

JEOL 2500SE (S)TEM

Operation Manual

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Introduction

The fundamental basis of electron microscopy is the interaction of electrons with matter. A beam of electrons hitting a thin sample will be transmitted and scattered at different angles and intensities giving rise to an image. This interaction, however, is very complex and several factors such as the coherence of the electron source, the inherent aberrations of the lenses, and many more greatly affect the quality of the sample image. A key step in minimizing these effects is to properly align the microscope prior every use.

Parts of the Microscope

Overall Schematic

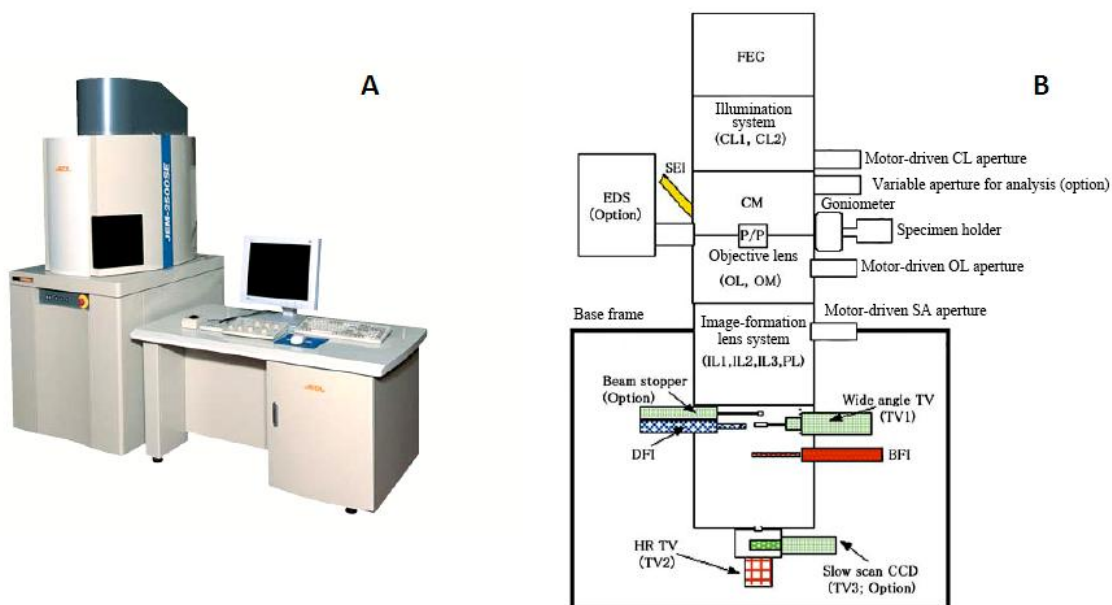
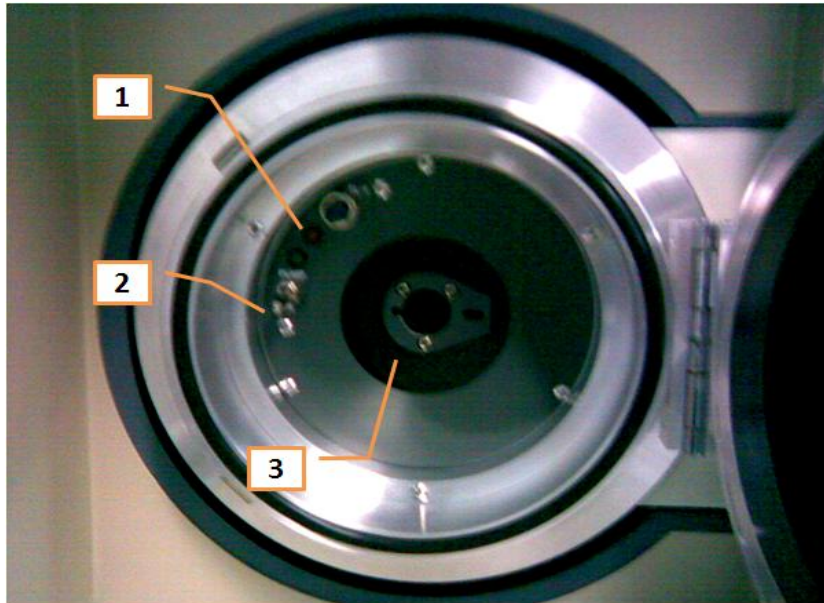


Figure 1 A) Picture of the JEM2500SE (S)TEM. B) Block diagram of the parts of the microscope.

