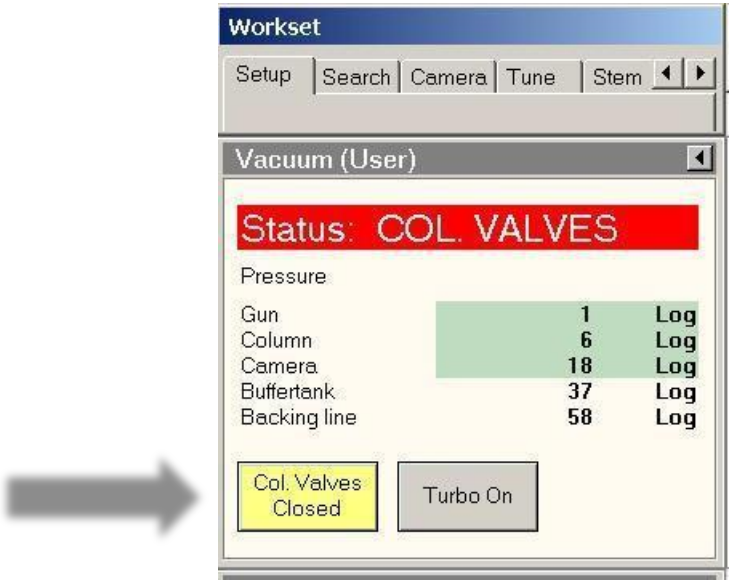
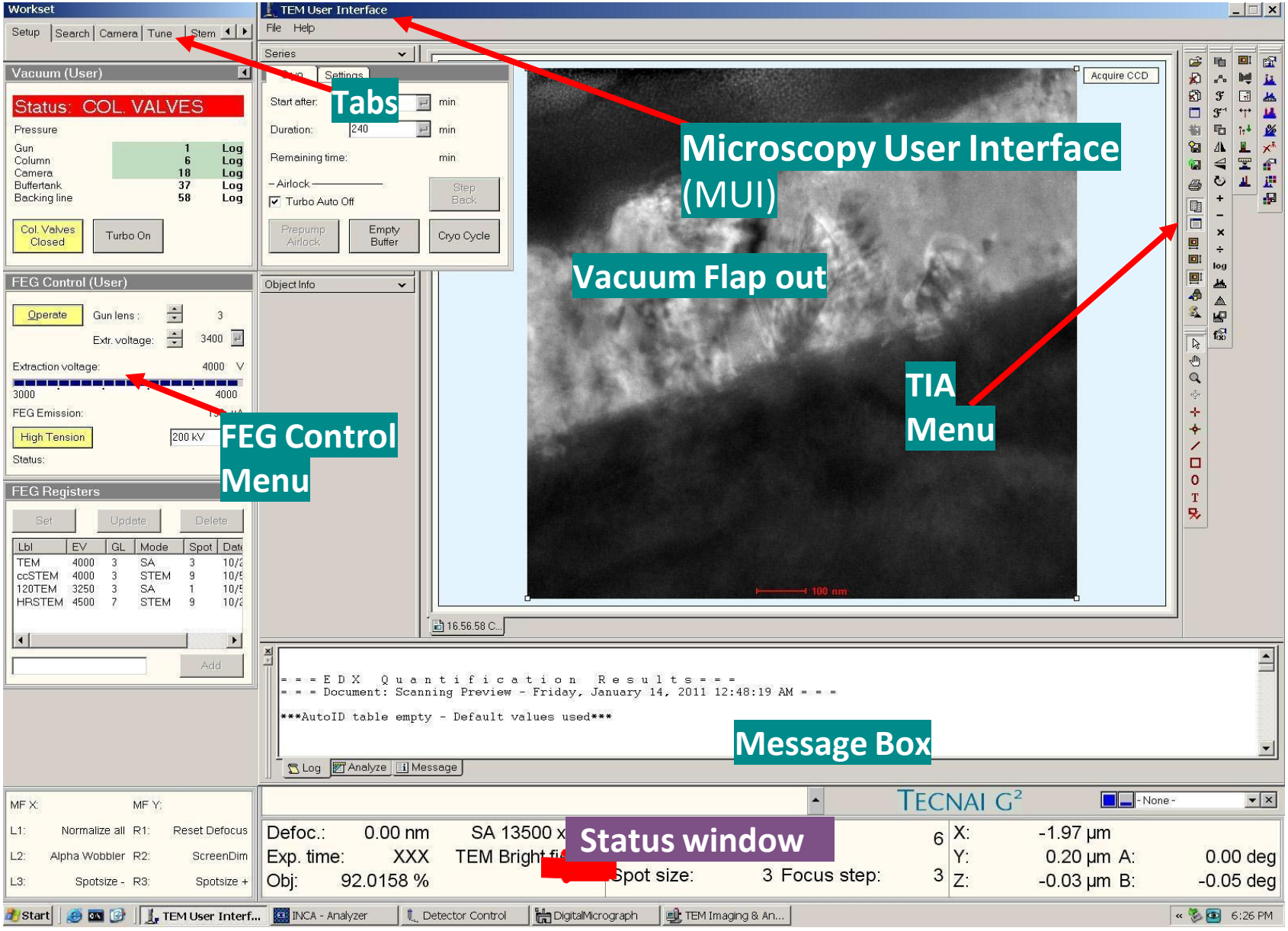
300kV FEG-TEM

FEI TECNAI G2 F30

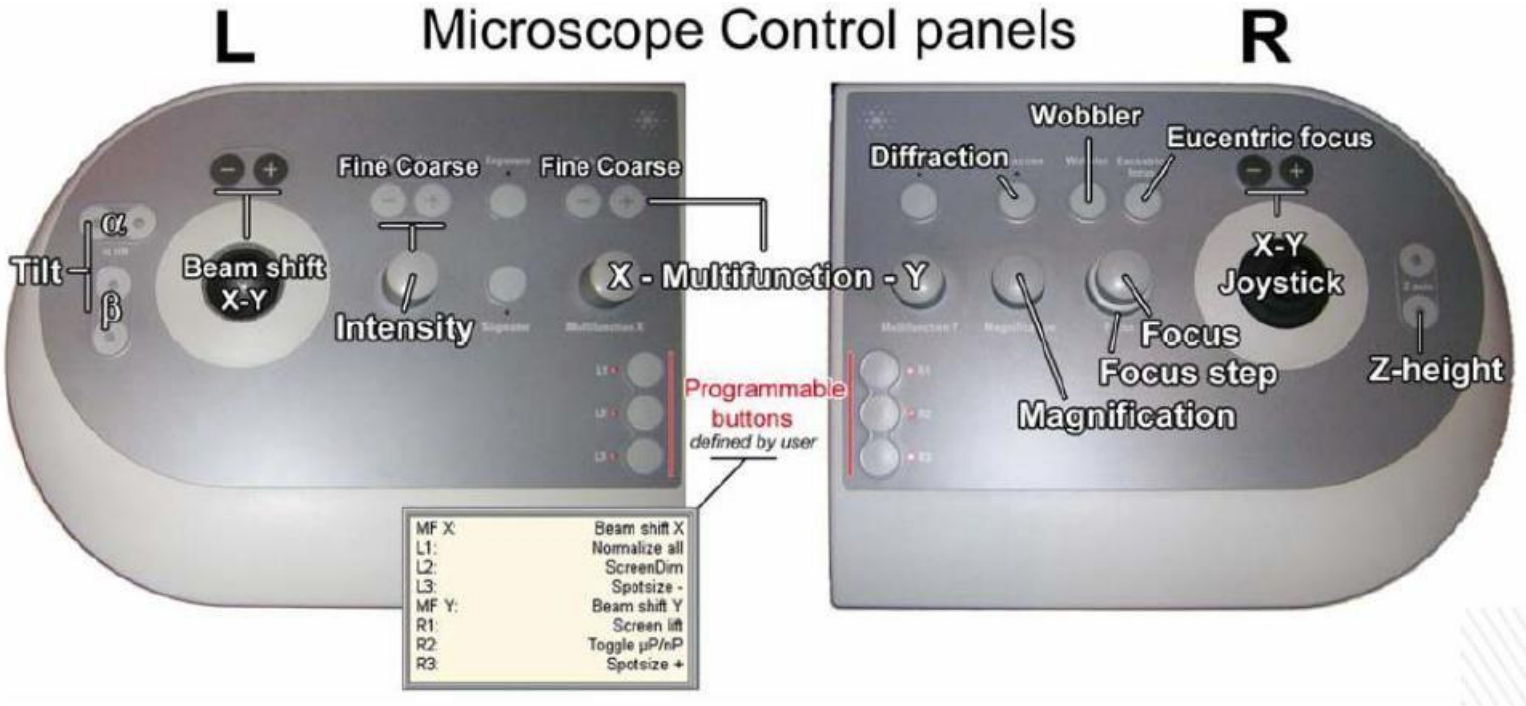
In Emergency, Simply Close the Col Valve and Leave



