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FEI Spirit Direct Alignments

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We use this protocol and it's working.

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Disclaimer

This protocol is intended for use by trained personnel familiar with the operation of electron microscopes. Misuse of equipment or failure to follow safety precautions may result in equipment damage or personal injury. Always adhere to institutional and safety guidelines.

Abstract

The protocol outlines the step-by-step procedure for direct alignment of the FEI Spirit microscope, ensuring optimal performance for electron microscopy. The process includes specimen holder setup, high tension adjustment, and alignment of various components such as the beam, gun tilt, condenser astigmatism, and objective aperture. Specific attention is paid to maintaining eucentricity and optimizing beam shift and focus for high-resolution imaging.

Guidelines

- Ensure proper specimen handling and alignment to avoid damaging the electron microscope.
- Maintain a clean and controlled environment for microscopy operations.
- Follow each alignment step carefully to avoid misalignment, which can affect the quality of imaging.

Materials

- FEI Spirit microscope
- Liquid nitrogen for the scope dewar
- Specimen holder and protective stand
- Tool pin for specimen clamp adjustment
- SerialEM software
- Calibration grid (TED PELLA, INC. Product No. 603

Safety warnings

- **Be cautious when inserting and removing the specimen holder to avoid holder damage and vacuum failure.**
- Ensure that the high tension voltage is set correctly to prevent equipment damage.
- Always verify the column valves are closed before removing the specimen holder.

Set up

1 Put dewar in holder and make sure copper cold fingers are inserted.

2 Fill the dewar with liquid nitrogen and cover with foam cap.

3 **Remove** specimen holder:

3.1 Before removing the holder, make sure the column valves are closed.

3.2 In the Search tab located in Workset, align the stage in the middle by double clicking the center of the grid.

3.3 Place your left fingers on the goniometer purple flange and grab the holder with your right hand.

3.4 Pull the holder straight back until it stops and make a small clockwise rotation. (holder at this point will not go back in the column)

3.5 Adjust your hand and keep turning clockwise until it stops.

3.6 Use your right thumb to push on the purple flange and take the holder straight out.

4 Put the holder in the clear protective stand and remove the cap to reveal the specimen holder tip.

5 While holding the handle with your hand, place the tool pin into the hole in front of the spring clamp, lift the specimen clamp upwards to 90 degrees, remove the tool pin, replace the tool pin in the stand and insert the grid (dark side facing down). With the same tool pin, place it in the hole and close the clamp downwards while holding the handle. Place the tool pin back into the stand.

6 Remove the holder from the protective stand.

7 **Insert** specimen holder:

7.1 Carefully orient the large guide pin at the 11 o'clock position & the small airlock pin at the 5 o'clock position, insert the holder as far as it can go. Slightly push to make sure it is in all the way.

7.2 Select 'single tilt' and press the arrow next to the rectangle box just above the SA magnification.

7.3 Wait until the pumping is finished. (Pumping count down timer reaches zero in the vacuum overview).

7.4 Once the stage red LED light is off, immediately turn the holder counter clockwise. Make sure to hold the holder tightly while inserting because the vacuum strongly pulls the holder in. Do not let go until it is secured inside.

8 High Tension set up

8.1 Start at 20Kv

8.2 Press 'High Tension' and wait for the emission to go down to 10.

8.3 Set to 40Kv

8.4 Wait for the emission to go down to 10.

8.5 Set to 60Kv

8.6 Wait for the emission to go down to 10.

8.7 Set to 80Kv

8.8 Wait for the emission to go down to 10.

9 Turn on Filament (takes approximately five minutes)

9.1 Log on book. Wait until the filament heats to 30 degrees.??? double check

Magnification Set up

10 Set magnification to SA 9700X

11 Set Spot Size to 1 by pressing L3 and R3 on control pad.

12 Set Focus Step to 5

13 Open Col. Valves.

Before Alignment

14 Before doing direct alignments one must set eucentricity to set lenses to factory set. Press “Eucentric Focus”.

15 Choose a window that is not broken by surfing through the simple with the stage trackball.

16 Select a latex bead and center it with the stage trackball.

17 Press L2 “Alpha Wobble" and press Z + or – to set eucentricity (the bead barely moves and is within the inner circle). Press L2 again to turn off “Alpha Wobble”.

18 Press L1 to set defocus to zero. (This tares the defocus to zero where the focal point is at the specimen. Focal point is the point at which parallel rays of electron converge at and diverge at

from the specimen)

Alignment Steps

19 *All alignments except astigmatism are accessible via the Direct Alignments Control panel in the “Workset” or from the popup panel.*

20 **Beam shift alignment.** Go to the cross-over using the “Intensity” knob on the left panel. Cross-over is the smallest beam spot possible. Click “Beam Shift” in the Direct Alignments. Using multifunction (MF) X-Y knobs, adjust to center the beam on the viewing screen. Click “Done” button. Do this after each alignment.

21 **Gun tilt alignment.** Click “Gun Tilt” in the Direct Alignments. Using one MF knob at a time, adjust the intensity of the beam to its maximum (Smallest exposure time). Click “Done” button.

22 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click “Done” button.

23 **Condenser aperture alignment** (not on screen, above objective aperture). Manually select an aperture size with the outermost aperture knob (1 through 4). 1 is the smallest and 4 the largest. Use the “Intensity” knob and spread the beam clockwise to see if the beam spreads evenly across the viewing screen. If the beam expands and contracts symmetrically, then the aperture is centered. If the beam sweeps off the screen, then adjust the two aperture knobs until the beam stays centered.