Side Entry Specimen Holder



- 1. LED indicator
- 2. Air/Pump Toggle Switch
- 3. Side entry
- 4. Black cap
- 5. Specimen cavity & clamp



Figure 2 The side entry goniometer stage & specimen holder.

The Control Panels



- | | |
|--------------------------------------|-------------------------------|
| 6 Z-shift control buttons | 16 STIG: stigmatation control |
| 7 Stage shift controls | 17 CRS for beam alignment |
| 8 Stage tilt controls | 18 Multi-function knobs |
| 9 CRS (coarse) button for stage | 19 Magnification knob |
| 10 Beam power button ON | 20 CRS for focus |
| 11 Image shift RESET | 21 Focus adjustment knob |
| 12 CRS for image shift | 22 Contrast adjustment knob |
| 13 x,y control knobs for image shift | 23 CRS for brightness |
| 14 WOB: beam wobble button | 24 AUTO brightness & contrast |
| 15 DEF: define multi-function knobs | 25 Brightness control knob |

Figure 3 The 2500SE control panels.

The Graphic User Interface

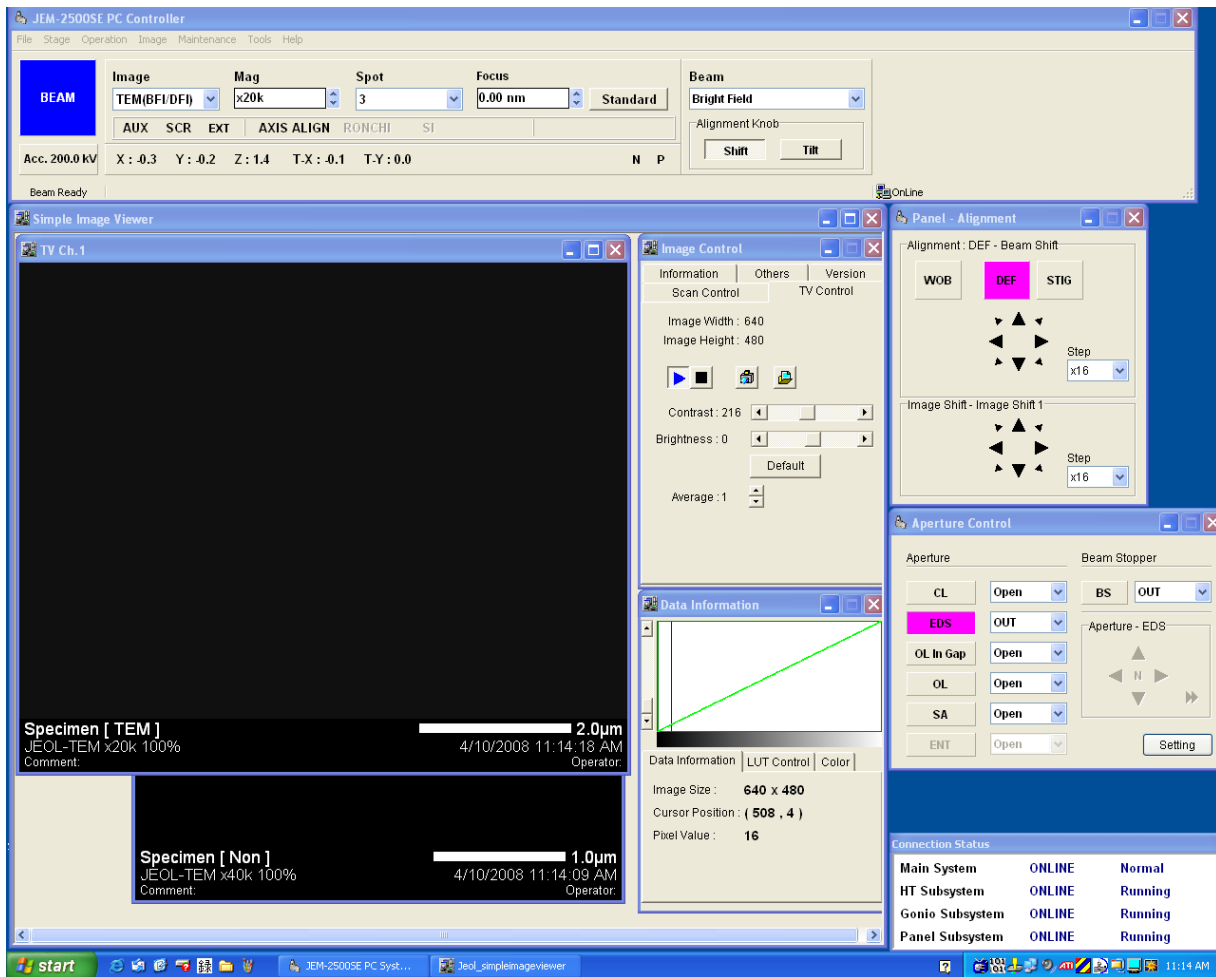


Figure 4 The JEOL 2500SE GUI.

Procedures

Sample Loading

1. With a pin or any sharp object, carefully open the clamp [5] of the specimen holder and insert the grid with the sample. Close the clamp back to secure the grid in place.
2. On the goniometer assembly, switch to *PUMP* [2] to activate the vacuum pumps. The yellow LED [1] should turn on at this point.
3. Holding the specimen holder by the black cap [4], slowly insert the holder into the side entry cavity [3] aligning the notch to the groove on the 9 o'clock position.
4. Twist the holder counterclockwise until a natural stop. Twist further counterclockwise while guiding the holder until the whole length is sucked in.
5. Wait for the green LED to turn on then close the side entry holder.

Column Alignment

Condenser Alignment

1. Turn the beam on either by pushing the beam *ON* [10] button on the control panel or *BEAM* on the software.
2. Insert the condenser lens aperture by clicking on the *CL* button on the *Aperture Control* window on the software and selecting an aperture size (typically 2) on the adjacent drop-down menu.
3. Check the alignment of the aperture by cranking the brightness knob [25] in both directions. Check to see that the beam spreads concentrically on opposite directions from the crossover.
