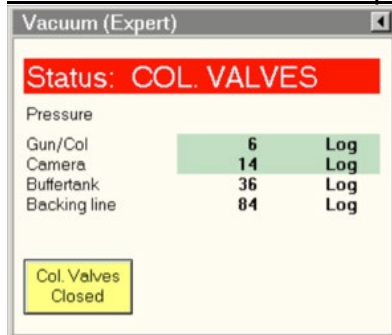


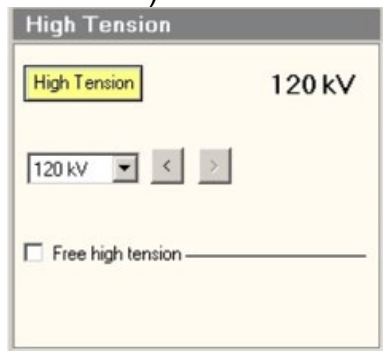


## Tecnai Spirit G2 Biotwin (Condensed Manual)

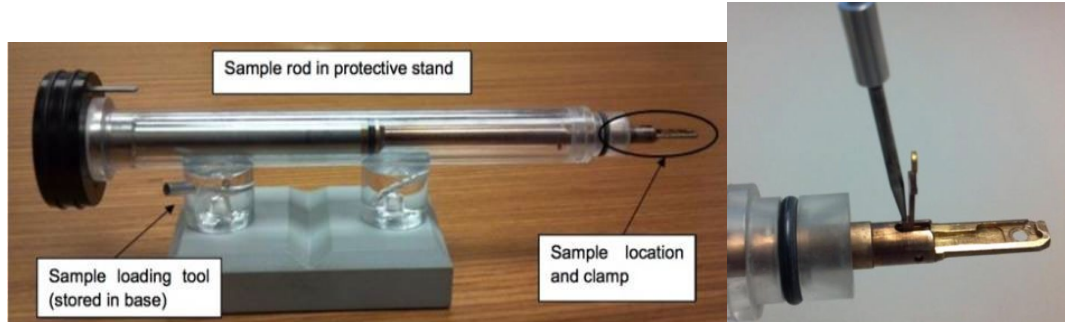
- 1. Check that the microscope status reads **“COL. VALVES”** and that the **“Col. Valves Closed”** button is pressed (buttons appear yellow when active)



- Fill LN (Liquid Nitrogen) cold trap
  - The first Dewar flask of the day should last ~30–40 minutes. Later Dewar will each last 2–3 hours. **Do not allow the cold finger to warm** or the vacuum will deteriorate significantly
- Turn on **High Tension** if not on (For menus, the grey box means off, yellow means on). The HT of BT is operated at **120kV**



- Once HT is fully activated, load the grid into the holder. Always use gloves to avoid contamination in the holder



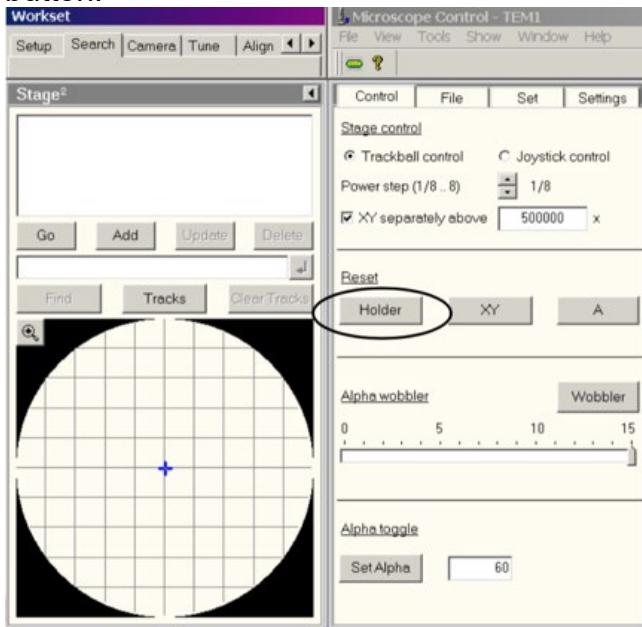
- Place the sample holder on the protective stand
- Remove the sample loading tool from the base of the stand
- Sample rod in the protective stand
- Using one hand to prevent the holder from slipping out of the stand, insert the tool into the hole in the specimen clamp and **gently** raise the clamp **straight up** until it stops

