FEI Tecnai T20

 Operating Procedure

**Samples should be prepared a day in advance of imaging and stored in a vacuum dessicator overnight.**

**Microscope Status Check – Pre start**

**1. Before adding liquid nitrogen (LN2) check:**

* Cryocycle is not active
* Vacuums are good
* Red light on stage is off

Ask staff for assistance if you see any of the above warning signs or do not understand what to look for

**2. Add LN2 to dewer on TEM 30 minutes before use**

* Add LN2 every 4 hours during use
* Fill LN2 dewer at start of your session
* Refill LN2 at end of your session

**3. High Tension**

* High Tension should be ON, and “High Tension” button should be yellow

If it is not ON let FMIC personnel know

**4. Filament – first user of the day will have to turn on the filament**

If filament is off;

1. Select “Set Up” tab (upper left corner left screen)
2. Click on “Filament” button, (when active it will change from Gray to Yellow)
3. Wait for filament guage to come on, it will change from gray to blue then stop at a pre-set point – do not change this setting.

**IF you see a note saying the cryocycle is on, review step 1. If you do not know how to tell that the cryocycle is active/inactive please ask a staff member.**

**General Operation**

**Load sample onto Single Tilt Holder** (STH)

1. Place sharp pin (stored on loading stage) into small hole of the STH and lift up clamp of holder to vertical position – do not remove pin
2. Load your grid sample side down
3. Gently lower clamp using pin, return pin to loading stage
4. Remove holder from plastic loading stage
5. Rotate holder upside down to check if sample is secured on the holder

**Center TEM Stage prior to inserting sample holder**

1. Go to; “Stage 2” tab
2. Click on “Control” side tab
3. Select “Reset” 🡪 Click on “Holder” button

**Insert sample holder**

1. Align pin on copper end of sample holder with line next to “Close” on airlock, insert gently until light comes on and pump starts.
2. If you see the message; “Select Sample holder”, click on arrow next to “Select Single Tilt”
3. Wait for the light on sample insertion disk to turn off (and for countdown to end)
4. Then turn holder toward “open” (counterclockwise), vacuum will try to pull holder into the airlock but don’t let it! Control the sample holder speed of entry or you will allow air into column. Alignment is correct when tab of holder fits into tab-hole on the air lock

**Beam Adjustments**

* 1. Look at Status Box – wait for Column to go below 10
	2. Click on yellow “Col. Valves Closed” button to open valves for viewing. It will turn gray when valves open.
	3. To view through main port; Push R1 button (RH panel) to lower the screen for viewing.
	4. Move grid around with joystick on RH panel to locate an area of interest – if you can’t see the beam you are probably on a grid bar or your sample is too thick, either move around more or ask for help.
	5. Set “Z” height
		1. Press “Eucentric Focus” button (Right Hand (RH) panel)
		2. Center an object on the view screen
		3. Press LH panel “L2” (alpha wobbler) or under Stage tab🡪 Stage2 pull out 🡪 bottom of “Control tab” alpha wobbler
		4. Adjust Z position using “**+**” and “**–**“ buttons on far side RH panel, until object movement is minimized
		5. Press “Wobbler” to stop movement
	6. Turn magnification knob to set mag at 8500x
	7. Set beam to crossover – turn “Intesity” knob until beam at smallest diameter
	8. Use “rollerball” to center beam, immediately turn Intensity knob clockwise to spread beam in order to avoid damaging viewscreen
	9. If beam spreads unevenly ask for help

**Bright-Field Imaging**

1. Cover veiw port
2. Select CCD/TV camera tab
3. Push R1 button to lift view screen so camera can display image
4. Click on “Search” button for live view
5. Find area of interest by moving grid with joystick, adjust magnification as desired
6. Lower viewscreen, remove cover, turn “Intensity” down so beam is 40 mm
7. Use “rollerball” to center beam, turn Intensity knob clockwise to spread beam
8. Focus image
	1. Click on “Live FFT” to bring up FFT focus assist
	2. Turn outer focus knob to change step size (larger number = larger change in focus)
	3. Turn focus knob so that lighter circle in FFT assist window is about 10 -15 mm from edge of window (approximately 1 finger width)
9. Check that bar under histograph is green to blue-green (no red, red = overexposure)
10. Click on “Acquire” button to capture image

