

JEOL 4000 EX II Handbook

General Guidelines for Booking the Microscope

1. Do not book time unless you are a qualified user.
2. **User Levels** There are two levels of user for each particular microscope and they may be defined as follows:
 1. **Operator**, unrestricted use, including high voltage changes.
 2. **User**, unrestricted use, includes days, nights, weekends and holidays.

NOTE: Users will be expected to be competent enough to operate the microscope without having to refer to the user handbook or manuals.

JEOL 4000 EX II Startup & Routine Alignment Procedures

Sign in the microscope log.

Enter the date, your name, sample identification, microscope voltage, the beginning film number and your research notebook identification in the spaces provided.

Accelerating Voltage

An Operator will bring the microscope up to the requested voltage prior to the beginning of your session.

Fill the anti-contaminator cold traps

(Always wear safety glasses when handling liquid nitrogen.) Be sure that the viewing port is covered and the binoculars are safely out of the way of excess liquid nitrogen drops. These traps need to be topped off every 4 hours. Take care not to let ice inside the cold traps when refilling; this could lead to microscope vibrations.

Specimen loading and Insertion

Most JEOL 4000 are side entry and standard JEOL insertion protocols should be followed.

Heating Up the Filament

Do not heat filament until gun vacuum is less than 6×10^{-6} Pa and the column vacuum is within 10% of value before inserting specimen.

1. Push **Airlock** release button (Opens gun to column and column to viewing port and camera.).
2. Check to be sure filament light comes on (20-30 second delay).
3. Turn on **Filament**. Slowly (30 seconds) turn up heater knob to position 4, wait 10 minutes. After 10 minutes, increase heater knob $\frac{1}{2}$ step every 30 seconds until emission is visible on screen, then slow to $\frac{1}{4}$ step every 30 seconds to saturation.

Note: During filament heating the column and gun vacuums should not exceed 2.2×10^{-5} Pa and 7×10^{-6} Pa, respectively, in winter these numbers should be much lower (about half) in cold climates.

The following chart lists the (suggested film sensitivity), proper standing currents, saturation currents and objective focus values (microscope specific) for selected operating voltages with the specimen at the standard z-height:

Film Sensitivity	Voltage (kV)	Standing Current (μ A)	Saturation Current (μ A)	Objective Lens Setting (specified by JEOL factory for each TEM)
12	400	122.7	128	7.15
	350	106.5		6.57
13	300	91.1		5.72
	250	75.9		5.01
15	200	61.1	68	4.28
	150	45.5		3.54
16	100	30.2	35	2.76

Setting Film Sensitivity This is shown on page 1 of the JEOL CRT.

1. Type **FSE** and return into the JEOL computer keyboard.
2. Enter the appropriate number from chart above and return.

Z Height Adjustment

1. Set spot size=2, Mag \geq 25K.
2. Turn **Obj** knob so that objective lens is just in the middle of the range for readout =7.15 (for 400kV) on CRT for magnifications between 25-100kx.
3. Change Z height control to move specimen up or down to minimize image contrast.

Note: This adjustment must be redone whenever the specimen stage is tilted.

Condenser Aperture Alignment

1. Focus beam to a small spot using **Brightness** knob (third condenser lens control).
2. Center illumination using **Shift X,Y** (beam deflectors, on panel).
3. Spread the beam with **Brightness** knob. Center the condenser aperture.
4. Repeat steps 1 - 3 until beam converges to center when focusing/defocusing.

Condenser Astigmatism Correction

1. Engage **Condenser Lens Stigmator**.
2. Sharpen filament image using **Def X,Y** (in drawer).
3. Disengage **Condenser Lens Stigmator**.

Gun and Condenser Lens Alignment

1. Ensure filament is under saturation (see table above).
2. Mag \geq 50K.
3. Select the C2 aperture and nominally center it.
4. Use **Gun Def** (in drawer) to tilt the gun for symmetric and bright illumination.
5. Set spot size=5
6. Center illumination using **beam shifts** (on panel).
7. Set spot size=1.
8. Center illumination using **Gun Shifts** (in drawer).
9. Repeat steps 3 - 6 until there is no shift with spot size change.
10. Disengage **Gun** button (in drawer).

Beam Tilt-Shift Purity

Note: Specimen must be set to the correct height / objective focus before making this adjustment.

Tilt purity

1. Set spot size=2, Mag \approx 100K.
2. Focus beam using **Brightness** knob.
3. Engage **Condenser Alignment Tilt** button (in drawer).
4. Toggle **Def Wobbler Tilt X** (in drawer, amplitude and frequency of wobbler should be set to maximum, 5).
5. Minimize motion of filament image using **Def X** and **Shift X**.
6. Repeat steps 4 - 5 for Y.
 - a. Disengage **Def Wobbler Tilt** and **Condenser Alignment Tilt**.
 - b. Center beam with beam deflectors.

