**Working Algorithm (TEM at Room Temperature)**

Note: ***Italic*** *-* microscope software; CAPTTAL LETTERS - microscope buttons

1. Check the log book to find out the status of the microscope.

2. Check the status of the microscope:

* ON/OFF main console- OFF, VAC and HT buttons should lit.
* ***Log in***and open the ***Tecnai user interface***(TUT), go to *vacuum overview* and check that the vacuum is o.k: Gun< 15; Camera< 30.
* If *HT* is off (gray) tum it on (drag the marker on the *HT* tracker to 20kV, click on the ***free high tension*** check box, change the step size to l0000V (or less depending on the emission jump) and gradually change the HT to 40, 70 and 80kV. Change the step size to 3000V and go up gradually till 100kV, change the step size to 1000 and go up till 120kV. At the end, click to close the free high tension).
* ***Column valves***V4 and V7 are closed (The Button is yellow).
* ***Filament***is off (gray).
* Cover the projection chamber glass (viewing screen) by the black plastic cover.
* Cover the microscope right control panel.

3. Fill the microscope dewar with liquid nitrogen (LN2) to cool the anticontarninator blades (keep the LN2 level as high as possible along the session).

**4. Check that coordinates (x,y,z,α.) are at zero position.**

5. Insert the holder with your grid.

**Inserting the holder** (check filament is ***off***, column valves are ***closed*** and the holder location is ***reset***, before taken out or inserting the holder):

Hold the specimen holder with the airlock trigger pin parallel to the small slit in the CompuStage front plate (at roughly three o'clock). Carefully insert the end of the specimen holder into the airlock cylinder and slide the holder in until a stop is reached (the red CompuStage light will be illuminated). Slowly turn the holder clockwise until it will go in a bit further (the airlock trigger pin now falls properly into its groove). Select the ***type of holder***(single tilt) and press ***enter****.*

When the red CompuStage light has been switched off, gently rotate the specimen about 120° counter-clockwise as far as it will, then allow it to slide in gently (while controlling it with your hand) further into the microscope.

**Alignment** (from # 6. Use X26500 mag and Spot size 3. Using MF, X,Y)

1. Wait for a good vacuum (IGP≤15).
2. Turn on the filament and wait until it will warm up.
3. Open the col. valves (V4 and V7) – press the button and wait for it to turn gray.
4. Find the beam and feature to look at and make sure the specimen is at **eucentric height** (in the stage tab click on the ***wobbler*** button. Minimize the image movement by changing the Z-Axis up or down in the microscope right control panel). It is advisable to ***add*** this location to your list.
5. **Pre alignment (Condenser) step**: Find an empty area on the grid, take out the objective (middle) aperture, **check centering of C2 aperture** by following the movement of the spot when going through focus *back* and *forth* with the C2 INTENSITY knob (microscope left control panel). If the position of the spot moves, center C2 aperture iteratively by minimizing beam movement using the ADJUSTMENT SCREWS on the aperture (the Condenser aperture, above one) itself and by going through focus repetitively. If the going back and forth with the Intensity knob opens the beam at different direction means we have Astigmation in the beam and we need to fix it with the ***Stigmator*** panel (bottom right), choose the ***Condenser*** and correcting the Astigmation with the MF, X,Y and by going through focus repetitively.
6. Go to ***direct alignment*** in the ***Tune*** tab and check the following alignments (spot size 3, first open the beam so that in the cross-over point it will almost cover the large circle (40mm circle) on the microscope screen (~X26500).
7. ***Gun tilt*** - continuously change each of the multifunction buttons (MF X,Y) until obtaining the brightest beam (minimal exposure time).
8. ***Gun shift -*** center the beam with the MF X,Y buttons: Minimize spot displacement when spot size is changed from spot size = 3 (center with MF X,Y) to spot size = 9 (center with beam shift TRACKER BALL).
9. ***Beam tilt PPx and PPy -*** change both MF X and Y alternatively till only one spot is visible in the image with minimum shake (if the beam moves center with the LEFT TRACKER BALL).
10. ***Beam shift -*** bring the beam to the cross-over point and center it using the MF X,Y buttons. At the end, press ***Done.***
11. ***Rotation center****-* Choose an area with sample (it is advisable to return to the Eucentric height location by pressing ***go*** in the search panel). Move to mag. X 26500, change with MF X,Y buttons till lateral movement stops (should move only in and out of focus – as if the sample is breathing). At the end of the direct alignments press ***Done***