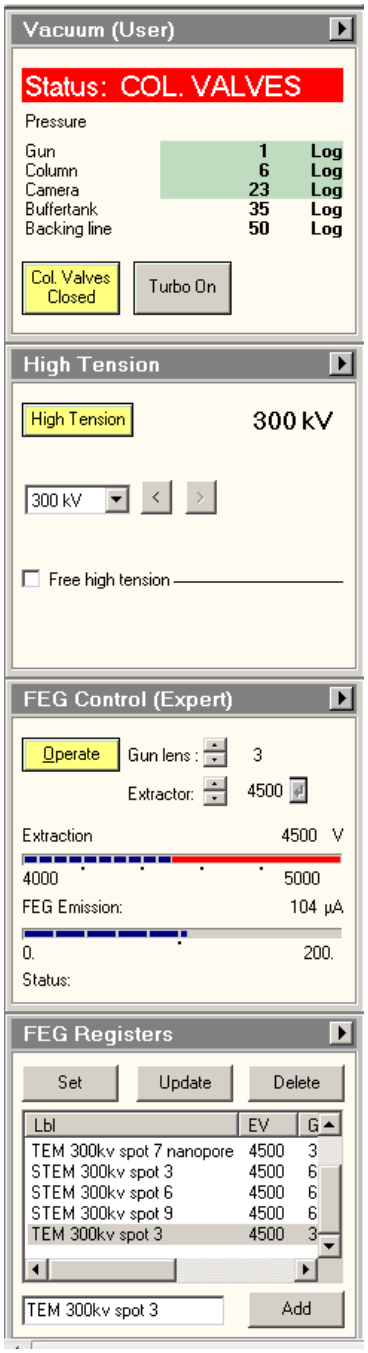
Snapshot – Microscope User Interface (MUI)



Snapshot - Left Hand Panel (LPH)
Right Hand panel (RHP)



Pre-start: Instrument



1. **Fill liquid nitrogen in the Dewar. Refill every four hours.**
2. **Check the following scope condition**

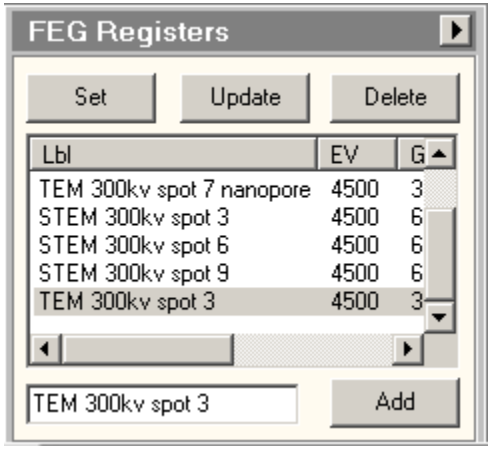
- **Left picture** - One “Red” and three “Yellow” on the Setup tab.
- **Vacuum state** – green, gun is 1, column is 6
- **Holder position** should be close to zero for X/Y/Z/A/B.
- **Apertures** - Aperture” is out (lever to the right). “C2 Aperture” is 2.

3. **Fill in the log book** (time, vacuum values and emission current).

Choose “FEG Register” and Pull Out Alignment File

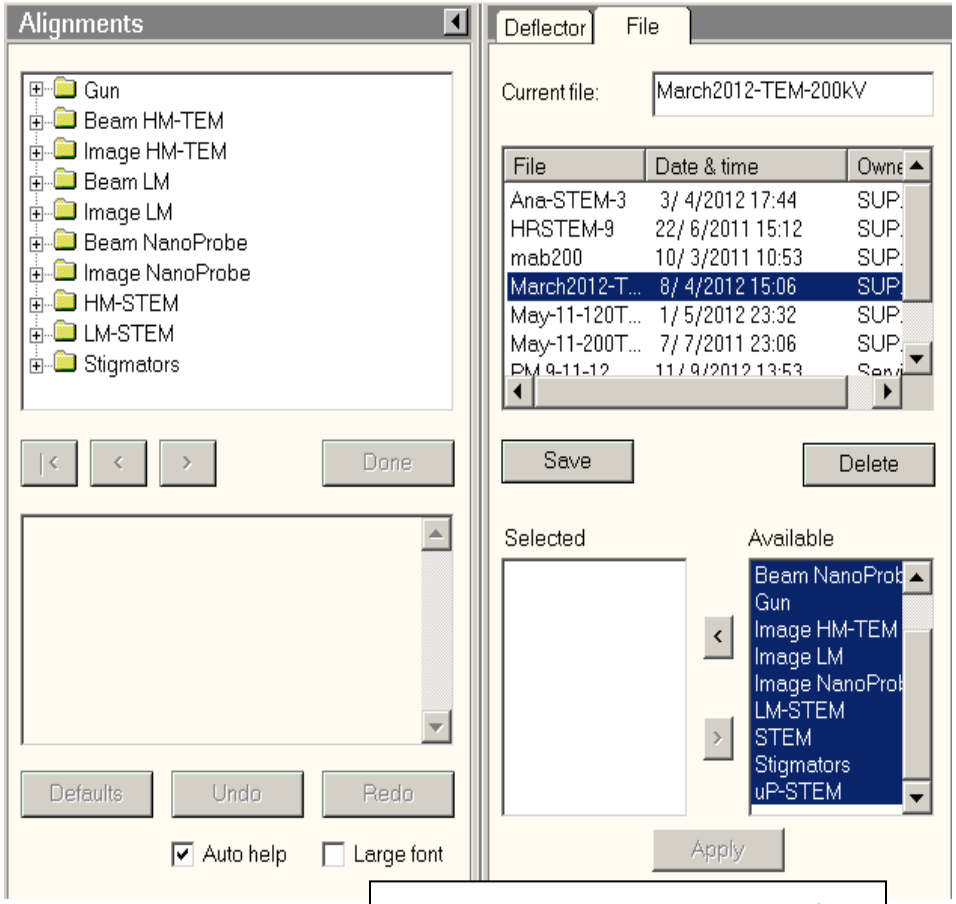
Do NOT rearrange the alignment files or FEG Register files

Step 1



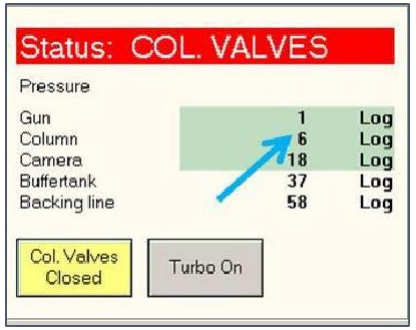
- Select a suitable FEG register. Click “Set”
- Go to flap-out of “Alignment” menu.
- Select a matching alignment.
- Select all alignment items move all items to the left column.
- Click “Apply”.

Step 2

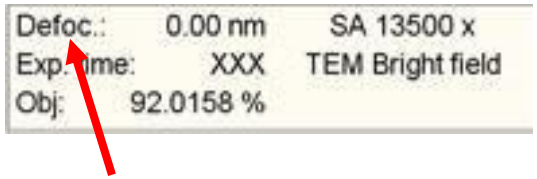


Move all items to the left

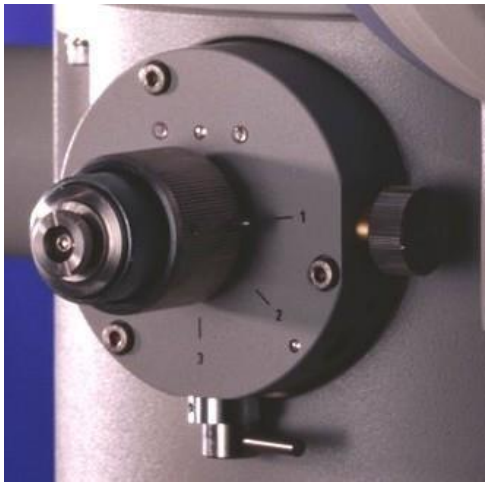
Bring Beam To Sample

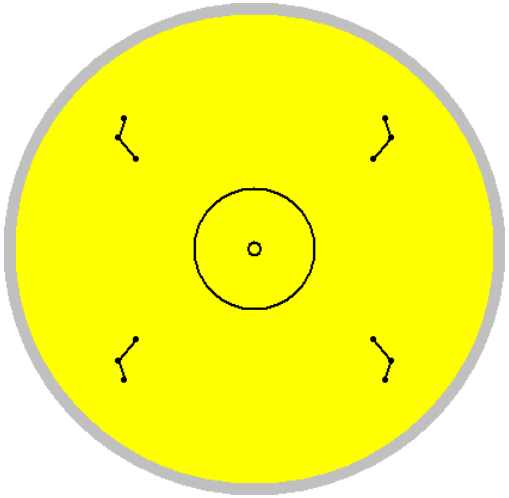


- Wait until column vacuum drops to 6.
- Click “Column Valve Closed” to open the column valve. The “Status” shall change to green color with “READY”.
- Click “Eucentric Focus”. The “Defocus” becomes zero nm.
- Confirm “Objective Aperture” and “SAD Aperture” both are out (*i.e.* obj aper. and SAD aper. lever to right direction)
- The electron beam should be seen on the big phosphor screen. Turn “Intensity” knob to spread beam to about the phosphorus screen size. Center beam using “Beam Shift” track ball.
- If no beam, lower magnification and move sample around to find the beam.