- Place the specimen grid into the recess (It does not matter whether the carbon side that contains the sample is upside or downside) at the end of the holder
- **Gently** lower the clamp **straight down** to hold the grid securely.
- Return the tool to the base of the holder stand
- Make sure the grid is secure in the holder

- **2. Holder Insertion**

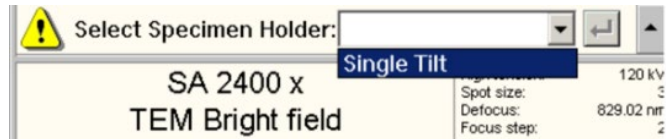
**Note:** the specimen holder, airlock, and compu-stage are made up of delicate, precisely machined components. **You should never have to exert significant force during any step of this procedure. Doing so may result in serious damage to the instrument or holder**

- **Before inserting or removing the sample holder**, make sure that the **column valves are closed, the objective aperture is not inserted**, and the **holder has been reset**. The Holder is reset in the “Stage<sup>2</sup>” tab (flapout), “Reset: Holder” button.



- Insert Holder into the chamber (**Video of process on the desktop of the computer**)
  - Carefully line the **pin** on the sample holder with the **5 o'clock** position on the goniometer and **gently insert the holder until it stops**. Be careful not to scrape the tip
  - You should feel some resistance as the holder O-ring seats in the airlock chamber.
  - The airlock will begin pumping, and the red LED on the compu-stage will light on. **Do not move the holder while the red stage LED is lit**

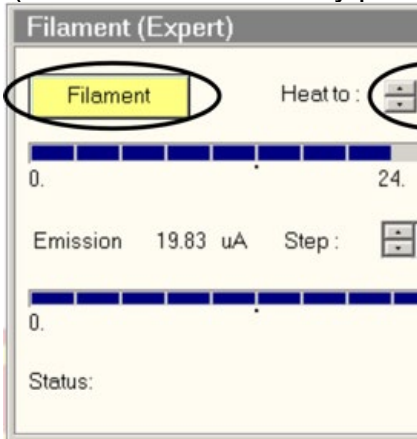
- The pumping time remaining will be visible in **the Vacuum Overview window** (~60s, can vary time as we need in the setting)
- Select the specimen holder type (**Single Tilt**) from the box in the interface. Be sure to click the 'enter symbol' button to confirm the selection.

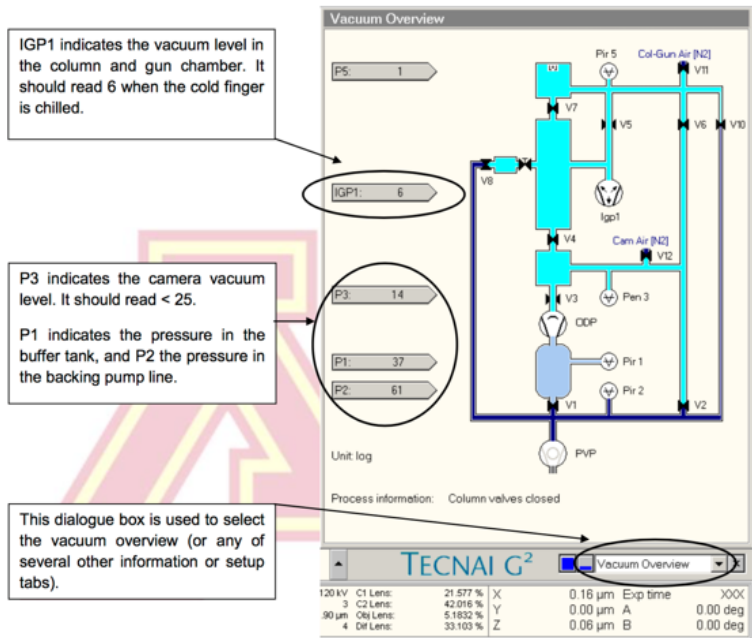


- When the **pump time ends** (status reads "**COL. VALVES**") and the **red stage LED goes out**, support the purple goniometer surface with one hand and **grip the holder securely** with the other. Slowly **rotate** the holder **counterclockwise** from 5 o'clock to **12 o'clock** and allow the holder to go inside. Gently tap the end of the holder to confirm.

