Quick Guide to Operate FEI Titan Themis G2 200 (S)TEM: STEM mode

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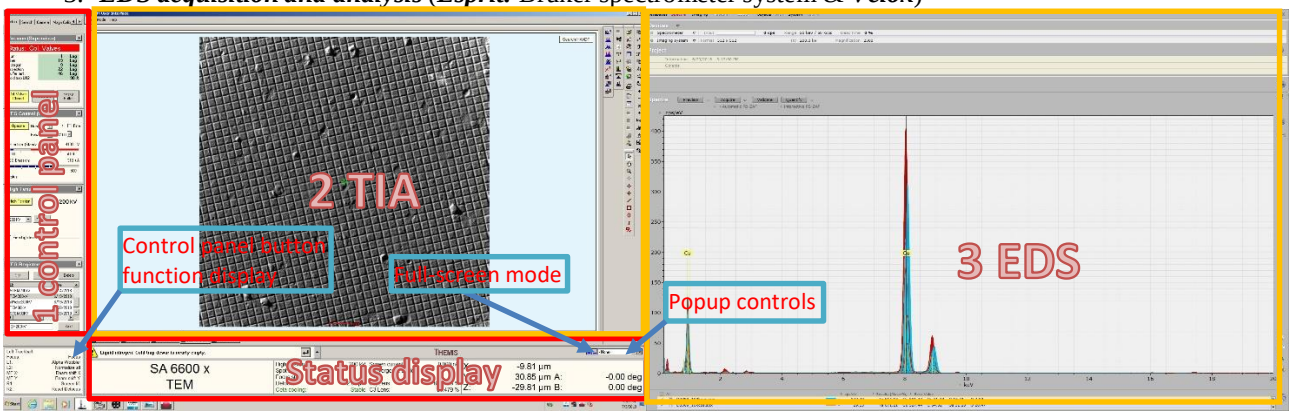
I. Before starting a session:

- Log in to your instrument session at https://fom.engr.pitt.edu

II. Microscope User Interface

The microscope operation interface consists of following programs:

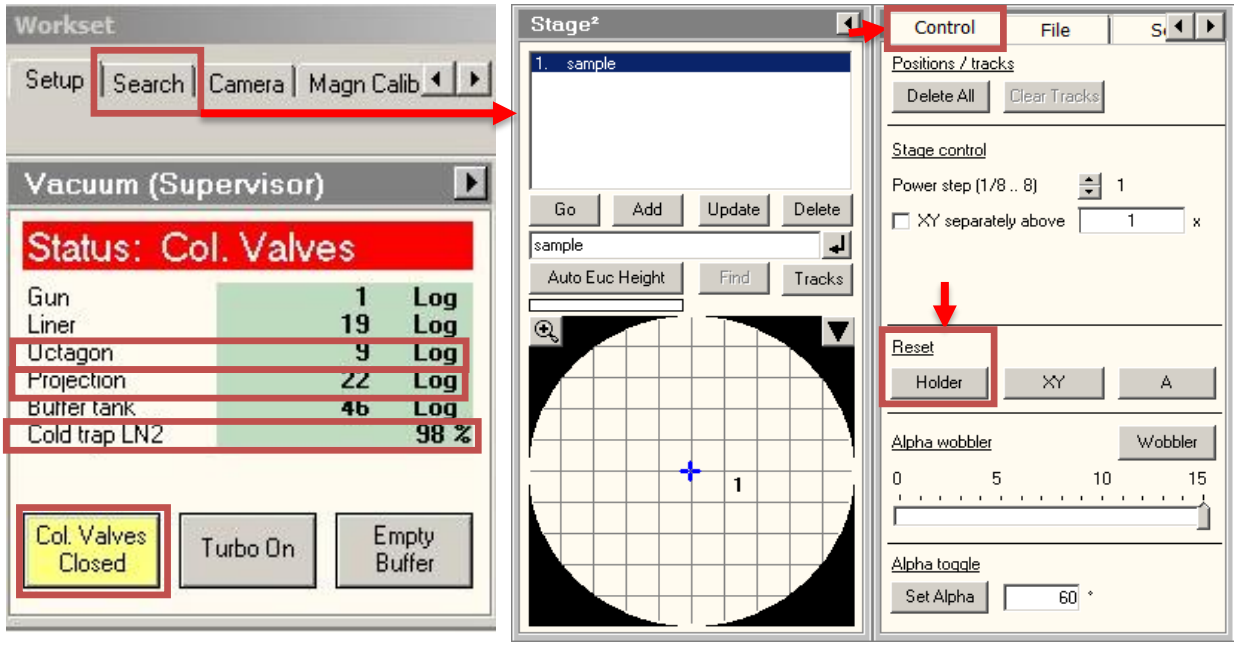
1. ***TEM User Interface***: general microscope operation and the display of live TEM image
2. ***TEM Imaging & Analysis (TIA) & Velox:*** image acquisition and analysis
3. ***EDS acquisition and analysis*** (***Esprit:*** Bruker spectrometer system & ***Velox***)



Overview of the microscope user interface, TEM User Interface and TIA on the left and EDS on the right

III. Check and confirm the following conditions:

a. Good **Octagon** (column) vacuum (< 20 log, <10^{-7} Torr), Projection (camera chamber, < 30 Log)
b. Good **liquid nitrogen** level (> 10%)
c. Close the **column valve** before sample holder insertion/removal
d. Reset the **stage** (***holder***) before sample holder insertion/removal



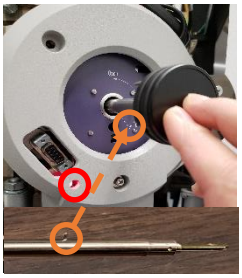
IV. Choose Sample Holder

Wear gloves when handling a sample holder to prevent contamination. Never touch the brass part of the holder.