Defocus value should be maintained not far away from zero during operation (< 2-3 µm).





TEM Alignment: 1 – Eucentric Height

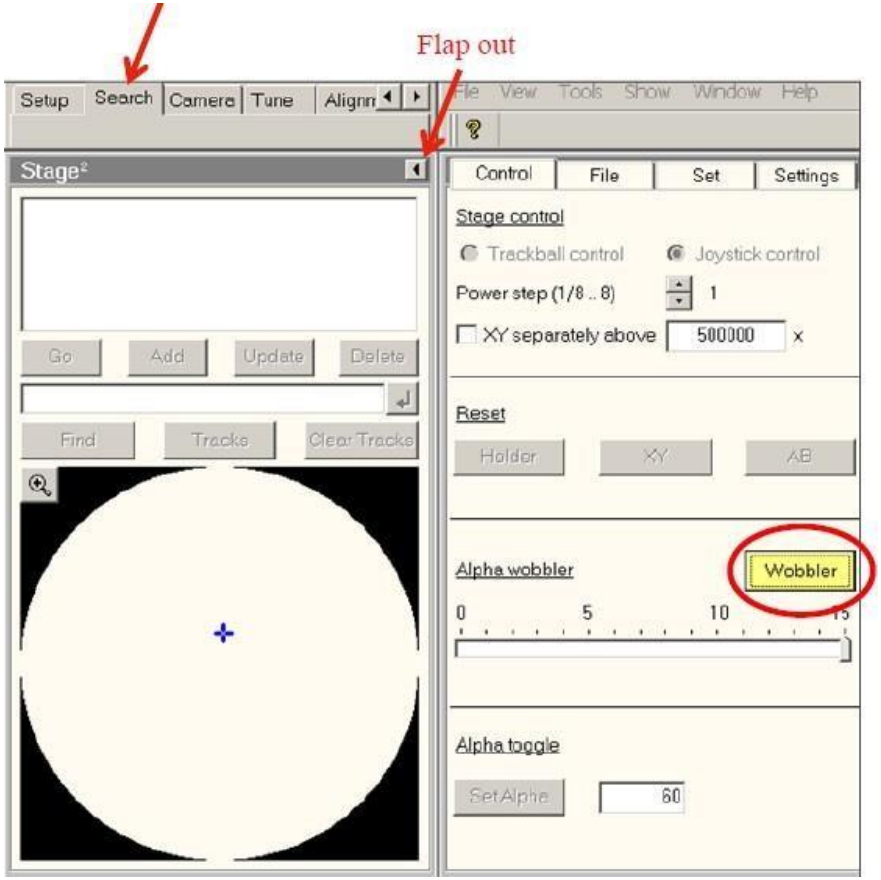
There are two ways to set up Eucentric Height. Click "Eucentric Focus" to start.

1. *Preferred way –*

   Condense beam on the area of interest. If the area of interest is not at the eucentric height, there will be a halo around the bright spot on phosphorus screen. Adjust "Z" to minimize the halo. Set magnification to 125k and repeat.

2. *Standard way –*

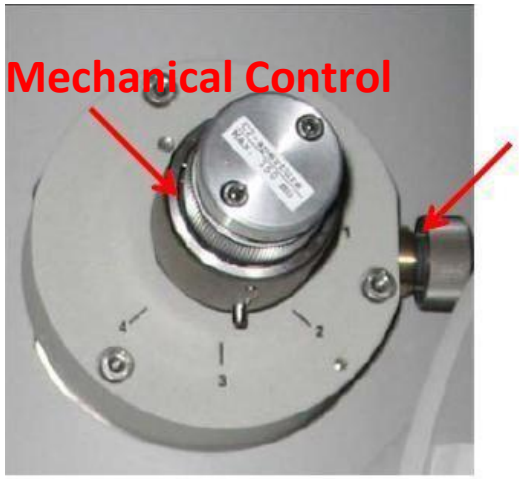
- Start Wobbler (Go to "Search", click the "flap out" arrow and click "Wobbler") or personalized button.
- Adjust "Z" on the right hand panel to minimize the sample movement.
- Set the magnification to 125k to do the fine adjustment.
- Turn the wobbler off.



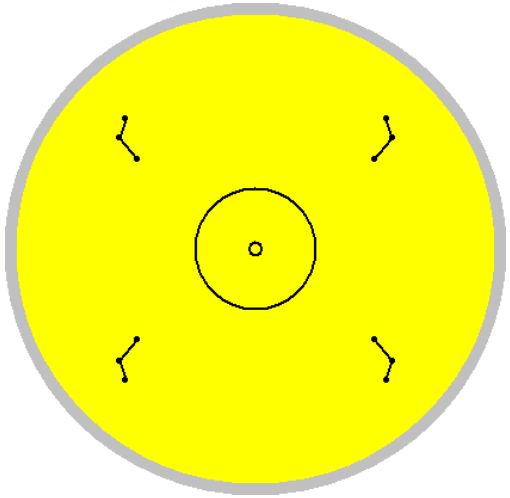
For magnetic specimen, only use the preferred way.

TEM Alignment: 2 – Center C2 Aperture

- Set an appropriate C2 aperture by turning the big knob to a numbered position. For TEM, number 2 or 3 is good.
- Set magnification to 125k.
- Turn “Intensity” knob on the left hand panel to condense the beam to a spot.
- Center the beam using “Beam Shift” (the trackball) on the left hand panel.
- Spread the beam by turning the “Intensity” knob *clockwise*.
- Adjust the two mechanical control at the C2 aperture to move the beam back to the screen center.
- Repeat the above steps until the beam remains centered.



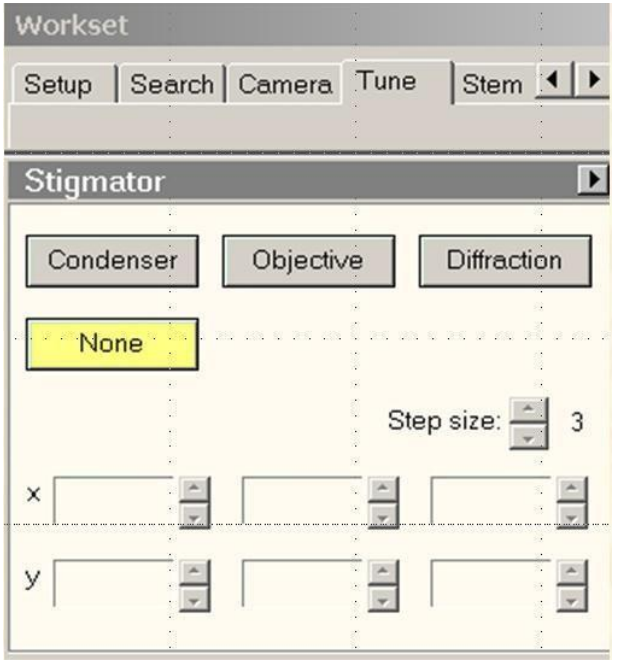
C2 aperture

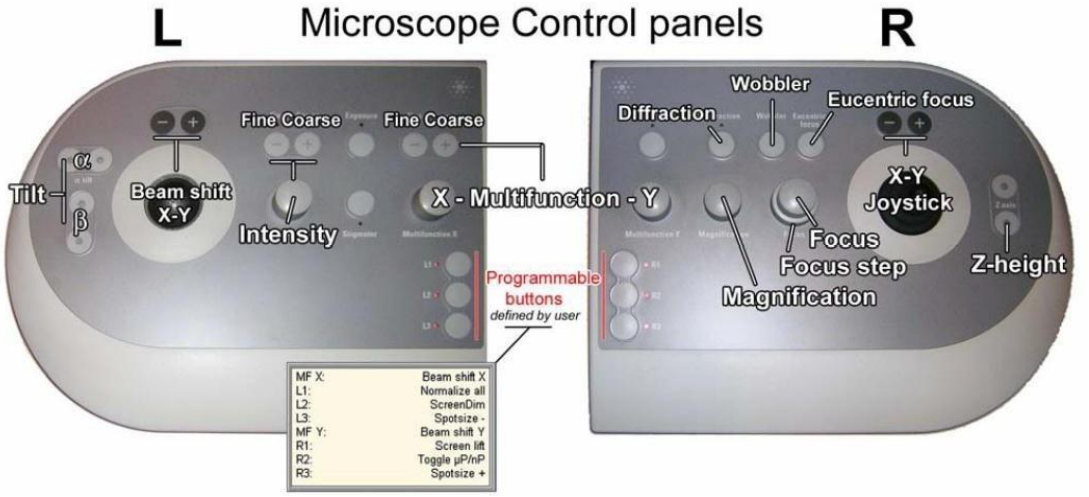


Phosphorus screen

TEM Alignment : 3 – Condenser Stigmation

- Adjust the condenser stigmation if the beam is not circular.
- Go to “Tune”, click on “Condenser” from the **Stigmator** menu.
- Use the “**Multifunction knobs (MF X/Y)**” to adjust the condenser stigmation in both x and y directions to ensure the beam is round and expands concentrically.
- Click “**None**” after finishing to exit stigmator.