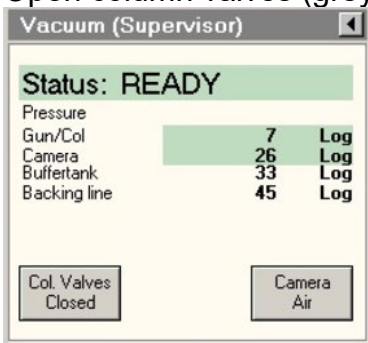
### 3. Filament heating

- DO NOT rush to **turn ON the Filament**. First wait for gun/col vacuum to recover to less than 10 log
- Once the gun/col vacuum is 10 log, **turn ON the Filament**. The computer will display the time needed for the filament to get warm. It takes a few minutes (follow the horizontal progress bar) to reach the designated values (29)
- (Turn on the filament by pressing the filament button)





- Open column valves (grey means open)



- Lower screen, move stage trackball around to find grid square, spread beam, and adjust mag to 1050x to view overall grid integrity

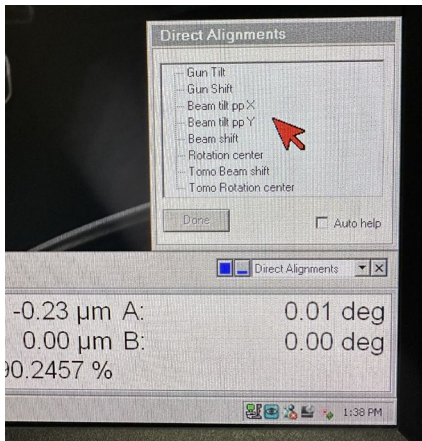
#### 4. Getting eucentric height using the stage wobbler and the Z +/- (Mag of 11500x is enough)



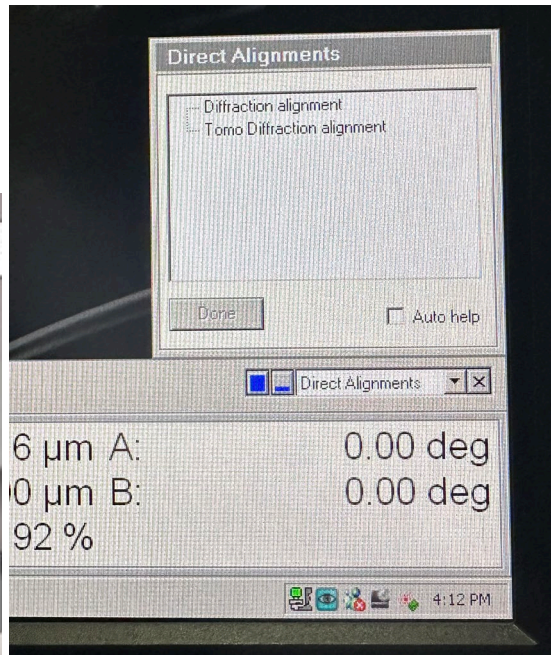
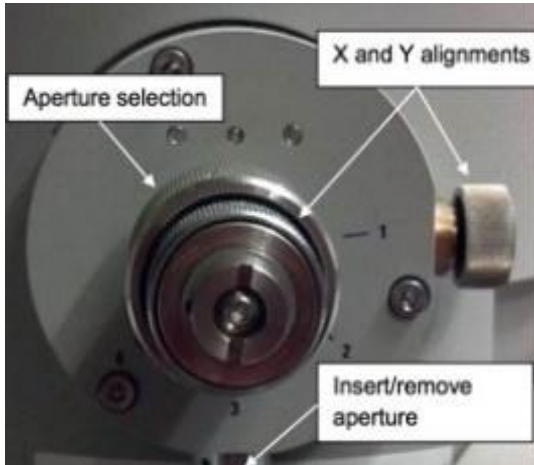
- Find a point of interest (say: a clear dark spot) on the specimen that can be tracked easily
- Activate the "Alpha wobbler" (LC button L2, by default). The stage will begin rocking through a tilt range of +/- 15°.