There are three types of sample holder available for different application:

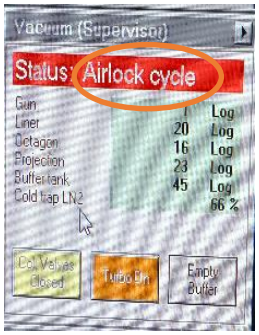
1. ***Single-tilt***: morphology observation, EDS acquisition (-30° ~ + 30°)
2. ***Double-tilt (low background)***: morphology, detailed crystalline material study, EDS acquisition (-30° ~ + 30°)
3. ***Tomography (low background)***: similar to single-tilt holder but with lower holder profile allowing high alpha tilt angle (-70° ~ +70°, depends on specimen Eucentric Z setting)

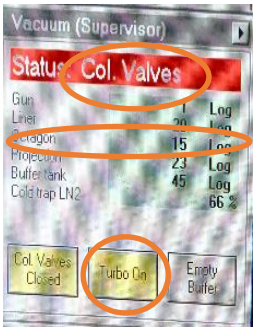
V. Holder Insertion/Removal



When Inserting a Holder

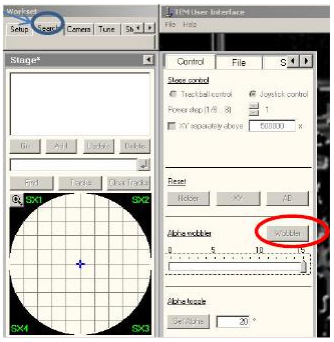
1. Align the holder guide pin with the **Close** line mark on the goniometer cover and insert the holder straight in to the stop of the stage load lock area (LED will light up in Red)
2. Select the apropriate holder from TIA Status display (*if double tilt holder, then connect cable to Y-connector on goniometer*)
3. Keep finger pressure on the holder (***no rotating***!!) to activate pumping process
2. Wait for the load lock to pump down (until **Airlock cycle** times out, ~3 minutes)
3. Slowly and smoothly rotate the holder *Counterclockwise* to its stop position and let it gently into the microscope column area (***Octagon: < 20 Log***)
4. Wait for **1 minute**, and then turn off the Turbo pump by clicking **Turbo On** button (color changes from yellow to gray)





When Removing a Holder:

1. Close column valves by clicking **Col. Valves Closed** button (color changes from gray to yellow!)
2. While pressing the purple stage cover down, ***slowly*** pull the holder straight out till its stop
3. Rotate the holder ***clockwise*** till its stop
4. Wait for ***30 seconds***, then
5. Slowly pull the holder straight out while you are still pushing the purple stage cover down



VI. Find Specimen Eucentric Height at TEM Mode

1. (***Optional***) Load the most recent alignment from → **Workset → Alignment → File →**, *e.g.* ***Routine 200kV***
2. → **Workset → Setup → FEG Registers**, select and set the ***most recent*** FEG Registry, e.g. ***RoutineTEM 200kV***
3. Set Specimen Eucentric Height (Z)
    1) Open column valves by clicking **Col. Valves Closed** button (color changes from **yellow** to **grey**!)
    2) Press the "**Eucentric focus**" button and ***normalize all*** **(L2)** button
    3) Set at a lower magnification (e.g. ~10,000x) and find a reference object
    4) Activate the stage "**Wobbler**" (**L1**) and observe object movement
    5) Reduce the movement by moving the Z up or down
    6) Repeat steps 3 – 5 at higher magnification (~50,000x) to fine tune the z position

    Note: *if your sample is crystalline, then converge the beam, move Z up or down until diffraction pattern converge to the center spot*

VII. Tune Probe Cs and Image in STEM Mode

1. → **Workset → Setup → FEG Registers** → set the most recent FEG Registry, e.g. ***RoutineSTEM 200kV***
2. Select ***spot size*** 9 and adjust ***Gun Lens*** value in **FEG Control** so as to reach a target Screen Current: ~30 pA
3. From **Workset → STEM** tab, set the ***camera length*** to ***135 mm***
4. Press the "**Eucentric focus**" button and normalize all lenses
5. Select a suitable magnification, *e.g.* 225,000x – 500,000x
6. Click **Search** to start scanning
7. Find amorphous area on a sample and bring it close to ***focus*** (within 200nm) by adjusting the **CompuStage Z**.
8. Optimize the ***contrast and brightness*** setting of the **STEM detector** (*adjust Contrast/Brightness sliding bar to make the Signal mean in the range of 6000 ~ 10000*)

