JEOL 1200-EX II TEM

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Components of the TEM		
Column Component	**Console Control***	**Function**
Illumination System		
Electron Gun (Filament, Wehnelt, Anode)	FILAMENT knob (LL); BIAS toggles (LL)	Electron source
Gun Alignment Coils (gun shift and gun tilt)	GUN SHIFT button and DEF X and Y knobs (LR). GUN TILT button and DEF X and Y knobs (LR).	Translates and tilts the beam beneath the gun to maximize illumination
Condenser Lens 1	SPOT SIZE toggle (UL)	Determines smallest illumination spot size on specimen
Condenser Lens 2	BRIGHTNESS knob (UL)	Varies amount of illumination on specimen
Condenser Stigmator	COND STIG button (UL) and DEF X and Y knobs (UL and UR).	Corrects for astigmatism in the condenser lens
Condenser 2 Aperture		Controls amount/coherence of illumination striking the specimen; reduces spherical aberration in convergent beam mode
Deflector Coils	SHIFT X and Y knobs (UL and UR).	Translates the entire illumination system onto screen center
Focus Wobbler	IMAGE WOBBLER button (UR)	Aids with low magnification focusing by alternating current to the beam deflector coils
Specimen Manipulation System		
Specimen Holder		
Goniometer		Tilts the specimen around the X-axis; Shifts the specimen vertically
Specimen Selector		Selects specimen grid 1 or 2
Imaging System		
Objective Lens	FOCUS knobs (UR)	Forms, magnifies, and focuses first image
Objective Aperture		Signal selection; Controls contrast and spherical/chromatic aberration
Objective Stigmator	OBJ STIG button (UL) and DEF X and Y knobs (UL and UR).	Corrects for astigmatism in the objective lens
Intermediate Lens	Magnification SELECTOR toggle (UR)	Magnifies image form objective lens and focuses diffraction pattern
Intermediate (Diffraction, Field Limiting) Aperture		Selects area to be diffracted
Projector Lenses	Magnification SELECTOR toggle (UR)	Magnifies image
* LL=lower left panel; UL=upper left panel; UR=upper right panel; LR=lower right drawer		

Routine Alignment and Operation for Independent User

Symbolism

Blue:	Console Objects: For ex, **FILAMENT** knob
Orange:	Column Objects: For ex, **Field Limiting Aperture**
"Black":	Imaging Program Objects: For ex, "Viewport Manager" window
Bold Black:	States objects can exist in. For ex, **IN**
	Processes used to change the states of objects. For ex, **Center** the beam

Starting Conditions:

- The N_2 Cylinder pressure should be 500 psi or higher; the regulated N_2 gauge should read between 5 and 8 psi. Contact the staff if readings deviate from these values.
- **Liquid Nitrogen Anticontaminator**. Add LN_2 carefully to anticontaminator dewar. Use funnel to avoid splashing. Top off after about 10 minutes.
- **FILAMENT** knob **OFF**.
- **PENNING GAUGE** reading at the bottom end of the "Green Zone"
- **HT** button **ON**. The green **READY** lamp **LIT**
- **Sample Holder** **IN** the column.
- **Condenser, Objective, and Field Limiting Apertures** **OUT** (aperture assembly levers turned to the right).

If you will be using **60, 80, or 100 kV** and the microscope is currently at a different kV: Slowly toggle the **ACCEL VOLTAGE** switch to the desired kV. Proceed to **"Electron Beam Generation".**

If you will be using **120 kV** and the microscope is at a lower kV: Proceed to **"High Voltage Generation and Conditioning"**.

Alignment:

High Voltage Generation and Conditioning.

1. Slowly toggle the **ACCEL VOLTAGE** switch to 100 kV.

2. **HT WOBBLER ON**.

3. SLOWLY toggle **ACCEL VOLTAGE** up, stepwise, to **120 kV**. Keep an eye on the **PENNING GAUGE** …watch for wobbling and allow the needle to bottom out before your next stepwise increase. At the same time watch the **BEAM CURRENT** meter. Don't let the Dark Current exceed 90 uA. If it jumps to 90 or more, slow down your stepwise increase.

