Basic (S)TEM Operation

Inserting the holder

1. Align the small pin extending from the metal part of the holder with the angled line next to closed on the faceplate.
2. Insert the holder such that the O-ring seals the airlock.
3. Connect the cable and select the toolholder in the interface. Wait for the timer to run out such that the vacuum is high enough.
4. Hold the holder in such a way that you can resist the pull of the vacuum and rotate the holder counter-clockwise until the pin extending from the holder is aligned with the hole in the faceplate.

Acquiring an image using the phosphorous screen

1. Check the vacuum in the octagon and make sure the vacuum is lower than 20 Log, if so you can open the column valves.
2. Make sure the phosphorous screen is inserted into the optical column.
3. Load the alignment files for the FEG and the operating mode of the microscope.
4. Open the column valves and check for a bundle.
5. Press the eucentric focus button to reset all displacements.
6. Using the sample z-height buttons try to minimise the contrast of the picture.
7. Further minimise the contrast of the picture by magnifying and then using the focus knob.

High Resolution TEM image using digital Camera

1. Make sure the screen current is roughly less than $10\,\mathrm{nA}$.
2. Retract the phosphorous screen using the R1 button.
3. In the Velox camera software press the play button to start acquiring.
4. Before changing the beam intensity or magnification always reinsert the phosphorous screen
5. Open the fast Fourier transform window on the top right.
6. By pressing the stigmator button and using the multifunction knobs correct the stigmator such that there are concentric circles visible in the FFT. Using the focus buttons make the circles larger. The flat inner section of the innermost circle should fill most of the FFT image.
7. The most detail can be achieved if there are bright spots or rings on the edges of the FFT.
8. Acquire a HRTEM picture using the camera button in the top toolbar.

Diffraction Image

1. Spread the bundle as to illuminate the whole sample on a small magnification
2. Insert the largest aperture and centre.
3. Insert smaller apertures until only the selected area is illuminated.
4. Insert the beam stop through the button at the top of the screen.
5. Position the centre beam behind the beam stop such that the rings show on the phosphorous screen.
6. Use the focus knob to tune the focus such that the outermost rings are sharp.
7. Adjust the Velox camera settings to minimal exposure time and take a short live image.
8. Make sure that the individual bright counts are not too high.
9. Adjust exposure accordingly.

STEM imaging

1. Load alignment file for high tension STEM. (STEM $300\,\mathrm{kV}$)
2. In the Velox software activate the HAADF detector by clicking it in the column overview.
3. Make sure the Titan PC is in control of the electron beam deflection by checking that the box under the monitor is set to ”INT SCAN”.
4. Refocus on the sample using the same method as in the HRTEM section.
5. Pause the beam and set it to illuminate amorphous material.
6. Activate the condenser stigmator and correct the Ronchigram to show a flat circle in the beam.

EDX

1. Re-check if the focus of is correct.
2. Select a region for the EDX inspection.
3. Select a region for the drift correction, make sure it has well-defined horizontal and vertical features.
4. Use the analysis toolset to analyse a region of interest.
5. Using the periodic table on the right of the screen you can select which elements you want to show.

EMPAD

1. Enter STEM mode by loading a register or creating your own (following alignment steps).
2. Create a reference circle by using the HAADF detector as a guide and note its centres
3. Create two lines a vertical and a horizontal one and create a crosshair through the centre of the circle.
4. Adjust the Ronchigram to lie in the centre of the crosshair-like shape.
5. Connect to the EMPAD PC by opening ”nomachine” and selecting ”EMAPD-PC (New), yes it is misspelled”. EMPAD PC is the second PC from the top in the rack.
6. Open the EMPAD GUI and open or create a project folder.
7. Make sure that the HAADF is retracted before inserting the EMPAD since the EMPAD doesn’t know about the HAADF sensor.
8. Switch beam control to ”EDX SCAN”
9. Align the Ronchigram to the centre of the EMPAD detector by using the ROI and Cross options in the video screen. You can mark this location for convinience in the TEM user interface.
10. While ”Acquire” is selected in the Scan Settings window you can take a image/dataset by pressing ”Take”.
11. Shutdown the EMPAD and retract the sensor from the column.

