Quick Guide to Operating FEI Titan Themis G2 200 (S)TEM: TEM mode

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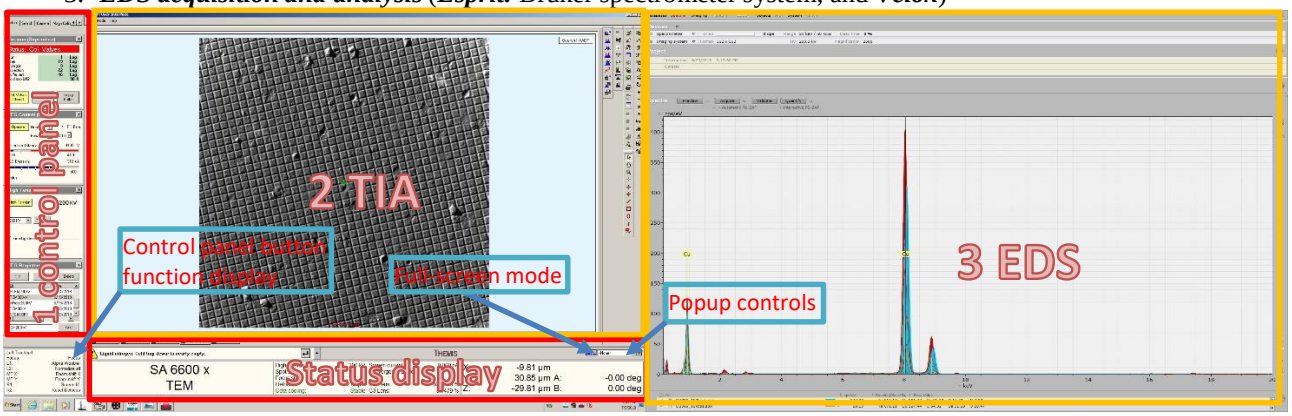
I. Before starting a session:

- Log in to your instrument session at https://fom.engr.pitt.edu

II. Microscope User Interface

The microscope operation interface consists of three programs:

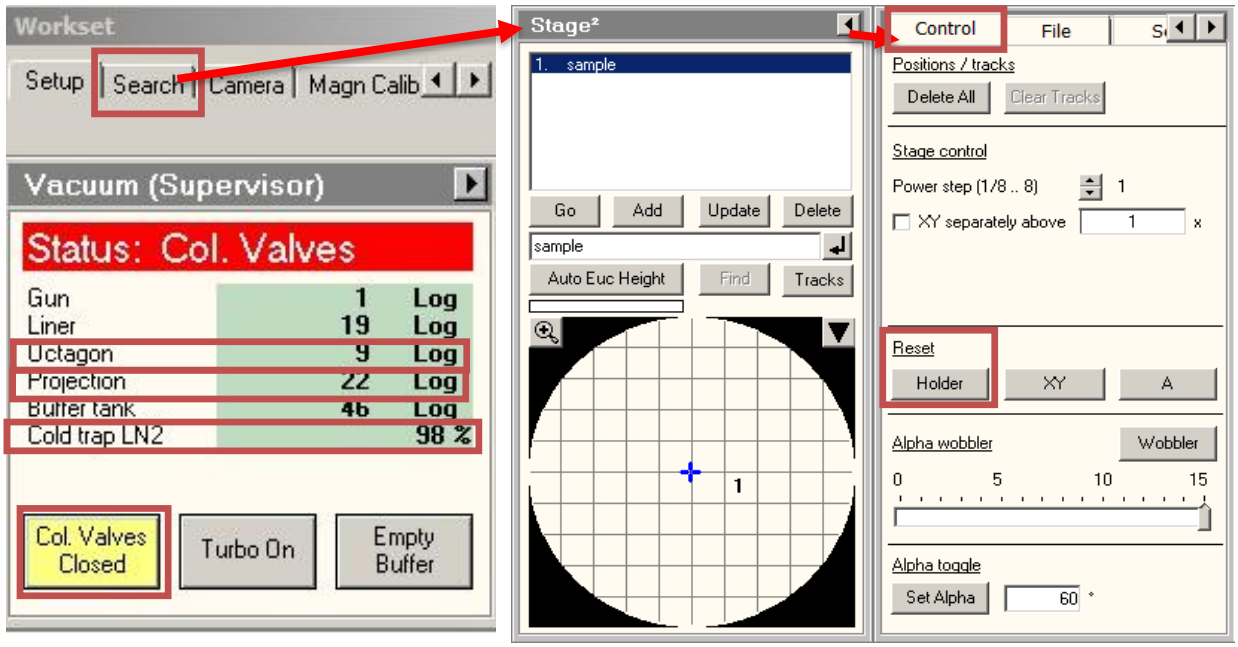
1. ***TEM User Interface***: general microscope operation and the display of live TEM image
2. ***TEM Imaging & Analysis (TIA) & Velox:*** image acquisition and analysis
3. ***EDS acquisition and analysis*** (***Esprit:*** Bruker spectrometer system, and ***Velox***)



