Chameleon Standard Operation Procedure version 1.2

Authors: Yan Liu, Mahira Aragon, Christina Zimanyi

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1. Purpose

1.1. Prepare cryo-EM grids using a chameleon (SPT LabTech)

2. Definitions:

2.1. chameleon is an automated grid freezing instrument from SPT LabTech

2.2. Liquid Nitrogen (LN_2) is a cryogenic liquid stored under pressure

2.3. Ethane is classified as a flammable gas

3. Supplies & Equipment

- ❏ PPE (BSL-1)
  - ■ Laboratory Coat
  - ■ Nitrile Gloves
  - ■ Goggles / Safety Glasses
  - ■ Cryogenic Gloves
- ❏ Chemicals/Reagents
  - ■ Liquid Nitrogen
  - ■ Ethane
  - ■ 100% Ethanol
  - ■ 100% Methanol
  - ■ Distilled Water
  - ■ HPLC grade Water
  - ■ Chamclean detergent
- ❏ Pipette and Tips
- ❏ Fine-tip Tweezers
- ❏ Chameleon/Self-wicking Grids
- ❏ TFS Autogrid Storage Box
- ❏ Autogrid Storage Box Opening Tool
- ❏ LN_2 Dewars for Transfer
- ❏ Long Forceps

4. Procedure:

4.1. Launch the chameleon application

4.1.1. Double click the chameleon icon on the desktop to open the control software and connect to the instrument. The main features of the interface are shown in Figure 1, below.

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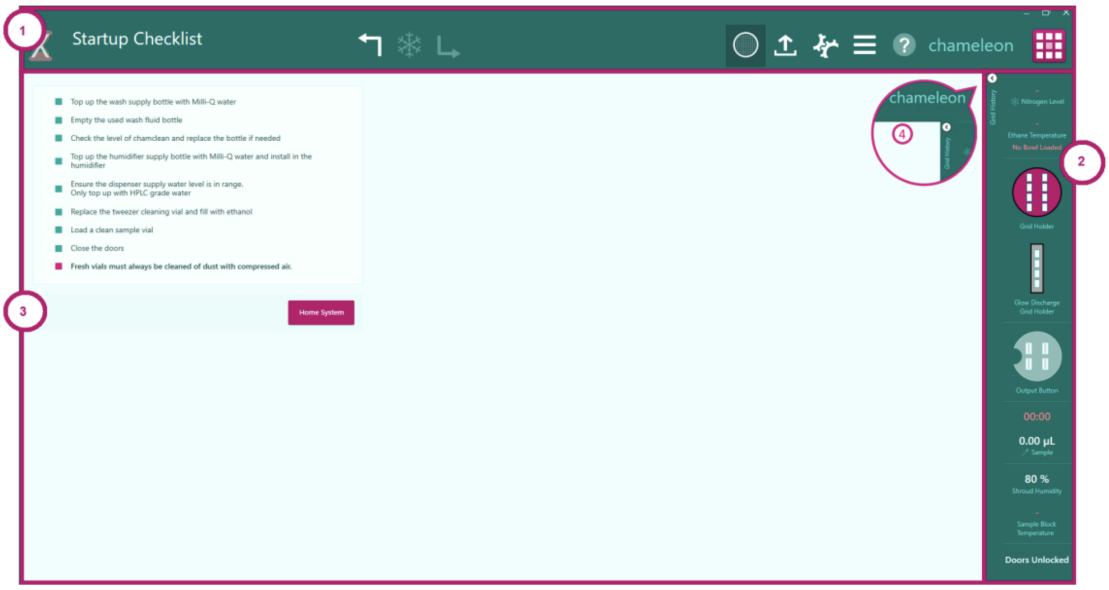
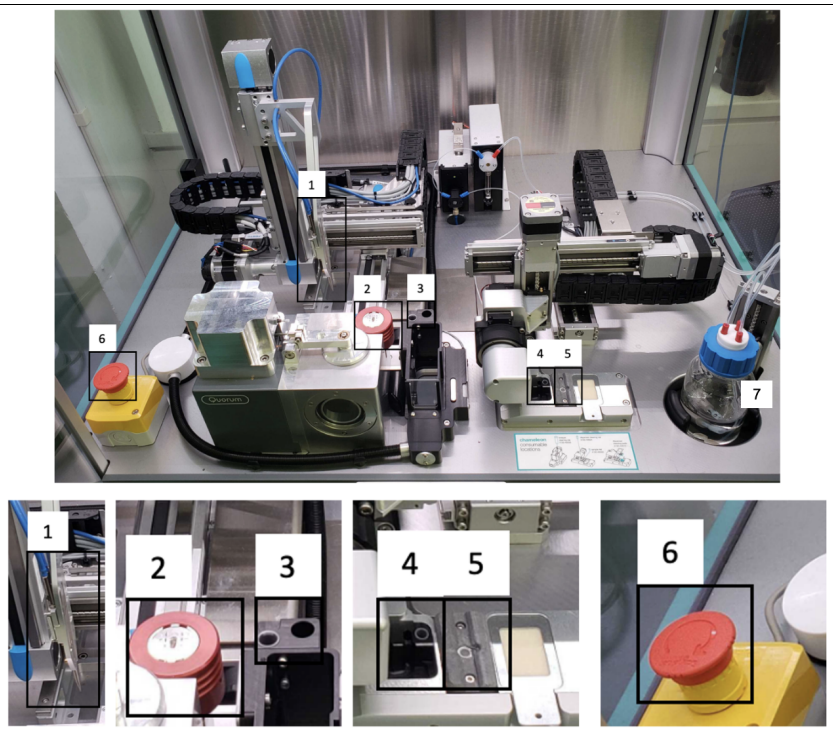


Figure 1. Main features of the chameleon software interface.
1. The toolbar at the top of the chameleon software window contains buttons concerned with high level functionality.
2. The status panel on the right displays environmental information from the system.
3. The contents of the main central area vary according to the step of the workflow.
4. The grid history "flip out" panel displays information about grids throughout the freezing session.



