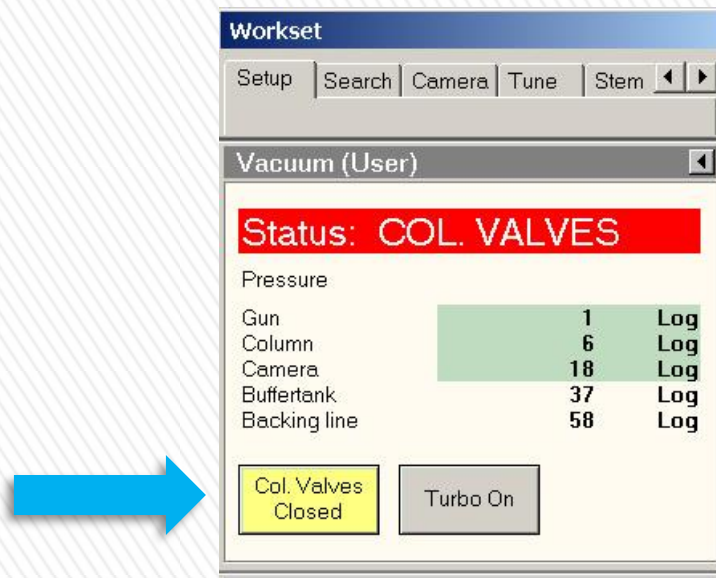# *FEI TECNAI G2 F20 S-TWIN TEM*

## *Training Manual*

*Advanced Materials Characterization Laboratory*

*Materials Research Center*

*Missouri University of Science and Technology*

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# In Emergency, Simply Close the Col Valve and Leave

# Snapshot – Microscope User Interface (MUI)

# **Snapshot** - **Left Hand Panel (LPH)** and **Right Hand panel (RHP)**



Microscope Control panels
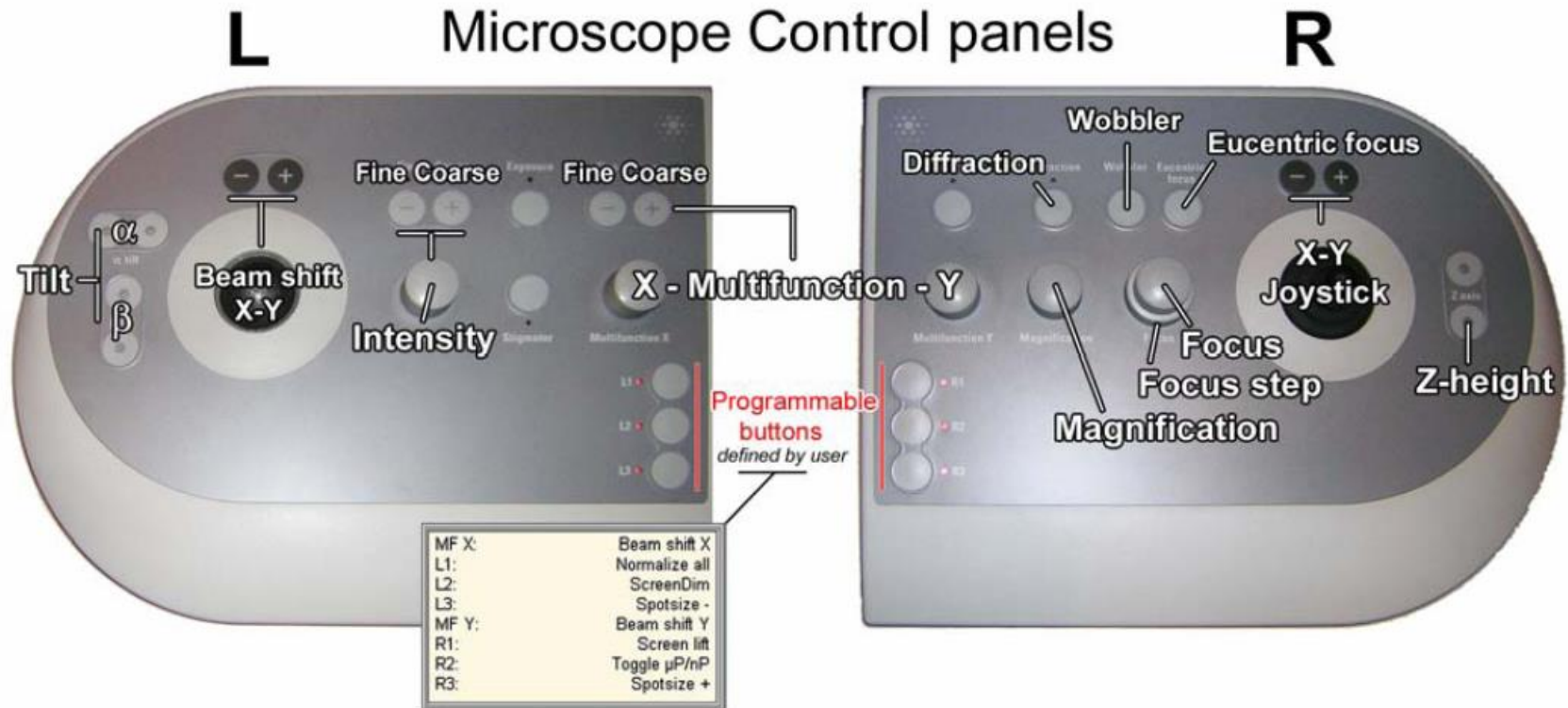
**Abbreviations** used in the manual: DL = Direct Alignment; Left Hand Panel = LHP; Right Hand Panel = RHP; Multi Function = MF; Bright Field = BF; Dark Field = DF

# Pre-start 1: Instrument
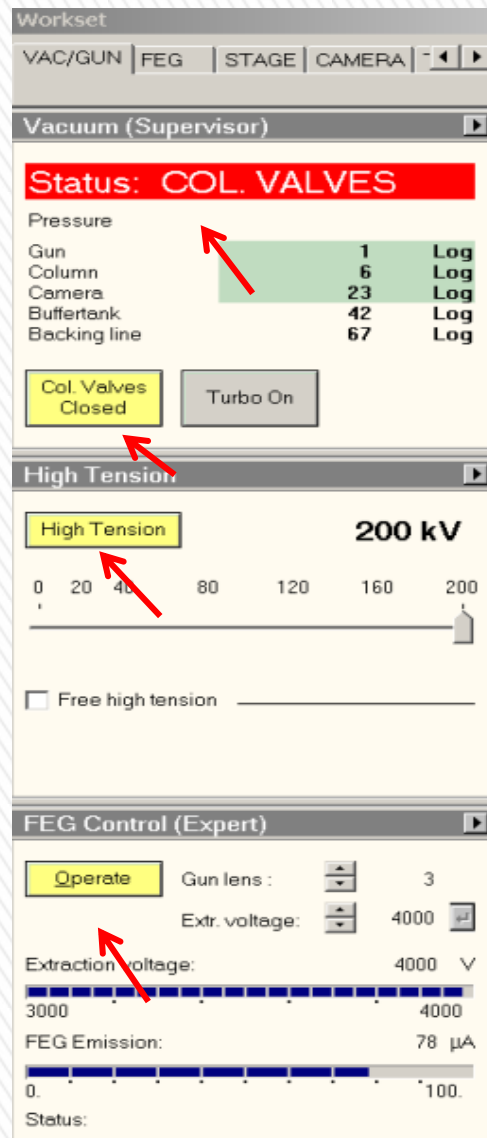




1. Top off **liquid nitrogen** in the dewar. Refill every three hours.

2. Check the following **scope conditions**

- **Left picture** - One "Red" and three "Yellow" as in the left picture.

- **Vacuum state** – green, gun is 1, column <10 (ideally 6)

- **Holder position** should be close to zero for X/Y/Z/A/B.  X may be close to 2 μm.

- **Apertures** - The "Objective Aperture" is out (on click 6). "SAD Aperture" is out (lever to the right). "C2 Aperture" is 2 or 3.

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

4. Vent plasma cleaner. Make sure the gas cylinder is open. It takes about 5 minutes to vent it.

# Pre-start 2: Mount Sample onto Holder (Single-tilt)

*Mount the sample onto a single tilt holder if you <u>do not need </u>double-tilt or EDX.*

- **Put on gloves.** Remove the holder from the plasma cleaner. You must handle the holder gently. The holder is fragile and dedicate.
- Safely place the holder onto the loading station.
- Before using, check the O-ring for cracks or dust. It must be clean. Do not touch any part of the specimen holder beyond the O-ring.
- Fit the tool into the hole in front of the clamp.
- Lift the clamp to its fullest extent.
- Place the specimen in the specimen carrier (the circular recess of the holder tip) face down.
- Lower the clamp with the tool onto the specimen carefully.

**Loading station**
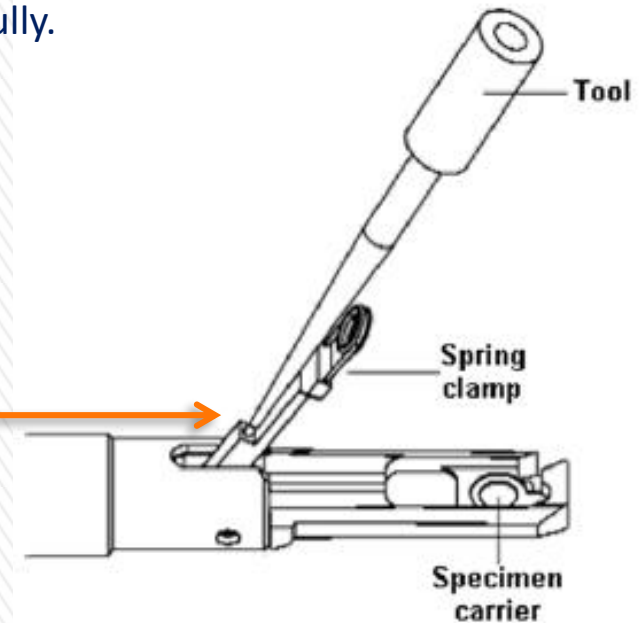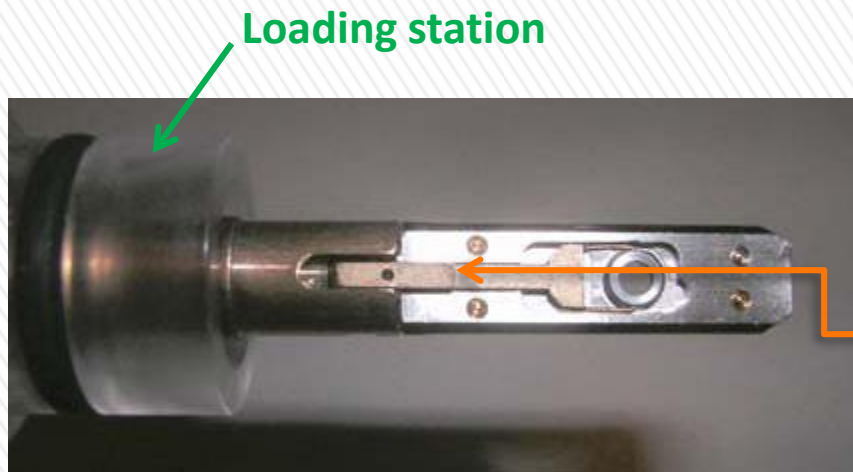
# Pre-start 2: Mount Sample onto a Holder (Double-tilt)

*Mount the sample onto a double-tilt holder if you <u>need</u> double-tilt and/or EDX.*

- Put on gloves. Remove the double-tilt holder from the plasma cleaner. You must handle the holder very gently. The holder is more fragile and dedicate than the single-tilt holder.

- Safely place the holder onto the loading station.

- Place the specimen in the specimen cartridge. The sample area should face *down*.

- *(Optional for thin specimen)* Place anti-twist ring above the specimen.

- Place the Be hex ring above your sample with **larger head facing up** (threaded side down).

- Use the wrench rod (hex head, not the round head) to screw in the hex ring. Do not over tighten!!

- Examine the hex ring under optical microscope. The ring must be flat and below the top surface of the specimen bowl. Also check the O-ring.
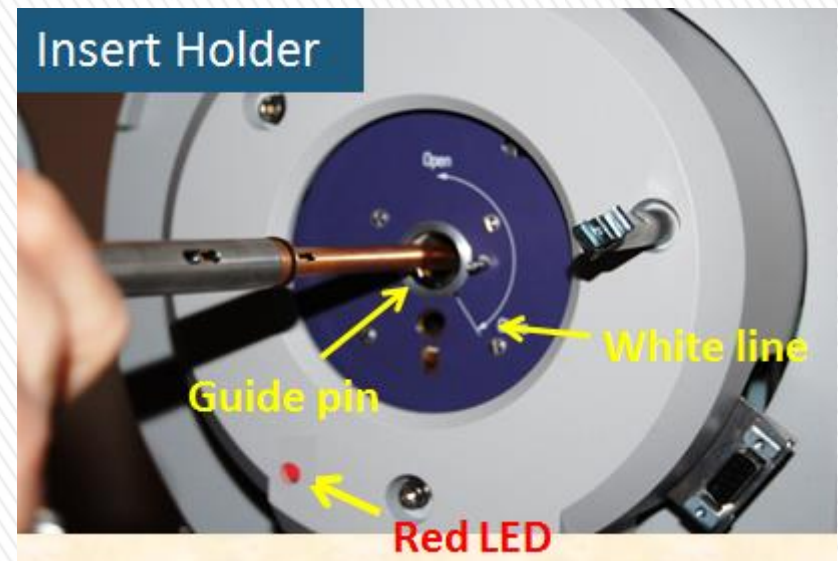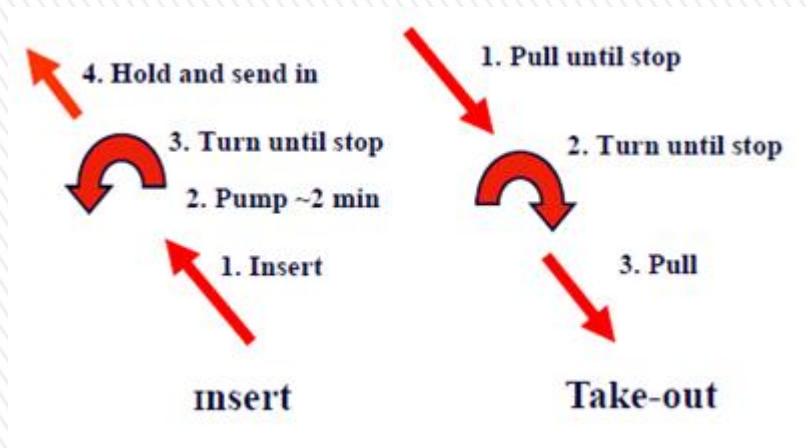
Do not stress this joint

