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# ELECTRON MICROSCOPY PROCEDURES MANUAL

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SPECIMEN PREPARATION PROTOCOL

1. FIXATION:

Tissue can be fixed by immersion or perfusion. The most commonly used method is immersion. Fixation time is variable, depending on tissue, but usually from 4 hours to overnight at 4 degrees (refrigerator).

- a. Primary Fixative: 1/2 strength Karnovsky's.
  - 2% Paraformaldehyde/2.5% Glutaraldehyde
  - This is buffered with a 0.2M Cacodylate Buffer

Note: Always use the fixative in the fume hood and wear gloves. Glutaraldehyde fixes skin and cacodylate buffer contains arsenic.

- b. 0.1M Cacodylate Buffer wash 3X for 10min. each
- c. Post Fixative: 2% aqueous OsO<sub>4</sub>/0.2 M Cacodylate Buffer
  - A 1:1 solution for 2 hours in the refrigerator
- d. 0.1 M Cacodylate Buffer wash 3X for 10 min each

2. DEHYDRATION:

Dehydration with ethanol:

35% ETOH	15 min. and decant (optional)
50% ETOH	20 min. and decant
70% ETOH	20 min. and decant
95% ETOH	20 min. and decant
100% ETOH	20 min. and decant
100% ETOH	20 min. and decant
Propylene Oxide	15 min. and decant
Propylene Oxide	15 min. and decant
1:1 PO/Epon resin	overnight

- The dehydrating times should be adjusted to size and kind of tissue.
- The resin is an Epon 812 recipe

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### 3. EMBEDDING:

Embedding with Epon:

- Next morning, change out to fresh Epon 812 for 1-3 hours.
- Embed (always) in fresh Epon 812
- Polyethylene capsules are placed in a holder and numbered strips of paper are inserted. A drop of fresh Epon 812 is placed in the capsules and the specimen is transferred to the appropriate capsule.
- The “blocks” are cured for 48 hours in a 60 degree oven.

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### 4. SECTIONING:

- 1 micron or thick sections are taken to find the area of interest
- Take ultra thin sections (~70nm) and place on grids

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### 5. POST STAINING:

- Stain grids on uranyl acetate for 2 hrs.
- Stain grids on lead citrate for 5-6 min.

\*\* This procedure is the basic TEM procedure. Each and every sample that comes to us is evaluated and the procedure is adjusted to make sure the best results will be obtained.

## FIXING CELLS FOR ELECTRON MICROSCOPY

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### METHOD 1: SCRAPE CELLS WITH CELL SCRAPER

**\*\*We need 1-5 million cells in order to do our procedure\*\***

Fixation:

Fixative = 1/2 Karnovsky's

- Use fixative (from refrigerator) and warm it up to room temp. or 37 degrees.
- Add fixative to media (1:1) and let it sit for 10 min to fix.
- Take off fix and media (don't let cells dry out) leave a small amount to cover cells and then add pure fix for 30 min - 1 hr.
- Scrape cells, put them into an Eppendorf tube, and spin down at a very low speed (1400 rpm).
- Pipette off fix (Keep a little fix on to not let the cells dry out) and put on new fixative and then re-suspend cells.
- Put cells in refrigerator overnight with fix or bring them down to the EM lab in fix.

**Or**

- Wash media off with 0.1M Cacodylate buffer quickly and add ½ Karnovsky's fixative and then scrape after 30min – 1 hr.

Wash:

- Wash cells 3 x 5 minutes in 0.1M Cacodylate buffer.
- Spin down each time.

Post Fixation:

- 1% Osmium Tetroxide in 0.1M Cacodylate buffer for 1 - 2 hr. at 4 degrees in the refrigerator.

Wash:

- Wash 3 x 5 minutes in 0.1M Cacodylate buffer.

Dehydration:

- In graded series of ETOH - 50%, 70%, 95%, 2 x 100% ETOH for 15 min. and 2 x Propylene Oxide for 15 min.