1.Tweezer
2. Grid Pedestal
3. Tweezer cleaning vial
4. Dispenser cleaning vial
5. Sample tube area
6. Emergency stop button
7. Dispenser supply water bottle

Figure 2. Key components of the chameleon instrument

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4.2. Initialize system:

4.2.1. Open the compressed air valve on the nitrogen tank. DO NOT ADJUST THE PRESSURE! (see site specific instructions)

4.2.2. Go through the startup checklist (Figure 3), ensuring each item has been completed. The location of supply bottles housed in the cabinet at the bottom of the instrument is shown in Figure 4.

Note:

- The liquid in the waste bottle (Figure 4, used wash fluid) needs to be disposed into a labeled biohazard waste container – see site specific instructions.
- Vials must be pre-cleaned using an air duster.

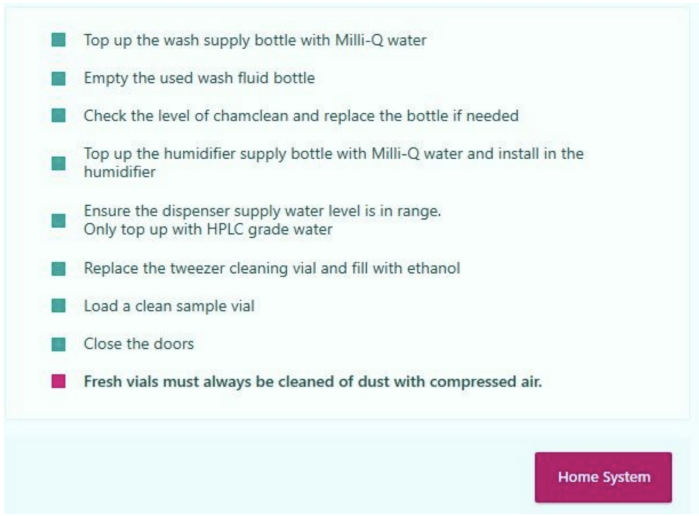

Figure 3. Start up checklist in the software interface.

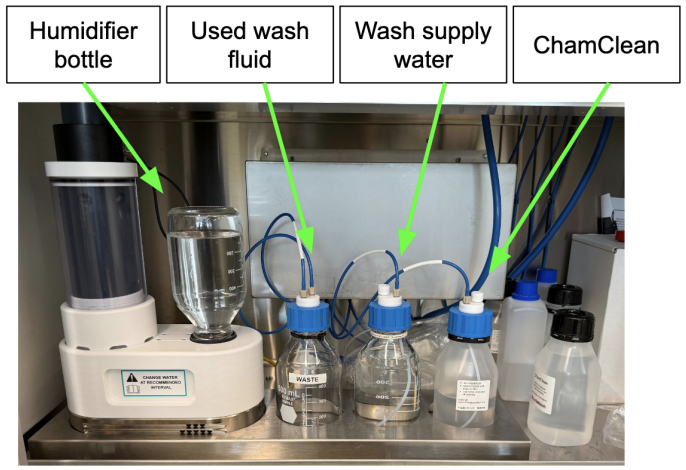

Figure 4. Supply bottles are located in the bottom cabinet of the chameleon.

When finished with the startup checklist, press the **Home System** button. The motors will be homed and tested.

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4.2.3. On the next screen, input the required information in Session Details (Figure 5): operator name and project. (E.g Operator: Yan Liu, Project: CD00 - see site specific instructions).

4.2.4. Select the radio button for the session type you want to use: **Screening Session** (when you plan to freeze grids at multiple different dispense-to-plunge times) or **Targeted Session** (when you plan to freeze at a single dispense-to-plunge time (i.e. 4 grids, all frozen at 101ms)).

4.2.5. Check if there are any grids in the glow discharge unit. If there are, use the **Retrieve Grids** button to remove them. When ready, press the **Prepare Grids** button.

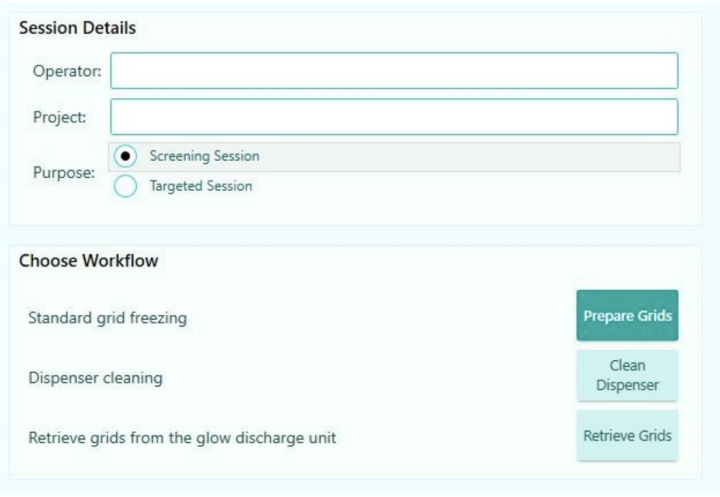

Figure 5. Session details interface.