**To Save image**

1. Click on image
2. Right click on the image and select “**Export Data**” or under “File” in menu bar select “**Export Data**”
3. Choose folder to save image in
4. Type in image name
5. Under “Save as type;” choose either;
6. “**PC TIFF w/scale marker (full re)(\*.tiff)**” **or** “JPEG w/scale marker (full res)(\*.jpg)” Warning; Micron bars will not be applied to image with any other option
7. Select “Save”

**If you need to take a short break;**

1. Leave filament on
2. Turn off “Search” icon
3. Close column valves
4. Leave a note on the scope so others know you are coming back

**Sample Exchange**

* 1. Screen down, Mag to SA 4400x, Spread intensity to cover screen
	2. Go to “Stage 2 Tab”
	3. Click on “Reset holder” icon
	4. Close valves (go to “set up” tab click on Close Valves button)
	5. Remove sample:
		1. hold plate with 2 fingers one on either side of sample holder
		2. pull gently until it stops
		3. turn clockwise until it stops
		4. then carefully pull out – the vacuum will suddenly release the holder
	6. Replace or remove sample

**End of session**

1. Screen down, Mag to SA 4400x, Spread intensity to cover screen
2. Follow Sample Exchange steps (but do not replace grid )
3. Return sharp pin to storage position on loading stage
4. **Close all image windows – but do not close TEM Imaging & Analysis window**
	1. **Click on “Window” in word menu bar (over images)**
	2. **Select “Close All”**
	3. **It will ask “Do you want to save “CCD Search?”🡪 “No to All”**
	4. **It will ask “Close all documents and discard changes?” 🡪 Yes**
5. During business hours – fill dewar with LN**2**
6. After business hours if **someone is** signed up after you fill dewar with LN**2**
7. After business hours or end of day and **no one is signed up** after you go to cryo-cycle/follow shut down/end of day procedure

**Image Transfer at end of session:**

1. Open “Users” folder or flash drive on HP support computer (left side of computer screen)
2. Open “FMIC Images (Tecnai-20…) (upper right corner support computer)
3. Transfer your images from the “FMIC Images…” folder to your folder on the HP support computer and/or your flash drive (remember to check your flash drive images later to be sure they are all there)
4. Delete your images from the “FMIC Images…” folder

 – **Important!! Too much data on the FMIC Images computer will interfere with the TEMs’ ability to function.**

**Shut down/End of day - Check with staff before proceeding**

1. Check Scheduler - if no reservation after you, continue with cryocycle
2. Reserve 4 hours cryocycle on scheduler:
	1. Login: cryocycle@wsu.edu Password “cryocycle”
3. Be sure the sample holder is returned to loading stage and place cover over end
4. Turn off filament (turns grey when off)
5. Remove liquid nitrogen dewer, empty, place upside down in box to dry
6. Select “Set-up” tab
7. Select “Cryo Cycle” icon on side tab - cryo cycle will start after 5 minutes

**Tips**

Often used Camera settings

Binning: 2

Readout area: Full

Image size: 2048 x 2048

Beam Brightness

* Intensity 🡪 changes beam brightness
* “+” and “-“ buttons under “Course” and “Fine”will increase or decrease how quickly the brightness changes

No micron bar on image? You didn’t select “PC TIFF w/scale marker (full re)(\*.tiff)” when taking image. No way to put on once you’ve Exported, you will need to re-take image.

 – **Important!! Too much data on the FMIC Images computer will interfere with the TEMs’ ability to function. Go to “Window” in menu bar to close these, please do not close TIA**