

Standard Operating Procedure I: FIB-SEM: Basic Operation and TEM sample preparation



Yale WEST CAMPUS
Material Characterization Core
ywcmatsci.yale.edu

ESC II, Room A119F
810 West Campus Drive
West Haven, CT 06516

Version 1.0, April 2019

Core Policy:

- > **FOLLOW** the SOP strictly to keep the instrument in good condition. **No** explorations allowed on software unless permitted by lab manager
- > **NEVER** use your own USB drive on instrument computer. Data can be either uploaded to Yale Box, or copied to the USB Drive provided by the Core.
- > **NEVER** surf the web on the XPS computer to minimize the risk of the computer being hacked
- > Users should **acknowledge** MCC in their publications. Please check the following link for details:
<http://ywc matsci.yale.edu/publications>
- > The core reserves the right to use the data for core promotion

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FEI Helios G4 Dual Beam Standard Operating Procedure

1 Introduction

1) Instrument features:

- > High quality ultra-thin TEM sample preparation: automated sample preparation using AutoTEM allows multiple lamella preparation for final polishing.
- > Slice and view: automated sequential mill and view to collect series of slices images. Allow sample 3D reconstruction for further analysis such as 3D segmentation.
- > EDS: allows simultaneous EDS mapping for 3D reconstruction
- > EBSD: provides grain orientation maps, grain boundary maps, phase maps and pole figures.
- > FIB patterning: creates small structures with feature size down to tens of nanometers via 3D ion milling and deposition.
- > E-beam lithography: e-beam writing on resist coated surface for device fabrication with feature size smaller than 10 nm.

2) Location

Materials Characterization Core
Room A119G
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3) Primary Staff Contact

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The Yale West Campus MCC Facilities are operated for the benefit of all researchers. If you encounter any problems with this facility, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.

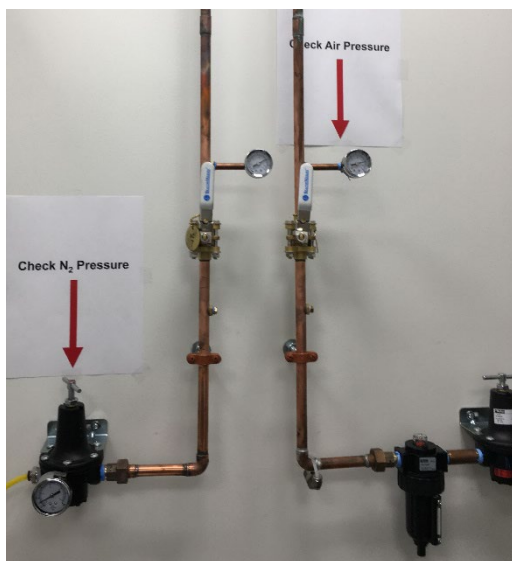
Notice: Please **follow** strictly the **SOP** to keep the facility under good condition. **No** explorations on program allowed unless approved by core manager.

2 Initial System Status Check

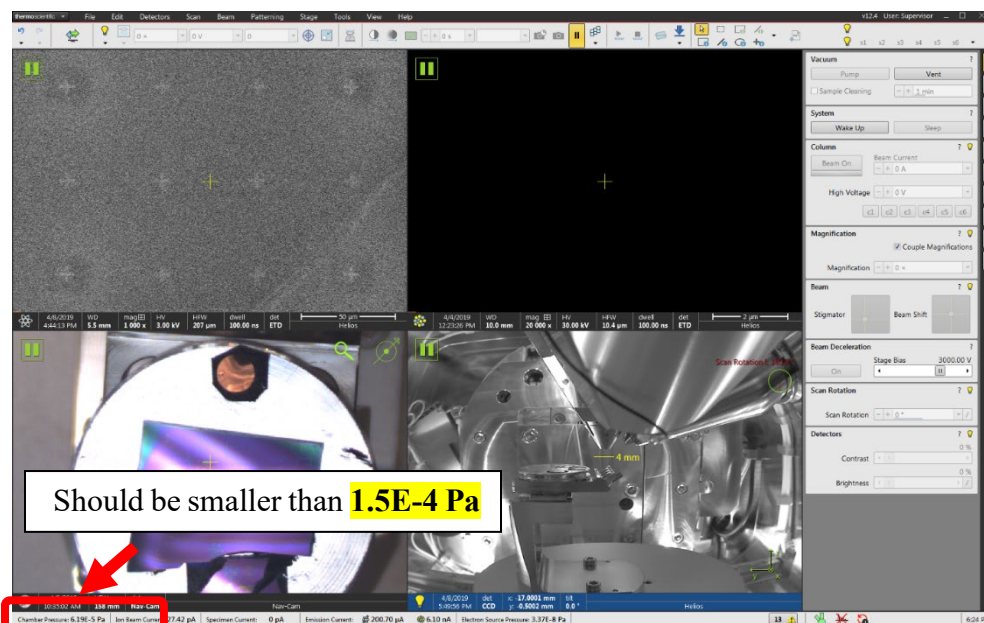
Warning: Before sample preparation and loading, the users are required to check the system status following the steps listed below:

- 1) Check the building supply N_2 and **Compressed Air (CA)** pressure gauges as labelled on the back wall and make sure the pressure reads **~100 psi** on both gauges. Report to manager immediately if any of those gauges reads **zero**.

Warning: Do not adjust the regulators on top of those gauges.

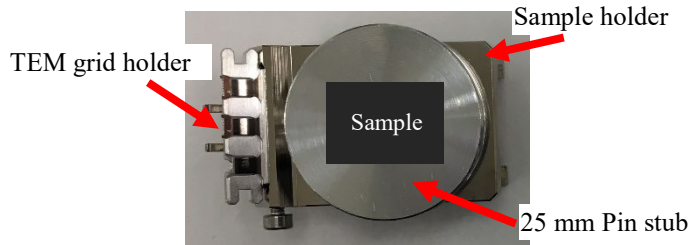


- 2) Check the **Chamber Pressure** at the bottom left corner of the **Microscope Control XT** window as shown below and make sure the main chamber pressure is below **$1.5E-4$ Pa**. **DO NOT** proceed if the pressure is high. This may indicate chamber leaking or outgassing. Report to manager immediately.

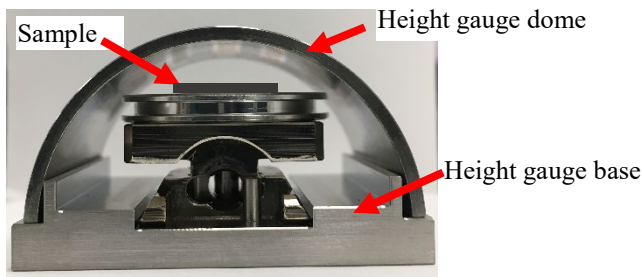


3 Sample preparation

- 1) **Always wear gloves** for vacuum sample preparation!! **Change gloves** if touched computer keyboard and mouse.
- 2) The sample for FIB should be **completely dried before coming**.
- 3) The sample can be fixed using double sided carbon tape directly onto the pin stub on the sample holder as shown below:



- 4) Sample height alignment: place the sample holder into the gauge as shown below and **MAKE SURE the sample is not touching the inner wall of the height gauge dome**.
Warning: misaligned/misplaced sample will hit the FIB pole piece causing server damage!