Embed:

- 1:1 PO/Epon resin overnight with Eppendorf tubes capped.
- Next day, spin down cells and uncap Eppendorf tubes.
- Add Fresh 100% Epon resin for 2- 4 hrs.

Polymerize:

- In 60 degree C oven overnight in Eppendorf tubes.

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**METHOD 2: PERMEABILIZE CELLS FROM PLATE**

Fixation: Do as above but permeabilize cells from plate instead of scrape.

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**METHOD 3: LEAVE ON PETRI DISH, PERMANOX CHAMBER SLIDES OR COVERSLIPS**

Fixation: Do as above but leave cells on the plate in the fixative for 1 hr.

Embed: Change 2x to fresh Epon resin and then invert beam capsules full of slightly hardened resin over relevant areas.

Polymerize: In 60 degree C oven overnight and then pop off beam capsules that contain the cells.

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**METHOD 4: TRANSWELL PERMEABLE SUPPORTS**

Fixation: Fix the attached supports containing cells in plate for 2 hr. or overnight.

Wash, Post fixation, Dehydration: Same as above.

Embed: 100% fresh Epon resin for 1 hr., then punch out supports with an 8mm biopsy punch on wax containing resin. Cut out relevant pieces and flat embed.

Polymerize: in 60 degree C oven overnight.

## LRW EMBEDDING

**\*\*Note:** Do not use OSO4 fixed tissue or cells

1. Fix tissue or cells in primary fixative.
2. Wash tissue 3X 10 min. in 0.1M Cacodylate buffer, pH 7.4.
3. Dehydrate in ethanol:
  - 50% - 1X-5 min. sit in hood/ 2 min. spin for cells
  - 70% - 1X-5 min. sit in hood/ 2 min. spin for cells
  - 95% - 2X-7 min. sit in hood/ 2 min. spin for cells
  - 100% - 2X-7 min. sit in hood/ 2 min. spin for cells
4. Infiltration with LRW:
  - a) Mix LRW Resin with ETOH – 1:1 in a scintillation vial. Put mixture in tissue or cells for 30 min.
  - b) After 30 min., make a 2:1 mixture of LRW to ETOH.
5. 100% LRW Resin – 3X-1 hour and leave overnight in the last change.
6. Embed in Ted Pella flat teflon mold or gelatin capsules. LRW will only polymerize anaerobically, seal tightly.
7. Next:
  - a) Cure in UV light cryo-chamber in 4 degree C walk in refrigerator overnight. Put pans of ice on the bottom of the container.

**OR**

  - b) Polymerize in gelatin capsules in 50 degree C oven overnight.

## POST-EMBEDDING IMMUNOGOLD LABELING ON LRW SECTIONS OF CELLS

**Example:** Cell pellets were fixed for 3 hrs. with 0.025%glut./3% paraformaldehyde. then worked up with short dehydration times (10 min starting at 50% ETOH) & polymerized at 4 degrees under UV light for 1.5 days with LRW in gelatin capsules.

### PRIMARY ANTIBODIES:

Rabbit Preimmune sera – Investigator supplies

### SECONDARY ANTIBODIES:

Goat anti Rabbit 10 nm, IgG (H+L) from EMS – Investigator supplies

### DILUTIONS:

- Primary - Preimmune sera – 1:30
- Secondary - 10 nm gold – Straight or 1:25
- Control - No Primary/ Straight gold

### PROTOCOL:

\*\*Everything is filtered with a 0.2 micron filter before use and purchased from EMS.