Hex ring

Anti-twist ring
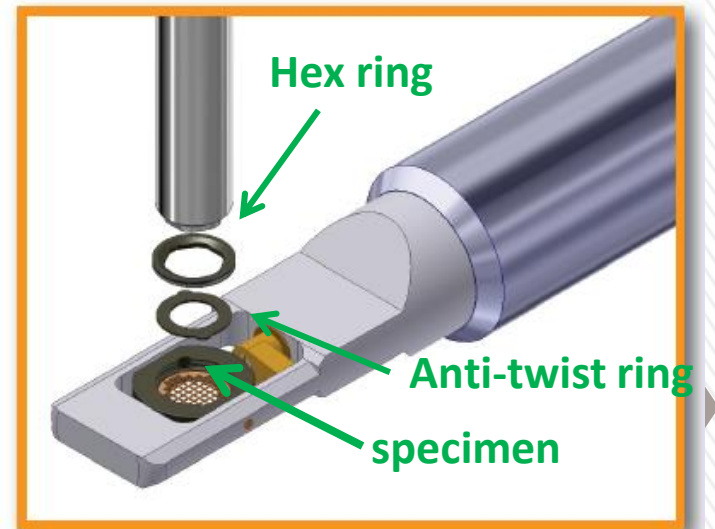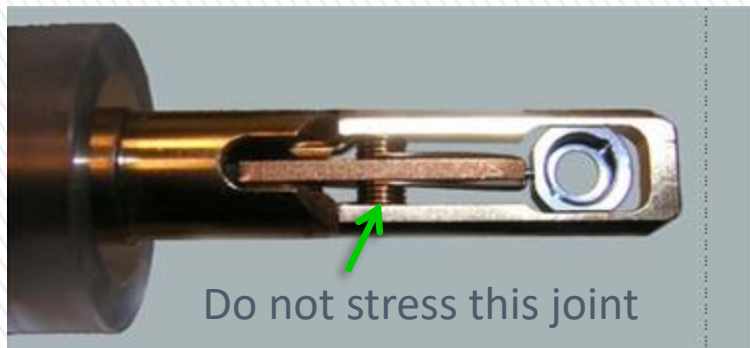
specimen

Specimen loading

# Pre-start 3: Insert a Specimen Holder

*Don't try to insert or extract a specimen holder if you were not trained.*

- Make sure "Column Valve" is closed in the software.
- At the bottom of the screen, select "Vacuum overview" to bring up the schematic.
- Insert TEM holder. Align the small guide pin with the white line on goniometer at about 5 o'clock position and insert the holder until it reaches a stop. Enter the correct holder type in MUI (*i.e.* single-tilt or double-tilt).
- *For double-tilt holder* - connect the holder cable and enter it in MUI.
- Turbo pump shall start. **STOP** and **wait until the "Red LED" turns off** (about 6 minutes).
- Turn holder *counterclockwise* for about 120 degrees (to "Open" position). Hold it and gently guide it in. Do not pause during this step (turn and insertion).   Check the Col vacuum reading.
- Turn off Turbo pump in Vacuum menu.

4. Hold and send in

1. Pull until stop

3. Turn until stop

2. Turn until stop

2. Pump ~2 min

1. Insert

3. Pull

insert

Take-out

Insert Holder

Open

White line

Guide pin

Red LED

# Extract a Specimen Holder

*For finishing or exchanging specimen*

- Make sure "Column Valve" is closed.
- Retract objective aperture (set to 6 or 7).
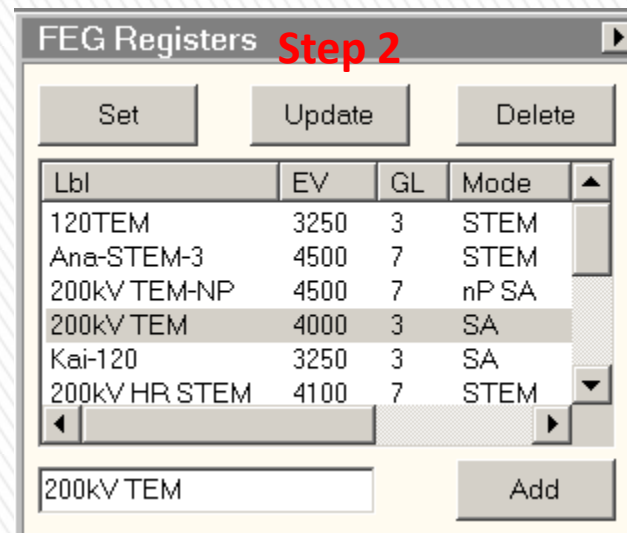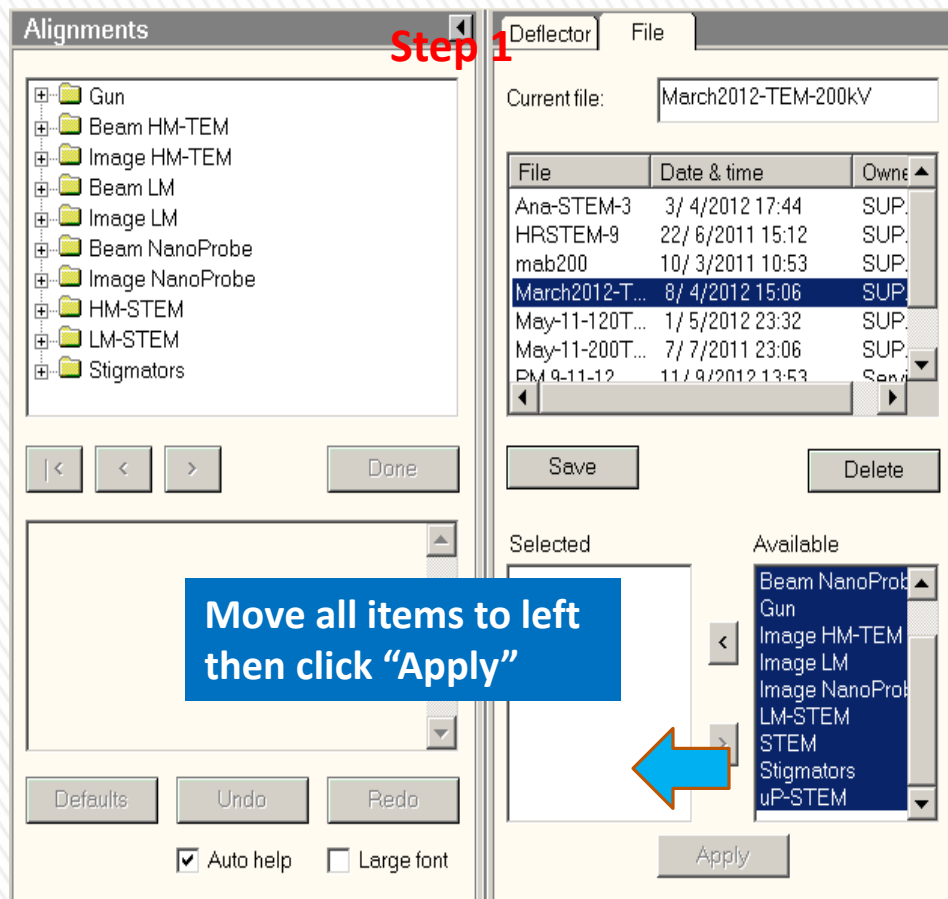- In the "Stage" menu flap-out, click "Holder" button to reset the holder's xyz position and tilt angles to zero.
- Press two fingers of your other hand against the purple plate firmly. DO NOT release your fingers in the whole process until you have removed the holder out of the column.
- Pull holder straight out gently until it reaches a stop. Rotate the holder **clockwise** until it reaches a second stop (about 120 degrees). *(Note: You can shift the hand on the holder after rotation is complete, if it's at an awkward angle for the rest of the extraction.)*
- Finally hold the holder firmly and pull the holder straight out of the column. (*Note: when starting the final removal, if it feels like the holder is caught, rotate it a very small amount counter-clockwise until freed*).



4. Hold and send in

3. Turn until stop

2. Pump ~2 min

1. Insert

insert

1. Pull until stop

2. Turn until stop

3. Pull

Take-out
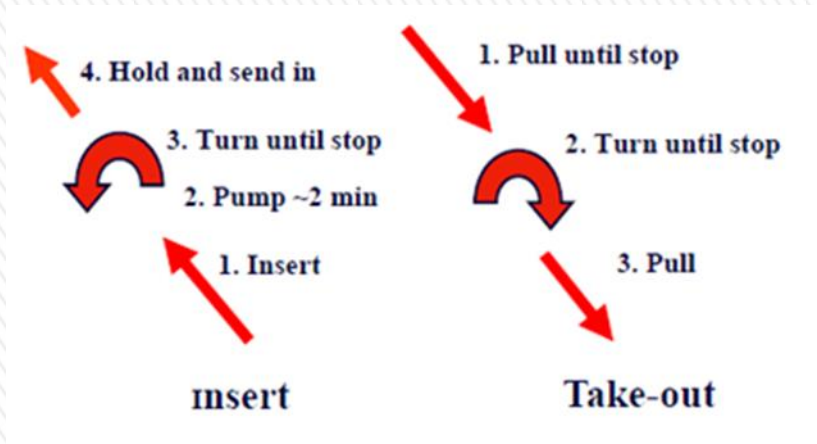
# Start up - Choose an Alignment File and "FEG Register"



**Step 1**

**Step 2**

Move all items to left then click "Apply"

- Go to File flap-out of "Alignment" menu.
- Select an appropriate alignment.
- Select all alignment items in the list below. Click the left arrow and move all items to the left column.
- Click "Apply".
- Select the FEG register matching the alignment file on "FEG" tab. Click "Set"

1. The alignments should be updated by the operator every half to one year.
2. TEM and STEM have different alignment files and FEG registers.
3. For different voltages separate alignment files are required.
4. For daily users, alignment files may not need to be reloaded each time unless the alignment was altered by the previous users.

# Start up – Find the Beam and Sample

- Wait until column vacuum drops below 10 and is relatively stable.

- Click "Column Valve Closed" to open the column valve. The button will turn gray. The "Status" shall change to green color with "READY".

- Click "Eucentric Focus". Reset defocus to zero if it is not*.

- Confirm "Objective Aperture" and "SAD Aperture" are both out (*i.e.* obj aper. = 6 or 7, SAD aper. lever to the right)

- The electron beam should be seen on the big phosphor screen. Turn "Intensity" knob to spread beam to about the size of the phosphorus screen. Center beam using the track ball.

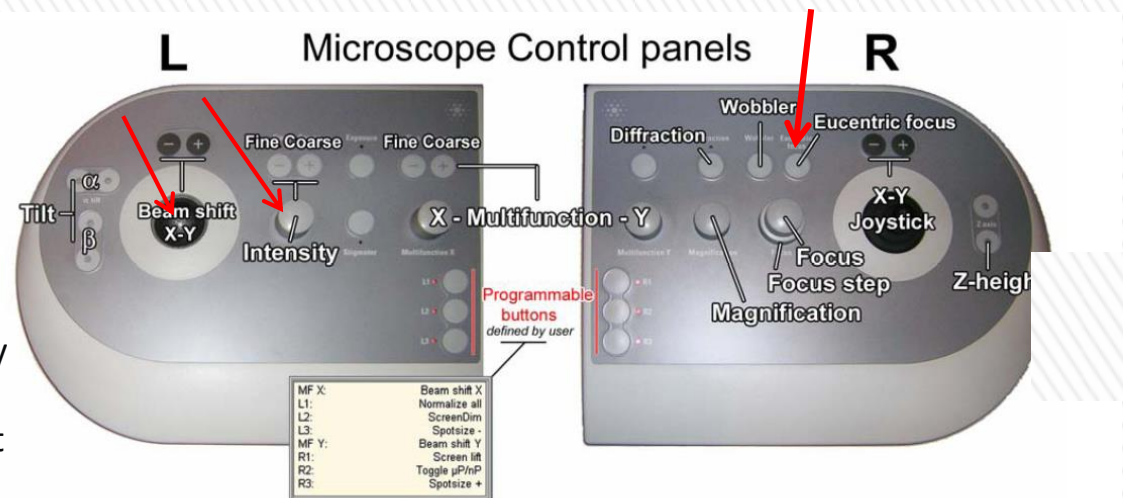- If no beam visible, lower magnification and move sample around to find the beam.

**Status: COL. VALVES**

Pressure

| Gun | 1 | Log |
| Column | 6 | Log |
| Camera | 18 | Log |
| Buffertank | 37 | Log |
| Backing line | 58 | Log |

Col. Valves Closed    Turbo On

**Vacuum (User)**

**Status: READY**

Pressure

| Gun | 1 | Log |
| Column | 6 | Log |
| Camera | 23 | Log |
| Buffertank | 34 | Log |
| Backing line | 51 | Log |

Col. Valves Closed    Turbo on    Camera Air

| Defoc.: | 0.00 nm | SA 13500 x |
| Exp. time: | XXX | TEM Bright field |
| Obj: | 92.0158 % | |

**Defocus value** should be maintained not far away from zero during operation (< 1-2 μm).

*The defocus value can be reset to zero by user at any objective value. So it is good practice to press eucentric focus and reset defocus to zero before the session.