Overview of the microscope user interface, TEM User Interface and TIA on the left and EDS on the right

III. Check and confirm the following conditions:

a. Good **Octagon** (column) vacuum (< 20 log, $<10^{-7}$ Torr), Projection (camera chamber, < 30 Log)
b. Good **liquid nitrogen** level (> 10%)
c. Close the **column valve** before sample holder insertion/removal
d. Reset the **stage** (***holder***) before sample holder insertion/removal

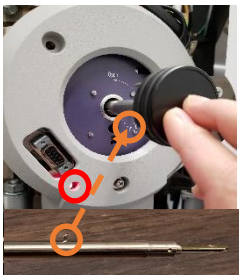


IV. Choose Sample Holder

Wear gloves when handling a sample holder to prevent contamination. Never touch the brass part of the holder.

There are three types of sample holder available for different application:

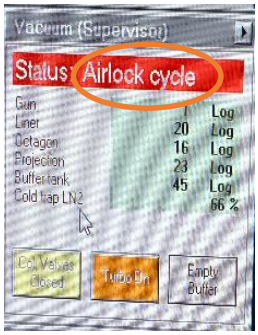
1. ***Single-tilt***: morphology observation, EDS acquisition (-30° ~ + 30°)
2. ***Double-tilt (low background)***: morphology, detailed crystalline material study, EDS acquisition (-30° ~ + 30°)
3. ***Tomography (low background)***: similar to single-tilt holder but with lower holder profile allowing high alpha tilt angle (-70° ~ +70°, depends on specimen Eucentric Z setting)

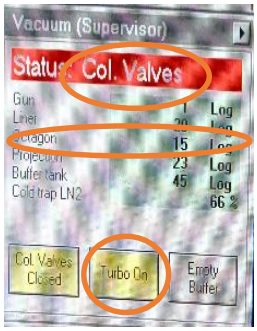


V. Holder Insertion/Removal

When Inserting a Holder

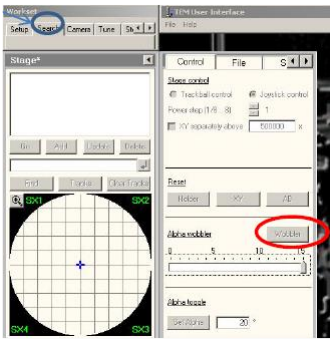
1. Align the holder guide pin with the **Close** line mark on the goniometer cover and insert the holder straight in to the stop of the stage load lock area (LED will light up in Red)
2. Keep finger pressure on the holder (***no rotating***!!) to activate pumping process
2. Wait for the load lock to pump down (until **Airlock cycle** times out, ~3 minutes)
3. Slowly and smoothly rotate the holder *Counterclockwise* to its stop position and let it gently into the microscope column area (***Octagon: < 20 Log***)
4. Wait for 1 minute, and then turn off the Turbo pump by clicking **Turbo On** button (color changes from yellow to gray)





When Removing a Holder:

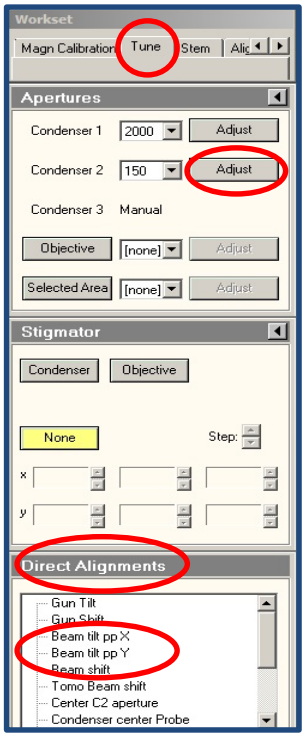
1. Close column valves by clicking **Col. Valves Closed** button (color changes from gray to yellow!)
2. While pressing the purple stage cover down, ***slowly*** pull the holder straight out till its stop
3. Rotate the holder ***clockwise*** till its stop
4. Wait for ***30 seconds***
5. Slowly pull the holder straight out while you are still pushing the purple stage cover down