1. 0.05 M Glycine – 20 min. at room temp.
2. PBS- 3 drops
3. 5% BSA – 45 min. at room temp. (optional)
4. BSA-C/PBS/Tween 20 – 3X5 min drops (PH 7.4)
5. Primary antibody – 1 hr - ? at room temp. or overnight at room temp. or at 4 degrees C in humidified petri dish. (spin Primary in a centrifuge for 5 min on high speed).
6. PBS – 3 drops **OR** Dip into beaker of PBS
7. BSA-C/PBS/Tween 20 – 6X5 min drops (PH 7.4)
8. Secondary – Gold – 1 hr. - ? at room temp. or overnight at 4 degrees C in humidified petri dish
9. BSA-C/PBS/Tween 20 – 6X5 min drops (PH 7.4)
10. PBS 3 drops
11. 2% glut/PBS for 10 min at room temp
12. Warm ddH<sub>2</sub>O wash
13. Post-stain with UA for 1 min and Lead citrate for 30 sec.



## NEGATIVE STAINING PROCEDURE

1. Use 200 or 300 mesh formvar/carbon coated grids. (Grids may need to be glow discharged for sample to adhere.)
2. Place a drop (approx. 25 ul) of specimen on wax plate and put the grid on top of the specimen. Leave for 15 min. to 1 hr.
3. Touch grids to drops of:
  - ½ Karnovsky's Fixative
  - 0.1M Cacodylate Buffer
  - 3 drops dH<sub>2</sub>O
4. Touch grids to a drop of UA and onto a moistened filter paper to draw off excess UA. The percentage varies with the sample.
5. Dry overnight in a Petri dish, in the desiccators, and view the next day in the microscope.

## SCANNING ELECTRON MICROSCOPY PROTOCOL USING HMDS

1. Fix tissue in ½ Karnovsky's Fixative and OsO<sub>4</sub> as usual.
2. Wash tissue in 0.1M Cacodylate Buffer
3. Dehydrate using a series of ethanol washes:
  - 50% Ethanol for 15 – 30 min.
  - 70% Ethanol for 15 – 30 min.
  - 95% Ethanol for 15 – 30 min.
  - 100% Ethanol for 15 – 30 min.
  - 100% Ethanol for 15 – 30 min.
4. Immerse tissue in pure HMDS for 15 min. x 2
5. Just cover the sample with fresh HMDS and leave under the hood until the HMDS evaporates off.
6. Specimens will be ready to mount onto stubs and sputter coated with Au/Pd in the morning or store desiccated until coated.

## OPERATIONS

### JEOL 1400 TEM OPERATION

The following procedure is a general operating procedure that the majority of users will apply when working on the JEOL TEM. The following should be used as a starting point for your particular work. The settings can be modified to produce the best images of your samples. With that in mind please remember to return the scope to the standard mode of operation for the next user.

#### SIGNING ON AND GENERAL SETTINGS:

1. Make sure that you sign on by recording your time onto the paper log sheet when you turn the beam on.
2. CL aperture: 2, OL aperture: 2, Spot size: 2 (recommend a new alignment when changing any of these – see alignment procedures), HT: 120kV
3. If system is logged into **JEM Administrator** account, log out and log into **JEM User**. Password: JEMUser
4. Bring up nanospace map and vacuum monitors, if not already onscreen.
5. Start Digital Micrograph software if it is not already on by double clicking DM icon on left monitor (Ultrascan camera will take approx. 20 minutes after software startup to cool down enough for good images)
6. Check to see that **HT** is at correct value (120kV normally). *If HT is off or at a different value, let an EM staff person know so they can re-establish it.*

#### SAMPLE LOADING AND REMOVAL-**FOR EM STAFF ONLY**:

**\*\*Please Note: If the 5-grid holder is in the Goniometer, be sure the “Exchange” white dot and the Number 1 (also with a white dot) are aligned before removing.\*\***

