Standard Operating Procedure for FEI Helios 660 NanoLab

General Rules

Helios 660 reservations may be made online using the NERCF FOM website. You need a valid cost object account to charge the reservation if you are an internal UNL user. Please do not cancel a reservation 24 hours before it starts. Also, please arrive on time for your reservation. You will be charged at least half an hour of use for late cancellations and missed reservations. The FOM system will allow other users to start using the Helios 660 if you are late 30 minutes after your reservation begin.

Users start with daytime access. You may reserve and use the Helios 660 Monday to Friday 8 a.m. to 5 p.m. Note that daytime access users will not be able to enter the NERCF Lab and therefore unable to use the Helios 660 during holidays when the UNL offices are closed despite being able to make reservations on FOM (e.g. MLK Day or winter holidays). Once you have used the instrument for 50 hours (excluding all trainings or technician-assisted sessions), you can apply for anytime access. Anytime access users can make reservations anytime and receive NCard access.

You may use the Helios 660 with other researchers, such as coworkers in your research group, during daytime hours. However, only trained users can operate the instrument. You are not allowed to let persons who do not have anytime access to enter the NERCF Lab during off hours (i.e. weekday nights 5 p.m. to 8 a.m. and weekends).

Please save all data (SEM images, EDS reports, etc.) on the local server – the “D” drive of the Support Computer. You can retrieve your data by using USB drive with the support computer or online via email or cloud service. Please do not connect your USB drive to the Microscope nor EDAX Computers.

If you have questions or technical difficulties while using the Helios 660, please contact the instrument administrators. Every week, there will be an “office hours” scheduled for users to seek help.

Special Notes and Warnings

- **Do not start the instrument** if either **the red OFF** or the **yellow STANDBY** power buttons on the front panel is illuminated. Contact the administrator to check if the instrument is operational.
- Always wear the nitrile **gloves** when touching any part of the chamber.
- Always view the **CCD chamber camera** when opening or closing the sample chamber.
- When screwing sample holders onto the stage or sample onto the sample holder, **do not over tighten**.
- **Never** use strong force to open the chamber when it is under vacuum. Wait until chamber is fully vented and the chamber door "pops" open.
- Ensure that samples are **properly secured** to the sample stubs/mounts. Only use the conductive tape or silver paste that are provided.
- **Do not** use samples that can outgas and/or can generate dust. If you have biological, archeological, or concrete/cement samples, please check with the instrument administrators first.
- **Do not** use any ferromagnetic samples with Mode 2 Immersion Mode. These include alloys with Fe, Ni, Co, and Dy. Even nonmagnetic alloys, such as stainless steel, that contain these ferromagnetic elements should not be used.
- **Never** insert the **EDS**, **EBSD**, **CBS**, nor **STEM** detectors when the standard **Universal Mount Base (UMB)** or when the **Nanoindentor** is mounted on the stage.
- Always make sure that the chamber has reached **high vacuum (i.e. the indicator turns green)** before ending your session and leaving the room.
- Be **very careful** of the **electron and ion beam pole pieces**. This is a **$30,000** replacement if damaged! It is equipped with a touch alarm, which will pop-up as an on-screen warning if tripped. If you see this, **click ok** and make sure to reverse whatever stage movement triggered it, because for the next move the alarm is disabled. Be very careful.

Part I: Basic Scanning Electron Microscopy (SEM)

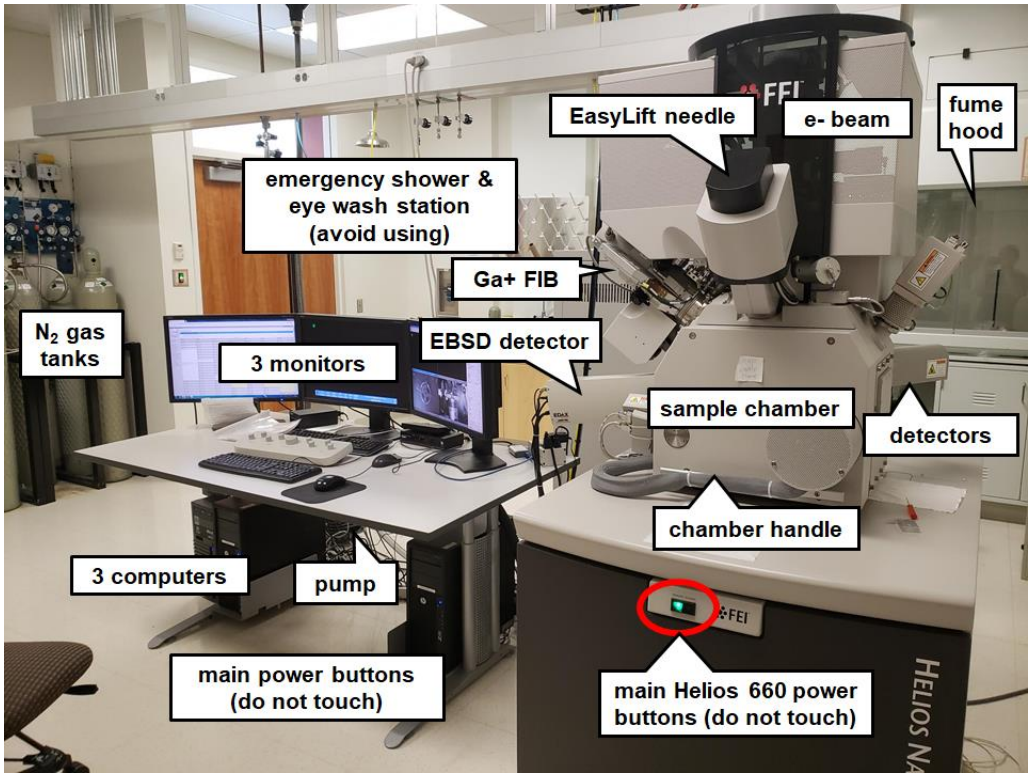


Figure 1: Helios 660 Lab – JH N223

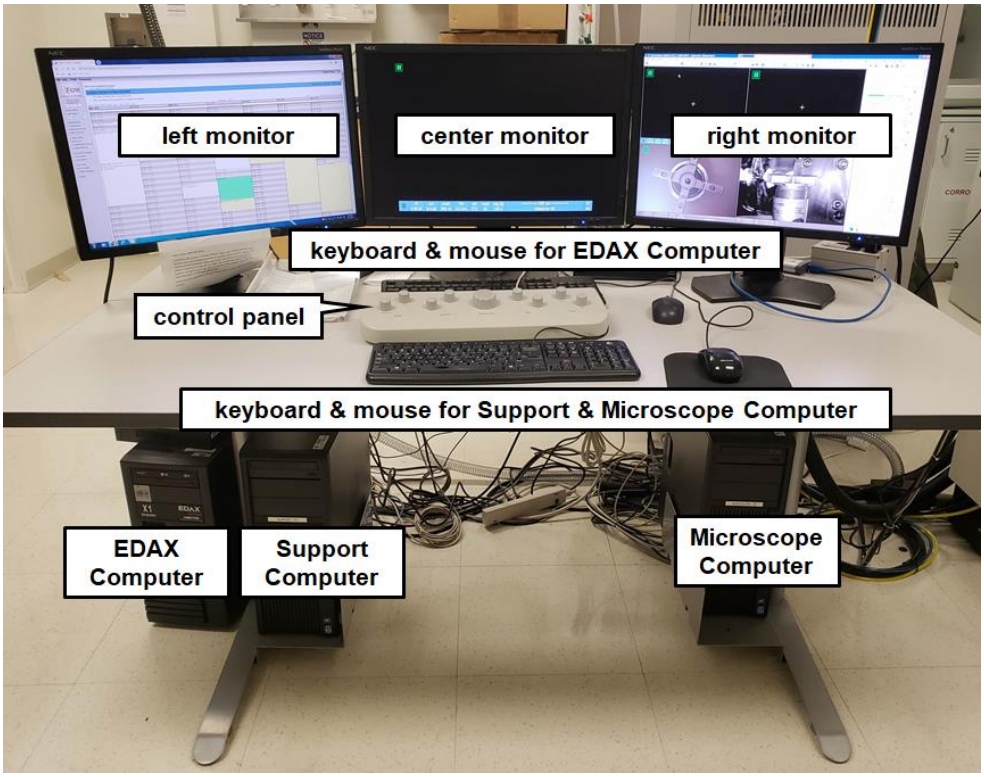


Figure 2: Helios 660 computers and control panel

System Startup

The previous user should leave the Helios 660 software running. If the software is not running and/or the computer(s) are shutdown, start the system:

1. Start and log in to each of the three computers:
    a. **Support Computer:** left monitor Username: supervisor, Password: supervisor
    b. **EDAX Computer:** center monitor (shared w/ **Microscope Computer**) Username: supervisor, Password: supervisor
    c. **Microscope Computer**: right & center monitor (shared w/ **EDAX Computer**) Username: supervisor, Password: supervisor
2. Double-click the **FeiSystemControl** icon on the desktop and press the **Start** button.
3. Once the server is running, click the **Start UI** button and login: Username: supervisor, Password: supervisor
4. Verify the vacuum status. All three vacuum lights (chamber, electron column, and ion column) should be green.

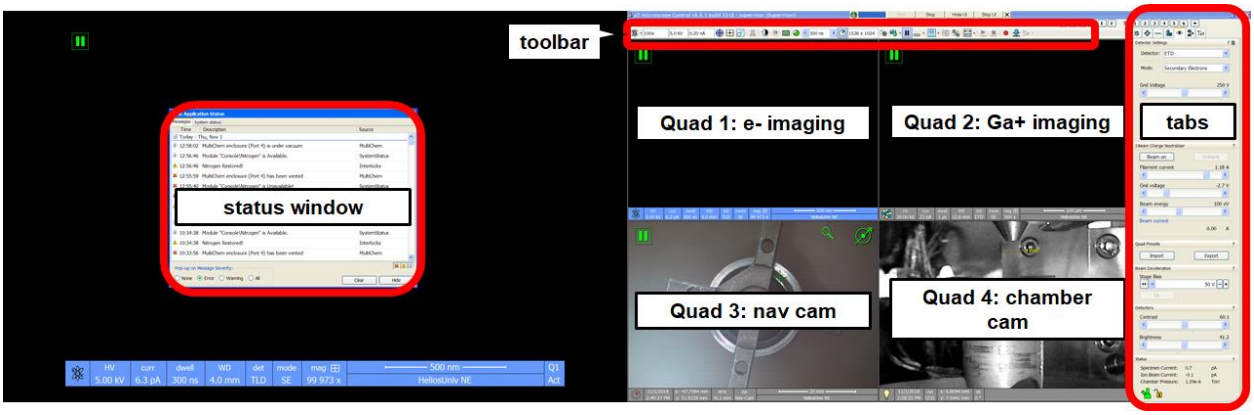


Figure 3: Microscope control program

Sample Load/Exchange

5. Press the **Pump** button
6. Once the chamber is vented, mount your sample on the stage. Check the chamber cam (Quad 4) to ensure that your sample is below the 4 mm mark, but not more than 10 mm lower than the mark. The stage could only be moved 10 mm upwards.

7. If you are loading multiple samples, they must all be **within 1mm of the same height** to prevent accidental collisions between the pole pieces and the top of your samples when imaging.
8. Close the chamber door and press the **Pump** button. Wait for the chamber vacuum status to turn green (wait time ~ 3-5 mins).
9. While waiting for vacuum you can take a Nav-cam image by clicking **Stage > take Nav-cam photo**. After frame integration is done, double click on the area of your sample you wish to move the stage to be centered on that location for SEM viewing.
10. Make sure **Quad 4** is active, if it's not, click inside quad 4 and press **F6** or the pause button to have live imaging.
11. Click and drag in the up direction with the mouse wheel to move the stage upward. Bring the top of your sample to a working distance of about 5 mm (about 1 mm below the yellow line). Be very careful not to go above the yellow line!
12. When the vacuum indicator for the chamber reaches ~5.5 x 10^{-5} torr it will go green, and the beam controls will become enabled.
13. Turn on the SEM by selecting Quad 1 and hit **Beam On** under the beam control tab**.** Only the SEM will turn on. There is no need to wake up the system.

Keyboard Shortcuts

1. F6: Pauses/activates scanning.
2. F5: Toggles between Quad Screen/Full Screen mode.
3. Ctrl + F5: Toggles between Quad/Full Screen on the middle screen.
4. F9: Starts auto contrast/brightness procedure.
5. Ctrl + F8: displays direct adjustments window.
6. Ctrl + click: Takes an Active Preset Snapshot from all quads with the same beam.
7. Shift + click: Takes a Photo from all quads with the same beam at once.

Be careful when operating the Helios as you may accidentally touch a keyboard shortcut. For full list of keyboard shortcuts see **pages 70-73** of **Helios 660 User Manual**.

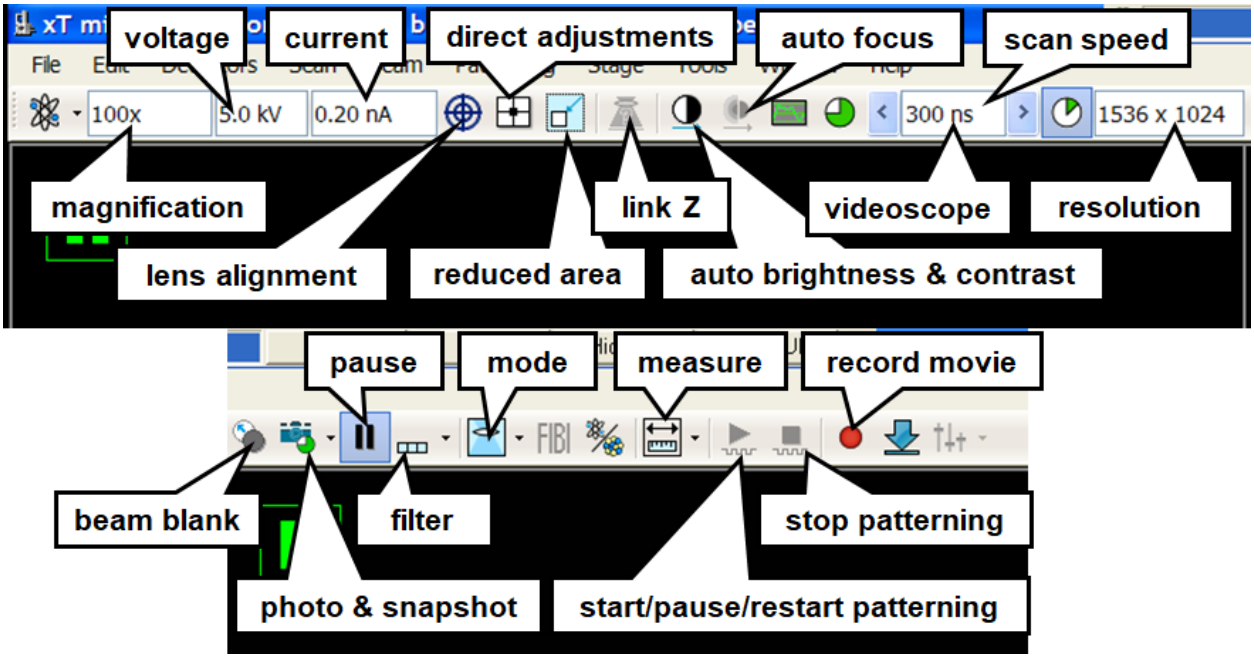


Figure 4: Microscope Control Program Toolbar

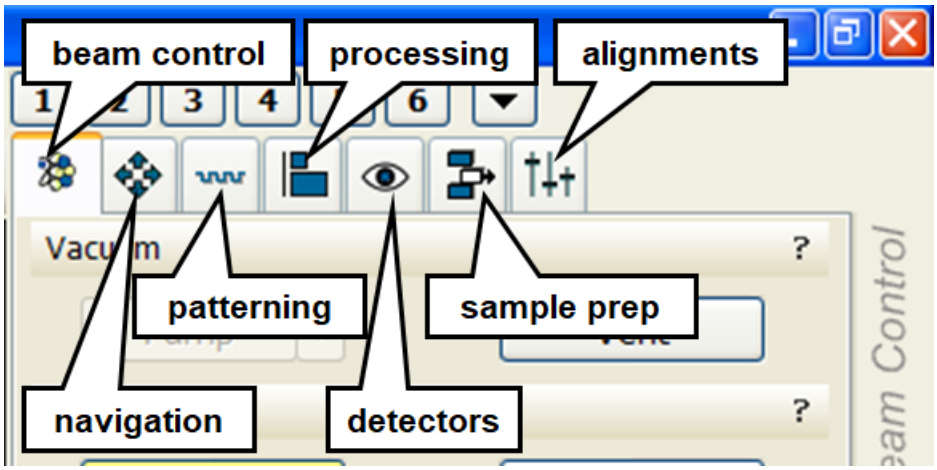
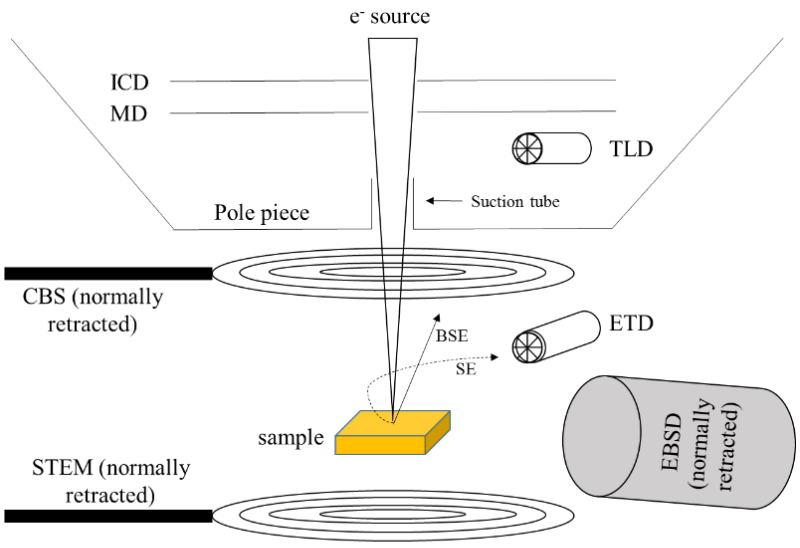


Figure 5: Microscope Control Program Tabs

Electron Beam Imaging Options



SE: Secondary Electron, less than 50 eV of energy
BSE: Backscatter electron, same energy as beam (1 - 30 keV)

Figure 6: Helios 660 detectors locations

With the beam already on, set the desired High Voltage and Beam Current for the electron column and the detector you wish to begin imaging with (usually ETD or TLD mode 1).