9. Stop STEM scan, retract HAADF detector
10. Carry out a Direct Alignment with Diffraction mode off
    1) Press Diffraction button to turn ***Diffraction mode off***
    2) Carry out **Direct Alignment**:
        i. Check C2 lens aperture centering: turn Intensity knob back and forth passing beam cross-over; if beam does not expand/contract concentrically, center C2 aperture by activating **Adjust** C2 aperture
        ii. **Pre-corrector Beam Tilt:** using MF-X and –Y to make the beam wobble concentrically (or stop wobble by setting the FOCUS adjust knob and then use MF-X and –Y to move the hot beam spot to the center of the beam disc)
        iii. **Pre-corrector Beam Shift:** using MF-X and –Y to center the beam
        iv. Click **Done** to accept the new alignment

11. Carry out a **Direct Alignment in Diffraction mode**
    1) Press Diffraction button to switch back to STEM in diffraction mode
    2) **Diffraction Alignment:** Center the diffraction disk using MF-X and –Y knobs
    3) **Pre-corrector Beam Tilt:** using MF-X and –Y to make the beam wobble concentrically (or stop wobble by setting the FOCUS adjust knob and then use MF-X and –Y to move the hot beam spot to the center of the beam disc)
    4) Click **Done** to accept the new alignment

12. Use the Ronchigram to correct condenser astigmatism (or go to next step 13 to use ***STEM Auto Tune/OptiSTEM+***)
    1) **Workset → Camera → Insert** CETA camera → Raise Phosphorus screen (R1) → **Search** (to view the Ronchigram)
    2) From **Stigmator OCX**, activate **A1** coarse Stigmator; Adjust FOCUS: if oriented lines are observed (Figure 1(a)), adjust A1 to correct astigmatism coarsely so that a symmetric pattern can be seen on both underfocus and overfocus sides (b) and at the right focus Ronchigram is flat
    3) From **Stigmator OCX**, activate coma **B2**; Adjust FOCUS cross underfocus and overfocus: if pattern changes asymmetrically (Figure 2(a)), then adjust Coma Free B2 to correct coma so that a symmetric pattern (b) can be seen on both underfocus and overfocus sides and at the right focus Ronchigram is flat
    4) Click **Done** to accept astigmatism correction values
    5) Stop CETA camera view

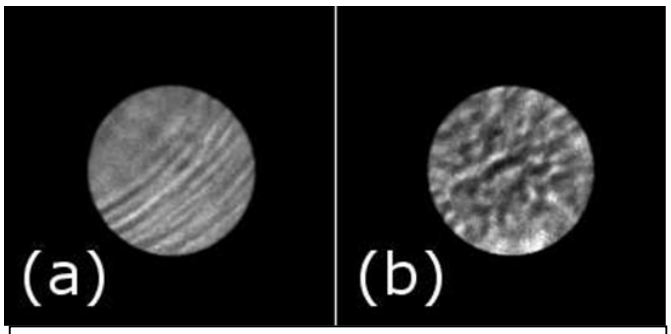


Figure 1: (a) image of the Ronchigram with a fairly large amount of A1 astigmatism; (b) the Ronchigram, slightly defocused, when A1 has been corrected to a good level. Further corrections are easier on the image at this level.

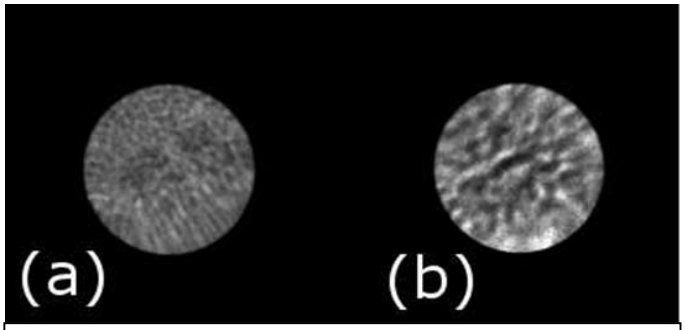
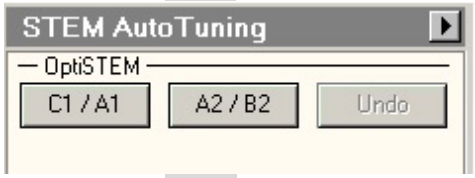


Figure 2: (a) image of the Ronchigram that shows comma as the area around 5 o'clock appears at much higher magnification than the rest; (b) the Ronchigram, slightly defocused, when *comma* has been corrected to a good level. Only the area around 11 o'clock is at slightly less magnification than the rest of the Ronchigram perimeter.

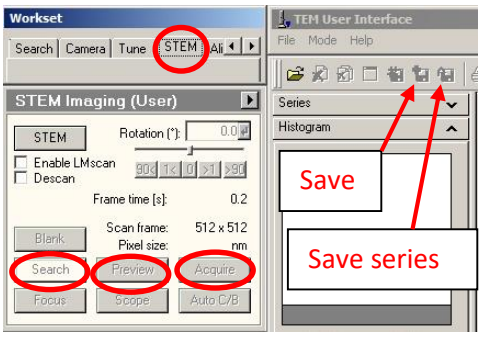
13. Insert HAADF detector
14. Click '**Search'** in **STEM Imaging** and bring the sample to focus using **Z** height control)
    1) → **STEM Auto Tuning /OptiSTEM**, click C1/A1 button to run automatic A1 astigmatism correction