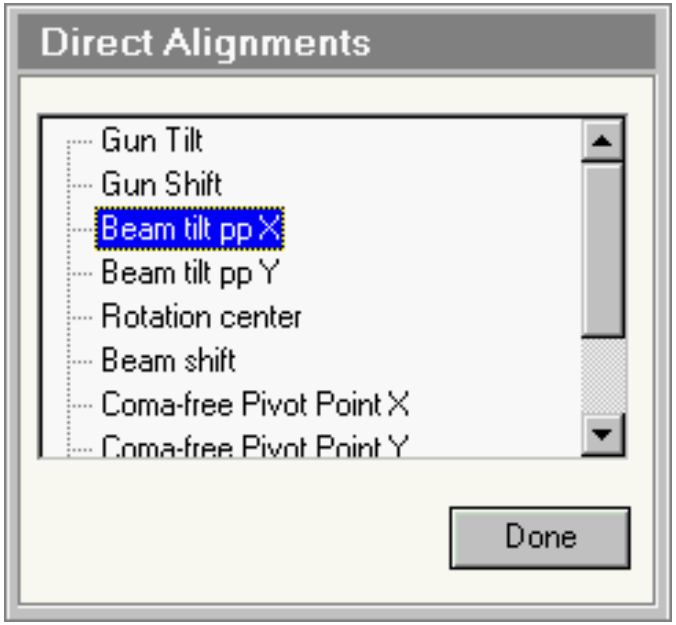


TEM Alignment : 4 – Direct Alignment (Gun Tilt)

- ***Caution* – if you are not familiar with gun alignments, don't do it.**

- *Purpose* - The gun tilt makes sure that the electron beam from the gun comes down parallel to the optical axis, so that no electrons from the beam are lost before they can be used for imaging, etc.

- *Procedure* - select a hole area without specimen. Set magnification at around 10k. Click "**Gun Tilt**" to activate. Adjust "Multifunction knobs" in both x and y directions to maximize the screen current (nA). Click "Done" after finishing. Correct condenser stigmation again.



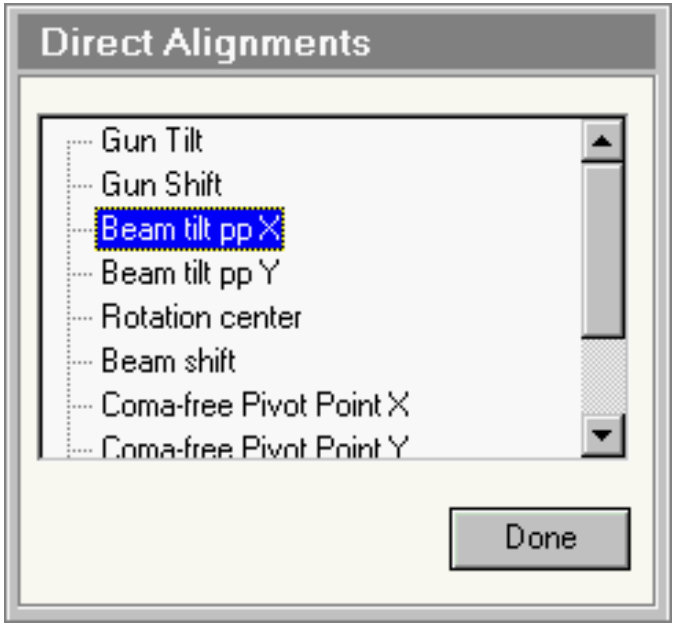
TEM Bright field		C2:	43.580 %	SA 175 kx	Em val: 80.00 µA
Defoc.:	0 nm	HT:	200 kV		
Spot size:	5			Screen: 0.000 nA	Focus step: 1

Status window

TEM Alignment : 5 – Direct Alignment (Gun Shift)

- *Purpose* - Shift the electron beam sideways so that it comes down along the optical axis.

- *Procedure* -Condense beam to a spot and center using track ball. Select "**Gun shift**" to activate. Change spot size from 3 to 9, center beam using Beam Shift (track ball in LHP); change spot size back to 3, center beam using MF X/Y. Repeat the above process until beam is centered at both spot size 3 and 9 (it is OK if the beam is slightly away from the center for other spot sizes). Leave spot size at 3 (normal TEM imaging). Click "Done" to exit. **Note: you must use track ball at spot size 9 and MF at spot size 3. Otherwise it won't work. Or Spot 9 with beam shift and Spot 3 with Gun Shift.**

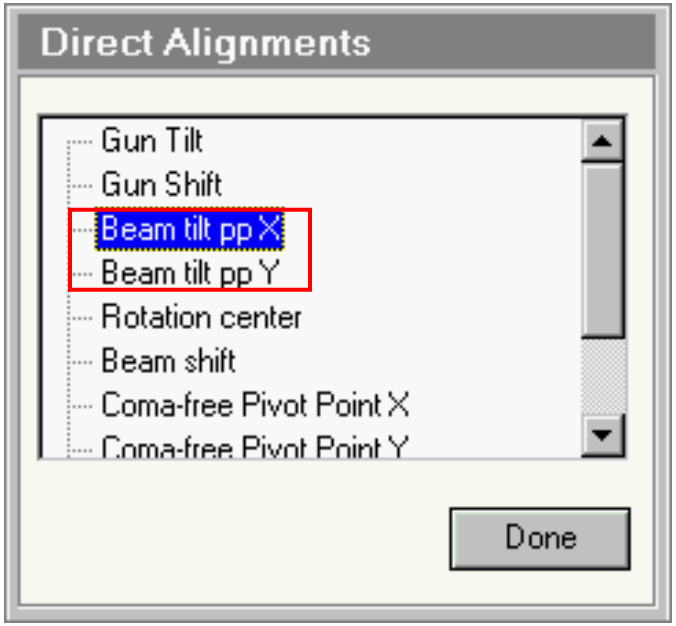


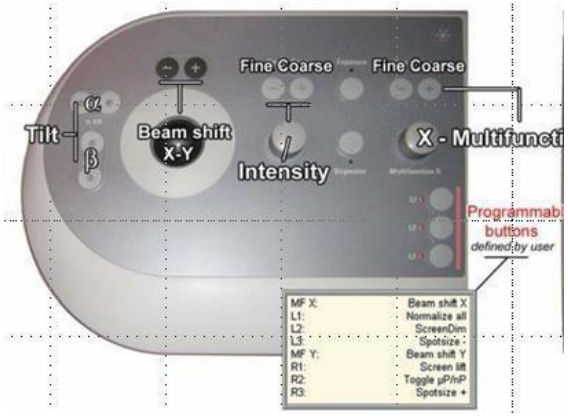
TEM Bright field		C2:	43.580 %	SA 175 kx	Em val: 80.00 µA
Defoc.:	0 nm	HT:	200 kV		
Spot size:	5			Screen: 0.000 nA	Focus step: 1

Status window

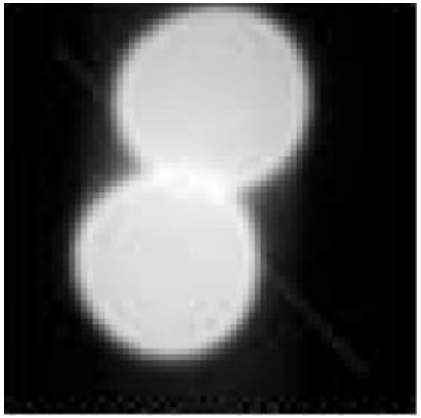
TEM Alignment : 6 – Direct Alignment (Pivot Point)

- *Purpose* – make sure that the beam does not shift when it is tilted.
- *Procedure* – Condense beam to a spot (Intensity). Center using track ball. Increase Mag to 125K. Center C2 aperture and correct condenser aastigmatism if needed. Condense beam. Select "**Beam Tilt pp X**" to activate. Using MF X/Y knobs to make the two beams to merge to one point. Click "Done" to exit.
- ***Beam Tilt pp Y*** - Mag 125K. Repeat the above step for Y direction. Click "Done" to exit.





Misalignment of beam tilt PP

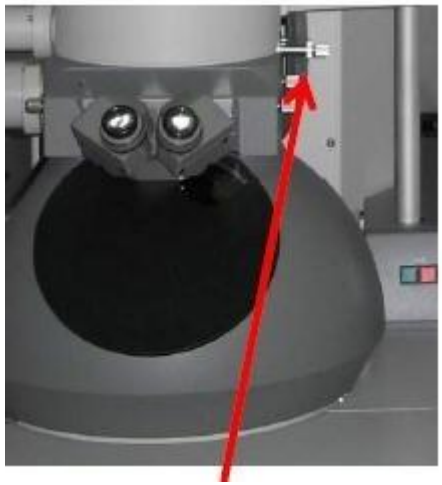


TEM Alignment : 7 – Focusing Binoculars

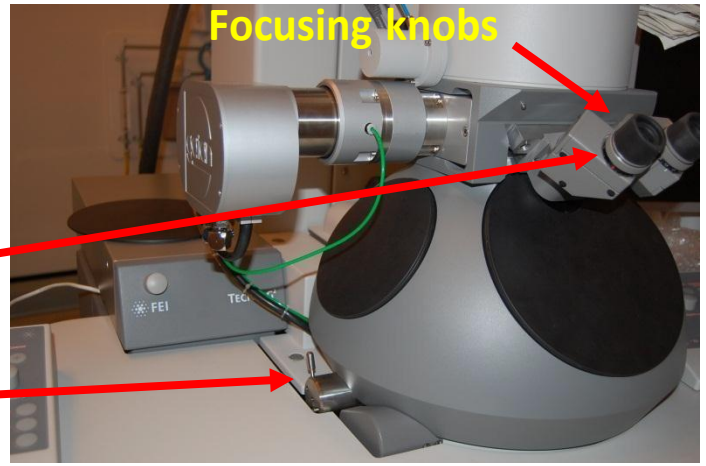
- *Purpose* – the binoculars provide additional magnification so that fine features can be better viewed.

