PHILIPS CM120 TEM

OPERATING INSTRUCTIONS

These instructions are intended to serve as a reminder for individuals who have had prior training on the operation of this microscope. If at any point you have a question or concern about the instrument's operation or performance, please ask for assistance.

The PHILIPS CM120 TEM is a shared instrument located in EICN/CNSI Lab B146B. It is dedicated to serve both UCLA and the outside EM community for their research and educational needs.

All users should follow common rules to ensure continued operation of the microscope. Please keep in mind that misuse committed by one user will have a direct effect on the next user. Serious abuse may disrupt the normal operation of the TEM and will affect all users. Failure to comply may result in revoking user access to the TEM.

Please check the standard settings of the microscope before imaging and restore it again when you have finished. Malfunctions or other irregularities should be recorded in the Log Book and reported to the facility manager for corrective action.

Facility Managers:

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Brief CM120 description:

- The CM120 is a 120 kV Transmission Electron Microscope (TEM).
- Accelerating Voltage 20, 40, 60 80 100 120kV
- Beam source: LaB6 filament.
- Vacuum system: Mechanical (MP), Oil Diffusion (ODP) and Ion-Giter (IGP) vacuum pumps.
- +/-70° fully computer controlled Goniometer stage
- Standard single tilt +/- 70° specimen holder
- Gatan 626 single tilt +/- 70° Cryotransfer Holder
- Liquid Nitrogen cooling system for CryoEM
- The CM120 is capable of recording images on either plain film or CCD camera.
- 2k x 2k TIETZ 214 CCD camera. 14 µm pixel size.
- CM120 operation software – CM Remote Control
- Digital Camera Software - EMMENU4, EM-Tools

Supporting Instruments:

- Gatan Dry Pumping Station Model 655
- Gatan SmartSet Model 900 Cold stage Controller
- Denton Vacuum Bench Top Turbo IV Coating System
- Vitrobot Automatic Plunger/Freezer
- Manual Plunger
- Cryo Holder

Application fields:

- Thin sections of fixed embedded specimens
- Negative staining, metal shadowing of biological (proteins, viruses, cell organelles, cells, etc.) and material science specimens.
- Immuno-electron microscopy
- Single Particle Cryoelectron Microscopy
- Cryoelectron Tomography
Standard setting of the instrument:

- Gun, column and chamber are permanently pumped. HIVAC and UHV green LEDs indicate that the Vacuum is READY.
- High Tension (HT) is set to 120kV (60, 80 and 100kV usable). Green LED above the HT button lit to indicate that the high voltage is on.
- LaB6 filament is desaturated.
- 100 µm condenser aperture is inserted.
- 100 µm objective aperture is inserted.
- MAG set to SA Mode 5600x
Simple/casual users:

- Check **High Tension** (i.e. 120kV) in Parameters, **Cathode** (LaB6 highlighted) and **Fil Limit** (highlighted, reading 20 to 30) in Configuration.
- To saturate the filament turn the **Filament** knob clockwise until **Filament Limit** is reached. The process is automated; every step will take 5s. The whole procedure should be completed in 2-3 min.
- From Page Menu set the Mode: **Low Dose TEM Bright Field**.
- Go to 5600x MAG and **Reset Holder** in **COMPUSTAGE/COMPUCTRL**. Set spotsize to 3-4, and find a small recognizable object on the viewing screen using the Joystick X/Y control, adjust illumination (**Intensity** knob) and press the **Auto Focus** knob.
- Press **A-WOBBLER** in **COMPUSTAGE** (this will initiate forth and back tilting of the goniometer to +/- 15 deg). Use the Z Control on the Joystick to align **Z High** by minimizing the apparent movement of the centered feature.
- Stop **A-WOBBLER** and focus desired object using the Focus knob.

Taking pictures on CCD Camera.

- Open and Start **EMMENU4**. Create new View Port (up to 16)
- Set Camera Format (2K x 2K, 2K x 1K, 1K x 1K), Bin (x1, x2)
- Choose or create Image Folder and Image Name.
- Find the object of the interest, set appropriate MAG, align illumination
- Press the Camera Button to acquire image on CCD camera.
- Rename images (if necessary) and save in your folder.

Ending your session

- Set MAG to 5600X.
- Spread the beam over the viewing screen
- Reset Holder
- Completely desaturate the filament
- Take out the holder and remove your grid.
Standard Operating Procedures (not Cryo)

Startup Procedures

1. Sign in the Log Book and log in to CM120 computer
   ❖ Take a look at previous comments made by the last few users.
   ❖ Write your name, username, date, and time in the Log Book.
   ❖ Samples have to be examined.
   ❖ Write down the IGP and P3 numbers (on vacuum page).

2. Check the following
   ❖ On VACUUM page: make sure the vacuum is ready (HIVAC and UHV should be lit).
   ❖ For normal operation IGP < 30 and P3 < 30.
   ❖ Check V3, V4, V5 and V7 are opened.
   ❖ On Configuration page make sure Cathode is set to LaB6 and Filament Limit is highlighted.

3. Loading and inserting specimen
   ❖ Load the specimen in the standard single tilt holder. Make sure the specimen is properly fastened in the specimen holder
   ❖ Check that the O-ring of the holder is clean.
   ❖ Make sure the filament is completely desaturated.
   ❖ Carefully insert the holder into the goniometer compustage with the pin on the holder in the 5 o’clock position. The prepumping cycle will initiate and the red indicator light will come on.
   ❖ When the red light goes off (about 1 minute), rotate the specimen holder fully counterclockwise until unable to rotate further and insert into the microscope carefully as it will be sucked into the vacuum of the microscope.
   ❖ Wait for IGP < 30 before proceed furthermore. Do not use the microscope if the IGP reading is higher than 30 to prevent damage to the LaB6 filament.
   ❖ Reverse these directions to remove the specimen holder from the microscope. The filament should be fully desaturated!

4. Bringing the microscope to operating conditions
Check **High Tension** (i.e. 120kV) in Parameters, **Cathode** (LaB6 highlighted) and **Fil Limit** (highlighted, reading 20 to 30) in Configuration.

To saturate the filament turn the **Filament** knob clockwise until **Filament Limit** is reached. The process is automated; every step will take 5s. The whole procedure should be completed in 2-3 min.

From Page Menu set the desired Mode: **TEM Bright Field** or **Low Dose TEM Bright Field**.

Go to 5600x MAG and **Reset Holder** in **COMPUSTAGE/COMPUCTRL**. Set Spotsizie to 3-4, find a small recognizable object on viewing screen using Joystick X/Y control, adjust illumination (Intensitity knob) and press the **Auto Focus** knob.

Press **A-WOBBLER** in **COMPUSTAGE** (this will initiate forth and back tilting of the goniometer to +/- 15 deg.) Using **Z** Control on the joystick, align **Z High** by minimizing the apparent movement of the centered feature.

Stop **A-WOBBLER** and focus desired object using the **Focus** knob.

5. **Taking pictures on the CCD Camera.**
   - Open and Start **EMMENU4**. Create new View Port (up to 16)
   - Set Camera Format (2K x 2K, 2K x 1K, 1K x 1K), Bin (no, 2, 4, 8)
   - Choose or create Image Folder and Image Name.
   - Find the object of the interest, set appropriate MAG, align illumination
   - Press the Camera Button to acquire image on CCD camera.
   - Rename images (if necessary) and save in your folder.

6. **Ending your session**
   - Set MAG to 5600X.
   - Spread the beam over the viewing screen
   - Reset Holder
   - Completely desaturate the filament
   - Take out the holder and remove your grid.