VI. TEM Operation

1. (***Optional***) Load the most recent alignment from →**Workset→Alignment →** **File**, e.g. ***Routine 200kV***
2. Set the ***most recent*** FEG Registry, e.g. ***RoutineTEM 200kV,*** from **FEG Registers**
3. **Set Specimen Eucentric Height (Z)**
    1) Open column valves by clicking **Col. Valves Closed** button (color changes from **yellow** to **grey**!)
    2) Press the "**Eucentric focus**" button and ***normalize all*** **(L2)** button
    3) Set at a lower magnification (~2,000x) and find a reference object
    4) Activate the stage "**Wobbler**" (**L1**) and observe object movement
    5) Reduce the movement by moving the Z up or down
    6) Repeat steps 3 – 5 at higher magnification (~50,000x) to fine tune the z position

    Note: *if your sample is crystalline, then converge the beam, move Z up or down until diffraction pattern converge to the center spot*



4. C2 aperture alignment check: condense the beam to a spot using **Intensity** knob, and use **Beam Shift** to move the beam spot to the center of screen; spread the beam and check if the beam expands concentrically.

    If not, align C2 aperture: from **Workset →Tune**, activate ***C2 aperture*** **Adjust** and adjusting **MF X/Y**. Condense the beam using **Intensity** knob, and use **Beam Shift** to center beam spot, expand beam on both sides of beam intensity and adjust C2 aperture to make sure beam expands concentrically using MF X/Y. Deactivate C2 aperture ***Adjust*** button after adjustment.

5. Carry out **Direct Alignments**
    1) **Gun Tilt (*Optional*)**
        a. Press **L3** (left panel) or **R3** (right panel) to select illumination Spot Size 3
        b. If beam is not at the center, go to ***step 2)*** **Gun Shift** and shift beam to center using MF-X and MF-Y knobs, and then return to **Gun Tilt**

alignment

c. Adjust **MF-X** and **MF-Y** to maximize **beam Current**

2) **Gun Shift**
   a. Press **L3** (left panel) or **R3** (right panel) to select the illumination **Spot Size 9**
   b. From **Direct Alignment** tab, select **Beam Shift** and use **MF-X** and **MF-Y** to shift the beam to the center
   c. Switch to **Spot Size 3**, select **Gun Shift** and use **MF-X** and **MF-Y** to shift the beam to the center
   d. Repeat *steps a – c* till both spot sizes beams are centered
   e. Center the other ***spot sizes*** in between ***(4 – 8)*** using **Gun Shift**

3) **Beam tilt pp X and Y**
   - Set the microscope at a high magnification (e.g. 500,000x) and locate an empty specimen area
   - Converge the beam using **Intensity** knob and use **MF-X** and **MF-Y** knobs to make the beams overlap

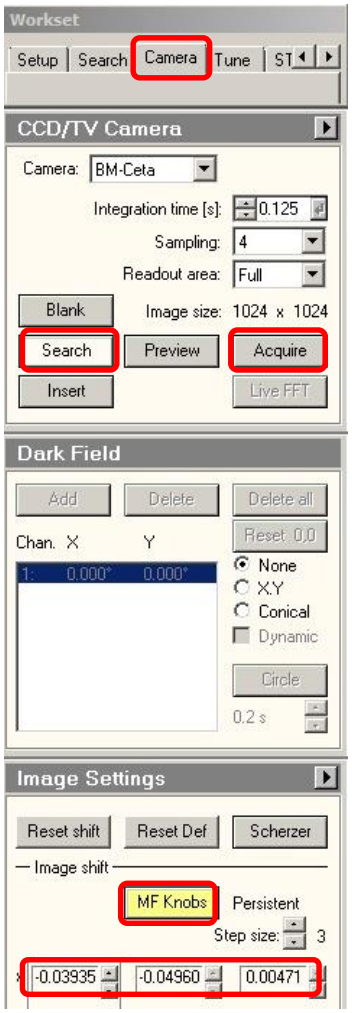
4) **Beam Shift**
   - Use **MF-X** and **MF-Y** to shift the beam to the fluorescent screen center

