TITAN S/TEM (FEI), 300kV
OPERATION MANUAL for Basic TEM

Overview.
TITAN is a 300kV high resolution Transmission Electron Microscope (TEM). The microscope’s interface contains three parts: the TEM Server, User Interface (UI), and control-panels (left and right). The sophisticated software that controls the entire microscope is called the TEM Server. Because the functionality of the TEM Server is vital to the Titan, the TEM Server is not accessible to ordinary users. Users communicate with the TEM Server (and therefore with the microscope) via the User Interface (UI), which is basically a second-level program. The UI provides access to the necessary microscope functions. Knobs and buttons in the control panels imitate the usual TEM controls – but in reality they interact with the computer, which in turn controls every function of the microscope. The extent of control depends on the position of the user in FEI’s user-account hierarchy: factory ➔ service ➔ supervisor ➔ user. Users have enough control to operate the microscope in all possible modalities, but are limited in ability to corrupt vital Titan settings, adjustments, etc. Every user has his or her own standard Windows account protected by password. Every new account inherits the supervisor settings at the time it is created. Later the settings can be altered and saved by the user under his/her account. Also, users may load other users’ settings at any time, including the supervisor’s – the advantage of digital interface.

The control panels contain a standard set of TEM controls: intensity (brightness), magnification, focus, focus-step, dark field, diffraction, wobbler, stage X-Y joystick, user beam shift trackball, Z-height and multifunction knobs X and Y (MF). The MF knobs’ default function is “user beam shift X & Y”. However, they can also be “bind” to other functions such as beam tilt, astigmatism correction, etc. The current function of the MF knobs is shown in the UI “Binding” window. Sensitivity of some knobs may be adjusted using the – and + buttons located just above. There is also a set of user-defined buttons (programmable buttons) that function as switches. The user may assign (bind) these buttons to frequently used functions such as screen lift, spotsizetc. MF knobs and programmable buttons may be bound to many microscope functions and therefore provide flexibility and convenience in microscope operation.
The **User Interface (UI)** links the microscope Control panels to the TEM server and therefore functions as the microscope interface:

The UI contains a number of panels and windows. They are arranged in a L-shape around a central empty space (black, above). The central space is reserved for additional programs like TIA, Digital Micrograph, etc. The horizontal, bottom part of the UI contains mostly information windows: **Message window**, **Status window** with current microscope parameters etc.

The vertical part of the UI contains numerous **Control panels** that control different microscope parameters. Every **Control panel** contains a set of controls related to a main microscope function such as **FEG** (Field Emission Gun) control, **Stage** control (CompuStage), **Camera** control, etc. All of these **Control panels** are organized in a **Workset** and may be called up by choosing the corresponding tab at the top. **Flap-outs** are used to extend the list of available options/choices on some Control panels. The **Workset** contains frequently used microscope controls and mainly functions as an organizer for the Control panels. The list of all accessible microscope controls is available through the **Popup panel** at the bottom-right corner of UI. The **Popup panel** may be used to open Control panels (in addition to the Workset) and to add/remove/rename items in the **Workset**.

The **Message window** shows messages regarding the current state of the microscope. The messages span a wide spectrum, from simple notifications to critical errors. Specific prompts are also sent to this window, in cases where additional information is necessary to continue microscope operation; for example, specifying the specimen-holder type when inserting a sample. Be sure to confirm your choice/action using the **enter** button at the right of the **Message window**.

**General note**: One can use the function key F1 to invoke the UI help pages from any Control panel. Position the cursor over a particular item and press F1. The relevant help pages for that item or Control panel will automatically pop up.
Overview of common Control panels in the UI.

Setup tab. The Control panels in this tab provide information on (and control over) physical conditions of the microscope: vacuum, high voltage (HT), FEG, aperture, etc. Individual Control panels may also be called from the Popup panel.

The Vacuum Control panel shows the vacuum level in the different parts of the microscope: gun, column (octagon) etc. Vacuum is very critical for this instrument, because of the FEG (Field Emission Gun). The Column Valves must be closed if microscope is not in use or if the vacuum reading in the Octagon is higher than 20 Log. Yellow color of the button indicates that the operation (Valves closed) is performing, gray – indicates that the function/device is not in operation. When the Col. Valves Closed button is yellow, the column valves are closed. Clicking this button makes it turn grey, indicating that the column valves are open.

Correct settings for the FEG are critical for optimal Titan performance. At 300kV, Extraction voltage = 4300 V, gun lens = 3. When the Operate button is yellow, it indicates that the FEG is Operating. Likewise, when the High Tension button is yellow, the high voltage is turned on.

