

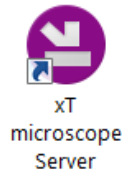
ThermoFisher Apreo C SEM SOP

April 2023

These instructions are for generic, basic use of the SEM. The system is capable of more advanced work: EDX detector, STEM detector, Low Vacuum detector (LVD); if you need access to those capabilities please request additional training.

BASIC OPERATING INSTRUCTIONS

1. Log into the tool by using FOM, it will turn on the monitor.
2. Start Microscope Control by clicking on **Start UI** (User Interface).






NOTE: The xT Microscope Server should be open and running all the time.

3. To login to the Microscope Control software for all microscope users:

User: user

Password: user

Loading a Sample:

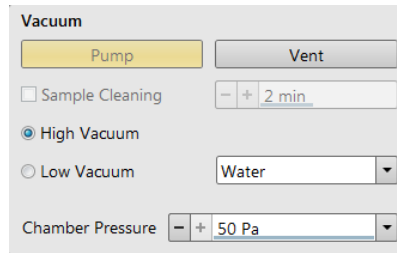
1. If **Sample Exchange Window** is not open, click on  to open it.
2. Click **Chamberscope** tab. If the icon  appears in the Chamberscope image, click to un-pause.
3. Click **Vent** to initiate the vent cycle. Confirm by pressing the vent button in the pop-up window.
4. A green status bar will appear at the bottom of the Sample Exchange Window, wait for the status bar to disappear, indicating completion of the vent cycle. 


NOTE: Wear gloves when handling samples!

5. Gently slide open the chamber door; place your sample stub in one of the holes in the stage. Make sure your sample is securely mounted to the stub. If the sample is placed in a position that has a set screw, do NOT over-tighten the screw. Only finger-tight. Notify the lab managers if the set screw is getting stripped.


NOTE: If loading multiple samples, limit stubs to one type per session. Do not mix flat stubs with 45° stubs or 90° stubs to avoid collisions with the pole piece due to different sample heights.

- While watching the live CCD chamber view, gently slide the chamber door closed. Ensure the chamber door is fully closed, then click **Pump** in the **Sample Exchange Window**.

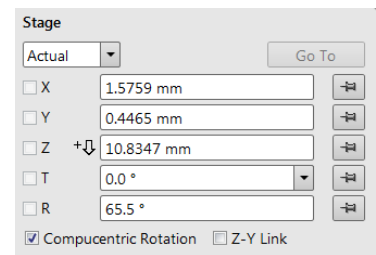



- Choose **“No Accessory”**, unless the low vacuum **PLA** (Pressure Limiting Aperture) cone is installed on the pole.
- Wait for the pumping status bar to reach the ‘full’ mark and then disappear, indicating the pump-down sequence is complete (~2 - 5min). Additionally, the chamber/column vacuum status icon at the bottom of the main UI window will appear fully green. 

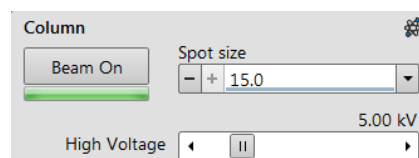
Preparing to Image: All steps must be completed!!!

- Lower the magnification to its minimum by rotating the Magnification knob on the control panel counterclockwise.
- Click the Stage Control button  and raise the stage to **15 mm**.






NOTE: The above step is optional **ONLY** if your sample is tall.



- At the top of the UI window, click **Stage** and select **Take Nav-Cam Photo**. Wait for the Nav-Cam operation to complete (~ 1min).
- An image of your sample should appear in Quadrant 3 (Q3, the lower-left quadrant).
- Navigate to your sample by double-clicking a location in the Nav-Cam photo.
- In the **Sample Exchange Window**, raise the sample stage to the **10 mm** marker by holding the middle mouse button and dragging upward; release the mouse button when your sample reaches the 10 mm marker.
- Set accelerating voltage and Spot Size/ Beam Current in the drop-down menus; recommended initial values are
 - High Voltage: **2kV or 5 kV**
 - Spot Size: **6.0/ Beam Current: 25pA**.
- Click the Beam Control  button on the side pane at the right of the UI.
- Click **Beam ON** to turn on the beam; the button will turn from grey to orange and you will hear a pneumatic valve actuate within the SEM column.



Basic Imaging: Steps 1 thru 9 must be completed before continuing with imaging!!!


