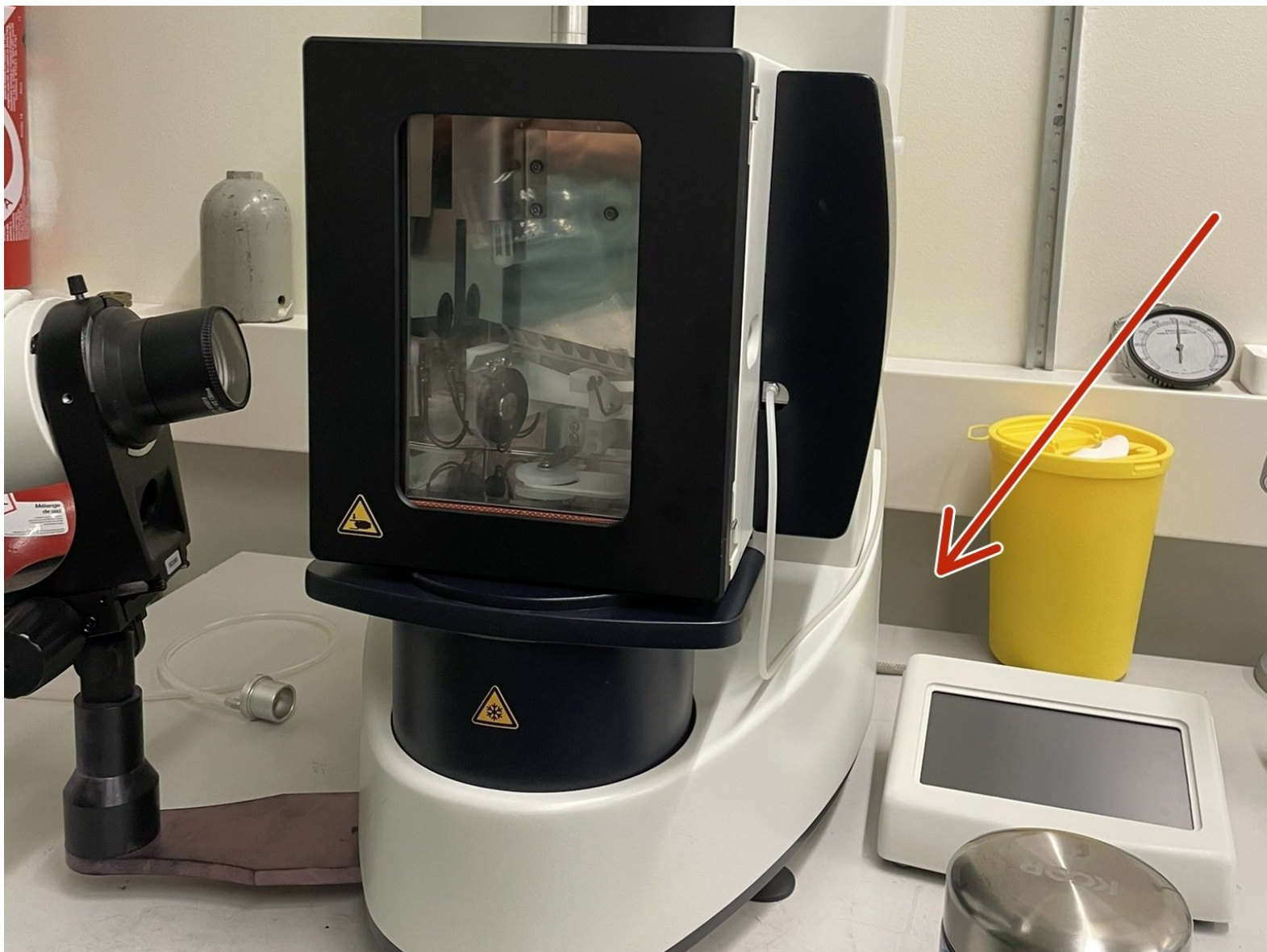
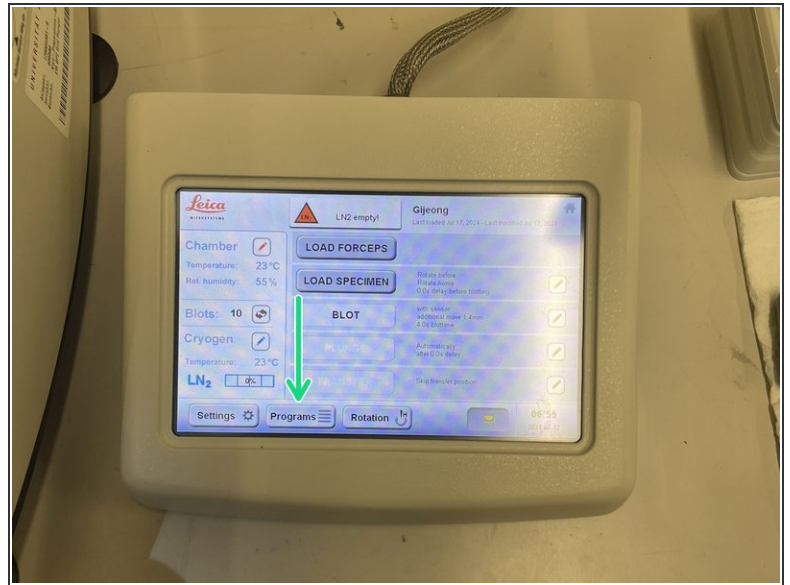
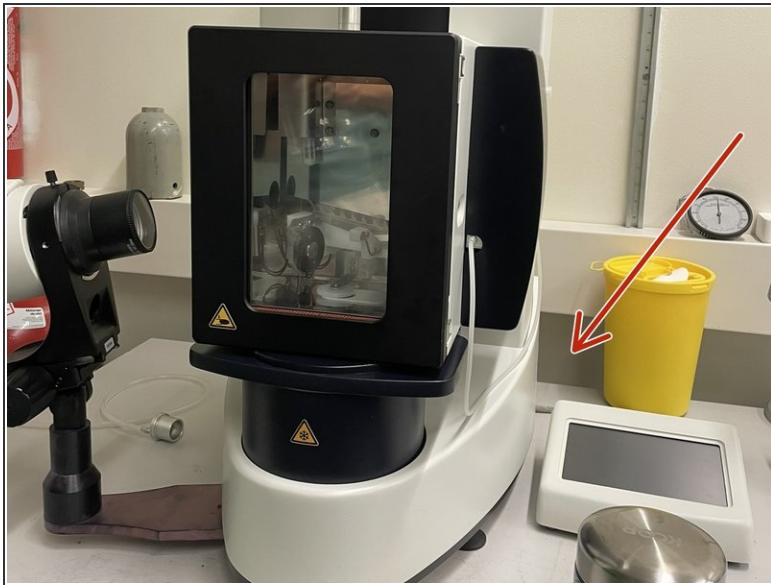