4. Reduce **ACCEL VOLTAGE** from 120 to **100 kV**. The **HT WOBBLER** should be left **ON.**

5. Wedge the "H.T Conditioning Stick" to push **in and hold** the **HT COND** button. This will raise the dark current about 10% and allow High Tension to go 10% above the value you are setting with the **ACCEL VOLTAGE** selector.

6. VERY SLOWLY repeat step 4, above. Allow the **PENNING GAUGE** to reach its lowest value with each kV increase Your goal now is to temporarily "overvoltage" the system, up to about 130-132 kV.

7. When you reach 120 kV, leave it there **5 minutes**, and then reduce back down to 100 kV. Decreasing kV can be conducted at a more rapid rate; however, be conservative.

8. Release the **HT COND** button by removing the stick and turn **HT WOBBLER OFF**.

9. SLOWLY toggle the **ACCEL VOLTAGE** to the value you will be using.

Electron Beam Generation

1. Toggle the Magnification **SELECTOR** to **2500X**. Set **SPOT SIZE** at **1.**
2. SLOWLY **saturate** the **FILAMENT** knob. When saturated, the beam at crossover will show no filament structure. Use the **FINE BIAS MODE** toggle to adjust the **BEAM CURRENT** reading so that it is 10 uA more than the Dark Current reading for the accelerating voltage you are using.

3. **Center** and **spread** the beam:
   - Focus the beam to "**crossover**" (smallest spot) with the **BRIGHTNESS** knob. The magnitude of knob increments can be increased by activating the **STEP** button.
   - **Center** the beam using the **SHIFT X and Y** knobs. Knob increments can again be controlled with the **STEP** button (UL).
   - **Spread** the beam with the **BRIGHTNESS** knob ***("spreading the beam" is accomplished by turning the BRIGHTNESS knob COUNTERCLOCKWISE. In the underfocus direction)***.

ILLUMINATION SYSTEM ALIGNMENT (at the beginning of a session)

Gun Alignment

1. Toggle the Magnification **SELECTOR** to **5000X**. Toggle **SPOT SIZE** to **1**.
2. Bring beam to **crossover** and **center** it.
3. Turn **FILAMENT** knob counterclockwise to create **"cat's eye" image**.
4. **DEFLECTOR-GUN TILT** button **ON -- LIT**. Use **DEF X and Y** knobs to attain **symmetry** in the filament image.
5. **DEFLECTOR-GUN SHIFT** button **ON -- LIT**. Use **DEF X and Y** knobs to **center** the filament image on viewing screen. **DEFLECTOR-GUN SHIFT** button **OFF**
6. Turn the **FILAMENT** knob to **saturation**.

Condenser Lens (Spot Size) Alignment

1. Magnification **SELECTOR** at **5000X**
2. Toggle **SPOT SIZE** to **5.** Bring beam to **crossover** and **center** with **SHIFT X and Y** knobs.
3. Toggle **SPOT SIZE** to **1**. Bring beam to **crossover** and **center** by turning the **DEFLECTOR-GUN SHIFT** button **ON – LIT** and using the **DEF X and Y** knobs.
4. Repeat steps 2-3 until the amount of beam shift when varying **SPOT SIZE** is minimized.
5. **DEFLECTOR-GUN SHIFT** button **OFF**.
6. From this point on, **center** the beam spot with the **SHIFT X and Y** knobs.

Condenser 2 Stigmation

1. Toggle the Magnification **SELECTOR** to **10,**000X Toggle **SPOT SIZE** to **5.**
2. Bring beam to **crossover** and **center**.
3. Turn **FILAMENT** knob counterclockwise to produce **"cat's eye" image** of filament tip. Adjust **BRIGHTNESS** to sharpen filament image.
4. **COND STIG** button **ON – LIT.** Use **DEF X and Y** knobs controls to **focus** filament image as sharply as possible. Use the small viewing screen and the binoculars to aid with focus.
5. Turn the **FILAMENT** knob to **saturation**.
6. **COND STIG** button **OFF**.