    2) → **STEM Auto Tuning /OptiSTEM**, click A2/B2 button to run automatic A2/B2 astigmatism correction
15. Watch the STEM image, carefully adjust ***focus*** (**C1**) and finely correct ***astigmatism*** with ***Condenser Stigmator*** to optimize image quality
16. Acquire and save STEM images

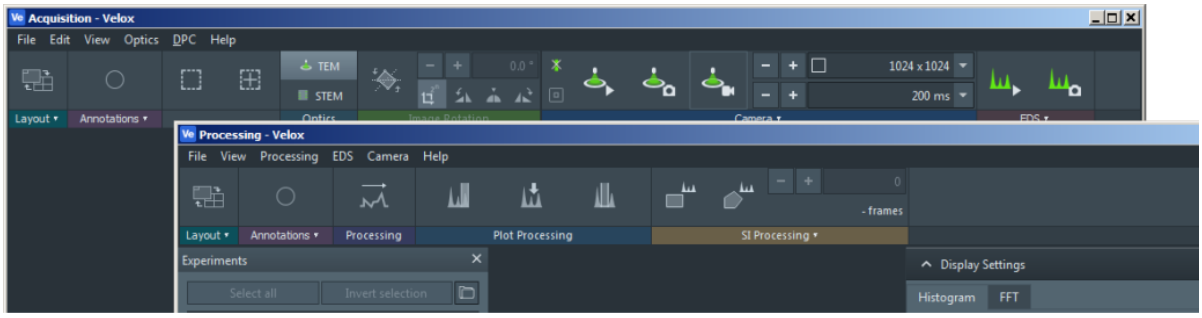
VIII. Acquire images using TIA

1. →STEM → STEM Imaging → Search:
    1. Carefully focus and stigmatize image
2. →STEM → STEM Imaging → Preview:
    1. (optional) preview image quality for acquisition
3. → STEM → STEM Imaging → Acquire
    1. To acquire high quality STEM image
    2. STEM image can be saved individually by clicking the save disk button or saved automatically by setting serious saving folder by clicking Save Series button

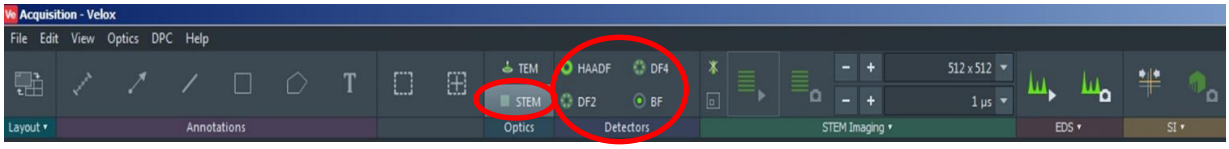


IX. Acquire images using Velox

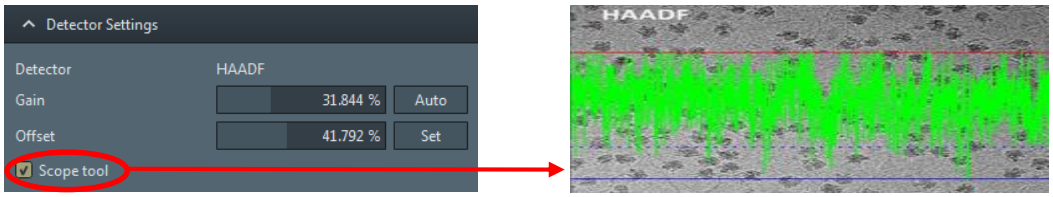
1. Start the **Velox** online software from the Windows Task bar. The **Acquisition** and **Processing** windows appear:



2. Select STEM from the Optics toolbar and Detector(s) from the Detectors toolbar



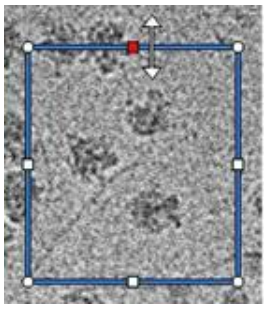
3. Optimize Detector Parameters (offset and gain)
    1) Tick **Detector Settings → Scope** tool



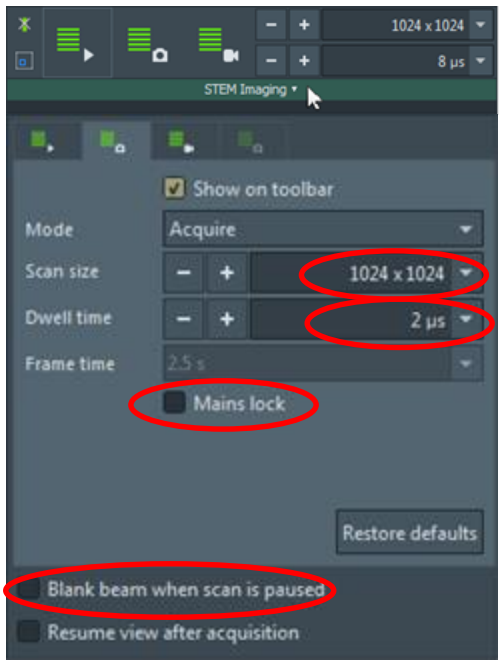
    2) For each detector, adjust the Offset and Gain values:
        a. Select **STEM Imaging → Beam Blank** 
        b. Adjust **Detector Settings → Offset**, so that the entire counts plot stays just above the lower blue line
        c. Select **STEM Imaging → Beam Blank** again to unblank the beam
        d. Adjust **Detector Settings → Gain**, so that the entire counts plot stays just below the upper red line
        e. Check the **Detector Saturation Indicator** to verify that the Offset and Gain are set properly