Shutting down

1. Close the column valves.
2. Center the stage using (Search) $\rightarrow$ (Stage) $\xrightarrow{\text{fly-out}}$ Reset Holder.
3. Take out the holder, remove the sample and reinsert it.
4. After the holder is fully inserted, turn of the turbo pump.

Aligning the column for High-Resolution TEM Imaging

FEG registers

FEG registers are configuration files that store the settings and/or positions of the lenses and apertures, loading such a register should therefore align the column for its respective imaging mode. Ideally a FEG register called ‘TEM 300kV ss5‘ will align the column for TEM imaging with $300\,\text{kV}$ and a spot size of 5. In reality due to vibrations or temperature differences the column will slightly misalign over time and will need to be realigned manually, below the steps for achieving alignment are outlined. If no FEG registers are available and you can not locate your beam make sure to:

- select the largest $150\,\mu m$ **C2** aperture
- retract the selected area aperture
- retract the objective aperture
- defocus the objective lens (intensity knob clockwise)

Locating the sample

To begin your alignment first locate the region of your sample that you want to image and magnify to an intermediate 'SA' magnification ($\geq$20 k$\times$). Bring the sample to eucentric height by minimising the phase contrast on the fluorescent screen, do this by pressing the **Z+** or **Z-** buttons on the right-hand side of the controls. Once phase contrast is minimised you can press the 'high contrast' button in the fluorescent screen toolbar to regain some contrast. Move your view and the beam to vacuum such that you can align over the vacuum.
See also: *Titan modes manual pages 5-7*

Choosing condenser system

The Titan microscope has three 'strongly excitable' condensor lenses; **C1**, **C2**, and **C3**; make it a three condensor lens system. This comes with some advantages and disadvantages. Having the extra condensor lens allows the system to provide a range of beam widths for which it is guaranteed to be parallel. Beam parallelicity can be checked in the Beam settings workset next to the TEM button. With the Titan in TEM mode and all three lenses active the user will also enjoy a higher beam current focused on the specimen, this is especially helpful in the 'Mh' magnification mode. A downside of the three condensor system is that a slight misalignment between the lenses is amplified, changing illuminated area and magnification might erratically move the beam. Therefore, some user would like to switch off either the **C3** or **C2** lens. This choice should be made before alignment! To turn the **C3** lens off: click on the fly-out button of the Beam settings workset and press the 'C3 off' radio button.
See also: *Titan condensor manual page 3, 6 & 17*

Direct Alignments

The 'direct alignments' panel is placed under the 'Align' tab in the workspace browser on the left-hand side of the screen. When pressing one of the direct alignments the behaviour of the controls will change. For example, the 'gun tilt' direct alignment will bind the multifunction knobs to changing the gun lens coils instead of the usual beam position. So, be careful when aligning. In case something went wrong, or you've lost the beam; you can reset and restart by reloading the FEG register. In the 'direct alignments' panel there is also a tick box for 'Auto Help' when toggled a help window will appear anytime you select a direct alignment. This window will give you a brief description on how to carry out the alignment.

Aligning the C2 aperture (Direct Alignment)

When active the multifunction knobs will control the placement of the **C2** aperture in the column. During the alignment the **C2** lens will be repeatedly over- and under-focused, appearing as a disappearing and reappearing circle. When the **C2** aperture is misaligned the two circles will appear on different sides of the caustic crossover point. The centres of the under focused and overfocused image of the aperture should coincide, this can be done by turning the multifunction knobs. It is advised to move one knob at a time until you are close to alignment after which the two circles might appear to "orbit" around one another, at this time one might need to turn both knobs at one. Alignment is achieved when the centres overlap, and it looks like you are viewing a bouncing ball from above. To make the alignment easier you can turn what would normally be the 'focus step' ring to slow or speed up the over- and under focusing. If one circle is consistently larger than the other you can turn the 'intensity' knob to balance the size of the circles.
See also: *Titan modes manual pages 3-4*