7. Check condenser astigmatism compensation by turning **BRIGHTNESS** knob from crossover to fully counterclockwise. Beam should be round or nearly so.

8. Toggle to the **SPOT SIZE** you wish to use (usually 2)

Condenser Aperture Centering

1. Insert the **Condenser Aperture** (lever all the way to the left). Rotate dial to select the desired aperture size (Usually #2 [200 uM]). Line up the white and black dots on the aperture assembly.

2. **Center** and **spread** the beam. If it spreads asymmetrically, it indicates that the **Condenser Aperture** is not well centered. **Center** the illumination, and thus the aperture, with the two black adjusting knobs on the **Condenser Aperture** assembly end and side.

3. Check that the beam is **centered** and ensure that the **Condenser Aperture** is **centered** once again.

Sample Insertion.

1. Slowly turn **FILAMENT** knob **OFF**.

2. Loading the **Sample Holder**: DO NOT touch the **Sample Holder** anywhere between the O-ring and the tip; the oils on your skin will contaminate the column vacuum. Use a pair of forceps to open and gently close the grid holders. The sample position nearest the tip of the dual sample holder is designated #1. Check the **Sample Holder** O-ring. It must be free of lint fibers or other contamination.

3. Insert the **Sample Holder** into the sample port with the alignment screw at 9:00 o'clock position. Push and wait for airlock pump to start. After the **Connector Box Lamp** goes **ON** (red) and **OFF** twice, turn the sample holder fully clockwise and gently let the vacuum pull it all the way in. Do this carefully; otherwise you could damage the sample holder's tip bearing if you insert the holder too fast. Use your thumb as a brake block against the sample port.

4. If you have a vacuum leak upon sample insertion and the High Tension turns off (the **BEAM CURRENT** meter will register no current): keep the **Sample Holder** **IN**; ensure that **FILAMENT** knob is **OFF**; allow the **PENNING GAUGE** to reach its lowest level; and reengage the **HT** (High Tension) button.

5. Adjust the sample position with the **Sample Select** wheel (left side of the microscope column) to select sample 1 or 2. Use the upper set of marks as the true positions.

Electron Beam Generation for Sample Observation

1. SLOWLY **saturate** the **FILAMENT**.

2. **Center** and **spread** the beam. If beam isn't seen, a grid bar may be blocking it. Adjust **Specimen Shifting Wheels** to find it.

3. Toggle the Magnification **SELECTOR** to a magnification that allows you to locate an area of interest in your sample…usually 4000 – 5000X.

4. Use **OBJ FOCUS** to focus your sample. Note that at this point your image will lack contrast because the objective aperture is not inserted.

IMAGE FORMATION SYSTEM ALIGNMENT (ideally for each sample)

Stage Centration

1. Press **PAGE** key to display **PAGE 2**.
2. Adjust **Specimen Shifting Wheels** to **X = 0, Y = 0.**
3. **LOW MAG** button **ON -- LIT**. **Objective Aperture** **OUT**.
4. Toggle the Magnification **SELECTOR** to **150X** and adjust **BRIGHTNESS** knob until grid is visible.

5. Adjust **Sample Select Wheel** until you approximately **center** the grid image.

Tilt Axis (Z Height) Alignment

1. **Stage Tilt** at **ZERO** degrees.
2. **LOW MAG** button **ON – LIT**. **Objective Aperture** **OUT**.
3. Toggle the Magnification **SELECTOR** to **500X** and focus your sample.

4. **Disengage** **Stage Motor** drive unit (push away).

5. Rotate the **Stage Tilt** **(+) 20 degrees**. (*CCW*)
6. Use the **Specimen Shifting Y Wheel** to locate and center on screen a conspicuous image (grid corner, piece of dirt, etc).

7. Rotate the **Stage Tilt** **(-) 20 degrees**. (*CW*)
8. Operate the **Z-Shift** knob to move the image to a point about ½ way between where it ended up in step 6 and the screen center.