4.3. Prepare grids

4.3.1. **Load grids:** Load the self-wicking grids into the pedestal with the nanowire side of the grids facing right, towards the dispenser. The nanowire side typically appears darker (matte, dull, or "waxy") whereas the non-nanowire side looks more "rainbow-like". The pedestal slot numbers increase from front to back, left to right. Position numbers are indicated on the pedestal. Grids should be loaded from the lowest numbered slot first.

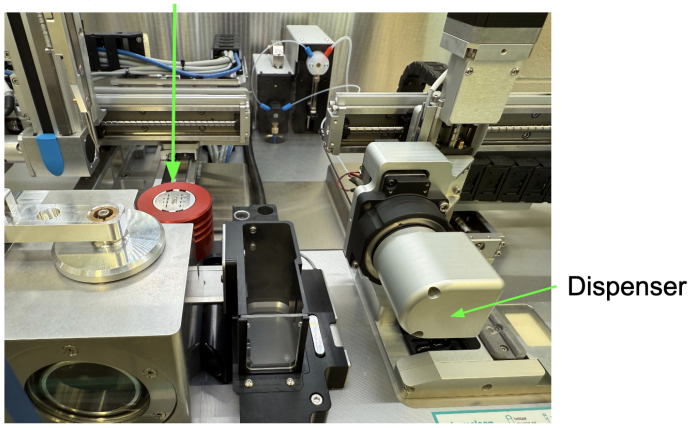

Figure 6. Grids are placed on the pedestal starting from position 1 (bottom left). The nanowire side should face towards the dispenser.

4.3.2. Enter the details of the grids in the software interface (Figure 7). Each grid requires a unique Batch Number and Position. Notes are optional.

4.3.3. Set the desired glow discharge parameters in the **Grid Activation** menu (default parameters are 12 mA for 20.0 s).

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4.3.4. Click **Continue** and the grids will be moved into the glow discharger. Note: The chameleon detects the presence of a grid on the pedestal. The **Continue** button is only activated when there is a label for each grid detected on the pedestal.

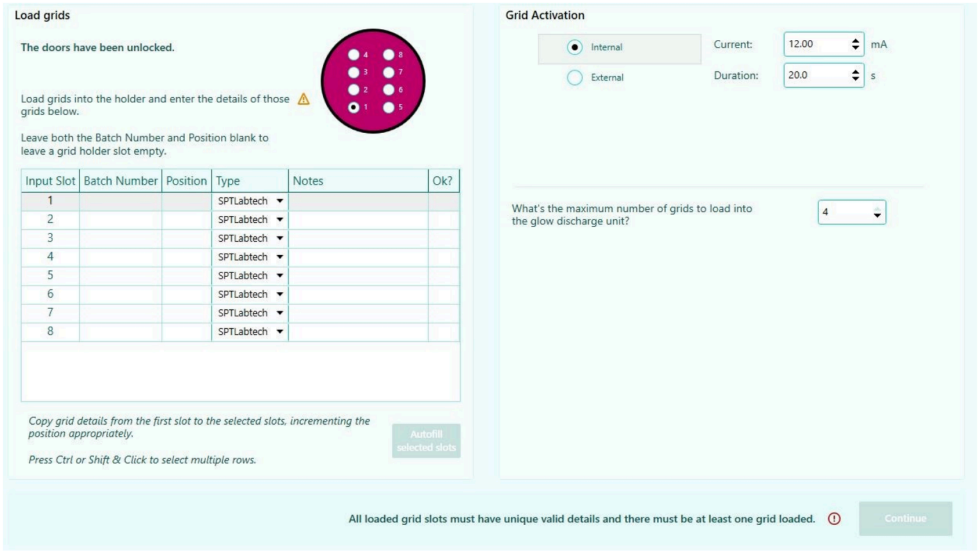

Figure 7. Grid information interface.

4.4. Prepare sample and instrument

4.4.1. Load sample: Follow the instructions on the **Preload Sample** screen (Figure 8):

Note: The minimum volume that can be loaded into the instrument is 5 µL: 3 µL to aspirate and 2 µL of dead volume. There is always 2 µL of dead volume but you may increase the volume to aspirate.

Clean a sample vial of any dust with an air duster before use. Open the instrument doors and place the clean sample vial into the sample holder. Pipette your sample carefully into the sample vial and ensure there are no air bubbles.

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4.4.2. Input sample details into the interface (Figure 8).

