

Start Up Titan Krios

1. Check LN₂ airtight, make sure all involved O₂ sensors are working properly
2. Check LN₂ level in **Temperature Control** tab, in flap-out tab of **State**, make sure there is no error to **Recover**, in **Filling** tab make sure **Fill Now** is available
3. Check system vacuum, **Gun/Liner/Octagon** should be 1/10/1 log or better at LN₂ temperature, or 1/20/1 log or better for Room Temperature
4. In **Setup** tab, the button **Operate** and **High Tension** (with 300 kV) should be ON check **Gun lens 4**, **Extractor 4650**, **FEG Emission ~330 uA**
5. Ask super-user to **Dock** AutoGrids into **AutoLoader**, and do the **Inventory**
6. Sign in the logbook, with registering the cassette & describing the slots
7. Log out the common user
Log on to the Titan Krios computer using your username and password
8. Start the software kit:
 - a) Start **Microscope Software Launcher** on Krios PC (to start UI & FluCam)
 - b) right click on **Remote DM** then left click on **Connect** on Krios PC
 - c) in **DM, Microscope** ↗ **Setup** → **Test** → **GetMagnification**, on K2 PC
 - d) Start **TIA** on Krios PC, check **Camera** with **BM-Ceta** and **EF-CCD** shown
9. **Krios PC** → **UI** → **FEG_Register** Tab (load the up-to-date alignments)
 - a) **FEG Registers** → Flap Out → **File** → **300kVrg021417.feg** → **Set**
 - b) **Alignments** → Flap Out → **File** → **300kVrg021717.alg** → **Apply**
(these two files are stored under **C:/Titan/Alg/**)
 - c) **C2** aperture, 70 um for SPA, 100 um TOMO
10. **K2 PC** → **DM** → **Health Status** → **Wrench & Hammer** → **Quick Scan**
→ All Status Lights should be green (except 5-8 Slot Sync Status stay gray)
(If Generate Log is shown, not Gear Button, Menu → **Help** → **UserMode** → **PowerUser**,
Default setting is stored under **C:/ProgramData/Gatan/prefs**
backup setting files are stored under **C:/Desktop/pref** and setting backup 09-12-16)
11. **Col. Valves Closed**, double check Objective Aperture or Phase Plate is retracted, click on Slot number and press **Load** to load AutoGrid to compustage

12. If Gun/Liner/Octagon is 1/10/1 or better (LN₂ T) or 1/20/1 or better (RT)

Open **Col. Valve**

13. Find the Eucentric Height by setting the right Z-Height, Press **Eucentric Focus** on right hand panel

14. Set SS 9, C2 100 for TOMO (SS 8, C2 70 um for SPA)

15. Center C2 Aperture, Beam Settings → Free Control → C3 off → C2 aperture Adjust; after centering the C2 press **TEM** to switch back

16. Direct Alignments

- a) Beam Settings, Flap-out Tune → Nanoprobe ON
- b) Correct **C2 Condenser** Stigmatism at 75 kx +
- c) Find True Focus at imaging mag (or slightly higher with a parallel beam)
- d) PP X, PP Y
- e) Rotation center
- f) Coma-free Alignment X
- g) Coma-free Alignment Y

17. Correct Objective Stigmatism at imaging mag (or slightly higher with a parallel beam)

18. Insert Objective aperture and center it under diffraction mode

19. Tune Filter, make sure select **Linear** Mode of K2, check **Slit in, Slit width** of 30.0 eV, go to an broken area, or unload the AutoGrid (double check Objective Aperture or Phase Plate is retracted)

Update K2 dark reference

SPA EFTEM_SA 105kx_SS 5-6_III Area 1.20uM_Bin 4_exposure 0.1s_~15,000 Counts

TOMO EFTEM_SA 26kx_SS 5-6_III Area 6.00uM_Bin 4_exposure 0.1s_~26,000 Counts

20. EPU, True Focus and fine tune of OBJ Astigmatism

set Preset of Data Acquisition to Parallel Beam Condition

Log Off Titan Krios

1. Bring magnification to 5000x.
2. Spread the beam to full CCD
3. Close **Col. Valve**
4. **OBJ** aperture out
5. **Unload** user's AutoGrid from compustage to AutoLoader, and **Load** slot #1 AutoGrid onto compustage, AutoLoader → Options → Leave cartridge on compustage (checked)
6. Reset the holder (Stage → Stage2 → Open flap-out → Reset → Holder)
7. Close the software kit:
 - a) Stop **Microscope Software Launcher** on Krios PC
 - b) Close **FluCam** on Krios PC
 - c) Close **TIA** on Krios PC
8. Log off your account
Log in to common user
9. Start **Microscope Software Launcher** on Krios PC