Microscope Control panels

L    R

Wobbler
Diffraction    Eucentric focus
Tilt    Beam shift X-Y    X - Multifunction - Y    X-Y Joystick
α
β
Intensity    Focus
Focus step    Z-height
Magnification

Programmable buttons defined by user

| MF X: | Beam shift X |
| L1: | Normalize all |
| L2: | ScreenDim |
| L3: | Spotsize - |
| MF Y: | Beam shift Y |
| R1: | Screen lift |
| R2: | Toggle μP/nP |
| R3: | Spotsize + |

# TEM alignment: 1 – Eucentric Height

*There are two ways to set up Eucentric Height.*
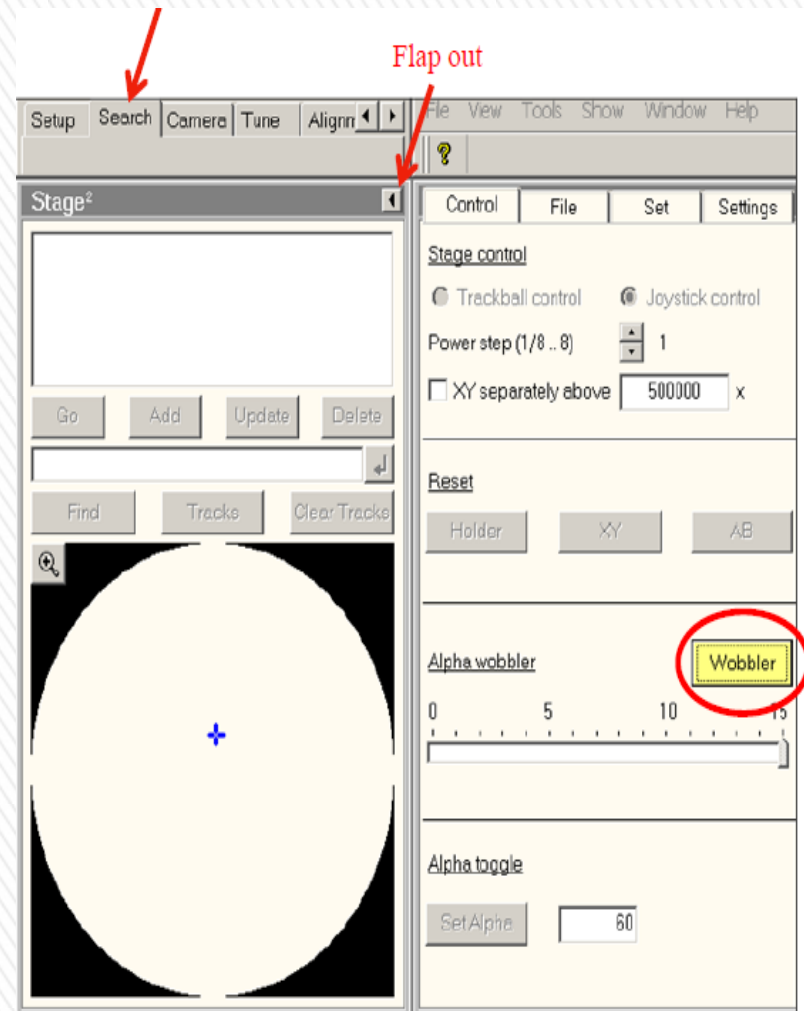
1. *Fast way* –

   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* –

- Go to "Stage", click the "flap out" arrow and click "Wobbler".  (*Note: the Wobbler button on the RHP is a different wobbler.  Do not use it here.*)

- Adjust "Z" on the right hand panel to minimize the sample movement.

- Set the magnification to 125k to do the fine adjustment.

- Click "Wobbler" again to turn it off.

*For strongly magnetic specimen, only use the first method because beam will always move during wobbling due to the magnetic field of the sample.*

*Click "Eucentric Focus" to start!*

Flap out



The fast way assumes that the procedure alignment (complete alignment) has been properly set up. Otherwise it won't be accurate.

# TEM Alignment : 2 – Center C2 Aperture

- Set an appropriate C2 aperture by turning the big (outer) 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 the trackball on LHP.

- Spread the beam by turning the "Intensity" knob *clockwise*.

- Adjust the two knobs at the C2 aperture (middle and side knobs) to move the beam back to the screen center.

- Repeat the above steps until the beam remains centered when turning the "intensity" knob on LHP.

**Adjustment knobs**

Size selector
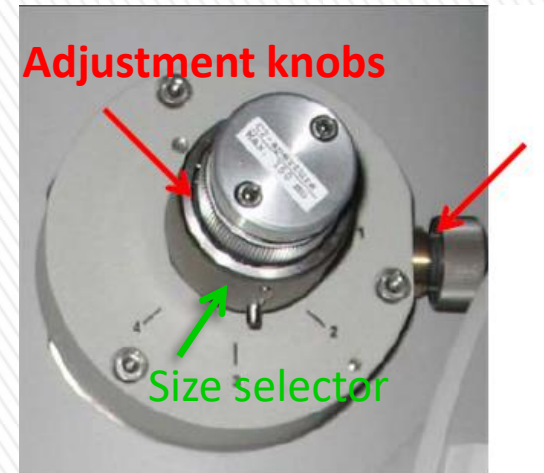
C2 aperture
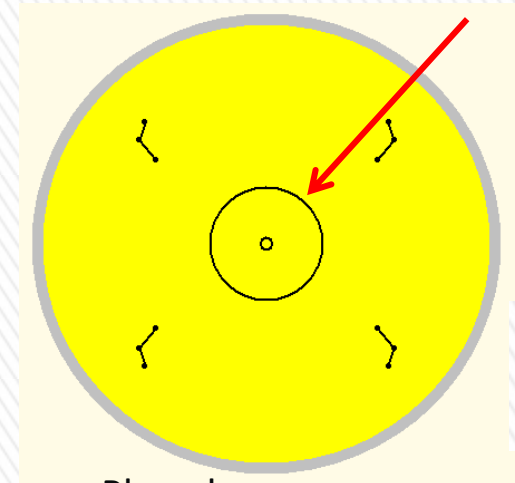
Phosphorus screen

# TEM Alignment : 3 – Condenser Astigmatism

- Adjust the condenser astigmatism if the beam is not circular.   This adjustment can be repeated at any time as necessary.

- Go  to "Tune", click "Condenser" from the **Stigmator** menu.  Or, push the "Stigmator" button on the LHP and make sure that "Condenser" is selected in the software.

- Use the "**Multifunction knobs (MF X/Y**)" to adjust the condenser Astigmatism in both x and y directions to ensure the beam is round and expands concentrically.

- Click "None" after finishing to exit the stigmator.
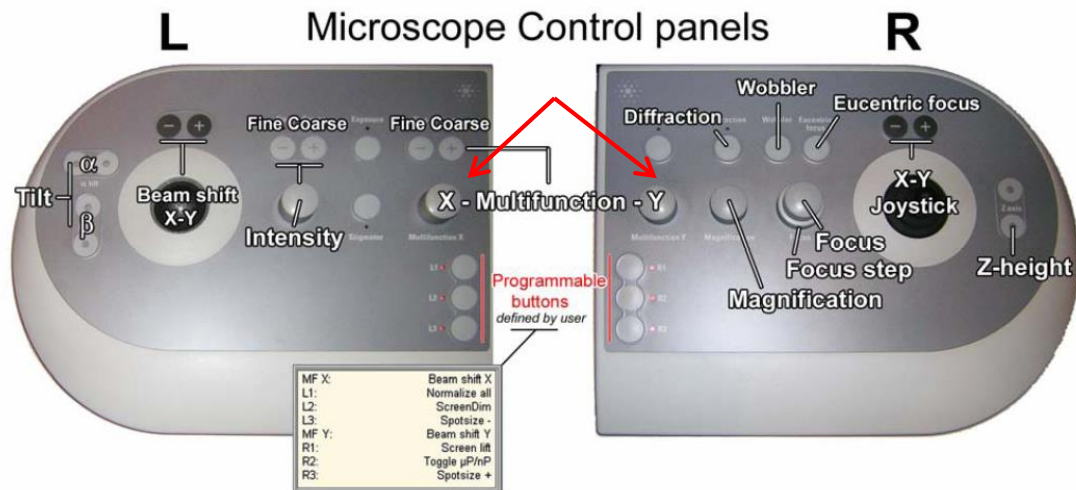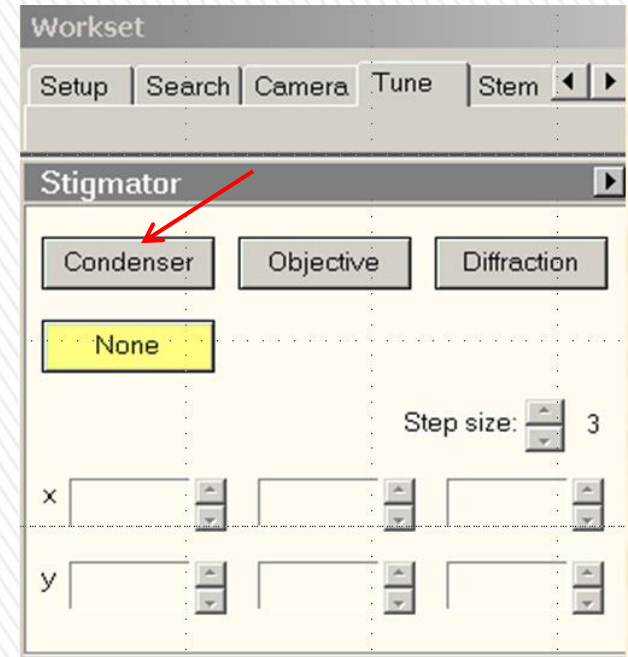
# TEM Alignment : 4 – Direct Alignment (Gun Tilt) (Optional)

- Note – Daily users do not need to do gun alignment, particularly if one was not trained.

- *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* - Find an area without specimen (e.g., hole).  Set magnification to around 10k. Click "**Gun Tilt**" on the "Tune" tab. Adjust "Multifunction knobs" in both x and y directions to maximize the screen current (nA) or minimize the "Exposure Time". Click "Done" after finishing. Correct condenser astigmatism again if necessary.
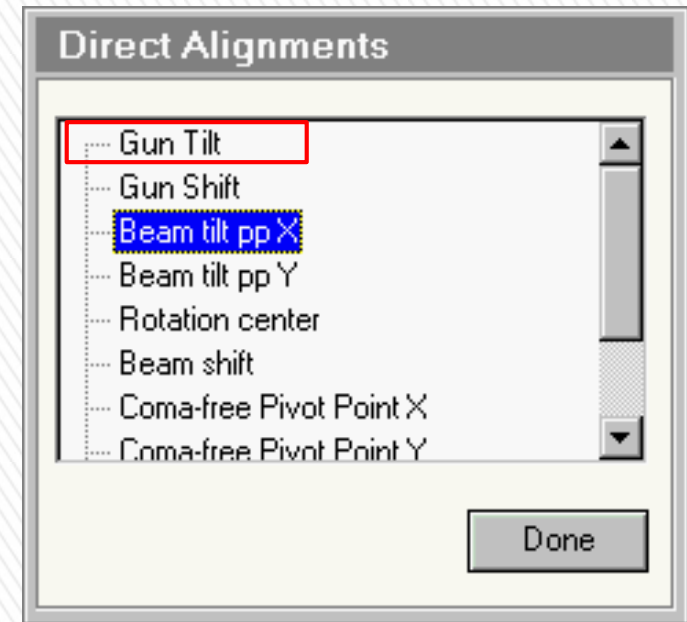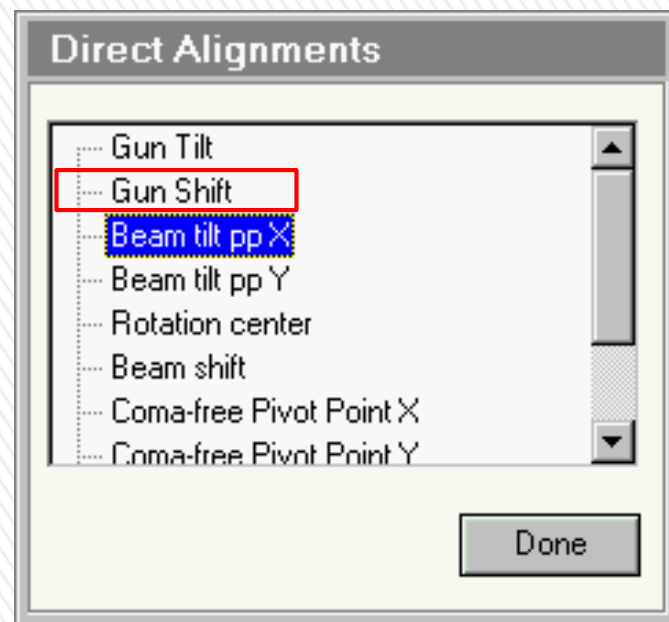


| TEM Bright field | C2: | 43.580 % | SA 175 kx | Em val: | 80.00 µA |
| Defoc.: | 0 nm HT: | 200 kV | | | |
| Spot size: | 5 | | Screen: 0.000 nA | Focus step: | 1 |

**Status window**

1.  The gun alignment (Tilt and Shift) is preferred to be conducted by the operator every a few days in the Procedure Alignment.  Daily users are not recommended to alter it on a routine basis.
2.  The holy cross method is better than the maximum intensity method for gun tilt.

# 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. Change spot size from 3 to 9, select "**Beam Shift**", and center beam using MF X/Y; change spot size back to 3, select "**Gun shift**", and 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.



Direct Alignments

- Gun Tilt
- Gun Shift
- Beam tilt pp X
- Beam tilt pp Y
- Rotation center
- Beam shift
- Coma-free Pivot Point X
- Coma-free Pivot Point Y

Done

| 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* – Increase Mag to 125K. Center beam using track ball. (Center C2 aperture and correct condenser astigmatism if needed.) Condense beam to a spot (intensity). Select "**Beam Tilt pp X**" to activate. Using MF X/Y knobs to make the two beams to merge to one point. Click "Done".  Select "**Beam Tilt pp Y**" to make two beams merge again and click "Done" when finished.
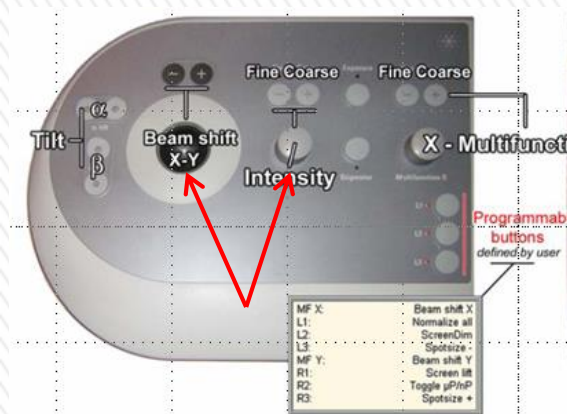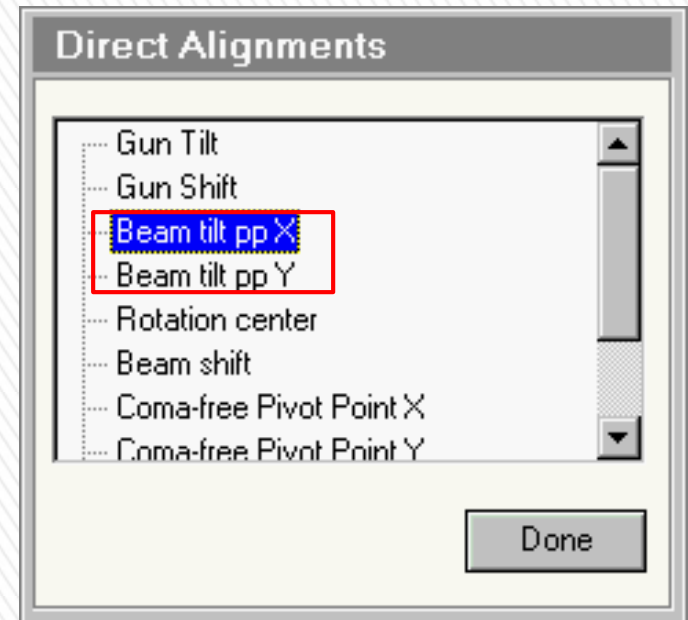






Misalignment of beam tilt Pivot point
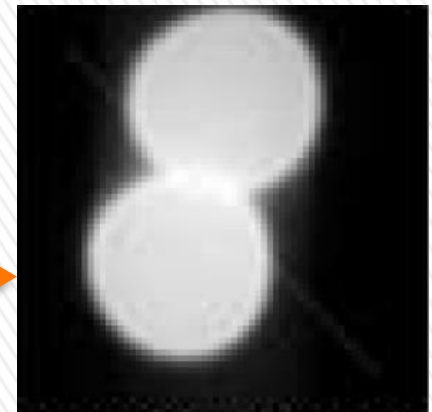
# TEM Alignment : 7 – Focusing Binoculars

- *Purpose* – the binoculars provide additional magnification so that fine features can be better viewed. It is necessary to adjust it so that the focus of the binoculars fits your eyes.

