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. Login to the microscope PC. That will restore your last used imaging conditions and will start EM CENTER – microscope control program
3. If EM CENTER complains that it cannot communicate with the microscope, make sure that TEM server (icon in system tray) is running; if it does not start it using desktop shortcut.
4. Check vacuum status of the microscope (PC screen), PEG vacuum should be < 15. Note and record the value in the logbook.
5. Fill ACD with liquid nitrogen.
6. HT status is Ready (PC screen). If it is not already up (HT OFF), open HT Control window from "Control" menu and lower HT down to 90 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. Wait 10 min. If the current is unstable, do not proceed.
7. Use 0.5 kV step and 3 s wait time to ramp HT up to 120 kV using HT scheduling program. Watch dark current. If unstable, do not proceed. If dark current value at 120 kV is higher than 68 uA, lower the voltage and notify Misha or Mike.
8. 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 or hydrocarbon contamination would be a problem.
9. Cool down a cryoholder in the cryo-transfer station and transfer a grid. Alternatively transfer a grid into standard holder. Take out blanking plug from the specimen airlock. Switch in the specimen airlock to "Air" position. 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 will 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, it should not go higher than 80. Never let the holder go until it stops against the stage.
10. In case of cryo-specimen wait 30-40 min before opening the holder's shield. Start the beam followed by daily alignment of the scope.
11. 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 3,000x simplifying next person's life in finding the beam.
2. Turn off the beam.

3. Close holder's shield.
4. Double click on LF2 on left hand panel; wait until the stage is re-centered and Z-height is back to zero and beam is off.
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. Put holder in dry pumping station, pump it and start warm-up cycle. Put the blanking plug into the specimen airlock, pump it and turn clockwise until it stops and the tab goes into the slot in goniometer stage. Leave the plug in there until next person's session.
6. If microscope was not reserved for a later time the same day, insert heater into ACD pipe. Go to "control" -> "maintenance" submenu, open ACD bake-out window. Start the bake-out, follow prompts. V1 and V2 will close; HT will be turned off allowing all the moisture from the ACD and EM column to be pumped by diffusion pumps.
7. Check emdash to ensure that all data were uploaded into EMEN2; log off from the emdash session.
8. Turn room lights off.
9. It is a good idea to come back in two hours after starting ACD heater and turn on HT.
10. Log off from the microscope computer
11. Kill DigitalMicrograph on Gatan computer

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. Select spot size 1. Open Alignment window from Control sub-menu. Maximize beam brightness (gun tilt controls).
3. Go to 10 - 15kx magnification
4. Align gun and condenser system (gun shift at big spots, beam shift at small spots; change between spot size 1 and 5). Do as many cycles, as needed.
5. Correct condenser astigmatism
6. Center condenser aperture
7. Align HV center. If you plan to use low magnifications (below 20 – 30kx) use current center alignment instead (OL wobbler)
8. Insert objective aperture and center it
9. Correct objective lens astigmatism
10. Set up low-dose procedure

Tomography

Typically working magnifications would be below 50,000x, in many 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 an Ultrascan US1000 camera.

1. Turn off low dose
2. Use TEM spot size 1. Do the same alignment as for high-resolution imaging. Instead of 6. ("Align HV center"), do current center alignment (use "objective wobbler" button in alignment panel).
3. Start Serial EM from Gatan PC. Make sure that DigitalMicrograph is already running.
4. Go to "Rough eucentricity" in "Tasks" SerialEM submenu
5. Start "fine eucentricity"
6. Set up low dose from SerialEM
7. Calibrate dose using an empty hole in the specimen
8. Set up "Tilt series"
9. Use "walk up" for the first time to go to high tilts. Check autofocus procedure in SerialEM.