1. There are 2 different imaging modes:
    a. **Mode 1**: Field-free mode. Useful for low mag (<10000X) imaging.
    b. **Mode 2**: Immersion. Used for high resolution (>10000X) of non-magnetic samples.
2. There are several detectors that can be used with the electron beam with recommended voltage and current:
    a. **ETD** (Everhart Thornley detector): BSE and SE detector. 5 kV/0.2 nA
    b. **TLD** (Through Lens Detector): BSE and SE detector. 3 kV/0.4 nA
    c. **ICD** and **MD** (In Column and Mirror detectors): Dedicated BSE detectors. 5 kV/1 nA
    d. **CBS** (Concentric Backscatter): Combination BSE and SE ring detector.
    e. **STEM** (Scanning Transmission Electron Microscopy): Transmitted electron detector for TEM specimens. 30kV/0.2 nA
    f. **EBSD** (Electron Backscatter Diffraction): For grain orientation mapping. 20 kV/1 nA
    g. **EDS: (**Energy dispersive spectroscopy): for chemical data. 15-30 kV/0.4-1 nA

*Note: using detectors **D-G** requires special training.

3. **Start image acquisition** (F6) in quad 1, and focus on the sample surface (Mag ≈ 1 kX).
4. Couple the **Z-axis** of the stage to the **working distance**. Verify the stage position and WD are the same and accurate.
5. Move the stage upwards to the regular working distance (**z = 4 mm)**.

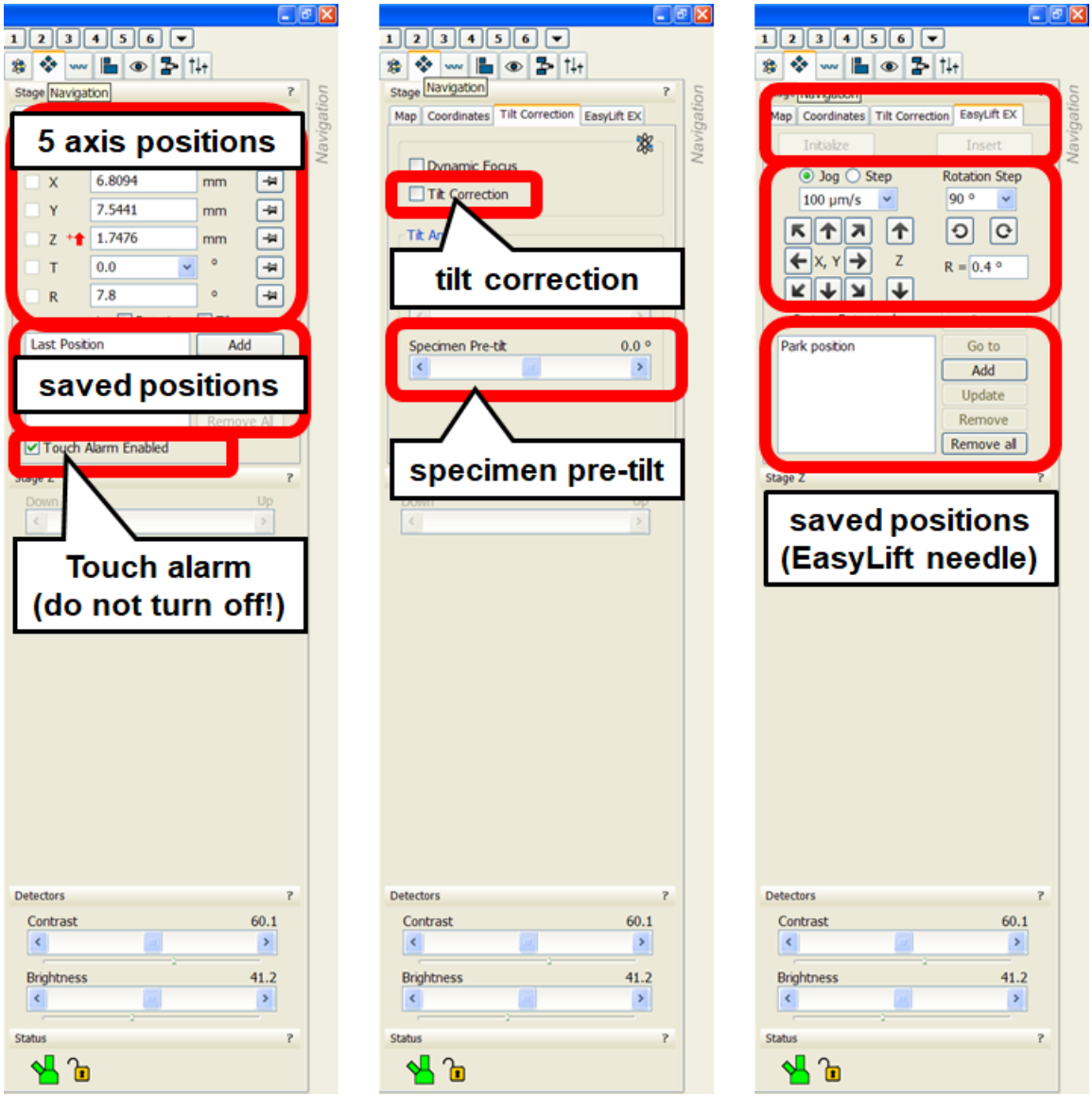


Figure 7: Navigation Tab with Coordinates, Tilt Correction, and EasyLift

Brightness, Contrast, Stigmation, & Focus

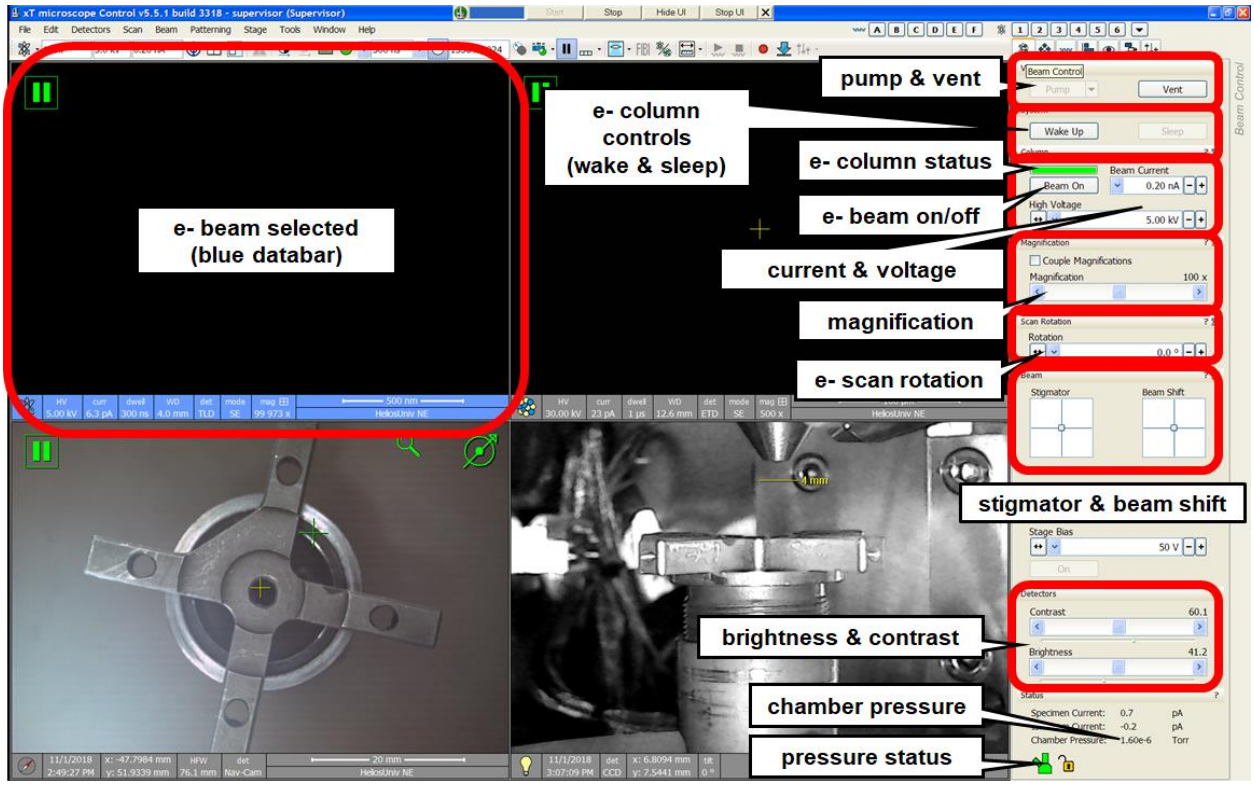


Figure 8: Beam Control Tab for Electron Beam (Note: it's completely different from Ion Beam)

1. **Auto-Contrast-Brightness** (F9): gets you close to optimal contrast/brightness very quickly.
2. If nothing appears, check crossover point by bringing up the **direct adjustments** window and entering **crossover mode**. Increase contrast until spot is visible then center spot.
3. **Reduced Area Window** (F7): Allows for rapid scanning (dwell time ≤ 300 ns) of small area, which gives good feedback for focusing – click and drag right mouse button.
4. After optimizing focus, while still in reduced area window, optimize **x and y stigmation**. It helps to treat them just like focus knobs. If they are way off, right click in the stigmation box on the **beam control** tab and **zero** them. Starting from zero often helps.
5. **Autofocus button**: will screw up your focus more often than it will fix it.
6. **Videoscope** (F3): Manual method to optimize contrast/brightness. Plots the greyscale values from 0 (black) to 255 (white) along the scan line. Easier to see if slow scan rate (dwell time ≥ 1 us). Want to utilize full range without clipping.
7. **Scan presets**: Predefined scan settings for 4 main imaging conditions: 1) live, 2) slow scan, 3) drift corrected frame integration (DCFI) good for charging samples, 4) DCFI + Scan Interlacing.

8. **xT Align Feature**: A great way to quickly rotate the stage so that lines that appear on your sample will align to the horizontal or vertical direction. Draw the line from left to right, select horizontal or vertical, then Finish. Can also do this in the Nav-Cam quad. **Scan Rotation Align Feature** works in the same way but rotates the beam scanning direction not the stage.

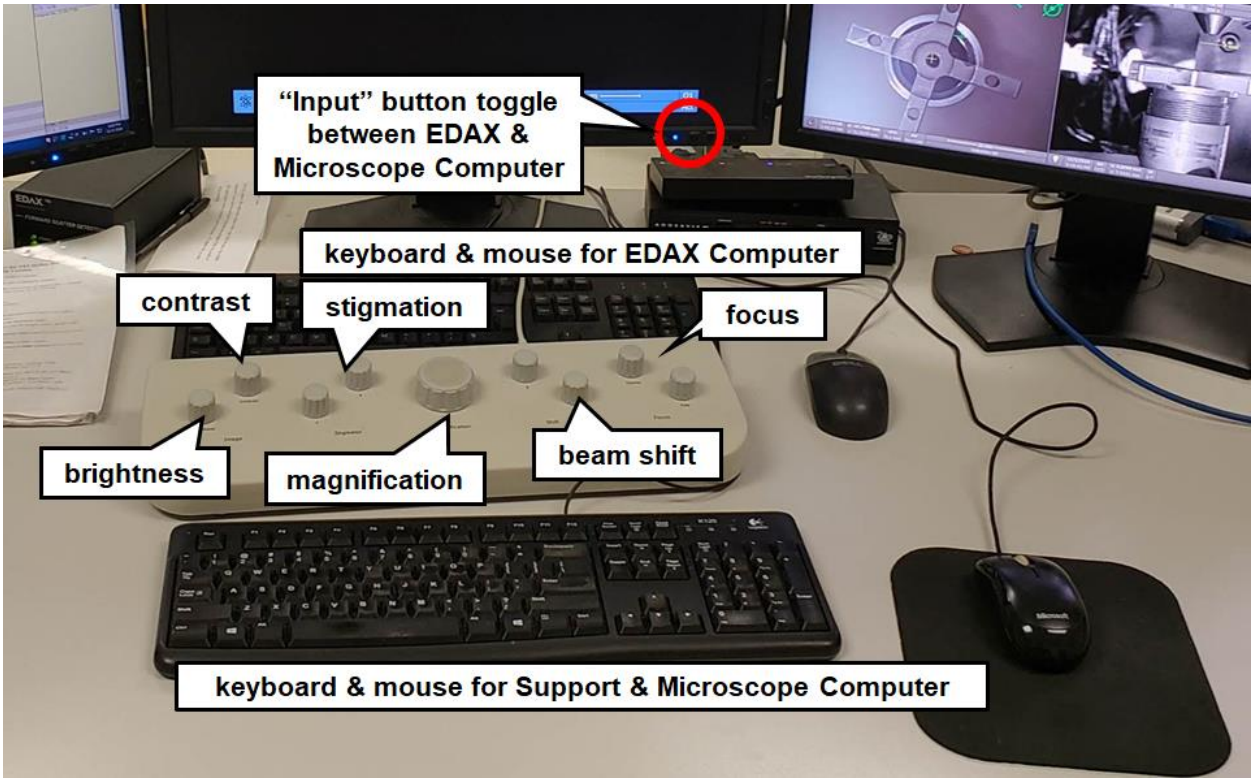


Figure 9: Helios 660 control panel

Alignment of Electron Column

1. Make sure all conditions are set (high voltage, beam current, working distance, etc.).
2. Select the E beam quadrant.
3. **Focus** on a small particle on the sample surface with magnification **>10,000X**.
4. Adjust the **Stigmator** (using front panel or the Beam Control Tab) to achieve the sharpest image and to minimize stretching during focusing.
5. Turn on **Crossover** (Fig. 4a). Adjust the Source Tilt (dragging the small rectangle using mouse) to bring the circle to screen center (Fig. 4b). Turn Crossover **off**.

6. (Optional) Turn on **Lens Align** (Fig. 4a). Adjust the Lens Alignment (dragging the small rectangle using mouse) to minimize translation of the image. Turn Lens Align **off**.
7. Focus and adjust stigmator again on the small particle. Note: using the reduced window helps
8. **Direct Adjustments:** Provides further optimization often needed for obtaining high quality high resolution images. Includes Lens Modulator, Stigmation Centering in X, and Y, which can be used to further optimize the normal focus and stigmator procedure.

High Resolution Electron Beam Imaging

1. Set desired high voltage and current. 5kV and 43pA is recommended for high resolution imaging in Immersion mode.
2. In Field-free mode (default mode), go to area of interest, focus sample and correct stigmation.
3. Make sure the sample is **in focus**. Link Z to FWD. Read the "Z" value in the Navigation tab. Lower Z to around 4-5 mm. Focus sample again.

4. Choose Immersion Mode. There are two modes available on the Helios:

a) Mode 1 – **Field-free** mode, useful for low magnification imaging, imaging during ion milling and magnetic sample.
b) Mode 2 – **Immersion** mode, useful for high resolution/high magnification imaging (sample immersed in lens field, better focusing of e beam, a powerful magnetic field between SEM-column and sample). For the immersion mode, the minimum magnification is about 1000x and the working distance usually is around or less than 5mm. A minimum working distance should be larger than 2 mm.

5. Focus specimen and correct stigmation in Immersion mode.
6. Align **Source Tilt** and **Lens Alignment** in Immersion mode. *Note:* alignment is separate for the Field-free and Immersion modes. Re-alignment is necessary when switching modes.

7. **Fine** Focus, correct stigmation and take high resolution images.

8. When you finish high resolution imaging, return to Field-free mode. Focus and correct stigmation again. Re-align Source Tilt and Lens Alignment in Field-free Mode.

***Note*: never load ferromagnetic particles in immersion mode!** The strong field can attract magnetic particles into the pole piece. If a bulk magnetic sample is to be examined in immersion mode, make sure the sample has been **firmly fastened to the stub** so that it won't bump onto the pole piece by the field!

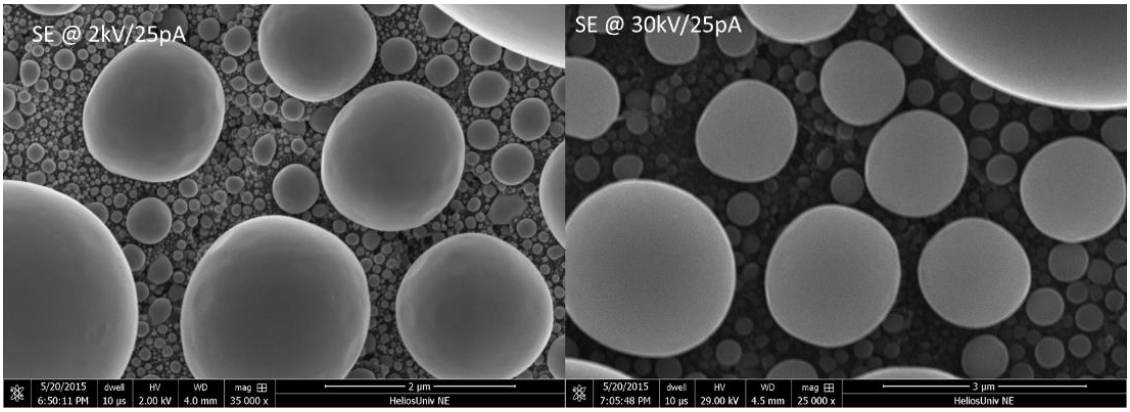


Figure 10: SEM secondary electron images taken with Mode 1 and 2.

Turn Off and Sample Removal

1. Shut off the electron beam, by clicking **Beam On**, wait for indicator to turn grey.
2. Press the **Vent** button to vent the chamber.
3. **Open** the door and remove your samples.
4. Close the chamber and press the **Pump** button to evacuate the chamber.
5. **Wait** for the chamber to pump down (vacuum status is green).
6. **Clean** any mess you made in the Lab. Please take your sample with you.

Part II: Focused Ion Beam (FIB)

Requirements

Part 1 training is **required** for Part II.

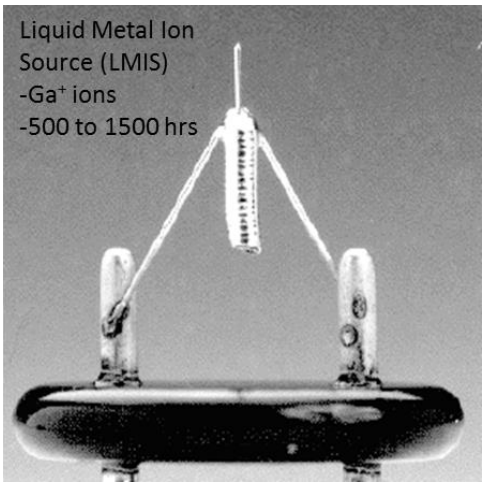


Figure 11: Ga Ion Source for the FIB

After sample installation, click **Wake up** to start the FIB heating process at the beginning of your session.