    3) Select the **STEM Imaging → Scan Size**
    4) Select the **STEM Imaging → Dwell Time** or specify a custom value
        a. For most acquisitions, a dwell time between 5 s and 40 sec. results in good quality images. A very short dwell time may cause noisy images
    5) If necessary, adjust focus and stigmation

a. Select **STEM Imaging → Reduced Area**  , a reduced area marker appears in the center of the image, and Velox starts scanning in only the marked area
b. Drag the **Reduced Area** handles to move it and adjust its size to an area of interested
c. While comparing the image quality inside the Reduced Area with the image quality outside the Reduced Area, adjust focus using the **Focus** knob and adjust stigmation using **Stigmator** button and the **Multifunction knobs**



4. Specify the Scanning Presets
   1) Select the **STEM Imaging** section title to expand the Presets section
   2) **Scan size**: 512 x 512 for View; 2048 x 2048 for Acquire
   3) **Dwell time**: 1 us for View; 5 us for Acquire
   4) Tick **Mains lock** to reduce the impact of an external electromagnetic (EM) field on the scanned image quality
   5) Tick **Blank beam when scan is paused**
   6) Tick **Resume view after acquisition** if you record continuous video using VirtualDub tool.



5. Acquire STEM Images
   1) Single image acquisition

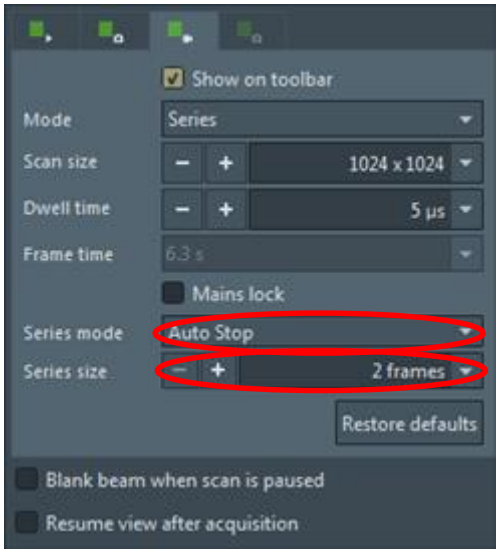
      In the toolbar, select **Start Acquiring**,  , to start acquisition. Velox simultaneously records a STEM image from each inserted detector into the Velox Experiment file.

   2) Series Acquisition (only for one detector)

      In Series mode, Velox acquires multiple back-to-back frames from a single detector.

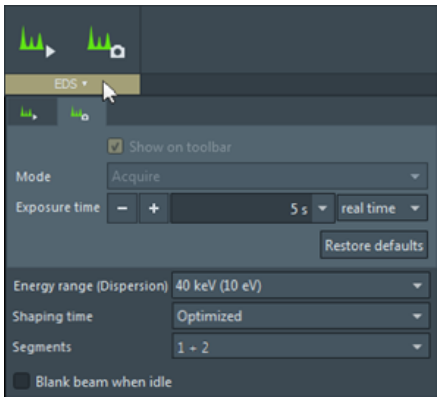
      The following additional parameters must be set:

      - **Series mode:**
        - **Auto Stop**: immediately starts recording a series of frames into the Velox Experiment file, until the specified number of frames is acquired.
        - **Circular:** starts recording frames into a cyclic buffer of the specified number of frames. When the series acquisition is stopped, the contents of the of the cyclic buffer is stored in the Velox Experiment file.
        - **Continuous**: immediately starts recording a series frames into the Velox Experiment file, until the acquisition is stopped by the user.
      - **Series size:** The number of frames that is recorded into the series. When Series mode is Continuous, the Series size parameter is not available.



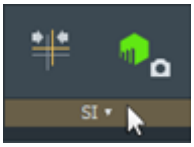
X. X-ray energy dispersive spectrum (EDS) acquisition using Velox

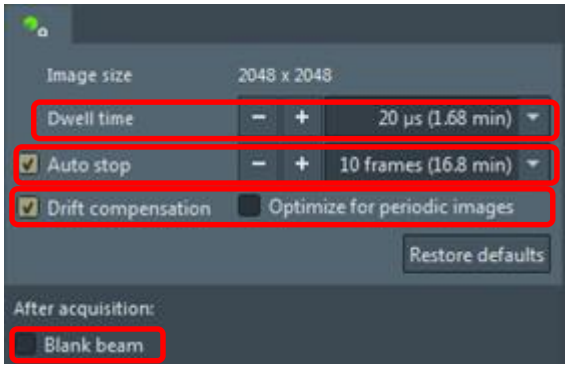
1. Acquire a single EDS
   a. Select the EDS section to expand the Presets section and define the Exposure time, Stop condition (Real time, or Live time), and Energy range (Dispersion)



   b. From the toolbar, select EDS→ Acquire EDS  to start acquiring data.
   c. Wait for the acquisition to complete.