- *Procedure* - Turn the outer knob of **Beam Block** towards you and insert the Beam Block from Park position (the Beam Block serves as a reference object for focus). Lift up **Focusing Screen**. Look at the Beam Block 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.
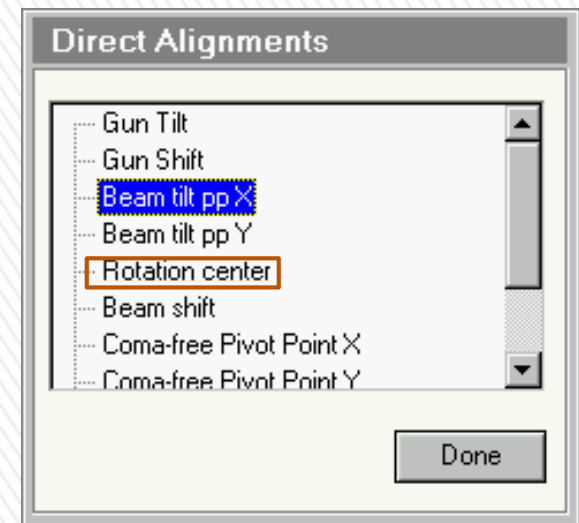


Beam Block



Focusing knobs
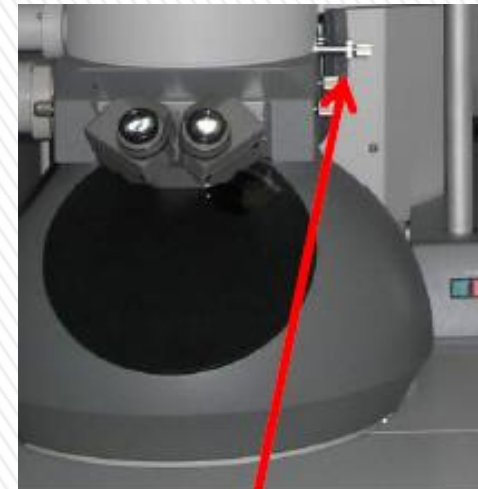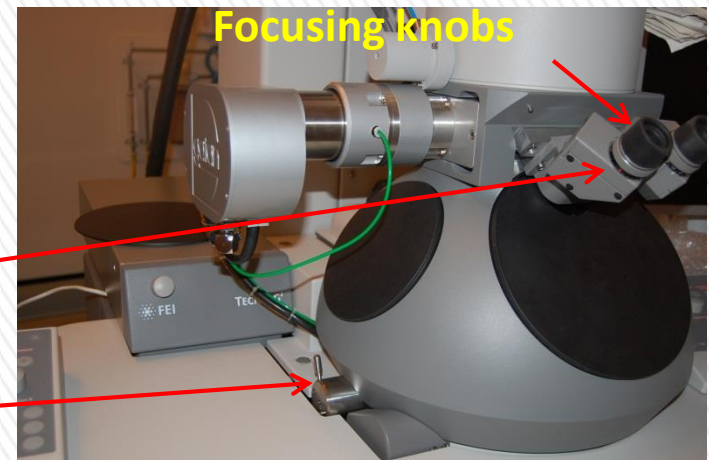
Binoculars

Focusing screen lever

# TEM Alignment : 8 – Direct Alignment (Rotation Center)

■ *Purpose* – make sure that the beam is along the optical axis of the objective lens and that small fluctuations in lens current don't affect the image.

■ *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 **MF X/Y**. The image should pop up like "heart beat" but not shift in x or 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 micrometer.

■ *Note* - If there is no suitable object to minimize the image displacement during the alignment of the rotation center, focus the beam (INTENSITY knob) and optimize the rotation center to get a symmetric opening and closing of the beam.

**Direct Alignments**

- Gun Tilt
- Gun Shift
- Beam tilt pp X
- Beam tilt pp Y
- Rotation center
- Beam shift
- Coma-free Pivot Point X
- Coma-free Pivot Point Y

Done

More precise alignment of the objective lens is done during the coma-free alignment, which is shown in the HREM section. For imaging at low to intermediate magnifications, rotation center is sufficient for alignment purpose.

TEM daily alignment is done now.

# Take TEM Image Using CCD Camera

- Choose the area of interest and move it to screen center.

- Focus specimen and spread beam. You may choose either WAC CCD or Slow CCD. Lift up phosphorus screen if Slow CCD is to be used. Be careful not to view a strong condensed beam using the CCDs.

- Click "Insert" to insert the CCD camera.

- "Search" with binning 4 and integration 0.07-0.2 sec.
  "Preview" with binning 2 and 0.5-1 integration.
  "Acquire" with binning 1 and integration 1-4 sec.

- Save image to .emi format. Right click on the image and export data in image format (.tiff, .jpeg, *etc*.)
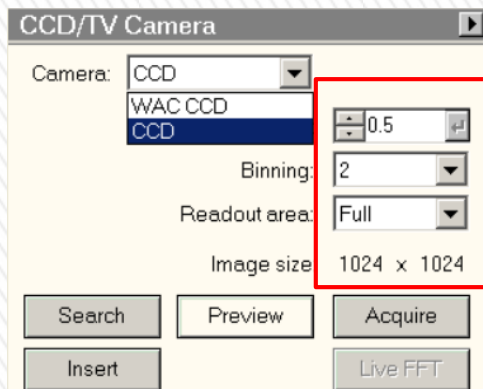


- The above are common setting for **BF imaging**. User can try and choose any camera setting best presenting his/her work.

- The setting should be *different* for Diffraction (short exposure) and DF imaging (longer exposure).
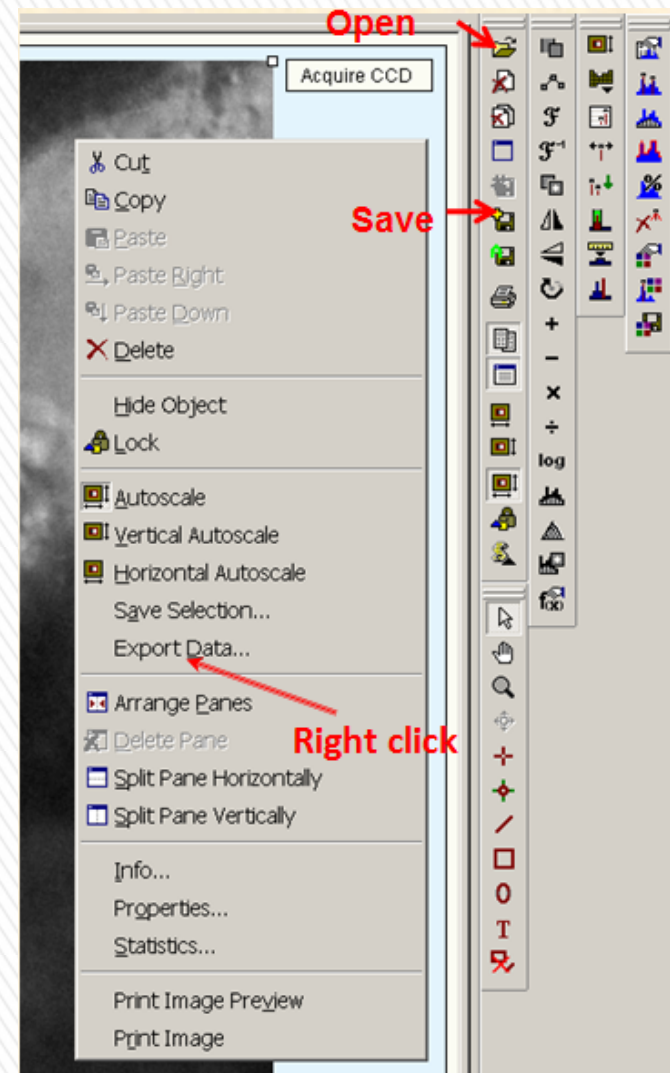
1. To reduce background noise or inhomogeneous intensities, the gain/dark reference needs to be checked/updated by the operator. Bias (dark) references are acquired with a blanked beam and Gain references with a homogeneous spread beam (however without sample).
2. Typically, Search is a fast, continuous mode, e.g., image frame 256 x 256, integration time 0.1 s using the full CCD chip at binning 4.
3. The Preview mode can be used for focusing under the suggested conditions: image frame 256 x 256, continuous integration time 0.2 s using only half of the CCD chip at binning 2.

# Save and Transfer Files

1. *For images taken with TIA* - "Save As" images in *.emi* format to the "**tecnai20 on 192.168.0.2**" folder. If you don't have a folder in the above folder, create one there. Then go to the support computer. Find the "**Shortcut to Tecnai F20**" folder on desktop, locate your saved files, and copy them to the "**Shortcut to AMCL**" folder (also on desktop). The latter is a network storage supported by MS&T. 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.

2. If you have not previously used the AMCL folder (also called Research Volume) , please follow the following instruction to set it up on your computer:-
   \\minerfiles.mst.edu\dfs\Research\MRC\amcl
   "Copy this link, go to desk top and open a new shortcut, paste this link in the shortcut path.  Give it a name and enter, it will ask you for your user name and password.  Make sure your use name starts with MST-USERS\. This link is also on the desktops of the F20 and Helios support computers. Once you upload your data to the research volume, you will be able to retrieve the data from any computer on campus by following the above instruction."

3. *For images taken using Digital Micrograph* - Go to "File" menu, "Save as" image in *.dm3* format and "Save display as" in image formats. Transfer files similarly as in Step 1.  Images can be batch converted to .tif or .jpg using Digital Micrograph, so it is not necessary to export each image.

   *If you have no access to the AMCL folder after following Step 2 , contact the lab manager.*

# HREM – Correct Objective Astigmatism

Proper correction of Objective astigmatism is important for HREM. There are two ways to correct Objective Astigmatism.

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. 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. Exit "None" after finishing.

Note: There are three saved settings for each stigmator.  It may be a good idea to copy the current settings before attempting to correct any astigmatism, so that you can return to the original conditions if astigmatism gets worse during correction.



(ii) Caustic curve

(iii) Aberration-free point

(i) Bright-field spot

100 nm

$\alpha_{in} = \sim 0$ mrad
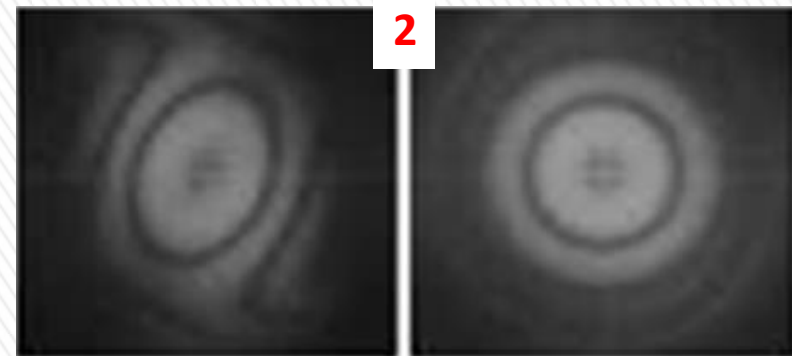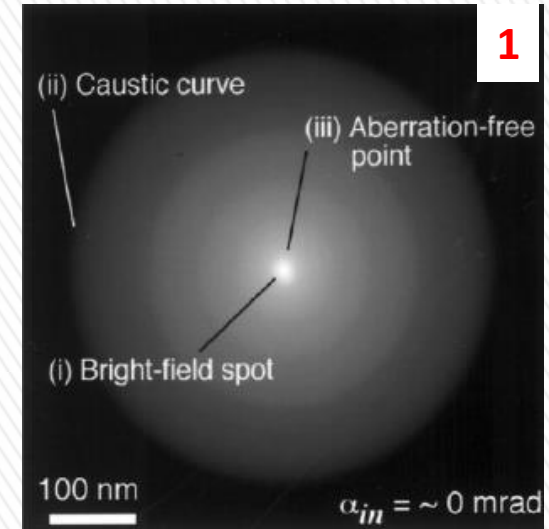


Astigmatic

Astigmatism corrected

# Focusing in TEM

Before focusing: (i) make sure eucentric height has been correctly set up. (ii) click "Eucentric Focus" on RHP.

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

1) **Minimum Contrast**. (recommended) This method applies to all magnifications. Look at image carefully. Focusing is achieved when the image has minimum contrast.

2) **Fresnel Fringe**. For all magnifications, works well for samples with particles, hole or edge area. 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). More precise. Click "Live FFT" in Camera menu to obtain FFT image. Correct Obj. Astigmatism. Turn Focus knob to maximize the diameter of the inner circle of FFT.