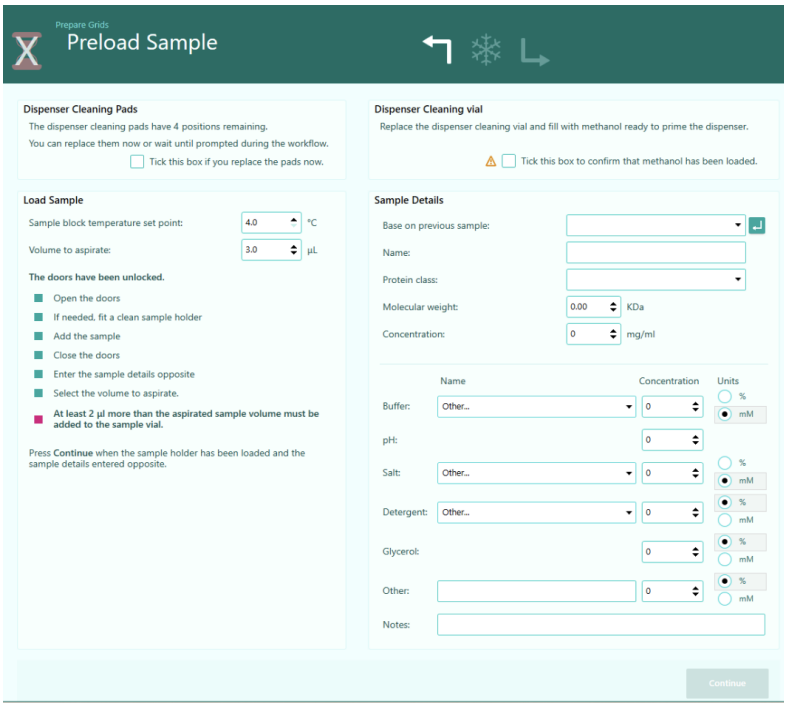

Figure 8. Sample loading and information interface.

4.4.3. Put a new dispenser cleaning vial that has been cleaned with compressed air into the holder and fill with methanol using the squirt bottle (Figure 9). Check the box on the Preload Sample screen to confirm you have done this and then click **Continue**.

Figure 9. Use the squirt bottle to fill the dispenser cleaning vial with methanol.

4.4.4. Load cryogens:

4.4.4.1. Press **Open** to open the cryogen drawer (Figure 10) and load a cryogen bowl (Figure 11). The side of the bowl with the electrical contacts should be facing away from the instrument (towards you).

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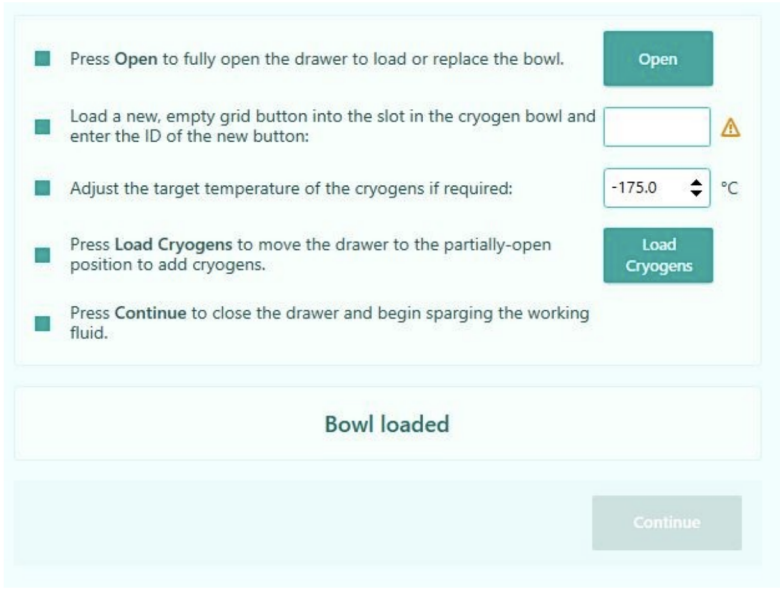

Figure 10. Cryogen loading interface.

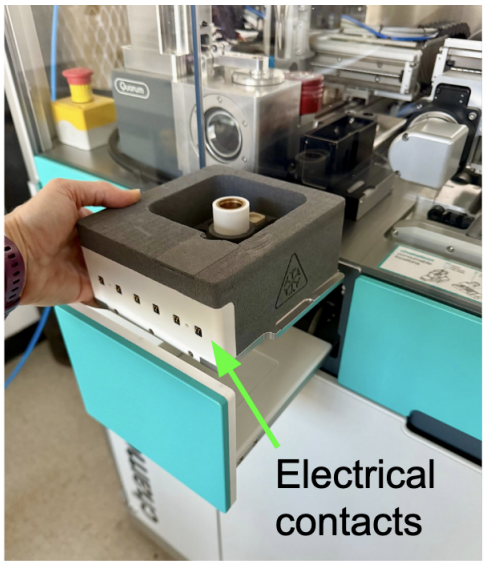

Figure 11. Cryogen bowl is placed into the cryogen drawer

4.4.4.2. Put your labeled grid box (Use Thermo Fisher buttons ONLY) into the holder in the bowl. Make sure your button DOES NOT have a lid attached. Type the button ID into the software interface.

4.4.4.3. Press **Load Cryogens**. Fill the bowl with LN_2. Check that the Nitrogen Level and Ethane Temperature on the status panel are reading (Figure 12). Make sure the ethane cup temperature is below -160°C and that there is no nitrogen in the cup before condensing ethane.