- **Minimize the specimen movement** by adjusting the “Z-axis” control buttons (RC). These buttons are pressure sensitive; pressing harder = faster change
- **Deactivate the “Alpha wobbler”**. For this, you need to press the L2 button again.
- Center the beam in the fluscreen. Minimize to a center spot and center using trackball if needed at the spot size 3
- Spread the beam and go to spot size 9 and repeat the same process to center the beam spot
- Revert back to spot size 4 and center the beam

## 5. Direct Alignments

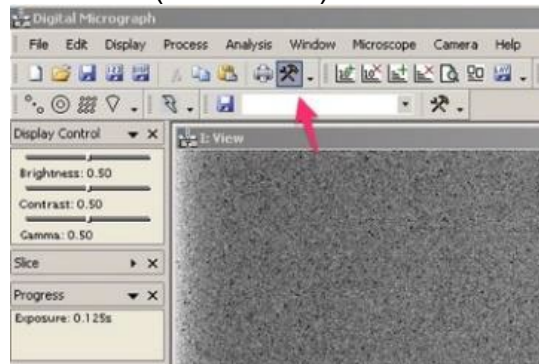


- Go to ‘Direct Alignments’ tab and select ‘Beam shift’ and center the condensed beam at the center of Fluscreen using MF X, Y knobs at SA 11500x mag.
- Also make corrections for ‘Beam tilt pp X’ and ‘Beam tilt pp Y’ using MF X, Y knobs
- Redo beam shift alignment to center the beam  
*Note: Never select ‘Gun tilt’ and ‘Gun Shift’ for your alignments. Users are found more likely to mess up the alignments*
- **Optional (using Objective aperture):** Minimize the beam and hit ‘Diffraction’. Insert the Objective aperture (position #1 for smallest aperture). In ‘Direct Alignments’ tab, select ‘Diffraction alignments’ to center the objective aperture using MF X, Y. click done and Toggle off diffraction by hitting the ‘Diffraction’ button.

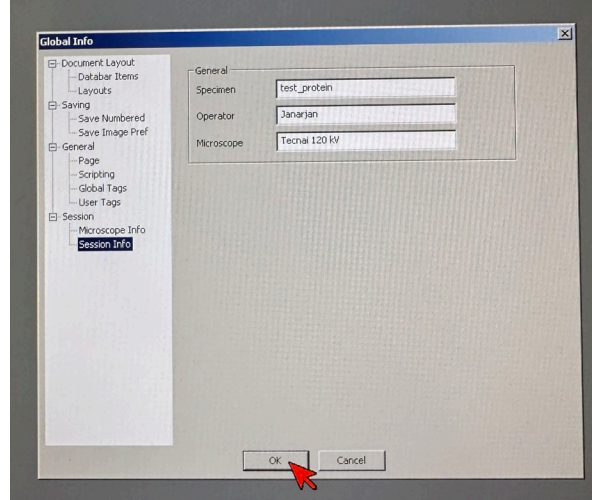
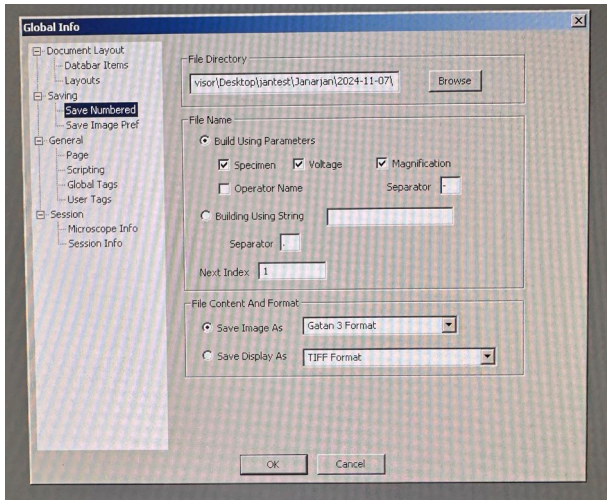