1. Click within Quadrant 1 (Q1, upper left) to activate the quadrant; the data bar at the bottom of Q1 should be blue, indicating Q1 is active.
2. Click the Detectors button  on the side pane at the right of the UI. Select ETD from the dropdown menu, this assigns the ETD to the active quadrant, Q1.
3. Click the green pause button  in Q1 to turn on the detector and begin imaging.
NOTE: You can press the grey/black pause button at the top of the UI at any time to pause or un-pause image acquisition for the *active* quadrant. **Please be aware that only two quadrants can be active at the same time.**
4. Adjust **Brightness and Contrast** manually by rotating the knobs on the control panel or perform the Auto Contrast & Brightness function by pressing  or **(F9)**.
5. Adjust the Focus knob, then increase magnification to at least 5,000x and re-focus.
6. Click the **Link Z to FWD** icon. 
7. With the sample in focus, pressing this icon will link the position of the sample to the focal length of the microscope establishing the Free Working Distance, which is the distance between the sample and the bottom of the pole piece.
8. Before **Link Z to FWD** is clicked, the icon appears with a question mark (as shown above); after clicking, the icon changes to  indicating successful linking.
9. After linking, the stage Z-coordinate will display the working distance between the pole piece and portion of the sample used during linking.

NOTE: If your sample is not flat or is mounted such that it is not parallel to the sample stage, you risk collision with the pole piece at smaller working distances. For *non-flat* samples, focus at the highest point on the sample and then **Link Z to FWD**.

10. You can now navigate across your sample to locate features of interest. Navigation can be accomplished in several ways:

Mouse: Double-click a point on the Nav-Cam image **or** the electron detector image to move the stage to that location. **NOTE:** Nav-Cam navigation is typically used at lower magnifications, to navigate to approximate locations on a sample (due to resolution limits of the optical Nav-Cam image)

Arrow keys: While imaging the sample with the ETD in Q1 (note: Q1 must be *active*), pressing the left /right /up /down arrow keys on the keyboard will shift the stage in a given direction. The magnitude of the shift is ~ 80% the field of view.

Stage coordinates: Click the Stage  button on the side pane. You can enter in specific x-and y-coordinates to drive the stage to that location. Sample Rotation and Sample Tilt are also available.



NOTE: The use of Tilt should be done with caution. Tilting samples greatly increases the risk of a pole piece collision. Always view the LIVE CCD image when tilting. Tilt with caution! **Maximum tilt angle** allowed is **30°**. Or use pre-tilted stubs (45° or 90°).

Some Notes on Working Distance:





You should enable the live CCD view whenever you change WD (the z-coordinate of the sample) or whenever you Tilt; this is to ensure there is NO chance of a pole piece collision.

NOTE: If you have initiated stage movement and you fear a pole piece collision is imminent, you can halt stage movement by pressing the **ESC key** on the keyboard. Be ready to press ESC!

Improving Image Quality:

1. Iteratively adjust Focus, then X-Stigmation and Y-Stigmation to achieve better images.
2. Single Image Mode (**F5**)  shows one display over the whole UI imaging area and is useful for observing details when focusing and correcting astigmatism.
3. Reduced Area (**F7**) mode is recommended to use, it is handy when focusing and correcting astigmatism, as the imaging update is faster in the smaller area.
4. Perform Lens Alignment:
 - a. Make note of some feature in the live image in Q1.
 - b. Click the Lens Alignment button. 
 - c. The image will begin to wobble. Drag the cross-hairs that appear on the wobbling image in the x- and y-directions. The goal is to minimize the wobbling in the image.
 - d. After minimizing wobbling, click the lens alignment button again.

Capturing Images:

1. Users can adjust frame averaging, dwell time and scan resolution by adjusting settings from the dropdown menus at the top of the UI: 
or users can apply one of three preset scans available by pressing the **s1**, **s2**, **s3** buttons at the top of the UI. 
s1 – TV scan rate with a 0.1s frame acquisition time
s2 – HDTV scan with a 0.2s frame acquisition time
s3 – this button can be edited / customized by the user; right click on the s3 button and select edit.
NOTE: Please do not edit **s1** or **s2**, only edit **s3**.
2. There are two preset image capture buttons; pressing either of these will scan and capture the image and prompt the user to save the image file:
 - The **Snapshot** icon  (**F4**)
 - The **Photo** icon  (**F2**)

NOTE:

- Save the un-annotated grayscale image as an 8-bit or 16-bit .tif image, 24-bit images are useful only for color. This file format is as uncompressed as the system can provide, while also including the metadata of the system parameters in the footer of the file.
- Annotated images may be saved as .jpg as a version of note-taking. These images are compressed, so data will be lost.