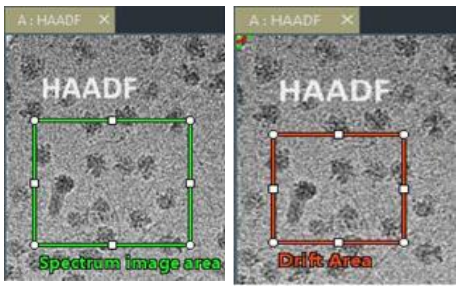
2. Spectrum Image Acquisition
   a. Select the SI section title to expand and specify the Presets parameters:





   - **Image size**: determined by the STEM Imaging toolbar > Scan Size value.
   - **Dwell time**: Recording time per pixel, excluding overhead. The time between brackets is the estimated frame time. Dwell times of 20 us or longer typically give good results for SI acquisitions.
   - **Auto stop**
     - Ticked: Acquisition stops after the specified number of frames.
     - Cleared: Acquisition continues until Spectrum Imaging is stopped by the user.
   - **Drift compensation:** Enable or disable Drift compensation.
   - **Optimize for periodic images:**
     - Ticked: The Drift compensation function suppresses periodic features in the FFT to make sure that it based on the displacement of unique features.
     - Cleared: Periodic features in the FFT are not suppressed.
   - **Blank beam:** When ticked, the beam is blanked automatically as soon as the acquisition is completed.

   b. Select **STEM Imaging → Start Acquiring**  to obtain a STEM reference image.
   c. Select **Spectrum Imaging Area**  , and draw an area of interest:
   d. Select **Drift Measurement Area**  and draw a Drift Area in the STEM image.

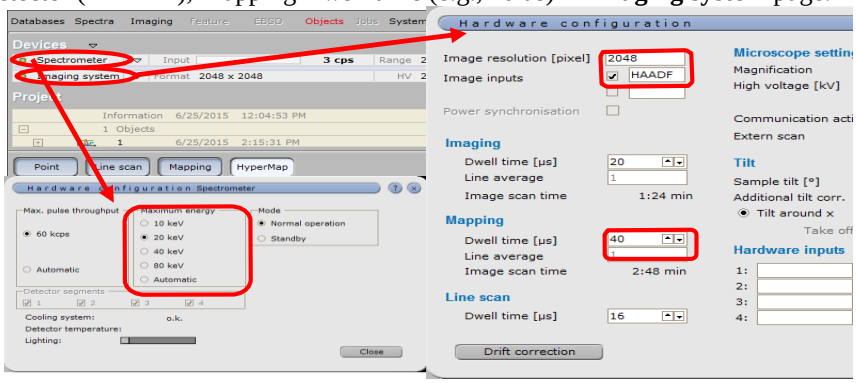


   e. Select **Spectrum Imaging**  to start acquisition.
   f. Select **Spectrum Imaging** again to stop acquisition if Auto Stop is not ticked.

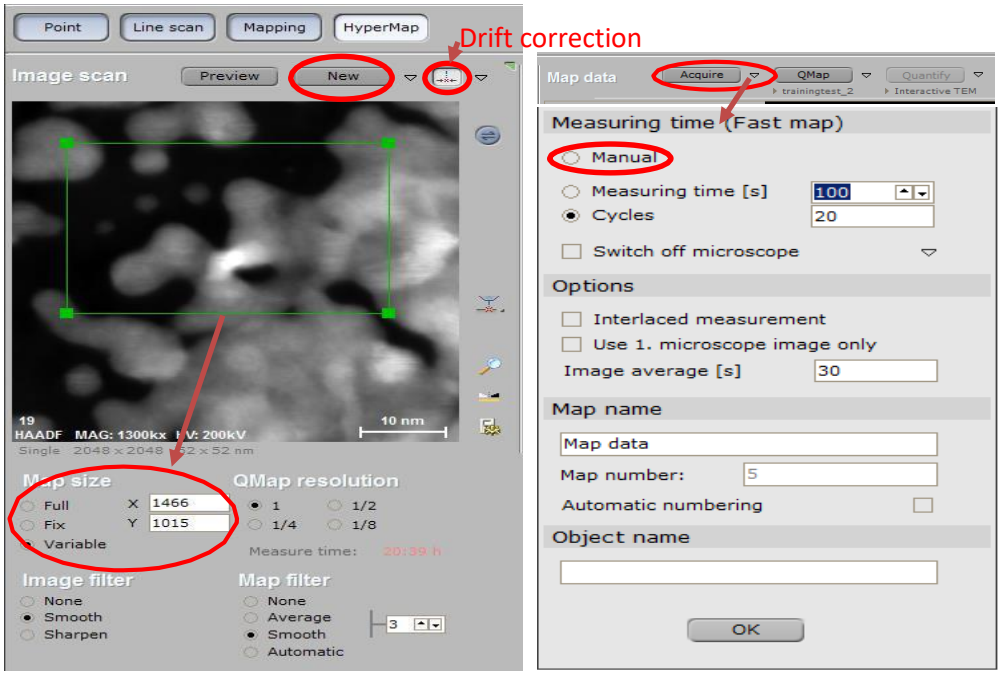
XI. EDS (spectrum, line, and map) Acquisition using Bruker Esprit

1. If EDS software has not been launched yet, start the Bruker **Esprit** software by clicking icon in the Windows quick launch panel, log in with default username (*edx*) and password (*edx*)

2. Set up EDS acquisition parameters: Maximum energy (e.g., 20 keV) in **Spectrometer** page and Image resolution (e.g., 2048), detector (HAADF), Mapping Dwell time (e.g., 40 us) in **Imaging system** page.