How to use the Leica Grid Plunger GP2

Written By: z mbstaff

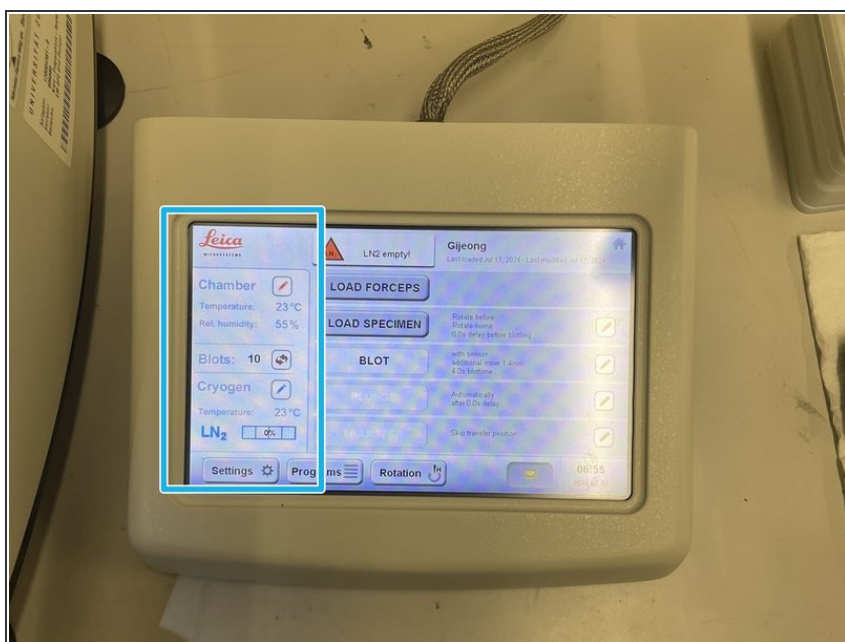


Step 1 — How to use the Leica Grid Plunger GP2



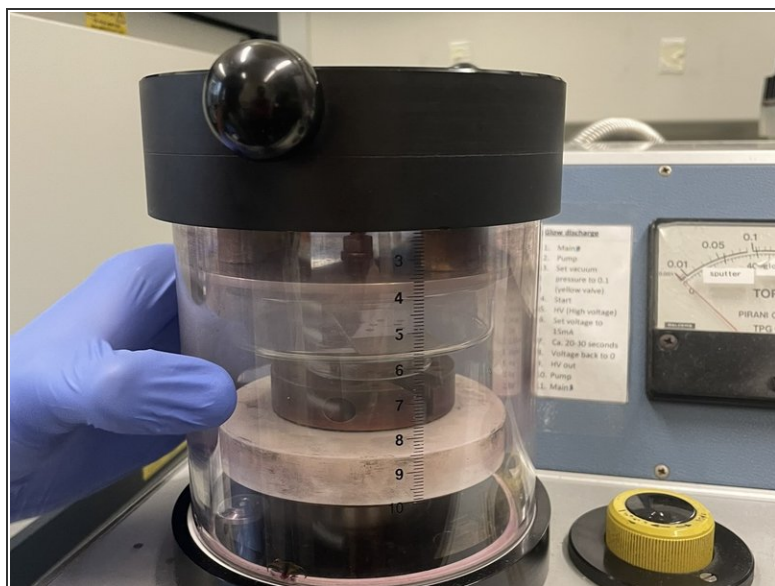
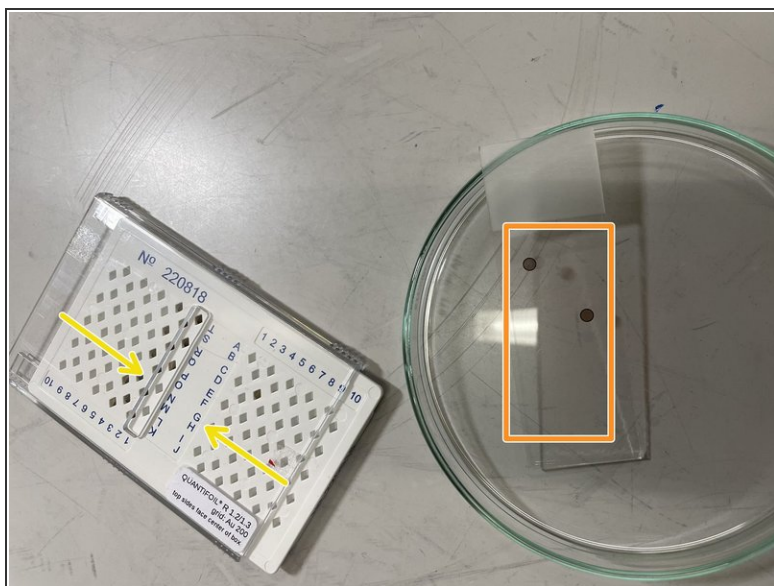
- Switch on the Leica Plunge freezer
- ① After startup the plunger will show warnings to replace the filter, water and nitrogen. Confirm these warnings and continue.
- Choose a program
- assemble the humidifier
- Fill a beaker with more than 100 ml MilliQ water.
 - ① The humidifier needs around **90 ml**
- Fill the syringe with **60 ml** of water.
- Connect the syringe and fill the reservoir.

Step 2 — Choose your settings



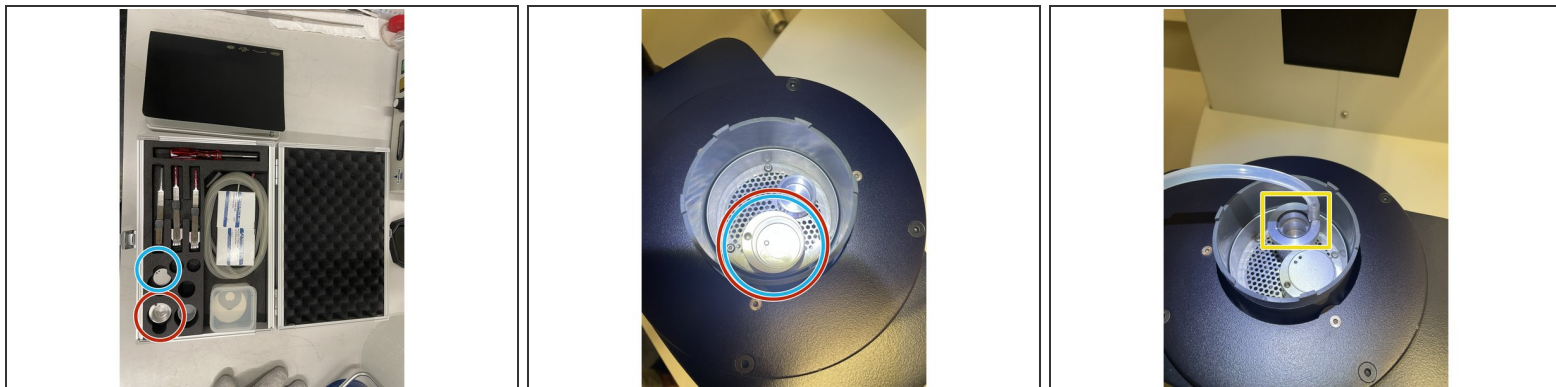
- Detach the syringe and fill it with another **30-40 ml of water**.
 - ① The plunger will give a warning, when a high level of water is reached.
- The saved programs contain settings for temperature and humidity, blotting time and other presets.
 - ① Typical values for single particle freezing are: **10°C** and **99% of humidity**. Blotting times between **3.5 to 4 seconds** work well. The cryogen should be at **-185°C**
 - ① Single particle grids are usually blotted from the side of the support film. There is the option to add an additional move of the blotting paper, which is guided by infrared light. A standard value is an additional move of 1.4 mm to follow the liquid drop.