Recommended setting for taking SEM photo

Type of the Sample	Most Conductive	Less Conductive	Poorly Conductive	Very Poorly Conductive
Resolution	1536 x 1024 or higher	1536 x 1024	1536 x 1024 or higher	1536 x 1024 or lower
Dwell Time	20us - 40us	1us - 5us	200ns - 500ns	100ns - 500ns
Scan Interlacing	1	1	1	4 - 8
Line Integration	1	4 - 16	50 - 200	1
Frame Integration with Drift Correction	Integrate = 1	Integrate = 1	Integrate = 1	Integrate = 50 - 200 Drift Correction=Yes
Total Frame Time target	30s - 90s +	30s - 90s	20s - 90s	20s - 90s

NOTE:

- In general, poorly conductive samples behave better with lower landing energy and lower beam current.
- The retractable CBS is a solid state detector and usually need > 1kV energy and >3uS dwell time for best contrast.
- In LoVac Mode, the LVD detector usually needs > 1uS dwell time for best signal integration.

Sample Removal / Ending Your Session

1. Click  and set Scan Rotation to 0° if not already.
2. Click  and set WD to 10mm or greater; If Tilt or Rotation were used, set both to zero; set stage translation X=0 and Y=0
3. Click  and then to turn off the beam.
4. Write down the info in LogBook.
5. Click **Vent** and confirm by pressing vent in the pop-up window.
6. If Q3 was changed, set back to Nav-Cam view, if Nav-Cam zoom was used, click **View > Undo Digital Zoom** at the top menu of the UI.
7. If Q4 was changed, set back to Camera view.
8. Wait for vent cycle to complete, then slide open the chamber and retrieve your sample.
9. Slide chamber closed and press **Pump**.
10. When the pumping is finished, click stop UI. It will close the SEM software.
11. Logout in the FOM from the SEM.



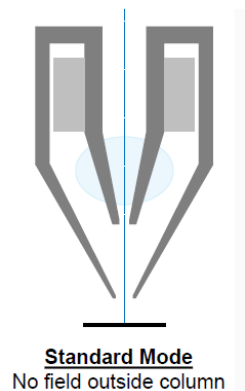
ADDITIONAL INFORMATION

Column Use Cases

The electron column can be operated in different **Use cases** optimized for specific applications. To select the desired Use case, use the Use cases module drop down list box. You can immediately switch from one Use case to another. If high voltage or beam current is out of the operating range of the new Use case, it is set automatically to the closest possible value. If the newly set conditions do not allow imaging, (e.g. it is not possible to focus), transition is not allowed, and a warning is shown in the application status window.

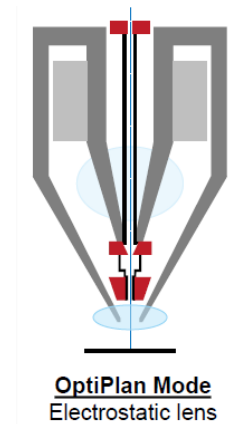
Standard

This is the survey mode ideal for navigating and reviewing sites at lower magnifications. The Acceleration tube (A-Tube) is on the ground potential, and the default detector is the ETD in the Secondary Electron mode. **Maximum probe current is limited to 13 nA.** Probe currents above 13 nA are available for analytical applications such as EDS, WDS or EBSD. It should be used when higher probe currents are required. Currents can be as high as 400 nA; the accelerating voltages above 13 nA are available within 5 to 30 kV. The A-Tube is at ground potential, the ETD detector should be used for secondary electrons and T1 or DBS (optional) for backscattered electron imaging.



OptiPlan

Here primary electrons are accelerated by the potential of the A-Tube and pass through the column at high energy. They are decelerated back in between the T1 detector and the sample. Secondary as well as backscattered electrons are also collimated into the final lens by the A-Tube electrostatic field and detected by the Trinity detectors (T1, T2, T3). This mode is used for ultrahigh resolution electron imaging of the sample at short working distances (**1 – 2 mm**). The A-tube is at the highest potential and the T1 and T2 detectors should be used. The full range of the Beam Deceleration mode is available for this Use case. In this mode, the maximal working distance depends on the landing energy (Beam Deceleration mode). It ranges linearly from 5 mm (at 200 eV) to 70 mm (> 10 keV). It is possible to use overview imaging at the maximal field of view, so there is no need to switch the Use Case for navigation purposes.