9. Repeat steps 5-8 until the image position no longer changes when the **Stage Tilt** is rotated + /- 20 degrees.

10. Repeat steps 5-8 at 10,000 X and 30,000. At these higher magnifications, again only adjust the object position by 1/4 to 1/3 when returning toward the center spot between tilts.

11. Return **Stage Tilt** to **ZERO** degrees. Re-engage **Stage Motor** drive unit (pull toward you).

Image Wobbler Balance

1. Toggle the Magnification **SELECTOR** to **10,000X** and obtain **TRUE FOCUS** (minimum contrast). Use the small viewing screen and the binoculars to aid with focus.

2. Bring beam to **crossover** and **center** it.

3. **IMAGE WOBBLER** button **ON**. Adjust the **IMAGE WOBBLER X and Y** knobs to merge separate spots into one and to minimize movement. **IMAGE WOBBLER** button (UR) **OFF**.

4. **Re-Center** and **spread** the beam.

Voltage Center Alignment

1. Toggle the Magnification **SELECTOR** to **60,000X**. Keep beam centered as you increase your magnification.

2. Locate a distinctive feature (hole) in sample and move it to screen center. Obtain **TRUE FOCUS** (minimum contrast). Use the small viewing screen and the binoculars to aid with focus.

3. **HT WOBBLER** button **ON – LIT.**
4. **BRIGHT TILT** button **ON – LIT**. Use **DEF X and Y** knobs controls to minimize movement in the X and Y plane. .

5. **HT WOBBLER** button **OFF. BRIGHT TILT** button **OFF.**

Inserting and Centering the Objective Aperture

1. Ensure that your sample is in the beam path.
2. Press the **DIFF** button.

3. Use the **IOS/DIFF** knob to obtain the sharpest/smallest central diffraction spot. Activate the **DEFLECTOR-PROJ** button and use the **DEF X and Y** knobs to center the central diffraction spot

4. Insert the **Objective Aperture** (lever all the way to the left). Select the desired aperture size by lining up the white and black dots on the aperture assembly.

5. **Center** the **Objective Aperture** around the central diffraction spot with the two black adjusting knobs on the assembly end and side.

6. Press **MAG 1** to get out of diffraction mode

Objective Lens Astigmatism Compensation

1. Toggle the Magnification **SELECTOR** to **75,000 to 125,000X**. Keep the beam **centered** as you increase your magnification. Locate a hole.

2. Use **OBJ FOCUS** to achieve slight **overfocus** (easily visible dark Fresnel fringe around the hole). At this point you may want to image using the flat panel monitors and AnalySIS software. See **Digital Imaging System** section

3. Adjust **OBJ FOCUS** in the underfocus *(CCW)* direction until the overfocus fringe just disappears at two opposing sides of the hole.

4. **OBJ. STIG** button **ON** -- **LIT**. Use **DEF X and Y** knobs controls to make the overfocus fringe uniform around the hole. It will become thinner as you do this.

5. Repeat steps 3-4, as many times as necessary. The amount you will turn toward underfocus will become less and less, until finally it will become very difficult to see any overfocus fringe at all.

6. When you have eliminated asymmetry in the overfocus Fresnel fringe, and can make it disappear entirely as you adjust toward underfocus, the edge of the hole will appear soft and lacking contrast -- you are now seeing **True Focus**. Adjustment to slight underfocus will produce a thin, uniform white Fresnel fringe inside the hole and the background film granularity will be enhanced -- this is the focus setting at which you should acquire your images

7. Alternatively, you can compensate for astigmatism by selecting an area of the holey carbon film that shows uniform density (no holes) and ensuring that the FFT is spherical at a high magnification (**150,000 to 200,000X**).

8. **OBJ. STIG** button **OFF**.

General Usage:

Basic Concepts

The goal of biological TEM is to obtain well focused images that: show high resolution; display a wide range of grey-scale values; and have adequate/uniform illumination/brightness. The Table below summarizes the main parameters a user can manipulate and their effects on these image properties.

