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Philips/FEI EM208S/Morgagni Operating Instructions (Transmission Electron Microscope)

A. Initial Standby Settings (the microscope should be left in this condition):

1. Large Haskris water recirculator in C-109 is on, showing 35-40 GPH water **flow**, approximately 28 PSI water pressure, and approximately 68° F. These settings do not need to be checked unless the microscope has been totally shut down
2. The **air pressure gauge** on the wall in C-106A shows 75-80 psi and the **water pressure gauge** shows 30-35 psi
3. The **power off** button is lit
4. The Morgagni computer is on and the monitor is on (though may be dark when in standby condition). The Morgagni icon is showing on the desktop
5. The **desk lamp** is off

B. To Shut Down the Microscope

1. Spread the beam (**Intensity**), reduce magnification to 1.8 kX and turn off the **HT**
2. Remove the **Specimen Holder Carrier**. Remove the **Specimen**. Re-insert the **Specimen Holder Carrier** into the microscope in the **park position**
3. **If there is a serious microscope malfunction, press the POWER OFF button to totally shut the microscope down and call FEI (1-800-432-1734). Tell FEI that it is a 208S TEM, with a D-703 serial number.**

C. Loading a Specimen Into a Specimen Holder

1. If the **Carrier** is in the **park position**, just pull it straight out to load it
2. Pick up a **Specimen Holder** with the **Specimen Holder Carrier**: Position the **Carrier** over the **Specimen Holder** while holding the **Carrier Button** (on the end of the carrier) in. Release the **Carrier Button**
3. Place the **Carrier** in the **Carrier Rack** with the **Clamping Device** up

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4. Insert the **Specimen Holder Pin (Pin)** (found in the center of the **Specimen Holder Rack**) into the small hole above the **Specimen Holding Ring** and gently raise the **Clamping Device**
5. Insert a **Grid**, shiny-side up, into the depression under the **Specimen Holding Ring**. Gently lower the **Specimen Holding Ring** with the **Pin**. Return the **Pin** to the **Carrier Rack**

D. Inserting a Specimen into the Microscope

1. Hold the **Carrier** so that the **Pin** is at approximately 20° clockwise
2. Gently insert the **Carrier** into the **Airlock** so that the **Pin** goes into the opening at 20° (**not the one pointing straight up**). Push it in until it stops (the mechanical pump will begin pumping)
3. Wait 7-10 seconds (until the **LED** is extinguished), then turn the **Carrier** counter-clockwise until the **Pin** is vertical. Push in until it stops (a few mm)
4. Turn the **Carrier** fully counter-clockwise until a firm stop is encountered (half a turn)
5. Gently push the **Carrier** into the microscope until it stops
6. Fully depress the **Carrier pushbutton** and (while holding the **pushbutton in**), pull the **Carrier** out as far as it will go. Release the **pushbutton** and rotate the **Carrier** clockwise until a firm stop is encountered (it is now in the **park position**)

E. Removing a Specimen from the Microscope

1. If the **Carrier** has not been removed from the **Airlock**:
 - a. Depress the **pushbutton** on the **Carrier**
 - b. While keeping the **pushbutton** depressed, gently push the **Carrier** into the microscope until it stops (i.e., turn counter-clockwise 180°).
 - c. Release the **pushbutton**

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- d. Pull the **Carrier** out until it stops, turn it clockwise until it stops (a half turn), and pull the **Carrier** straight out of the airlock
2. If the **Carrier** has been removed from the air lock, perform steps D.1-D.3 above, before continuing with step E.1.a-E.1.d above

F. Removing a Specimen from a Specimen Holder

1. Place the **Carrier** with an attached **Specimen Holder** in the **Carrier Rack** with the **Specimen Holding Ring** up. Raise the **Specimen Holding Ring** as described in section B above. Invert the **Carrier** over a piece of clean filter paper so that the **Grid** falls onto it. If it does not fall out, use the **Specimen Holder Pin** to gently push the **Grid** out of the **Specimen Holder**. **Never bang the Specimen Holding Ring on a surface to dislodge a specimen (it will break the wires holding the Ring). Also avoid twisting the Ring with the Specimen Holder Pin**

G. Beam Generation (Beam time is located at the bottom of the High Tension page)

1. Activate the **monitor** and turn on the **desk lamp**
2. If the log on screen is up, Type <**Supervisor**> and then type **laelom** as the password
3. If the Morgagni icon is on the desktop, click on it
4. The **Vacuum screen** allows you to check the vacuum status. **Vacuum ready** means the microscope is ready for use
5. Look at the information bar at the bottom of the screen and check that the operational parameters are set properly:
 - a. **HT** (high tension) is set to 80 kV
 - b. **Spot** (upper condenser lens) is set to 3
 - c. **Magnification** is set to 1.8 kX