SEM column Use case	Spot size	Best Use for
Standard /OptiPlan	1 - 4	Very low currents, charging and sensitive samples
Standard /OptiPlan	4 - 6	High resolution
Standard /OptiPlan	6 - 12	Standard imaging
Standard /OptiPlan	12 and more	High current imaging, X-ray analysis with SDD detectors
Standard /OptiPlan	10 - 17	High current imaging, X-ray analysis, EBSD detector, cathode-luminescence
Standard /OptiPlan	18 - 20	Very high currents, fast analysis

Detectors T1 / T2

Electrons generated by a primary beam can be collected by the in-lens Trinity detectors T1 and T2, which are located inside the final lens.

NOTE: Whenever the T1 or T2 is selected, the optical display is paused (the CCD camera infra-red LED's are switched off to keep emitted photons from supersaturating the detector).

T1

The T1 detector is primarily designed to collect backscattered electrons. It provides composite sample contrast. In the OptiPlan Use case, it detects backscattered electrons throughout the whole range of accelerating voltages thanks to the A-Tube at high potential that accelerates the BSE towards the detector. In the Standard column Use case, the T1 provides strong BSE contrast at accelerating voltages of 5 kV and higher. The Compound Lens Filter is optimized for this detector especially.

The functional detector area is split into two halves, and the detector can be operated in four modes. Apart from the composite mode A + B, the detector can be operated in the topographical mode A - B, where a pseudo-topographical imaging with suppressed atomic number contrast and maximum topographical contrast is obtained. Signals from each half can be also collected separately in modes Segment A / Segment B.

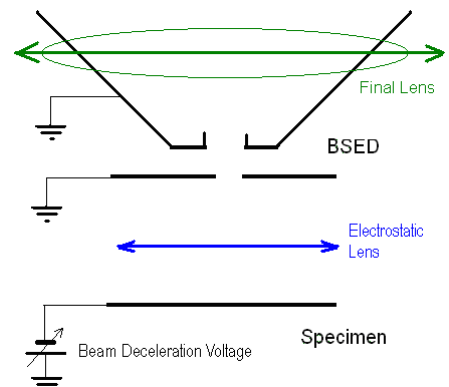
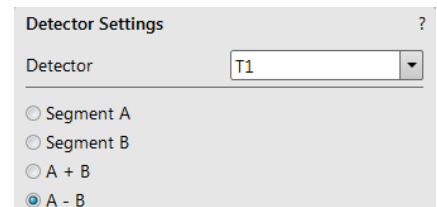
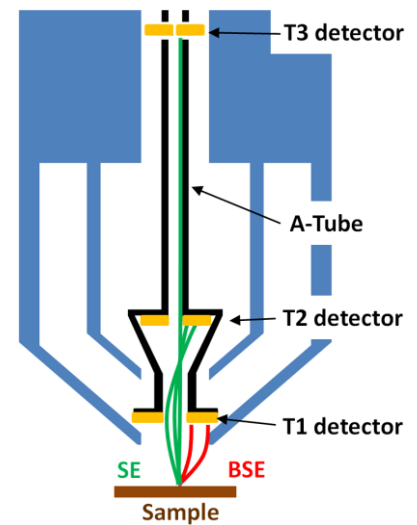
T2

The T2 detector is primarily designed to collect secondary electrons and provides information of the sample topography in the OptiPlan Use case. Thanks to the high potential of the A-Tube, the SE are accelerated and collimated to the T2 detector. It can be operated with the grounded A-tube as well - Standard column Use case. In this case, backscattered electrons are collected, but the accelerating voltage must be at least 5 kV. The intensity of the signal in the Standard column Use case is dependent on the beam current and working distance – the higher the beam current and the shorter the WD, the higher the T2 signal.

Beam Deceleration

The Beam Deceleration (BD) mode is based on a negative voltage (bias) applied to a stage (i.e. a sample). An electrical field is formed between the sample and the nearest surface over it (a column bottom or a detector). This field acts as an additional electrostatic lens.

