

# INSTRUCTIONS FOR ZEISS EM10 TRANSMISSION ELECTRON MICROSCOPE

## A. TURNING ON THE MICROSCOPE (from a cold start): Note: During normal working hours, the microscope is already turned on.

1. Depress the **red "O/T"** button located on the upper left-hand main console panel.
2. Open **main** nitrogen tank valve. **DO NOT** adjust pressure regulator.
3. It will take approximately 15-20 minutes of pumping before microscope is ready to use. Microscope is ready for use when the **PV** (0.1mBar) light goes off permanently (middle of the upper left-hand panel) and the **green HIGH VOLTAGE** button light is on.

## B. GETTING STARTED:

1. Make sure microscope is turned on and the **green HIGH VOLTAGE** button light is lit.
2. Fill cold trap (left-hand side of column) with liquid nitrogen. The liquid nitrogen tanks are located in the hallway outside the lab (room 037 LSB).
3. Reduce the microscope **MAGNIFICATION** to approximately **2500**.
4. Center the **STAGE CONTROLS**. The two stage micrometers should be set around **500**.
5. Depress the **DESICC** (desiccator) button so that the **red button** light comes on and appears lit. This will speed up specimen exchanges.

## C. INSERTING A SPECIMEN:

1. You should find the specimen holder already inserted into the microscope. First, retract the specimen holder by pulling the specimen holder rod all the way out toward you, then move the rod up to approximately the one o'clock position, and then pull the rod out slightly toward you.
2. Rotate the large silver dial at the back of the chamber counterclockwise. The specimen holder should now be exposed.
3. Remove the silver tools from the **blue plastic box** with the white cloth. Using the threaded aluminum handle, remove the specimen holder from the chamber and lay the brass specimen holder in the silver holder support. Use the fork to lift up the end of the brass holder and swing the support lever over to hold the specimen holder in place. Unscrew the top (cap) of the brass specimen holder with the cap removal tool and lay aside. After loading a grid in to the holder, screw the cap back on to the holder. While lifting holder up slightly with fork tool, swing support lever out of the way and lay specimen holder back down in support.
4. Using already attached handle, insert specimen holder back in to the chamber by lining up pins. Now unscrew threaded handle from holder and **tap holder with handle to ensure that holder is seated properly in chamber.**
5. Rotate large silver dial clockwise until it stops. Push down on small **red button** on specimen rod (it will move forward) and then move rod down to the three o'clock position (should be pointing toward the right). In this position, a small **red pre-vacuum light (PV)** comes on (middle of the upper left-hand panel). When **red light** goes off, wait a couple of seconds and then move the rod lever all the way down to the six o'clock position (pointing toward the floor). Move the specimen rod to the first stop position. Push the **red button** and move the rod to the second stop position. Push the **red button** again and move the rod to its final position. The rod and holder should now be all the way in and in the proper position.

#### **D. TURNING ON THE ELECTRON BEAM:**

1. Depress the **green HIGH VOLTAGE** button (upper left-hand control panel). The **green HIGH VOLTAGE** light will go off and the **red HIGH VOLTAGE** button light will come on.
2. Depress **red FIL** button (upper left-hand control panel). Filament will already be pre-saturated so no need to adjust. Microscope should be set on **60kv** (upper left-hand panel). **Do Not Change.**
3. The **CONDENSER 2** knob (upper left-hand panel, larger inner knob) should be set on **#5**. **Do Not Change.** With the smaller center knob of **CONDENSER 2**, bring the beam to cross-over. Center beam in the middle of the screen using the **X** and **Y** knobs on the lower right-hand panel, left side as a pair **OR** the pair found on the lower left-hand panel, right side **BUT DO NOT** mix (i.e. do not use Y knob from one side and X knob from the other side). After centering the beam, de-condense beam by turning the smaller center knob of **CONDENSER 2** clockwise slowly to expand the beam. Then entire field of view should now be illuminated with the beam.
4. Calibrate focusing binocular scope to main viewing screen.
  - a. Swing binocular in to place so it is pointing at the main screen and specifically at the small focusing screen in the middle of the large screen.
  - b. Firmly grasp each binocular barrel/tube and rotate clockwise or counterclockwise in order to adjust them to your inter-pupil distance. After doing this you should be able to look down the tubes with both eyes comfortably to see one image on the screen.
  - c. After adjusting the inter-pupil distance properly, now focus each eye independently using the focus adjustment on each ocular/eye piece. Close your left eye and focus your right eye on the tiny scratch located in the middle of the smaller focusing screen. Once your right eye is in focus, now close your right eye and focus your left eye on the small scratch. The tiny scratch should now be in focus with both eyes.