The Titan may operate in a range of HT from 80 to 300 kV. Every voltage requires complete alignment of the microscope’s optical system. At EICN, the microscope is only aligned for 80 and 300 kV. Therefore, there are only two voltages currently available: 80 and 300 kV.

Apertures in Titan are motorized and controlled via this interface. Choose the size and mark the appropriate box to insert the aperture:

To center – check Center and use the MF knobs to center. Uncheck Center when done. The larger the C2 aperture, the brighter the beam. 150 μm is the largest Condenser 2 (C2) aperture. The most common settings are 50 μm for C1 and 100 μm for C2. For HRTEM, the objective aperture usually is not used.
Vacuum Overview panel.

This Control panel may be accessed only from the Popup panel (bottom-right corner of the UI). It shows the Titan’s vacuum diagram. Different colors represent different qualities of the vacuum: cyan represents good vacuum for the specific component highlighted with that color. For instance, in the case of the FEG, it corresponds to the ultra-high-vacuum essential for the proper operation of this component.

Abbreviations:
IGP – Ion-Getter Pump; TMP – Turbo-Molecular Pump;

![Valve in closed position.](image1)

![Valve in opened position.](image2)

**Note:** Certain events are not displayed in the current version of this diagram. In particular, the cartoons of the gun and projection chamber valves do not change according to the open/closed state of these components. The state of these valves is reported on the Vacuum Control panel (see above).
Stage tab.
On this picture, Stage$^2$ is shown in the **flap-out** configuration.

This panel controls the Titan’s CompuStage. On the left side is the representation of the specimen. Click on **Add** to save the current X-Y position of the stage. Click on **Go** to recall a previously saved position. On the right side - **Control:**

- **Stage control** ★ **Power step** (adjusts speed of stage movement);
- **Reset** zeroes the XYZ positions and AB tilt angles of the CompuStage.

It is a good idea to reset the holder (**Reset** ★ **Holder**) before starting work and when you have finished. Alternatively, it is possible to reset just XY or tilt (A/B). **Wobbler** is used for Z-height adjustment when a new sample is introduced.

**Note:** The button turns yellow when wobbler is on.
Alignment tab.
The Titan is a high-resolution electron microscope. Because of hysteresis of the magnetic lenses, the microscope needs to be aligned from time to time during its operation. Therefore, it is expected that users are capable of performing alignments appropriate for their tasks.

There are two different panels in the Alignment tab: Alignments (Procedure Alignment) and Direct Alignments. The **Procedure Alignments** are only performed when the microscope is seriously misaligned. The Direct Alignments are usually performed at the beginning of each microscope session.

**Alignments (Procedure Alignments).** Each folder contains several alignment procedures. Every **Procedure** contains a sequence of manipulations that must be completed. In the **flap-out**, there is a **File** option where user may load/upload saved microscope alignment files. If there is serious misalignment, it may be helpful to reload the standard Titan alignment file from **File**. User may also save his/her best alignment in a separate file for future use. **Note:** set C2 aperture to 50 μm before loading the file (bug in UI); you may change aperture size later.

These buttons go to the next or previous step in **Procedure Alignment**. **Do not forget to hit “Done”, after each step.**

In this window, the computer will guide you through **Procedure Alignment**. Read carefully and follow the directions.

**Direct Alignments.** These are the common alignments done during the TEM session. The Gun Tilt/Shift, Beam tilt pp X/Y, Beam shift, and Rotational center must be checked often. Fine alignment is necessary for the best TEM performance. Select alignment from the list, then use MF knobs to adjust alignment and click **Done** when done. Each alignment may be performed independently and many times. These alignments will be saved only if the user chooses “save changes” when exiting the UI.

**Note:** For additional information, please refer to the Direct Alignments Quick Guide by Agustin Avila-Sakar (FEI Company), located in a laminated sheet by the microscope.
Loading the specimen into the holder.
For those who have had previous experience with JEOL microscopes, the Titan holder will feel very fragile and delicate. Please pay special attention when handling the FEI holders – they ARE really fragile and delicate! Before using, inspect the holder: check the O-ring for cracks or dust. It must be clean. Check the conical area next to the O-ring – it must be clean and without visual scratches, etc. Do not touch any part of the specimen holder beyond the O-ring. The oil from your fingers will contaminate the vacuum as well as your sample. Inspect the specimen-holding mechanism; make sure it is not damaged. Please report any problem immediately! Users are responsible for any damage to the holder.