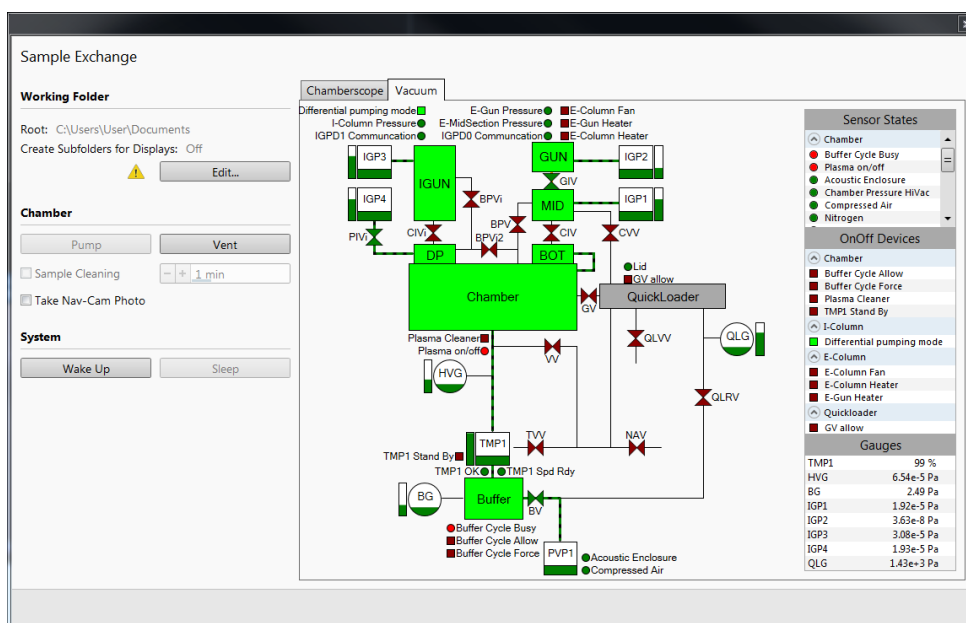
- 5) **Clean sample holder:** bring the specimen stub inside the fume hood and **blow off** loose particles on the sample surface using the **N₂ gun**.


4 Starting Instrument

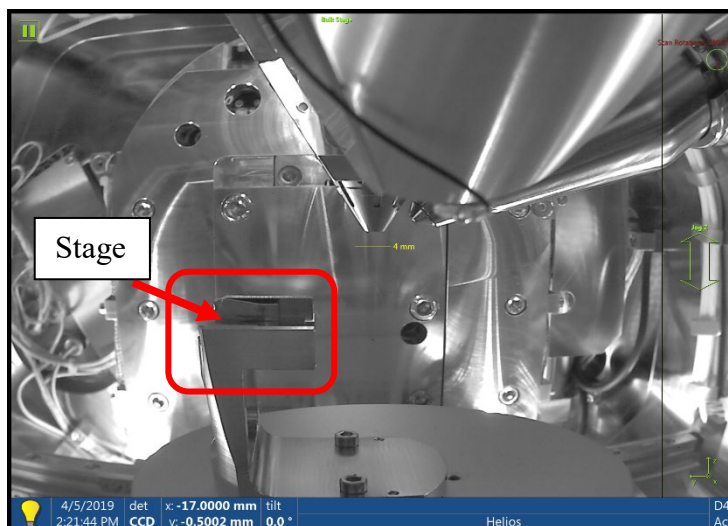
- 1) **Sign in** on the **logbook** and put down date, usage time, sample materials, Chamber pressure, working modes (FIB, EBL, EBSD or EDS), and report any issues during measurement.
- 2) Log into **FOM** on the Support PC monitor on the left side by clicking **Click here to login with NetID:**


5 Sample Loading

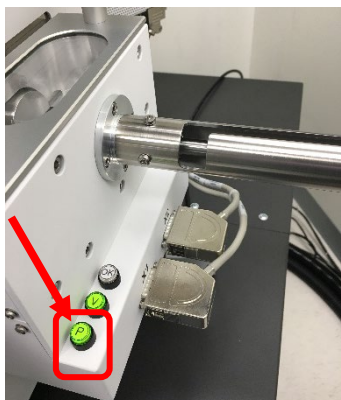
- 1) Click  button on **XT** window tool bar to open the **Sample Exchange** window below:



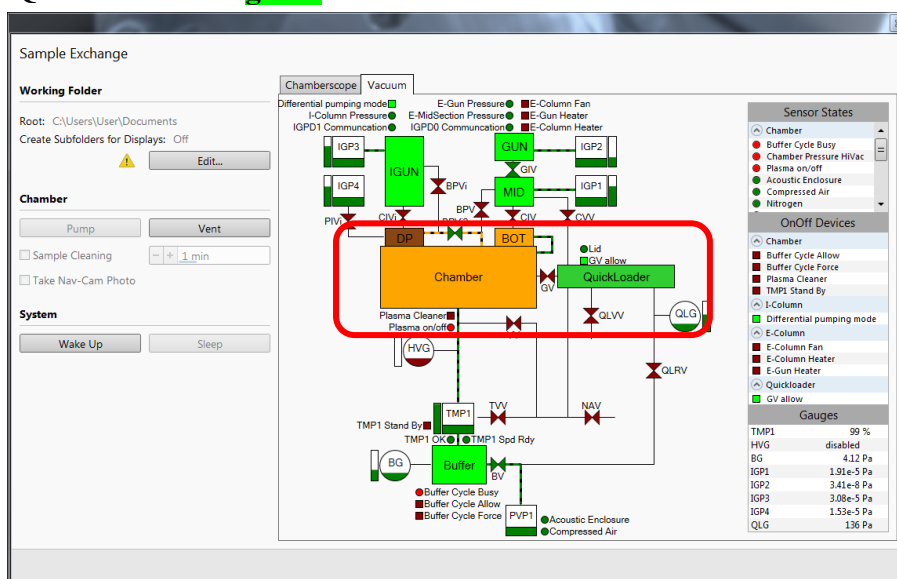
- 2) Click  inside **CCD** window (bottom right window) to unpaused/activate **CCD** view inside main chamber.
- 3) Click top menu **View > Large Image Window** to amplify the **CCD** window onto the computer monitor on the right side.:




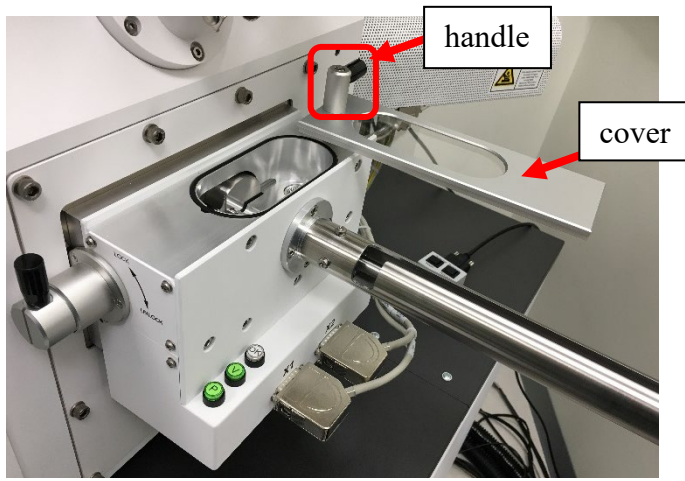
- 4) **Put on gloves.** Press and hold  button for one second on **Quick Loader** as shown below:



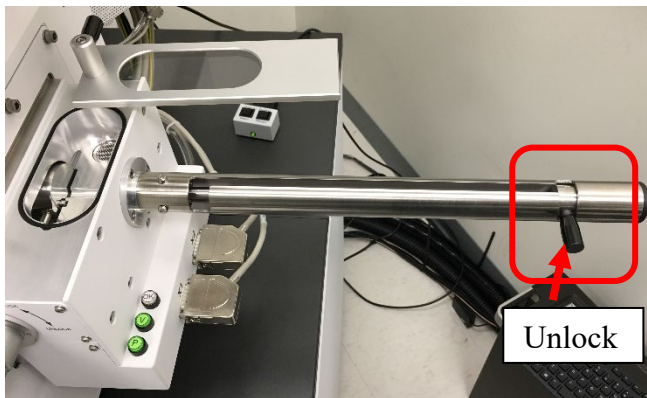
- 5) Watch the **Sample Exchange** window till the **Chamber** color changes to **orange** and **Quick Loader** to **green** as shown below:



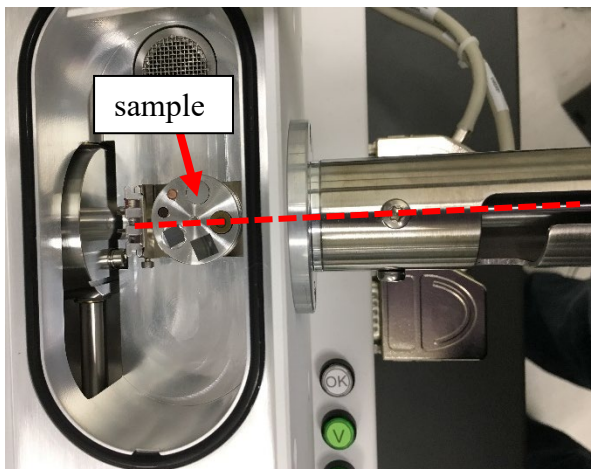
- 6) Right after **Quick Loader** changes into **green** and **Chamber** stays in **orange**, press and hold  button for one second on **Quick Loader** and wait to hear an air sucking sound, indicating the **Quick Loader** is vented.
- 7) Wait for **5 seconds**, grab the handle on top of the **Quick Loader** and **SLOWLY** lift the cover upward and turn it **90 degrees** to expose loading area as shown below. **Never force to break the vacuum** if the **Quick Loader** is not fully vented.