1. **In the SEM**, zero the beam shift, find a feature, focus, link Z to working distance, double click on feature to move it under center cross, and find the **Eucentric height**!
2. Check the "Couple Magnification" box. This is to maintain the same magnification between E and I column.
3. Set the desired voltage and current for the ion column. Although there is a wide range of voltage selection, for ion imaging, low current is commonly used to minimize sample damage.
4.
5.
6. Keep in mind that ion imaging simultaneously causes surface damage and Ga implantation. For ion milling, higher currents will allow for better throughput, but result in a larger beam.

Eucentric Height & Coincident Point

1. Move the stage (x & y) by holding down the mouse wheel or double clicking the screen.
2. Align a feature on the sample to the center cross on the screen.
3. Tilt the stage to 5 degrees.
4. Adjust the **Z-axis** of the stage to bring the feature back to the same position. Tilt to 10 degrees, and adjust z again. Tilt back to 0.
5. Tilt to 30 and correct height, and then 52 degrees and correct height. This is **eucentric height**: the height at which tilting the stage does not shift the image position in the SEM.
6. Now you are ready to image your sample at tilt angles between 0 – 60 degrees.

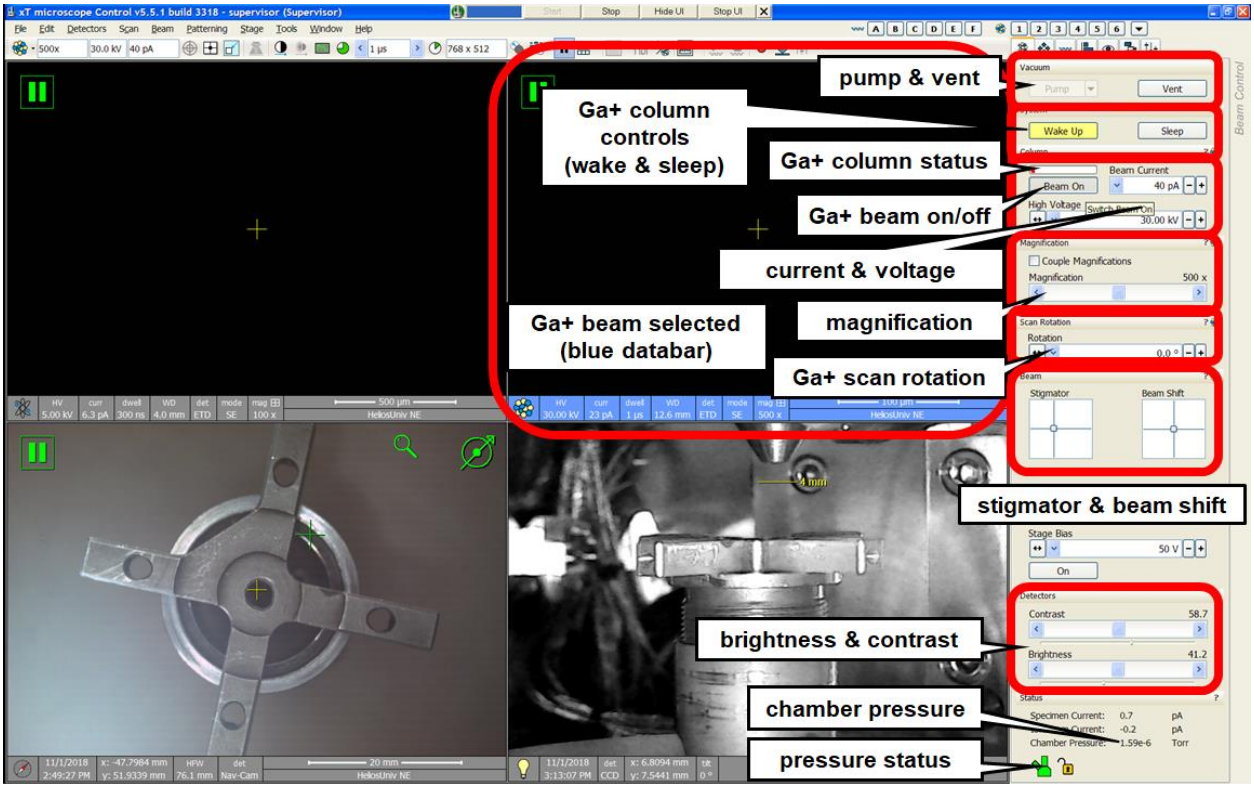


Figure 12: Beam Control Tab for Ion Beam (Note: it's completely different from Electron Beam)

Setting Up the Ion Column and Beam Coincidence

1. The stage should be at a tilt angle of 52°.
2. Set the desired High Voltage and Beam Current for the ion column. For general imaging, start with 30 kV and 40 pA with a 300 ns dwell time and lowest image resolution.

3. Run the **auto contrast brightness** to adjust the ion beam imaging.
4. Take a **snapshot** with the FIB by activating Quad 2 and pressing the F6 key twice, quickly.
5. The contrast should be ok, but focus/stig might need adjusting. Optimize the focus and stigmatism between snapshot iterations.
6. Use **snapshot** imaging setting to minimize beam damage and use **camera** setting sparsely to obtain a high quality FIB image.
7. Find the feature you centered in the SEM and use the **Beam Shifts** to center it in the FIB. Now the SEM and FIB are looking at the same sample area. This is "**Coincidence**". Even if you change the stage (x, y, tilt, rotate) both beams will remain coincident.

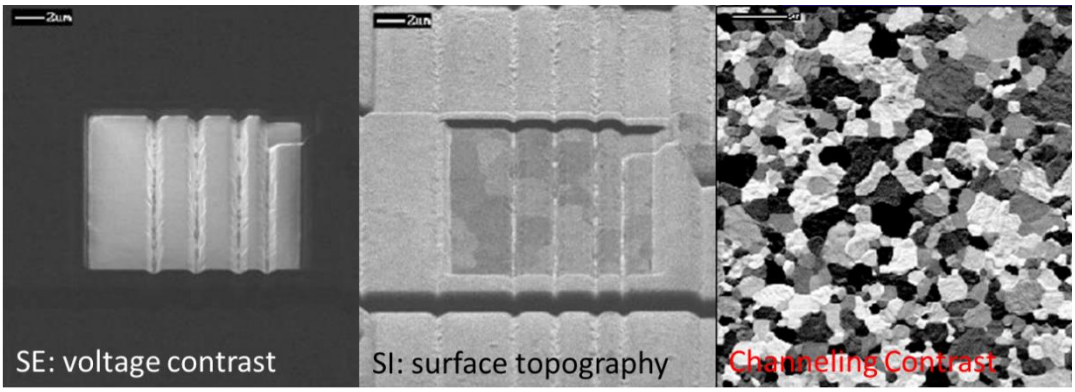


Figure 13: Comparison of SEM voltage, topographical, and channeling contrasts

Ion Beam Imaging

1. There are 2 detectors for ion imaging:
    a. ETD – general purpose detector to detect ion-induced secondary electrons.
    b. The In Chamber Electronics (ICE) Detector is a charged particle detector mounted near the end of the ion column. It collects secondary ions (SI) or electrons (SE, BSE) to form an imaging signal. For advanced imaging.
2. The ion beam can be used to show excellent channeling and voltage contrast and surface topography.
    a. Channeling: SE imaging on ETD, 30 kV, shows grain orientation contrast in single phase, or phase contrast between two phases.
    b. Voltage: SE imaging, signal comes from top 5-10 nm, ETD/ICE detector biased positive, insulators dark, conductors bright.

c. Surface topography: SI imaging, signal comes from top 5-10 Å, ICE detector biased negative, shows channeling too, good for imaging oxides.

Milling/Patterning

1. **Tabs** – located under the scan presets.
2. Select the 3rd tab for patterning. Here you have access to:
    a. Predefined patterns (e.g. rectangle, regular xsec, cleaning xsec, line…), and you'll notice you can change many of the settings for these patterns under the **Basic** and **Advanced** subtabs. Some of these options are trivial, such as the pattern dimensions or application, while subtle changes to others can mean the difference between milling and patterning itself
    b. **MultiChem** Gas Injection Module: Inserts needle that injects metalorganic gases that destabilize under the beam (SEM or FIB) and deposit one or more component materials (W, Pt, C, TEOS, H_2O).
    c. **Endpoint Monitor**: Provides a way to alternate between milling/patterning in FIB and monitoring progress with snapshot SEM images (iSPI).
3. **Milling** speed is a function of beam energy and current. The **beam energy** controls how much of the surface is damaged by the beam, and the **beam current** controls how often these beam damage events occur, so it changes the rate of milling.
    a. **Dwell time** also affects milling time, longer dwell may help mill through tough materials
    b. **Overlap**: Each beam current has a different spot size, positive overlap means each position is adjusted so that these spot sizes overlap each other, which provides an even milling profile along each scan line and from line to line. 50 % overlap in both x and y will usually be sufficient.
4. **Patterning** only works when the right beam conditions are set. Beam energy is usually 30 kV, dwell time should be low (~500 ns), and overlap should be 0 or -0.2. There is a "rule of thumb" formula for finding the right amount of beam current. First approximate the area of

your pattern in μm^2, then multiply that by **5** (Pt), **10** (C), **100** (W), and **3** (TEOS). This product is a rough estimate of the beam current to use in pA.

   a. Ex: To make a 15 µm x 2 µm Pt bar, use (30 μm^2) x (5 pA/μm^2) = 150 pA.

5. **Curtaining** occurs when grains mill at different rates, and are generally unavoidable – especially under Pt protective layers – but can be reduced by lowering the beam current, increasing overlap or dwell time, and even defocusing the FIB image slightly.

MultiChem operation

1. Deposition can be carried out with either the electron or ion beam. Electron beam induced deposition will typically provide higher resolution features, but with lower efficiency and less complete precursor dissociation. Ion beam deposition is more common for most applications.
2. Etch enhancements (H_2O) are designed for use with the ion beam.
3. Before inserting the MultiChem nozzle at either SEM or FIB position, make sure the stage is at eucentric height. The nozzle is aligned to insert 200 µm above eucentric height. Needless to say, we don't want it to crash into your sample!
4. Insert the needle by clicking the appropriate box under the patterning tab.
5. After you have drawn your pattern(s) and set up all parameters, click the **play** button.
6. It is generally a good idea to wait a bit, **pause** the pattern, and then take a snapshot of the pattern and check to make sure it's working ok before **finishing** the rest of the pattern.
7. When you are done patterning, retract the MultiChem nozzle.
8. Once retracted, the stage can be moved again.

Shut Down and Sample Removal

7. Set FIB beam current to 3rd lowest preset.
8. Shut off both electron and ion beams.
9. Retract all nozzles, needles, and detectors.
10. Press the **Vent** button to vent the chamber.
11. When the chamber vents, open the door and remove your samples.
12. Close the chamber and press the **Pump** button to evacuate the chamber.
13. Wait for the chamber to pump down (vacuum status is green).

14. **If you are the last user** of the day (check calendar), put the system to **Sleep**.
15. Clean up any mess you made in the Lab.

Part III: TEM Sample Lift Out Procedure

Requirements

Part 1 and II training are **required** for Part II.

EasyLift Needle Check & Calibration

1. Before starting, the EasyLift Needle needs to be checked and calibrated.
2. **Zero beam shift** for both e- and Ga+ beams.
3. Wake Ga+ column and turn on Ga+ beam.
4. Your sample is not required to be installed. However, there needs to be at least something (i.e. the sample mount) to properly focus the electron beam and set **Z height**.
5. Click the Alignments tab and select **EasyLift Needle Exchange/Calibration** program.

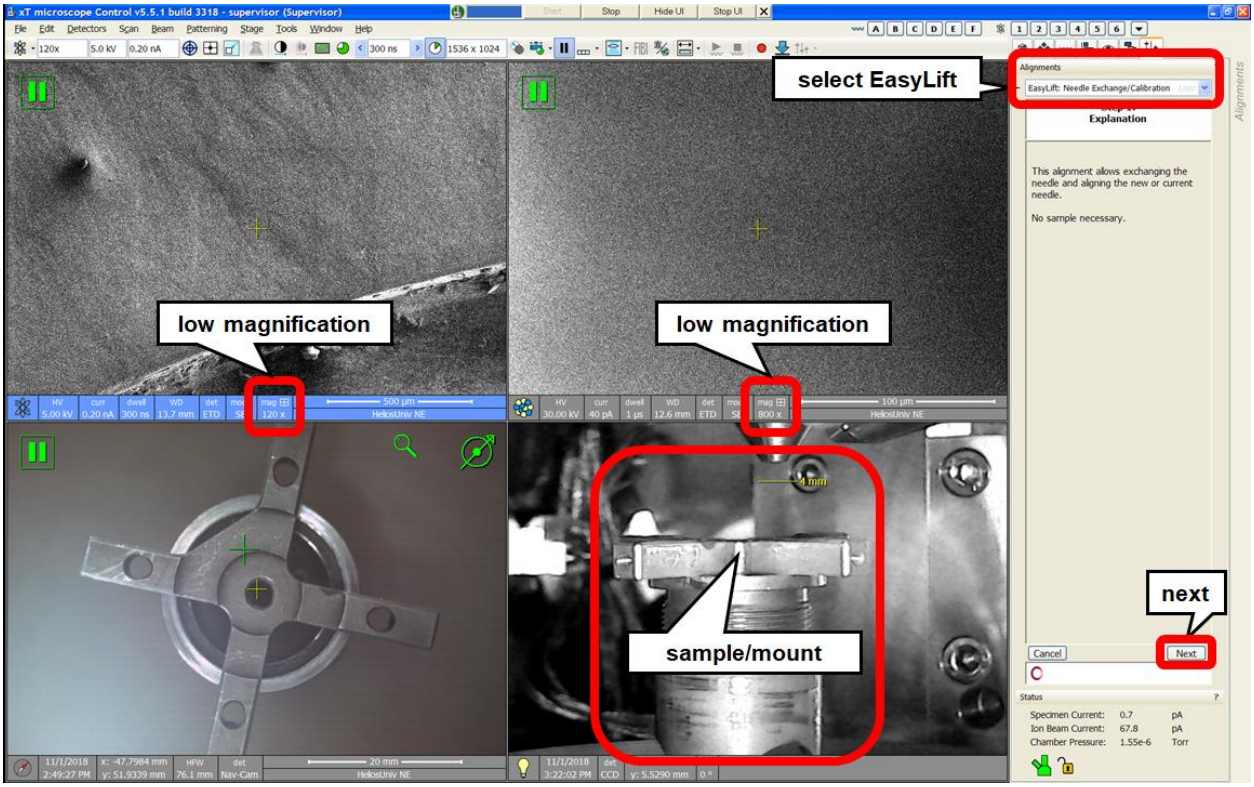


Figure 14: Step 1

6. **Step 1 Explanation:** Set both e- and Ga+ imaging to low magnification (100 to 500X). Click **Next** button.

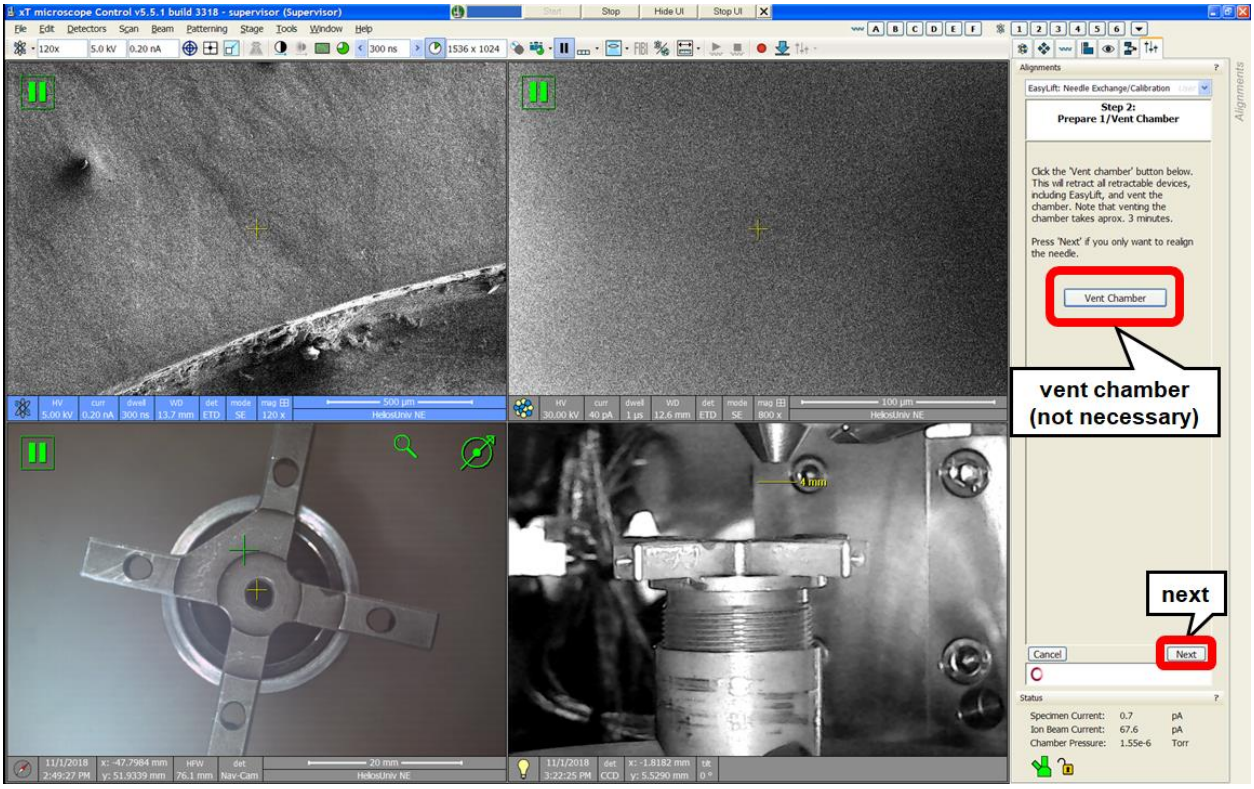


Figure 15: Step 2

7. **Step 2 Prepare/Vent Chamber:** Not necessary to vent chamber if you already have set z height. Click **Next** button.