- *Procedure* - Turn the outer knob of **Beam Block** towards you and insert the Beam Stop from Park position. Bring in **Focusing Screen**. Look at the Beam Stop on the Focusing Screen through the binoculars. Focus the Beam Block by adjusting the two **focusing knobs on the binoculars**. *Attention*: - not the Focus knob on RHP!

  After finishing, return the Beam Block to park position by retracting and then turning the outer knob of the Beam Stop away from you. Lower the Focusing Screen.



Beam Block



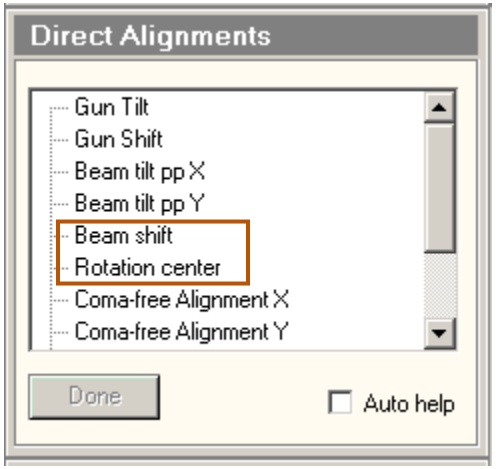
TEM Alignment : 8 – Direct Alignment (Rotation Center & Beam Shift)

- *Purpose* – make sure that the beam is along the optical axis of the objective lens.

- *Beam shift* - Mag 125K. Condense beam to a spot. Click "**Beam Shift**" to activate. If the beam is away from screen center, center it using MF X/Y. If the beam becomes invisible, reduce magnification to find the beam and then bring it to screen center by using MF X/Y. Click "Done" to exit.

- *Rotation Center* – Mag 175K or above. Spread beam to cover screen. Correct condenser astigmatism if needed. Find a sharp feature and move it to screen center using joystick. Lift up **Focusing Screen** and watch the feature at screen center through **binoculars**. Click "**Rotation Center**". Minimize the image shift of the central feature using the two "**Multifunction knobs**". The image should pop up like "heart beat" but does not shift. For samples with no apparent sharp features, condense beam to a point, center the beam and minimize beam shift using MF X/Y Click "Done" to exit.

- ***Note*** **- It is good practice to check Rotation Center regularly before taking a high resolution picture, especially when you have moved to another location on the specimen or have changed focus value more than 1 µm.**

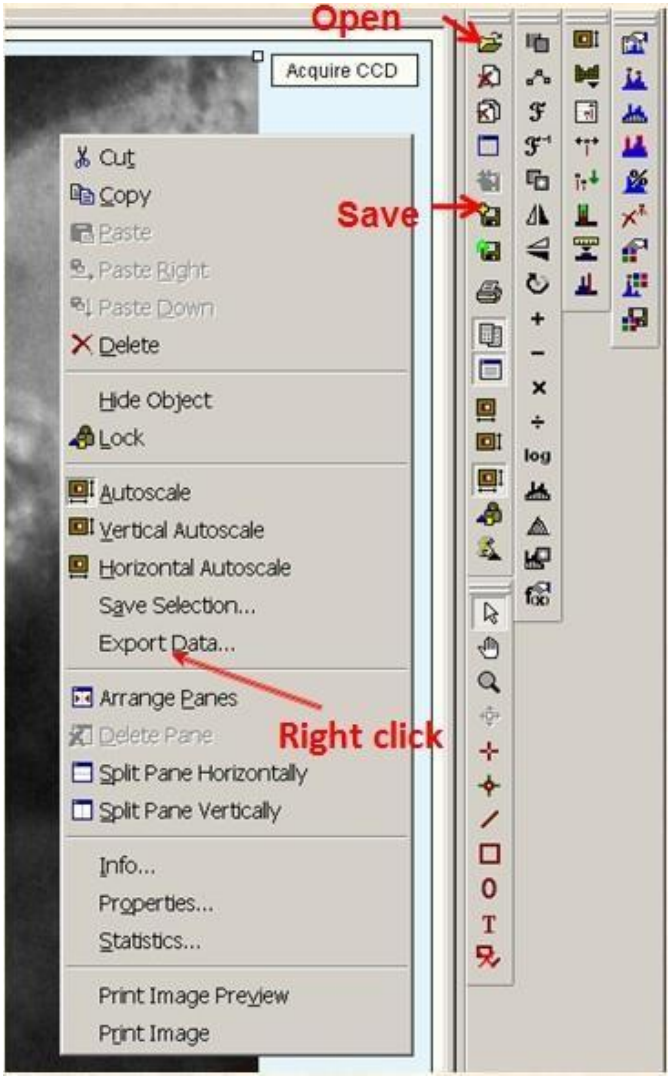


Take TEM Image Using CCD Camera

- Choose the area of interest and move it to screen center.
- Focus specimen and spread beam. **Never view a strong condensed beam using CCD**.
- You may use BM-Ultrascan to take regular TEM images. Click “Search” to start viewing samples on CCD.
- “Search” with binning 4 and integration 0.07-0.1 sec.

  “Preview” with binning 2 and 0.5-0.7 integration.

  “Acquire” with binning 1 and integration 1-2 sec.
- Save image to .emi format. Right click on the image and export data as image format (.tiff, .jpeg, *etc*.)



Save and Transfer Files

First, “Save As” images in *.emi* format (original data) to the “Images on Fei-e1390631eac” under “data” folder. If you don’t have a folder in the above folder, create one there. Then go to the Support PC. Find the “Images” shortcut on desktop, located your saved files, and copy them to the “Userdata” folder (right next to “Image” shortcut). The latter is a network storage supported by CVM IT. You can download your files from any computer on campus. After you have saved the file in *.emi* format, you may right click the image and “Export data”. Choose a format “w/scale marker (full resolution)”. By doing this, you can save the image in a *.tiff* or *.jpeg* format. TIA can be installed on individuals’ PC upon request so .emi files can be read directly and manipulated.

If you have not previously used the UserData folder, please ask any staff member to help set up your own folder. To retrieve the files from your office PC, also ask staff for the instructions.

If after you set it up but find you have no access to the AMCL folder, contact lab manager.

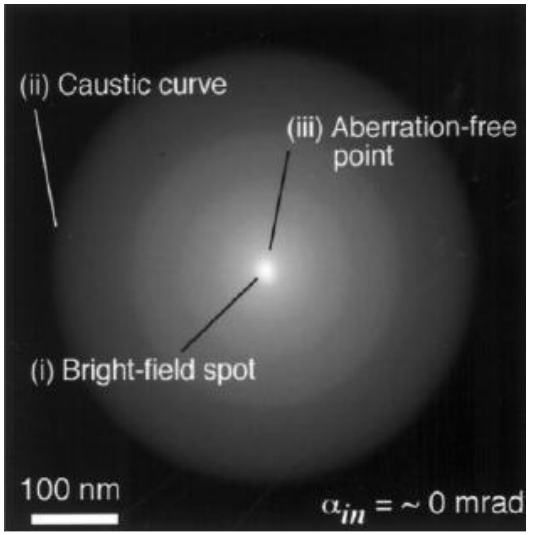
If you take images using Digital Micrograph, go to “File” menu, “Save as” image in *.dm3* format and “Save display as” in image formats. Then save and transfer files in the same way.

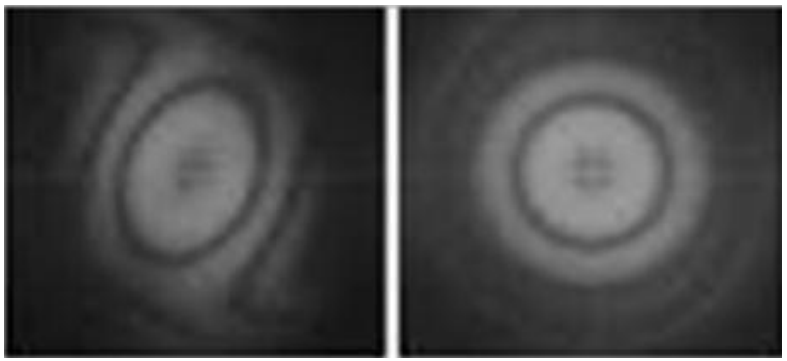
Correct Objective Stigmation

There are two ways to correct Objective Stigmation.