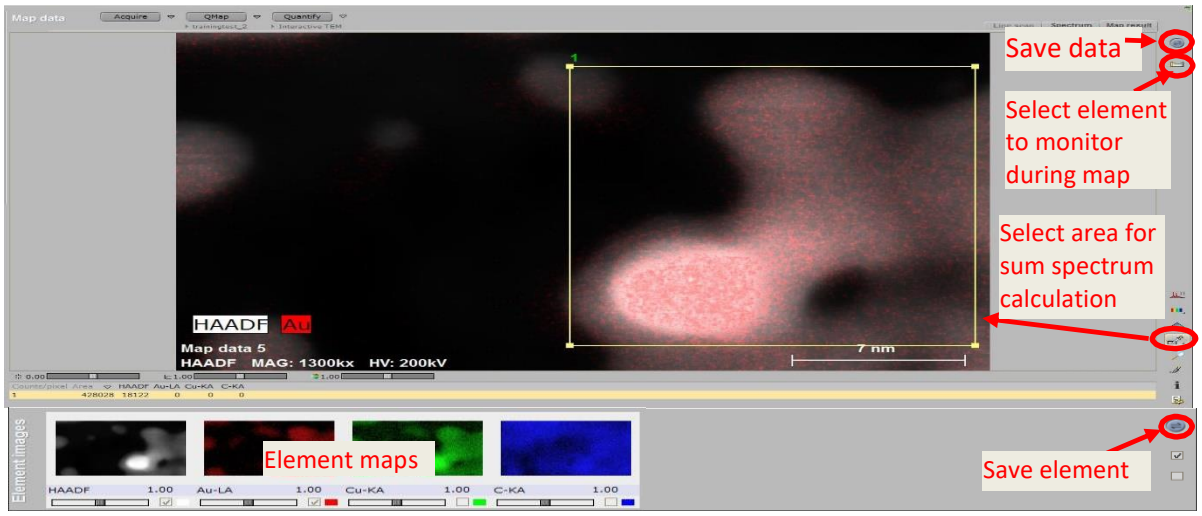
3. Navigate to the EDS operation, e.g., **Objects/HyperMap**, click **New** in **Image scan** panel to obtain a STEM reference image. Drag the corner of the box to define an area to collect fast map and activate **Drift** correction button.



4. Click the triangle icon to the right of **Acquire** button to set up Measuring time.
   Manual: acquire EDS hypermap until user clicks **Stop** *(recommended choice).*
   Measuring time: set up total acquisition time.
   Cycles: set up number of frames to acquire and integrate for EDS hypermap.
   Note: if **Cycles** is chosen, clicking **Stop** once will terminate acquisition after the current frame and double-clicking **Stop** will terminate acquisition immediately.
   Note: reducing the gun lens number and beam spot size number can increase the beam intensity, thus

enhances the x-ray counts per second.

5. Click **Acquire** to collect hypermap of selected area. A status bar with remaining time is displayed in the upper right corner of the software interface. Select elements (multiple selection) from flap-out periodic table to monitor hypermap acquisition progress.
6. Save hypermap database and individual element images.
7. Use area selection objects to select full or partial map for spectrum calculation and click Spectrum tab to show spectrum.
8. Use Quantify to calculate the weight or atomic percentage of selected elements: click the method in grey under Quantify button, check Element identification, you can choose "Automatic" or select elements using "Preset list". Click **Quantify** button to calculate the elemental percentage.



XII. Finish your microscope session

- Retract HAADF detector and return to TEM mode by simply loading the TEM registrer, e.g. ***RoutineTEM 200kV***
- Click **Col Valves Closed** button in **Setup**>**Vacuum** to close the column valves
- Retract CCD (BM-CETA) camera
- Lower the fluorescence screen (**R1** button). Put the rubber cover on.
- From **Reset** panel of **Search> Stage>Control** page, click **Holder** to reset stage to center
- While pressing the purple stage cover down, ***slowly*** pull the holder straight out till its stop
- Rotate the holder clockwise till its stop
- Wait for ***30 seconds***
- Gently pull the holder straight out while you are still pressing the purple stage cover down
- Remove and save your specimen
- Insert the empty *single tilt holder* in to goniometer
- Transfer your data to your shared drive folder
- Log off your microscope session via FOM

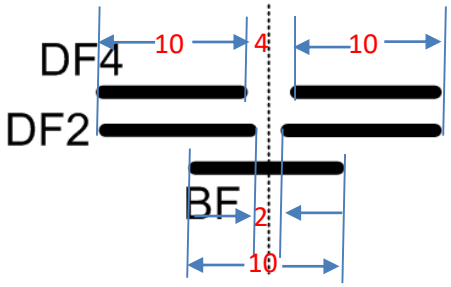
STEM detectors

Bright-Field (BF) detector

The retractable BF detector consists of a solid-state detector with a diameter of 10 mm.