*Note* – "Z-height" control is usually used for coarse focus while "Focus" knob is used for fine focus.
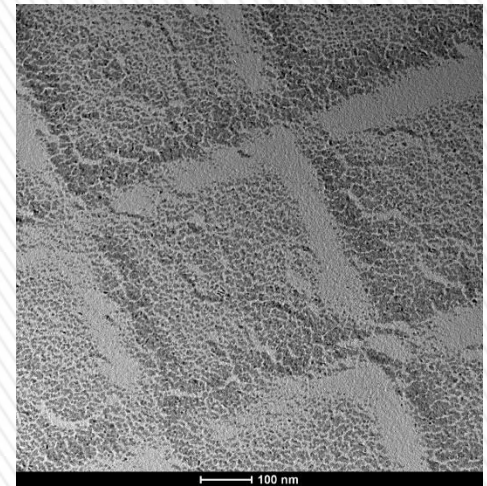
In-focus; minimum contrast
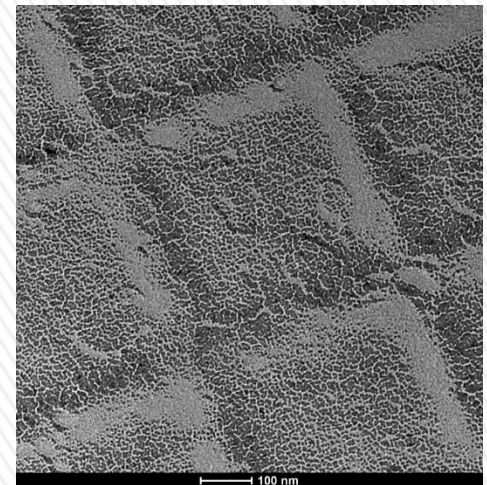


Out-of-focus; stronger contrast
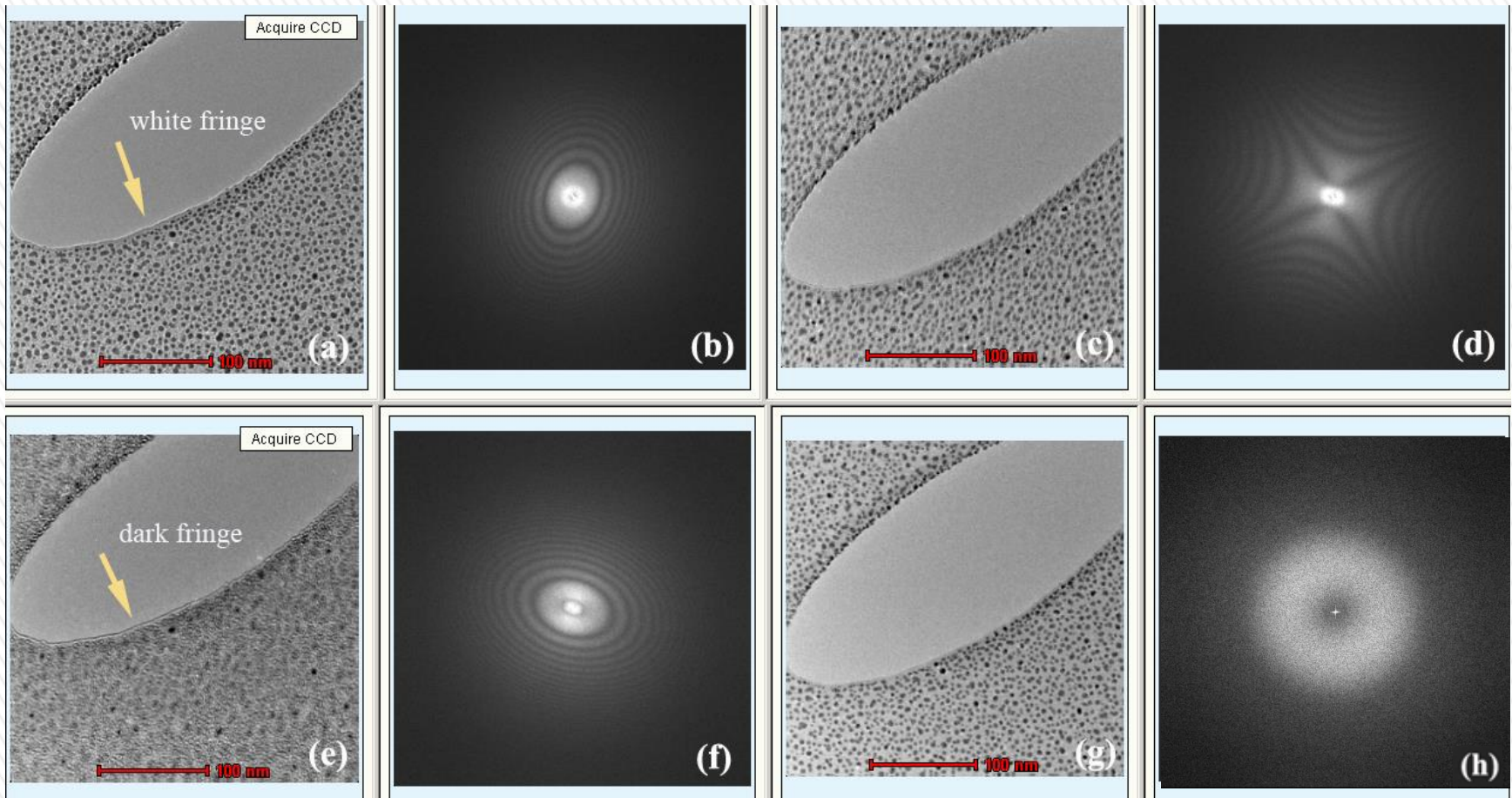
**Figs.** (a) under focus with objective astigmatism; (b) FFT for (a); (c) in focus with objective astigmatism; (d) FFT for (c); (e) over focus with objective astigmatism; (f) FFT for (e); (g) in focus without objective astigmatism; (h) FFT for (g).

**Example of Focus and Objective Astigmatism**

# HREM – General Procedure

*Note*: HRTEM generally requires sample thickness of less than 50 nm. The thinner the better.

- Retract Objective Aperture if inserted before.
- Carefully align the scope as described in previous slides.
- For crystalline material, accurately tilt the sample onto a major zone axis using alpha and beta tilts.
- Adjust the illumination so that it is slightly larger than the inner circle on the phosphorus screen (the red dashed circle).
- If possible, locate an amorphous area close to the area of interest. (This is to assist focus and particularly the correction of the objective astigmatism.)
- Lift up screen. "Search" or "Preview" using CCD.
- While viewing the amorphous area, focus and correct Objective Astigmatism with FFT.
- Optimize coma-free alignment in the "Direct Alignment" menu with aid of FFT (next slide).
- Move back to the area of interest. Carefully adjust the focus knob to achieve the best lattice fringes.
- Minimize mechanical vibrations. Acquire HREM image.
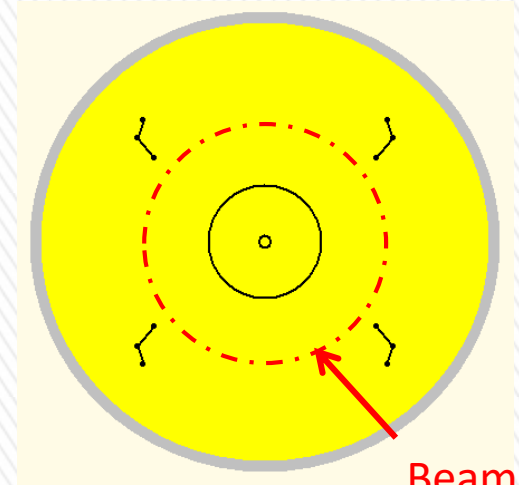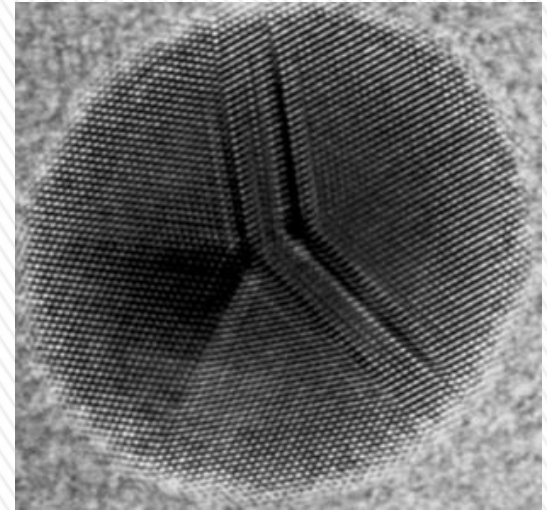




Beam size

Inserting an objective aperture limits the HREM transfer approximately to the information limit (Tecnai F20 ST), thereby cutting off the fast oscillating parts of the contrast transfer function. Objective astigmatism must be corrected when switching the objective aperture.

# HREM – Coma-free Alignment

Optimize coma-free alignment: focus the image at 350 kX or higher, select COMA-FREE ALIGNMENT X&Y in Direct Alignments, and make the contrast as similar as possible for both beam orientations. This can be done by either looking at the images (as shown in the example below) or at FFT. The latter may be more convenient to use.
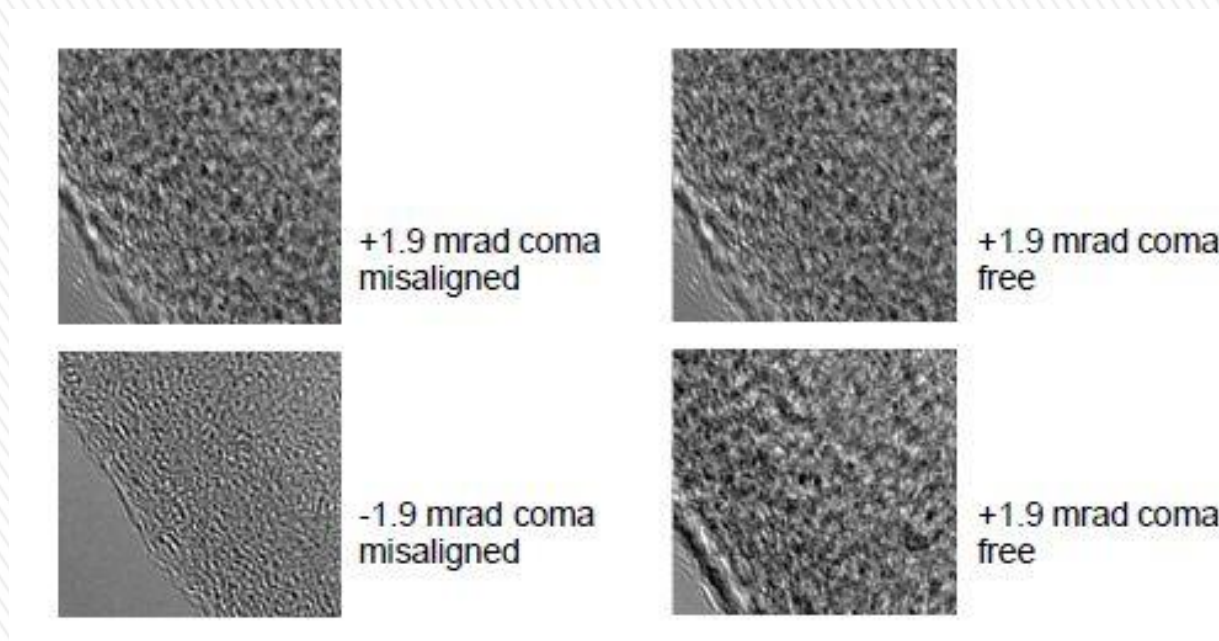


**Example of Coma-free Alignment**

# TEM Diffraction Mode

*Note – do not view an intense direct beam of a diffraction pattern using CCD without Beam Stop.*

- Choose the area of interest.
- Set sample at eucentric height and properly focus image.

## CBED (Convergent Beam Diffraction)

- Converge beam to a spot, click "Diffraction" button on RHP. View pattern on screen.
- Focus diffraction pattern with "Focus" knob.

## SAED (Selected Area Diffraction)

- Insert SAD aperture by turning the lever from right to left. The aperture should cover the area of interest. Adjust aperture size with big (outer) knob and center with other knobs.
- 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 …*
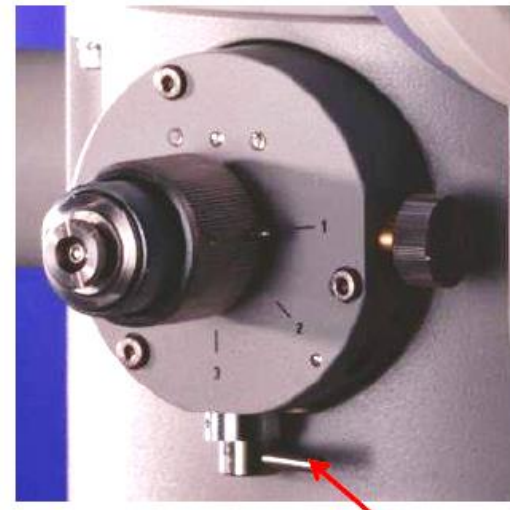


Selected area aperture lever
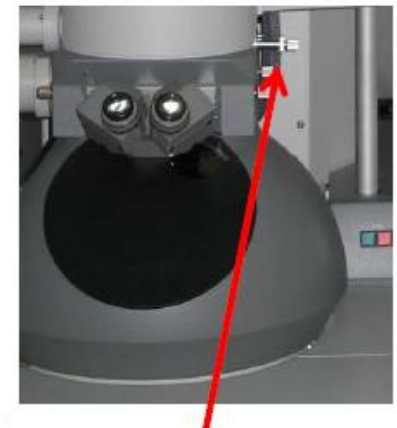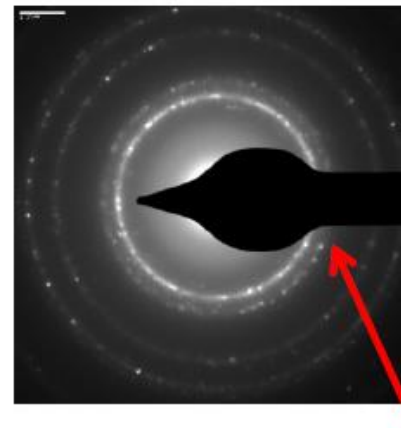


Beam Block

# 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. *For highly symmetrical SADP from a crystalline sample, the direct beam intensity usually is low and so Beam Block may not be required.*

- Lift up the phosphorus screen. Use Digital Micrograph. Insert bottom mounted CCD.

- "Search" and "Start View" with **0.1 sec** exposure time. Increase exposure only if the pattern is dim. Stop "Search" and directly save the image.

***Note:-***

- In Digital Micrograph, "**Save as**" *.dm3* format and "**Save display as**" in 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.

- Turn "Magnification" knob to change camera length.