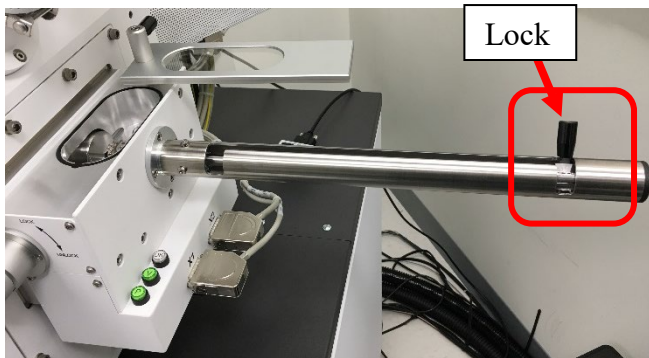
8) Pull the handle on the transfer rod downward to **Unlock** position as shown below:



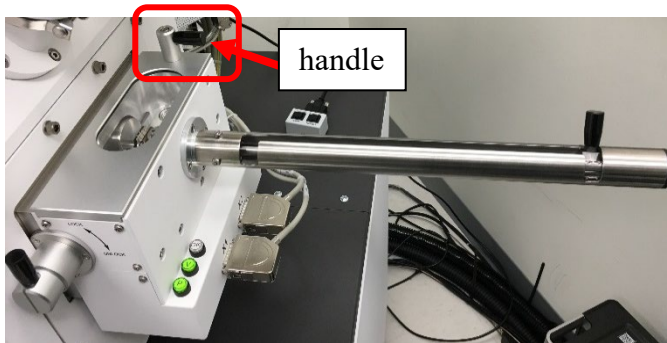
9) Place the sample holder inside **Quick Loader** and align with the transfer rod as shown below:



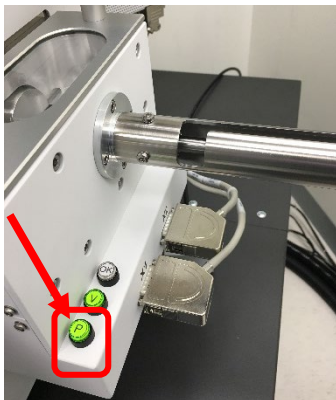
- 10) **Left hand** holds and pushes the sample holder rightward into the transfer rod meanwhile **right hand SLOWLY** turning the **Quick Loader** handle upward to the **Lock** position as shown below. **Never force the handle to break the parts.**



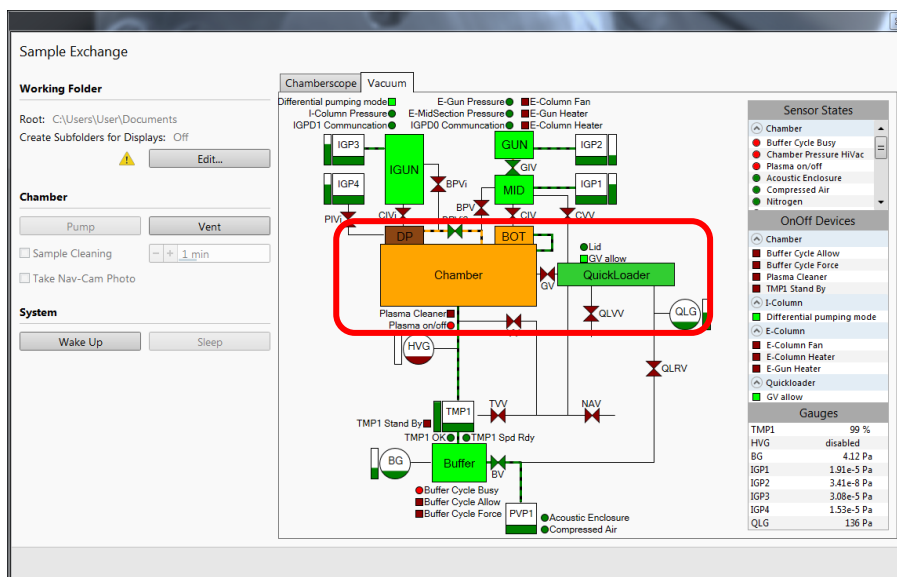
- 11) Turn the cover handle back and press down to seal the **Quick Loader** chamber as shown below:



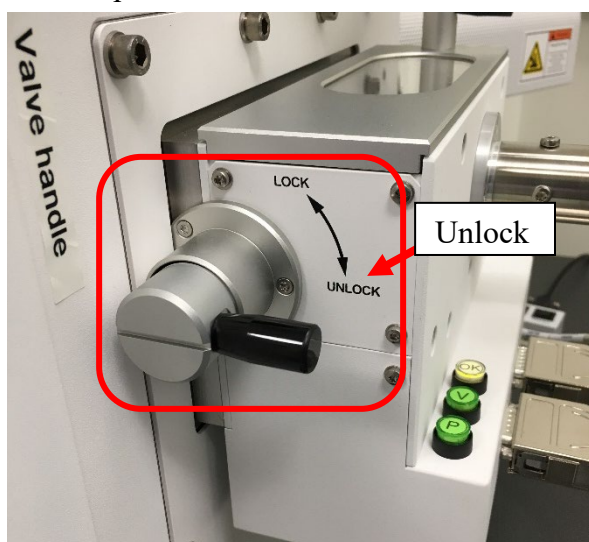
- 12) Press and hold  button for one second on **Quick Loader** as shown below:



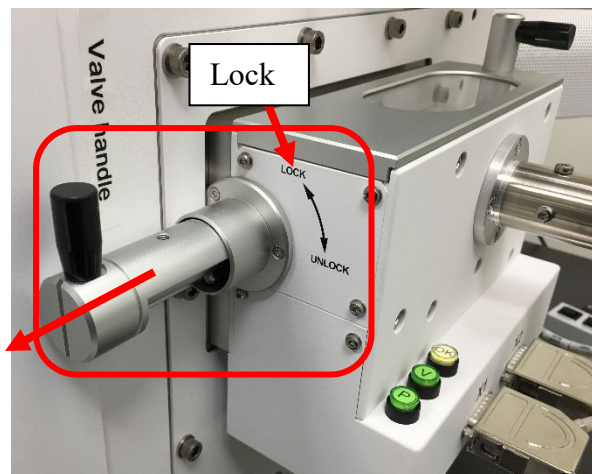
- 13) Watch the **Sample Exchange** window till the **Chamber** color changes to **orange** and **QuickLoader** in **green** as shown below:



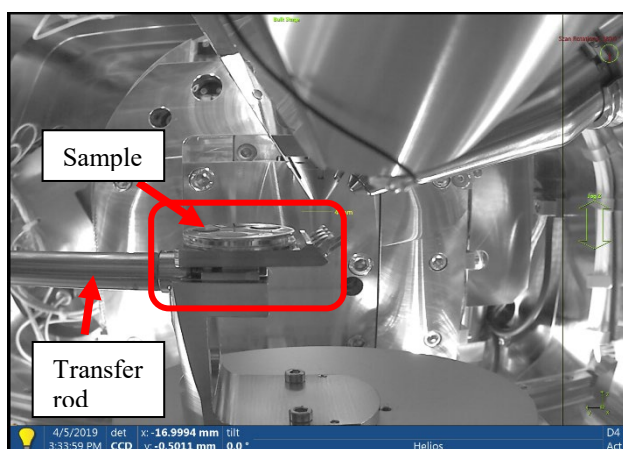
- 14) Right after **Quick Loader** changes into **green** and **Chamber** stays in **orange** turn the **Quick Loader Valve handle** to **UNLOCK** position as shown below. Hold the handle for next step.