WARNING: POTENTIAL DAMAGE! The retractable BF detector can be damaged by a very high electron dose. If you are using the detector with a very high beam current (e.g. for X-ray mapping), change to a high camera length so the central-beam disk is large and the electron dose per detector area is not too high.

Important note on the retractable BF detector. The area covered by the BF detector is larger than the holes in the DF2 and DF4 detectors. Consequently, when you set contrast and brightness with the BF inserted but the DF retracted and then insert one of the DF detectors, the area of the BF detector receiving signal will be much reduced and typically no BF signal is observed any more. Set the contrast and brightness again (AutoCB) and the signal will re-appear.



Schematic drawing of the retractable BF/DF detectors.

Dark-Field (DF) detector

The retractable DF detectors (there are two with different diameters) are annular solid-state detectors with outer diameters of 24 mm and inner diameters of 2 (DF2) and 4 (DF4) millimeters. The acceptance angles of the DF detectors (inner and outer) are determined on the one hand by their dimensions (inner and outer radius) and on the other hand by the camera length chosen (the values are given by the arc tangent of the relevant radius divided by the camera length - in mm of course). These detectors are located above the retractable BF detector.

High-Angle Annular Dark-Field (HAADF) detector

The HAADF detector is an annular detector consisting of a scintillator-photomultiplier. This detector is placed in a housing above the projection chamber and is moved in and out. This detector has been optimized for two aspects, in order to allow atomic-resolution STEM imaging (on FEG instruments) :

- Single-electron sensitivity
- High acceptance angles

The inner acceptance angle can be seen directly from the shadow of the detector on the fluorescent screen (when it is inserted), the outer acceptance angle is five times the inner angle.

STEM Acceptance angles

The table shown below gives the minimum (inner) and maximum (outer) acceptance angles of all four STEM detectors for the current situation (highlighted in red) on the microscope when run on-line.

Detector angles in mrad

Detector		HAADF		DF4		DF2		BF	
		Min	Max	Min	Max	Min	Max	Min	Max
Diameter (mm)		4.25	26	4.3	24	2.3	24	0	10
Camera length									
(mm)	32	200	200	55.6	200	29.4	200	0.0	126
	41	185	200	43.4	200	22.9	200	0.0	98.6
	51	149	200	34.9	195	18.4	192	0.0	79.3
	65	117	200	27.4	153	14.5	151	0.0	62.2
	86	88.4	200	20.7	115	10.9	114	0.0	47.0
	110	69.1	200	16.2	90.3	8.55	89.2	0.0	36.8
	135	56.3	200	13.2	73.6	6.97	72.7	0.0	29.9
	165	46.1	200	10.8	60.2	5.70	59.5	0.0	24.5
	210	36.2	200	8.47	47.3	4.48	46.7	0.0	19.3
	255	29.8	182	6.98	38.9	3.69	38.5	0.0	15.9
	320	23.8	145	5.56	31.0	2.94	30.7	0.0	12.6
	400	19.0	116	4.45	24.8	2.35	24.5	0.0	10.1
	510	14.9	91.2	3.49	19.5	1.84	19.2	0.0	7.93
	650	11.7	71.6	2.74	15.3	1.45	15.1	0.0	6.22
	800	9.50	58.1	2.22	12.4	1.18	12.3	0.0	5.05
	1000	7.60	46.5	1.78	9.93	0.94	9.82	0.0	4.04
	1250	6.08	37.2	1.42	7.94	0.75	7.85	0.0	3.23
	1600	4.75	29.1	1.11	6.21	0.59	6.13	0.0	2.53
	2050	3.71	22.7	0.87	4.84	0.46	4.79	0.0	1.97
	2550	2.98	18.2	0.70	3.89	0.37	3.85	0.0	1.59
	3500	2.17	13.3	0.51	2.84	0.27	2.80	0.0	1.16

Note: The maximum acceptance angles for the DF2 and BF assume that the DF4 or DF2 and DF4, respectively are NOT inserted. If these are inserted the maximum acceptance angles for the DF2 or BF become equal to the minimum acceptance angles of the detector blocking the other one (for DF2 the DF4; for BF the DF2 or DF4).

SPECIFICATIONS (Highlights):

- Accelerating voltages: 80 and 200 kV
- Ultra stable, high brightness X-FEG (brightness: 1.8 x 10^9 A/cm^2/sr)
- X-FEG probe current: 0.9 nA in 0.2 nm spot and 14 nA in 1 nm spot
- Cs DCOR probe corrector for sub- Å resolution in STEM mode
- TEM point resolution and information limit: < 0.11 nm
- STEM resolution: 70 pm (Cs corrected)
- EDS energy resolution (Windowless Super-X EDS detector system with output count rate up to 200 kcps):
    - ≤ 136 eV for Mn-Kα and 10 kcps (output)
    - ≤ 140 eV for Mn-Kα and 100 kcps (output)
- Detectors:
    - High angle angular dark field (HAADF) detector for STEM
    - 4 on-axis ADF/BF detectors including 4 Quadrant ADF for differential phase contrast (DPC) imaging
    - Ceta 16M CMOS camera
- Specimen Holders:
    - FEI CompuStage single tilt holder
    - FEI CompuStage high-visibility, low-background double tilt holder (±35° tilt range for alpha and ±30° tilt range for beta)
    - Fischione tomography holder model 2021 (up to ±70°)