SIMONS ELECTRON MICROSCOPY CENTER

NEW YORK STRUCTURAL BIOLOGY CENTER

J1230 User Manual

Simons Electron Microscopy Center (SEMC) at the New York Structural Biology Center (NYSBC)

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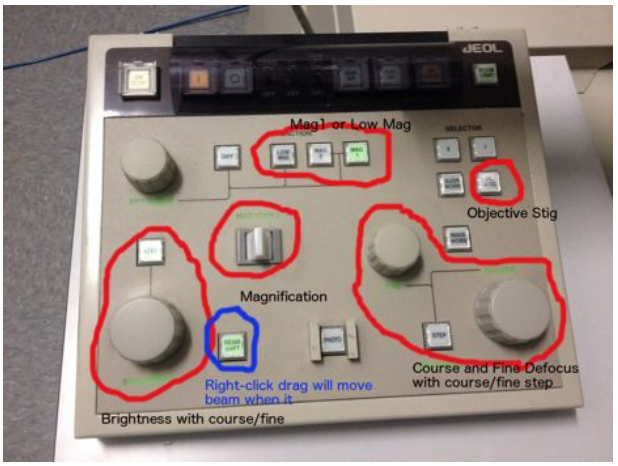
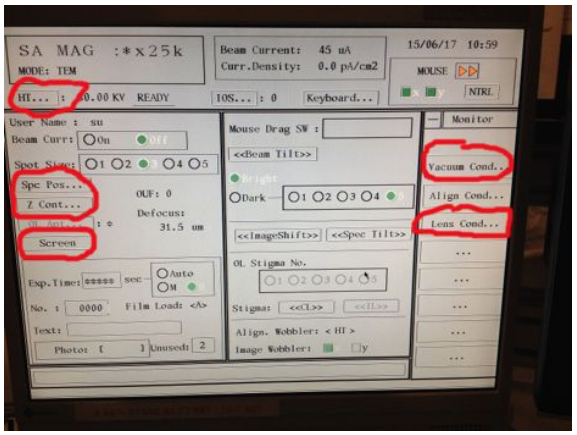
J1230 Information Sheet

- Apertures
  - Condenser = 300, 200, 100 um
  - Objective = 80, 50, 20 um



- Important buttons:
  - Use LOW MAG or MAG1 mode (not MAG2)
  - When OL STIG lig, right-click drag will adjust
  - When BEAM SHIFT lift, right-click drag to move beam
  - When STEP is lit, adjustments are coarse (BRIGHTNESS, Obj Focus)

- Important software controls:
  - General controls needed for operation are circled
  - DO NOT adjust other alignments without consulting staff first
  - You can leave the Monitor panel open (far right)



J1230 User Guide

1. Beginning of session:
    a. Camera at -25℃
    b. Beam = OFF
    c. HT = 80 kV and "READY"
    d. Recenter stage
        i. Spc Pos… → Position → click center of stage map (make sure Point option is ON)
        ii. Tilt/Rot selected → click center of stage map
2. Retract holder
    a. Objective aperture OUT, flu screen DOWN, stage recentered, filament OFF
    b. Open vacuum window
        i. Monitor → Vacuum Cond…
        ii. should see "Vac. Ready" in all 5 chambers (COL, GUN, CAM, SPC, RT)
    c. Switch from PUMP to AIR on goniometer
    d. Pull straight out until you can't any further
    e. Turn counterclockwise until you can't any further
    f. Pull slightly out until you can't any further
    g. Turn counterclockwise until you can't any further
    h. Wait for vacuum
        i. Watch V37 and V34 (turns from white/closed to black/open, and then finally back to white/closed during retraction)
        ii. SPC turns from "Vac ready" to "Air" (will go to the ~200 range)
    i. Pull holder straight out; you should feel no resistance
3. Transfer negative stain grid to holder tip
4. Insert holder
    a. Objective aperture OUT, flu screen DOWN, stage recentered, filament OFF
    b. Open vacuum window
        i. Monitor → Vacuum Cond… (should see "Vac. Ready" in all 5 chambers)
    c. Switch from AIR to PUMP
    d. Insert holder with pin lined up at 9 o'clock position and insert holder straight in; you should hear the pump start; light near toggle (on goniometer) turns yellow
    e. Wait until SPC switches from "Air" to "Evac." to "Vac. Ready" (vacuum should drop from ~200 to ~40 range)
        i. If pump times out, toggle from PUMP to AIR and then back to PUMP
    f. Insert holder turning clockwise
    g. Close vacuum window when done
5. Turn beam on
    a. On scope PC, click HT
        i. HT should be at 80 kV and ON
        ii. Beam should be OFF
    b. Click beam ON
        i. Should see "Beam Current" (at top of window) go from 45 uA to 58 - 59 uA

