

If you do not have badge access to BSL-3 containment in the lab, sign in and out in the log book every time you enter or leave the containment. The logbook is located in 1.204A (change room) on a table to the left from the door. Fill all the fields, with your name, enter and leave times etc.

The microscope was heavily modified from original design by JEOL. Do not attempt to modify existing imaging and other protocols without consulting first with Misha Problems? Do not attempt to fix them by yourself. Find or call Misha or Michael. Microscope is very expensive, as well as downtime.

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, it should be $< 2.0 \cdot 10^{-5}$ Pa. Note and record the value in the logbook.
4. HT should already be at 200 kV. Check if FEG emission is on (TEMCON window). If there is no emission, do not proceed; immediately notify Misha or Michael.
5. Fill ACD with liquid nitrogen.
6. 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.
7. Cool down a cryoholder in the cryo-transfer station and transfer a grid. Switch in the specimen airlock pump switch 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 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 ~ 2 mm. Turn holder further **slowly** clockwise until it stops and let it gradually into the column, not to break the vacuum. Watch vacuum gauge, it should not go out of scale of 10^{-5} Pa. Never let the holder go until it stops against the stage.
8. Wait 30-40 min before opening the holder's shield. Start the beam (by opening beam valve) followed by daily alignment of the scope.
9. 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. **Leave the microscope in mag mode**, not in diffraction or LM.
2. Close the beam valve.
3. Close 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 at least two clicks coming from valves. That means that specimen airlock was, 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, 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; insert heater's connector into receptacle on the microscope side. Go to "maintenance" submenu, open ACD bake-out window. Start the bake-out, follow prompts. That will allow 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. Delete all the image files/folders acquired during the session from DE PC' SSD ("Raw Frames\DE20\" folder) **EXCEPT** for "References" folder.
10. Empty the dewar labeled "SPECIMEN" and turn it upside-down to dry overnight.

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.
3. Select TEM alpha mode 2 and spot size 1. Open Alignment window from Maintenance sub-menu. Start anode wobbler; make beam to breeze evenly (**gun tilt** controls). Stop anode wobbler.
4. Align gun and condenser system (**gun shift** at big spots, **beam shift** at small spots; change between **spot size 1 and 4**). Do as many iterations, as needed.
5. We modified spot size 5 conditions (Jan 2016) to use it only for low mag (1,500x – 2,500x) imaging on DE-20 and focusing mode in low dose imaging; use lower spot sizes otherwise.
6. Correct condenser astigmatism
7. Center condenser aperture
8. Adjust tilt compensator
9. Align current center

10. Insert objective aperture and center it
11. Correct objective lens astigmatism
12. Set up low-dose procedure
13. Beam is parallel at 2 μ m diameter
14. If you plan to use DE-20 camera for imaging, make sure DE server is running on DE computer, if not, start it using desktop shortcut. Same for DE-WEB interface to ensure that the server knows about the microscope state. Start DE-IM data acquisition program. It is a good idea to acquire new dark and gain references at the beginning of session. Be careful with DE-20 camera; avoid excessive exposure of it with electron beam. It is easy to damage DE sensor with electrons. Avoid exposing it with intense electron beam, avoid uneven exposures of the sensor when beam covers only part of it; the sensor should always be completely covered by the beam. You might try using DE-20 survey sensor for search and focus modes, it is much faster than the main sensor and much less sensitive to the beam. Use survey sensor in live mode. Do not forget to check "Final image" and "Raw data" in DE-IM if you plan to save images in movie mode; remember that saving raw frames will increase amount of data stored on disk by factor of 25 if you are making 1s exposures or even higher for longer exposure or higher acquisition rates; think of available disk space. Empty the solid-state drive at least by the end of the session or earlier if the disk is getting low on space. Starting data uploading into EMEN2 is critical for DE imaging, do it as early in session as you can.

Note. Omega filter operation: do not degauss omega filter, we found it to be very stable; do not adjust its isochromaticity. If needed touch up filter lens to center zero-loss peak, and slit position; the rest is normally very stable.

Tomography

Use lower magnifications for alignment and imaging compared to high-resolution imaging. 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 a Slow-scan US4000 or on DE-20 cameras.

1. Use TEM alpha mode 3. Do the same alignment as for high-resolution imaging but in alpha 3.
2. Start Serial EM from DE PC. Make sure that DigitalMicrograph is already running.
3. If you plan to use DE-20 camera for imaging, make sure DE server is running on DE computer, if not, start it using desktop shortcut. If MicroManager or DE-IM are running, kill them before starting SerialEM since DE server supports currently only a single client.
4. For US4000 tomography switch in the "Camera Properties" to that camera, SerialEM will use DE-20 by default
5. Go to "Rough eucentricity" in "Tasks" SerialEM submenu, execute that
6. Start "fine eucentricity"

7. Set up low dose in SerialEM
8. Calibrate dose using an empty hole in the specimen
9. Set up “Tilt series”
10. Use “walk up” for the first time to go to high tilts. Check autofocus procedure in SerialEM.

Magnifications and pixel sizes for DE-20 camera, April 2015

Nominal EM Mag	DE-20 pixel size, Angstrom	DE-20 Magnification
500000	0.106	603773
400000	0.134	477612
300000	0.179	357542
250000	0.217	294931
200000	0.273	234432
150000	0.377	169761
120000	0.478	133891
100000	0.575	111304
80000	0.717	89261
60000	1.032	62015
50000	1.209	52936
40000	1.466	43656
30000	1.863	34408
25000	2.22	28862
20000	2.79	22926
15000	3.58	17852
12000	5.06	12657
10000	5.74	11150
8000	7.64	8377
6000	8.7	7356
5000	11.22	5704
4000	16.58	3860
3000	19.61	3264
2500	23.76	2694
2000	30.87	2073
1500	39.68	1613
	Low magnification	
1500	22.72	2817
1200	36.78	1740
1000	43.14	1483
800	77.38	827

600	101.3	632
500	122.1	524
400	151.5	422
300	188.5	339
250	226.9	282
200	296.7	216
150	387.9	165
120	488.2	131
100	562.9	114
80	711.2	90
60	939.7	68
50	1075.2	59