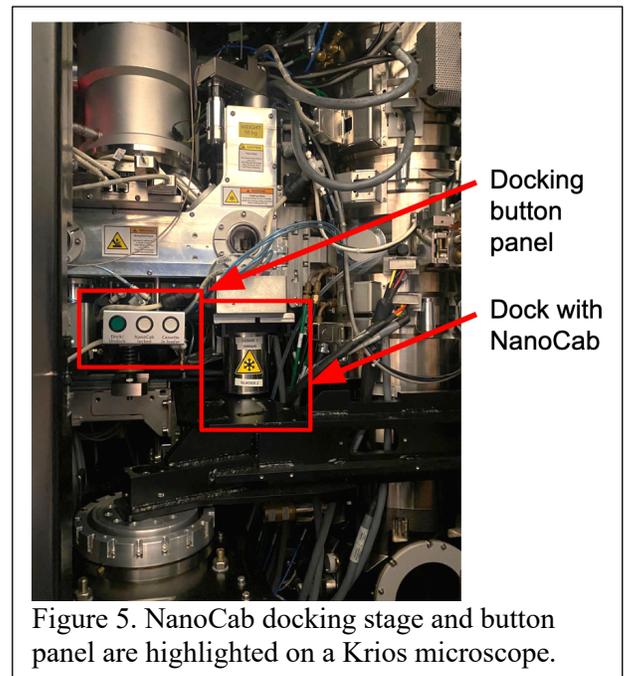


Figure 5. NanoCab docking stage and button panel are highlighted on a Krios microscope.

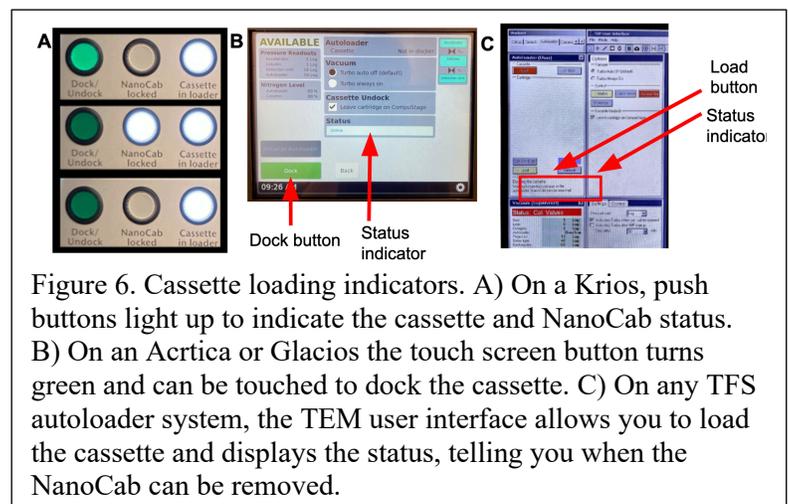


Figure 6. Cassette loading indicators. A) On a Krios, push buttons light up to indicate the cassette and NanoCab status. B) On an Arctica or Glacios the touchscreen button turns green and can be touched to dock the cassette. C) On any TFS autoloader system, the TEM user interface allows you to load the cassette and displays the status, telling you when the NanoCab can be removed.

4.4. If the Cassette is Stored Warm: Cool Cassette



Avoid contamination: always wear gloves when handling anything that enters the microscope vacuum, including the cassette

4.4.1. Retrieve a dry cassette from storage. The cassette must be for the specific microscope you are loading.

4.4.1.1. Using the cassette tweezers, securely grasp cassette at the top position (fig 7). [There are two indentations at the top of the cassette for the cassette tweezers. The Silver shield (foot) is the bottom of the cassette.]