5) **Rotation Center**
   - Set the microscope at a high magnification (*e.g.* 500,000x) and locate a reference object
   - Expand the beam to illuminate the entire screen area
   - Minimize the image movement by turning MF-X and MF -Y knobs

6) **Coma-free Pivot Point X and Y**
   - Set the microscope at a high magnification (e.g. 500,000x) and locate a reference object
   - Converge the beam and use **MF-X** and **MF-Y** knobs to make the beams overlap

7) **Coma-free Alignment X and Y**
   - Raise the fluorescent screen and start camera view on an amorphous specimen area at *e.g.* 500,000x
   - Turn on ***live FFT*** and defocus the microscope until ring patterns are visible
   - Adjust **MF-X** and **MF-Y** knobs so that the ring patterns do not change in size and shape

6. Search samples and take images using ***TIA*** (fine tune *Obj Stig* and *Focus* as needed)
   1) Navigate stage and find your area of interest
   2) Optimize image quality using **Focus** knob (right panel) and **Obj. Stigmator** (left panel)
   3) Switch to **Camera** page through the TEM User Interface:
      - **Inset** camera, and raise the fluorescent screen by pressing the **Screen Lift** button (**R1** on right panel)
      - **Search**: carry out fine focus and objective stigmation adjustment to optimize image again
      - **(optional)** ***MF Knobs*** can be switched to ***Image Shift*** in **Image Settings**
      - **Acquire** image
   4) Data Saving
      - Save the data on the **support PC** (network attached ***Z drive***)
      - It is recommended to save the images in FEI's raw format (**.emi**) as the format contains information about the experimental condition (magnification, camera length, voltage, etc.)



VII. Selective Area Diffraction

1. Obtain a TEM Bright Field image in the SA magnification range.
2. Select required field of view.
3. Insert a Selected-Area aperture of appropriate diameter.
4. Remove the objective aperture.
5. Press the **Diffraction** button (LED on).
6. Select required camera length (Magnification).
7. Focus the diffraction pattern.
8. Adjust **Intensity** of illumination to a suitable level by turning ***clockwise*** to reduce the intensity.
9. Refocus the diffraction pattern if necessary.
10. Insert the beam stop to block the central diffraction spot to avoid Damage to the camera.
11. Record the diffraction pattern with manual exposure-time

VIII. Dark-field Imaging

A. Axial dark-field imaging

1) Before activating dark field, execute the following procedure:
   - Center the beam.
   - Press **Dark-field** while in TEM mode in the SA magnification range.
   - Set the dark-field tilts to 0.00 by pressing **Reset**.
2) Obtaining a dark field image: In TEM BF mode, select required field of view in the SA magnification range.
   - Obtain a diffraction pattern of the chosen area.
   - Center the diffraction pattern on the screen with the diffraction shift.
   - Decide from which Bragg reflection (or section of a polycrystalline ring) a dark field image is to be obtained.
   - Press the **Dark-field** button (LED on).
   - Use **Multifunction X and Y** knobs to bring the Bragg reflection opposite to the one selected to the point where the central beam was originally positioned (this should be the center of the screen).
   - Press **Dark-field** to return to Bright Field Diffraction mode.
   - Introduce an objective aperture and center it accurately around the central spot.
   - Press **Dark-field** to return to Dark Field Diffraction mode.
   - Center the chosen diffraction spot accurately in the objective aperture using the Multifunction X and Y knobs. The objective aperture must be small enough to isolate the chosen spot from neighboring diffracted beams.
   - Press **Dark-field** button (LED off) and a dark field image of those crystal planes causing the selected diffraction spot will now appear.
   - Carry out Magnification, Intensity, beam shift and Focus adjustments as for a Bright Field image.

Note: Switching between Dark and Bright Field images may be achieved simply by successively pressing the Dark-field button.

