1. **Login to microscope computer with your uniqname and Kerberos (level 1) password.**

2. **Turn on the two camera systems.**
   - Turn on power strip and side mount camera module.

3. **Turn on second computer.**
4. **For negatively stained samples only** – fill anti-contamination device with liquid nitrogen.
   Remove white cap from anti-contamination device, insert funnel.
   Slowly fill device with 1 dewer of liquid nitrogen from tank in hallway.
   **Press white switch upwards to ensure yellow light does not brighten.**

5. **Remove specimen holder.**
   Pull specimen holder out to first stop (large pull)
   Turn counterclockwise until a stop (large turn)
   Pull holder out to second stop (small pull)
   Turn counterclockwise until next stop (small turn)
   Set PUMP/AIR switch to AIR.
   **WAIT** as chamber releases vacuum
   Pull holder out fully

6. **Mount grid specimen side up (dark/shiny side) in specimen cartridge of specimen holder.**
   Do not touch metal rod with your fingers!

7. **Insert specimen holder.**
   Insert specimen holder, aligning gold screw on rod with indentation, to first stop (yellow light will illuminate.)
   Set PUMP/AIR switch to PUMP.
   **After green light illuminates** –
   Turn holder clockwise until first stop (small turn)
   Control holder as vacuum pulls in (small insertion)
   Turn holder clockwise until next stop (large turn)
   Control holder as vacuum pulls in (large insertion)

8. **Select “single tilt holder” in specimen box that pops up in JEM software.**

9. **Turn on filament**
   Check that V1 and V2 squares in software Valve/Vacuum monitor are green.
   Beam current in software Beam controller should be ~half current HT.
   Push Grey “ON” button under Filament tab.
   Filament will saturate automatically.
   Beam current should be 50-70uA.

10. **Center your beam.**
    Lower magnification to 2000x (use the MAG1 arrows in TEM software Task Bar,
or use the magnification knob on the right hand control module.)

Use the brightness knob on left hand control module to bring beam to crossover (twist counter-clockwise.)

Use x and y knobs on left and right hand control modules to center beam.

11. Spread beam just beyond field of view on phosphor screen. ALWAYS USE BEAM TO THE RIGHT OF CROSSOVER.

12. Check that appropriate objective aperture is in.
We recommend the second to largest aperture for most applications.

13. Find an object in your grid.
Set magnification to 5000x.
Use the wheel to change locations on your grid.

14. Eucentric Positioning –
Increase magnification to 15,000x.
Press the “Standard Focus” button on the right hand control module.
Press “Z-Focus” and “Image Wobbler” buttons on the right hand control module.
Use the Focus knob (now set to move through the Z plane) to focus image.
Deselect “Z-focus” and “Image Wobbler.”

15. Only if you have switched objective apertures –
Bring beam to crossover.
Press “Diff” button on right hand control module.
Center the caustic spot in the diffraction pattern using the objective aperture translation knobs.
Press the “MAG” button on right hand control module to return to magnification mode.

16. Find a region of interest.

17. Spread your beam to lower brightness so as not to damage cameras.

18. To use side mount camera – put camera in position by flipping switch left.
“IN” tab will appear.
19. To use bottom mount camera – press “Screen Up” button on right hand control
module.
   Phosphor screen will move out of beam path.

20. Cover viewing screen.

   Bottom mount camera version will initially open. If you are using the side mount
camera, press “Go To Sidemount” at top of the page.

22. Perform a background correction.
   Remove sample from beam path – pull sample holder out (long pull), twist
counterclockwise (large turn.)
   In AMT software, click Corrections Tab.
   Select “Acquire a Background”
   Use brightness knob to center red histogram in chart in software.
   Press “Proceed.”
   When completed, image will say “Background Image Correction Complete.”
   Return sample to beam path (large turn, long pull in.)

23. Press “Click for Live Image” button.
   Press red “Speed Live” button to change image to Quality Live.

24. Focus image using “Image Wobbler” and Focus knob.
   Deselect Image Wobbler when complete.

25. Be sure red histogram is centered by adjusting brightness knob.

26. Take image by pressing “Click for Final Image” button.

27. Set Brightness Mag Link to adjust brightness automatically as magnification is
increased or decreased.
   In TEM software, click on Control Tab > Illumination System Controller
   Click green “ON” button under Brightness Mag Link.
28. **Save image as Tif file.**
   Create a folder with your uniqname in the desktop “Users Shortcut” folder.
   Label new folder with today’s date.

29. **To save a location on your grid:**
   Click “Memory” button in nanospace map (TEM software.)
   Highlight and click “Go” to return to memory location.

   **To remove/switch sample grids –**
   1. Press “Abort” to stop live image.

   2. **Turn off filament.**
      Press grey “off” button in TEM software Beam Controller box.

   3. **Neutralize the stage**
      Press “Stage Neutral” in TEM software Stage box.

   4. **Remove sample holder.**
      Large pull
      Large turn
      Small pull
      Small turn
      **WAIT**
      Pump → air
      ~20 sec
      Remove sample holder

   5. **Replace sample holder**
      Air → pump
      Insert to first stop
      **WAIT for green light**
      Small turn
      Small controlled pull in
      Large turn
      Large controlled pull in

   6. **Turn filament on.**
      If there is initially no beam, check side mount camera.

**For each new sample –**
   1. Perform eucentric positioning (see #15)
   2. Focus image (see #25)
SHUT DOWN

1. **Remove camera from beam path.**
   - Flip switch for side-mount camera.
   - Press “Screen Up” button for bottom mount camera.

2. **Turn brightness knob clockwise until it beeps.**

3. **Turn off filament.**

4. **Neutralize stage.**

5. **Remove sample and replace sample holder.**

6. **Close AMT software, turn off 2nd computer and monitor.**

7. **Exit JOEL software, log out of 1st computer. LEAVE COMPUTER ON.**
   - HT is always left on at 80kV.

8. **Turn off monitor.**

9. **Turn off side-mount camera and power strips (in back.)**

**Please use an encrypted device or M-Box to transfer your data files.**