Diffraction Alignment

1. Using **SAM/ROCK** (selected area magnification) mode choose and center the area selecting aperture.
2. Engage **Diff** button on panel.
3. Focus using **Diff Focus**.
4. Engage **Proj** (in drawer).
5. Center spot using **Shift X,Y** (in drawer).
6. Remove the area selecting aperture and spread the beam.
7. Engage **Int Stig** (in drawer), correct astigmatism in caustic figure using **Def X,Y**.

Shift purity

1. Set camera length to preferred length for selected area diffraction.
2. Defocus beam using **Brightness** knob (*fully clockwise*).
3. Obtain caustic with **Diff Focus**, use **DEF-Proj Shift** to center the spot.
4. Correct astigmatism using **Intermediate Stigmators** (in drawer).
5. Engage **Condenser Alignment Shift** button (in drawer).
6. Toggle **Def Wobbler Shift X** (in drawer, amplitude set to 3 and frequency set to 5).
7. Minimize motion of filament image using **Def X** and **Shift X**.
8. Repeat steps 4 - 5 for Y.
9. Disengage **Def Wobbler Shift** and **Condenser Alignment Shift**.

High Voltage Centering

1. Center point of interest on specimen (mag at least 200K)
2. Defocus illumination with **Brightness** knob.
3. Engage **HT Wobbler** (right panel).
4. Engage **Bright Tilt** (left panel).
5. Using **Def X,Y** (on panel) eliminate image translation.

6. Disengage **Bright Tilt** and **HT Wobbler**.

Correct objective astigmatism

1. Engage **Mag**.
2. Increase Mag to something high 200-500kx.
3. Turn **Obj** knob to minimize contrast. (Should occur at values listed in table on page 2, if not, re-adjust z-height).
4. Engage **Obj Stigmator** button (in right drawer).
5. Use **Def X,Y** (in drawer) to eliminate contrast in an amorphous region.

Note: Ideal contrast in amorphous region should be matted (black and white dots) at over or under focus, with no streaking or directionality. At minimum contrast condition no contrast should be present. If acquiring images on digital camera, the FFT (fast fourier transform) will have concentric rings when under or over focused and will be without structure when in-focus.

6. Disengage **Obj Stigmator** button.

Notes on using the Gatan Slow Scan CCD Camera

1. Turn on camera (if it is not already on) and turn on the cooler switch (the green light comes on above switch). Launch **Digital Micrograph** software. Allow at least 2 hours for the camera unit to reach thermal equilibrium. The camera may be used without cooling but the images will be noisy
2. Set the microscope magnification to a suitable value. A suitable magnification will depend on exactly what you are trying to image. Typically magnifications around 200K to 400K are most suitable for high resolution since they will allow you to set the image astigmatism by monitoring the thin amorphous contamination film that is found at the edge of most specimens.
3. To view images via the Gatan camera engage **Screen** button (the viewing screen lifts allowing the electrons to reach the CCD camera).
4. Use **Image Shift** (on panel) when small image shifts are needed.

Note: To minimize the number of X-rays hitting the camera the CCD camera is offset to the left of the center of the viewing screen. The center of the CCD camera is at the 9 o'clock position just of the edge of the small viewing screen.

5. Since the phosphor grains in scintillator of the camera are sensitive to thermal variations the gain reference image should be checked each session. This is done by collecting a **Gain Normalized Image** of uniform illumination through a hole in the specimen. If structure is observed in this image then collect a new **Gain Reference** image. Click on **Collect Gain Reference** and follow the instructions.
6. So that Digital Micrograph will automatically read the microscope magnification, set the JEOL computer to external control. Using JEOL keyboard type **EXT 1** and return.

Notes on Recording Micrographs

The JEOL camera holds 50 plates. Beware that after the 50th plate has been used the counter for the number of unused plates resets to 50.

1. Micrographs may be recorded by either automatic or manual exposure.
2. The Automatic exposure should only be used for bright field images. Dark field images and diffraction patterns will not usually expose correctly if you attempt to use automatic exposure. Some users have developed a way of using the automatic exposure setting to estimate the exposure of diffraction patterns (and/or dark field images). Such a system will vary from sample to sample and user to user there are no hard and fast rules. The best rule for recording diffraction patterns and dark field images is to bracket several exposures.
3. The automatic exposure system measures the electron current that is incident on the screen and calculates the exposure assuming that the image covers the whole screen.
4. If the small screen is in position the metering system will calculate the exposure based on the illumination on the small screen. If your sample is not of uniform contrast, it may be better to locate your feature of interest on the small screen and let the microscope determine the exposure from the small screen.
5. Manual exposure is selected when the Auto/Shutter button is in the released position and it is not illuminated. Manual exposure times are selected using the rocker switch above the button. Automatic exposure is selected when the Auto/Shutter button is engaged and illuminated.

Note that is advisable to leave the Film Advance button in the manual position. The green Auto light should be off. If you use the AUTO position, there is always a plate left under the screen. This plate is wasted when you raise the screen to use the Gatan camera.