1. *Caustic curve* – Focus image. Condense beam to a spot. Reduce "Z" so that a halo forms around the central bright spot. Go to "Tune", click on "Objective" from the **Stigmator** menu. " Adjust "MF x/y" to make the boundary of the halo round. This method is easy to perform but less accurate than the following FFT method.

2. *Fast Fourier Transform (FFT)* – Magnification 125k or above. Find and center an amorphous area. Acquire a live view in slow CCD (see next section for imaging with CCD camera). Click "Live FFT" in Camera menu to obtain a FFT image. Click on "Objective" from the **Stigmator** menu. " Adjust "MF x/y" to make the FFT round.





Astigmatic

Stigmation corrected

Focusing in TEM

Before focusing: (i) make sure eucentric height has been correctly set up. (ii) click “Eucentric Focus” on RHP. (iii) make sure C2 aperture is properly centered.

Besides making features sharp, there are three ways to assist focusing.

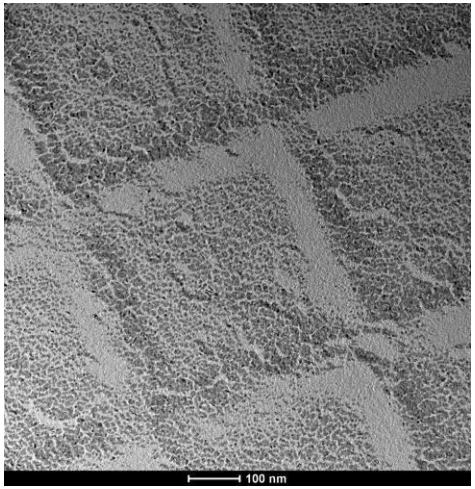
1) **Minimum Contrast**. (recommended) For all magnification. Look at image carefully. Focusing is achieved when the image has minimum contrast.

2) **Fresnel Fringe**. For all magnifications, look for particles, hole or edge area of the sample. Sample is out of focus if there is white (under-focus) or dark (over-focus) fringe around the edge. Adjust focus to minimize the fringe.

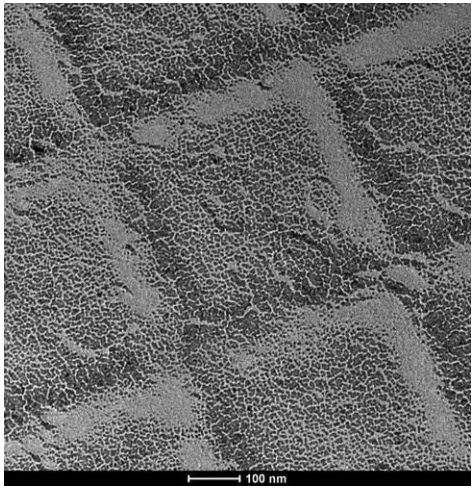
3) **FFT**. Preferred for high magnifications (>125k). Click “Live FFT” in Camera menu to obtain FFT image. Correct Obj. Stigmation. Turn Focus knob to maximize the diameter of the inner circle of FFT.

Note – use “**Z-height**” for **rough focus** and “**Focus**” knob for **fine focus**.

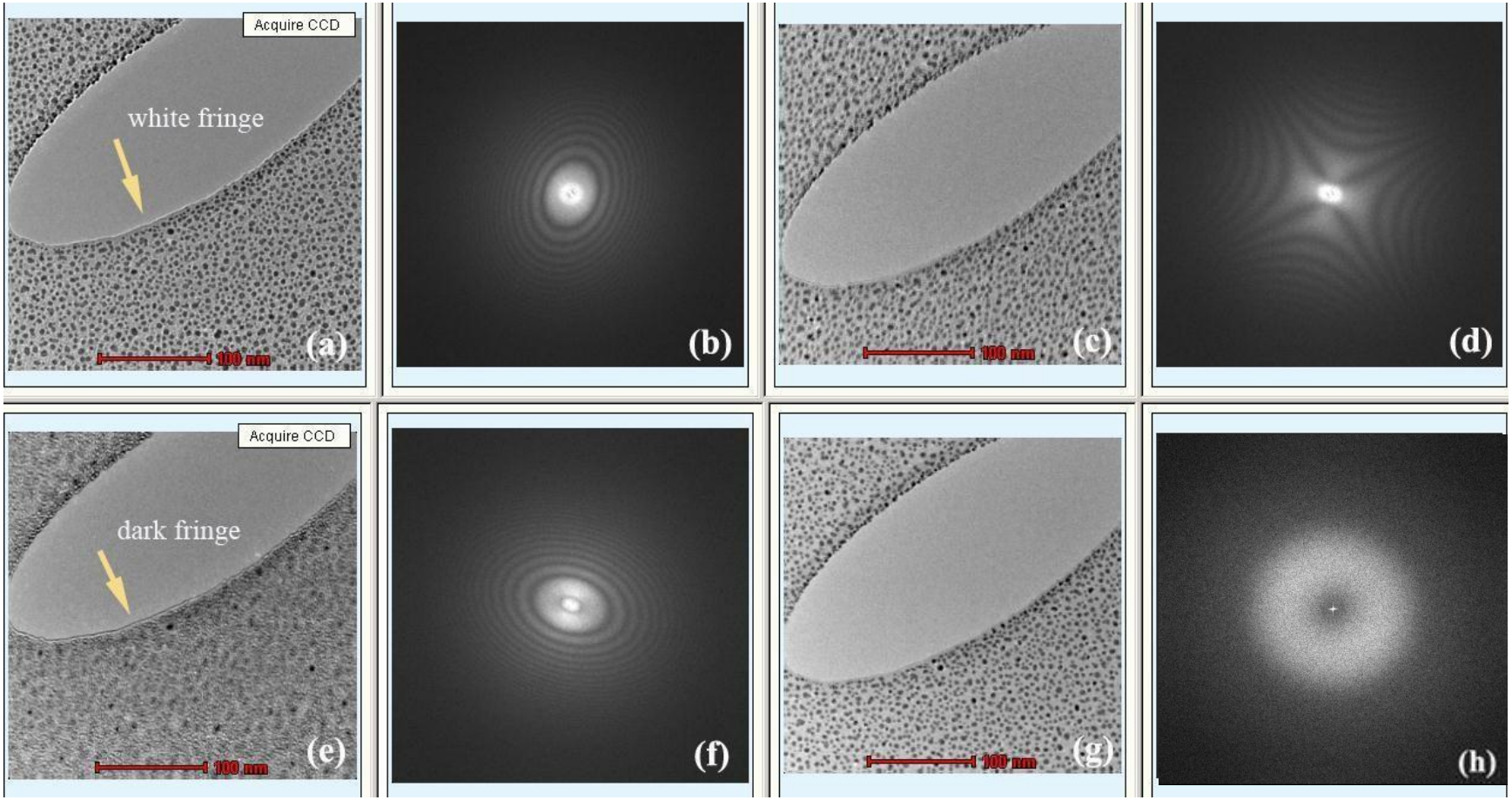
Continued on next page



In-focus; minimum contrast



Out-of-focus; strong contrast



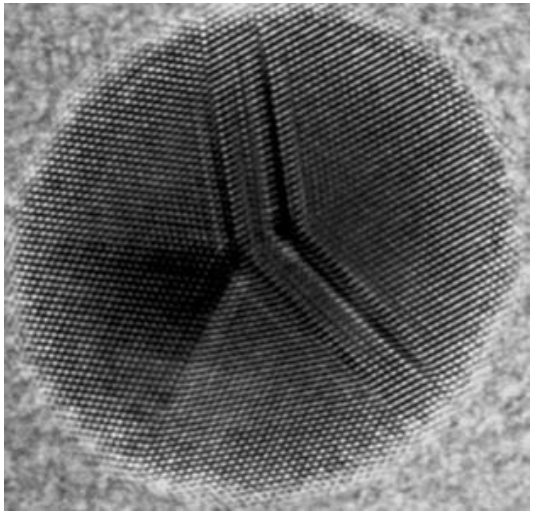
Figs. (a) under focus with objective astigmatism; (b) FFT for (a); (c) in focus with objective astigmatism; (d) FFT for (c); (e) over focus with objective astigmatism; (f) FFT for (e); (g) in focus without objective astigmatism; (h) FFT for (g).

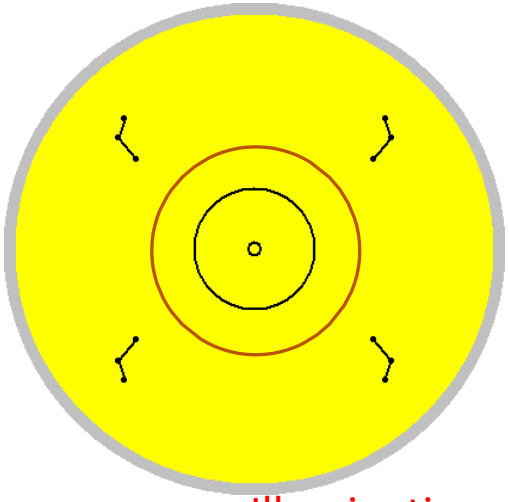
Example of Focus and Astigmatism

High Resolution TEM

Note: HRTEM generally requires sample thickness of less than 50 nm.