Step 3 — Glow discharge grids



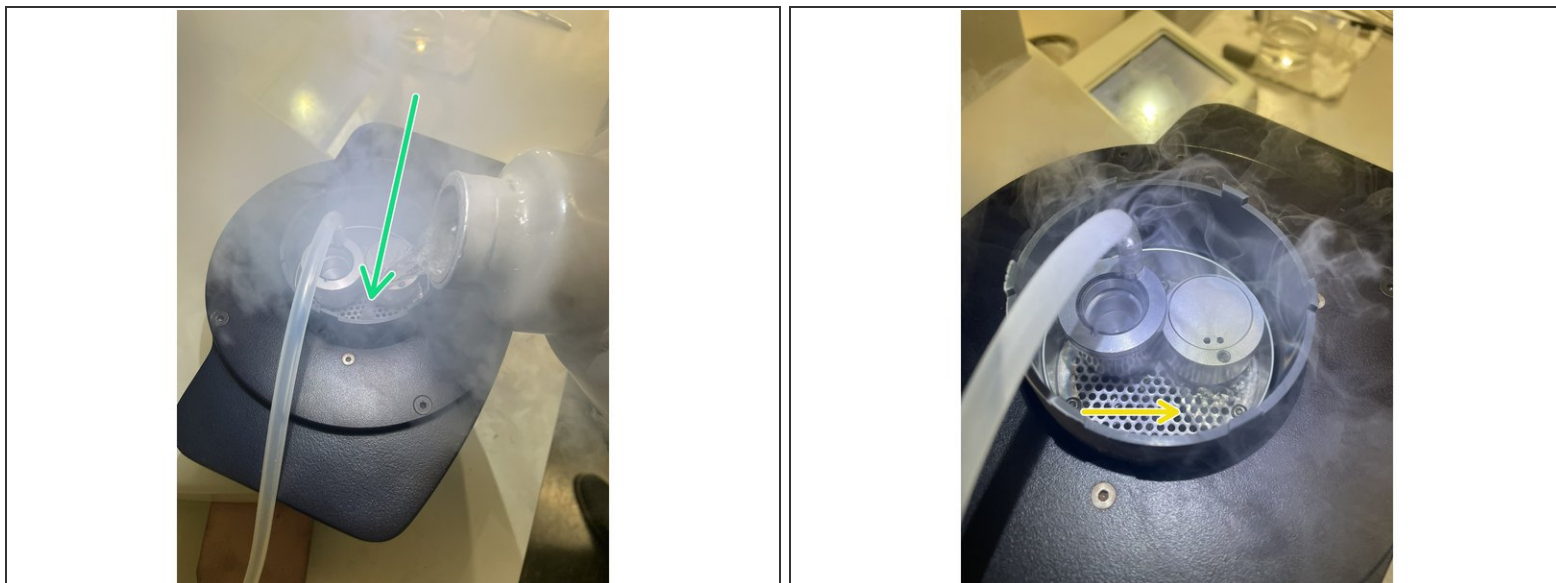
- The carbon side of the cryo EM grid is typically oriented towards the center of the grid box.
- Place the grids with the carbon side facing upwards on a glass slide.
- ① Typical grids are **Au 200/1.2/1.3** grids (*number of mesh squares/diameter of holes/distance between holes*)
- Place the large petri dish without a lid on a small petri dish as a spacer in the chamber of the glow discharger.
- follow the instructions on the glow discharger
- Set it to **20 mA** and glow discharge for **90 seconds**

Step 4 — Prepare the plunge freezer



- Take the metal container out of the accessory box and place it in the Plunge freezer.
 - Place the lid on the container.
 - Add the cryogen supply lid as well.
- ❗ A typical cryogen is a mixture of ethane and propane gas, that is liquid at -185°C

Step 5 — Cooling down



- Pour liquid nitrogen into the dewar of the plunge freezer to cool down the system.
- ⚠ Avoid pouring liquid nitrogen into the cryogen container
- Add nitrogen until the level is reaching the grid at the bottom and until the nitrogen stops boiling.

Step 6 — Fill in the cryogen



- Open the main valve first.
- Open the second valve carefully and watch the cryogen container through the little window.