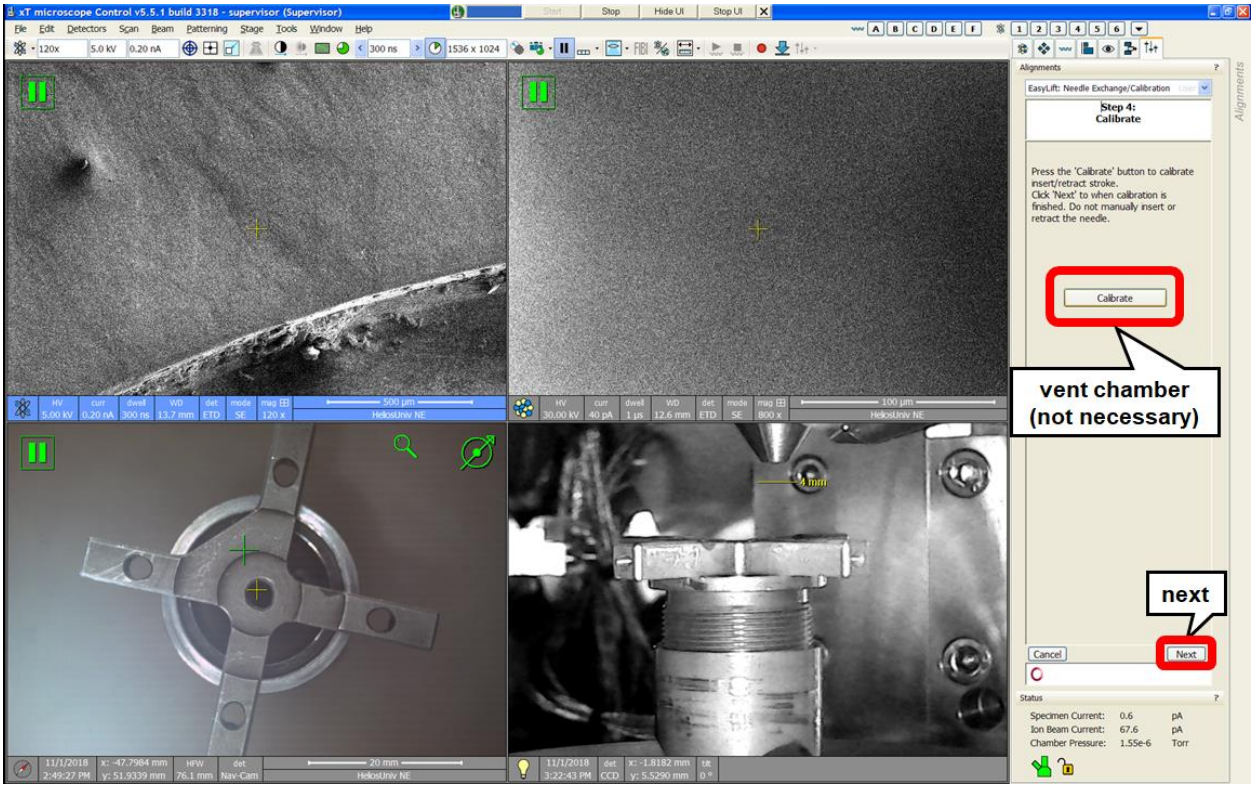


Figure 16: Step 4

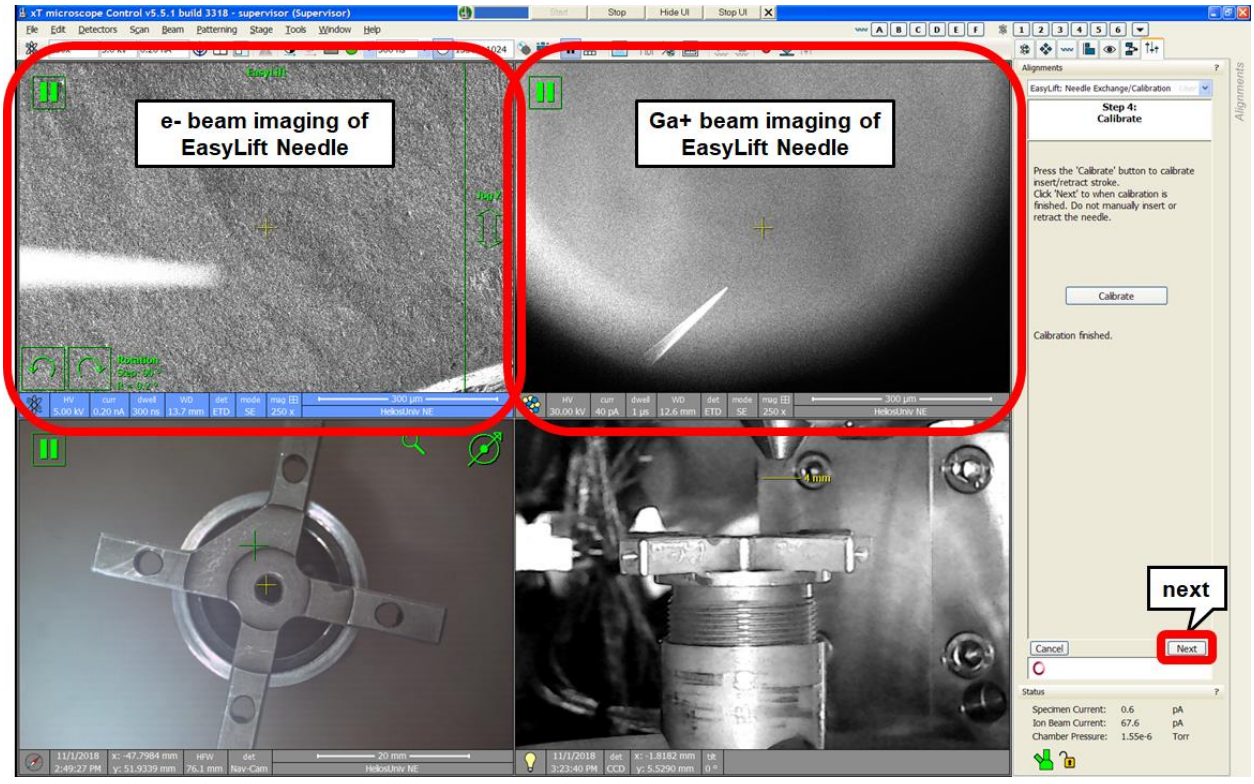


Figure 17: Step 4 After Insert Needle

8. **Step 4 Calibrate:** Click **Calibrate** button to insert the EasyLift Needle. Check the needle sharpness (tip radius <5 μm) with both e- and Ga+ imaging. Click **Next** button. If you cannot see the EasyLift Needle even using the lowest magnification or if the needle is too dull, you cannot perform the TEM Sample Liftout Procedure. Click **Cancel** button and notify the Helios administrator immediately.

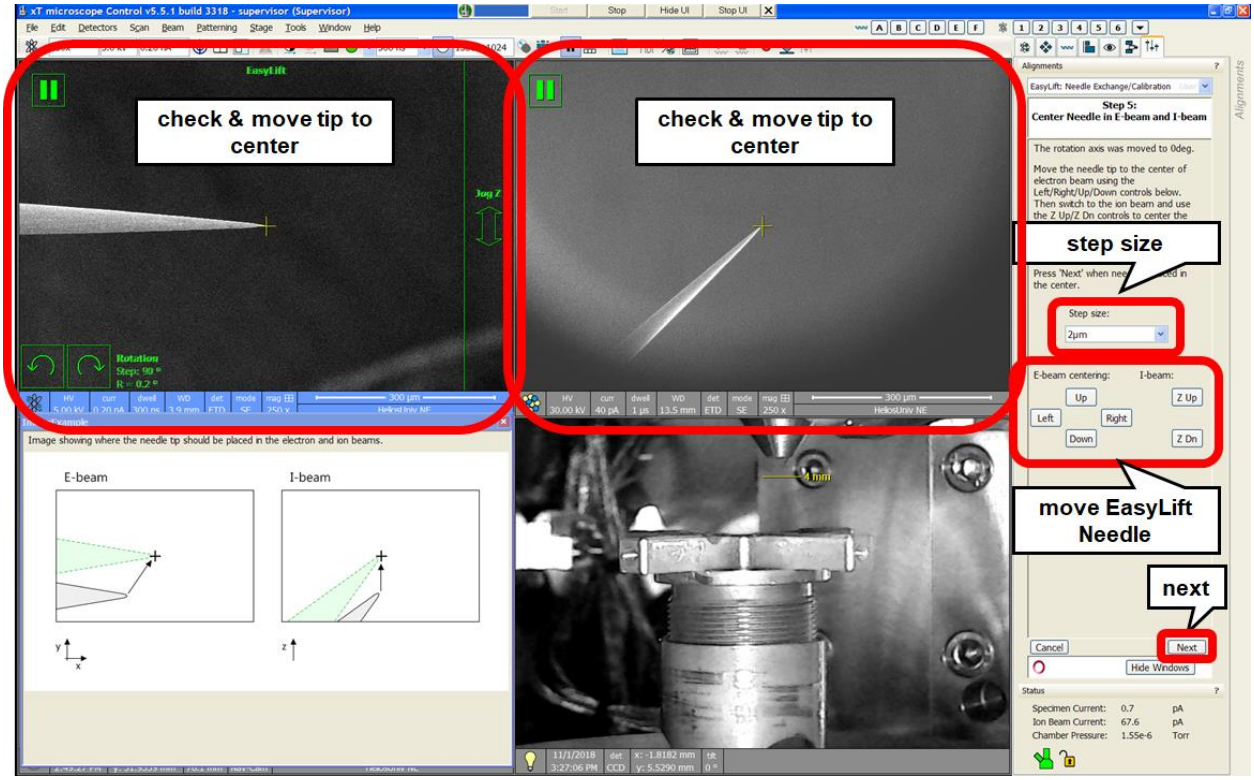


Figure 18: Step 5

9. **Step 5 Center Needle:** Move the EasyLift Needle tip to the center of the e- and Ga+ screen. Select step size and click the buttons (Up/Down for X axis, Left/Right for Y axis, and Z Up/Z Down for Z axis). Do not use beam shift. Click **Next** button. If you cannot move the EasyLift Needle, you cannot perform the TEM Sample Liftout Procedure. Click **Cancel** button and notify the Helios administrator immediately.

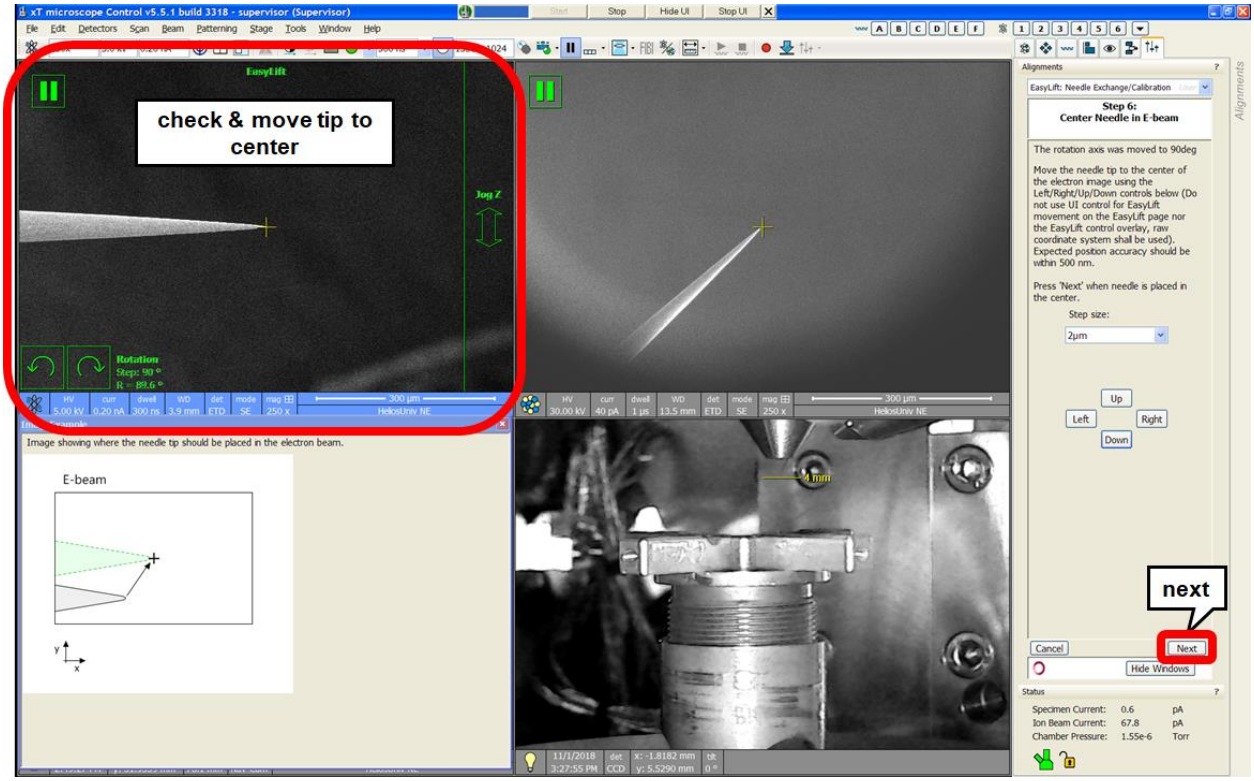


Figure 19: Step 6

10. **Step 6 Center Needle:** The EasyLift Needle will rotate 180 degrees. Once again, center the tip to the center of the e- screen. Click **Next** button.

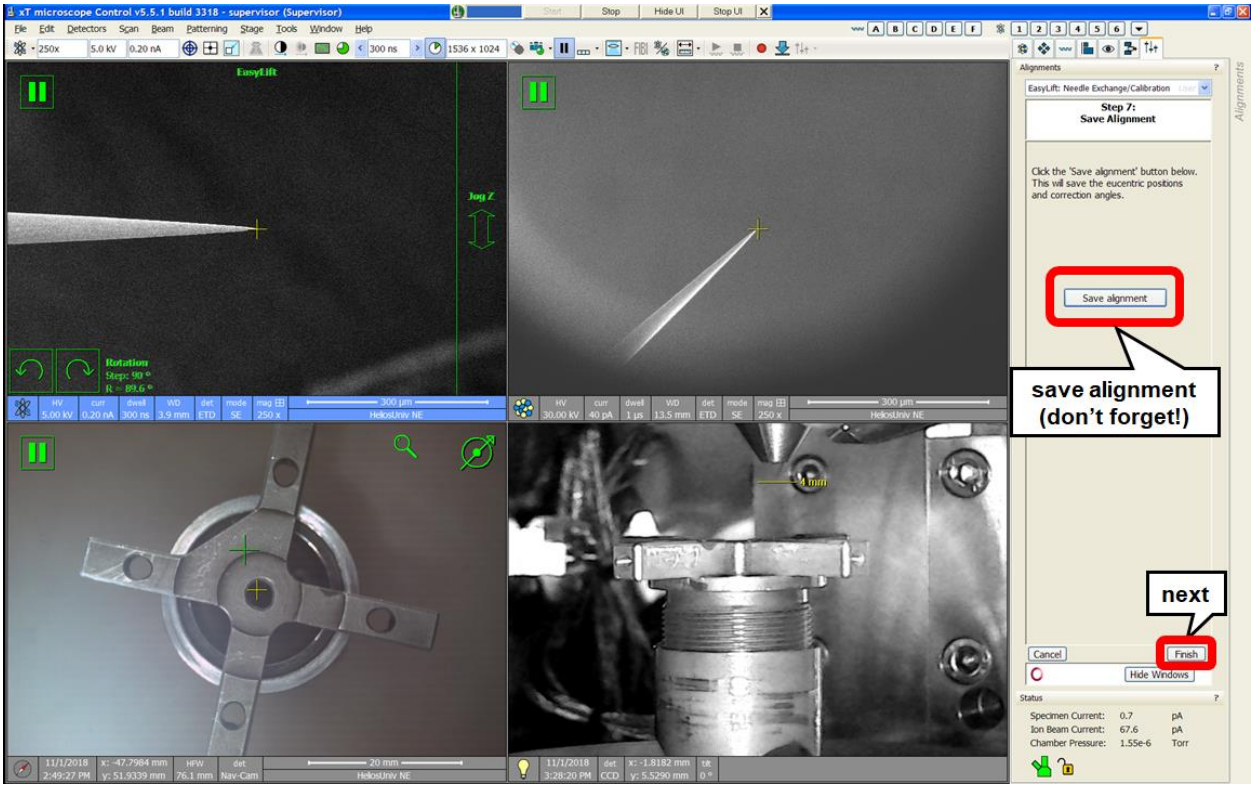


Figure 20: Step 7

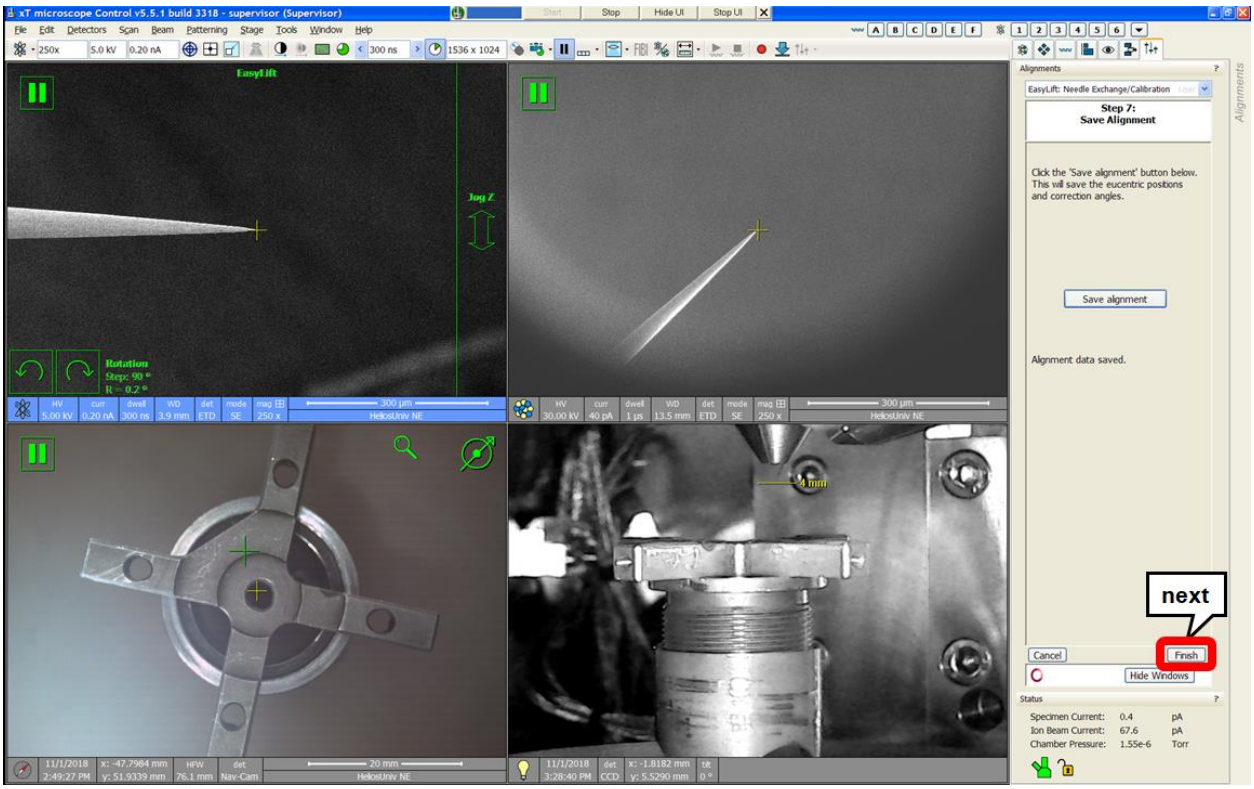


Figure 21: Step 7 After Save Alignments

11. **Step 7:** Click Save Alignment. Click **Next** button.