4.4.2. Place cassette in the loading position of the metal block oriented as shown in figure 8.

4.4.3. Top off LN₂ level in transfer station, as required until the cassette is cooled.

4.5. Attach NanoCab to Cassette Loading Station and Position Cassette for Loading

4.5.1. Remove the window cover from the left side of the loading station by flipping the red clamp.

4.5.2. Remove the NanoCab lid.

4.5.3. With the NanoCab label facing you, rest the side edge on the pivot ledge of the loading window. Rotate the NanoCab opening flush with the transfer station. Secure by flipping up the red clamp (fig 9).

4.5.4. If cassette is in NanoCab: Remove the two covers and lid from the top of transfer station.

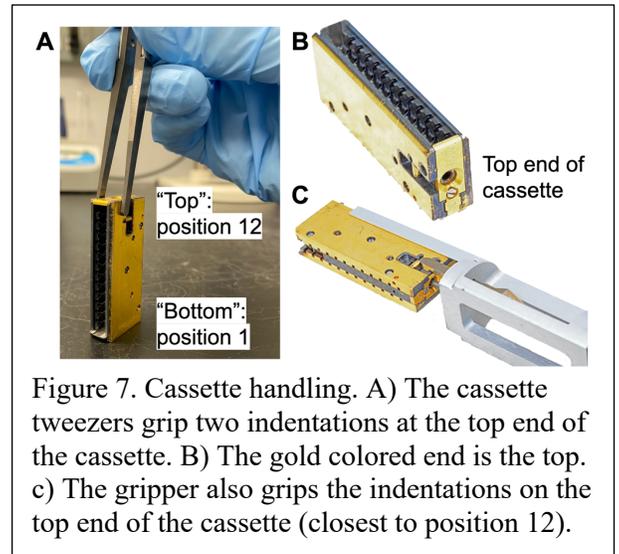


Figure 7. Cassette handling. A) The cassette tweezers grip two indentations at the top end of the cassette. B) The gold colored end is the top. c) The gripper also grips the indentations on the top end of the cassette (closest to position 12).

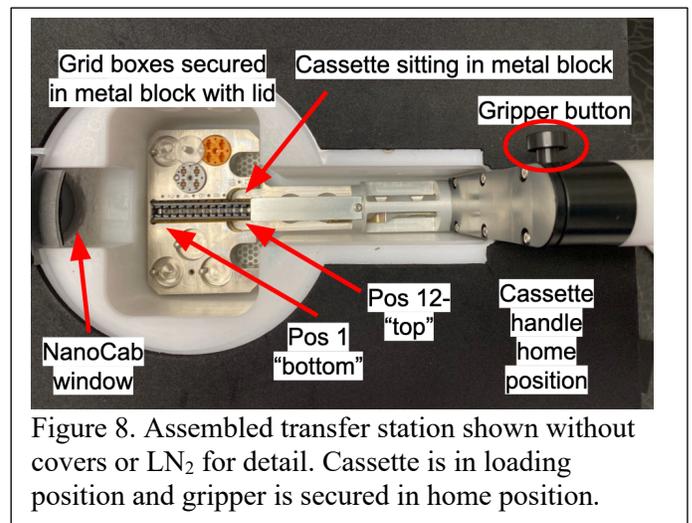


Figure 8. Assembled transfer station shown without covers or LN₂ for detail. Cassette is in loading position and gripper is secured in home position.