#### **E. PHOTOGRAPHY**

##### **1. Acquiring the Image:**

- a. Acquire a well focused image of your sample on the main TEM viewing screen. Make sure the illumination is well centered and evenly distributed by adjusting the beam with the **CONDENSER 2** knob and with the **XY BEAM ALIGNMENT** knobs.
- b. Make sure the computer is turned on. In most cases the computer will be on already but with the screen saver being displayed. Move the mouse to reactivate the computer and monitor. A desktop with a black background should now be displayed.
- c. Turn on the digital camera by turning on the white power strip switch located just to the right of the TEM column. Red toggle switch will be lit when power strip is on.
- d. Wait for the computer to recognize the camera. A faint double "bell sound" will be heard when this is done.
- e. From the main computer desktop, double click on the **MaxIm DL5** icon. Once loaded the main display will be visible.
- f. With an image of interest showing on the main TEM viewing screen, insert the scintillator in to the beam path (right-hand side of TEM column) by first depressing (pushing in) the small locking button and then slowly sliding the scintillator in to place. Once inserted, the square scintillator will be visible through the front viewing port.
- g. From the main computer screen restore the **Video Window** which is initially minimized to the lower left-hand corner of the screen.
- h. Once the **Video Window** is open, click on the **Run** button (lower left side of video window). An image will be visible in the video window. Adjustments to the Exposure Control, Gain Control,

Contrast & Brightness can be made but will only affect the appearance of the displayed video image and not the final image. Do not spend much time with these adjustments.

- i. If a faster image refresh rate is desired, then select either **2x2** or **4x4** under **BIN**. This will allow for a more responsive image display but the image will be increasingly pixelated.
- j. Under **ROI** (region of interest), click on the **Select** button. While holding down the left mouse button, draw a small box around any object of interest. An enlarged view of the image will now appear in the **Video Window**. Use this enlarged view to focus on the image using the microscope's **FOCUS** controls. If you had selected a higher binning rate (e.g. 2x2 or 4x4) earlier, I would suggest switching back to a BIN rate of 1x1 while focusing. Once the image is as focused as possible, click on the **Reset** button under the **ROI** to return to the normal view.

Focusing Tips:

- Smaller ROI means a faster refresh rate but don't forget about exposure duration too (i.e. even very small ROI will not run faster than 2 fps if exposure = 500msec or faster than 4 fps if exposure = 250msec).
  - Do not select too large ROI for focusing (too slow video)
  - Do not select too small ROI for focusing (pixelated)
  - Optimal ROI is somewhere around 300x300 pixels (up to you) at BIN = 1
  - More illumination during focusing will increase video rate
- k. Now click on the **Stop** button (lower left side of panel).
  - l. Locate and click on the **AutoSubtract** button. This is found in the **Noise & Background** section of the **Camera & Image** panel.
  - m. From the **Camera and Image** panel, click on the **Single** button under **Image Capture**. A 'snapshot' of your image will now be displayed.
  - n. From the **Screen Stretch** panel, select "**Range**" from the drop-down menu.

**2. Saving the Image:** Recommendations - Save a copy of the initial image 'snapshot' and then save a new copy after every change to the image (i.e. adding annotations such as text, scale bars or measurements, change histogram settings, etc.). Treat this initial captured image as if it were your negative!

- a. Locate and click on the **Save As icon** found in the **File** section of the **Camera & Image** panel. A **Save As** box will appear.
- b. Choose a file location and name your file.
- c. You have the option of saving your image either as an **8 bit** or **16 bit** image. While the 16 bit image contains a much higher number of values per channel and is by far the better option, some programs such as older versions of PhotoShop or PowerPoint may not be able to handle 16 bit images. If you are unsure, select 8 bit or else save the image both ways.
- d. Click on the **Save** button. If you chose the 8 bit option, a warning box will appear asking you to confirm your choice. Click **Yes** to continue.
- e. If you want to apply additional annotations to the captured image, proceed to the next section. Otherwise, you can close the displayed image.
- f. Remove scintillator from microscope and lock in to place by retracting slider until a 'click' is heard. The scintillator is now fully retracted.

### **3. Annotations: Adding Text to the Data Strip**

- a. Locate and click on the **Annotation icon** (small tablet with pencil) found in the **Process** section of the **Camera & Image** panel. A small **Annotate** box will appear.
- b. Click on the "A" icon. (Note: do not change font type, size, and color. Font must be Arial, size

20-24 and black color.)