Getting Started

1. Use SEM (2 kV, 0.2 nA) to find the area of your sample you want to view in the TEM and save the stage position under the **Navigation** tab.
2. Move away from that area, and find a feature to set **Eucentric Height** and beam coincidence.
3. Increase the beam current to 1.6 nA and focus/stig at a low dwell time.
4. Move back to your **region of interest (ROI)** without imaging (blind).

E-beam Deposition (Pt or C) Deposition

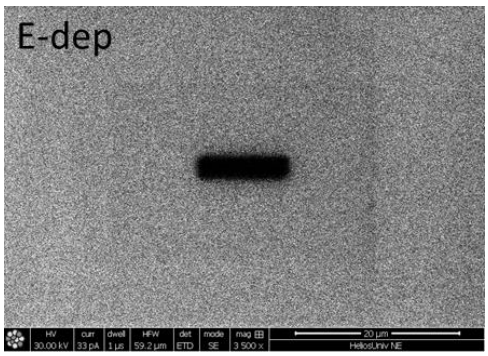
1. Insert the **MultiChem (MC)** nozzle.
2. Take a snapshot of your ROI. Change contrast if needed.
3. Select the **rectangle** tool from the Patterning tab and draw a 10 x 1.5 µm rectangle in the SEM quad. Mag should be high enough so that the pattern takes up about 1/2 of the image.
4. Hit **Play** to start patterning. Check after a ~30 seconds to make sure it's working.
5. When done, **retract** the MC nozzle and return beam current to 0.2 nA.

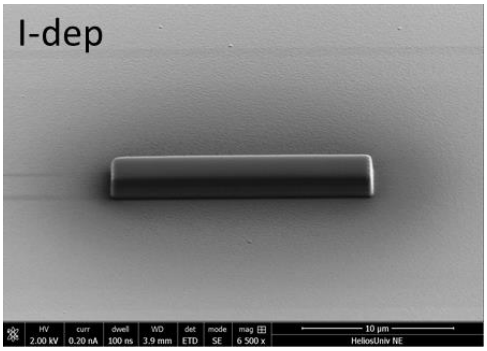
2 kV	10 µm x 1.5 µm x 100 nm
1.6 nA	50 % overlap (x and y)
Pt_M_e-dep surface / C_M_e-dep surface	1 µs dwell time
Electron mode	Deposition time ~ 5-7 mins

I-beam Deposition (Pt or C, but be consistent with E-Deposition)

1. Tilt the stage to 52˚ (Ctrl-i).
2. Take a snapshot (F6 key x2) with the FIB set at 30 kV and 40 pA. Optimize the focus/stig/contrast between snapshots (blind). The E-beam deposition should be in the center of the image if the beams are coincident.

3. Change the beam current to 0.43 nA, and optimize the image with snapshots. Place another rectangle pattern on top of the E-deposition pattern with the dimensions below.
4. For the pattern, the overlap should be 0 or -0.2, and the dwell time should be about 500 ns.
5. Can use iSPI to view the progress of your deposition.
6. After you are done with this pattern, retract the MC nozzle.

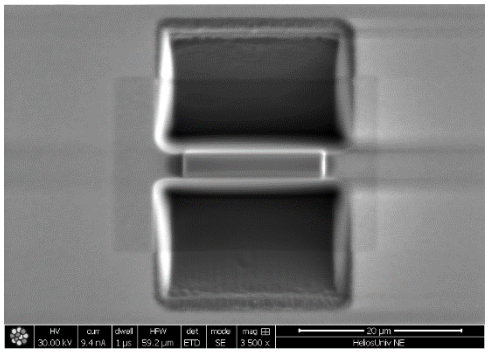


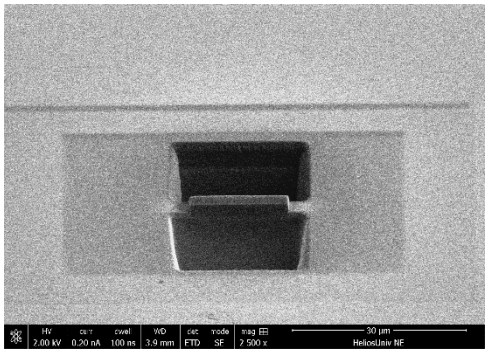


30 kV
0.43 nA
17 x 2.5 x 2 µm
Pt_M / C_M
Rectangle
Time ~ 7 mins

Rough Cuts

1. Place two Regular Cross Sections (Reg. X-Sec) on either side of your Pt or C bar with the thick yellow line nearest the bar.
2. Change the beam current to 21 nA and use snapshots to optimize the beam blind.
3. Use iSPI to view the progress of the rough cuts every minute or so.
4. Delete the patterns when done and **tilt** the stage to 50 degrees.

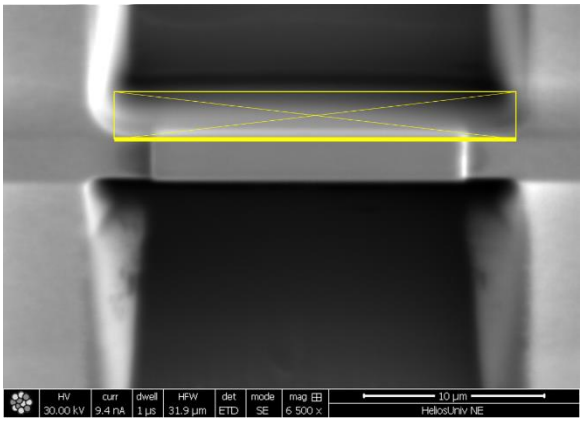




30 kV
21 nA
20 x 12 x 25 µm
Si_New
Reg. X-Sec
Time ~ 10-15 mins

Cleaning Cross Sections

1. Place a Cleaning X-Sec above the bar as shown below, milling from top to bottom.
    a. X: should be equal to that of your Reg. X-Sec x dimension.
    b. Y: should be thick enough to encompass the entire face.
    c. Z: 2*(Reg. X-Sec depth)
2. **Play** the Cleaning X-Sec.
3. Tilt to 54 degrees and move the box to the opposite side of the lamella. This time milling from bottom to top.
4. You want your lamella to be about 1-2 µm when you are finished with this step.



30 kV

2.5-9 nA

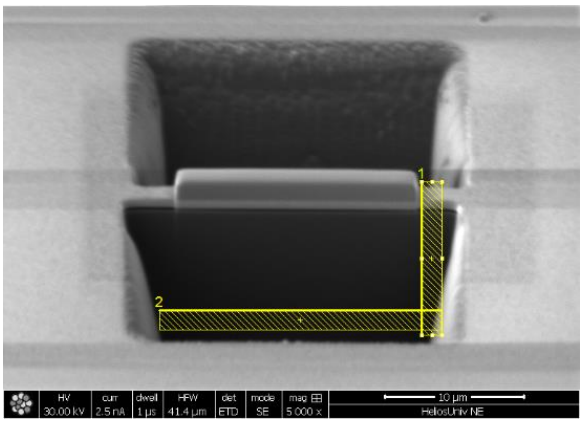
Si_ccs_new

Tilt: ± 1-2˚

20 x1.5x50 µm

Lamella Cuts

1. Tilt the stage to 0˚.
2. Select Quad 2, and go to the Beam Control tab and under scan rotation select 180 degrees from the drop-down menu. This inverts the FIB image.
3. Make an “L” shape out of rectangles to cut one side and the bottom, but leave the side opposite to where the needle is attached intact. Set up all the rectangles to mill according to the parameters listed below and play.
4. The **Auto-Start Real-Time Monitor** should be enabled, so you should automatically be able to view the progress in the FIB (after you adjust contrast/brightness). You can also use iSPI in the SEM. **Stop** milling when you get all the way through. Bottom takes the longest.



30 kV

2.5 nA

Si_new

Rectangles

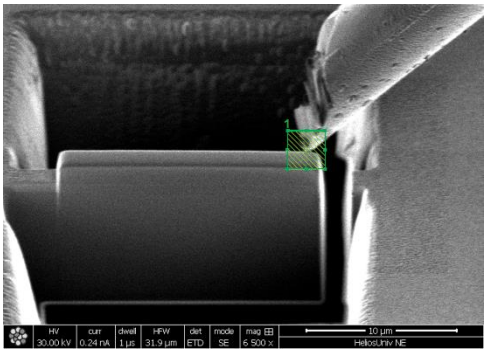
z = 10 µm (arbitrary)

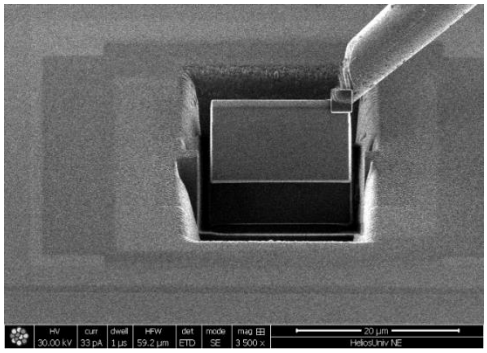
Parallel Milling

Time ~ 3-5 mins

Needle Attachment and LiftOut

1. Go to the Navigation tab and select the EasyLift sub-tab and insert the needle to park position. After the needle is inserted, two green boxes appear in the SEM and FIB quads.
2. Control the needle with a **left click and drag** along the direction you wish to move. The larger green box controls motion in x and y. To raise and lower the needle, click in the smaller, vertical rectangle and drag up and down.
3. Use the SEM to manage the needle's x and y position. Move it over to the bottom left corner of the lamella.
4. Live viewing in the FIB can be done at low beam currents (40 pA), where it is easy to observe z motion.
5. Lower the needle to within 50 – 100 µm of the sample, and insert the MC nozzle.
6. Continue lowering the EasyLift needle, by viewing in the FIB, until it is near the surface of the lamella. Increasing the Mag reduces the EasyLift's jog speed. Keep pausing to ensure the x and y position of the needle is where you want it.
7. Bring the needle down and slightly behind the lamella without touching.
8. Weld needle to lamella with C/Pt deposition (see below).
9. Cut the remaining side using a rectangle at 30 kV, 2.5 nA, z = 10 µm.
10. Use the EasyLift needle controls to raise the sample (up direction in FIB quad). Once fully cleared of the sample surface, you may retract the needle and the MC nozzle.

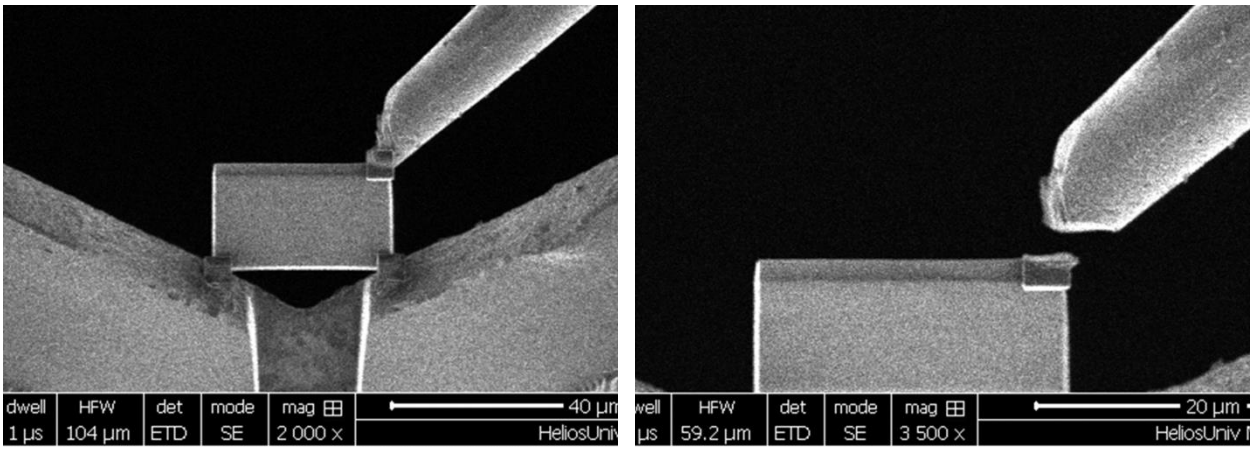




30 kV
0.23 nA (Pt)
0.43 nA (C)
Pt_M / C_M
z = 1 µm
x = y = 2.5 µm

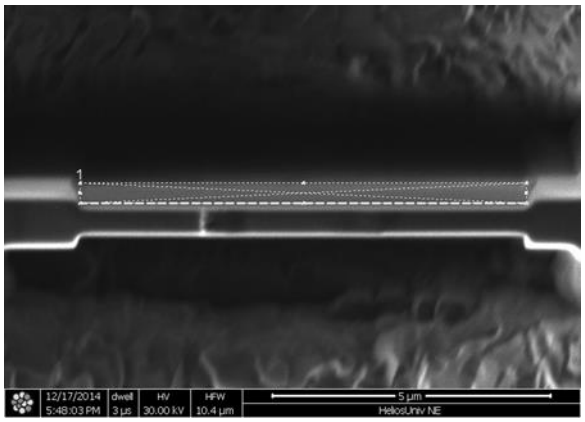
Lamella Attachment to Cu TEM Post

1. Lower the stage 1-2 mm and use Nav-Cam to find a flat Cu TEM grid to use for this next step.
2. Ensure 0˚ tilt, zero beam shifts, link z to WD, use XT align feature to align grid with horizontal, and reset Eucentric height and beam coincidence.
3. After coincidence is reset, and still at 52°, use a 10 x 10 x 10 µm rectangle to mill out some of the Cu grid at 21 nA. This prevents Cu re-dep during final thinning.
4. Tilt back to 0°. If sample is non-magnetic, you may want to switch to Immersion mode in the SEM.
5. Go to lowest magnification for both beams. Insert the needle to park position. Image at 2 kV and 0.2 nA in SEM, and 30 kV and 40 pA in FIB.
6. Lower the needle toward the Cu grid. Look for lamella to overlap the edge of the Cu grid first then the electron shadow. Attach the lamella to the grid and weld with C/Pt at same conditions as you used for the needle attachment.
7. Cut the needle off at 30 kV, 2.5 nA, and z = 5 µm. This should only take a few seconds. Retract needle to park position and retract MC nozzle.
8. If some of the W tip remains on the lamella it is best to mill it off as best you can to prevent strain and uneven milling during final thinning. It isn’t necessary to weld the other side of the lamella.
9. Remove the FIB scan rotation by setting it back to 0°.



Final Thinning to Electron Transparency

1. This is the most customizable step in the LiftOut procedure, and requires experimentation and experience because it depends largely on the material and your starting shape. Here are some tips that will help you fine tune the procedure for your sample:
    a. Tilt angle: higher angles will create a thin-bottomed wedge shape.
    b. Cleaning X-Sec depth (z): higher z ensures longer cleaning time per line, lower times result in a thick-bottomed wedge shape.
    c. Box position: overlapping the thick line of X-Sec box with the top surface of the sample will lead to a thick-bottomed wedge, and take off more material.
    d. Minimum Sample Thickness: Measure it throughout this step. Key thicknesses are 500 nm and 300 nm. At 500 nm, switch to 0.23 nA, and at 300 nm go to last step.
    e. For most samples, you want the lamella to be about 400 nm thick at the bottom and about 200 nm thick at the top when you move on to the last step.
2. **Start** at 30 kV and 0.77 nA, z = 50 µm, and a 10 µm wide box. Tilt = ±1.5°. Alternate both sides until lamella is about 750 nm thick.
3. Drop beam current to 0.43 nA and box width to 9 µm. Do both sides until 500 nm thick.
4. Drop beam current to 0.23 nA and box width to 8 µm box width). Stop at 200 nm thick.
5. The C/Pt protective layer should start to mill towards the end of this step.



Tilt ± 1-2˚ from chosen starting point

30 kV

0.77 => 0.43 => 0.23 nA

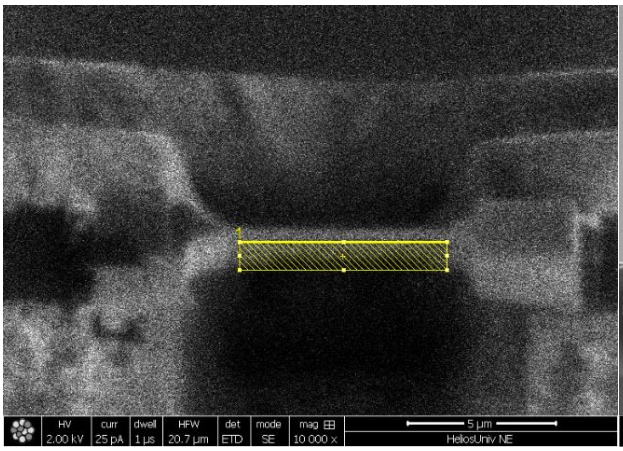
Si_ccs_new

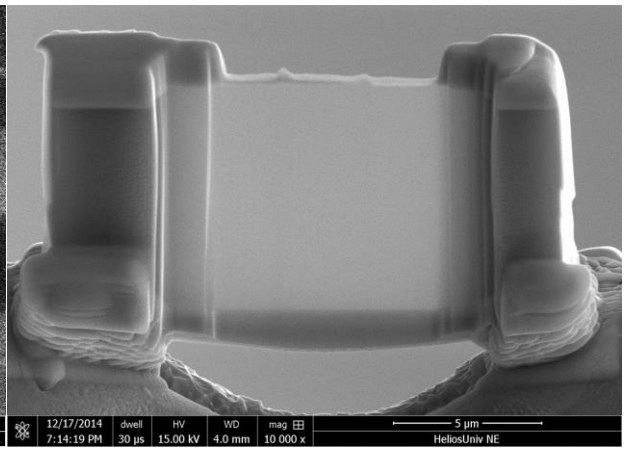
x ≈ 10 µm

y ≈ any

z = 50 µm

Final Cleaning





1. The final step is to clean both sides of the lamella with a low beam energy (2 kV and 23 pA).
2. Zoom out slightly and image live. Adjust contrast, brightness, and focus as best you can.
3. Tilt the stage to ± 7˚ from your starting point and set z = 0.1 µm. Milling shape is rectangle and application is Si new.
4. Set the box so that it encompasses the entire thinned region, then move it down from the surface so that it only overlaps ½ the lamella.
5. If it is too slow move the box so that it overlaps more of your sample. If it is still too slow (e.g. this step takes you longer than 30 mins) increase beam current.
6. If it is too fast – protective layer is removed in a couple of minutes – change the milling direction so the thick line isn't overlapping the sample.
7. Milling at this beam setting will only damage about 2-3 nm of your sample. It effectively removes re-deposited material like Cu form the lamella surface.