1. Center the holder by tapping the LF2 button two times on the left console to turn off the beam and reset the holder.
2. Prepare to remove the specimen holder by making sure the beam is off.
3. Switch Goniometer from “Pump” to “Air”. Nothing should happen.
4. Pull Specimen holder straight out of Goniometer ~7cm until it stops then turn ~75 degrees Counter-Clockwise until it stops. You can now let go and reposition your hand.
5. Pull Specimen holder straight out of Goniometer ~0.5cm until it stops then turn ~15 degrees Counter-Clockwise until it stops. Let go. The square “seat” should be at 12:00. You will hear the Goniometer venting to air and green light will turn off. On the CPU the “Vacuum” chamber field should now read “Not Ready” and will climb to ~241.
6. Gently remove the holder straight out from the Goniometer. If there is resistance wait another ten seconds. Be careful not to touch the holder beyond the screw/collar, as this will introduce grease and dirt into the microscope.
7. Raise clamping mechanism on the specimen holder. Place your grid in the holder, then lower and clamp the mechanism.

**\*\*Again Please Note: If you are using the 5-grid specimen holder, be sure the “Exchange” white dot and the Number “1” (also with a white dot) are aligned.\*\***

8. When carefully replacing the specimen holder, align the small pin with opening in side of Goniometer until seated in Goniometer.

9. With slight pressure pushing specimen holder in Toggle switch from “Air” to “Pump”. You should hear the valve opening and the CPU “vacuum” SPC field should still read “Air” but will drop to about 50uA.
10. Select the specimen holder you are using. Ex. Penta for the 5 grid specimen holder EM-11170.
11. Once the specimen chamber (PG4) reaches 50uA (Evac Ready) get a good grip on the specimen holder and turn clockwise ~15 degrees until it stops, then allow it to go into the chamber ~0.5cm.
12. Still with a good grip turn clockwise ~75 degrees until it stops and slowly allow it to go into the Goniometer until the square “seat” nestles into the Goniometer at 3:00. Eventually you will do both of these steps as one fluid step. (Please note: indicator will change from “Air” to “Ready” and then you can go ahead and turn the beam on)
13. Confirm the HT is at 120 KV and is READY.

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#### TURNING ON THE BEAM AND CORRECTING Z-AXIS (HEIGHT OF SAMPLE):

1. **Turn Beam on:** After seeing that the HT is at the set value (120 kV), turn Beam on (on screen or tap **Beam** button on left console). Beam will take approximately 3 minutes to come up to target intensity. Often it is a good idea to beam blank the sample by pressing **F6** key (key will illuminate) to protect your samples from beam damage while waiting for the beam to ramp up. Turn off beam blank when ready to view specimens (**F6**).
2. **Set Z height:** Set mag to 10,000x and click **std focus** on right console to neutralize the focus. Use **Z controls** and **x** or **y wobbler** on right console to bring image into fine focus. This can be done with stage binoculars or with a live camera image. Sample is now at x, y, z focal crossover (esp. important for tomography). When using the Penta holder, position 1 focuses at a Z value of about 200 - 250. Each specimen position will need a new Z height set and each successive position reduces Z by about 50.
3. Refocus image if necessary.

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#### VOLTAGE CENTER ADJUSTMENT (IF NEEDED):

Click **HT wobbler** (right console), **Bright Tilt** on (left console) and use **def/stig** knobs (yellow) to align. The image should pulse on center (may have to bring beam to center with green **Shift** knobs).

The Voltage Center will most likely have to be checked if the **Neutralize** is used to neutralize all settings to home.

If the image shifts significantly while focusing, check Voltage Center again.

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#### USING THE GATAN DIGITAL IMAGING CAMERA AND SOFTWARE:

1. Make sure the Gatan PC screen is on and make a folder on the desktop. Label with username or Hutch account\_date. Example: jdoe\_011814. No spaces please.
2. Open the Gatan Digital Micrograph application (if not open already) and insert the camera.
3. For the Ultrascan 1000XP bottom mounted camera-Lift up the screen by selecting [F1] on the right console. For the Orius side mounted camera-Make sure illumination is very dim and then insert the camera. Then select [Start View] on the right panel of the Gatan software and you will see a continuous, quick scan of your sample taken at low resolution (search mode). Be sure in the “intensity box” that the box is within the green field with the brightness knob.