Single-tilt specimen-holder.

Loading the specimen.
There is a special tool to open the clamp, which holds the specimen in the carrier. In order to open the clamp, (1) locate the small hole at the base of the clamp. (2) Insert the tool into the hole and slowly move the clamp up into a vertical position (make sure the clamp is secured in this position). Do not apply excessive force! (3) When clamp is in the vertical position, load the specimen into the carrier; make sure it is centered well and slowly move clamp down into the closed position. Control this movement with the tool. Do not remove the tool from the hole unless clamp is completely closed. The orientation of the specimen should be with the sample face down, since the holder undergoes a ~140 degree rotation upon insertion into the column (see next section). Do not use tweezers etc. to manipulate the clamp mechanism. Improper operation will damage the mechanism.
Double-tilt specimen-holder (DTSH).

To load the specimen into the holder you need special tools, which you can find in a small plastic container inside of the plastic bag. These tools are the anti-twist washer, hexring, and exchange tool. Keep in mind that the hexring is made of Be and it is very expensive piece which can be easily lost if special attention is not paid. Users are responsible for its replacement in case of loss due to negligence!

For your convenience you can use the loading station, which is equipped with the light, magnifying lens and vacuum tweezers.

Procedure for loading specimen.

First, remove plastic cover from the tip of DTSH. Using vacuum tweezers place your sample inside the cup of DTSH.

Put anti-twist washer on top of the sample making sure that both pins on the anti-twist washer go into special notches made in the cup.
Place hexring on a flat clean surface, covered with filter paper. Make sure that its wider side is facing up. Grab it with exchange tool, transfer it to the DTSH cup and carefully screw it in (clockwise direction). 

*Don’t apply extensive force while screwing the hexring.* **Note:** There is only one turn of thread in the cup – before screwing hexring in, make sure that thread in the cup and screw of the hexring are correctly aligned.

Verify that the top of the hexring is at the same level as the top of the cup. The orientation of the specimen should be with the sample face down, since the holder undergoes a ~140 degree rotation upon insertion into the column (see next section). Do not use tweezers etc. to manipulate the specimen-holding mechanism. *Improper operation will damage the mechanism.*

Turn the holder 180° to flip the cup up side down and make sure specimen is held in place, though do it while keeping holder above the desk/filtering paper. Keep in mind that it is not a big deal if your sample drops at this point, but it will be a lot of trouble if you lose your sample, anti-twist washer, or the hexring inside the column of the microscope.
Insertion of Specimen-holder into the microscope column.

1) Load the specimen in the holder as described above. Make sure the specimen is properly fastened in the holder. Check that the O-ring and conical surface of the holder are clean and intact.

2) Make sure that the Column Valves are closed (Col. Valves Closed button is yellow).

3) Turn ON turbo-molecular-pump (TMP) by clicking Turbo on. The color of the button will first change from grey to orange, then to yellow. Once it is yellow, the TMP is ready.

4) Locate the small pin on the end of the holder closer to the tip. Carefully insert the holder into the CompuStage with the small pin on the holder in the 5 o’clock position (large pin at the handle in 11-o’clock position). The pre-pumping cycle will initiate and the red indicator light will come on. Note: If double-tilt sample-holder is used, plug holder connector to the CompuStage. In the Message window, select double-tilt holder and click enter button:

5) When the red light goes off (2-5 minutes – the remaining time may be checked in Vacuum Overview - Popup panel), rotate the specimen-holder counterclockwise until it stops. Then guide the holder into the microscope carefully as it is sucked into the vacuum of the column.

6) Turn off the TMP by clicking Turbo on button (it will turn grey).

7) Check the vacuum level in Octagon (Workset ➔ Setup ➔ Vacuum).

It should be 20 log or less:

Removing specimen holder from the microscope. If double-tilt sample-holder is used, unplug the holder connector from the CompuStage, gently pull out the holder from the column until it stops, then rotate clockwise to the stop and carefully remove from the CompuStage with two hands (as was shown in training).

General note: Do not apply any lateral and/or excessive force to the specimen-holder during insertion or removal!
Step-by-step Instruction for microscope use

1. Fill up the Dewar flask with liquid nitrogen and install in place, as shown during microscope training. If the Cold Trap was at room temperature, you may need to allow it to cool for at least 30 minutes. Re-fill liquid nitrogen during the session if needed.