4.4.4.4. Condense ethane into the ethane cup (see site specific instructions)

4.4.4.5. After condensing ethane, close the ethane valve. Press **Continue** to close the cryogen drawer.

4.4.4.6. If you need to refill the LN_2 in the cryogen bowl at any point, press the snowflake button (Figure 13) in the top toolbar to open the cryogen drawer. There are a small number of steps where this button is not available (i.e. when the glow discharger is in use). If you allow the LN_2 to drop to 55% or below and request the system plunge a grid, it will require you to top up the LN_2 before plunging.

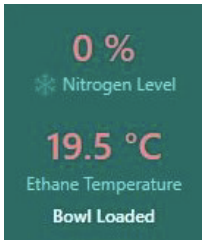

Figure 12. Cryogen status is shown in the right panel.

Figure 13. The snowflake icon allows you to open and close the cryogen drawer.

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4.4.5. Helium sparge: Follow the instructions on the software interface (Figure 14):

4.4.5.1. Open the helium supply valve.

4.4.5.2. Press **Sparge** to begin helium sparging to degas the dispenser water (HPLC water). This will take a couple of minutes.

4.4.5.3. After the sparge is complete, the system will automatically start testing the dispenser. While it does this, ***close the helium supply valve***.

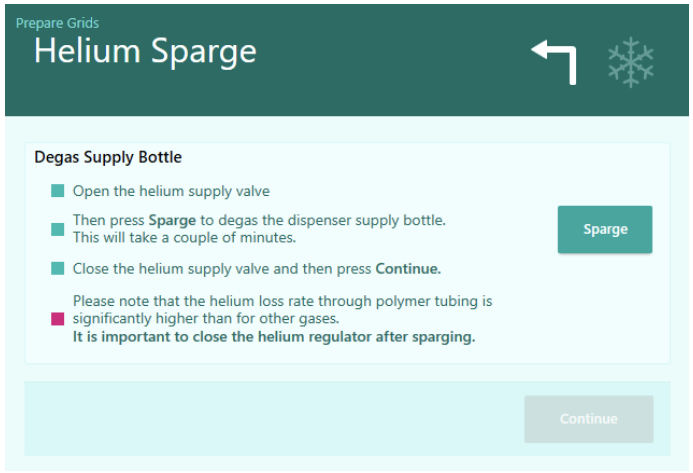

Figure 14. Helium sparge interface.

4.4.6. Dispenser setup & testing: This step will start automatically. The dispenser will be tested using water. Images will be taken and analyzed. If the test is good (there is a consistent and vertical pattern of droplets), a box displaying "Dispenser tested ok" appears (Figure 15). If it does not dispense ok, you can use the troubleshooting tab to try to get a consistent dispensing from the tip, then manually test the dispenser as described in the next steps.

4.4.6.1. Troubleshooting dispenser: If necessary, use the **Troubleshooting** tab (Figure 16) to diagnose and address issues with the dispenser. After running a troubleshooting step, manually test the dispenser.

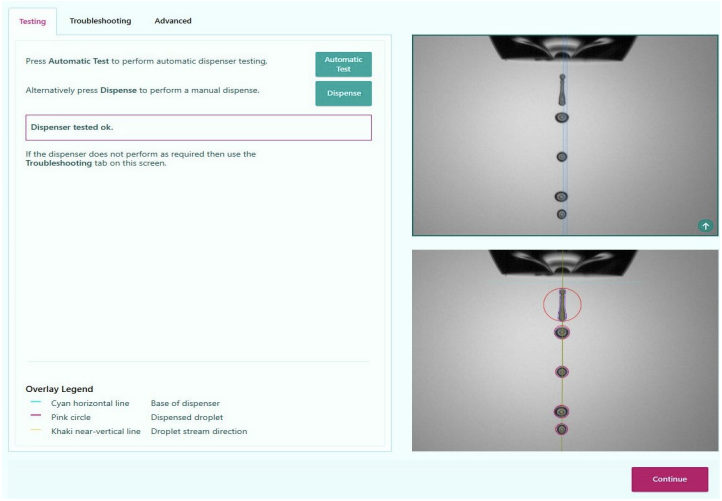

Figure 15. Dispenser testing interface. This test uses water.

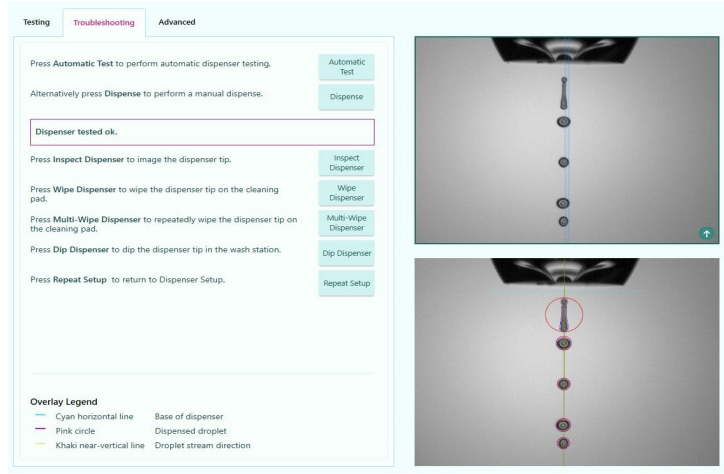

Figure 16. The troubleshooting tab will walk you through options to clean the tip until the dispenser tests ok.

