

**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 NanoCab that holds the cassette and docks with Autoloader.
- 2.4. Liquid Nitrogen (LN2) is a cryogenic liquid stored under pressure.

**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 and Covers
- NanoCab & Lid
- Autogrid Cassette
- Autogrid Tweezers
- Cassette Tweezers
- Standard Tweezers
- Autogrid box(es)
- Large Forceps
- Autogrid Opening Tool
- Cassette Gripper Handle

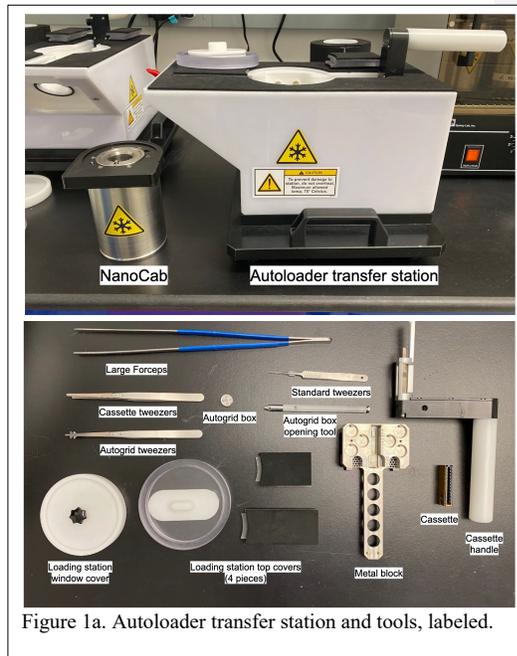


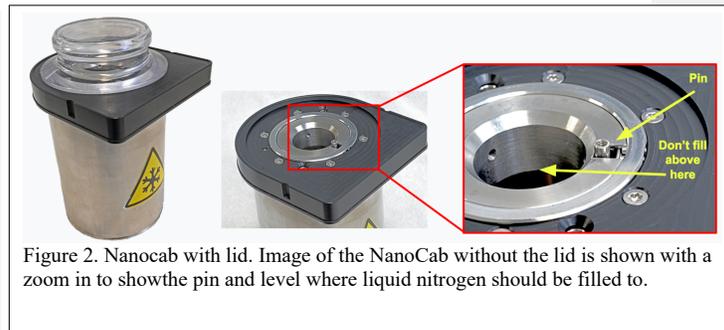
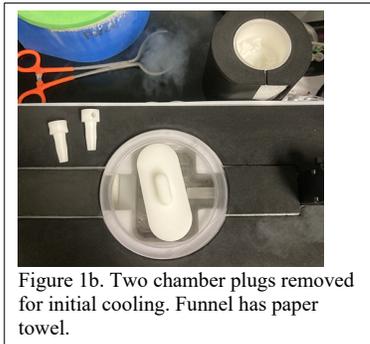
Figure 1a. Autoloader transfer station and tools, labeled.

**4. Procedure:**

**4.1. Prepare the Transfer Station**

- 4.1.1. Gather the cassette transfer station and parts (fig. 1a)
- 4.1.2. Assemble the cassette transfer station with the loading station window cover, metal block, the four transfer station covers.

- 4.1.3. Remove the two chamber plugs and white center cover. Cool the transfer station with LN<sub>2</sub> without overfilling (Fig 1b).
  - 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. 2)
- 4.1.4. Once the liquid nitrogen has finished bubbling, the temperature has stabilized.
- 4.1.5. Reattach the chamber plugs. The third outlet always remains unobstructed.



#### 4.2. Cool the NanoCab

- 4.2.1. Retrieve the dry Nanocab, with lid, for the microscope you'd like to load. (fig. 2)
- 4.2.2. Cool the NanoCab with liquid nitrogen.
  - 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. 2)
- 4.2.3. When the liquid nitrogen has stopped bubbling, the NanoCab is cooled.
- 4.2.4. Verify the **cassette lock pin** (fig 2) is not stuck frozen. Pulling upward on the pin, it's spring loaded. This pin's operation is essential for docking and undocking cassettes from the microscope.