**Astigmatism**

Check objective lens astigmatism: find an amorphous material, start at mag. X 97000, exposure time=l sec. Open ***Digital******Micrograph***(DM), choose *CCD,* arrange *Preview integration time= 0.0626sec,* lift screen, press ***preview****,* in the tool bar choose *process-live-reduced FFT,* go from underfocus to overfocus and check if there is an astigmatism (in FFT beam is not round). To fix astigmatism: ***stigmator***window, choose ***objective****,* copy 1 to 2, with MF X,Y make the FFT beam round.

**Working with the Objective Aperture:**

When working with the objective aperture for the first time it is necessary to see that the beam is focused in the center of the small circle. If the beam is not focused, correct it with the ADJUSTMENT SCREWS of the aperture. It is advisable to do this correction from time to time when inserting and taking out the aperture.

**Taking images:**

* choose a feature you would like to take an image of and roughly focus it
* Open Digital Micrograph and choose the relevant camera (CCD or WAC CCD).

For CCD (bottom camera):

* **With INTENSITY knob arrange the exposure time = 1 sec**

Arrange ***preview***integration time = 0.0626 sec; ***acquire***integration time = 1 sec. If intensity value is higher than 5000, reduce integration time or intensity (brightness).

Press ***preview***and focus the image. Press ***acquire***to take the image.

For WAC CCD:

* With INTENSITY knob arrange the exposure time = 10 sec

Arrange ***preview***integration time = 0.05 sec; ***acquire***integration time = 0.25 sec. If intensity value is higher than 5000, reduce integration time or intensity.

Press *preview* and focus the image. Press ***acquire***to take the image.

**Saving images:**

At the DM screen open ***file*** and ***global info*** and chose ***numbering…*** find the folder, give a name to the images and the first number you want copy the name and exit the panel. Go to the ***global info*** at software buttons at the lower right side of the screen give a name for the images and operator. Do the same in ***image info*** after imaging you can press the save button (disc with 1,2,3).

**At the end of the session:**

1. Put down the screen (and leave the microscope with a reasonable intensity and magnification (10000-30000 for finding the beam next time)
2. Turn off the***filament***
3. Close ***column valves***(V4 and V7)
4. Retract camera and exit ***Digital Micrograph***
5. Reset coordinates (stage workset *-****holder*** *)*
6. Retract the holder from the CompuStage, take out your sample and insert the holder back.

**Retracting the holder:**

* Always close the column valves before taking out the holder!
* Turn off the filament
* Reset the holder position (***Search***)

Note**: In all stages it is important to give contra on the purple wheel!** Pull till first stop, tum to right until it stops (here you can leave it), pull out all the way by holding the axis itself and giving contra on the purple wheel.

1. Cover the projection chamber glass by the black plastic cover.
2. Cover the microscope right control panel.
3. Fill in the LN2 Dewar for the next session.
4. **At the end of the day**: Activate the ***cryo cycle***button (should be set for 240 min.). Empty the microscope dewar (put it upside down to dry) and put a plastic beaker under the copper wires to collect the melting frost
5. Burn your images to a CD. It is your responsibility to transfer the images to your own computer.
6. Add notes to the log book (including: Date, Operator name, User name and group. Sample imaged. Holder number, Filament time (to be read from the flap out of filament window), session time, problems in operation of the TEM or other notes.
7. Exit ***Tecani user interface*** (save changes) and log *out.*
8. Turn off the lights and leave the room in a state that we would like to find it