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#### IMAGING:

1. Using the **Brightness** knob, establish a comfortable illumination for imaging the sample. If using the camera to scan your sample, make sure to keep the intensity in the green zone! **Staying in the red zone will damage the camera!**

2. If the **LF1** is not lit, click it once to set a brightness zoom to keep illumination in the same range at all magnifications
3. To center the beam, use the green **Shift** knobs, **X** and **Y**, on the left and right consoles. It may help focus the illumination before doing this.
4. Find your region of interest on the grid using the track ball, arrow keys on the track ball box or with the **nanospace map**.

- **Focus:**

Use **coarse** and **fine** knobs (right console).

Any controls are in the coarse mode when the **CRS** button nearby is illuminated.

Click CRS off to return to fine mode. Use **X** or **Y wobbler** to help with focus.

Turn off the **wobbler** when focus is established and before recording an image.

- **Magnification:**

Change magnification up or down with the **mag/cl** knob.

Illuminating **LF1** will set a brightness zoom to keep illumination in the same range at all magnifications.

- **Using the Nanospace Map:**

Click on a target area on the graphic and click **Shoot** to move to the new area.

The area can be saved for future navigation in the window next to the map by clicking **Memory**.

Positions can be recalled by highlighting the location and clicking **Go**.

- **Image Orientation System (IOS):**

You can use this system for rotating your sample for the best picture at certain magnifications.

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#### TO COLLECT IMAGES:

To collect images, center the beam and bring the beam to a reasonable intensity (see above).

Bring the image to a final focus and click **Start Acquire**.

Set up your image file parameters under **Global Info Setup**.

Save images with either **Save As** or **Save Numbered** making sure you have the relevant information in the image title, including magnification. Save images to a folder you have set up on the desktop.

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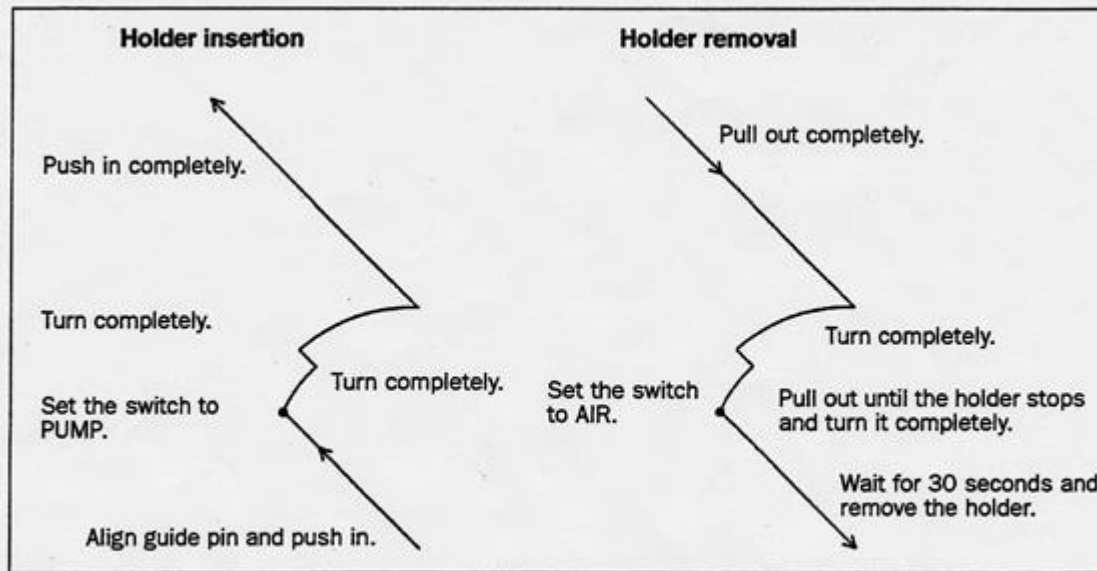
#### END OF SESSION:

1. Lower the magnification to ~1000x.
2. Spread the beam maximally.
3. Tap **F6** to beam blank the specimen.

4. Turn off the beam on the screen or by tapping the **Beam** button on the left console. Note that the **Beam** button on the monitor turns darker green indicating the beam is beginning to ramp down.
5. Return the specimen rod to position 1.
6. **Note the time off on the paper log sheet.**
7. The staff will remove your sample from the specimen holder and reinsert it back into the microscope.
8. **Batch convert** images to **TIFF's** by selecting **Batch Convert** under the **File** menu. Be sure to "Browse" to choose the correct folder in the "**Save To**" box. Make sure **Save Display As** is selected or you will convert to the wrong bit depth.

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SAMPLE HOLDER LOADING AND REMOVAL FOR THE JEOL 1400 MICROSCOPE



**Specimen holder motion diagrams**

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SPECIMEN LOADING AND EXCHANGE:

To Load Samples:

1. Screw in stubs to a specimen holder.

To Exchange Samples:

1. **Vent** the specimen chamber.
  - a) Click the HT icon to get HT OFF
  - b) Click the **Sample Setting** of the operation menu tab
  - c) Click the **Removing the specimen** button.
  - d) Click the **Vent** button
  - e) After the light of the Vent button turns ON, the stage can be withdrawn to remove the specimen holder.
  - f) Perform the "Sample height setting". Set the height of the sample according to the procedures.
2. **Evacuate** the specimen chamber.
  - a) Click the **Evacuating the Chamber** button.
  - b) Close the specimen chamber and click the **EVAC** button. Evacuation in the specimen chamber will start.
  - c) Click the **Sample Setting** (or **Stage**) in the operation menu tab.
  - d) Click the **Navigation** button, and capture the navigation image according to the operational navigation.
  - e) Click the **Capture** (or **SNS Capture**) button to capture the image and the stage will move automatically to the photographing position.
  - f) After the **HT icon** is turned on you can start image observation.

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SETTING THE SPECIMEN:

- a) Click the **Sample Setting** in the operation menu tab
- b) Click the **Setting** button.
- c) Select the specimen holder used, and click the Select button.
- d) If the specimen protrudes above the holder, make sure to input the protruding height in the dialog box.
- e) Set the specimen holder onto the specimen stage.
- f) Close the door and hit the **EVAC** button.

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CHOOSING A RECIPE:

- a) Click **Recipe** in the operation menu tab.

- b) From the displayed list of Standard recipe, select a recipe applicable to the sample. Select Universal if you are not sure.
- c) The operation navigation is changed to the setup observation condition menu. Set observation conditions according to the questions you will be asked.
- d) Click the **OK** button.