- Retract Objective Aperture if inserted before.
- Carefully align the scope as described in previous slides.
- For crystalline bulk material, accurately tilt the sample into a major zone axis.
- Locate an amorphous area. For crystalline specimen, move to the edge of the specimen and find some amorphous area at the edge.
- Adjust the illumination so that it is in between the inner circle and the markers on the phosphorus screen.
- Lift up screen. “Preview” using CCD.
- Focus sample and correct Objective Astigmatism with FFT.
- (For expert user) conduct coma-free alignment in “Direct Alignment” menu.
- Acquire HREM image.

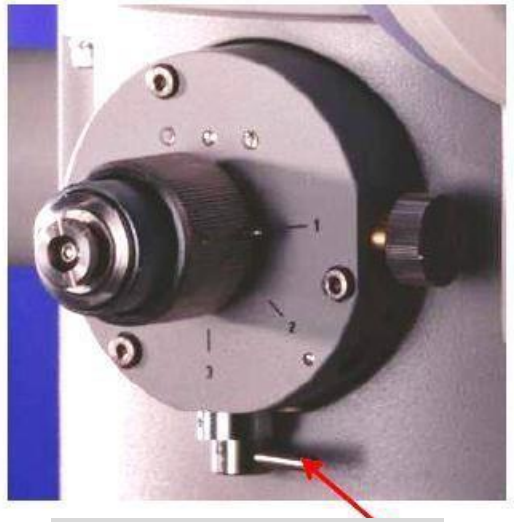




Illumination

TEM Diffraction Mode

Caution – never view a strong direct beam of a diffraction pattern using CCD without Beam Stop.



Selected area aperture

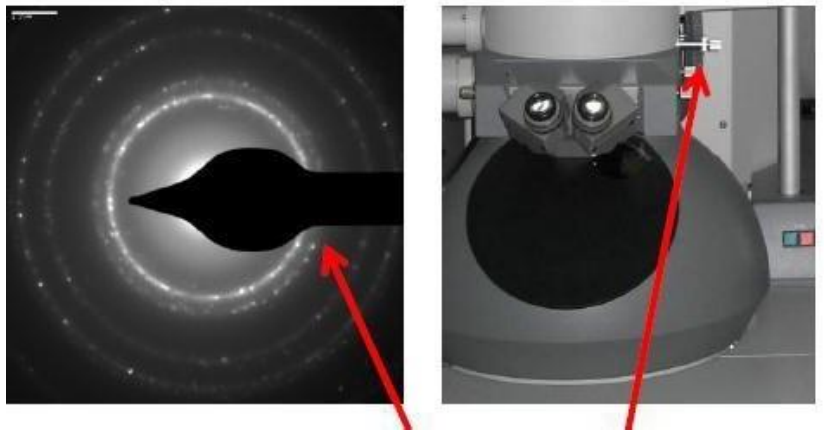
CBED (Convergent Beam)

- Choose the area of interest.
- Converge beam to a spot, click “Diffraction” button on RHP. View pattern on screen.
- Focus diffraction pattern with “Focus” knob.

SAED (Selected Area)

- Choose the area of interest.
- Insert SAD aperture by turning the lever from right to left.
- Click “Diffraction” button on RHP.
- Spread the beam with “intensity” knob until the pattern contains spots.
- Focus diffraction pattern with “Focus” knob.

Continue on next slide …



Beam Block

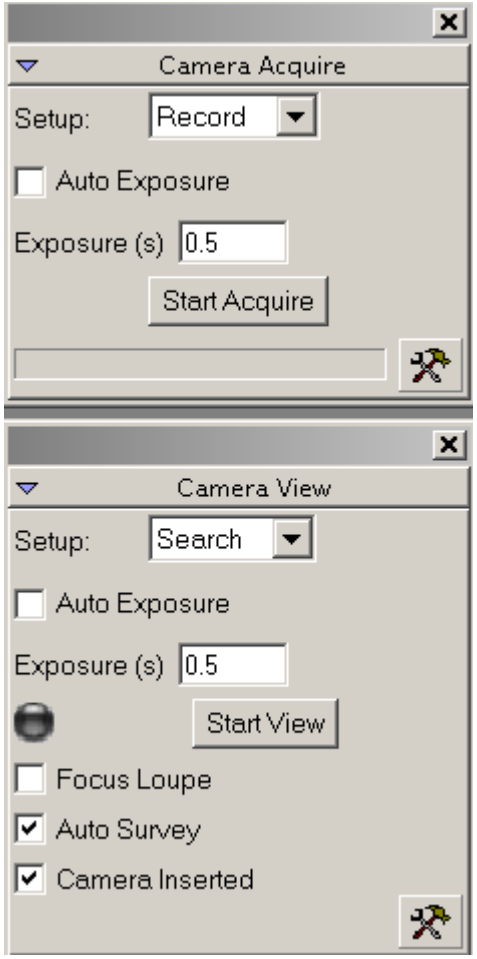
TEM Diffraction Mode (Cont'd)

SAED (Cont'd)

- If there is a strong central direct beam, insert the "Beam Block" and block the direct beam.
- Lift up the phosphorus screen. Use Digital Micrograph. Insert CCD.
- "Search" and "Start View" with **0.01 sec** exposure time. Increase exposure if the pattern is dim. Stop "Search" and directly save the image.

Note:-

- In Digital Micrograph, "**Save as**" *.dm3* format and "**Save display as**" image formats.
- If the direct beam spot is not at screen center, use "MF X /Y" to center it. If it is not circular, adjust "Stigmator-Diffraction" to make it round.
- Use "Magnification" to change camera length.
- The maximum tilt angle is 35° for A and 25° for B without Obj. aperture.

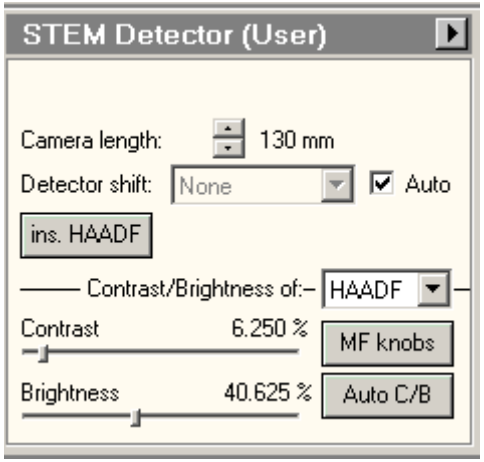


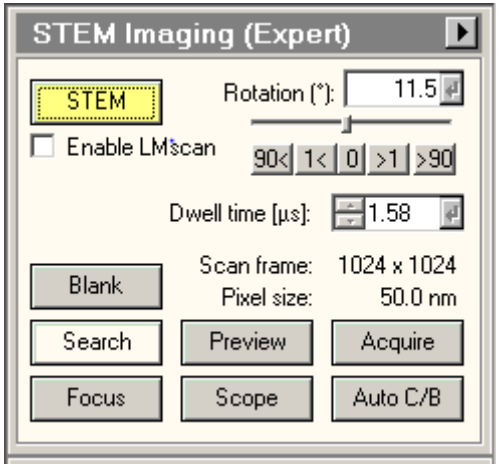
Camera menu in Digital Micrograph

STEM Alignment

***Note*: For good STEM imaging, it is important that the specimen is *clean* and column vacuum is below 10.**

- Carefully align the scope in imaging mode. Make sure Objective aperture and SAD aperture are out.
- Go to “FEG Registers”, select a suitable STEM register. For HR-STEM, use spot size 9. For analysis, use spot size 3. For both, use spot size 6.
- Recall a corresponding STEM alignment in “Alignment” flap out. Wait for one minute to stabilize beam.
- The scope should be in diffraction mode. Otherwise click “Diffraction” on RHP.
- Find an amorphous area on the sample, adjust camera length to 130 mm. Watch Ronchigram with binoculars.
- Adjust “Z” to achieve focus in Ronchigram (next slide).
- Move condenser aperture and center it around the Ronchigram.
- Adjust “Condenser Stigmator” to make the Ronchigram round (next slide).
- Move diffraction disk to center position (next slide) Click
- “Insert detectors” to insert detector. Lift up screen. Use
- “Search” and “Preview” to watch STEM image.
- Focus image using “Focus” on RHP. Correct astigmatism using “Condenser Stigmator”. Acquire image.