	↓Accelerating Voltage	↓ Sample Mass/Thickness	↓ Objective Aperture Size	↓ Spot Size
Contrast	↑	↓	↑	--
Resolution	↓	↑	↑	↑
Illumination	↓	↑	↓	↓

Accelerating Voltages of 60 kV and 80 kV are appropriate for biological TEM

Sample Mass (Z) is dependent on the nature of the specimen and its staining; Sample Thickness derives from the ultramicrotome cutting thickness.

The Objective Aperture on the JEOL 1200 has settings of 50, 100, and 150 microns. The 50 micron setting is best able to produce the contrast desired for biological samples.

A Spot Size of 2 is commonly used on this TEM for biological imaging. Generally speaking, use the smallest spot size possible consistent with adequate illumination, in order to increase beam coherence and thus improve resolution. However, if you find you have to focus BRIGHTNESS nearly to crossover for adequate illumination to record an image, you should use a larger spot size.

Focusing

Unlike an SEM which is parfocal, the focus for a TEM must be adjusted every time there is a change in magnification. Use the small pop-up screen and binoculars to aid with focusing. Ensure the binoculars are in sharp focus for your eyes (when the black dot in the viewing field is in sharp focus).

Low Magnification Focusing (up to 20 kX): **IMAGE WOBBLER** button **ON -- LIT**. Use the **OBJ FOCUS** knob to minimize object movement in the image. The microscope is set to automatically apply a small amount of underfocus to your image once you subsequently disengage this feature.

High Magnification Focusing: There are two approaches you can use to focus at high magnification. One is to observe the behavior of fine particle detail. When this background granularity is just underfocused, it becomes sharp to the eye. The other approach is to use Fresnel fringes associated with holes/objects in the specimen which show discrete edges/boundaries. Overfocus will be seen as dark fringes; underfocus appears as white fringes; true focus will reveal no interference lines. Remember that you want a small amount of underfocus.

Beam and Histogram Centering

Even with the microscope well aligned, you may need to ensure even illumination by centering the beam when you change magnification. Get in the habit of centering and spreading the beam as you make changes in magnification (especially large changes). Care must be taken in beam centering, as delicate sections are likely to be destroyed at cross-over. Likewise, beam brightness will change with changes in magnification. Activating the **ZOOM** button will help to keep the histogram distribution centered. Additional histogram centering is accomplished with the **BRIGHTNESS** knob.

Shut Down

1) **MAG 1** button **ON – LIT** and Magnification **SELECTOR** to **7500X**
2) Toggle **SPOT SIZE** to **2.**
3) Beam **centered** and **spread**.
4) **FILAMENT** knob **OFF**.
5) **Condenser, Objective, and Field Limiting Apertures** **OUT** (levers all the way to the left).
6) Camera **OUT**.
7) Remove the **Sample Holder**, remove your sample from it, and return the holder to the microscope.
8) **HT** button **ON**.
9) MegaView Camera Control Unit **OFF**; **exit** "iTEM" computer program; Computer and Flat panel displays **OFF** (if you are the last user of the day).
10) Turn down the microscope CRT display down.
11) Fill in all requested information in the logbook and log out of your session on the computer

Digital Imaging System

Basic Operation:

1. MegaView Camera Control Unit **ON**. (It is found on the box on the top of left console of the TEM.) Camera out: the green lamp will light on the In/Out Hand Switch unit.

2. Select "User" to log into the computer.

3. **Select** "iTEM" icon on desktop to load anaySIS program.

4. **Select** "DUAL" button under "Viewport Manager" window and highlight the first open Image Buffer.

5. **Insert** the camera by pressing the green lamp on the In/Out Hand Switch.

6. **Left click** on camera of choice.

	Camera 1	**Camera 2**	**Camera 3**
Name	Snapshot	Search (1)	Diffraction
Resolution	1376 x 1032 *	688 x 516	688 x 516
Exposure Time	200—1000 ms **	50 ms	50 ms
Image Acquisition	Yes	No	Yes
Live Histogram; Live Overlay	Yes	Yes	Yes
Offset & Gain Shading Correction	Yes	No	Yes
Function	Final focus; High resolution image capture ***	Identifying areas of interest; Initial focus	Diffraction: image acquisition***

* 1376 x 1032 is the largest resolution available for our camera setup.