4.4.6.2. If needed, you can manually test the dispenser, from the "testing" tab: Press either **Automatic Test** or **Dispense** to run the automatic dispenser testing or to manually test the dispenser, respectively. If **Automatic Test** is pressed, the lower image, overlay legend, and results in a pink box will appear. To assess the consistency of the dispense manually, click **Dispense** 3-5 times in a row. The dispense pattern here should be vertical and consistent. Once dispensing is satisfactory, press **Continue**.

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4.4.7. After dispenser testing, the chameleon will glow discharge the grids and then aspirate your sample automatically. You do not need to push any buttons.

4.4.8. Test dispenser with sample: Press the **Dispense** button multiple times in a row to assess the dispenser performance with sample (Figure 17). If necessary, use the **Troubleshooting** tab to diagnose and address issues with the dispenser. Once the dispenser is consistently dispensing the sample in a vertical line, press **Continue**. A grid will be picked up.

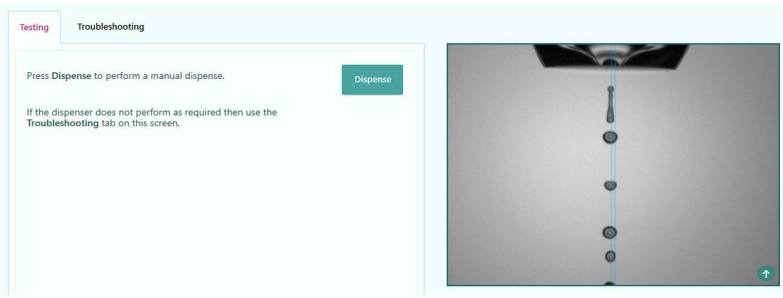

Figure 17. Dispenser testing interface. This test uses your sample.

4.4.8.1. Some troubleshooting tips: Use Increase/Decrease Dispenser Back Pressure to compensate for a more or less viscous sample/buffer solution. Next, adjust the Amplitude with small step-wise (step = 50) increments to avoid formation of bubbles in the tip. If there is debris or liquid stuck to the tip, use the Wipe Dispenser and Multi-Wipe Dispenser options. If a film has formed at the tip, use the Dip Dispenser option. Note: After a Dip Dispenser action, the Mini-Prime action automatically pushes 0.5 µL of sample out of the dispenser tip. Once the dispenser is consistently dispensing the sample, press Continue.

Note: if the available troubleshooting steps do not lead to an acceptable dispense pattern. Ask staff for assistance. In the worst case, you may need to use the Finish Session button on the main toolbar to end the current freezing session and clean the dispenser. This will cause the aspirated sample to be lost.

4.5. <u>Freeze Grids</u>

4.5.1. Use the Plunge Control workflow. Select the radio button to make grids using either Two-strip or One-stripe mode:

a. *Two-stripe dispense mode (Figure 18)*: Select **Two-stripe** under the **Dispense Mode** section. Press the **Characterise** button to perform wicking characterization. Based on the **Wicking time**, a **Suggested plunge time** will be calculated automatically, taking into account the slowing that is generally observed when dispensing a second stripe of sample onto the same grid. The user can again adjust this value by dragging the **Actual plunge time** slider under the wicking video to a desired value. Pressing the **Return arrow** button next to the **Suggested plunge time** will reset

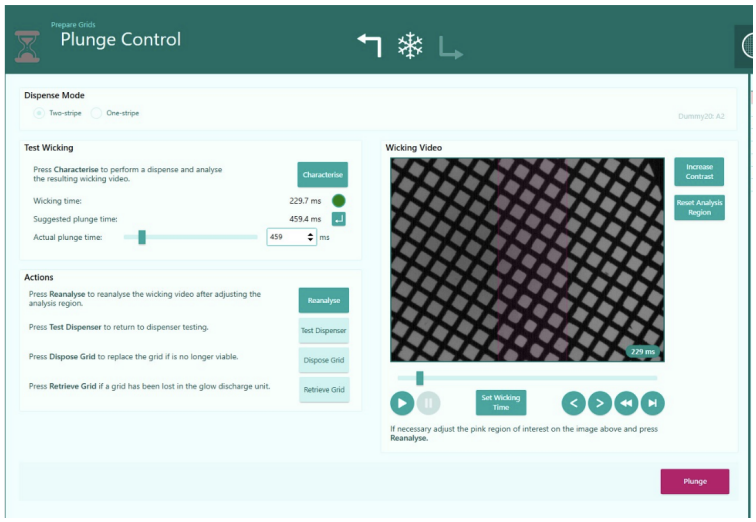

Figure 18. Plunge control interface for two-stripe mode.

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the **Actual plunge time** to the suggested plunge time value. Press **Plunge** to freeze the grid using the value displayed using the **Actual plunge time** slider.

b. *One-Stripe Dispense Mode (Figure 19)*: Press the radio button next to **One-stripe** under the **Dispense Mode** section. Use the slider to set the desired **Plunge time** (101 ms – 2,500 ms) or the tick box to choose 54 ms. Press **Plunge** to freeze the grid using the value displayed on **the Plunge time** slider.