4. Use the arrow keys on the same window to align the aperture.

Eucentric (Z) Height Adjustment

1. Spread the beam using the brightness knob until the sample is visible. Use the trackball to center on a relatively large hole on the carbon film.
2. Click on *WOB* on the control panel [14] or on the GUI. The image should start wobbling at this point. Right click on *WOB* and select *Image XY*.
3. On the GUI click on *DEF* then right-click to select *Beam shift*.
4. Use the z-shift buttons [6] on the specimen stage control panel to adjust until the wobbling is minimized.
5. Click on *WOB* again to turn off the wobbling.

Stigmation Adjustment

1. Select a hole on the specimen and increase magnification [19] to about 500K.
2. Narrow the beam enough to fit the screen and center it using the multifunction knobs [18].

3. If the shape of the beam is not circular, the condenser lens has astigmatism. Start adjusting by clicking on *STIG* either on the control panel [16] or the GUI then right click to select *CL Stig*.
4. Use both the X & Y multifunction knobs to modify the shape of the beam until it becomes circular.
5. Spread the beam just enough toward the other direction of the crossover and do the same.
6. Click on *STIG* to exit stigmatism mode.

Tilt Balance

1. Lower the magnification to about 100K.
2. Narrow the beam and center it on the screen.
3. On the GUI, click on *DEF* then right click and select *Tilt Balance*.
4. Click on *WOB* then right click to select *Image X*. The beam should now wobble in the X direction.
5. Use only the X multifunction knob to minimize the wobbling.
6. Subsequently, right click on *WOB* and select *Image Y*. The beam should now wobble in the Y direction.
7. Use the Y multifunction knob to minimize the wobbling.
8. The beam will move quickly along the Y direction while adjusting the Y wobble. Switch between *Beam shift* and *Tilt Balance* from the DEF menu to alternately center the beam and fix the tilt balance.
9. As a final check, right click on *WOB* and select *Image XY*.
10. Use both X and Y multifunction knobs as needed to further minimize the wobbling.
11. Click on *WOB* to end the wobbling and switch back to *Beam shift* under *DEF*.