2. Sign in to the logbook.

3. Start the Titan TEM User Interface (UI):
   a) Log on to the Titan computer using your username and password.
   b) Open User Interface (UI) from Windows quick launch toolbar or from start menu. Microscope Control-TEM1 will pop up.
   c) Workset ➔ Setup ➔ FEG Control (User). Make sure that FEG Control parameters are set correctly: HT=300kV; Extraction voltage=4300V and Gun lens setting (GL) is 3. If you need to change the extraction voltage value, set the value first and then click the enter button next to the value.
   d) Workset ➔ Setup ➔ Vacuum. Check vacuum in the system: Gun vacuum should always be at 1; Octagon (column) vacuum should be below 20 log. Verify that Col. Valves Closed button is yellow (valves are closed). The column valves should always be closed if the Octagon vacuum is greater than 20 log or when microscope is not in use. The field emission gun will be damaged if the column valves are opened when the Octagon vacuum is too high. This will result in an extremely costly and time-consuming repair.
   e) The state of the microscope’s vacuum system also may be observed in the Vacuum Overview - Popup panel, right-bottom corner of the UI.

4. Start digital camera controlling software: Digital Micrograph (DM) or/and TIA. Note: TIA requires DM to be running (start DM before TIA).

5. Insert specimen-holder into the microscope column in accordance with procedure described above and demonstration during the training session. Improper insertion will cause vacuum leak and damage to the vital microscope components, IGPs and/or FEG.

6. If Octagon is 20 log or less, open the gun-valve by clicking Col. Valves Closed button. The button will turn gray and the Setup tab will indicate Status shown: liner opened. If everything is fine you should see the beam on the screen and microscope is ready for operation. If sample is blocking the beam move it using the X-Y joystick. Note: When the column valves are opened, the CompuStage light will turn red. Do not touch the CompuStage or specimen holder when the red light is on!

7. Set microscope parameters: magnification – 5600x, spot size –3-4; aperture: C1 - 50 μm and C2 - 100 μm (the most common settings).
8. Reset the holder: **Workset ⇐ Stage ⇐ Stage² ⇐ Control ⇐ Reset ⇐ Holder**. It is a good habit to reset the holder when starting and ending a microscope session. Also, if there is a problem controlling the CompuStage, sometimes resetting the holder may fix the problem.

9. Center the beam: Condense the beam to its smallest size using **Intensity** knob. Center the beam using multifunction (MF) knobs – Beam Shift X & Y.

10. Center the C2 aperture: Spread the beam just beyond the outer circle on the fluorescent screen. Open aperture control panel (**Workset ⇐ Setup ⇐ Motorized Apertures**). Check the **Center** box next to Condenser 2 and use the MF knobs to adjust the position of the C2 aperture. The C2 aperture is centered well when the beam spreads symmetrically around the center of the screen.

11. If necessary, insert and center objective or/and selected area aperture(s).

12. Adjust the Z-height: Find an easily recognizable feature and position it in the center of the screen. In **Stage ⇐ Stage² ⇐ flap-out ⇐ Control** click the **Wobbler** button. The button will turn yellow and the image will start shifting back and forth. Minimize amplitude of this movement by pushing one of two Z-axis buttons. Turn **Wobbler** off and make sure that A&B are close to “0”. **Press “Eucentric focus”-????.**

13. Observe image on the screen. Adjust **Intensity** to spread the beam over the screen.

14. Use **Digital Micrograph** or **TIA** to record images with the digital camera.

15. In **Digital Micrograph** (DM):
   a) **Microscope ⇐ Global Microscope Info** – fill in the **Specimen** and **Operator name**, leave other fields as is.
   b) **File ⇐ Global Info ⇐ Save Numbered** - set up directory path where your files will be saved (**File Directory**); create rules for **File Name** (do not use special symbols like # / \ % # @); choose the file format, normally – Gatan Format) in **File Content and Format**. Choose **Save Image As** for high-resolution, 16-bit images. **Save display As** – low-resolution, 8-bit option. **Note**: Save images on Titan STEM support computer- C support computer on TITANSTEM/users/your directory. **Do not save your data on the Titan computer!**
   c) Make sure that beam is spread: CCD chip of the digital camera will be damaged by condensed beam.
   d) On right (DM) panel – click **Camera Inserted** to insert the camera.
   e) Uncheck **Auto Exposure**. Set exposure time manually – 0.1 sec. **Note**: Auto Exposure slows down the camera’s readout. Occasionally, this option may be used in case of difficulties with imaging.
   f) Lift up the microscope’s big screen (usually, R1 programmable button).
   g) Choose **Preview** or **Search** in **Workset ⇐ TV/Camera (UI)**. Alternatively, start **View** in **Camera View** (right panel) in **DM**. Adjust microscope **Intensity**, so intensity indicator in **DM** is in green zone. To accelerate the camera’s refresh rate, use 2x or 4x
binning (setup icon). Note 1: The CCD chip of the digital camera is very expensive and sensitive to beam damage. Always keep the intensity of the beam so that the DM Intensity indicator is in the green zone. Note 2: The digital camera may be controlled from either the FEI User Interface (CCD/TV Camera) or Digital Micrograph (DM). In either case, the image will be displayed in DM.