B. Off-axis imaging procedure

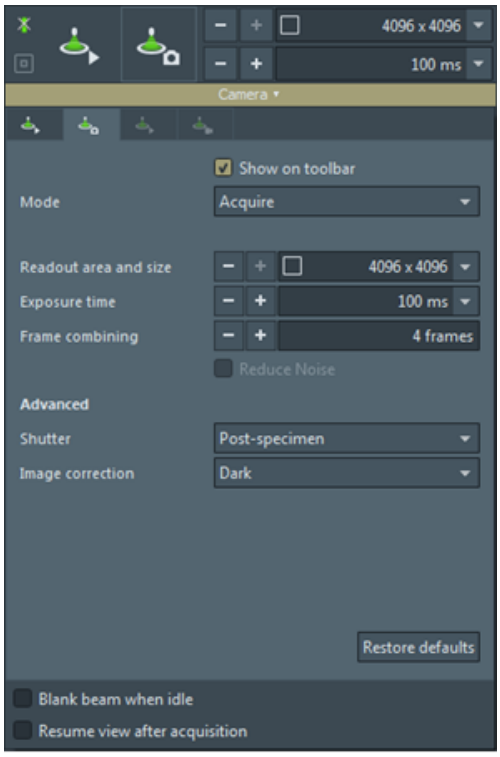
1. Switch to diffraction.
2. Center the objective aperture around the diffracted beam required for dark-field imaging.
3. Switch back to imaging.

IX. Finish your microscope session

- Stop Camera View (search) and Retract CCD (BM-ceta) camera
- Click **Col Valves Closed** button in **Setup**>**Vacuum** to close the column valves
- Lower the fluorescence screen (**R1** button). Put the rubber cover on.
- From **Reset** panel of **Search→ Stage → Control → Reset** page, click **Holder** to reset stage to center
- While pressing the purple stage cover down, ***slowly*** pull the holder straight out till its stop
- Rotate the holder clockwise till its stop
- Wait for ***30 seconds***
- Pull the holder straight out while you are still pressing the purple stage cover down
- Remove and save your specimen
- Insert the empty *single tilt holder* in to goniometer
- Transfer your data to your shared drive folder
- Log off your microscope session via FOM

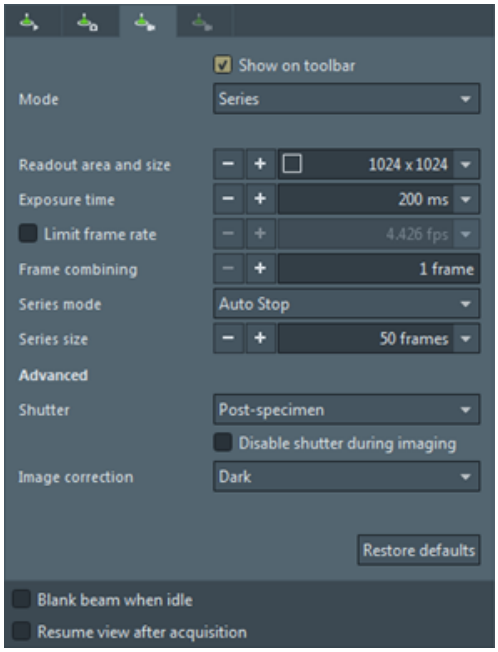
SUPPLEMENT I. Acquire TEM Images using Velox

1. Specify the Camera Preset Parameters for Ceta
   - Select the **Camera** section title to expand the Presets section and specify the parameters for the selected Preset.
     - **View**: navigating to the ROI.
     - **Acquire**: acquires a single image.
     - **Series**: acquires a series of frames.
     - **High Speed Series**: acquires a series of an indefinite number of high -resolution, high-speed MRC images, limited by the amount of free disk space on the Storage Server. The High Speed Series mode is only available for Ceta cameras with Speed Upgrade.



2. Specify the generic parameters for all Camera Presets.
   - Tick **Blank beam when idle** to minimize specimen damage due to exposure to the electron beam while no viewing or acquisition is taking place.
   - Tick or clear **Resume view after acquisition**.
     If Resume view after acquisition is Ticked, Velox will automatically return to view mode as soon as the acquisition is completed.

3. Series Acquisition with a Ceta Camera
   - **Limit frame rate:** The maximum frame rate depends on the combined values of various acquisition parameters.
     - When *Limit frame rate* is disabled, the camera will acquire frames back -to-back, at the maximum rate.
     - When *Limit frame rate* is enabled, the camera will not acquire frames back -to-back. Instead, it will skip an integer number of intervals in between acquisitions.