**OBSERVATION:**

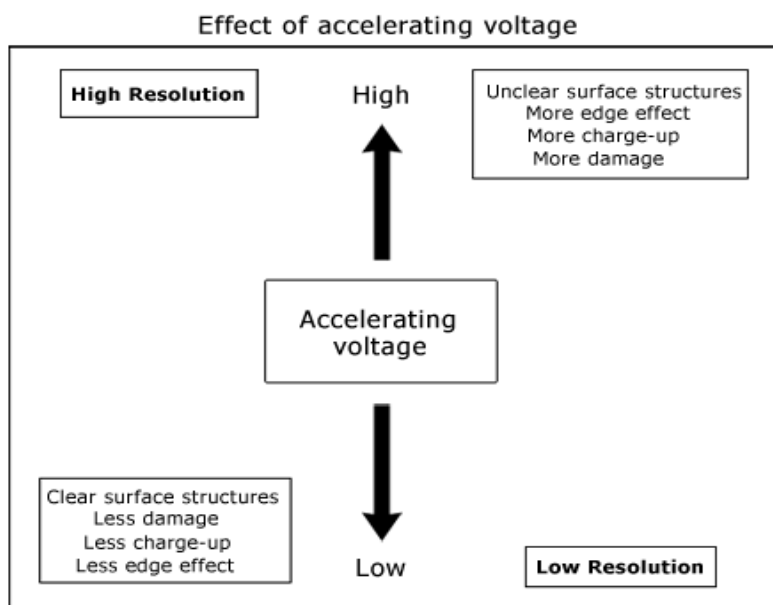
1. Specimen(s) are loaded and **HT icon** has been pushed and is ready.
2. Click the **ACB**, **AF**, and **AS** icons to observe the image.
3. Move view of interest to the center of the main screen with “Click center”.
  - a) Double-click the left mouse button at any position in main screen. The double-clicked position moves to the center of the screen.
4. Find view of interest.
5. Adjust the image quality by using the **Contrast**, **Brightness**, **Focus**, and **Stig X**, **Sig Y** buttons.
6. For observing at high magnification it is recommended to focus at low magnification first and then increase magnification gradually.

**OBSERVATION CONDITIONS:**

- For the observation conditions of a specimen, you must select optimum values from various factors, such as accelerating voltage, probe current, aperture, working distance, and specimen tilt. Also, the brightness adjustment, astigmatism correction and focus adjustment become important.

Choosing the Accelerating Voltage:

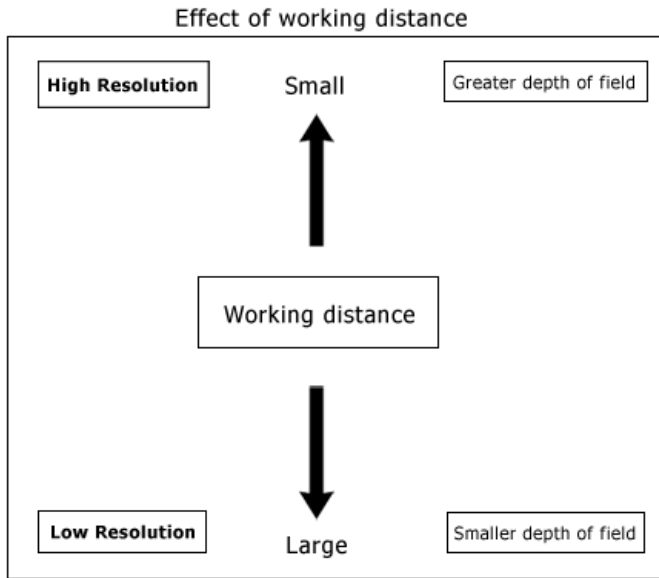
1. Generally, the more fine structure of the specimen surface appears when using a low accelerating voltage than using a high accelerating voltage.



2. Click the Accelerating voltage in the image data display and double-click on the desired accelerating voltage in the list.

Set Z (WD) and T (tilt) as desired:

1. WD is the distance from the underside of the objective lens to the specimen surface. See Figure for explanation:



Set Spot Size as desired:

1) Higher magnification and higher resolution for the SEM image can be obtained by smaller probe diameter (spot size) to irradiate the specimen.

2) Spot sizes range from 1 to 99.

<u>Spot</u>	<u>Size</u>	<u>Current</u>	<u>Resolution</u>	<u>Noise</u>
1	Small	Low	High	Most
49.5	Medium	Medium	Medium	Medium
99	Large	High	Poor	Least

3) Click the Spotsize (SS) on the image data display.

4) Click one of the preset buttons or use the slider button.

Objective Aperture Size:

- #1 = 20uM (High Resolution)
- **#2 = 30uM (Normal)**
- #3 = 100uM (High Current for EDS)

Centering Objective Aperture:

1. "OL" **WOBB** on.