Correcting condenser astigmatism

To correct the condensor astigmatism tighten the beam using the intensity knob and place it next to the reference circle of the FluCam overlay. The after pressing the 'condensor' button in the 'stigmators' workspace use the multifunction knobs to get the beam as circular as possible.
See also: *Titan modes manual page 10*

Gun tilt (Direct Alignment)

The goal of the gun tilt alignment is to maximise the intensity of the electron beam on the fluorescent screen. Turn the intensity knob to focus the beam to a caustic spot and centre it on the fluorescent screen. By clicking 'gun tilt' the multifunction knobs will be bound to the deflection of the electron gun image in the focal point of the **C2** lens. The goals are to turn the knobs in such a way that the screen current, which can be seen in the info section at the bottom of the screen, is maximised. To make the tuning easier you can press the fine button over the left multifunction knob to decrease sensitivity.

Gun shift (Direct Alignment)

Gun shift alignment serves the purpose of decreasing the distance the beam "jumps" when changing spot sizes. As changing spot sizes changes the strength of the **C1** lens it can move the image of the gun on the **C2** lens' focal plane. To align switch to spot size 9, focus to a caustic spot, centre the spot using the 'beam shift' direct alignment. Then switch back to spot size 3, focus to a caustic spot, and bring to the centre of the fluorescent screen by selecting the 'gun shift' direct alignment. Changing spot sizes is done by pressing the 'R3' button to increase and the 'L3' button to decrease. If you lose the beam you can demagnify the image to correct the beam position and then magnify again to perfect the cantering. After this recheck your **C2** alignment.

Pivot point purity (Direct Alignment)

Setting the pivot points for both the 'x' and 'y' lens pair correctly ensures point purity of the beam shift and tilt lenses thus ensuring that a beam shift will only shift the beam and not tilt it. Engaging the point purity alignment will wobble the beam tilt and shift lens pairs such that the image will jump between two positions that will overlap with no movement once point purity is corrected. The goal of the alignment is then to use the multifunction knobs to have both images appear in the same position.

Beam Shift (Direct Alignment)

Before carrying out the beam shift, it is best to recentre the **C2** aperture. The beam shift alignment will bind the multifunction knobs to 'beam shift (alignment)' which is different from the normal beam shift as this alignment will set the zero point for the beam to which it will return upon switching spot sizes. The goal of this alignment is to focus the beam to a caustic spot and centre this caustic as good as possible on the fluorescent screen.

Rotation Center (Direct Alignment)

The alignment will make the rotation centre coincide with the optical axis of the column the result be that the FOV does not shift when changing the intensity. To align the rotation centre you will need to line up your crosshair with a distinct feature and press the 'Rotation Center' direct alignment, this will start moving your view around the aligned feature. Using the multifunction knobs try to minimise the movement.

Aligning the column for High-Resolution STEM Imaging

Consideration

Scanning Transmission Electron Microscopy (STEM) in the Titan is very flexible with a lot of knobs to turn and options to consider. We have the flexibility to choose between *Microprobe* and *Nanoprobe*, change the *semi-convergence angle* α, switch *spotsize* and lastly choose a *camera length*. A brief explanation of each setting is given below.