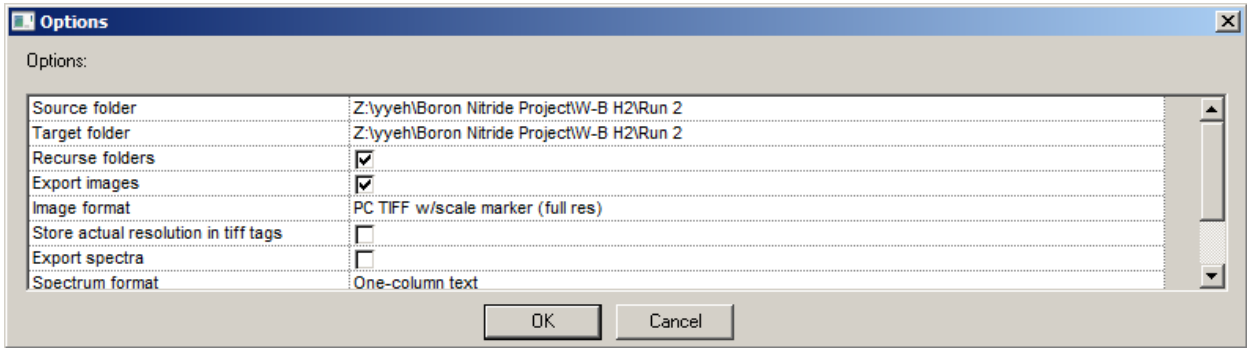
   - **Series mode:**
     - **Auto Stop**: immediately starts recording a series of frames into the Velox Experiment file, until the specified number of frames is acquired.
     - **Circular**: starts recording frames into a cyclic buffer of the specified number of frames. When the series acquisition is stopped, the contents of the of the cyclic buffer is stored in the Velox Experiment file.
     - **Continuous**: immediately starts recording a series frames into the Velox Experiment file, until the acquisition is stopped by the user.
   - **Series size:** The number of frames that is recorded into the series. When Series mode is Continuous, the Series size parameter is not available.

4. From the toolbar, select **View**, **Acquire**, or **Acquire Series**  to start viewing or acquiring image.

Note 1: Use TIA ***Folder Export*** function to batch convert the files to .jpg, .tif, etc.

This can only be done from the support PC – an offline TIA is available on the support PC

- From menu **Components → Folder Export → Settings** to set up the export parameters (Source and Target folder path, image type, scale bar, etc)



- Click **Components → Folder Export → "Export"** to execute batch file conversion

Note 2: Titan Themis STEM SPECIFICATIONS (Highlights)

- Accelerating voltages: 80 and 200 kV
- Ultra stable, high brightness X-FEG (brightness: 1.8 x 10^9 A/cm^2/sr)
- X-FEG probe current: 0.9 nA in 0.2 nm spot and 14 nA in 1 nm spot
- Cs DCOR probe corrector for sub- Å resolution in STEM mode
- TEM point resolution and information limit: < 0.11 nm
- STEM resolution: 70 pm (Cs corrected)
- EDS energy resolution (Windowless Super-X EDS detector system with output count rate up to 200 kcps):
  - ≤ 136 eV for Mn-Kα and 10 kcps (output)
  - ≤ 140 eV for Mn-Kα and 100 kcps (output)
- Detectors:
  - High angle angular dark field (HAADF) detector for STEM
  - 4 on-axis ADF/BF detectors including 4 Quadrant ADF for differential phase contrast (DPC) imaging
  - Ceta 16M CMOS camera (acquisition speeds: 4k x 4k – 1fps; 2k x 2k – 8fps; 1k x 1k – 18fps; 512 x 512 – 25 fps)
- Specimen Holders:
  - FEI CompuStage single tilt holder (+/- 30°)
  - FEI CompuStage high-visibility, low-background double tilt holder (±35° tilt range for alpha and ±30° tilt range for beta)
  - Fischione tomography holder model 2021 (up to ±70°)

***Note**: the actual tilts depend on the Eucentric Z setting. It may not reach the highest tilt when the Eucentric Z is far off zero (0).*