Axis Align (Rotation Center)

1. Click on *AXIS ALIGN* from the top menu of the GUI.
2. Alternately spread the beam in both directions from the crossover to check whether the beam spreads concentrically from the center.
3. Otherwise, use the multifunction knobs until the beam spreads and narrows concentrically.
4. Click on *AXIS ALIGN* again to exit this mode.

Image Focus

1. Spread the beam until features of the specimen become visible.
2. Insert the objective lens aperture by click on *OL* on the *Aperture Control* window and select an aperture size, again typically "2."
3. Adjust the Focus knob [21] until the fringes on the edges of a hole disappear.

Finding the Zone Axis

1. Using the trackball, center on the thinnest part of the crystalline sample.
2. Focus the image on the screen.

3. Insert the Selected Area Diffraction (SAD) aperture by clicking on *SA* on the GUI and selecting *2* from the drop down menu.
4. Switch to diffraction mode by selecting *DIFF* from the *Image* drop down menu on the top of the GUI.
5. Remove the beam stopper as necessary by clicking on *BL* then selecting *OUT* from the menu.
6. Note the pattern on the screen. If the pattern is symmetric, then the specimen is on the zone axis (which is highly unlikely for most samples). If not, find the zone axis by tilting the specimen using the *TILT* adjustment buttons on the control panel. Stop when the current pattern has the brightest beam on the central spot and the spots around it has symmetric brightness.
7. Capture the diffraction pattern by clicking on the camera icon on the *Image Control* window.
8. Switch back to TEM mode by selecting *TEM* from the *Image* drop down menu.

Objective Astigmatism Correction

1. Use the trackball to move to an amorphous part of the specimen.
2. Change the magnification to ~400K to 500K.
3. Switch to the MSC camera by clicking on *AUX*. Check that the exposure times are initially low in the *Setup* menu.
4. Click on the rabbit icon to start image acquisition.
5. Display the Fourier transform of the image by selecting *Process > Live > FFT* on the GUI.
6. Click on the bottom of the histogram to update the FT image.
7. Switch back to the CCD camera image and adjust the exposure time until the thon rings on the FT plot becomes visible. If the ring is not circular around the origin, then the objective lens has astigmatism.
8. Correct the astigmatism by right-clicking on *STIG* then selecting *OL stig*. Use the multifunction *XY* knobs to adjust the FT image until the rings become circular.
9. Select the CCD image once more after astigmatism correct and stop image acquisition.

Image Capture

Camera Setup

1. On the second monitor, check that the correct camera for image capture is used by going to *Camera* then selecting *MSC*.
2. Click on *AUX* on the GUI menu on the first monitor to turn on the auxiliary camera.
3. Click on the *SETUP* icon then select the *Search* tab. Check that the exposure time is about 0.01 so that the camera does not get damaged by the electron beam.
4. Switch to the *Capture* tab and select that the exposure time is on the same order of magnitude as that of *Search*. Click *OK*.

Image Search & Capture

1. Click on the *rabbit* icon to start the image search. A pop-up window will appear for the scale bar size. Make sure that the scale bar matches that on the TV camera.
2. Once the pop-up window disappears, the auxiliary camera will start taking pictures. Adjust exposure time by clicking on the arrow keys one at a time on the keyboard.
3. Use the trackball to go to a desired area on the specimen.
4. To stop search, press the *SPACE* bar.
5. Once ready for image capture, click on the *camera* icon.
6. Save the image as a high resolution TIFF file by going to *Custom* and *Save as HR-TIFF*.