- The maximum tilt angle is 35° for A and 25° for B without Obj. aperture.
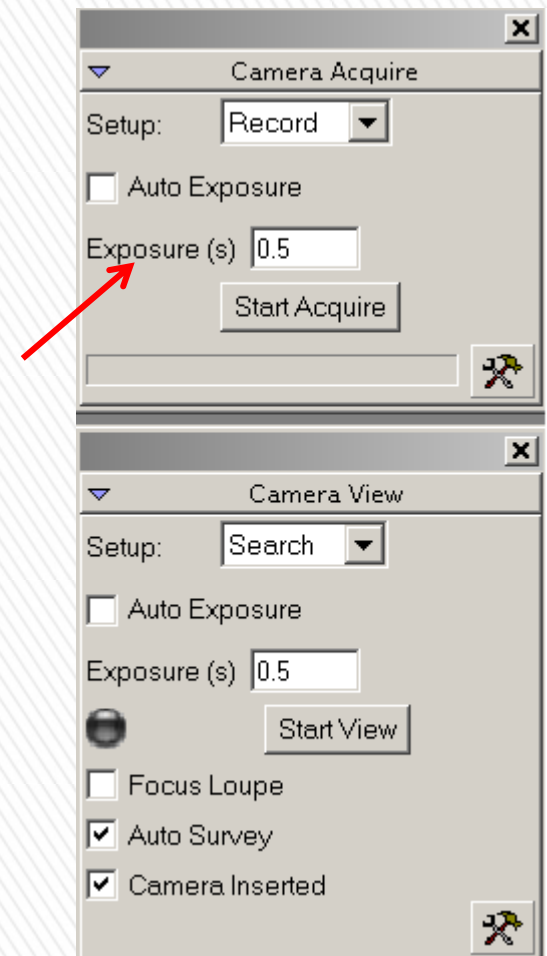


Camera menu in Digital Micrograph

# TEM Bright Field (BF) and Dark Field (DF) Imaging

- Conduct alignments and focus image. Obtain a CBED or SAED pattern.

- **BF Imaging** - Insert and center a suitable objective aperture on the central direct beam of the diffraction pattern.

- Click "Diffraction" button again to exit Diffraction mode. If a SAD aperture is inserted, remove it. Adjust "Intensity" to see Bright Field image. Adjust focus if necessary.

- **DF Imaging** - A dark field (DF) image is formed using a diffracted beam or a group of adjacent diffracted beams.

- Focus image in bright field. It is hard to focus in DF mode.

- Click "Diffraction" and acquire a diffraction pattern. Insert and center objective aperture around a desired diffraction spot for Simple Dark Field image.

- Exit diffraction mode, adjust "Intensity" and acquire a DF image. Increase exposure time if image is dim (may need 3-10 sec exposure).

BF Imaging

DF Imaging

Sample

Sample

Objective lens

Direct beam

Diffracted beam

Objective aperture

Screen
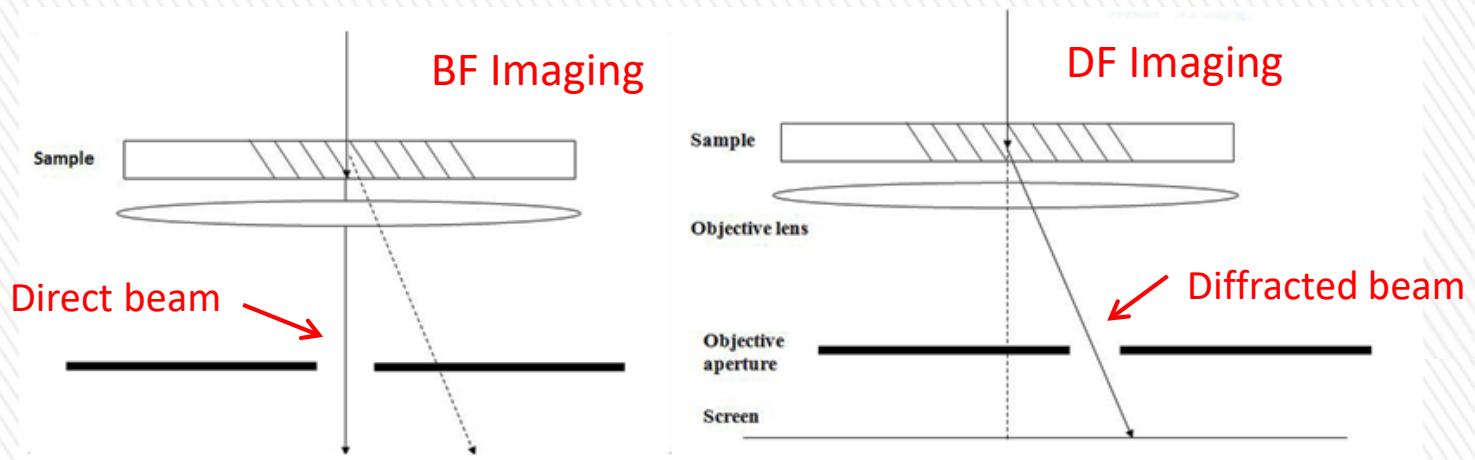
# Centered Dark Field (CDF) Imaging

CDF imaging is almost always preferred because it has better resolution and contrast than simple dark filed imaging.

- Tilt sample to a zone axis or a two-beam condition.

- Click "Diffraction" on RHP and view a live diffraction pattern on screen.

- Move the central direct beam to screen center using "MF X/Y".

- Insert the "Beam Block" on the direct beam to indicate screen center.

- Click "Dark Field" on RHP. Go to "Camera" tab, check "X/Y" on "Dark Field" menu.

- Move a desired diffraction spot to screen center using "MF X/Y" (see next slide).

- Insert and center objective aperture around the diffraction spot (see next slide).

- Exit diffraction mode, adjust "Intensity", remove "Beam Block" and SAD aperture (if inserted), and acquire a CDF image.

- After finishing DF imaging, click "Reset 0.0". Push "Dark Field" to exit DF mode.
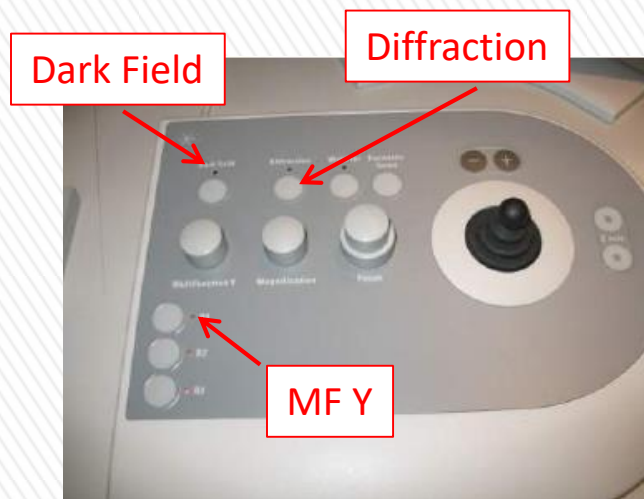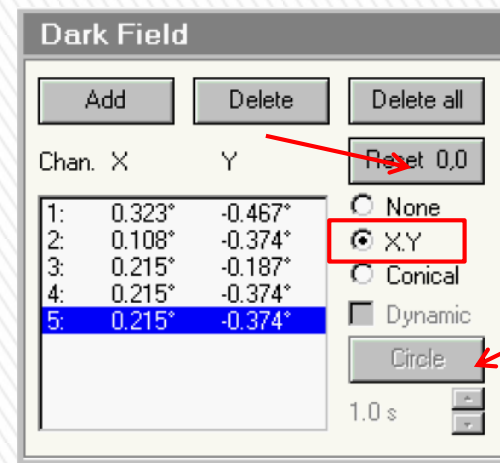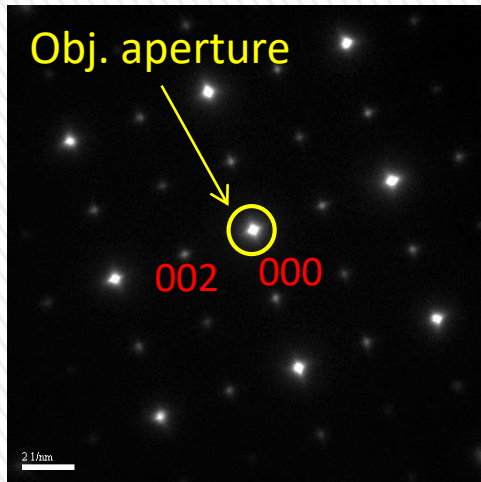
Obj. aperture around direct beam to form BF image
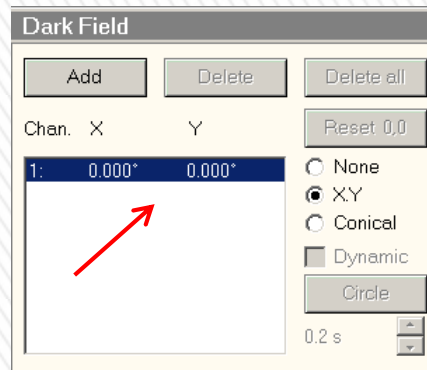
No DF tilt. The direct beam is at screen center
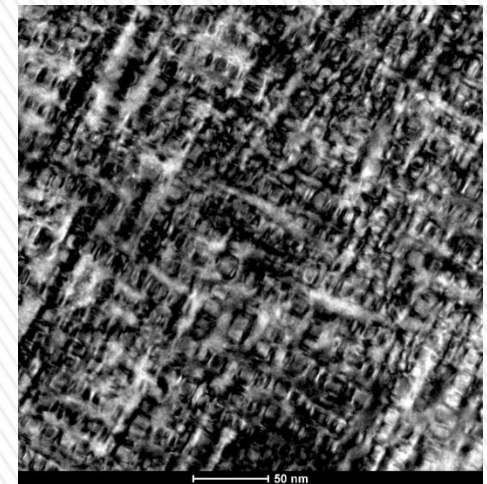
Bright field image
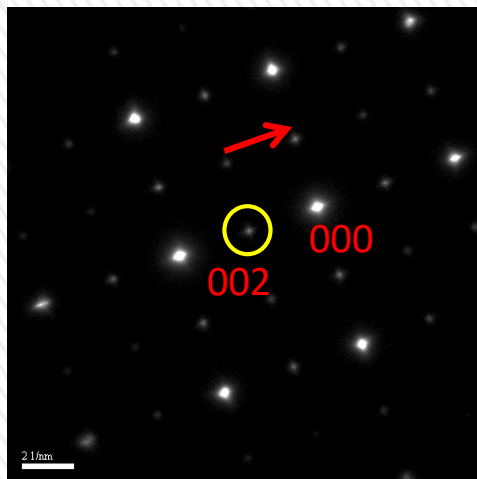
Obj. aperture is around (002) spot to form a CDF image. Diffraction pattern was shifted as indicated by the red arrow.
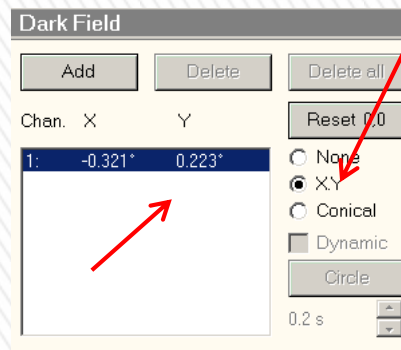
(002) spot is tilted to screen center
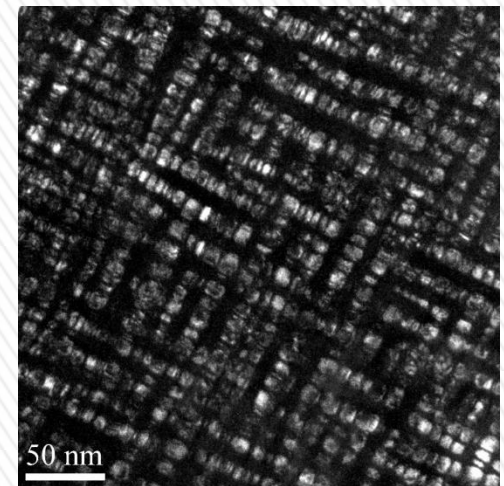
**Example of CDF**

Centered dark field image of the same area

# STEM Alignment 1 – Initial Focus and Stigmation

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

A spot size of 6 is recommended for analytical work.

- Remove Objective aperture and SAD aperture if they are in.

- Locate area of interest in TEM mode. Bring sample to eucentric height with Z control.

- Recall a STEM alignment in "Alignment" flap out. Load the corresponding STEM Feg register. Default mode in STEM is "Diffraction".

- Insert DF4 or BF or both detectors. Set camera length to 30 mm. *Camera length affects contrast of BF/DF images. A small camera length like 30mm is good for Z contrast.*

- Start STEM live viewing by pressing "Search". Lift up the projection screen to see an image. Click "Auto C/B" if the STEM image is too bright or dark.

- Press "Eucentric Focus" on RHP. Bring sample to focus using Z again. (There is a few µm difference in focus between TEM and STEM modes)

- Adjust Focus knob and Condenser Stigmator to make the image sharp. Click "Focus" to bring a reduced window for fine adjustment.
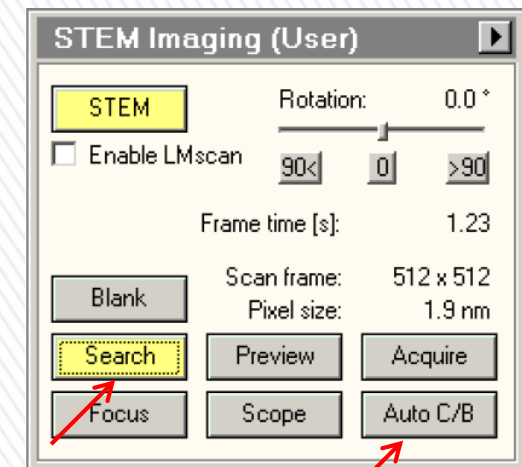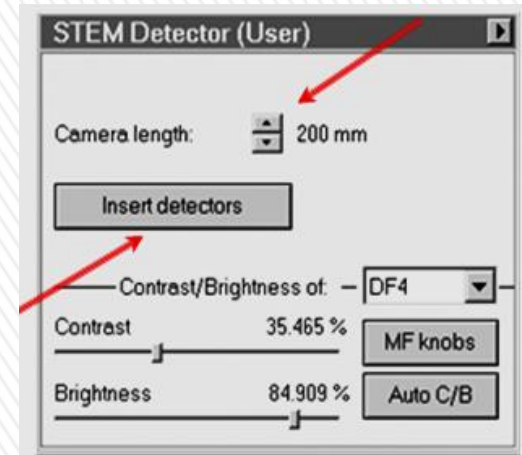
Click "Auto C/B" to correct bad contrast

# STEM Alignment 2 – Probe Alignment

1. Move sample to a vacuum area. Put a Beam Position Marker close to screen center. Stop "Search" or "Preview".

2. Push the "Diffraction" button on RHP to change STEM from diffraction mode to Imaging mode.

3. Set magnification to maximum SA mag (≈ 400K).

4. Move beam to screen center using "Beam Shift" in Direct Alignments. If beam is not visible, reduce magnification, find the beam and move it to screen center. Then change the mag back to the highest value in SA mode.