8. Each side should take about 5-10 mins.
9. View the top surface at high mag (immersion mode) using iSPI every 15 seconds.
10. Stop when only a few 100 nm of C/Pt are left on the lamella. Watch the protective layer constantly during this step to see how much is being removed by each scan.

Part IV: EDS Version

Requirements

Part 1 training is **required** for Part II.

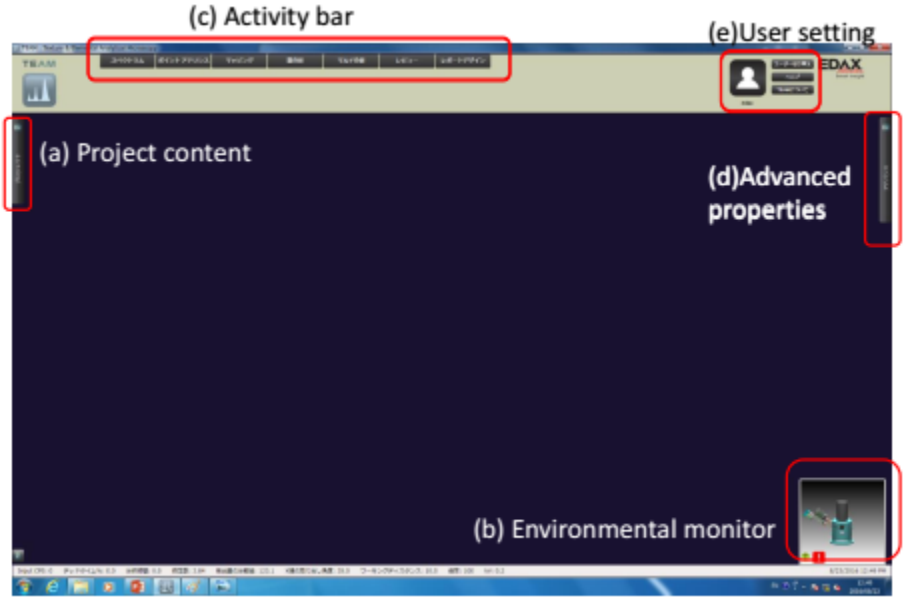
The “EDAX” system mounted on our Helios is an X-Ray analysis system capable of producing an energy spectrum from X-Rays emanating from a specimen material that is struck by energetic electrons and analyzing the data to determine what elements are producing the X-rays. This principle is termed Energy Dispersive (X-ray) Spectroscopy (EDS).

Step 1, Check SEM parameters, Select an appropriate electron acceleration voltage for your material (normally 10 kV - 30 kV) by checking the K, L, and/or M characteristic X-rays of your elements of interest.

Step 2, Log in to EDAX TEAM 

User ID: Administrator Password: apollo

Step 3, Create a “New Project” for new analysis and insert specimen name



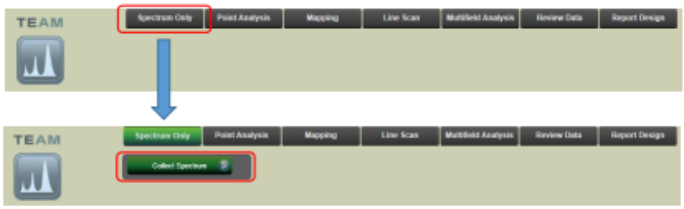
User interface after a new project is created

Step 4, Cooling EDX Detector

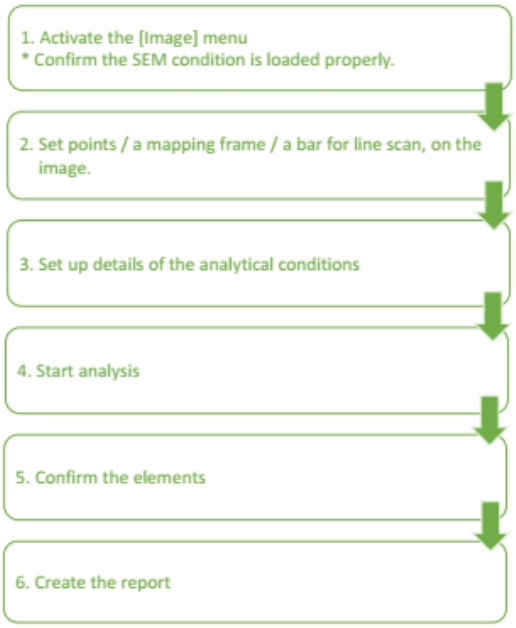
Cooling down EDX detector is compulsory before analysis. (1) Click the [Advanced Property]→ the [EDS detectors]→ the [Det1- Detector Status]; (2***) Click the red[Cooling off] button***; (3) ***Wait until the indication becomes the green***, which will take about 2 mins.

Step 5, Select “Spectrum Only” and run, and Click the “Collect Spectrum”,

*Check the CPS counts is more than 10000, and Dead time should between 20-40%.



Step 6, Analyze Your Specimen--Point Analysis



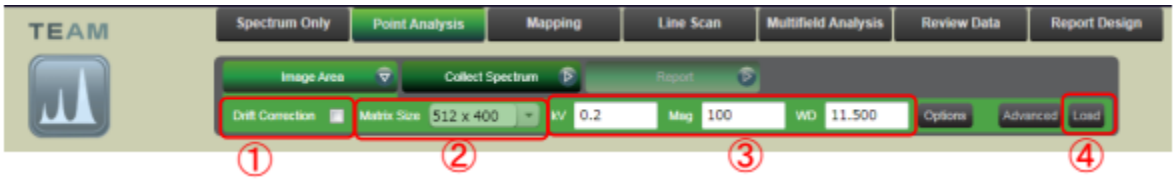
1. Activate the [Image Area]

① Select the [Drift Correction]

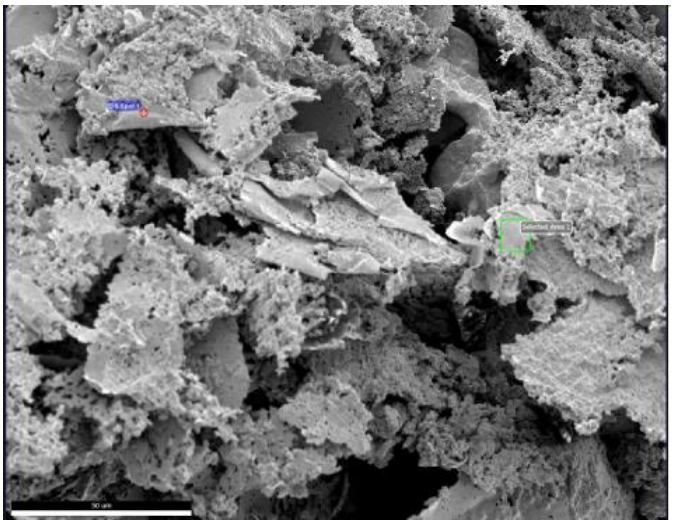
② Select the[Matrix size]

③ Confirm the displayed [Conditions of SEM] accords with the actual SEM conditions

④ Click the [Load] when ③is not accurately reflected



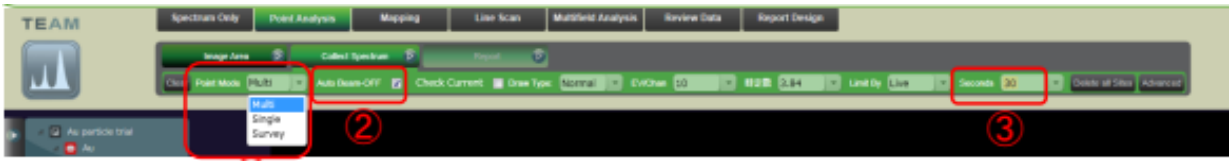
2. Set points or frames for the area analysis on the image.



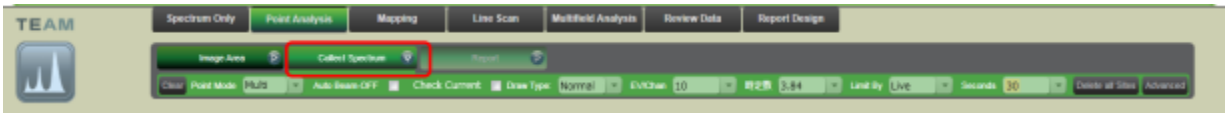
3. Select ①the [Point Mode]

②the [Auto Beam － OFF] and

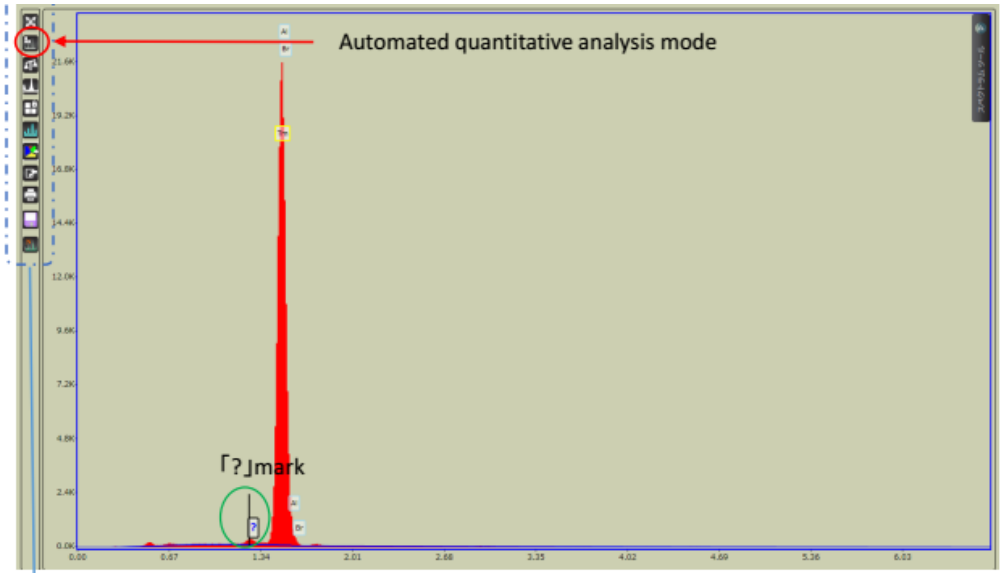
③the [Seconds]



4. Click the [Collect Spectrum]



5. Identify the element, Select the [Automated qualitative analysis mode] from the list which is on the left side of the spectrum.



Step 7, Analyze Your Specimen-- Mapping

1. Activate the [Image Area] menu.

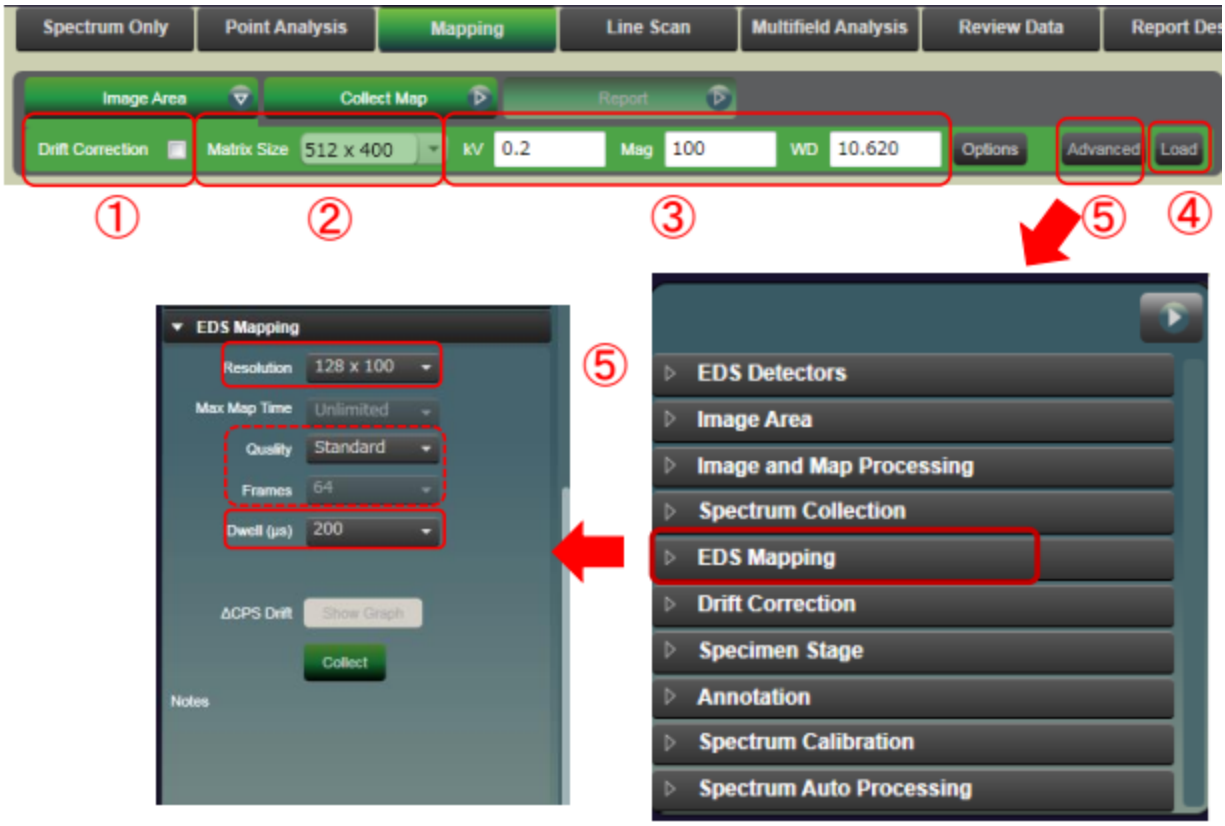
①Select the [Drift Correction]

②Select the [Matrix Size]

③Confirm the displayed [Conditions of SEM] accords with the actual SEM conditions

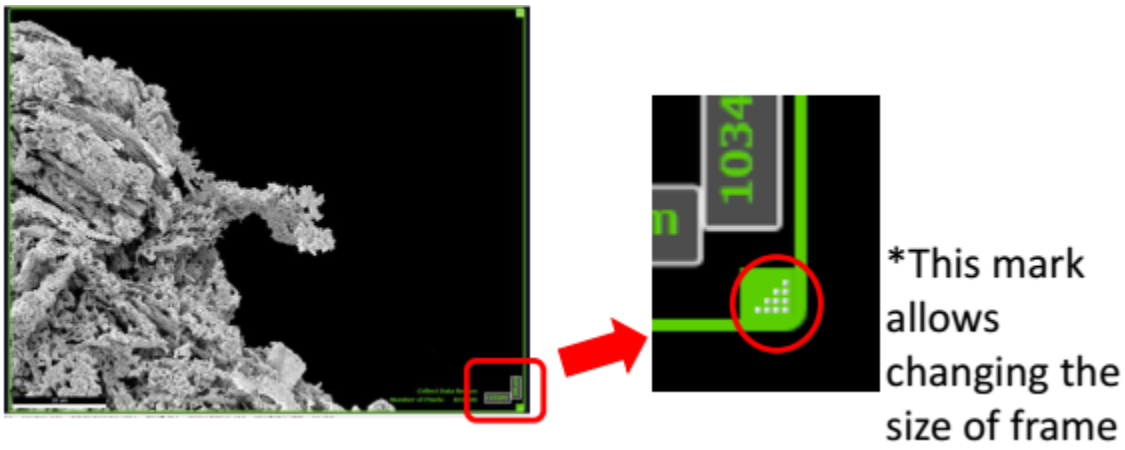
④Click the [Load] when ③is not accurately reflected.

⑤Select the [Resolution], [Frames], and [Dwell time] in the [Advanced]



Note: *Analysis time will be increased when the high resolution of the [Quality], the large number of the [Frames] and the long [Dwell time] are selected. *The number of the [Frames] can be changed when the [Manual] is selected from the [Quality].

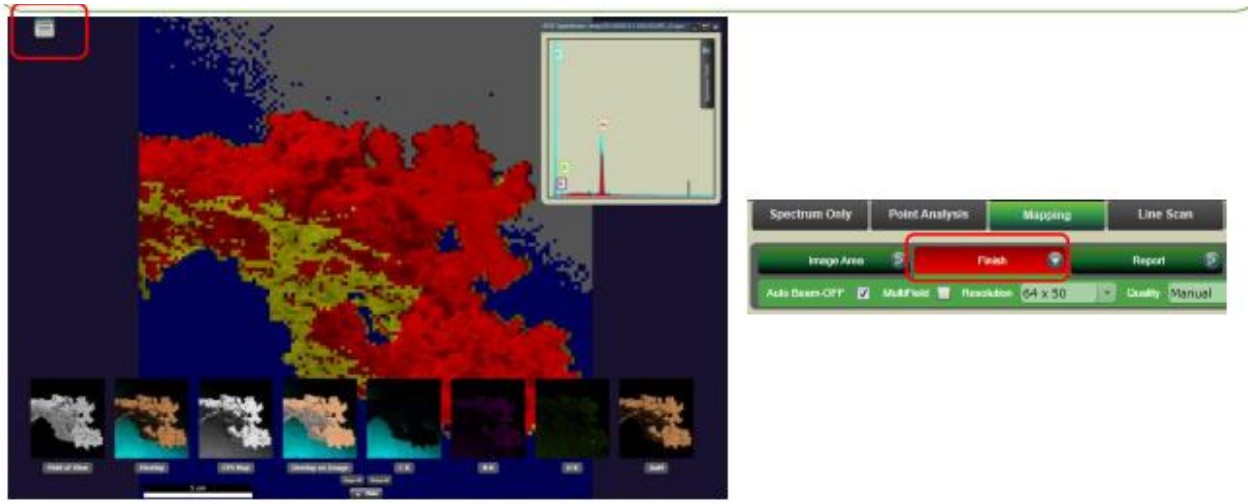
2. Set a mapping frame on the image.*The size of the frame can be changed when the cursor is on the green square.