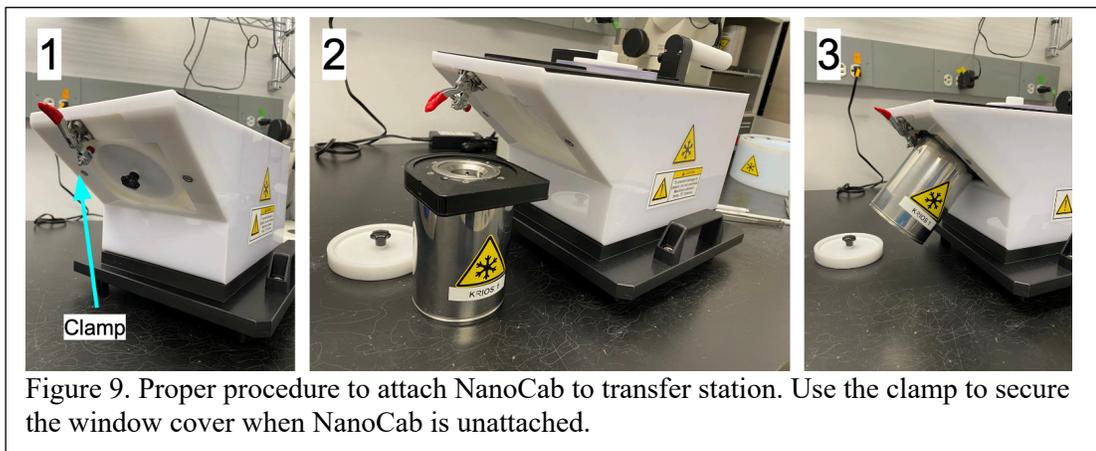


Figure 9. Proper procedure to attach NanoCab to transfer station. Use the clamp to secure the window cover when NanoCab is unattached.

- 4.5.5. Use the cassette gripper handle to move the cassette from the NanoCab to the loading position.
- 4.5.5.1. Holding the cassette gripper handle, push the cold end into the NanoCab.
- 4.5.5.2. Press and hold the side gripper button (see fig 8) to grip the cassette.
- 4.5.5.3. In one fluid motion, being careful not to lift out of nitrogen vapor, remove the cassette from the NanoCab and place it in the loading position of the workstation (fig 8).
- 4.5.5.4. Release the gripper button and slide the handle back to the home position. [The metal block has two support pins that fit the underside of the gripper (fig 10). Failure to place gripper on these pins will result in the end popping up out of the LN₂ vapor and warming up.
- 4.5.6. Once the gripper is in the home position, replace the transfer station covers and top-off the LN₂ level.

4.6. Add Autogrids to Cassette

- 4.6.1. Cool autogrid tweezers in LN₂.
- 4.6.2. Add autogrid boxes to the transfer station chamber. You can add multiple or work one at a time.
- 4.6.3. Remove the autogrid box lid(s) with the lid opening tool and screw one into the metal block to hold down the grid box(s) you will be loading to/from (fig 11A).

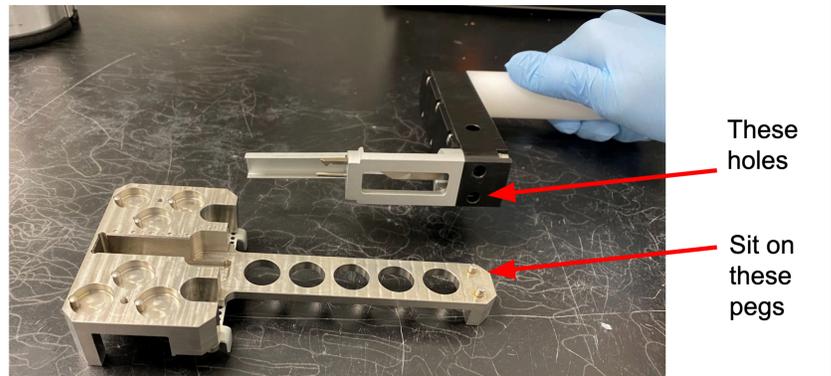


Figure 10. Metal block and gripper are shown outside the loading station at room temperature to highlight the holes in the gripper that position it in a stable “homed” position on the metal block.