- **6. Set up a Digital Micrograph session**

- Hammer and wrench (Global Info) icon next to the printer icon



- Click on 'Save numbered section'
  - Browse to C:\Documents and Settings\supervisor\Desktop\jantest\..., Your PI Name, Session Folder. [For the first time, you have to create your name folder inside the C:\Documents and Settings\supervisor\Desktop\jantest]
  - Under File name, checkmark the 'Build Using Parameters' and check the boxes for Specimen, Voltage and Magnification
  - **Save image as Gatan DM3 Format**
- Click on 'Session' → 'Session Info' for naming your specimen
  - Specimen naming convention is Date\_Sample\_Stain used: ex. 2024-10-29\_Phage\_UF
  - Fill in your name for Operator



- Lift screen (L1). [Pressing the L1 button again will close (reverse) the screen]
- Insert Ultrascan on DM and Start View

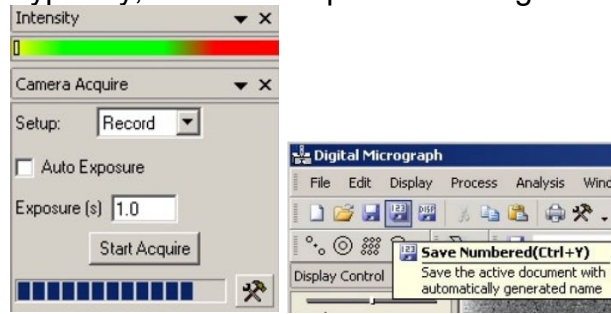


- Adjust intensity with brightness on scope until the indicator is in the green zone
  - Be **extra careful not to overexpose the camera** to too bright of a beam as this can damage the CCD. For this, spread the beam to the full area of fluscreen before retracting the screen
- Focus image



- Focus step of '2' is suitable while tuning the focus knob
- Use live FFT feature (Process → Live → FFT) during fine focusing. At focus, Thon ring is almost gone and at a good defocus few thon rings are visible
- Start Acquire

- Typically, 1-second exposure for negative stain images is enough



- Click save numbered
- Again hit 'Stop View' and insert the Fluscreen by pressing L1 button
- Go to the area of interest in the grid square and when you are ready spread the beam and retract the Fluscreen to take another image. Click save numbered as above to save the acquired image
- Again hit 'Stop View' and insert the Fluscreen by pressing L1 button to go to the grid sample selection. The above steps are repeated

- **7. After your session:**

- Hit 'Stop View' and uncheck the box for 'Camera Inserted' to remove the camera
- Remove objective aperture (if inserted)
- Lower screen (Fluscreen)
- **At spot size 4, lower magnification to 2550x**, this is essential to maintain a stable objective lens current and prevent thermal drift for the next user
- At 2550x magnification, adjust the intensity knob so that the beam **covers ¾ of the Fluscreen (at spot size 4, C2 lens is ~ 42% for this setting)**
- Neutralize Stage by resetting the holder. **ALWAYS make sure to hit 'RESET HOLDER'**
- Turn off filament
- Close Column valves

- **8. Remove sample holder and start cryo-cycle (if needed)**

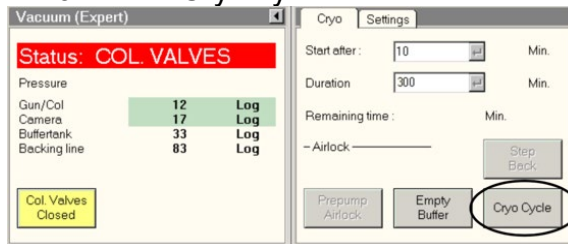
- Steps for Removing the Holder:
  - **Always keep light pressure on the purple goniometer surface** when removing the sample holder. Pull the holder **straight back without rotating** until it stops moving.
  - **Rotate** the holder **clockwise** until it stops. This rotation moves the guide pin (see steps 3 and 4) approximately from the 12 o'clock position to 5 o'clock.
  - **Gently, while keeping pressure on the goniometer**, pull the sample holder back to break the airlock vacuum. This will require a **small** amount of force applied using your index finger.
  - Remove the holder **straight back** out of the column while being careful not to scrape it along the inside of the airlock.