    ii. Don't change filament current percentage from 66%
6. Start Leginon
    a. Type betaleginon in terminal
    b. Connect client to j1230-cam1
    c. Open leginon client on camera J1230CAM1 PC
    d. Condenser aperture = 200 um (should be)
        i. as of 4/15/19 it is the 300 um aperture
    e. Application = J1230MSI-Raster Screen2 (3.2)
        i. Main = j1230leginon
        ii. Scope and camera = 1230-cam1
7. Import presets
    a. J1Presets_Manager → Import presets from another session (*)
        i. TEM = Jeol1230
        ii. Digital Camera = Gatan
        iii. Find
        iv. Load most recent users presets
        v. Import → Done
8. **<u>We DO NOT recommend taking a grid atlas</u>**
    a. Leginon works best in a semi-manual mode
    b. If you must take an atlas, do so at gr = 120x mag, radius = 0.009 = 43 targets
    c. After atlas, beam needs to be re-centered at SA mag
        i. To center, make sure BEAM SHIFT button is lit
        ii. Right-click drag EM mouse to move beam
9. Find a good square
    a. **<u>Make sure objective aperture is out</u>**
    b. Send gr preset to scope
    c. Move stage using trackball on right to find an area with carbon / good stain
    d. Send sq preset to scope
    e. **<u>Insert objective aperture now</u>**
    f. Using fluorescent screen, center over a good square
    g. Lift screen (close the furthest left panel on the scope monitor; click "screen" to lift/lower flu screen)
10. Adjust preset intensity - this should be done for every square as stain thickness varies
    a. Send sq preset to scope
    b. With the flu screen down, adjust current density (on monitor) to be between 20 and 25 pA/cm2 using the brightness knob on the control panel
    c. In Leginon, click "edit selected preset parameters", and click the icon "import from scope" for intensity and save
    d. Repeat for hl and en.
        i. After adjusting current density for en, you can import the intensity for fa and fc presets (they are at the same magnification: 60K)
11. Adjust high mag beam shift
    a. Send en preset to scope

   b. With beam shift selected on the control panel, right click and drag mouse to center beam
   c. Through leginon, click “edit selected preset parameters”, and click the icon “import from scope” for beam shift and save
      i. You can also import the beam shift for fa and fc presets
12. Simulate square target
   a. In the J1Square node…
   b. Click simulate target (icon with three x’s)
13. Submit subsquare targets
   a. In the J1Subsquare_Targeting node…
   b. Use the acquisition mouse to select subsquare area of interest
   c. Use the focus mouse to select eucentric height target
   d. Click “Submit Targets”
   e. Click “Submit Queued Targets”
   f. At this point, you should see action happening in the J1Z_Focus node and J1Subsquare node
14. Submit exposure targets
   a. In the J1Exposure_Targeting node...
   b. You should see a template of 5X5 raster targets for exposures and 1 focus target
   c. Click “Submit Targets”
   d. You should see action in the Drift_Manager, J1Focus and J1Exposure node
15. Repeat steps 7 - 10 until you are done collecting data
16. End of session:
   a. Grid has been removed from holder and holder stored inside column
   b. Recenter stage
      i. Spc Pos… → Position → click center of stage map (make sure Point option is ON)
      ii. Tilt/Rot selected → click center of stage map
   c. Fluorescent screen down
   d. Objective aperture out
   e. Beam off
   f. Send scope to en preset (SA MAG x60k)

Troubleshooting

- EN/FA/FC beam should be at least as large as flu screen, otherwise images will sensitive to beam movements
- EN preset
    - Brightness 20 pA/cm2
    - 500 ms at 2048 x 2048, bin2
    - If quadrants show up in images, then the beam is too dim or too bright
    - Should be ~8000 counts
- FA/FC presets
    - Brightness 20-30 pA/cm2
    - 200 ms exposure at 1024x1024, bin4
    - If beam is too condensed, focus will fail because the image will move too much
- If focus is off
    - Pause in J1Exposure node
    - In J1Focus node, simulate target; verify that focus is good
    - Resume collection in J1Exposure node (play button)
- Exposure and Focus node should use “Image Beam Shift” to move to target
- Grid, Square and Subsquare node should use “Stage position” to move to target
- MUST USE THE J1230 Raster app!