Beam Deceleration influences both primary and detected electrons.

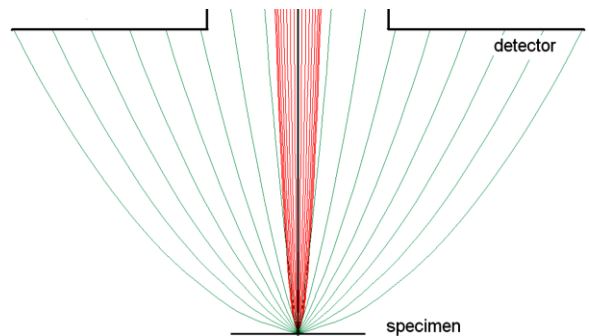


As the sample is at a negative potential with respect to the ground and detectors, the initial SE and BSE energy (when leaving the surface) is accelerated by the Stage Bias before the detection. The higher the Immersion Ratio is, the lower the difference between SE and BSE energies is when detected.

Signal electrons are accelerated upwards and deflected towards the column axis.

The SE's have a low initial speed. They are usually absorbed into the detector central hole, continue through and can be detected by in-column detector T2.

Conversely, the BSE, heading nearly parallel to a surface (which normally cannot be detected), are driven to a detector. By changing the Stage Bias, the output angle of electrons leaving a surface can be changed.



Detectors most convenient for Beam Deceleration are BSE detectors placed closely under or directly inside the column. Their efficiency depends on their active area – the smaller the inner diameter of the active area is, the better. The standard ETD can also be used, but its efficiency is low.

Restrictions

In the LoVac mode, the chamber environment is rather electrically conductive, and the BDM is not available.

Sample preparation and limitations

In general, all types of samples can be imaged using the Beam Deceleration mode. However, there are several types with certain limitations that should be mentioned:

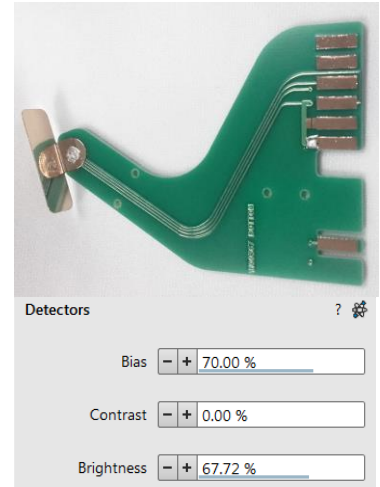
- **Tilted samples** Tilted samples, such as non-horizontal fractures, may cause distortion of the electrical field deformation, which adds non correctable aberrations and an image distortion. In general, the flatter the sample the better for the resolution and ease of use (fewer corrections of astigmatism and lens alignments). An acceptable sample tilt is about a few degrees; for higher immersion ratios, it is preferably less.
- **Particulate samples** All samples consisting of small (micro- or nanoparticles) particles must be prepared to minimize presence of volatile particles on the stub when loaded into the chamber. The sample can be prepared either from solution (dilute the powder in ethanol or isopropyl alcohol, disperse and put few droplets on a clean stub or piece of wafer) or fixed by carbon paste or tape. When pumping and venting, keep the sample at long working distance (10 mm) and ideally off the pole piece. Imaging can be done on any working distance. For the systems with T1 detector, the shortest working distances are interlocked by the software.
- **Non-conductive particles, fibers, wires and rods** These samples are very sensitive to charging induced by an electron beam. When charged, they have tendency to be attracted by the electric field of the Beam Deceleration and move around the stub or travel towards the final lens pole piece. Therefore, these samples must be securely fixed to the stub. Longer working distance or lower Stage bias is another way that prevents the column from the particle contamination. Decreasing of a sample exposition to the electron beam (by setting of low voltage, low current, and short dwell time) is recommended.

Low Vacuum Detector (LVD)

This detector is standard and optimal for general imaging in Low Vacuum mode over the entire pressure range from 10 – 500 Pa (0.08 to 4 Torr). Its signal contains both secondary and backscattered electrons. The LVD detector limits the field of view to about 1 mm at the 10 mm working distance. The minimum working distance is limited to about 3 mm.

LVD Settings

After the pump down and purge processes, switch on the accelerating voltage. The modified Detectors module / Bias slider is automatically activated. Adjust Bias if necessary to increase the signal or to stop possible discharges. At the lowest possible magnification, adjust the Contrast and Brightness, if necessary, until the bright circle of the LVD cone can be seen in the center of the image. Increase magnification and adjust Contrast and Brightness more precisely.