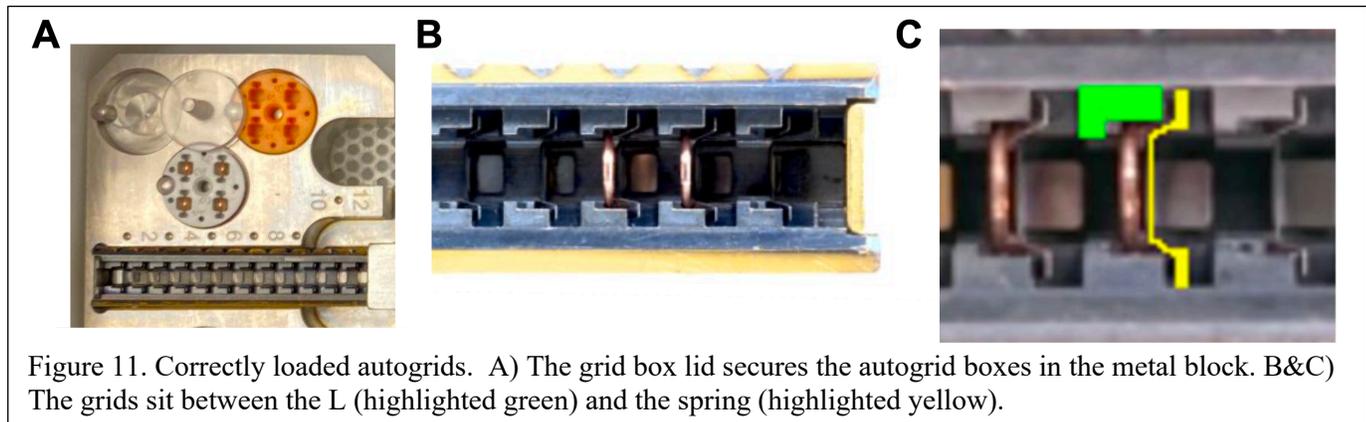


Figure 11. Correctly loaded autogrids. A) The grid box lid secures the autogrid boxes in the metal block. B&C) The grids sit between the L (highlighted green) and the spring (highlighted yellow).

- 4.6.4. If old grids are present, remove them (to autogrid boxes for storage or trash, as appropriate). Always insert & remove the autogrid tweezers into the cassette perpendicular to the cassette face.
- 4.6.5. Place grids, one at a time, correctly in the cassette (see **site specific instructions for correct loading orientation**). They should be placed between the L and the spring in each position (fig 11B&C). Between each grid:
- 4.6.5.1. Re-cool the autogrid tweezers by briefly submerging in LN₂.
- 4.6.5.2. Add LN₂ as required.
- 4.6.5.3. Check for secure insertion in cassette by **gently** tapping the top edge of the autogrid with the autogrid tweezers.
- 4.6.6. Repeat until all grids are loaded.
- 4.6.7. If a grid is on the microscope stage, leave one empty position (see **site specific instructions**).



4.7. Add Loaded Cassette to the Attached NanoCab

- 4.7.1. Working in the reverse direction of 4.5, you will use the gripper to place the cassette into the NanoCab: Remove the top covers from the transfer station.
- 4.7.2. Slide the cassette gripper onto the cassette and hold by pressing down and holding the gripper button. The shield should fully cover the cassette.
- 4.7.3. In one fluid motion, without lifting too high, place the cassette into the NanoCab. Once it is fully inserted, release the gripper button.
- 4.7.4. Remove the gripper from the NanoCab and return it to home position.
- 4.7.5. Replace transfer station covers.
- 4.7.6. While holding the NanoCab with one hand, unsecure the red clasp with the other hand and rotate the NanoCab down off the loading station.
- 4.7.7. Top off the LN₂ inside the NanoCab.
- 4.7.8. Pull the cassette lock pin up to make sure cassette is properly seated (you may hear or feel a small thud).
- 4.7.9. Replace the NanoCab lid.
- 4.7.10. Replace the transfer station window and top covers.