#### 4.3. If Cassette is in the Microscope: Retrieve Cassette

- 4.3.1. Dock Nanocab with the Autoloader
- 4.3.2. Before docking the cold NanoCab to the Autoloader, check:
  - 4.3.2.1. The microscope is not doing any operations.
  - 4.3.2.2. Column valves are closed.
  - 4.3.2.3. Temperature of all components is cryogenic.
  - 4.3.2.4. Position 1 (cross grating) is loaded onto the stage (or no grid is loaded)
- 4.3.3. Open the enclosure door and locate the **autoloader docking position** (Figure 3).
- 4.3.4. With the NanoCab label facing you, remove the cap and slide the NanoCab into Autoloader dock.
  - 4.3.4.1.1. Krios: Push green dock Button
  - 4.3.4.1.2. Arctica/Glacios: Push touchscreen dock Button
- 4.3.5. If docking button is not available, remove the NanoCab and check cassette lock pin (fig. 2)
- 4.3.6. Wait for autoloader to complete the docking procedure. When complete:
  - 4.3.6.1. Krios: "NanoCab locked" light off, "cassette in loader" light on.
  - 4.3.6.2. Arctica/Glacios: "NanoCab can be removed" displayed on the screen.
- 4.3.7. Remove NanoCab from Autoloader dock and replace cap.
- 4.3.8. Close enclosure.
- 4.3.9. Proceed to step 4.5

Commented [SM1]: Picture of the autoloader docks?

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 the dry cassette from storage. The cassette must be for the specific microscope you are loading.
- 4.4.2. Using the cassette tweezers, securely grasp cassette at the top position (fig 3).
  - 4.4.2.1. There are two indentations at the top of cassette for the cassette tweezers (fig. 3).
  - 4.4.2.2. Silver shield (foot) is the bottom of the cassette.
- 4.4.3. Place cassette in the loading position of the metal block oriented as shown in figure 4.
- 4.4.4. Top off liquid nitrogen level in transfer station, as required until the cassette is cooled.

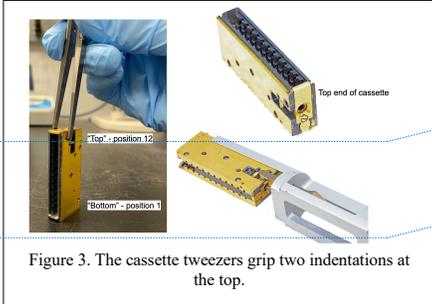


Figure 3. The cassette tweezers grip two indentations at the top.

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Commented [SM3]: Need good pic showing how the cassette sits in the metal block "loading position"

4.5. Attach NanoCab to Cassette Loading Station and Position Cassette for Loading (Fig 5)

- 4.5.1. Remove the outlet cover from the left side of cassette 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 point of the transfer station loading window. Rotate the NanoCab opening flush with the transfer station. Secure by flipping up the red clamp.
- 4.5.4. Remove the two covers and lid from the top of transfer station.
- 4.5.5. If cassette is in NanoCab: use the cassette gripper handle to move the cassette from the NanoCab to the loading position.

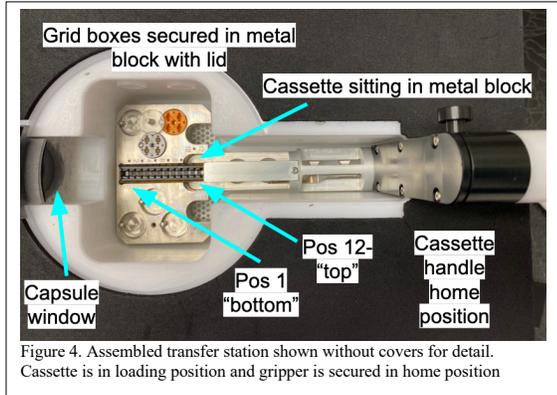


Figure 4. Assembled transfer station shown without covers for detail. Cassette is in loading position and gripper is secured in home position

- 4.5.5.1. Holding the cassette gripper handle, push the cold end into the NanoCab.

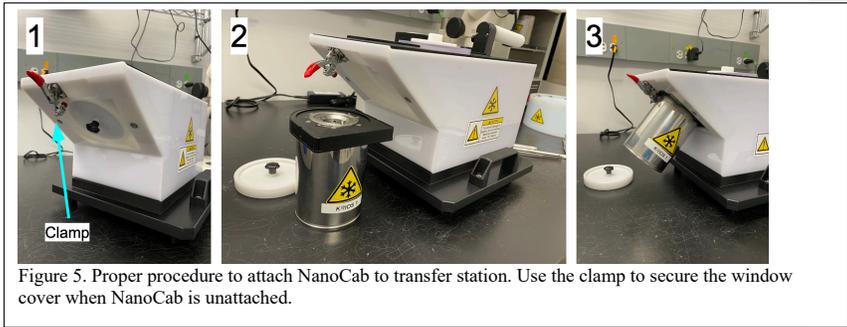


Figure 5. Proper procedure to attach NanoCab to transfer station. Use the clamp to secure the window cover when NanoCab is unattached.

