

Quick Guide for ESEM (Fei Quanta 200)

- 1 Chiller On, 10°C Switch on water chiller and cooling (set to temp – green). We start with 10°C.
- 2 Set 15kV, Spot 3.5 On the Microscope user interface set the Accelerating voltage (15) and Spot size (3.5).
Vent chamber Switch to temperature control tab (thermometer icon). Vent the chamber.
- 3 Remove stage, Remove specimen stage. Insert Peltier stage mount and then Peltier stage with water
Insert Peltier stage lines and ribbon cable extending into chamber. Lightly tighten set screw (helicoil).
mount and stage. Remove water plugs on left hand side of chamber and push on the water tubes.
Connect Control Depending on sample, determine whether or not you need a sample holder insert.
and water lines. Attach 9 pin connector of ribbon cable between the water lines. When the stage is
plugged in, the Cooling button should highlight (become active). To insure proper
cooling and humidity conditions, samples should fit completely into holder (<5mm).
Sample size < 5mm
- 4 Valve box On. Switch on the water valve box at left of column. The tone will start. Press and hold the
Fill water lines. green button while watching water move up the tubing from the chiller, through the
box and into the small tubes entering and leaving the chamber. Release the button. If
tone continues press and hold a few more seconds. Check for leaks in chamber.
- 5 Insert GSED, BSED. Insert gaseous (GSED) or backscattered (BSED) electron detector axial to pole piece.
No sample in yet Close chamber door. Pump and go to step 6. Note: No sample has been inserted.
Close chamber, If Cooling did not highlight, the ESEM will need to be rebooted as follows:
Pump. A – Close user interface.
 B – On right hand monitor, press stop in the Server window. DO NOT PRESS
SHUTDOWN. Wait until all the green icons have turned brown. Exit server. Tab is
at bottom of middle screen.
 C Press Start: restart
 D When computer reboots, enter the password <QuantaD8680>. Press on FEI Server
icon. When this opens Login: Supervisor, password: QuantaD8680. System
should start.
 E Hide the comments page that opens and Press Yes to Home the Stage. When this
finishes, Press Beam On. Cooling tab should be yellow when pressed.
- 6 Scan: Preferences: There are two steps that need to be done to aid in quickly changing and locating
a- General, No to subsequent samples. Under Scan: Preferences, go to General tab and scroll near the
"Lower stage on bottom to find "Lower stage when venting". Change this to No and Apply. Still in
Venting"
b- ESEM, set to Preferences go to ESEM tab. Normally the humidity in the chamber will cycle from
<1 to 9 Torr (automatic). This is called purging and is done to equilibrate the
<NO Purge> humidity. We find that it is often better to either set the purge limits to a smaller
Range for our conditions (e.g., 5 to 9 in Custom mode) or to turn off the purge
Altogether and setting the Pressure go to a preset desired value.
- 7- In Temp Tab, ESEM should now be pumped down and cooled. In the Temperature Control tab, push
Cooling On. On the Cooling button (yellow). Pushing the Humidity button will show the Triple Point
Humidity tab for Graph. The Temperature will be at or near 10°C (vertical line on graph), pressure (as set
Triple pt graph. by previous user– horizontal line on graph) and Humidity (also as left by previous user).
These will be set next.

- Startuup suggestions: Notes: The graph shows the real time triple point representation. When the Temp (V) and Pressure (H) lines cross above the curved line, the sample will be at 100% Humidity. If the lines converge on the white area above the curve, the Temp is at freezing or below. Setting the Humidity is best done by changing the Temperature or Pressure. A reasonable starting point of Temp = 5°C, Pressure = 5.5 Torr will give a Humidity of about 75-78%. This will allow slow evaporation /sublimation and will keep samples moist. The image will degrade dramatically at humidities much above this. Very small changes will be required to change the humidity.
- Temp = 5°C
 Pressure = 5.5 Torr
 Humidity = 78%
- WD = 10 mm
 <Temp = >Humidity
 >Press. = >Humidity
- 8 - Adjust Temp or Pressure to reach desired Humidity. Input the desired Pressure in the Pressure box (Main Menu). To change the Temperature select the Temperature button, input desired temp and Press Go To button. Switch back to Humidity tab and observe the temp and pressure change on the graph.
- 9- Center stage WD to 10mm
 Beam On, B/C at 400x. Focus, Stigmatte, and Lens Align now. In main tab, press Beam On. Be sure the stage is centered. Raise the stage (joystick) so that the edge of the Peltier stage is just above the yellow 10mm line on the chamber image. At 100x most of the field will be covered by the GSED with only a small circle of image showing. Increase the magnification to 400x and press Auto Brightness and Contrast. Find the bottom of the specimen holder and focus. This will make locating the specimen much easier and the temp, press and humidity will have a chance to equilibrate to your chosen settings while pumping your sample. You can do a lens align now because it is very difficult in the ESEM mode.
- 10- Beam Off, Vent. Push off beam. Vent the chamber. Have your sample ready to insert when chamber is opened.
- 11- Load sample, Pump. Load sample*. You can add a little extra water if you like. Some experience will indicate how much to add. Close chamber. Press Pump.
- 12- Beam On, 400x, Auto B/C, Find a feature, Focus, Link, Set WD = 8.0mm
 Beam on. Go to 400x for Auto B/C. Hopefully something will be in field to focus. ESEM images can be very different from high vacuum images (i.e., low contrast, lower resolution). Find and focus an object. Link the stage height and change the Working Distance to no lower than 8.00 mm**. As long as the samples are not taller than the holder, the chamber can be vented without adjusting the working distance.
- 13- Set all back to start conditions
 When finished with session, set all conditions back to original setup (cooling Off, Pref:Scan:ESEM – “Lower Stage on Vent”, WD all the way down, drain water lines, remove water and control lines, remove Peltier stage, replace single stub holder, remove GSED).

*Please clean all holders, counters and equipment when ESEM experiment is finished. Be sure that all traces of samples (particles, tubes, cells) are completely removed from specimen holders. After imaging biological material, especially if unfixed, wipe down all surfaces and equipment with 70% ethanol.

**When removing Peltier stage, completely lower stage before inserting specimen holder to avoid striking the pole piece on closing the chamber door.