Click “Auto C/B” to correct bad contrast

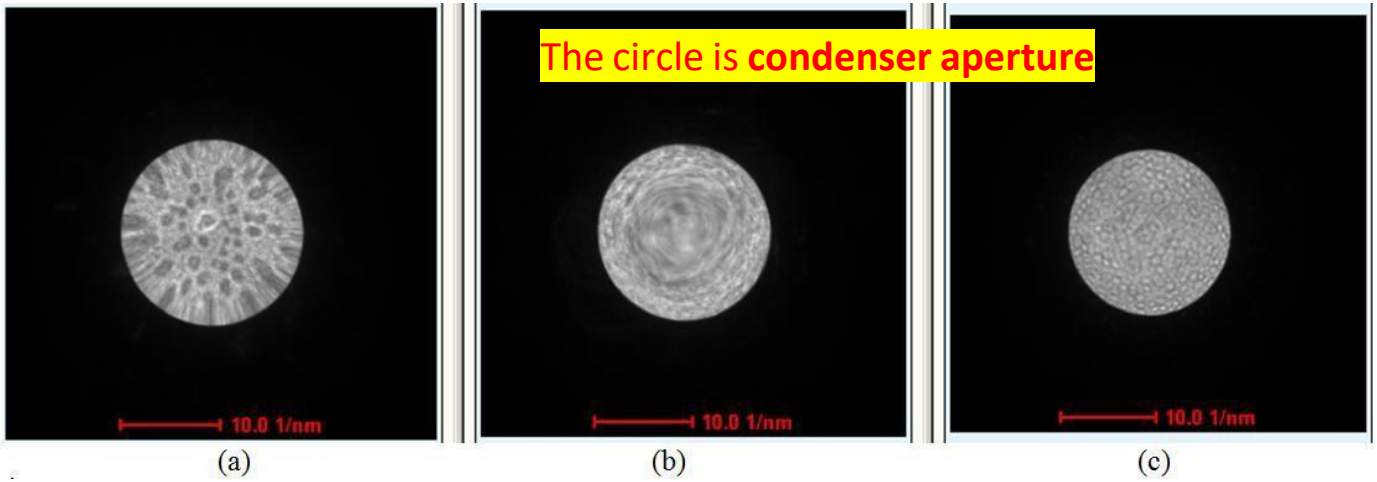


Figure: example of Ronchigram (Au nanoparticles on carbon film). (a) overfocus; (b) in focus; (c) under-focus. (a) and (c) show shadow image of the illuminated region on the specimen. (b) Focus is achieved when no feature can be seen from inside the Ronchigram.

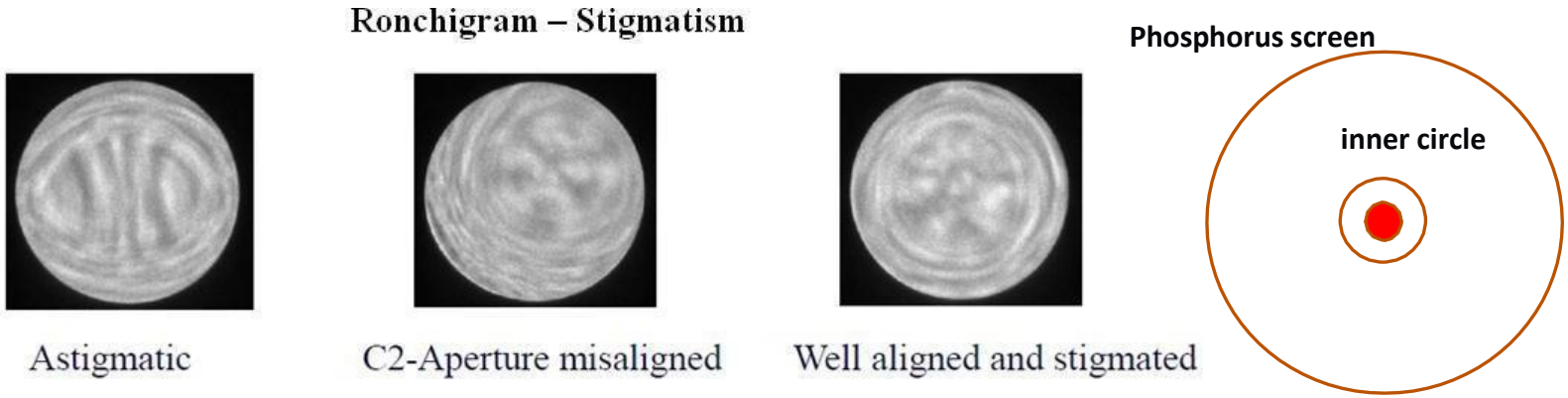
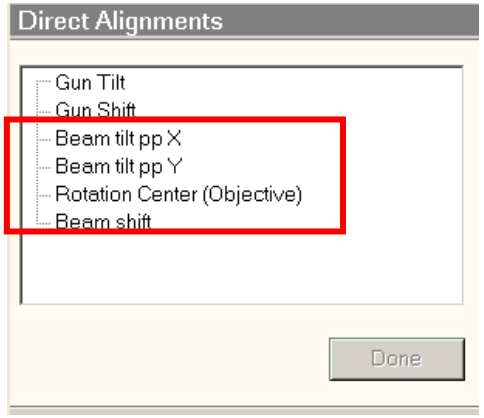
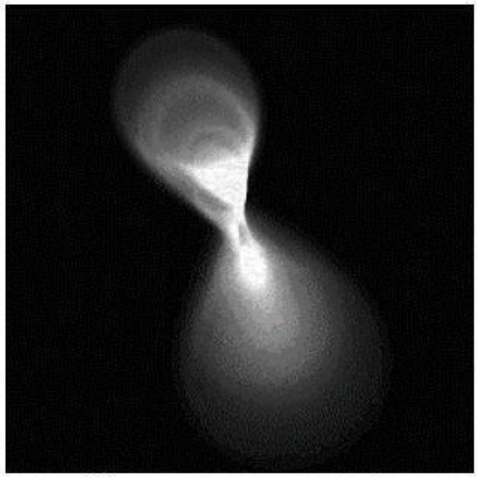


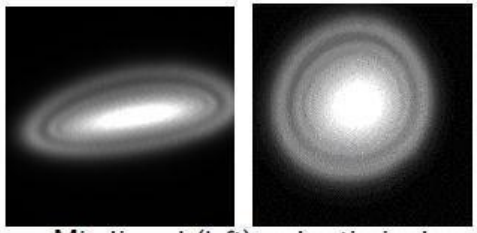
Figure: from left to right – Ronchigram with Condenser Astigmatism; Ronchigram without Condenser Astigmatism but C2 Aperture is not centered around it; Well-centered C2 aperture and no Condense Astigmatism; schematic diagram for correct position of STEM diffraction disk at 30mm camera length.

1. Push the “Diffraction” button on RHP to change STEM from diffraction mode to Imaging mode.
2. Use “trackball” to move beam to screen center.
3. Set magnification to 175kX or above.
4. Use “Focus” knob on RHP to condense beam to a minimum.
5. In “Direct Alignments”, select “Beam tilt ppX”. Use “MF X/Y” to make two spots merge to one. Repeat on “Beam tilt pp Y”.
6. Select “Rotation Center”, Use “MF X/Y” to minimize overall movement of the beam (like a heartbeat).
7. Select “Beam Shift” and move beam to screen center again.
8. Click “Done” to finish direct alignment.
9. Push “Diffraction” on RHP, back to Diffraction Mode.
10. Increase “Camera Length” to 100 or 150 mm. Watch Ronchigram.
11. Focus Ronchigram using “Z-height”. Re-center condenser aperture around the Ronchigram.
12. Lower camera length to 30mm. Move diffraction disk to center position.
13. Repeat 5-9 once for further tuning. Pay particular attention to #6.
14. “Search”, “Preview” image, focus with “Focus” knob & acquire



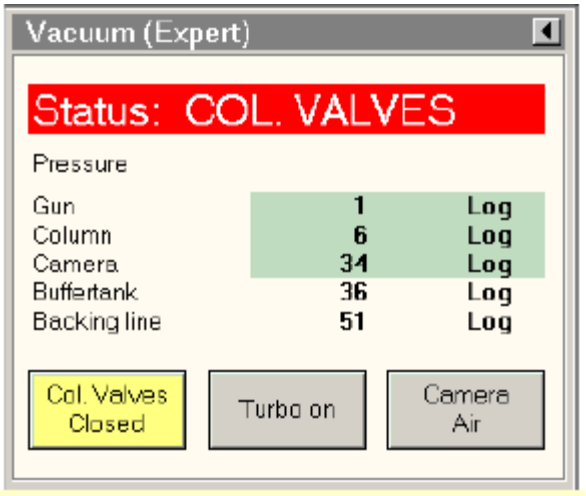


Misaligned rotation center



Misaligned (left) and optimised condenser stigmator

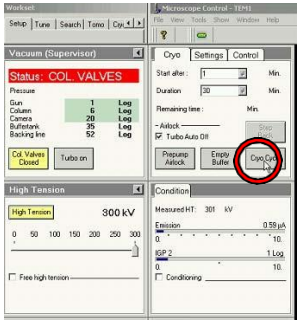
Finishing Procedure



- **Click "Col. Valves Closed" button (color changes to yellow) to close column valves. Always close the column valve before inserting/removing the specimen holder!!**
- Switch back to TEM-Imaging mode (if you are using STEM or Diffraction).
- Go to "Search", click "Holder" button to reset the holder's xyz and tilt angles to zero. (This is very important step. Failure to do so, the sample holder could be damaged during taking out).
- Fill in the log sheet (time, the vacuum values and the emission value).

AT THE END OF YOUR SESSION

If someone has booked the microscope after you , fill the LN2 dewar and leave



If you are the last one, remove the LN2 dewar and start the cryo cycle (the turbo should start)