HRTEM Image Capture

1. Click on *AUX* to switch back to the TV camera.
2. Use the trackball to move to a crystalline area on the specimen.
3. Increase the magnification to 6M.
4. Switch to the CCD camera once more by clicking on *AUX*.
5. Start the image search and adjust the exposure time until the best image is shown.
6. Save the image by clicking on *Custom > Save as HR-TIFF* on the menu.
7. To stop search, press the *SPACE* bar.
8. Once ready for image capture, click on the *camera* icon.
9. Save the image as a high resolution TIFF file by going to *Custom* and *Save as HR-TIFF*.

STEM Mode

1. On the *Image* drop-down menu on the top of the PC Controller window, change the image mode to *STEM DFI*.
2. Click on *RONCHI* on the top menu.
3. Change the spot size to a desired setting. For low resolution imaging, *1.0 nm* is sufficient. For high-resolution mode, *UHR* is chosen.
4. Increase the magnification to see the ronchigram.
5. Change the condenser to *CL1* to see the whole specimen area if necessary.
6. Adjust the focus to find the ronchigram.
7. If the ronchigram is not centered, select *Image Shift* from the *DEF* menu and use the multifunction knobs to center.
8. If the ronchigram is not circular, correct the condenser lens astigmatism by clicking on *CL Stig* and using the multifunction knobs.
9. Switch to *CL2* to see only the ronchigram. Use *Beam Tilt* and the multifunction knobs to center the condenser aperture.
10. Switch to *CL3* and center if necessary. Adjust the size of the ronchigram through *FOCUS* such that the disc of infinite magnification is inside the aperture with a little space between the edges of the disc and the aperture.
11. Click on *RONCHI* to switch back to image mode.

12. Adjust the *Contrast* knob to see the image and use the trackball as necessary to select an area on the specimen.
13. Capture the image by clicking on a scan rate of 4 on the *Image Control* window then followed by the *snapshot* button.
14. Save the image.

EELS

1. On the *Digital Micrograph* window, find *Autofilter* then *Technique* then select *TEM*. Then click on *GIF* (Gatan Image Filter) > *ON*.
2. Switch to the *PC Controller* window then go to *Operation* then select *GIF*.
3. Change the magnification to around 30K and remove the sample from view. Bring the beam to the center.
4. On the second monitor, remove the TV Camera by selecting *Camera* > *GIF Multiscan*.
5. Check the *SETUP* to ensure that the exposure time is ~0.001.
6. Go to *Commands* then select *Align ZLP* then click on *Tune GIF*. At this point more *Autofilter* controls will not be grayed out.
7. Select the following choices: *Technique* > *EELS*, *View* > *Turbo*, *Energy* > *Zero Loss* then click on *Idle*. The button then changes to *Active*.
8. Adjust focus X by double clicking on *FOCUS X*. Move the mouse left and right until the peak height of the ZLP is maximized then click.
9. Do the same for *SX* and *SY*.
10. To zero the ZLP, double click on *Adjust* on the *GIF Trididem Control* window. Use the mouse to move the peak to zero. Change the step size if necessary using the scroll wheel and use *Ctrl* the mouse to zoom in.
11. To start collecting EELS data from the sample, switch back to the TV camera then select *OFF GIF*.
12. Find an area on the sample where the spectra will be collected.
13. Turn off the TV Camera and then click *ON GIF*.
14. See the sample in the GIF camera. Select *Technique* > *TEM* then click on the *rabbit* button.
15. On the second monitor, make sure the following are selected: *EELS*, *Turbo*, *Custom*. Click on *Acquire* to start collecting the EEL data.
16. Once acquisition is done, analyze the spectra by assigning electronic transitions to significant peaks. Remove the background as necessary by pressing *Ctrl-Left Click* on a peakless area of the spectrum then select a 50eV window.
17. Save the spectra by clicking on *File* > *Save As* followed by an assigned filename.

Sample Unloading

1. To unload sample, first select "N" to neutralize.
2. Toggle the switch to *AIR* so backfill the chamber.
3. Wait for three swooshing sounds before removing the specimen holder.

References

Ohsaki, M.; Matsusita, M.; and Kondo, Y. *Development of Nano-Analysis Electron Microscope JEM2500SE*. 40 (2002).

Williams, D. B. and Carter, C. B. *Transmission Electron Microscopy*. Springer: New York, NY, 1996.