2. Increase Mag. to see image movement.
3. Adjust aperture knobs to minimize movement.
4. “**OL**” **WOBB** off.

#### Correct Stigmatism:

1. The image is astigmatism-free if it has no unidirectional defocusing when the objective lens is changed to under- or over-focus.
2. This is done by adjusting the two knobs, x and y, of the stigmator with the **STIG** button pushed on the operation keyboard or on the main window of the menu bar.

#### Adjust Brightness and Contrast:

1. Brightness and Contrast can be set by pushing **Scan 1** and the **Contrast and Brightness** buttons on the main window menu bar and then adjusting with the mouse.
2. Adjustment also can be made with the knobs on the operation keyboard.
3. Bring the two bars in the middle.

#### Adjust Focus:

1. Place a mouse pointer on the **Focus** button:  
Course Adjustment – While holding down the right mouse button move the mouse up (right) & down (left)  
Fine Adjustment – While holding down the left mouse button move the mouse up (right) and down (left)
2. Focus can also be adjusted by using the focus knob on the operation keyboard or using the **AF button** (automatic focus) on the main window menu bar

**\*\*Note: Set Desired Magnification, Focus, and Stigmatism before taking a photo.\*\***

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#### SETTING UP TO TAKE A PHOTO:

A folder will be created for you on the desktop with your name and date Ex. Jdoe\_012407.

1. Go to **Set Up** in the operation menu tab.
2. Go to **Scan and Auto Save** tab.
  - Set up the **Auto Save Config**.
  - Select for the photo speed (resolution of the image) – Select from 40s to 160s. The slower the speed the higher the resolution of the image.
3. Go to **SEM Data Display** tab to display selected SEM data on the image. Select **Photo** button (main menu or keyboard) and wait for the scan to finish and then select **txt paste** (optional) and **Save**.
4. Select for **Image List** tab to see images taken.
5. Click on **L** button and **EXT ON** button to switch over to the microscope.
6. Transfer files to your fred account or copy onto CD.

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#### MICROSCOPE SHUT DOWN:

1. HT button should be pushed off.
2. Push the **Vent** button.
3. Take the samples out when venting is completed or ask the EM Staff to do this.
4. Minimize main JEOL SEM software.
5. Turn off the monitor screens.

## ADDITIONAL INFORMATION AND RESOURCES

### GENERAL ELECTRON MICROSCOPY RESOURCES

For more information on the topics listed below, please check the websites or publications listed below.

#### INTRODUCTION TO ELECTRON MICROSCOPY

- <http://www.sci.sdsu.edu/emfacility/555class/class1.html>
- <http://www.amc.anl.gov/> (Select the PDF slides/lecture notes by Nestor Zaluzec)

#### ELECTRON MICROSCOPY – WEB COURSE NOTES AND REFERENCES

- <http://em-outreach.ucsd.edu/web-course/toc.html>

#### TRANSMISSION ELECTRON MICROSCOPY (TEM) BASICS

- <http://www.matter.org.uk/tem/>

#### LISTSERV FOR ELECTRON MICROSCOPY INFORMATION

- <http://www.microscopy.com>

### SPECIFIC PROTOCOL OR TECHNIQUE RESOURCES

For more information on the topics listed below, please check the websites or publications listed below.

#### EM INFO FOR *CAENORHABDITIS ELEGANS*

Electron Microscopy and Three-Dimensional Image Reconstruction.

By David H. Hall, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

Methods in Cell Biology, Vol. 48, Ch. 17, pages 395-436.

#### IMMUNOGOLD LABELING

For additional information and literature, including company newsletters: <http://www.aurion.nl/>

#### IMMUNOGOLD QUESTIONS

<http://www.emsdiasum.com> or Electron Microscopy Sciences Catalog 2007-2010

#### OPTICAL IMAGE PROCESSING

<http://microscopy.fsu.edu/primer/digitalimaging/russ/index.html>

Course notes by John Russ, Florida State University