6. When you have selected the area you wish to photograph, set the exposure value (upper right panel), manually or automatically, and press the Photo button once.
7. The microscope advances a plate under the viewing screen, the Photo button light will now be on.
8. Press the Photo button a second time, this exposes the plate. The small green LED above the Photo button will be illuminated while the exposure is taking place.
9. During this process the information field (descriptive text, your film number, voltage and magnification) is also written on to the film.
10. Upon completion of the exposure the plate will be retracted and drop into the exposed plate container. The Photo button light will now be off.
11. If you wish to make multiple exposures you should press the Photo button twice when exposing the plate for the first time, this will disable the plate withdrawal system and allow you to expose another image or diffraction pattern on the same plate. Pressing the Photo button twice for the second exposure allows you to record three exposures on one plate. You may make as many multiple exposures as you wish, simply by pushing the Photo button twice each time you expose the plate.

Note: In the event of a **camera jam** there will be a shrill alarm sound coming from the microscope, often *changing the magnification will quiet the alarm*. If this occurs follow steps 1 and 2 for **Shutting down the JEOL 4000 EX**. If the alarm sound continues, turn the high voltage **off**, depress the **reset** button (red) below the JEOL 4000 keyboard; this will shut off the alarm. Then **call for assistance** for fixing the camera jam.

Shutting down the JEOL 4000 EX at the end of the day.

1. Turn down the **Filament** heater.
2. When filament is off push the **Airlock** release button (Isolates the gun from the column and the column from the viewing port and camera.).
3. **Remove Objective and Area Selecting apertures.**
4. Return sample to **HOME** position.
5. If you are a qualified user or operator, remove your specimen. Otherwise ask a qualified operator to remove it or leave it in the microscope and put a note on the column stating that your sample is in the microscope.
6. Replace the photographic plates in the camera as described in **Changing Plates in the 4000 EX**. Always leave a full 50 plates for the next user! The only exception is if the next user agrees to fewer plates.
7. After the camera is back to high vacuum (<50 μ A). Place the heaters in the cold traps for the anti-contaminating devices (make certain that the hoses for the nitrogen boil off are directed away from the microscope towards the back and that the heaters are pushed all the way down), then depress the **ACD HEAT 1** and **ACD HEAT 2** buttons (in the left drawer).
8. **Turn off the panel lights and lights on the keyboard in the drawer.**
9. Set **Magnification** to a **500kx**; this keeps the column warm and stable.
10. **Turn down the brightness and contrast on the JEOL CRT.**
11. **Complete the Login form, including film numbers and comments.**

Changing Plates in the 4000 EX

Filament should be off and the airlocks closed, high tension stays on. Always wear rubber gloves when handling the film boxes and cassettes.

1. Turn the valve on the front of the camera chamber clockwise from vertical to horizontal to allow the nitrogen to fill the camera chamber.
2. When the camera door opens withdraw the camera and replace the film catch box with an empty one and replace the film box with a new one. Make certain that both boxes are seated properly.
3. Push the camera back into the chamber. **Inspect the o-ring on the camera door, wipe off any hairs or dust using your glove.**
4. Close the camera door and turn the valve back down to the vertical position.
5. Check the camera vacuum level on the meter below the high voltage tank on the west side. The camera should reach high vacuum conditions (<50 μ A) in 15 minutes.
6. To reset the unexposed film counter, type in **FUN** and enter 50 <return>.

SPECIAL CASE:

If you only use the microscope in the early part of the day and there is another user booked for later that same day:

Turning down the JEOL 4000 EX in the middle of the day.

1. Turn down the **Filament** heater.
2. When filament is off push the **Airlock** release button (Isolates the gun from the column and the column from the viewing port and camera.).
3. **Remove Objective and Area Selecting apertures.**
4. Return sample to **HOME**.
5. If you are a qualified user or operator, remove your specimen. Otherwise ask a qualified operator to remove it or leave it in the microscope and put a note on the column stating that your sample is in the microscope.
6. **Change the film and contact the next user.**
7. **Do not run the ACD heaters. Top off the anti-contaminators with liquid N₂.**
8. **Turn off the panel lights and lights on the keyboard in the drawer.**
9. Set **Magnification** to a **500kx**.
10. Turn down the brightness and contrast on the JEOL CRT and the sleep or power off the PC monitor.
11. Leave the Gatan camera electronics box **on**, the **Peltier cooler on** and the PC **on**. If using the TV camera, shut off the electronics box. Remember to save any images recorded using Digital Micrograph onto external media and delete your files from the PC.
12. **Complete the Login form, including film numbers and comments.**

Department of important information:

1. Buttons to never push:

The **HT COND** on the far left side of the left panel. This conditions the high voltage with a 10K overvoltage.

The **reset** button in the right drawer on the far right. The resets the JEOL computer and results in a high voltage shut down.

2. Earthquake or Tornado or building evacuation

Turn the **Filament** heater to off.

Leave room.