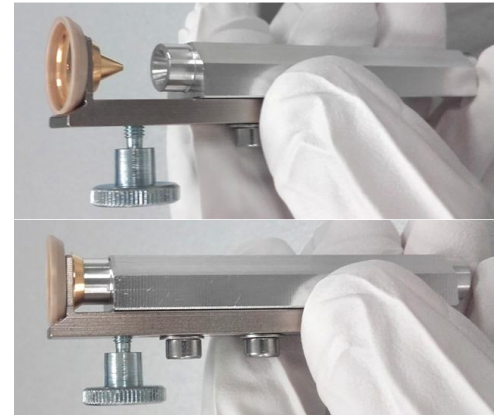


X-Ray Cone Mounting / Dismounting Procedure

Under normal operation, the system will know the PLA cone size for the detector installed in the LoVac mode. This will allow the system to set automatic pressure range limits for the aperture installed, thus avoiding vacuum errors when setting the chamber pressure. The X-Ray cone is stored in the provided installation tool. The large arresting screw releases the working part so it can slide along its skid.

To mount the **X-Ray Cone** follow this procedure:

1. Push the cone attached in the tool to the final lens pole in its axis direction.
2. Release the arresting screw, slide the working part down, and pull the tool to the side.
3. Turn the tool by 180° and push the cone again to ensure it is sealed well. To dismount the cone, follow **Steps 1 and 2** in reverse.



NOTE: Do not touch the cone or the mounting tool by hand. Store it attached to the mounting tool in a clean plastic bag.

4. Select an appropriate cone and click the Vacuum module / Pump button. While pumping, choose the highest specimen point and bring it to the 10 mm Working Distance (yellow line in optical display).

Low Vacuum (LoVac) mode

In this mode, the column section is under lower pressure than the specimen chamber, where the pressure ranges from 10 to 500 Pa (0.08 to 4.0 Torr) depending on the Use case and PLA used:

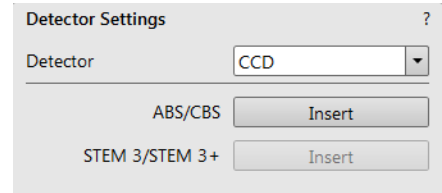
- Standard Use case without PLA: **10 – 50 Pa**
- OptiPlan Use case with PLA: **10 – 150 Pa**
- Standard Use case with PLA: **10 – 500 Pa**

High Vacuum (HiVac) mode

For High Vacuum, the system achieves the lowest pressure possible throughout the column and specimen chamber. The typical pressure value is within the order of 10^{-2} to 10^{-4} Pa.

Retractable detectors control

When any retractable optional detector is installed in the system, the appropriate **Insert/Retract** button is added to the Detector Settings module/Detector CCD to enable control of the equipment while observing the optical imaging display. The same button is also present in the appropriate detector module. To insert any retractable detector, a confirmation dialog appears. When insertion is not possible, tooltip gives a reason why.