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- d. Click on the **Beam** button and make sure that **Intensity Zoom** is highlighted
6. Depress the **HT** button on the right console panel. The high tension will come up to 80kV and the filament will automatically saturate. Center the beam spot with **Shift X** and **Shift Y** controls and spread the beam to cover the screen with the **Intensity** knob on the left console panel
- H. Routine Alignment (done at each sitting for best resolution):**
1. **Gun Tilt:** Click on pull down menu box at the lower right of the screen. Select **Alignments**. Click on **Gun Tilt and Saturation**. Desaturate filament with the **Filament Knob** until a halo is visible. Center with **Multi X** and **Multi Y** knobs. Click on **Done** and then on **Green Check**. Click on **X** next to pull down menu box at lower right of screen to close **Alignments** screen.
 2. **Condenser Aperture** alignment: Insert the first aperture (**300 μm**). Sweep through crossover with the **Intensity** knob. If the illumination enlarges symmetrically, the aperture is centered. If not, open it slightly to see which direction it “tails” to, then move it in the opposite direction with the appropriate aperture centering knob. Enlarge it to fill the entire screen and center it again in a similar fashion. For **Condenser stigmation**, desaturate filament at 1.8 kX to 4.4 kX to see the filament halo. Depress **Spot button** on left console panel. Use **Multi X and Y** controls to sharpen image of filament imperfections. Depress **Spot button** again to shut off condenser stigmation mode
 3. **Do not** use the instructions for **Objective Aperture** alignment on this page. Select **Cancel** button, followed by **Green Check** button
 4. **Objective Aperture** alignment:
 - a. Insert **specimen** and **objective aperture #1** (50 μm)

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- b. **Select 1.8 kX** with **magnification** knob on right console panel
 - c. Center beam with **Shift X/Shift Y**
 - d. Center the **Objective Aperture: Use the first aperture (50 μm)**. Put a plastic section under the beam. Focus the beam spot using the **Intensity** knob. Depress the **Diffraction** button and use the **Objective Aperture** knobs on the column to center the beam spot in the aperture. Turn off the **Diffraction** button
 - e. Stigmatize the **Objective Lens**: while viewing a specimen, bring the magnification to about 110,000X. Press the **Image** pushbutton (LED will be lit). Focus on the grain in the specimen image until it is sharp, using the **Multifunction X and Y knobs**. **Stigmatism values** appear under column 1 on the **Stigmaters Objective** page
5. Click on **Camera** button to check settings:
- a. **3.25 X 4" camera** selected
 - b. **Auto** selected
 - c. **Stock** set at 54
 - d. **Exposure** shows number of photo **coming up**, with the first two digits designating the year (i.e., 04000004 means the 4th photo taken in 2004 on the Morgagni system)
 - e. **Mode: single** (the normal setting for individual photographs)
series (for through-focus series)
exp. select (to photograph stored positions automatically, with conditions set up the way they were when stored)

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- f. **Camera options:** all should be checked except **stage position** and **mode**. All of the information checked will be stored in the computer for each photograph
 - g. **Date format:** mm.dd.yy should be checked
 - h. **Data on Plate:** mirror should be checked
 - i. **User code:** 04 (this is to indicate the year the photograph was taken)
 - j. **Set stock:** this should read 54
 - k. **Settings:** Flap out to see these (carat in upper right of **Camera** screen)
 - 1. **Numeration Type:** unique (all photographs taken, regardless of user will be numbered in sequence)
 - 2. **Film type:** Kodak 4489
 - 3. **Emulsion:** 10
 - 4. **Data int.:** 2.5
 - 5. **Link to HT:** unchecked
6. Taking a **Photograph**
- If the pumping system cycles when taking a picture, the program may freeze the Exposure button. If it happens, close the program and reopen it to unfreeze the function.**
- a. Bring the **Focusing Screen** (the small screen) forward by pulling the small lever on the left side of the **Viewing Chamber**
 - b. Insert the **Beam Stop** (on the right side of the **Column**) and focus each ocular on it. Pull the **Beam Stop** out and turn it clockwise or it will be in your photograph
 - c. Select the **Specimen** magnification desired and focus the image, using the **Focusing Screen**. Push the **Wobbler** button in and sharpen the image (for photographs below 5.6 kX). Make sure you turn the **Wobbler**

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off before you take a photograph. If the wobbler is not working well, check that the **Wobbler amplitude** is set to 13-14% in the **Wobbler box** on the screen or on the **Tune** screen.

- d. Frame the **Specimen Image** by using the 4 marks on the **Viewing Screen**. The right side of the area circumscribed will lose almost 8 mm due to the numbering block printed on the negative. In addition, the side of the negative closest to the user will not include all that is within the circumscribed area (about 4 mm is lost on that side)
- e. Metering is most accurate using the **Focusing Screen** for samples of tissues or cells. If it is pushed back, the meter reads the illumination on the **Viewing Screen**. Metering with the **Viewing Screen** is recommended for negative stain preparations or unstained preparations
- f. Bring the **Beam Spot** toward crossover (turn the **Intensity** knob counter-clockwise) and center with **Shift X** and **Shift Y** knobs
- g. Spread the beam so that **Exp. meter time** shown in the box at the bottom of the monitor screen 1.5-2 sec. If the illumination is too low or too high, the **Exposure/Transport System** is disabled. In addition, the **microscope will beep at you if the speakers are on**
- h. Depress the **Exposure** button to take a photograph. Wait until the second pneumatic sound is through before touching anything so that the numbering can take place
- i. Note that the negative magnification appears at the bottom of on the monitor **after** the large screen lifts (viewing and negative magnifications are different)