4.8. Dock Nanocab with the Autoloader

- 4.8.1. Before docking the cold NanoCab to the Autoloader, check:
 - 4.8.1.1. The microscope is not doing any operations.
 - 4.8.1.2. Column valves are closed.
 - 4.8.1.3. Temperature of all components is cryogenic
 - 4.8.1.4. Position 1 (cross grating) is loaded onto the stage (or no grid is loaded)
- 4.8.2. Open the enclosure door and locate the autoloader docking stage (fig 5).
- 4.8.3. With the Nanocab label facing you, remove the cap and slide the Nanocab into Autoloader dock.
 - 4.8.3.1. Krios: Push green dock Button (fig 6).
 - 4.8.3.2. Arctica/Glacios: Push touchscreen “Dock” button.
 - 4.8.3.3. Any autoloader: Click “Load” on the microscope computer autoloader interface (fig 6B).
- 4.8.4. If docking button is not available, remove the NanoCab and check cassette lock pin (fig 4). If there is an error (cassette is not docked or undocked) often the pin needs adjustment. Notify staff and use a different NanoCab should solve the issue while the defective one is warmed up.
- 4.8.5. Wait for autoloader to complete the docking procedure. When complete you will see:
 - 4.8.5.1. Krios: “NanoCab locked” light off, “cassette in loader” light on.
 - 4.8.5.2. Arctica/Glacios: “NanoCab can be removed” displayed on the screen.
 - 4.8.5.3. Any autoloader: Interface says you can now remove the NanoCab (fig 6B)
- 4.8.6. Remove NanoCab, visually confirm there is no cassette in the NanoCab, and replace cap.
- 4.8.7. Close enclosure.

4.9. Clean-up / Shutdown

- 4.9.1. See **site specific instructions** for clean-up procedures for the center you are working at.
- 4.9.2. Empty the remaining LN₂ from the NanoCab, place NanoCab with lid in the incubator/oven to dry.
- 4.9.3. Remove gridboxes from transfer station and store appropriately (you will need them to unload).
- 4.9.4. Remove all tools from the transfer station and return them to their respective warm-up locations.
- 4.9.5. Remove all transfer station components (4.1.1.) and return them to their respective warm-up locations.
- 4.9.6. Empty remaining LN₂ from the transfer station and store the station.

5. Additional Figures:

5.1. Figure 12. Autoloader Dock Highlighted

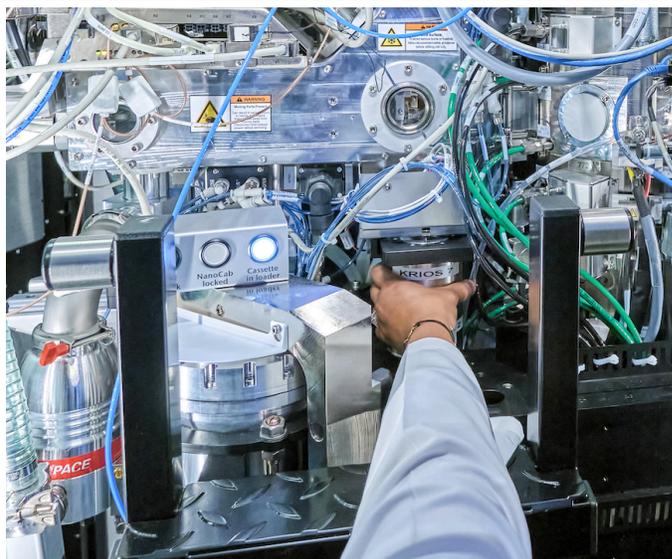


Figure 12. Highlight of Krios autoloader dock, with hand placing NanoCab for docking. Indicator lights and loading buttons are to the left.

6. Chemicals:

6.1. Liquid Nitrogen

7. Waste Disposal:

- 7.1. Follow facility procedure for proper disposal (see [site specific instructions](#)).
- 7.2. Biohazardous waste will be collected in designated bins lined with red biohazard bags.
- 7.3. Chemical hazardous waste will be segregated by hazard class (e.g. flammable, corrosive) and state (e.g. solid, liquid), appropriately labelled, and placed in the laboratory's hazardous waste collection.