- c. Now move your cursor to the data strip. A **'hand'** symbol will appear as the cursor passes over the text areas. To add mag value to the TEM Magnification label, position the **'hand'** over the words TEM Magnification and right button click. Select **Properties** and a **Shapes** panel will appear. Add the correct magnification value in **Text Caption** area of this box. Click on the **OK** button when finished. To add additional text, click anywhere on the captured image, enter desired text in the **Text Caption** box, click **OK**, and then move the text to the desired location with mouse.
- d. To make text changes permanent, click on the **Paste to Image** button on the **Annotate** box and then click **Yes** to confirm.
- e. Click on **OK** to complete this process.
- f. Save annotated image under a different file name. Remember, do not save these annotations on your original image but rather save this changed file as something other than your original image!

#### **4. Annotations: Adding a Scale Bar to Image**

- a. Locate and click on the **Annotation icon** (small tablet with pencil) found in the **Process** section of the **Camera & Image** panel. A small **Annotate** box will appear.
- b. Click on the **"Ruler" icon** and then click on the **Properties** button. A **Shapes** panel will appear.
- c. Under the **Ruler Properties** section, select the proper microscope magnification from the drop-down list. This will ensure the correctly calibrated scale bar is added. If you would like to change the scale bar line thickness or the size and font of the scale bar text, you can do this by adjusting the **Line Width** and/or **Font Size** values on the **Shapes** panel.
- d. Once you have selected the proper magnification, click the **OK** button.
- e. Click on the image to add the scale bar. Hold down the left mouse on the scale bar and move the mouse to reposition the scale bar. To delete it, use the right mouse button and select **Remove**.
- f. To make scale bar permanent, click on the **Paste to Image** button on the **Annotate** box and then click **Yes** to confirm.
- g. Click **OK** to complete this process.
- h. Save annotated image under a different file name. Remember, do not save these annotations on your original image but rather save this changed file as something other than your original image!

#### **5. Annotations: Adding a Scale Bar to the Data Strip (Legend):**

- a. Locate and click on the **ShowLegends** button found in the **Measurements** section of the **Camera & Image** panel. A **red** scale bar will appear on the image.
- b. From the drop down list, select the proper microscope magnification for the image. This will ensure the scale bar is accurately calibrated for your image.
- c. Left mouse click on the **red** scale bar to make it active (should turn **green**). Using the left mouse button, click and drag the scale bar to reposition it. Once it is in place, click elsewhere on the image to deactivate the scale bar (**green** bar will turn **red**).
- d. To make the scale bar permanent, click first on the **Stamp Annotation icon** and then on the **Stamp Measurement icon** found in the **Process** section of the **Camera & Image** panel. If only one stamp icon is active, then click on that one. Choose **Yes** when asked to confirm.
- e. Click on the **Show Legends** button to deactivate this feature. The **red** scale bar should now appear as a single line with text below.
- f. Click on the **Convert to Mono icon** found on the left-hand side tool bar. This will convert the **red** scale bar to **black**.
- g. Save annotated image under a different file name. Remember, do not save these annotations on your original image but rather save this changed file as something other than your original image!

## 5. Annotations: Measuring Objects

- a. Locate and click on the **Show Measurements icon** found in the **Measurements** section of the **Camera & Image** panel.
- b. From the drop down menu, select the proper microscope magnification.
- c. Click on the **Tape Measure icon**. A **green** measurement bar will now appear on the image. Left mouse button click and hold on the bar to reposition. Measurement bar can be resized by left mouse holding on the small **red** dot. Measurement text can be repositioned in a similar manner. Use **Pan** zoom window to highlight objects for more accurate bar placement and measurement.
- d. Repeat step 'c' for applying multiple measurement bars to the image.
- e. When finished, click somewhere off of the **green** measurement bar to deactivate (bar will turn **red**).
- f. To make measurement bar(s) permanent, click on the **Stamp Measurement icon** located in the **Process** section of the **Camera & Image** panel.
- g. Save annotated image under a different file name. Remember, do not save these annotations on your original image but rather save this changed file as something other than your original image!