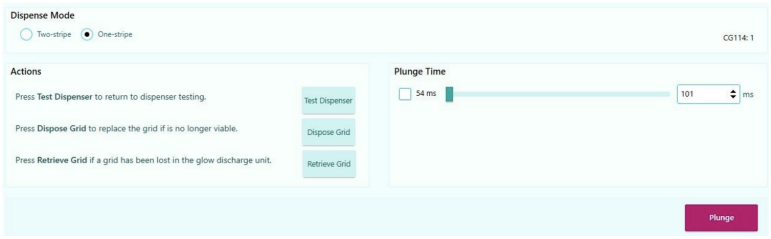

Figure 19. Plunge control interface for one-stripe mode.

4.5.2. Plunge review (Figure 20): Review the wickedness of the grid (move the slider to examine the strip frame by frame and compare the last frame prior to the tweezers entering the field of view to the reference images). Decide if you will **Accept Grid** or **Reject Grid** button to remove the grid from the cryogen bowl and place it in the disposal area of the grid holder, followed by the drying of the tweezers. Make sure the grid is released from the tips of the forceps.

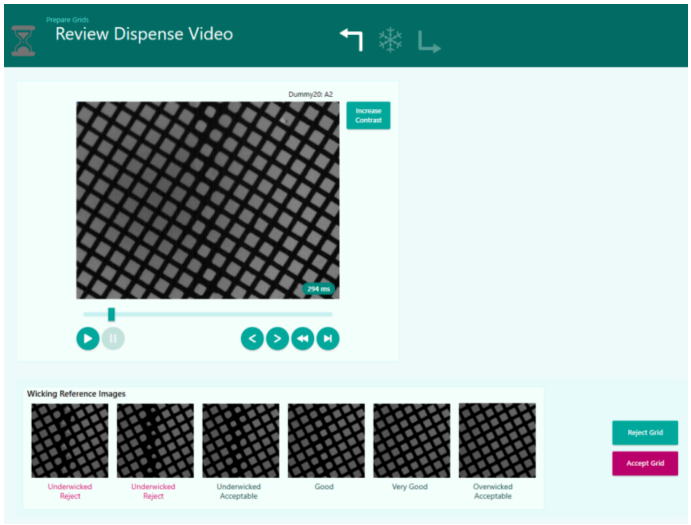

Figure 20. Plunge review interface.

4.5.3. Accept or reject the plunged grid.

a. **Accept Grid**: the grid will be placed into the next available autogrid button slot. Once grid release has been attempted, the tweezers will hover above the LN_2. Visually inspect the tip of the tweezers. If the grid release was successful, press **Lift Tweezers**; if it was unsuccessful, press **Retry** (Figure 21). Provide a reason for acceptance (Figure 22) and then press the **Continue** button.

b. **Reject Grid**: Confirm that the grid has been successfully disposed of. Press the **Retry** button if it has stuck to the tweezers. Once this step is complete, press **Continue.** Fill in the reason for

Figure 21. Confirm the grid has been released before continuing.

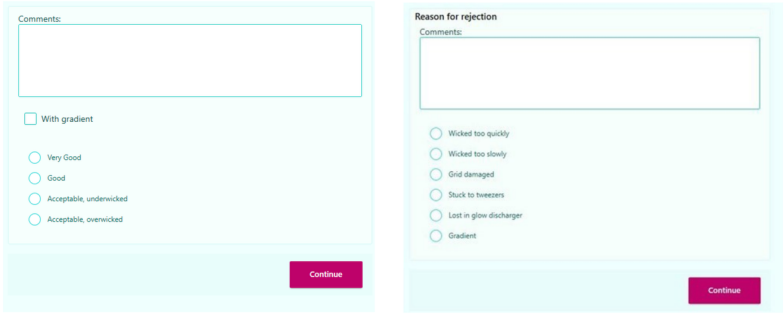

Figure 22. Interface for providing grid quality and other notes for accepted grids (left) or rejected grids (right).

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rejection and press the **Continue** button.

4.5.4. Prepare more grids or finish session (Figure 23): **Prepare Grid** will continue the freezing workflow. If no more slots are available in the button, you will be prompted to load another button. If no more grids are loaded, you will be prompted to load more grids. **Finish Session** can be used either to end the session or to change to a new sample.

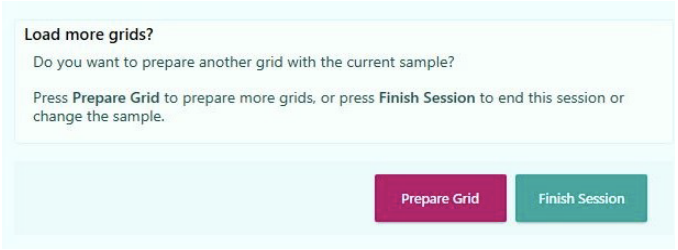

Figure 23. After all loaded grids are used, choose whether you will prepare more grids (using the same sample) or finish the session.

