Problems? Do not attempt to fix them by yourself, find or call Misha or Mike. Microscope is very expensive, as well as downtime. If you need to modify existing protocols, consult with Misha or Michael first.
No food or drinks are allowed in the microscope room

Daily start up procedure:

1. Make sure the microscope is ON (at least some lights on the panels are lit).
2. Make sure that TEMCON and TEM server (icon in system tray) are running; otherwise start those using desktop shortcuts.
3. Check vacuum status of the microscope (PC screen), vacuum gauge on the power supply panel. SIP should be ON and vacuum should be < 2.5 10^{-5} Pa. Note and record the value in the logbook.
4. Fill ACD with liquid nitrogen.
5. HT status is Ready (PC screen). Open HT Control window from "Dialogue" menu and lower HT down to 100 kV. Push "ON" button in the HT window. Wait until HT is ramped up to that value and check dark current "beam current" in the window. That current should be roughly half of HT value, close to 50 - 51 uA in this case.
6. Wait 5 min. If the current is unstable, do not proceed. Use 0.1 kV step and 2 s wait time to ramp HT up to 140 kV using HT program. Watch dark current. If unstable, do not proceed.
7. Wait 10 min. Use 0.1 kV step and 3 s wait time to ramp HT up to 160 kV using HT program. Watch dark current. If unstable, do not proceed.
8. Wait 10 min. Use 0.1 kV step and 5 s wait time to ramp HT up to 180 kV using HT program. Watch dark current. If unstable, do not proceed.
9. Wait 15 min. Use 0.1 kV step and 5 s wait time to ramp HT up to 200 kV using HT program. Watch dark current. If unstable, do not proceed. If dark current value at 200 kV is higher than 104 uA, lower the voltage until the current is roughly half of the voltage, and notify Misha or Mike.
10. ACD should be cold for at least half an hour, if not, wait. Top it off. It usually lasts for at least 8 hrs after that. Refill it as necessary; otherwise ice contamination would be a problem.
11. Cool down a cryoholder in the cryo-transfer station and transfer a grid. Switch in the specimen airlock to "Air" position. Take out blanking plug from the specimen airlock. Put the holder into specimen airlock, slightly push on the holder. Wait for 7 s, and then put switch in the specimen airlock to "pump" position. You should hear a click and rotary pump would start evacuating the airlock. Wait for 3 min; green light should be on by that time. Turn holder ~15 deg clockwise and let it into the column by ~2mm. Turn holder further clockwise until it stops and **slowly** let it into the column, not to break the vacuum. Watch vacuum gauge on the power supply, it should not go out of scale of 10^{-5} Pa. Never let the holder go until it stops against the stage.
12. Wait 30-40 min before opening the cryo-holder's shield. Start the beam followed by daily alignment of the scope.

13. On GATAN computer start EMDASH, fill the forms and setup watch directory for emdash to upload acquired images into EMEN2.

Daily close down procedure:

1. Bring the magnification down to 6,000x – 10,000x and spread the beam simplifying next person's life in finding the beam.
2. Turn off the beam.
3. Close cryo-holder's shield.
4. Double click on "neutralize stage" in TEMCON menu; wait until the stage is re-centered and Z-height is back to zero; acknowledge the prompt.
5. Extract the cryo holder from the microscope. It is critical to wait after switching pump switch in goniometer to "AIR" position to hear hissing sound coming from nitrogen gas cylinder. That means that specimen airlock is, in fact, vented.
6. Put holder in dry pumping station, pump it and start warm-up cycle. Connect plastic tubing to the dewar valve. When warm-up cycle on the holder is completed start "zeolite" cycle, setup time until next session (if known), or for 99 hrs if it is not known when the next session will start. Wait until vacuum in the station recovers to better than $6*10^{-5}$ Torr, then open dewar valve to pump the dewar.
7. Put the blanking plug into the specimen airlock, pump it and leave it there until next person's session. Pay attention to turn the plug 15 deg. clockwise to let the flap into the slot, otherwise V2 will never open and the camera chamber will not be pumped.
8. Insert heater into ACD pipe. Go to "maintenance" submenu, open ACD bake-out window. Start the bake-out, follow prompts. SIP and HT will be turned off allowing all the moisture from the ACD and EM column to be pumped out by turbo pump.
9. Check emdash to ensure that all data were uploaded into EMEN2; log off from the emdash session.
10. Empty the dewar labeled "SPECIMEN" and turn it upside-down to dry overnight.
11. Turn room lights off.
12. It is a good idea to come back in two hours after starting ACD heater and turn on HT.

Daily alignment:

Usually microscope requires only alignment touch-up, unless it was used previously in a non-standard mode. Expect larger changes in high-resolution alignment if tomography was used in the previous session and vice-a-versa.

High-resolution imaging.

1. Push "**Standard Focus**" button to make sure objective lens is at optimal conditions. That will standardize magnification as well. Focus image with **Z-height** adjustment buttons.
2. Go to working magnification intended for current EM session.

3. Select TEM **alpha mode** 2 and spot size 1. Open Alignment window from Maintenance sub-menu. Maximize beam brightness (**gun tilt** controls). Set beam at the crossover using brightness knob. Go to "filament image" in HT window and lower the filament current if needed to observe filament image. LaB6 filament has characteristic image shape with bright central spot and four satellite spots coming from crystal faces. Make the image as symmetric as you can without losing beam brightness (**gun tilt** controls)
4. Align gun and condenser system (**gun shift** at big spots, **beam shift** at small spots; switch between spot size 1 and 5). Do as many iterations, as needed.
5. Correct condenser astigmatism
6. Center condenser aperture
7. Advanced users:
   Adjust pivot points in x- and y-directions (check "**tiltX**" and "**Tilt**" or "**Angle**", then switch to "**tiltY**" and do the same in the other direction)
8. Align rotation center
9. Insert objective aperture and center it
10. Correct objective lens astigmatism
11. Set up low-dose procedure

Tomography

Use lower magnifications for alignment and imaging compared to high-resolution imaging. Typically working magnifications would be below 50,000x. In most cases 30kx and below is enough for tomography. Keep in mind that higher magnifications lead to increased dose per tomogram. Typically, tomography is done on a Slow-scan CCD camera.

1. Use TEM **alpha mode 3**. Do the same alignment as for high-resolution imaging but in **alpha 3**.
2. Start Serial EM from Gatan PC. Make sure that DigitalMicrograph is already running.
3. Go to "Rough eucentricity" in "Tasks" SerialEM submenu, start the procedure
4. Start "fine eucentricity"
5. Set up low dose from SerialEM
6. Acquire new gain reference for the camera (camera menu), calibrate dose using an empty hole in the specimen
7. Set up "Tilt series"
8. Use "walk up" for the first time to go to high tilts. Check autofocus procedure in SerialEM.