h) Find the area of interest. Adjust magnification; make sure that DM intensity indicator is in the green zone.

i) Focus the image using the Wobbler button (not the A-wobbler that was used to adjust the z-height) at magnification range up to 50-80xK. At higher magnification use the “minimal contrast” approach or live FFT (Fast Fourier Transform or “power spectrum”) in DM: Process Live FFT.

j) If necessary, use Display Control on the DM left panel to adjust brightness/contrast of the image. The quality of the image may be judged based on the histogram (left panel) shape: a good histogram has smooth, bell-like shape and symmetrical.

k) Take a picture: Camera Acquire Start Acquire. It is a good idea to use Auto Exposure option at this time. If you are planning to take many pictures at the same magnification and under similar illumination conditions – you may uncheck Auto Exposure to accelerate the process of taking pictures. Note: All your pictures will be named based on information provided in Global Microscope Info: Specimen name.

l) When sample is changed, do not forget to change Specimen name in Microscope Global Microscope Info. DM will automatically associate typed in Specimen name with all pictures taken after its the last change.

m) When all pictures have been collected, save them using “save numbered” (diskette 1-2-3 icon) option: select the window with the picture and click Save Numbered, close the window (Ctrl-W), repeat for every picture. All pictures will be saved in the directory specified in File Global Info Save Numbered. Note: Save images on the Titan STEM support computer - C support computer on TITANSTEM/users/your directory. Do not save your data on Titan computer!
Shutting Down the System.

1. Bring magnification to 5600x.
2. Spread the beam.
3. Reset the holder (Stage \(\Rightarrow\) Stage\(^2\) \(\Rightarrow\) open flap-out \(\Rightarrow\) Reset \(\Rightarrow\) Holder).
4. Close Digital Micrograph and TIA.
5. Close column valves by clicking Col. Valves Closed button in Setup \(\Rightarrow\) Vacuum. The button will turn yellow and the Setup tab will indicate Status shown: liner closed.
6. If you are using the double-tilt holder, disconnect the cable from the CompuStage.
7. Remove the specimen holder from the microscope column.
8. If you are the last person operating the microscope for the day, make sure that you start a cryo cycle. Otherwise, proceed to the next step.

Cryo cycle procedure: (1) remove the liquid nitrogen dewar from the cold trap as shown during the training session; (2). Open Vacuum tab on the Workset. Open its flap-out. Click Cryo Cycle On button. The TMP will start its operation and the Cryo Cycle On button will turn yellow. Note: If you are the last user of the day, don’t leave before starting a cryo cycle.
10. Log off from Titan computer.
11. Complete the record in the logbook.

*Many thanks for following these instructions!*
Appendix:

Basic Alignments.

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

1. **Gun tilt alignment.** Click Gun Tilt in the Direct Alignments. Using MF, adjust intensity of the beam to its maximum. Click Done button.

2. **Gun shift alignment.** Use L3 programmable button to set the spot size to 3. Condense the beam. Select Gun shift. Use MF knobs to bring the beam to the center of the screen. Switch to the spot size 9 (R3 programmable button). Condense the beam. Select Align beam shift. Use MF knobs to bring the beam to the center of the screen. Repeat until beam is in the center for both spotsizes 3 and 9.

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

4. **Rotational center alignment.** Perform this alignment on a visible feature on the sample. Use high magnification for this alignment. Select Rotation center alignment in the Direct Alignments. Image of the feature will start moving. Use MF knobs to minimize the movement. Click Done button.

5. **Astigmatism.** Select Stigmators at popup panel (right-bottom corner of the UI). Choose Condenser, Objective or Diffraction. Use the multifunction (MF) knobs to do the correction. For the condenser, adjust the beam shape to become a circle. For the objective, make the granularity of a high magnification image isotropic (use CCD + live FFT for optimal results). For diffraction, make the central spot of the defocused diffraction pattern circular.

For additional information, please refer to the Direct Alignments Quick Guide by Agustin Avila-Sakar (FEI Company), located in a laminated sheet by the microscope.

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