4.5.5. Prepare more grids for the same sample: Pressing **Prepare Grid** above gives you options for the next grid (Figure 24). **Additional glow discharge** can be applied to all grids remaining in the glow discharge unit by ticking the box and modifying the **current** or **duration** as appropriate. If the glow discharge unit is empty, this option will be disabled. Press **Continue** to prepare more grids

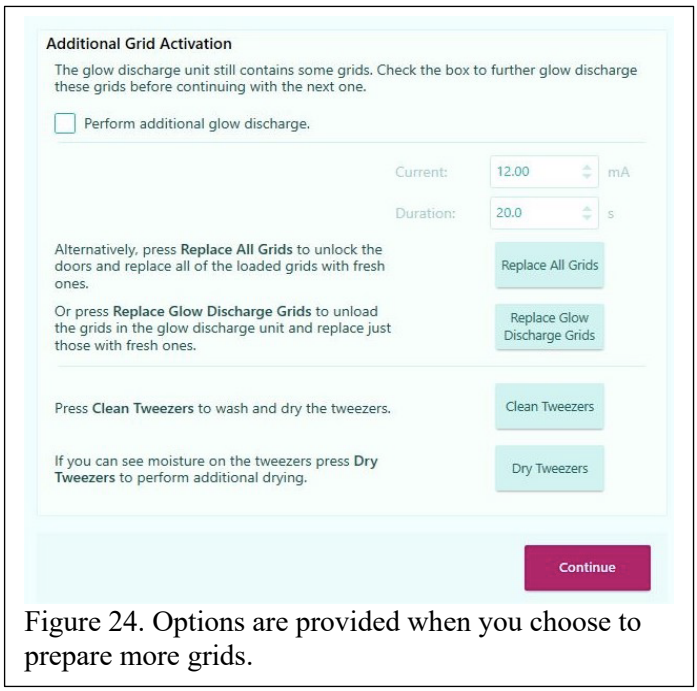

Figure 24. Options are provided when you choose to prepare more grids.

4.6 Freezing grids of another sample

4.6.1 Pressing **Finish Session** above (step 4.5.4) begins the dispenser cleaning process. ***This will take 7-8 minutes.***

4.6.2 Test dispenser: refer to 4.3.10. If performance of the dispenser is satisfactory, either press **Continue** to **Load New Sample** to jump back in the workflow to the Load Sample page.

4.6.3 A new sample can then be placed into a new sample vial and the workflow rejoined as usual.

4.6.4 The cryogen bowl stays inside of the chameleon throughout this process. The ethane temperature is controlled, the LN_2 level is detected, and you may top up the LN_2 as needed.

4.6.5 Any grids still in the system will be used with the new sample. If no grids are left, you will be prompted to load more.

4.6.6 Remove grids: If unused grids are in the chameleon at the end of a session, the following page will display to allow the user to remove these grids and store them as appropriate. Press **Continue** when ready.

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4.7 Shutting down: Remove cryogens

4.7.1 Press **Open** to remove the cryogen bowl from the instrument. Once the bowl has been removed, press **Continue**. Transfer the buttons to a transfer dewar or storage dewar.

4.7.2 If continuing to use the instrument, press **Go to Initialise** (Figure 26) or use the **Reset Arrow Button** in the main toolbar to reset the system.

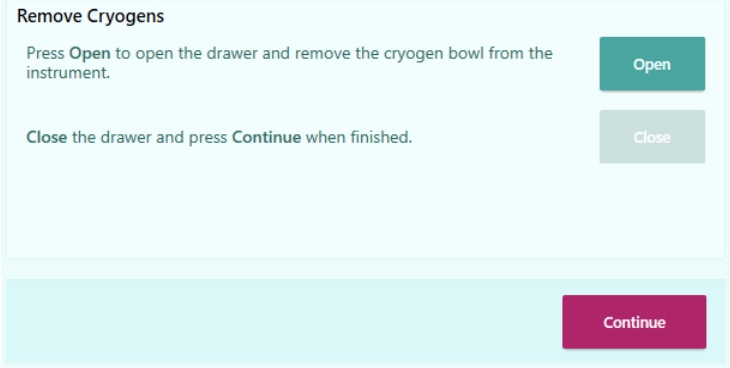

Figure 25. Remove cryogens interface.

4.8 End of session

4.8.1 Press **Create Reports** to generate reports or to view or export videos from this or previous freezing sessions (See site specific instructions).

4.8.2 The workflow is finished, and the application may now be safely closed by either pressing the **Close** button (Figure 26) or by pressing the X in the upper right-hand corner of the application.

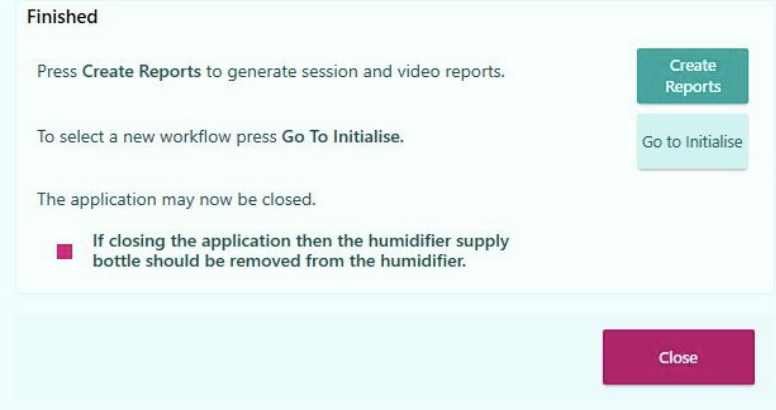

Figure 26. End of session interface.

4.8.3 The humidifier supply bottle should be removed from the humidifier to enable the humidifier to be kept dry between sessions.

4.8.4 Make sure all gas tanks used are closed if not needed by other equipment.

4.8.5 See site specific instructions for other shut down procedures.

5. Chemicals:

5.1 LN_2
5.2 Ethanol
5.3 Methanol
5.4 Chamclean

6. Waste Disposal:

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

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