Be careful not to touch the holder O-ring or any part past it with bare hands

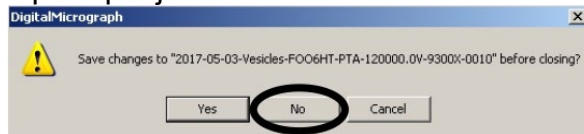
- If you are not the last user, fill the LN in the cold trap Dewar
- If you are the last user:
  - Remove cold trap Dewar
  - Place foam box underneath the cold fingers
  - Insert the blank plug into the specimen chamber by pushing straight in (select 'Single Tilt')
  - Dump out LN in a foam box kept in the main room and place the Dewar next to the oven



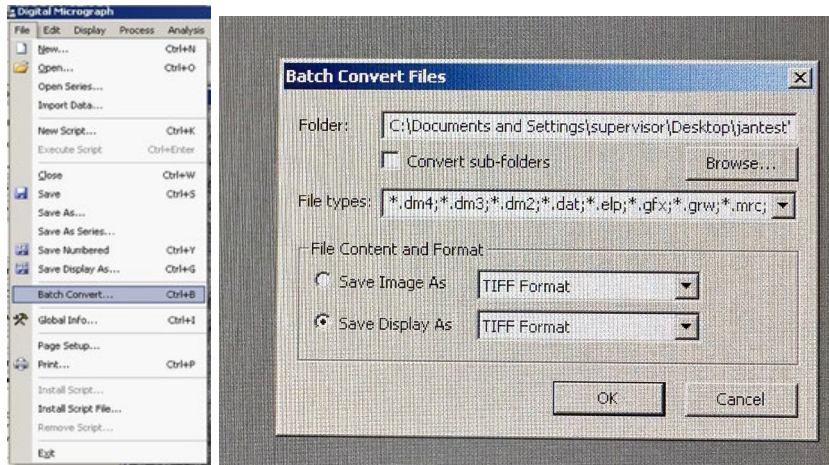
- Start Cryo cycle



- **9. Saving and retrieving your micrographs:**
- Close images in DM (if you've done all save numbered, you can close, and when it prompts just click no when it asks to save changes)



- Batch convert files
- Open Batch convert: Digital micrograph → File → Batch Convert
- Choose the working folder as input and save display as TIFF Format
- Access your data files by contacting the cryo-EM staff. All files will be uploaded to a shared folder in Google Drive by the cryo-EM staff



### Useful tips and tricks:

1. Image is drifting for a long time during focusing:  
 This indicates that the image is not properly focused  
 Click 'Stop View' and Insert the Fluscreen  
 Redo the eucentric height to bring the object at eucentric focus  
 Perform Direct Alignments at imaging magnification: 1. Beam shift to center the beam 2.  
 Minimize the beam and make pivot X and pivot Y corrections. Again perform beam shift  
 Always use 'Live FFT' as a reference to focus the image  
 Also note the defocus value and stick with the focus step of 2 while tuning the focus knob. It is  
 very easy to lose focus using the higher value of focus step
2. Do not change the spot size (usually 4) and accelerating voltage (always 120 kV)
3. Do not rotate intensity knob to pass the cross-over. The intensity knob should always spread the  
 beam when rotated in the clockwise direction
4. A good image is the combination of a suitable defocus and a suitable amount of beam intensity
5. Make a habit of using the low beam intensity (greener zone in the intensity scale bar) at the  
 imaging magnification
6. Keep in mind that the beam intensity is increased when you revert to lower magnification.  
 Always insert Fluscreen if you are about to switch to a significantly lower magnification. This  
 prevents camera saturation and potential damage of camera
7. Electron beam is lost:  
 Make sure column valve is open and HT and filament are active  
 Click 'Stop View' and Insert the Fluscreen  
 Switch to lower mag. (2550x) and move the stage  
 Still beam is not visible, make sure the Objective aperture is not inserted  
 Hit 'Eucentric Focus' and then hit 'Diffraction'. Unless blocked or the beam is significantly  
 moved, at diffraction, we expect a beam at the center of Fluscreen  
 Toggle off diffraction and turn the magnification knob to further lower value to get the beam.  
 Inform facility team immediately if the electron beam is still lost  
 Note: Always perform direct alignments (pivot point corrections for X and Y, and beam shift  
 once you retrieve the beam