- 15) Pull the **Valve handle** outward to open the valve and turn the handle back to **LOCK** position as shown below. The valve should be kept open for sample transfer.



- 16) Rotate the **Quick Loader** handle in mid position between **LOCK** and **Unlock** position and **SLOWLY** push the transfer rod forward to the main chamber. Meanwhile watch the **active** CCD window when the sample holder is approaching the stage as shown below:

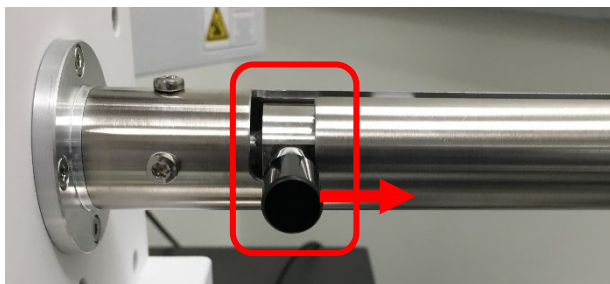


- 17) When the transfer rod is **stopped** turn the transfer rod handle **SLOWLY** downward into the slot until **stopped** as shown below:

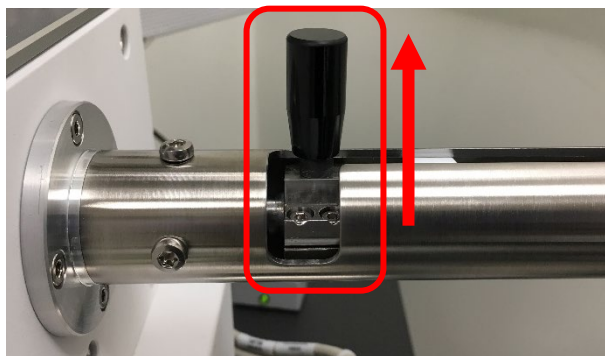


- 18) **VERY SLOWLY** pull the handle **backward** in the slot as shown below and meanwhile **watch** the transfer rod being retracted from sample holder in the **active** CCD window. **Stop** retracting if the sample holder is being pulled out.

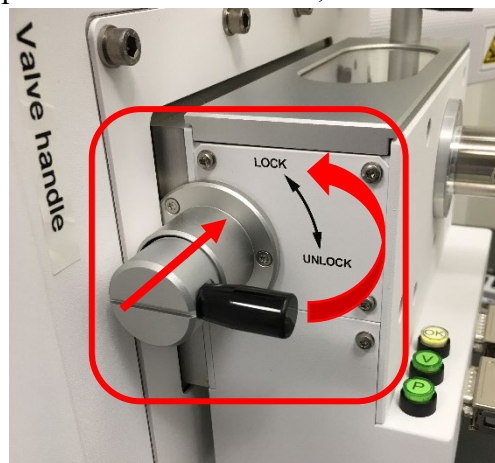
Warning: **Never force the handle to damage the parts.** Report to manager immediately for any issues.



19) **Hold** the handle and **SLOWLY** turn **upward** as shown below and pull the transfer rod all the way to the end and turn **upward** to the **LOCK** position.

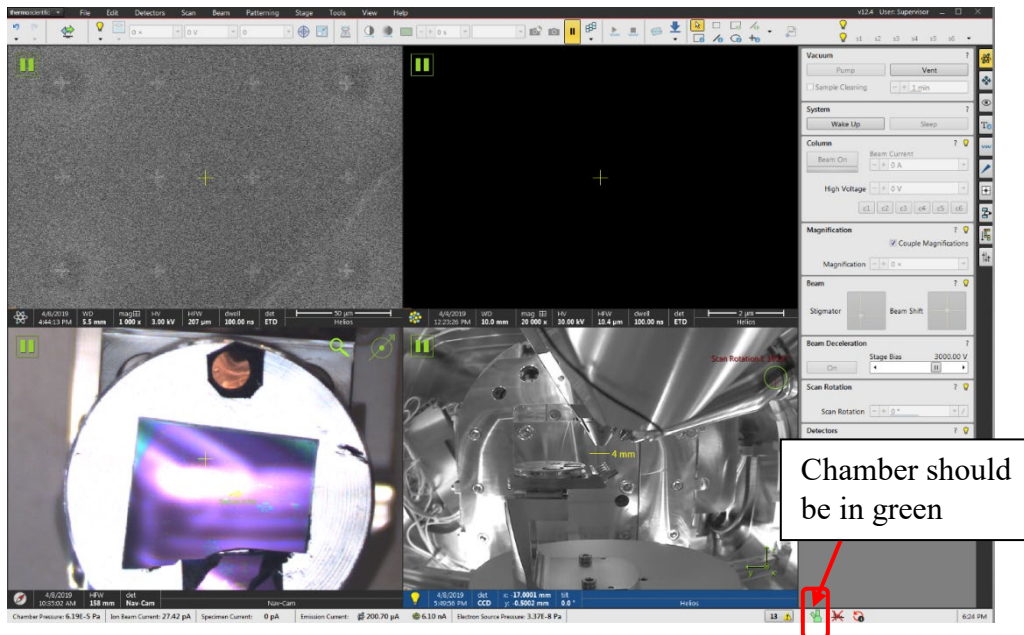


20) Turn the **Valve handle** downward to **Unlock** position and push the valve into sealing position as shown below, and turn the handle upward to **Lock** position.



21) Make sure the

22) **Monitor** the **Chamber Pressure** indicator at the bottom right corner of the **XT** window as shown below.




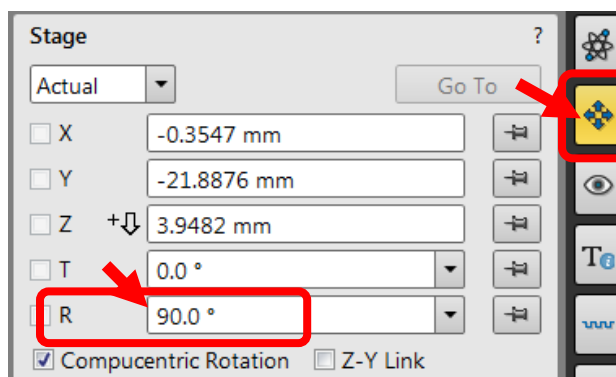
- 23) **NEVER** turn on electron or ion beam. Wait until the chamber color turns into green to proceed to next step.

Warning: early e-beam or ion beam start will lead to electron or ion column contamination.