3. Activate the [Collect Map]

4. Click the [Collect Map], 

5. Click the [Finish] button



Step 8, Analyze Your Specimen-- line scan

1. Activate [Image Area] menu

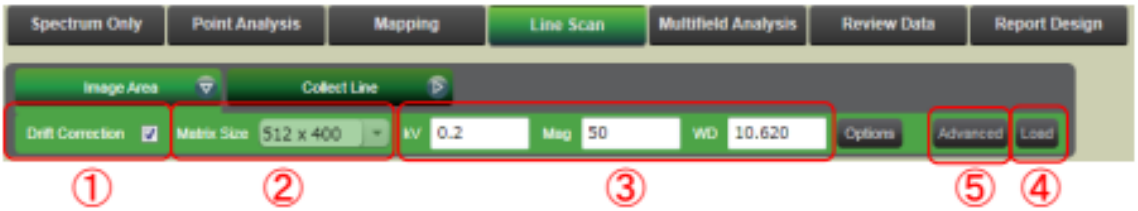
①Select the [Drift Correction]

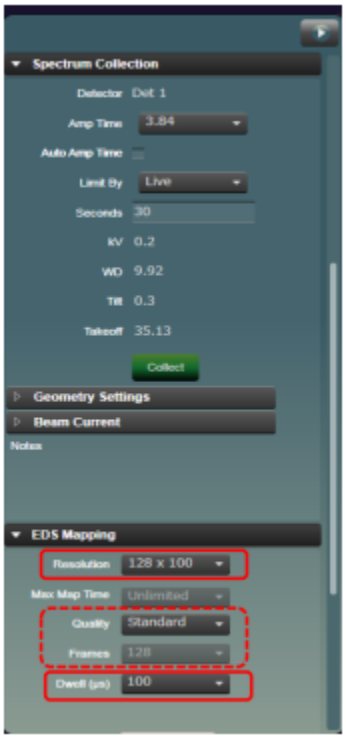
②Select the [Matrix Size]

③Confirm the displayed [Conditions of SEM] accords with the actual SEM conditions

④Click the [Load] when ③is not accurately reflected

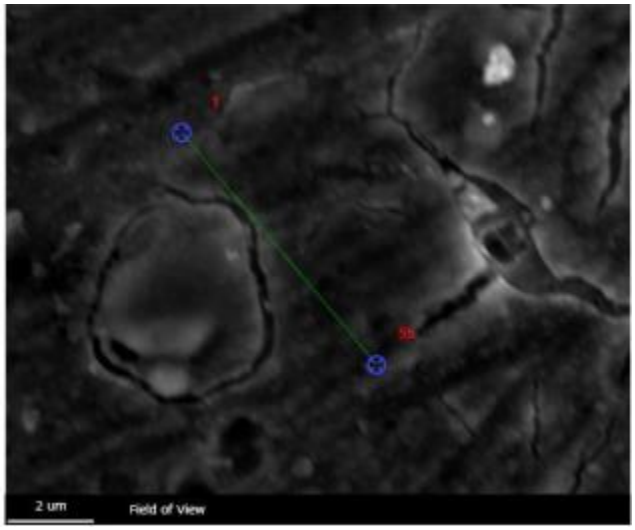
⑤Select the [Linewidth], [Resolution], [Frames], and the [Dwell time] in the [Advanced]





Notes: Analysis time will be increased when the high resolution of the [Quality], the large number of the [Frames] and the long [Dwell time] are selected. *The number of the [Frames] can be changed when the [Manual] is selected from the [Quality].

2. Set a bar on the image

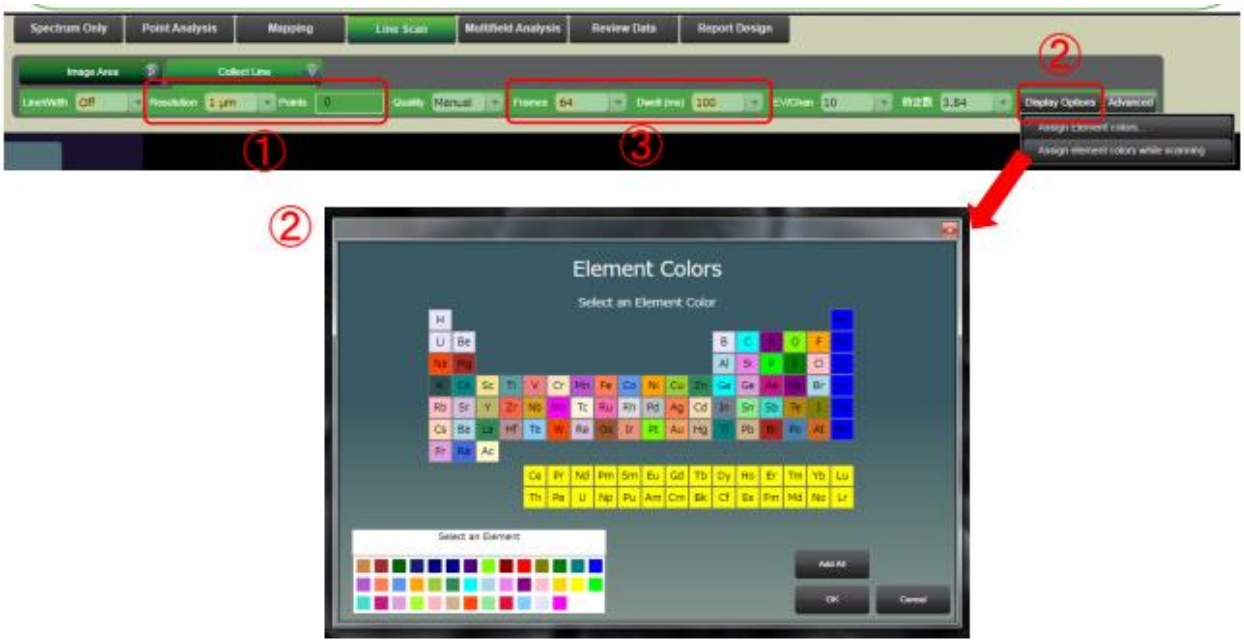


4. Activate the [Collect line].

①Select the [Resolution] Then, the [number of points] will be automatically indicated.

②Change the color through the [Assign element colors] of the [Display Option]

③ Indicated number of Frames and Dwell time are already selected in advanced menu previously .



5. Click the [Collect Line].

6. Click the [Finish] button

Step 9 Create the report

1. Select the[Export] in the [Project Content], and Select data you wish to save and click [OK]

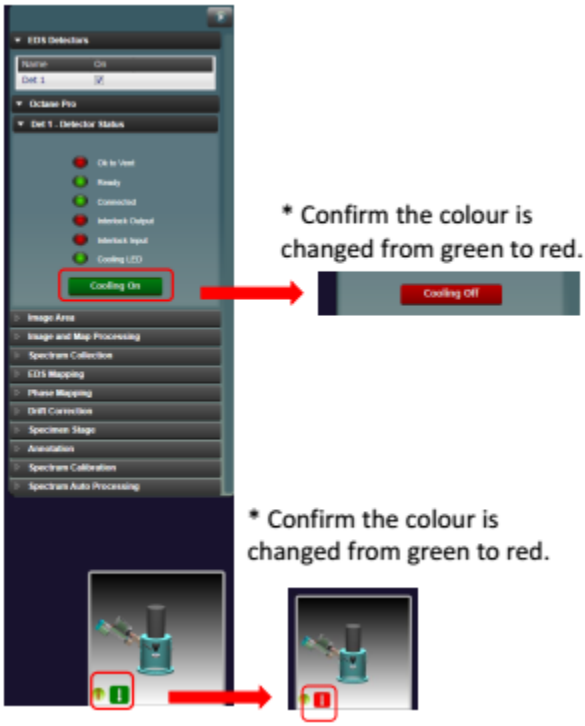
3. Save the [ZIP file] as the raw data

Step 10, Close TEAM Software, make sure cooling of EDX detector is now off.

1. Stop cooling the detector

① Click the[Cooling On] of the [Advanced] menu or

② Click the [green thermometer] icon



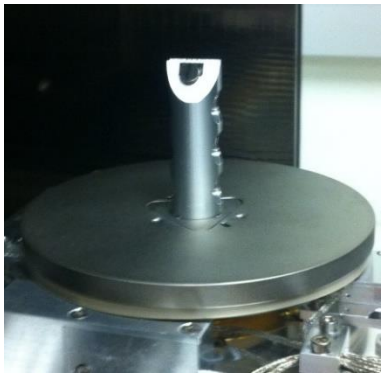
Part V: EBSD Operation Manual

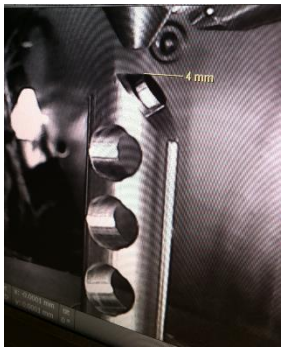
Requirements

Part 1 training is **required** for Part V.

Sample Set-up

In order to use EBSD, the sample must be mounted at a 70° angle to the electron beam. To accomplish this in the Helios, **use the 54° and 36° pre-tilt holder** for general samples or the **special 54° tilt holder for 1 inch polished samples**. Long side for plan view and short side for cross-section view. Before closing the door, ensure that your sample is at the right height. Close the door very slowly and watch in the chamber camera to make sure your sample will clear the objective lens.



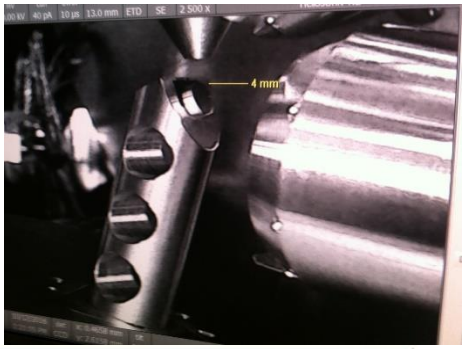


Find interest area and take SEM images

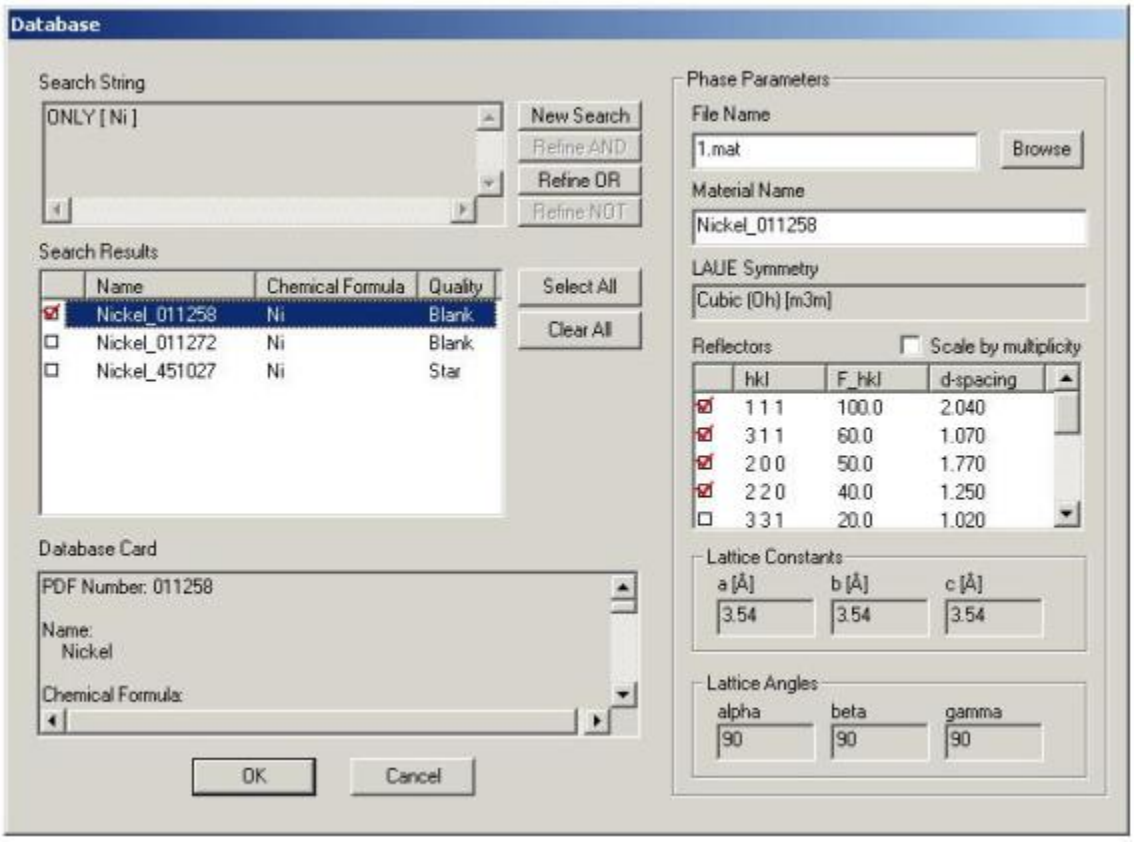
1. Ctrl +0, move stage to (0,0) position. Take Navigation photo, Z distance is 4 mm. Follow the Helios SEM instructions to set up and align the microscope. Find the area of interest and focus the image following routine SEM operation.
2. A general guide to conditions for EBSD is below: a) Accelerating voltage. In general, higher voltage is useful for surfaces that have more damage or contamination. Lower voltage will improve the spatial resolution, but will be much more sensitive to surface finish. b) Spot size/Objective aperture – Larger spot sizes and larger apertures (smaller aperture number) will increase the beam current. Higher beam current will allow you to map more quickly. For example, set electron beam acceleration voltage at 30kV, 0.8 nA for better resolution; 1.6 nA or 3.2 nA for better signal;
3. XT align feature, set eucentric height; find a spot feature then tilt at 16˚degree;

III EBSD Data Acquisition

1. Open OIM analyzer, insert EBSD detector, and click toggle stage control, to switch between EBSD and main computer.



2. Load Elemental information, enter space group, as well as atom position, occupancy. For example, Ni, im3 bcc structure,

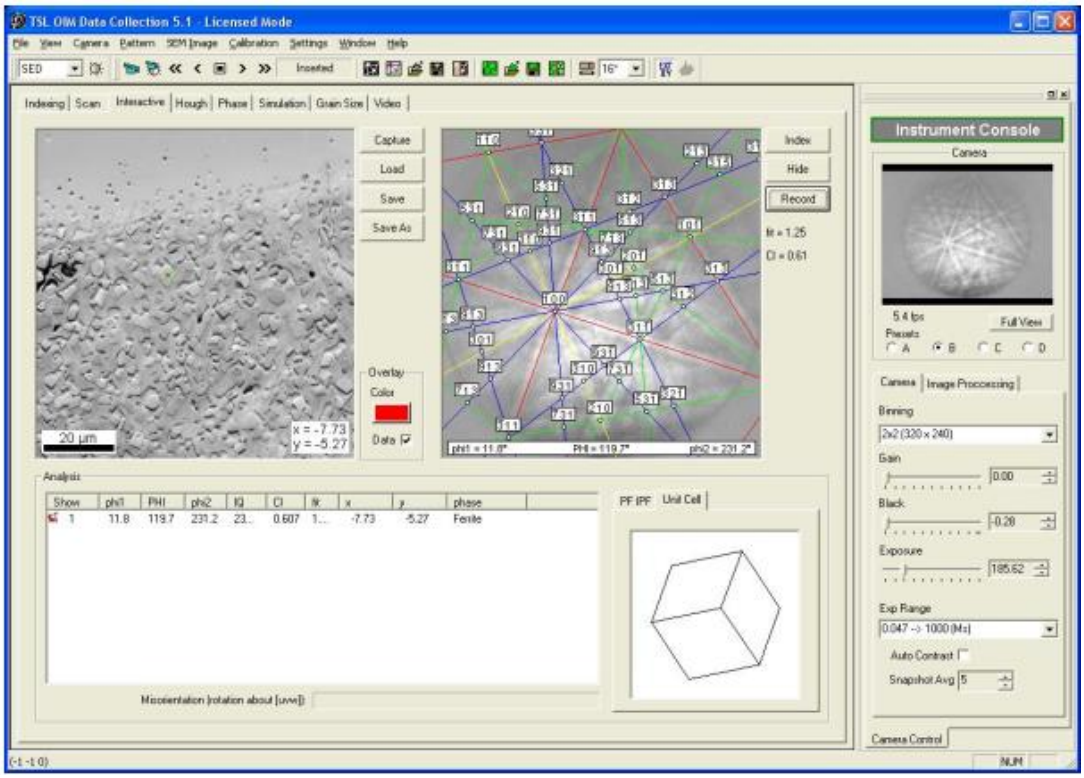


3. The EBSD camera is setup with 4 preset buttons, A, B, C, and D. These buttons are programmed with different resolution or binning settings: A: 1x1, B: 2x2, C: 4x4, D: 8x8 The A and B presets are typically used for good quality patterns when speed is not important, low gain and long exposure time. The C and D presets are used for faster pattern collection during EBSD mapping, higher gain and short exposure times. For example, Select Binning 4*4, Decrease scanning dwell time, such as 100 ns, spot+beam unblank. Lower the magnification so you are scanning many grains.