24 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click “Done” button.

25 **Condenser Astigmatism.** Select “Stigmators” in the popup panel (right-bottom corner of the UI). Choose “Condenser”. Use the multifunction (MF) knobs to do the correction. For the condenser, spread the beam to half the diameter of the viewing screen. Adjust the beam shape to become a circle. Then click “None” when finished.

26 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click “Done” button.

27 **Gun shift alignment.** Use the L3 programmable button to set the spot size to 3. Select “Gun Shift”. Use MF knobs to bring the beam to the center of the screen. Switch to the spot size 9 (R3 programmable button). Select Align beam shift. Use MF knobs to bring the beam to the

center of the screen. Repeat until the beam is in the center for both spot sizes 3 and 9. **Bring back to spot size 1 when finished.**

28 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click the “Done” button.

29 **Beam tilt pivot point X and Y alignment.** Click “Beam tilt pp X” in the Direct Alignments. The beam will begin to wobble. Using MF knobs, stop the beam wobbling, so that beam is stationary in the center of the screen. Click “Done” button. Select “Beam tilt pp Y” in Direct Alignments. Repeat the above procedure for the Y pivot points. Click “Done”.

30 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click the “Done” button.

31 **Objective aperture alignment.** Press “Diffraction”. Manually select an objective aperture size (1 through 4). 1 is the smallest and 4 the largest. Center the aperture using the two aperture knobs until the aperture is centered over the diffraction spot (not the center of the viewing screen). After aligning, press “Diffraction” to turn off diffraction mode.

32 **Cross-over and beam shift alignment.** Use the “Intensity” knob on the left panel to go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click the “Done” button.

33 **Rotational center alignment.** Use high magnification (23Kx or 26Kx) for this alignment. Perform this alignment on a visible feature (region of interest) on the sample that is centered on the viewing screen. Turn the focus knob counterclockwise (underfocus) and see the direction shift of the visible feature. If the feature is moved, select “Rotation Center” alignment in the Direct Alignments. Image of the feature will start wobbling in and out. Use MF knobs to center visible feature. Click the “Done” button and press “Eucentric Focus”. Repeat this alignment again to check if the feature is centered when focusing.

34 Go back to magnification 9700X. Cross-over and beam shift alignment. Use the “Intensity” knob on the left panel, go to cross-over and click “Beam Shift” in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click the “Done” button.

35 **Repeat objective aperture alignment.** Press “Diffraction”. Center the aperture using the two aperture knobs until the aperture is centered over the diffraction spot. After aligning, press

"Diffraction" to turn off diffraction mode.

36 **Cross-over and beam shift alignment.** Use the "Intensity" knob on the left panel, go to cross-over and click "Beam Shift" in the Direct Alignments. Using MF, adjust to center the beam on the viewing screen. Click the "Done" button.

37 **Gain reference.**

37.1 Open SerialEM.

37.2 Take a pencil and place it between the specimen holder end and the goniometer. (One does not want a specimen in the beam path).

37.3 Go to (Camera & Macro Controls) and open "Setup".

37.4 Make sure "Record" exposure time is set to 1 second at bin 1, "Focus" exposure time is set to 0.2 second at bin 2 and "View" exposure time is set to 0.2 second at bin 2.

37.5 Press "OK". Place the focus screen in the viewing chamber and spread the beam clockwise to exposure time 0.40s on the "Set Up" page in FEI software.

37.6 Go to the "Camera" pull down menu on Serial EM and select " Prepare Gain Reference".

37.7 Make sure "Target number of counts" is set at 1800 and "Number of Frames" is set to either 5 or 10.

37.8 Press "Yes" to spot position aligned. (This means the electron beam is aligned).
After gain reference, press "Record" to check image gain reference image.

37.9 Remove the pencil.

38 **Objective Astigmatism.**

38.1 Place the focus screen in the viewing chamber and spread the beam to exposure time 0.40s on the “Set Up” page in FEI software.

38.2 In Serial EM, press “View” in Camera & Macro Control.

38.3 Go to the “Process” pull down menu in Serial EM and select “Live FFT”.

38.4 Slightly defocus using the focus knob counterclockwise to spread the FFT ring.

38.5 Go to “Stigmators” at the popup panel (right-bottom corner of the UI).

38.6 Choose “Objective”. Use the multifunction (MF) knobs to do the correction (make sure the ring is circular/isotropic).

38.7 Press “None” when finished correcting for objective astigmatism.

38.8 Uncheck “Live FFT” in the “Process” pull down menu in Serial EM.

38.9 Press “Stop” in Camera & Macro Control.

Shut Down Procedure

39 Make sure viewing screen is down.

40 Make sure condenser and objective apertures are inserted.

41 Magnification is set to SA 9700x.

42 Hit 'Eucentric Focus' and reset the defocus to 0.

43 Uncheck low dose button in SerialEM to revert the microscope to normal imaging (only for those using low dose operation).

44 Set the spot size to 1.

45 Spread the beam clockwise till the exposure on the FEI set up screen shows 0.60s.

46 Beam/Column Valve is closed (should turn yellow).

47 Filament is turned off.

48 Go to 'Stage' tab and double click at the center of specimen position to bring the stage to neutral position.

49 Remove the sample holder by pulling out the rod slowly and then turn clockwise and pull out again.

50 Insert specimen rob back into the microscope.

51 Remove dewar containing liquid nitrogen and dispose appropriately.

52 Go to 'Set Up' tab and find 'Vacuum Supervisor'.

53 At the Cryo tab, press 'Cryo Cycle'.

54 Record session time in log book if applicable.