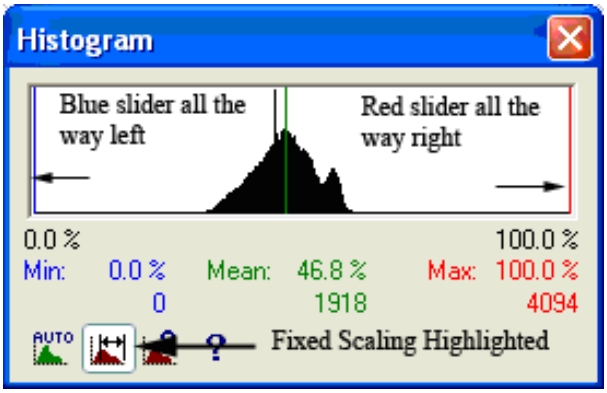
** To change the Exposure Time for Camera 1: Choose "Set Input" under the "Image" pull down menu. Highlight the Camera labeled "Snapshot" and left click the "Configure Input" button on the top of the dialogue box. Select the "Input Tab" and adjust the "Exposure Time".

*** Shading Corrections MUST be made for the "Cameras" used for image acquisition. This will require the acquisition of an "Offset" and "Gain Image"

7. Live image comes up on “left screen”. “Right screen” will show previous image (may be black if first capture coming up).

8. Adjust “histogram” to **50%** (Range=45-55%, with wide spread of distribution) with **BRIGHTNESS** knob.

   There is a “Fixed Scaling” and “Auto Scaling button at the bottom of the “Histogram” window. Auto Scaling is fine for most applications. Selecting Fixed Scaling with the blue/red sliders all the way left/right will ensure maximum information capture. Histogram stretching gamma adjustment of the captured images should be done subsequently with programs such as Photoshop and ImageJ.



and

9. **Left click** the “Acquisition Button” (Button 1, 2, or 3) corresponding to the camera chosen. If you take a snapshot of Camera 1 (high resolution), you will be prompted to enter the magnification. Doing so will ensure a “micron marker” in your image. The size and format of the micron marker can be adjusted in the “Properties” dialogue box within “Scalebar” under the “Image” pull down menu.

10. If you wish to save the snapshot, highlight the corresponding buffer and do: “File”> “Save As”. Save your images in your own folder in the “My Documents” folder on the desktop (This folder is a shortcut to: “C” – “Document and Settings” – “Characterization Fac” – “My Documents”. You will need to know this location for FTPing your documents—see below). If you wish to save your file as an 8 bit rather than 12 bit: When you select “Save as” from the “File” pull down menu there is an “Options” button. Select that button and check “Convert 12 bit images to 8 bit”

11. Click and highlight the next open “Image Buffer” …will turn blue. Then click on one of the camera icons to return to live imaging.

Shading Correction

Acquiring the “Offset” image: **FILAMENT** current **OFF;** Sample Holder **OUT** of the column. Camera **IN** the column. Cover the viewing window of the microscope. **Activate** the Camera of choice by selecting the “Camera” icon followed by its “Acquisition” button. **Select** “Acquire Reference Images” under the “Image” pull down menu. **Click** the “Acquire” button adjacent to “Offset Image”.

Acquiring the “Gain” image: **FILAMENT saturated**; Sample Holder **OUT** of the column. Ensure the beam is **centered** and **spread**. Cover the viewing window of the microscope. Camera **IN** the column. **Activate** the Camera of choice by selecting the “Camera” icon followed by its “Acquisition” button. Select “Camera Control” under the “Image” pull down menu and ensure that the “Exposure Time” value there has the same value as it does within “Configure Input” for

the camera. Select "Acquire Reference Images" under the "Image" pull down menu. Adjust **BRIGHTNESS** so that the "histogram" median is 50%. Click the "Acquire" button adjacent to "Gain Image" (Do not select "Automatic intensity adjustment").