- **Probe Mode**
  The probe can be set to either microprobe or nanoprobe mode in the Beam Settings workset. TEM illumination uses, by default, microprobe mode. In microprobe mode the maximum illuminated area is $5\times$ larger but the divergence and/or convergence is $5\times$ weaker. In nanoprobe the mini-condenser lens that is below **C3** is turned off whereas in microprobe mode it is turned on. Even though it is off the mini-condenser lens' stigmator can be used in nanoprobe mode.
- **Semi-convergence angle** The semi-convergence angle α determines the angular radius of the conical probe shape and is mainly influenced by the aperture. In normal range nanoprobe mode to semi-convergence angle is continu-

ously adjustable between roughly 6 mrad - 30 mrad, a larger range is available in large range mode. This can be selected, when *Free Ctrl* is active, in the *Beam Settings* workset. The Titan Condenser manual notes that the optimal resolution is achieved at a semi-convergence angle of 10 mrad. Note that this is the actual radius of the Ronchigram not the semi-convergence angle listed in the software as this is most likely the selected aperture.
The semi-convergence angle sets the electron modes which can be used for probe forming in the STEM system, selecting the 'nice' modes should be done with an appropriately sized aperture.

- **Spotsize** The spot size number is an indexing number between 1 and 11. It is an aligned preset for the first condenser lens **C1** and determines its strength. The spot size number tells you roughly how much the image of the electron gun is magnified by the **C1** lens, spot size 1 is the 'weakest' lens-setting and yields the smallest magnification of the gun image. This small demagnification means that a large part of the image of the gun can be selected with the aperture thus yielding a large 'spot' with high brightness. The higher the spot size number the greater the magnification of the gun image thus the smaller the part of the gun you are selecting with the **C2** aperture with the result being a smaller intensity but a smaller 'spot' after demagnification by the rest of the condenser system.
- **Camera length** Since in STEM mode the magnification knob is bound to change the scan size area we can not use this to enlarge the image on our fluscreen and later on the image on our detector. To achieve this in STEM mode we use the *Camera length* in the 'STEM Detector' workset. To visualise, imagine a cone whose tip is coinciding with the sample and a plane intersecting this cone at a right angle as a camera. Moving this plane closer or farther away will change the image of the sample on the plane, thus (de)magnifying the image. By changing the camera length when using a dark-field detector one can select which reflections of the crystal (disks in the CBED) are incident on the detector, when using the EMPAD or fluscreen the camera length will this magnify or demagnify the image on the respective screen or detector.

Pre-alignment

Tilting to zone axis, SAED, dark field imaging

Energy Dispersive X-ray Spectroscopy

Electron Energy-Loss Spectroscopy techniques

4D-STEM techniques with the EMPAD

Diagnostics

Correcting dark and gain (Removing horizontal bars)

1. On the top left of the screen open the camera settings page.

2. Then select the CETA camera and open the fly-out.
3. In the fly-out menu open the ”dark and gain” settings tab.
4. Press the singular button and load a correction file or the most recent one.

No beam control in STEM mode

No image using the HAADF or other dark field detectors $\rightarrow$ check that beam control is on the Titan PC by having ”INT SCAN” pressed.
No image using the EMPAD detector $\rightarrow$ check that the beam control is given to the EMPAD PC by having the ”EDX SCAN” button pressed.

Stage Disabled

Symptoms of a disabled stage are as follows: can not move stage with stick, no dialogue option to select holder, can reset holder position or red light on loading point. Resolve by unloading the holder from the Titan, then:

1. Move to the Stage workspace
2. Click on the fly-out arrow and navigate to the rightmost tab called ‘settings‘
3. Verify that the ‘enable‘ button is gray
4. Remove any holder and then press the ‘enable‘ button. It should turn orange.
5. Wait until the countdown of 5 min has elapsed, and the button has turned yellow. During the countdown the stage will move to all its extremities and back. The grinding noise is therefore ‘normal‘.
6. When the ‘enable‘ button is yellow, you can reinsert the holder.

High Tension Off

High tension is off, and acceleration voltage is set to 60 kV.

1. Ensure gun vacuum is of high quality. To check click the fly-out arrow on the workspace and verify that ‘igpi‘ is 1 log.
2. Press the ‘High Tension‘ button.
3. In the dropdown menu click the next voltage step. Verify that vacuum stays at same level, otherwise halt operation and toggle off high tension. If the voltage has stabilised repeat.