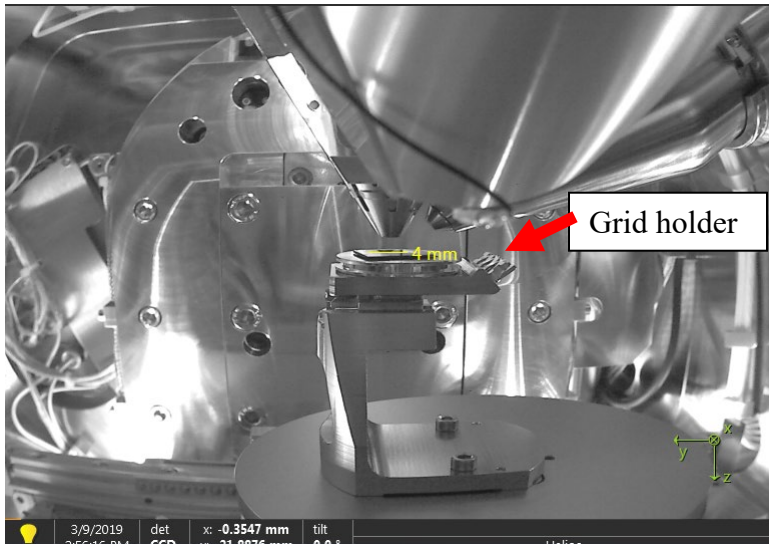
6 Sample Z link to Working Distance




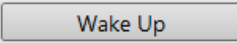
- 1) Click on  Navigation button on the right side of the XT window and make sure the Rotation angle is around **90.0°** as shown below:

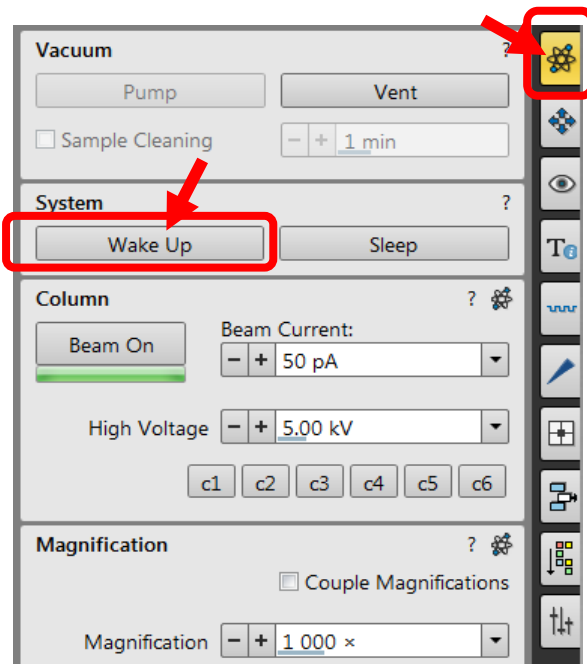


- 2) Check the CCD window and make sure the TEM grid holder is on **the right side** of the sample holder as shown below:

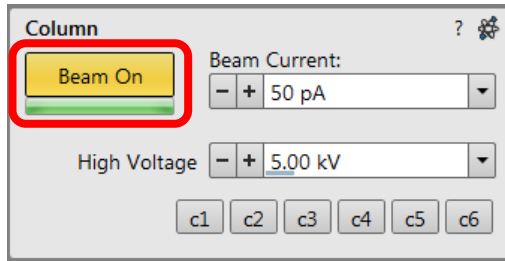



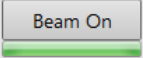
3) Check to make sure the Chamber pressure is below **1.5E-4 Pa**.



4) Click  button on the top right corner of **XT** program window to enter **Beam control** window as shown below and click  to turn on both electron and ion beams.





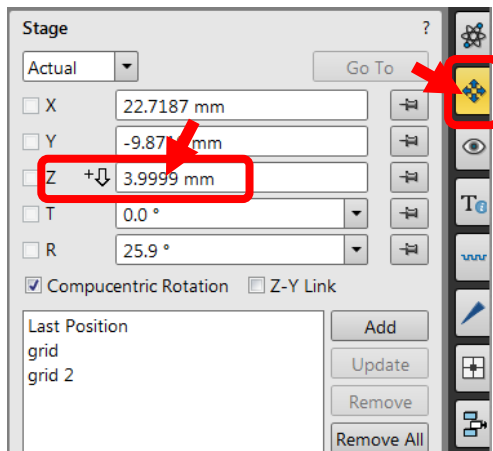
- 5) It takes a short time to turn the ion beam on. Wait till the **Beam On** button turns into **orange** in **Column** control below:



- 6) **If only electron beam is needed** for SEM imaging, e-beam lithography or EDS/EBSD:
- Click on the top left electron imaging window with an  icon on its bottom left corner to switch **Beam control** to electron beam.
 - Single click** on  in **Column** control below to turn on the electron beam:
- 7) Click **Stage** on top menu and select **Take-Nav Cam Photo** to take a sample picture on the bottom left Nav-Cam window,
- 8) **Double Click** on the sample surface inside Nav-Cam window to drive stage to selected area. Zoom in and adjust focus on selected area.

- 9) Click  **Link Z to FWD** button on top tool bar to link **Stage Z** to working distance. The **Link Z to FWD** button changes into  once linked.

- 10) Click on  Navigation button on the right side of the **XT** window and **input 4** and hit **Enter key** on the keyboard to move the stage to 4 mm. Make sure the  appears next to the number, indicating increasing number brings the stage down.

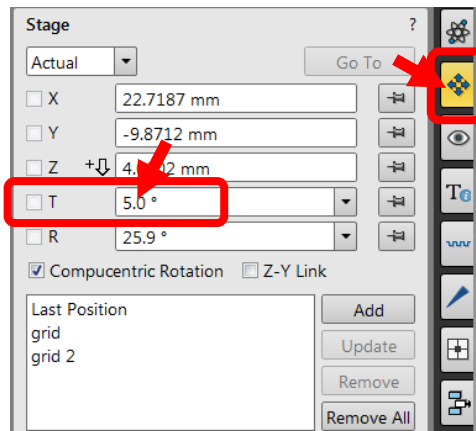


- 11) Eucentric tilt adjustment **for ion beam application only**:

- a) Double click on a reference feature on the sample to center the in the image indicated by a hair cross as shown below:



- b) **Input 5** and hit **Enter** key in **T** (tilt) space below:



- c) Click **Up** or **Down** arrow in **Stage Z** control below to change stage height and bring the reference feature back to the center of the image.





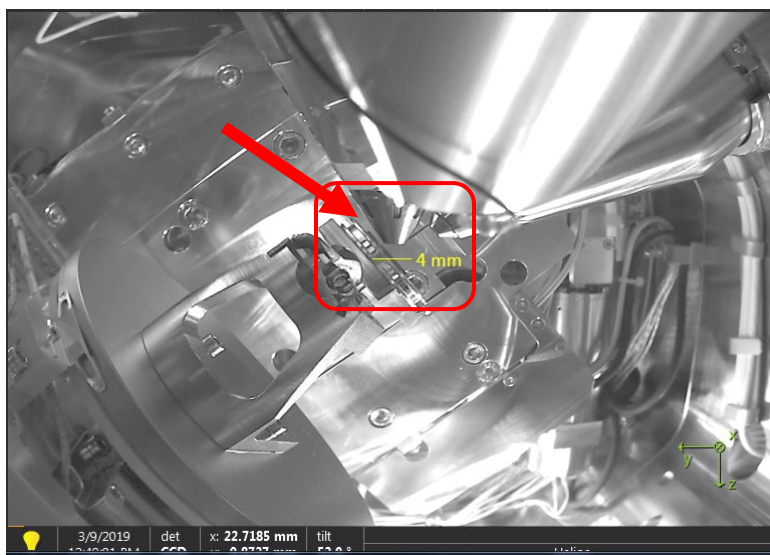
- d) Tilt stage to **20°** and finally to **52°** and repeat Step c) above to center the reference feature as shown


7 AutoTEM Applications

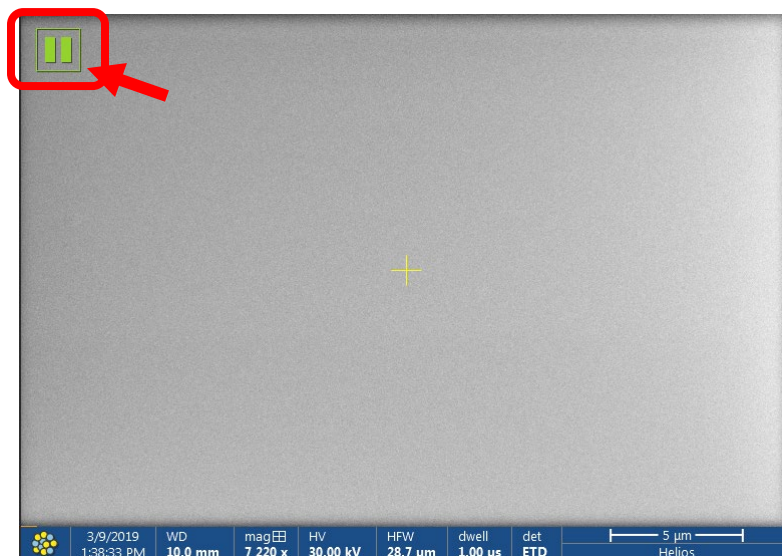
8.1 In-situ lift-out

Note: The final TEM sample thickness using AutoTEM is above **130 nm**. The manual thinning is still required after AutoTEM.