- 4.5.5.2. Press and hold the side gripper button 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 center of the workstation at the loading position.
- 4.5.5.4. Release the gripper button and slide the handle to the right to remove the support pin and shield.
- 4.5.5.5. Place the gripper in the home position (fig 4). The metal block has two support pins that match the underside of the gripper. Failure to place gripper on these pins will result in the cold end popping up, out of the LN2 vapor (fig 5d).
- 4.5.6. Replace the transfer station covers and top-off the liquid nitrogen level.

Commented [SM4]: Close up of the pins on the metal block / the gripper properly "homed". Could show what it looks like when you miss is with 5d but not necessary.

#### 4.6. Add Autogrids to Cassette

- 4.6.1. Cool autogrid tweezers by submerging tip in liquid nitrogen.
- 4.6.2. If old grids are present, you must first remove them before adding new.
- 4.6.3. Add gridboxes to the transfer station chamber. You can add multiple or work one at a time.
- 4.6.4. Remove the gridbox lid with lid tool and attach it to metal block so that it will hold down the gridbox you will be loading. (fig. 6c)
- 4.6.5. Remove the grids, one at a time, and place them correctly in the cassette. Add between the L and the clip (fig 6b).  
Between each grid:
  - 4.6.5.1. Re-cool the autogrid tweezers by briefly submerging in liquid nitrogen.
  - 4.6.5.2. Add liquid nitrogen 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 center specific policies).

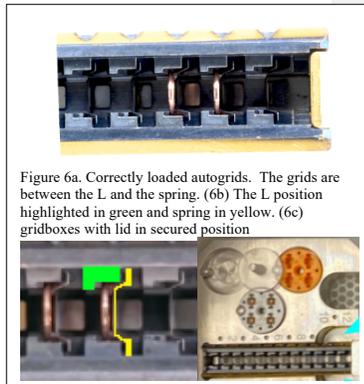


Figure 6a. Correctly loaded autogrids. The grids are between the L and the spring. (6b) The L position highlighted in green and spring in yellow. (6c) gridboxes with lid in secured position

#### 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.
- 4.7.2. Remove the top covers from the transfer station.
- 4.7.3. Slide the cassette gripper rod into the cassette and hold the gripper button securely. The shield should be covering the grids.
- 4.7.4. In one fluid motion, without lifting too high, place the cassette into the NanoCab and release the gripper button once the cassette is fully inserted.
- 4.7.5. Remove the gripper from the NanoCab and return it to home position.
- 4.7.6. Replace transfer station covers.
- 4.7.7. While holding the NanoCab with your opposite hand, unsecure the red clasp and rotate the NanoCab off of the loading station.
- 4.7.8. Top off the liquid nitrogen inside the NanoCab.
- 4.7.9. Pull the pin, the cassette lock, up to make sure cassette is properly homed inside the NanoCab. You may hear or feel a small thud.
- 4.7.10. Replace the NanoCab lid.
- 4.7.11. Replace the transfer station window cover.

#### 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 position. (fig. 10)
- 4.8.3. With the Nanocab label facing you, remove the cap and slide the Nanocab into Autoloader dock.
  - 4.8.3.1.1. Krios: Green dock Button
  - 4.8.3.1.2. Arctica/Glacios: Touchscreen dock Button
  - 4.8.3.2. If docking is unavailable, remove NanoCab and check that cassette is secured by pulling up on pin (4.2.5 - fig 2c).
- 4.8.4. Wait for autoloader to complete the docking procedure. When complete:
  - 4.8.4.1. Krios: “NanoCab locked” light off, “cassette in loader” light on.
  - 4.8.4.2. Arctica/Glacios: “NanoCab can be removed” displayed on the screen.
- 4.8.5. Remove NanoCab and replace cap.
- 4.8.6. Close enclosure.

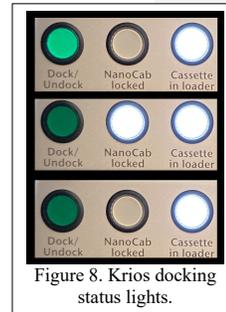
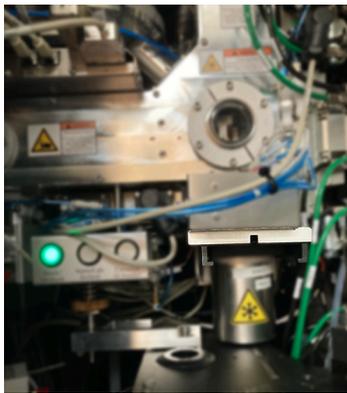


Figure 8. Krios docking status lights.

#### 4.9. Clean-up / Shutdown

- 4.9.1. See site specific policies for detailed clean-up procedures for your specific center.
- 4.9.2. Empty the remaining liquid nitrogen 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 liquid nitrogen from the transfer station and store the station.

#### 5. Additional Figures:



5.1. Figure 10. Autoloader Dock Highlighted

5.2.

#### 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.