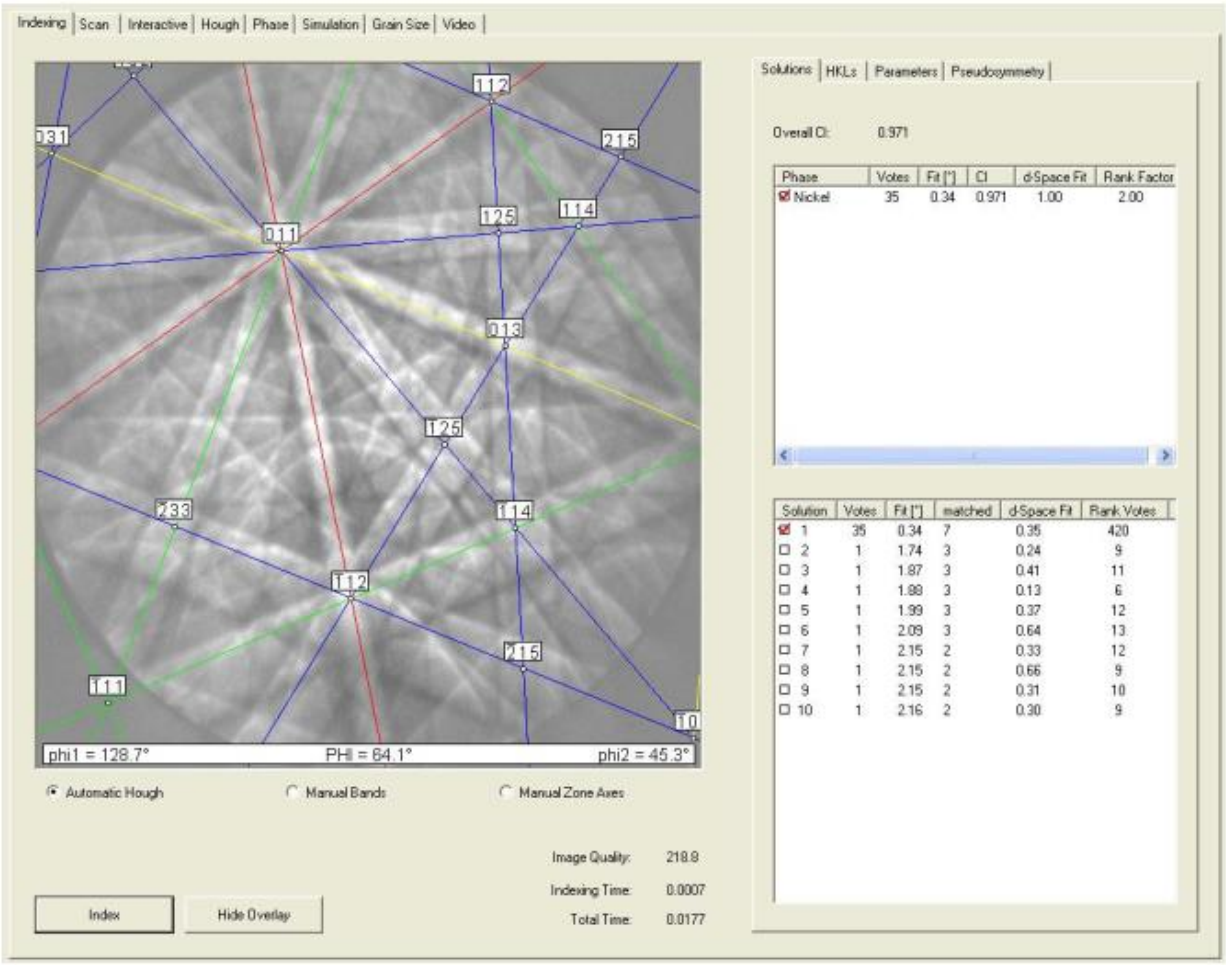
4. Go to the Live EBPS tab and uncheck background subtraction. Now you should have the raw EBSD signal showing up – It should be more intense in the center of the screen, fading to the edges and you may see some Kikuchi bands. Adjust exposure 0.100-100 ms, 50 fps (fram per second), Gain 300, below true saturation, close to 1.

5. Compare background, frame 100; apply background, subtract.

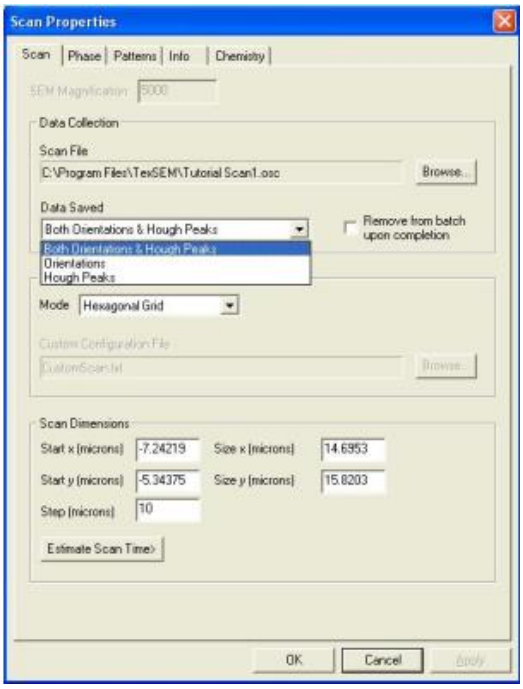
6. Switch to spot mode, to check the pattern; reduce the gain, may be helpful to improve the pattern.



7. Notice that there are three options for indexing: Bands, Hough, and Zone Axis. The Bands method involves manually drawing in the position of the diffraction lines in the pattern. The Zone Axis method required the user identification of multiple poles in the diffraction. The most commonly used method is the Hough option, which uses a Hough transform to automatically detect the position of the lines in the pattern. Make sure this option is selected. Then press the Index button. The pattern you have obtained should be automatically indexed as seen below.

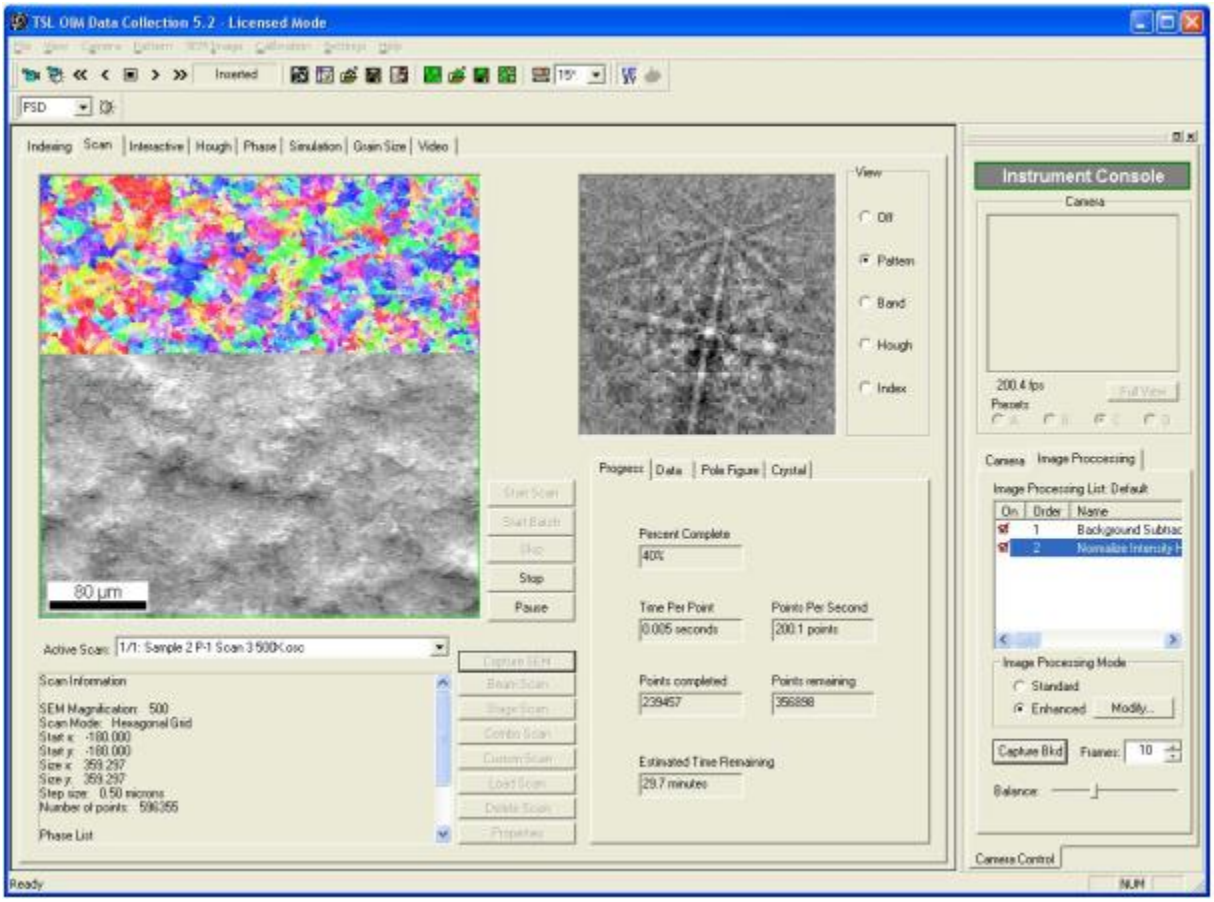


8. Capture an SEM image using the Capture SEM button. Set SEM voltage as 30 kV. You can adjust the contrast and brightness of the SEM image using the button on the OIM toolbar. Rectangular scan areas can be defined in this window by left clicking and dragging to define the area of interest. Polygon scan areas can be defined by left clicking on initial point of the polygon scan area, left clicking on additional points to define corners of the scan area, and then closing the scan area with a right click. For a line scan, left click on the initial point, and right click on the terminating point. Experiment with these different scan parameters. Notice that after you define a scan

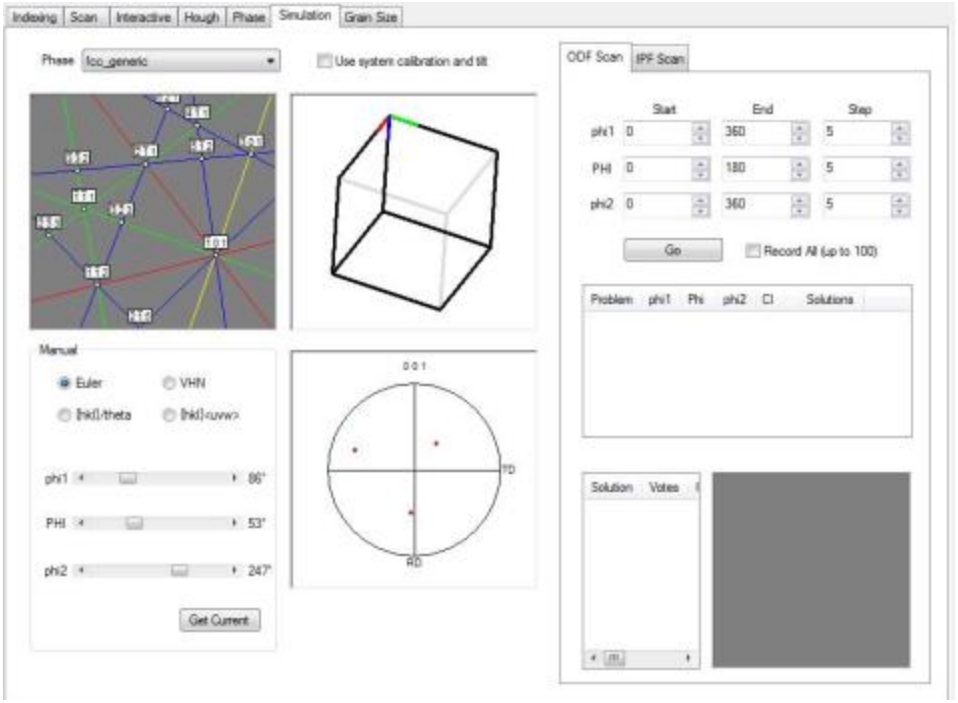


area, the Scan Properties dialogue box appear. Save data both Orientations & Hough Peaks. Orientation means that the Euler angles describing the orientation associated with each point in a scan will be recorded. Hough Peaks means that the parameters associated with the peaks in Hough Transform will be saved for each point in the scan. Scan type-hexagonal grid for Ni, scan resolution-fine/medium.

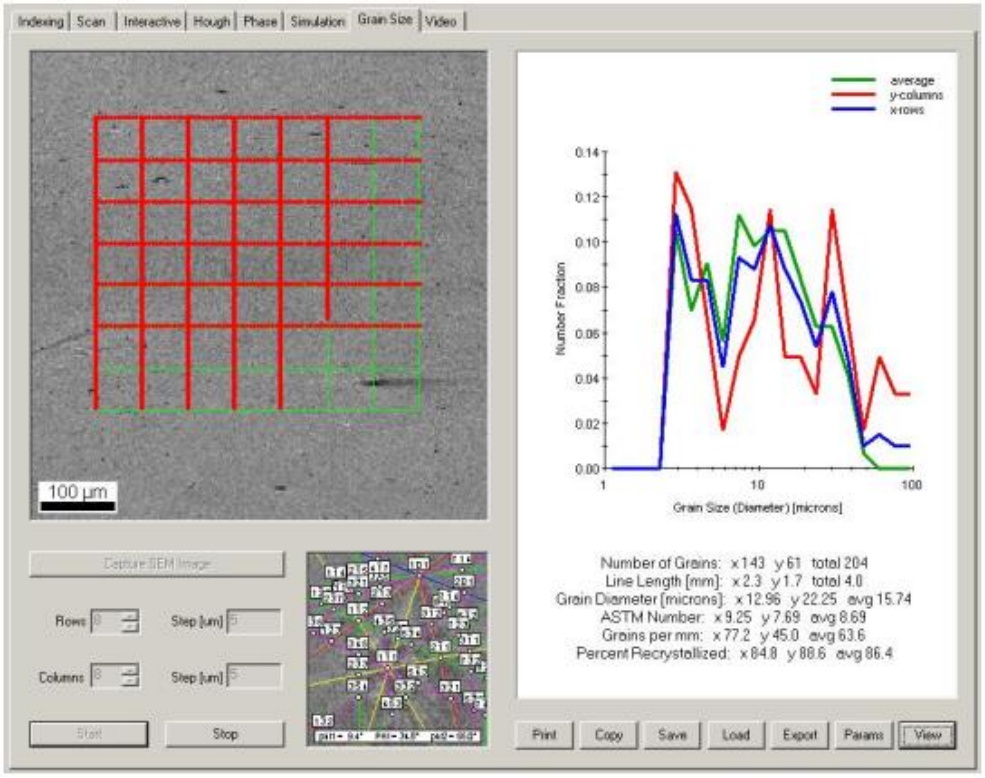
9. Set tolerance between 3 and 10. Higher tolerance, better fitting. Adjust Interactive parameters to improve the index.



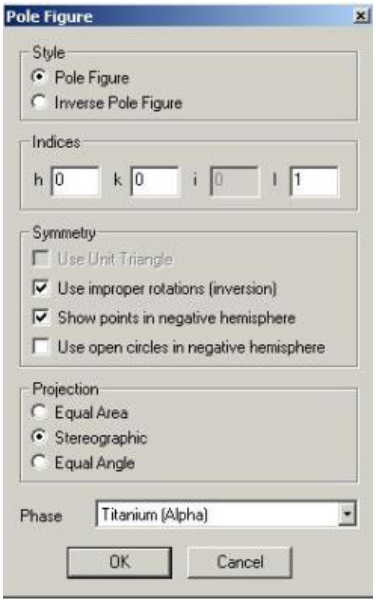
10. The simulation page allows the crystallographic orientation to be visualized in physical space, as diffraction patterns and as projections in pole figures or inverse pole figures.



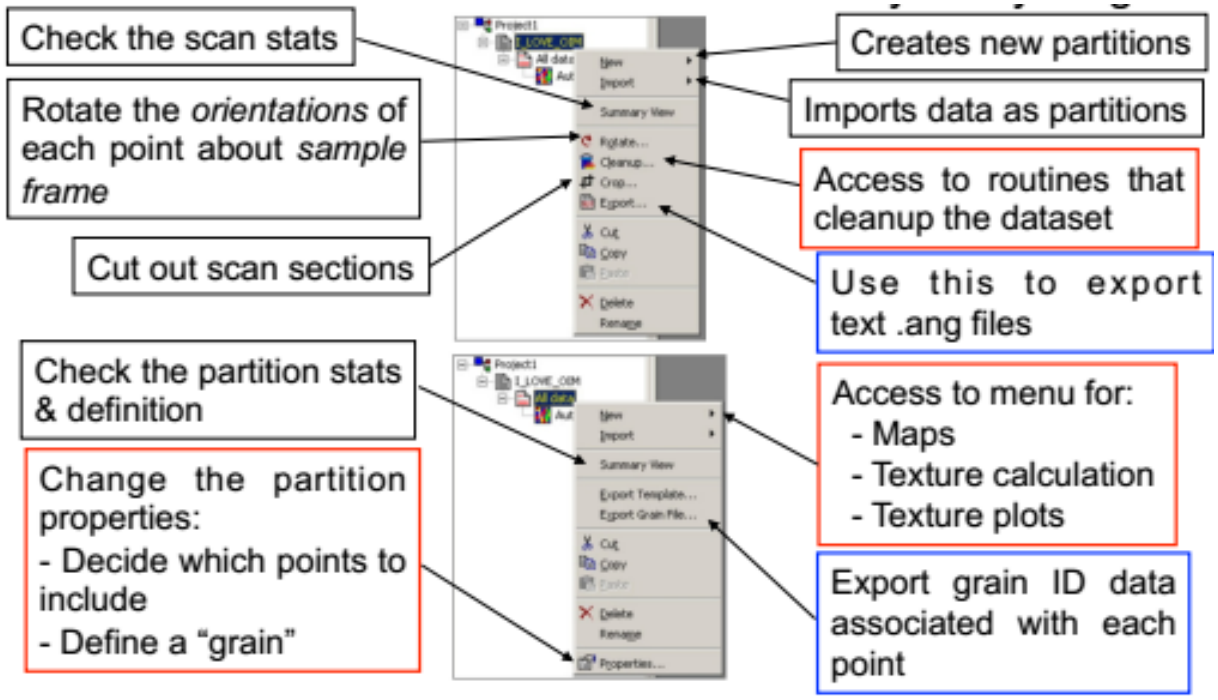
11. The grain size page is designed to help the operator determine grain size statistics for a given sample. A scan is defined by selecting the number of rows and columns, and drawing a rectangle to form a grid. A step size is entered for the rows and columns, and the scan is started. A grain size histogram is automatically updated after each row/column is completed.

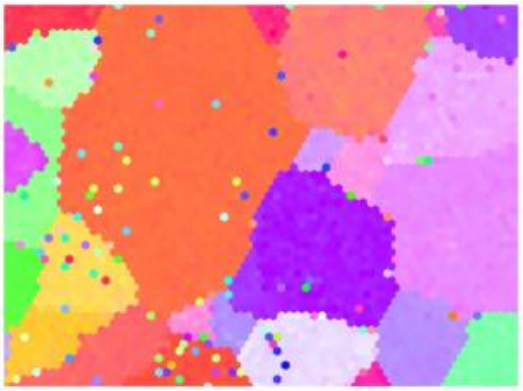


12. By clicking on a pole figure window with the right mouse button a menu is displayed. Two options are available, the first is the pole figure properties and the second is to save the pole figure as a bitmap. Upon selecting the properties menu item the dialog box shown below is displayed. This dialog allows you to select the phase to display, as well as change the style, indices, symmetry, and projection of the Pole Figure.

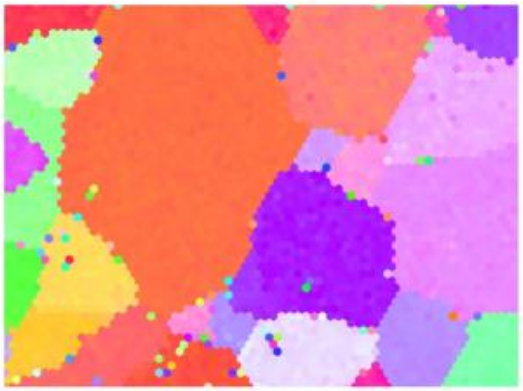


12. Go to analysis software, open the data file. Click on the data, IPF quick map, Auto IQ to show grain and grain boundary. Right click, clean up, tolerance 3, minimum 10-20.





No Cleanup



Level 0

Level 3

Note that Higher cleanup levels are iterative (i.e. Level 3= Levels 0,1,2,3)