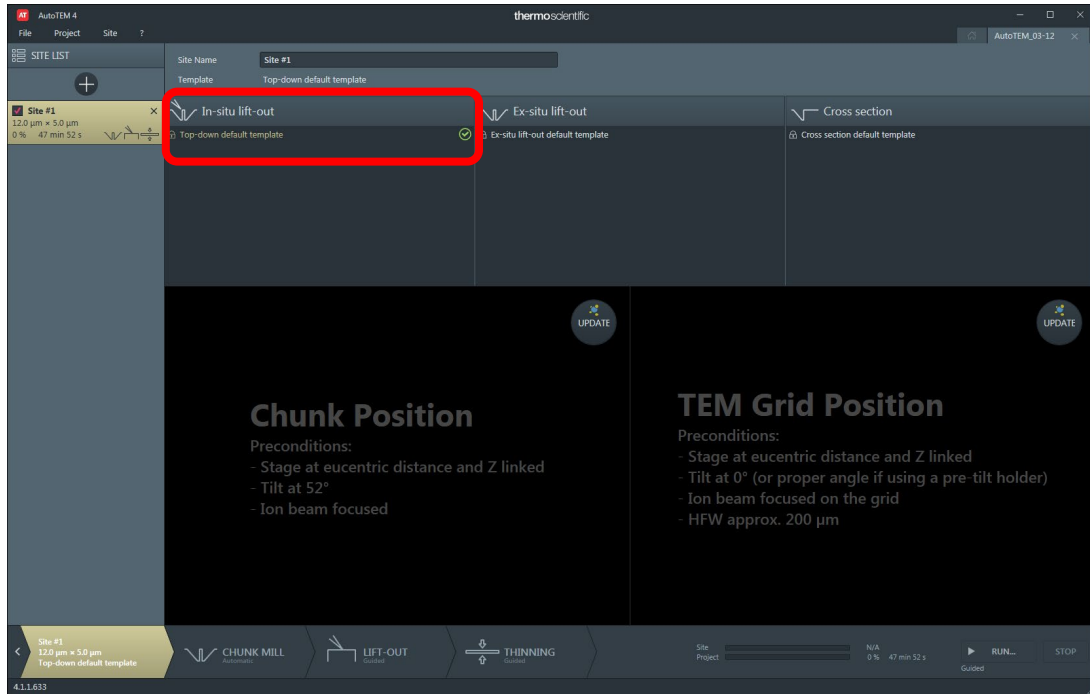
- 1) Click  button on the top left corner of the **XT** window and click to open **AutoTEM 4.1**.
- 2) Inside **AutoTEM 4** window, click  to create a new project.
- 3) Sample registration in AutoTEM:
 - a) Inside **ELECTRON** window, choose an area on the sample surface for the project. **Do NOT** move too far from reference feature to **hit** electron column which is less than 1 mm from sample surface as shown below:




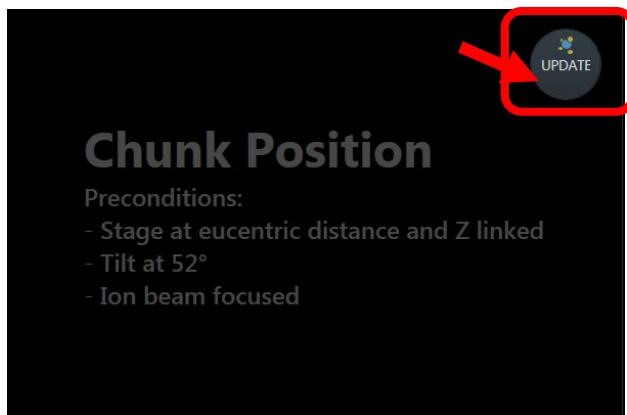
- b) Click on ion imaging window on the right side of **ELECTRON IMAGE** window and unpause the ion beam by clicking the  sign on its top left corner. Adjust contrast and focus if necessary.



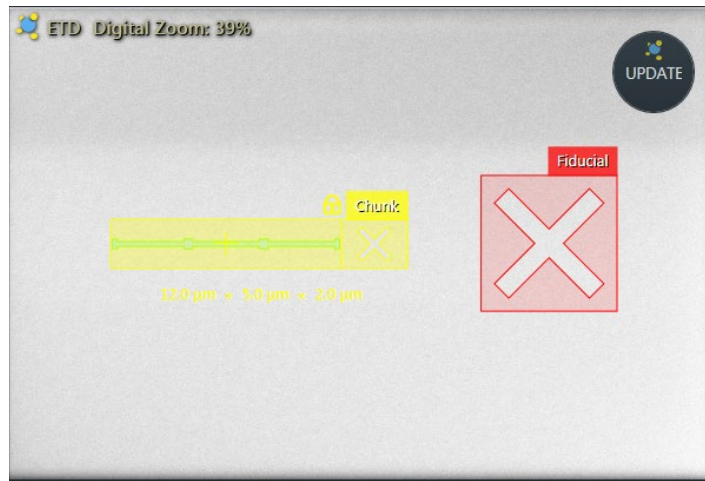
- c) Go back to **AutoTEM 4** window and check highlighted area below to make sure In-situ lift-out process is active.



- d) click  in the **Chunk Position** to register project working area on sample in AutoTEM.



- e) The **Chunk** area and **Fiducial** marks will appear on selected surface area below:

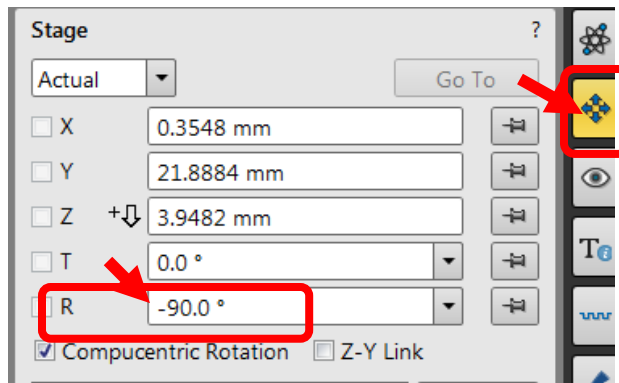


- 4) TEM grid registration in AutoTEM:

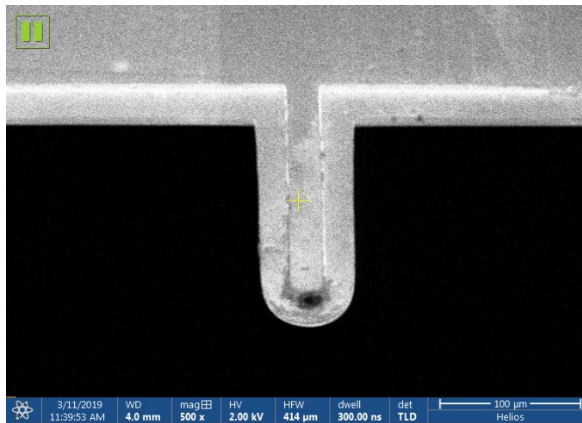
- Inside Navigation control window in **XT**, **input 0** in **Tilt** space to put the stage back to flat position. **Never move stage in Z before stage is flat**. Otherwise the stage will **hit** the electron column.
- Press the **P4 Rotate 180** button on the left side of the microscope control panel to rotate stage 180 degree so the grid holder is on the **LEFT** side of the stage as shown below:



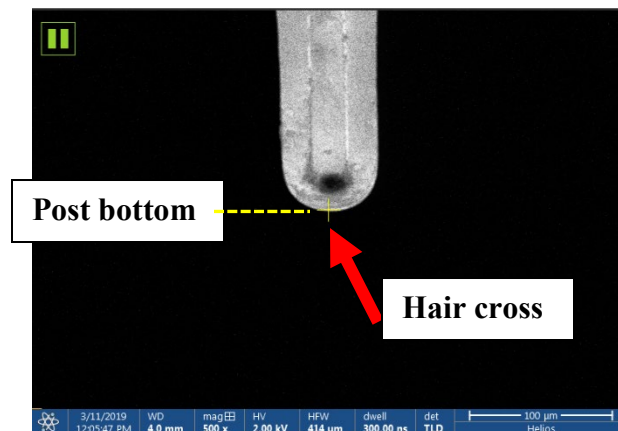
- Check the Stage panel in Navigation and make sure the Rotation angle has been changed to **-90.0°**:



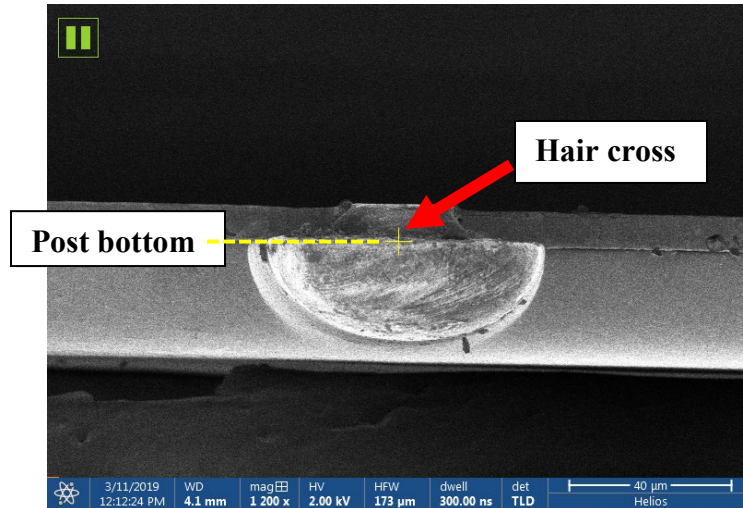
- d) **Double click** on intended TEM grid position in Nav-Cam to move the grid down to electron column.
- e) Find the intended grid in electron image and get a good focus. The mounting finger should face downward as shown below:




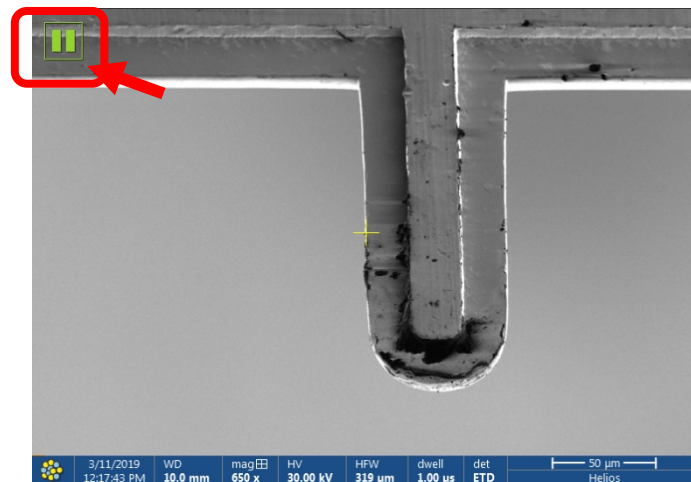
- f) If the finger edge is not aligned straight downward, go to top menu **Stage> Align Feature...** to do realignment.
- g) Eucentric tilt adjustments for grid position:
 - > find a reference feature, the bottom of the finger in ELECTRON image, double click to center the finger bottom in the image indicated by the hair cross as shown below:




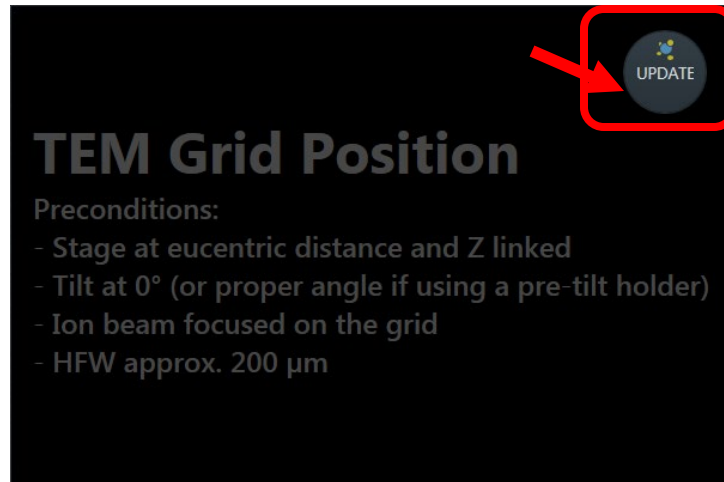
- > **Input 5** and hit **Enter** key in T (tilt) space in **Navigation>Stage** panel.
- > Click **Up** or **Down** arrow in **Stage Z** control to change stage height and bring the reference feature back to the center of the image.
- > Tilt stage to 52° and repeat Step h) above to center the reference feature.



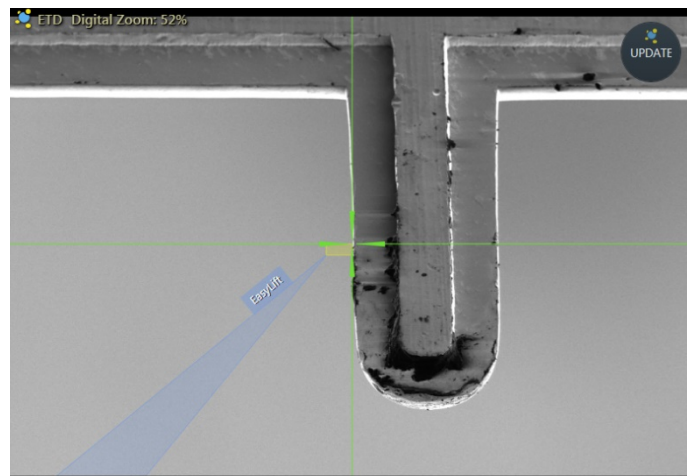
- > Click on ion imaging window on the right side of ELECTRON image window and unpause the ion beam by clicking the  sign on its top left corner. Adjust contrast and focus if necessary.



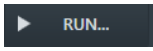

- > Go back to **AutoTEM 4** window and click  in the **TEM Grid Position** to register the grid in AutoTEM.