## F. REMOVING AND/OR EXCHANGING GRIDS:

1. Release the **red FIL** button (**red FIL** light goes out).
2. Depress **red HIGH VOLTAGE** button. The **red button** light goes off and the **green HIGH VOLTAGE** button light comes on.
3. Make sure camera scintillator is fully retracted from microscope. To do this move scintillator slider to the right until it 'clicks' in to place. The scintillator is now fully retracted.
4. Retract the specimen holder by pulling the specimen holder rod all the way out toward you, then move the rod up to approximately the one o'clock position, and then pull the rod out slightly toward you.
5. Rotate the large silver dial at the back of the chamber counterclockwise. The specimen holder should now be exposed.
6. Using the threaded aluminum handle, remove the specimen holder from the chamber and lay the brass specimen holder in the silver holder support. Use the fork to lift up the end of the brass holder and swing the support lever over to hold the specimen holder in place. Unscrew the top (cap) of the brass specimen holder with the cap removal tool and lay aside. Remove your grid and insert a new grid. After loading a grid in to the holder, screw the cap back on to the holder. While lifting holder up slightly with fork tool, swing support lever out of the way and lay specimen holder back down in support.
7. Using already attached handle, insert specimen holder back in to the chamber by lining up pins. Now unscrew threaded handle from holder and **tap holder with handle to ensure that holder is seated properly in chamber.**
8. Rotate large silver dial clockwise until it stops. Push down on small **red button** on specimen rod (it will move forward) and then move rod down to the three o'clock position (should be pointing toward the right). In this position, a small **red pre-vacuum light (PV)** comes on (middle of the upper left-hand panel). When **red light** goes off, wait a couple of seconds and then move the rod lever all the way down to the six o'clock position (pointing toward the floor). Move the specimen rod to the first stop position. Push the **red button** and move the rod to the second stop position. Push the **red button** again and move the rod to its final position. The rod and holder should now be all the way in and in the proper position.

## **G. END OF SESSION:**

1. Remove your grid from the microscope (See Section F: Removing and/or Exchanging Grids).
2. Make sure specimen holder is returned to the microscope.
3. Return the specimen exchange tools to the blue box with the white cloth.
4. Make sure camera scintillator is fully retracted from microscope. To do this move scintillator slider to the right until it 'clicks' in to place. The scintillator is now fully retracted.
5. Shut off digital camera by turning off white power strip switch (red light is not lit)
6. Close **MaxIM DL5** software but leave computer on.
7. Record your time in the TEM usage log book.
8. If you had problems or difficulties with the microscope, please inform the Director before leaving from your session.

**H. SHUTTING DOWN MICROSCOPE:** *(Do this only if told to do so OR if you turned on the microscope. Otherwise, the microscope is left on during normal working hours.)*

1. Remove any specimens from the microscope. Reduce magnification to 1000 and center stage controls (micrometers should be around 500). Make sure specimen holder is inserted in to the microscope.
2. Release the **red O/I button** (**red light** goes out).
3. Close the **main** nitrogen tank valve. **DO NOT** adjust pressure regulator.
4. Turn off safe-lights and turn off room lights.
5. Leave chiller on (left-hand side of room). It stays on for an additional 30-45 minutes after the microscope is turned off to continue to cool the oil diffusion pump and will go off automatically.

**I. UNLOADING FILM:** *(Do this only if you were told to do so. In most cases, film will be developed for you so no need to unload and load fresh)*

1. Make sure filament is off by depressing **red FIL** button. **Red light** will go off.
2. Make sure **red HIGH VOLTAGE** button is off by depressing **red HIGH VOLTAGE** button. **Red light** will go off and the **green HIGH VOLTAGE** button will come on.
3. Lock TEM door. Plug in the 2 room red safe-lights.
4. Make sure room lights and main desk (console) lights are OFF. Depress **red X** button (upper right-hand console panel) to turn on **red panel safety light**.
5. Depress **red CAM** button (upper left-hand panel). Camera begins to vent with nitrogen.
6. Once camera cover is free, remove film receiver box by pulling the lever just underneath the front toward you. Be sure to have your hand on the box just behind the lever to prevent box from falling out.
7. Remove box by lifting it off support rails. Remove exposed film from the box and place film cassettes on the counter..
8. Replace receiver box. Line up side pins on box with support rails, slide box up rails, raise rails and lock in to place.
9. Release the **red CAM** button (upper left-hand panel). This will evacuate the camera.
9. Remove individual negatives from the metal cassettes and place each piece of film in to a free slot in the plastic developing racks.
10. Process film according to instructions posted on wall near sink.