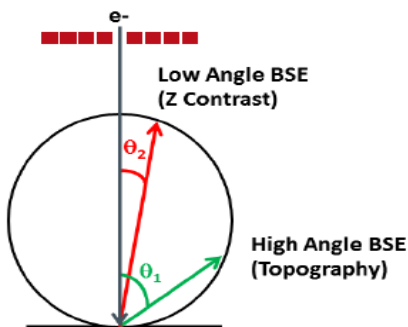


Detector insertion not allowed:
Please link Z to FWD

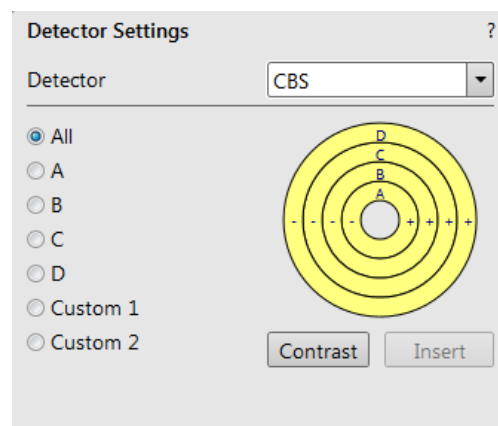
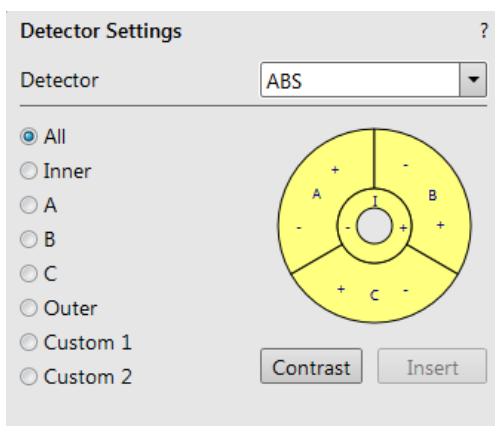
Detector insertion not allowed:
Please calibrate the rotation axis first

Directional Backscattered Detector (DBS) – Angular Backscattered Detector (ABS) Concentric Backscattered Detector (CBS)

The ABS uses angular segmentation of the detector diode to distinguish topographic contrast from different signal directions. The CBS uses concentric segmentation of the detector diode to distinguish between BS electrons scattered close to the beam axis - inner segment (preferentially composite contrast) and electrons scattered far from the beam axis - outer segment (more topographical signal).



Low angle scattered electrons are captured by the inner rings.
Wider angle scattered electrons are captured by the outer rings.



Retractable Annular Scanning Transmission Electron Microscopy Detector (STEM 3 / STEM 3+)

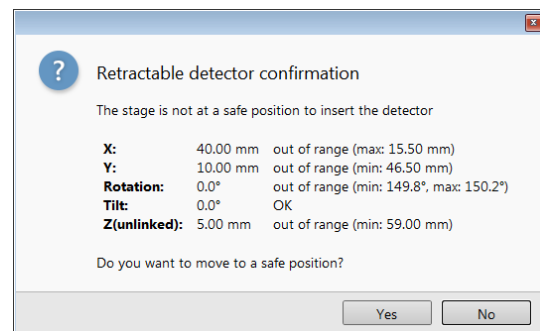
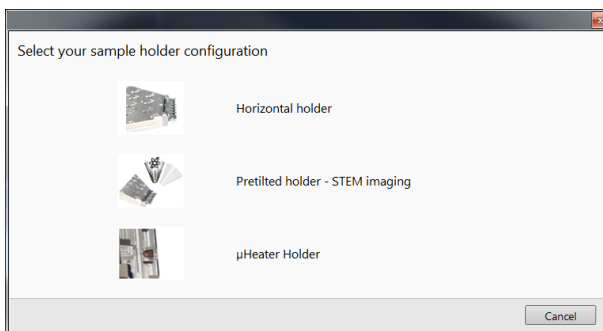
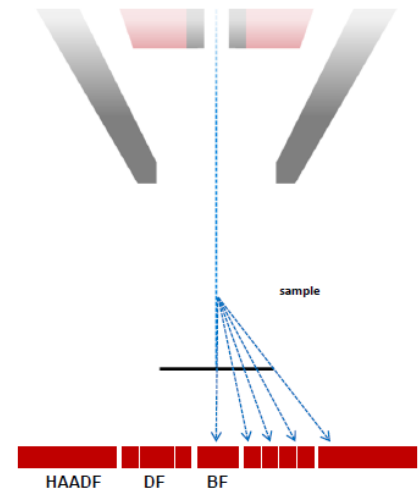
This is a solid-state diode mounted on a retractable arm. It works best at a slow scan conditions.

Inserting and retracting STEM 3 / 3+ detector

Before insertion of the retractable STEM 3 / 3+ detector, the chamber must be pumped, otherwise the Insert button is not active (a tooltip occurs under mouse cursor). When the STEM 3 / 3+ detector is inserted stage movements are restricted automatically for the safety.

When clicking on the **Insert** button, the **Select your sample holder configuration** dialog requires determination of the correct sample holder configuration to enable safe detector insertion. When the stage is not in the correct position for insertion, another dialog appears requiring confirmation of moving it to the safe position.

Retraction of the STEM 3 / 3+ is automatic when Stopping / Starting the server or venting the chamber. Otherwise a user can use the Retract button. When the detector is retracted, this information text is shown in each display that uses it.