Checking the reference images: Sample Holder must still be out of the column. Ensure the beam is **centered and spread**. **Select the** "Camera" for which you acquired the "Gain" and "Offset" image followed by its "Acquisition" button. The only thing you should be able to see is homogeneous noise.

Taking Your Work With You

Burn a CD:

1. Insert CD
2. Select the "file/folder" of interest
3. Select "Copy this File/Folder" under "File and Folder Tasks".
4. Select the "DVD/CD Drive" and click "Copy".
5. You will be prompted with a "You have files waiting to be copied" balloon. Click on the balloon.
6. Select the "File/Folder" of interest in the dialogue box which appears and click "Copy to CD"

FTP to Server:

1. Open FileZilla on the desktop
2. Select "Site Manager" under the "File" menu. Select "CFServer" and enter your username and password.
3. Select and drag the file/folder of interest from the "Local Site" to the "Remote Site".

Multiple Image Alignment

1. Select the sample area and magnification of interest. In order to create a composite image you will be increasing your magnification and dividing the area of interest into "subimages" that will later be aligned. The subimages will be displayed in a matrix of rows and columns. You can have up to six rows and six columns. Obviously, the larger the number of subimages, the higher your magnification increase will need to be. The following example shows the numbering system for a 2 x 3 matrix.

1	2
4	3
5	6

2. Select/highlight the first open "buffer". Choose "Camera 2" and position the sample so that you are viewing subimage 1. Choose "Camera 1" to acquire a high resolution image in the buffer (histogram median should be at 50%).

3. Select/highlight the next open "buffer". Choose "Camera 2" and position the sample so that you are viewing subimage 2. Ensure an overlap of about 20% with subimage 1 so that the computer program can pattern match and subsequently align the two subimages. Continue this process through the final subimage.

4. Select/highlight the first buffer subimage captured
5. In the "Image" pull down menu select "Acquire Multiple Image" and then "Arrange".

6. Set up the horizontal and vertical parameters to match the matrix of subimages you just captured.

7. Make sure that "Meander" and "Horizontal" are selected. The highest "Correlation quality" is a value of 1

8. Click OK. An "Arrange Multiple Images" dialogue box with the composite image will appear

9. Select "side by side" as the "Play with Overlap Area Options". To reposition a subimage –left click, hold and move with the mouse. Checking "Original Size" will increase the screen magnification and allow for fine tuned alignment.

10. Checking "Equalize" will align the grayscale values of the composite subimages
11. Selecting "Cut Margins" will eliminate image borders that jut out as well as black areas.
12. Click OK and the composite image should be saved to the next open buffer where it can be saved in the usual way

Diffraction

Selected Area Electron Diffraction

1. Locate sample area of interest.
2. Turn DIFF on and set camera length to 250 cm.
3. Use the IOS/DIFF knob to obtain the sharpest spot
4. Activate the DEFLECTOR-PROJ button and use the DEF X and Y knobs to center the spot.
5. Turn SAM/ROCK on.
6. Select desired magnification and field of view
7. Insert the Field Limiting Aperture and center (if image disappears, decrease magnification)
8. Use the IOS/DIFF knob to focus the Field Limiting Aperture.
9. Center the Field Limiting Aperture.
10. Remove the Objective Aperture.
11. Turn DIFF on and set camera length to 10-250 cm.
12. Use the IOS/DIFF knob to focus the diffraction pattern.
13. Insert the beam stopper.
14. Capture the image (need sections on digital and film)

Microbeam Electron Diffraction

1. Locate sample area of interest.
2. Turn DIFF on and set camera length to 250 cm.
3. Turn MAG 1 on. Set desired Magnification.
4. Center precisely the feature of interest and focus.
5. Remove Objective Aperture.
6. Select smallest Condenser Aperture.
7. Minimize Spot Size.
8. Bring beam to crossover and center.
9. Turn DIFF on and set camera length to 10 cm.
10. Turn the IOS/DIFF knob to focus diffraction pattern.
11. Insert the beam stopper
12. Capture the image (need sections on digital and film).