5. Use "Focus" knob on RHP to condense the beam to a spot. Bring it to screen center with "Beam Shift" in DL.

6. Make beam circular with a three fold caustic using **Objective Stigmator**.

7. Spread the beam with "Focus" knob. If beam moves away, adjust C2 aperture to bring the beam back to screen center. *This is similar to centering C2 aperture in TEM mode.*

8. Condense beam to a spot using "Focus" . Select "Beam tilt ppX" in DL. Use "MF X/Y" to make two spots merge to one. Repeat on "Beam tilt pp Y".



Direct Alignments

Gun Tilt
Gun Shift
Beam tilt pp X
Beam tilt pp Y
Rotation Center (Objective)
Beam shift

Done
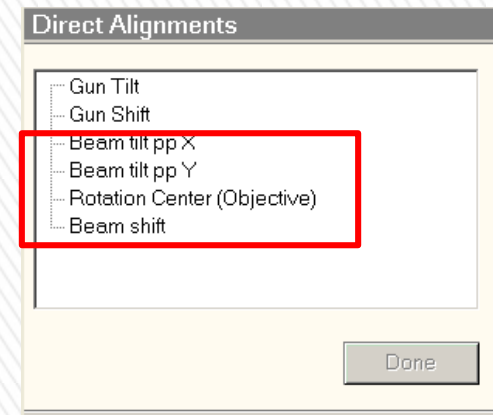


Three-fold caustic
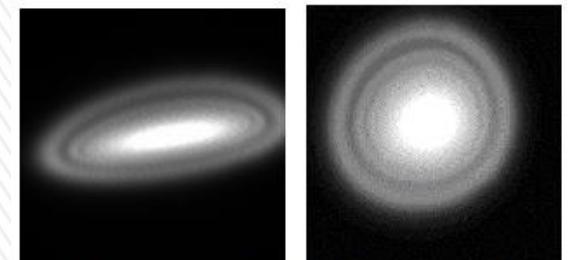


Misaligned (left) and optimised condenser stigmator

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1. Step 6 – correct objective astigmatism; Step 7 – Center C2 aperture; Step 8 – tilt purity alignment;
2. Daily users shall not adjust Gun tilt and shift alignments because of the possibility of messing up the probe. It is for the operator to set up correct gun alignment in the Procedure Alignment .

# STEM Alignment 2 – Probe Alignment (Cont'd)

9. Select "Rotation Center" in DL, Use "MF X/Y" to minimize overall movement of the beam. *The magnitude of the rotation center wobbling can be changed by turning "Focus Step" knob. Note that X and Y directions are reversed compared to TEM mode.*

10. Select "Beam Shift" and move beam to screen center again.

11. Repeat the above steps once to improve alignment.

12. Finally focus probe to a smallest observable spot using "Focus"

13. Push "Diffraction" on RHP, back to STEM imaging.
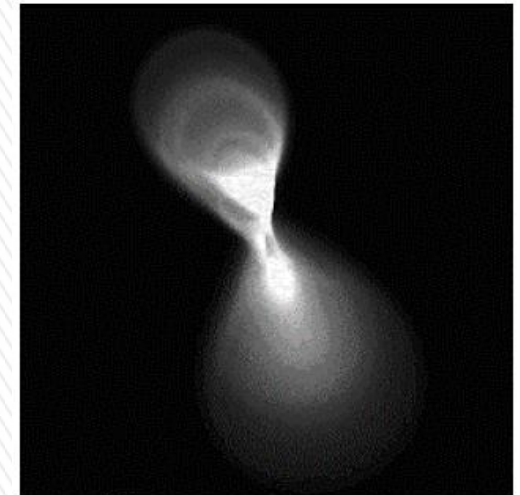


Misaligned rotation center
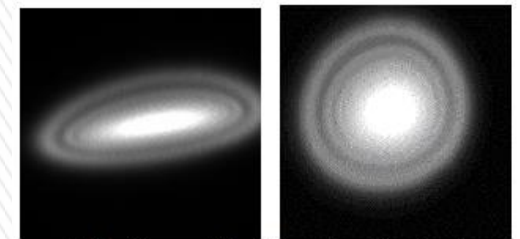


Misaligned (left) and optimised condenser stigmator

# STEM Alignment 3 – Alignment with Ronchigram

- Center an amorphous area. Stop "Search". Put a **Beam Position Marker** on the amorphous area and close to screen center.

- Increase camera length to 100 or 150mm (and a larger condenser aperture if necessary). Lift up the focusing screen. Watch Ronchigram with binoculars. *These steps are to enlarge the view of the Ronchigram*.

- **Focus the Ronchigram** with "Focus" knob. The criterion is as follows - at over or under focus, you will see a shadow image of the sample; When approaching the focus, the image seems coming toward you, becomes larger and larger and eventually has an infinite magnification. When properly focused and stigmated, the central part of Ronchigram should be featureless and look like water wobbling. Please see next slide for examples of Ronchigram.

- Correct **condenser astigmatism** by adjusting condenser stigmator. To do this, focus Ronchigram, then go through focus and minimize any stretching inside the Ronchigram.

- Lower camera length to 30mm (*better Z contrast*). Center condenser aperture around the Ronchigram. Remove focusing screen.

- On the phosphorus screen, move **diffraction disk** to 7 O'clock position using MF X/Y (next slide). *The 7 O'clock position is where the STEM detector is located on this scope*. Lift up phosphorus screen.

==See next slide for examples of Ronchigram==

1. STEM alignment includes more steps than TEM alignment. So it is necessary to store the alignments via alignment file and feg register so that users can quickly switch between TEM and STEM.
2. As of 09/2012, the 7 o'clock position gives best contrast in DF4 at 30 mm camera length. The operator may need to adjust the position of the diffraction disk for other camera lengths or with time.

Note: Ronchigram can only be clearly viewed from amorphous areas.



**The circle is condenser aperture**

10.0 1/nm    (a)    10.0 1/nm    (b)    10.0 1/nm    (c)
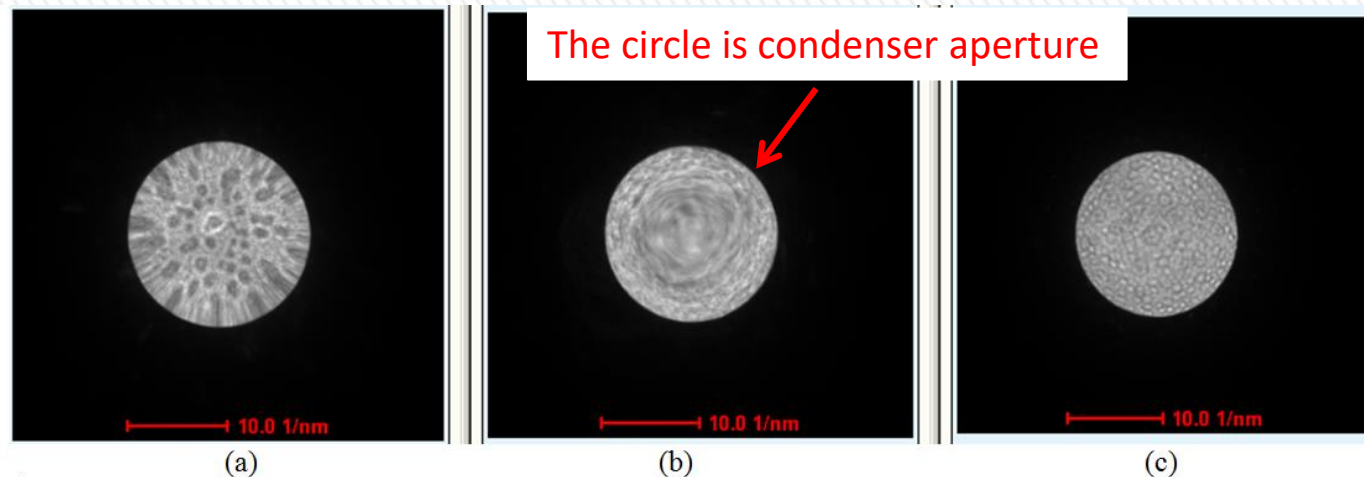
**Figure**: examples of Ronchigram (Au nanoparticles on carbon film). (a) overfocus; (b) in focus; (c) under-focus. (a) and (c) show shadow image s of the illuminated region on the specimen. (b) Focus is achieved when no feature can be seen from inside the Ronchigram.
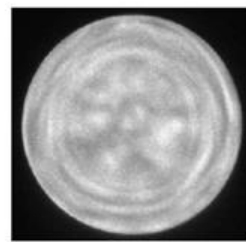


Ronchigram – Stigmatism

Phosphorus screen

inner circle

diffraction disk at 7 o'clock
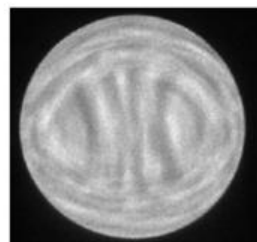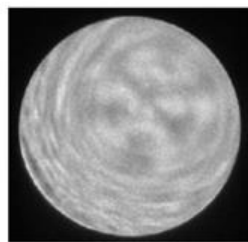
Astigmatic        C2-Aperture misaligned        Well aligned and stigmated
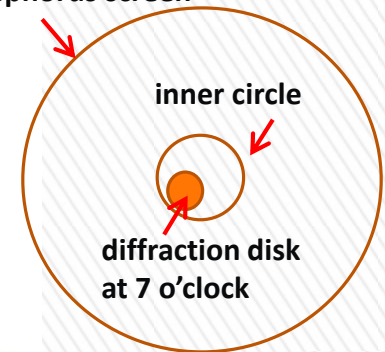
**Figure:** from *left to right* – Ronchigram with Condenser Astigmatism; Ronchigram without Condenser Astigmatism but C2 Aperture is not centered; Well-centered C2 aperture and no Condenser Astigmatism; schematic diagram for correct position of STEM diffraction disk at 30mm camera length.

# STEM Alignment 4 – Final Tuning

14. Start STEM live viewing. Center an amorphous area.

15. Increase "Camera Length" to 100 or 150 mm. Watch Ronchigram.

16. Focus Ronchigram using Z control. Carefully correct condenser astigmatism using condenser stigmator.

17. (Optional) Insert small C2 aperture (position 2 or 1) and center it with Ronchigram on small phosphor screen

18. Change camera length to 30mm. Move diffraction disk to 7 o'clock position if it is not there.

19. Move to area of interest. Focus feature with "Focus" knob. You are now ready to take STEM images.

20. Adjust contrast and brightness of the images by activating "Auto C/B" on "STEM DETECTOR" tab or manually tune contrast and brightness values.

Step 9 – align rotation center; Step 14-15 – precise correction of condenser astigmatism using Ronchigram, which is more accurate than by looking at the image.

# EDX – General

EDX can be performed in either TEM or STEM mode. STEM mode is always preferred because of its accurate positioning and much less spurious X-rays.

- Mount sample to the low-background double-tilt holder. Find a thin specimen area.

- The Objective Aperture **must** be removed if in (set to 6 or 7).

- Go to "INCA", select "Option" and open "Detector Control" (if not open already).  Open "Shutter".

- Click "View" to observe a spectrum in TIA software. Click "Acquire' to collect a spectrum.

- Check the "Dead Time".  If it is too high (from green to red), try to reduce it by using a smaller spot size or condenser aperture, or move sample to a thinner region.

- For automatic peak identification, click 🔬 .

- For manual peak identification, go to analysis mode ▭ .

- Use the marker tool ⚖ to select a peak in the EDX spectrum. Select Object Properties in the Menu View to get a list of all chemical elements matching that peak. Manually label the peaks in the spectrum by selecting/unselecting the element in the marker tab 🔹 Markers  of the periodic table ▦ .
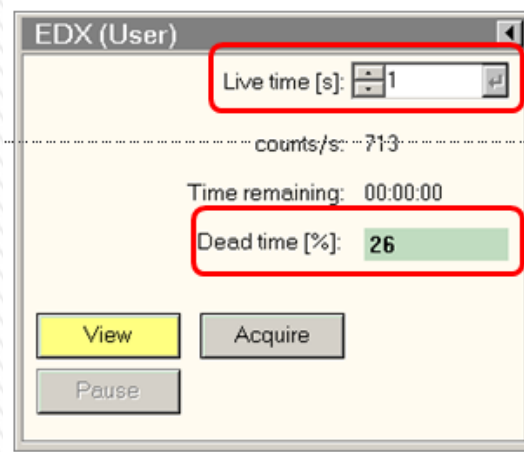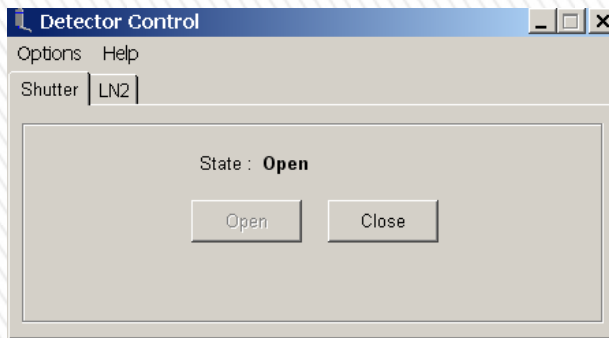
Switch to analysis mode
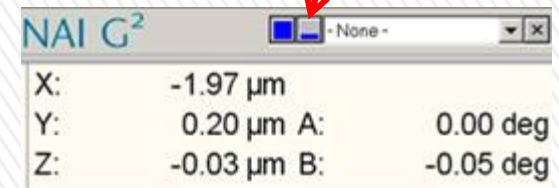
# EDS in STEM Mode – Single EDS Spectrum

- Switch to STEM mode. Conduct proper STEM alignment.

- Acquire a STEM image of the area of interest.

- Open EDS Shutter.

- Use the position ⊕ , line ╱ , or rectangular ☐ marker tool in TIA to select a spot, line, or area of interest for EDX analysis.

- Set EDX acquisition time (typical 1 sec for view and 15-100 sec for acquisition) in the EDX Control Panel.

- Select the position marker and view the EDX spectrum.