**J. RELOADING CAMERA:** *(Do this only if you were told to do so. In most cases, film will be developed for you so no need to unload and load camera)*

1. Open the desiccator box (lower right-hand drawer) by depressing the **red DESICC** button (next to **CAM** button). When depressed, the **red DESICC** light will come on.
2. Pull the desiccator drawer toward you. While holding the drawer out, lift up on the knob located on top of the desiccator box to vent the chamber. Once the desiccator is vented, carefully open the box by lifting up on the lid. The lid will lock in to place when completed opened.
3. Remove a packet of film from the desiccator.
4. Refill the empty film cassettes with fresh film. Make sure the film is loaded correctly in the cassette (notch in film goes in the upper right-hand corner).
5. Place unused film back in to its silver foil package and place this back in to the desiccator. While holding on to the desiccator lid with your right hand, carefully pull up on the lid hinge with your left hand just until the lid becomes free. Lower the lid gently down and slowly push the entire desiccator box back in to the microscope.
6. Depress **red CAM** button (upper left-hand panel) once again to vent the camera. Lift up on the round camera cover plate found just in front of the viewing port. Align replacement film cassettes and place them back in to the camera making sure the raised edge of the cassette is to the left. Slide

your hand down each side of the stack of film to ensure all cassettes are aligned properly.

### Special note: Completely empty camera

The TEM camera hold 30 pieces of film. If the **green START** camera light goes out (is not lit), the camera is completely empty. In order to reset the camera, do the following:

- a. Put 5 refilled film cassettes in to the camera. Make sure they are properly aligned and sitting correctly inside the camera holder.
  - b. Replace the round camera cover plate. Release the **red CAM** button and evacuate the camera (wait until the **red PV** light goes off and the **green HIGH VOLTAGE** button comes on).
  - c. Depress the **red MAN** button (upper right-hand panel). The **red light** will come on.
  - d. Depress the **green START** camera button once (upper right-hand panel). This will cause the viewing screen to rise and will load a piece of film in to the camera.
  - e. Depress the **green STOP** camera button (upper right-hand panel). The viewing screen will lower and the **green START** button light will appear lit.
  - f. Depress the **red MAN** button again. **Red light** will go out.
  - g. Now finish refilling the camera. Depress **red CAM** button (upper left-hand panel) once again to vent the camera. Lift up on the round camera cover plate found just in front of the viewing port. Put the remaining 25 replacement film cassettes back in to the camera. Make sure they are aligned properly and that the raised edge of the cassette is to the left. Slide your hand down each side of the stack of film to ensure all cassettes are aligned properly.
7. Replace the round camera cover plate. Release the **red CAM** button and evacuate the camera (wait until the **red PV** light goes off and the **green HIGH VOLTAGE** button comes on).
  8. Once the camera is completely evacuated (**red PV** light goes off permanently), evacuate the desiccator by releasing the **red DESICC** button. As desiccator evacuates, the **red PV** light will come on again and then go out when the desiccator is under sufficient vacuum.
  9. Make sure all film is put away or properly processed before turning on regular room lights.
  10. Unplug 2 red safety lights. Release **red X** button (upper right-hand console panel) to turn off **red panel safety light**. Unlock TEM door.

## TEM Alignment

C2 at 15

Mag at 1000x            pp. 415 of brief operating instructions by Zeiss

V=1.5 or 1.6 (controlled by filament heating)

1. Set bias at 30 to 40 (beam current knob, each point = 10 starting at 12:00 position)
2. Remove objective aperture (do not move X and Y knobs for alignment)
3. Push in P2 lens button
4. Push in buttons I, II, III
5. Get brightest spot with furthest left dial then release button I; get brightest spot with 2<sup>nd</sup> dial from the left and release button II; same for button III; same for button IV but no button need be released.
6. Release P2 lens button
7. Slightly reduce filament heating knob to get image of filament
8. Now use X's in pairs and Y's in pairs to put filament image in center of bright spot.
9. Now use either one pair of X and Y or the other pair to center spot on screen.
10. Spread beam until it almost reaches edge and adjust CONDENSER stigmators if necessary.
11. Put in objective aperture and center if necessary.
12. Check stigmation of objective lens

## Centering Objective Aperture

1. Put in grid, focus on small hole (if using holey grid)
2. Change magnification to 6000 (20,000 works better)
3. Use the 2nd or middle aperture of the intermediate aperture. Align aperture with X & Y knobs.
4. Set on diffraction mode and focus
5. Condense beam to tiny spot (smaller inner knob) and adjust intensity with outer knob (condensor 2)
6. Introduce objective aperture, center around spot on screen with X & Y centering knobs.
7. Switch back to imaging mode
8. Remove intermediate aperture

## Centering CONDENSER Aperture

Set magnification to 2000-2500

Expand beam (clockwise)

Center aperture with X and Y knobs on the aperture