Settings for STEM 3 / 3+ Detector

1. Position the desired sample grid in the field of view using the ETD. Focus and link Z coordinate to FWD. 2.
2. Select the Detector Settings module / Detector list box / STEM 3 / STEM 3+ detector.
3. Insert the STEM 3 / 3+ detector

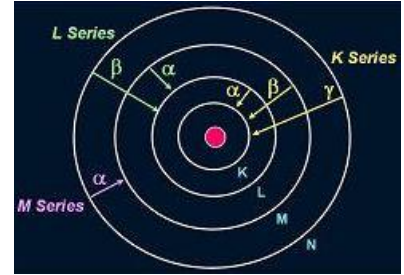
Custom segments setting option is available for STEM 3/3+ detector. Click on the Custom Angular radio button. You can select / deselect any combination of outer segment partitions by clicking on them in the detector diagram. Selected segments are highlighted in yellow.

- + sign – segment signal is added (**yellow color**)
- - sign – segment signal is subtracted (**blue color**)
- no segment signal (**gray color**)

Energy Dispersive X-ray (EDX) Analysis

High Vacuum HiVac operation gives the most accurate X-ray results, but the sample must be electrically conductive.

The EDX (sometimes also referred to as EDS analysis) is a technique used for identifying the elemental composition of the specimen, or an area of interest thereof. It works as an integrated feature of a scanning electron microscope (SEM), and cannot operate on its own without the latter. The specimen is bombarded with an electron beam inside the microscope column. These electrons collide with the specimen atoms' own electrons, knocking some of them off in the process. Positions vacated by ejected inner shell electrons are occupied by a higher-energy electron from an outer shell, while giving up some of its energy by emitting an X-ray. The amount of energy released depends on which shell it is transferring from / to. The atom of every element release X-rays with a unique amount of energy. This identifies it. The output of an EDX analysis is an EDX spectrum, which is just a plot of how frequently an X-ray is received for each energy level. The higher the peak in a spectrum, the more concentrated the element is in the specimen.

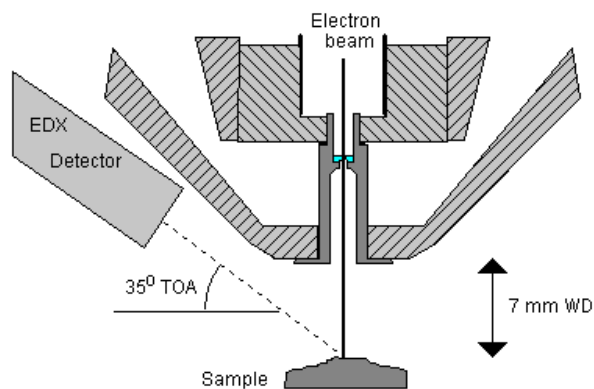


Low Vacuum EDX Analysis

X-ray analysis in LoVac mode is possible in combination with the standard LVD, the LVD detector offers the largest field of view for the LoVac operation. The CBS is compatible with EDX. The X-ray analysis should be performed at the lowest possible gas pressure to minimize interaction of the electrons with the chamber gas. Normally, it is performed with a relatively high beam current so that there will be enough signal for a good LVD image even at very low gas pressures.

STEM EDX Analysis

Set the sample surface to **7 mm WD**. Select the area of interest in the STEM mode and perform X-ray analysis, mapping, or line scans as appropriate. Because the samples are not bulk in nature, the beam spread normally associated with SEM samples is greatly reduced, and therefore higher spatial resolution can be obtained with the STEM detector. This also provides less background in the spectrum and allows better separation of peaks as well as more accurate lower count rate mapping. The high voltage chosen for the analysis still depends mainly on the composition of the sample.



Recommended SEM settings for different Detectors

Detector	Beam	Spot size/ Current	Working Distance
ETD	2-5 kV	6 – 8 / 25 - 100pA	10 mm (recommended) or less
BSD	5 - 10 kV or greater	8 - 10/ 100-400pA	5.5 - 6 mm
STEM	30 kV	3 - 6/ 50pA	8 - 10 mm
T1 & T2	2 - 5 kV	6 - 8/ 25 - 100pA	3 - 5 mm, 1 mm for HiRes
LVD	5kV w/o PLA, 2kV with PLA	>7 - 9 /50pA	8.5 - 10 mm, min 3 mm
EDS	20 kV	10-12 / 1.6 nA - 400nA (adjust for 30-40% dead-time)	8.5 mm (recommended)