- If the dead time appears red (above 40-50%), try reducing beam intensity (probe size, condenser aperture).

- Acquire a spectrum. Automatically or manually identify the spectrum.

- *Quantification* – see next slide for illustration
  - Go to analysis mode ▭ .
  - In "View" menu, open "Periodic Table" ▦ .
  - Go to "ROI" in "Periodic table". Select elements for quantification. Right click an element to choose which peak is used for analysis (*i.e.* K, L or M)
  - Click the spectrum for analysis.
  - In "EDX Quant" side bar menu, click "Quantify".
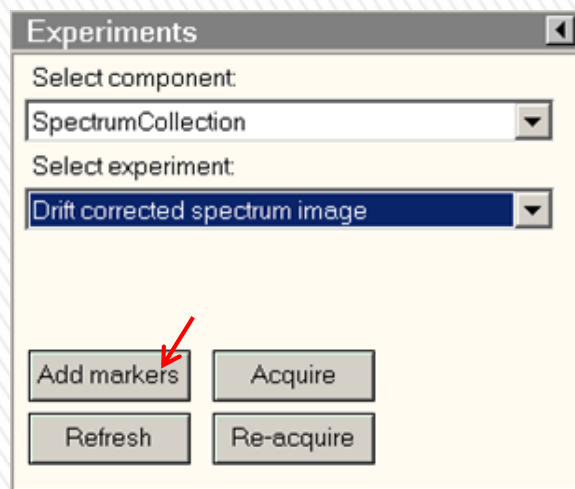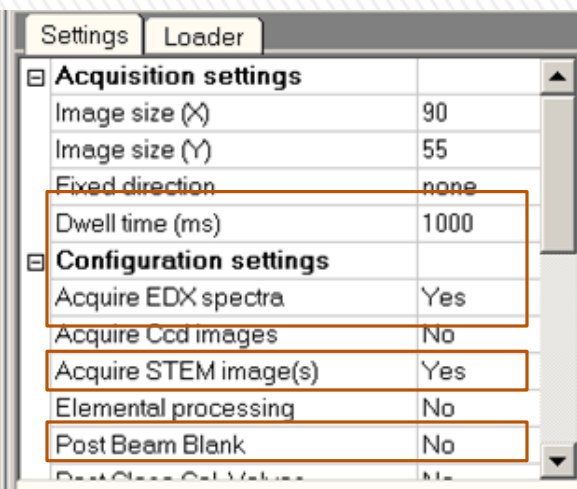  - The quantification result will appear in the message window. These results can be exported as text.
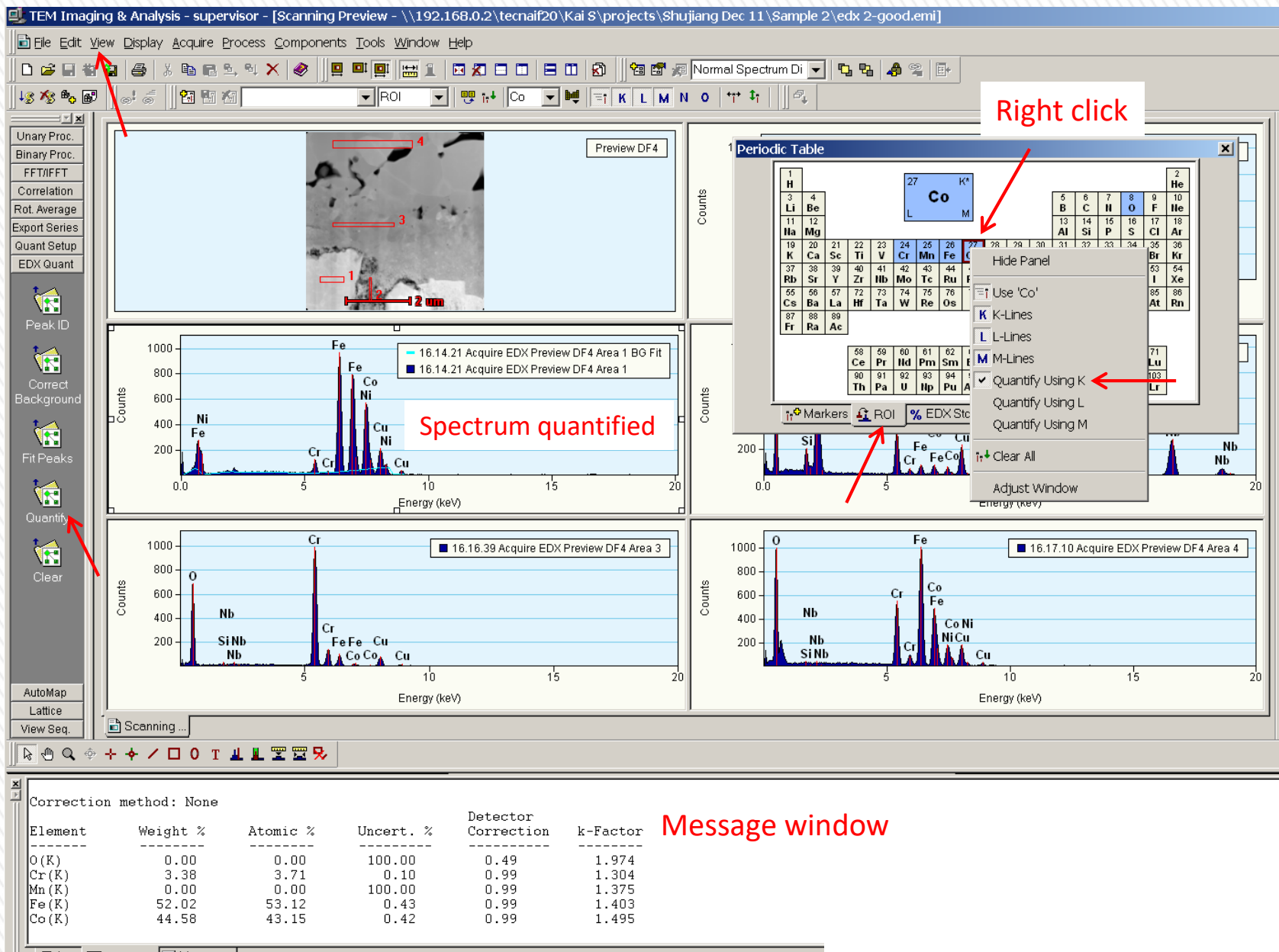
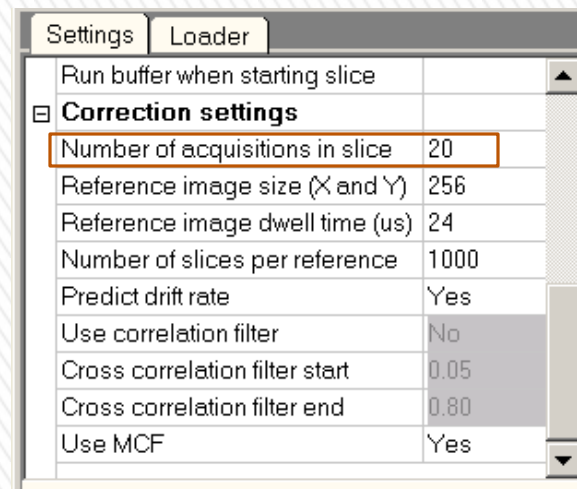**TIA Analysis Window**

# EDS in STEM Mode – Map and Profile

- Preview or acquire a STEM image as described in previous slides. See next slide for an example.

- Go to "EDX" tab, at "Experiment", select "Spectrum Collection" from "Select component" menu.

- Select "Drift corrected spectrum image" for mapping or "Drift corrected spectrum profile" for line scan.

- Click "Add markers" to add the spectrum position marker (orange) and drift correction marker (yellow).

- Open the flap-out of "Experiment".

- Set desired image size or profile size. Set "Dwell time". Usually 1000 for mapping, and 5000 for line scan.

- Check the following options - "Acquire EDX spectra" = "Yes"; "Acquire STEM images" = "Yes"; "Post Beam Blank" = "Yes". The last item means that the beam will be automatically blanked once the map or line profile is completed.

- Scroll down the flap-out menu and choose the number of acquisition in slice for drift correction. Example – 20 for mapping and 5 for line scan. Press "Acquire" to start.

**Experiments OCX**

**Configuration settings**

**Drift correction settings**

The "number of acquisitions in slice" means drift correction to be performed for how many spectrum acquisitions. For example, "20" means that after collection of 20 spectrum a drift correction will be performed. If drift is too great, the acquisition will be stopped early.

**Setting up a drift-corrected spectrum image**

# Finishing Procedure



- Switch back to TEM-Imaging mode (if you are using STEM or Diffraction).

- Click "Col. Valves Closed" button (color changes to yellow) to close column valves. Always close the column valve before inserting/removing the specimen holder!!

- Remove objective and diffraction apertures if inserted .

- Go to "Stage", click "Holder" button to reset the holder's xyz and tilt angles to zero. (This is an important step. Failure to do so could damage the sample holder during removal).

- Take out the specimen holder (see "Extract Holder" slide). Unplug the cable first if using the double-tilt holder.

- Remove the specimen from the holder.

- Open main valve of gas cylinder.  Switch on plasma cleaner. Wait until the cleaner reaches atmosphere on front panel (~ 5 minutes). Insert the holder back into the cleaner. Pump the system back to high vacuum (~ 3 minutes).  Close the valves and switch off the plasma cleaner.

- Fill in the log sheet (time, the vacuum values and the emission value etc.).
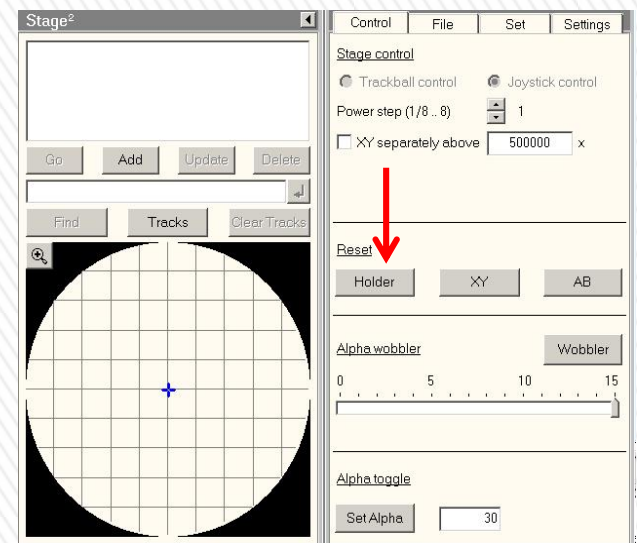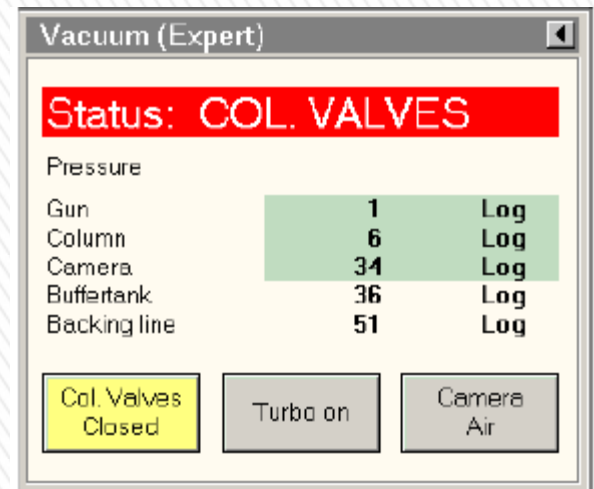
# Last User of the Day

> **If you are the last user of the day, please don't forgot to do the Cryo Cycle.**



- Finish the procedure in the previous slide.
- Remove the liquid nitrogen dewar. Place the towel back on the drip tray.
- Open the "flapout" of the "Vacuum" tab. Select "Cyro".
- Set "Start After" time to be 3 min. Then click "Cryo Cycle".
- Notice the scope status becomes "Cryo Cycle" from "Col Valves". The column vacuum will become disabled (gray).
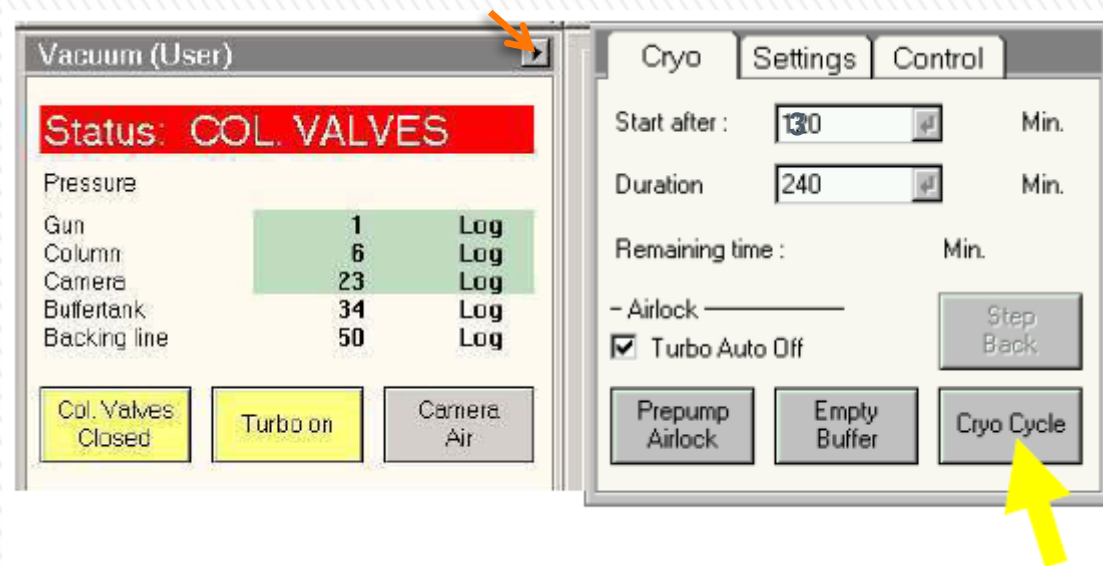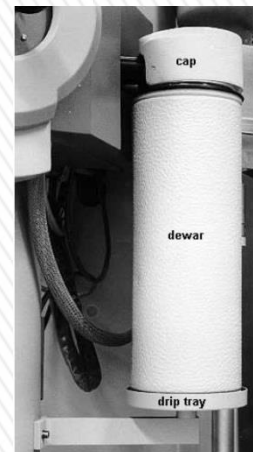
Note: please make sure you are the last user! The complete cryo cycle takes 4 hours and should not be stopped after the initial 3 min delay!