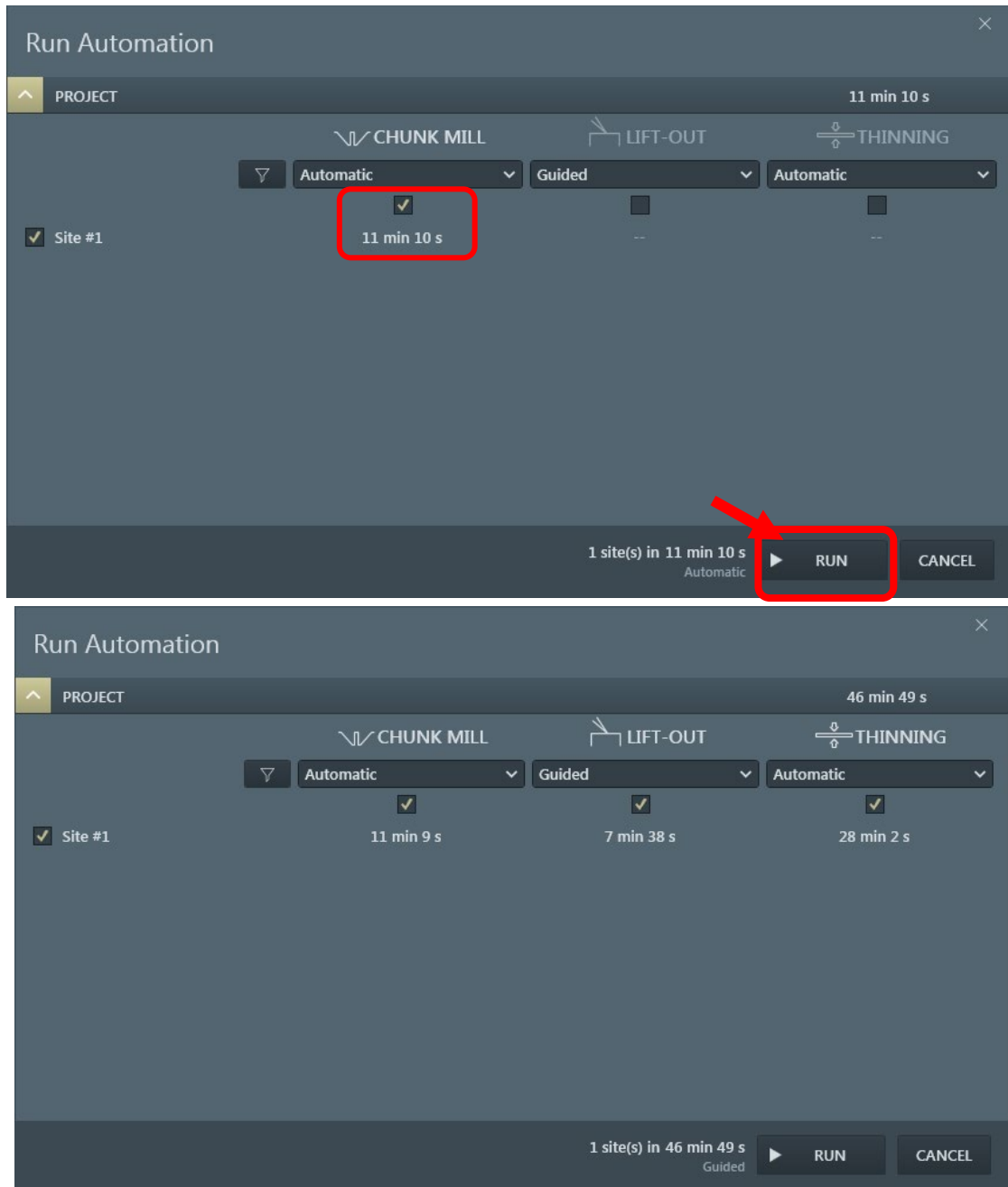


> The EasyLift needle and mounting position guideline will appear on selected finger below:

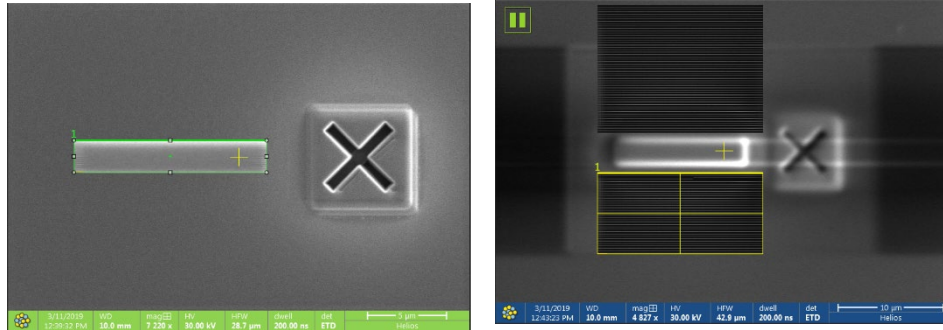


5) Start In-situ lift-out:

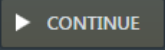
- a) Click  button on the bottom right corner of AutoTEM grid window to start the **Run Automation** window below. Select **CHUNK MILL** and click  in the window to start chunk mill process.

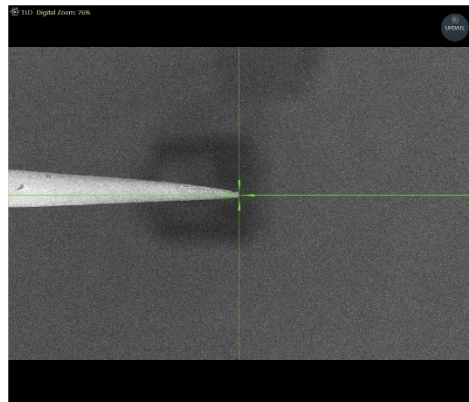


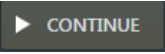
- b) Click Run button on the bottom right corner of AutoTEM to start auto CHUNK MILL process first. Watch the processes in AutoTEM including Electron Beam Deposition, Create Fiducial, Protective layer, rough milling, needle trench, medium

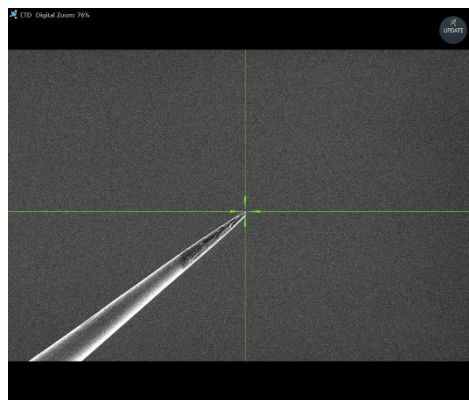


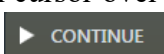
c) Follow AutoTEM instruction to start lift-out:

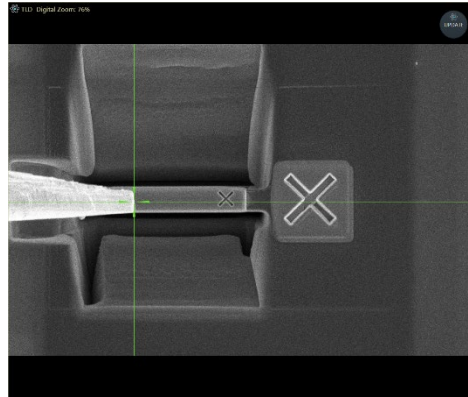
> EasyLift needle edge alignment in electron imaging: place green cursor over to the most right visible edge of the tip as instructed and click  in AutoTEM window after finish.



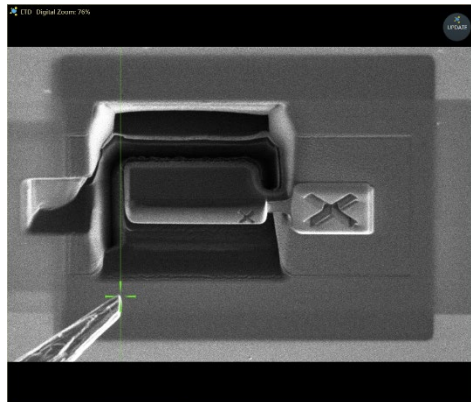
> EasyLift needle edge alignment in ion imaging: place green cursor over to the most top-right visible edge of the tip as instructed and click  in AutoTEM window after finish.



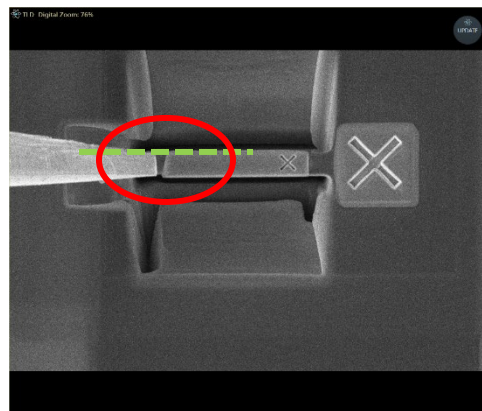
> EasyLift needle edge alignment in electron imaging over sample: place green cursor over to the most right visible edge of the tip as instructed and click  in AutoTEM window after finish.



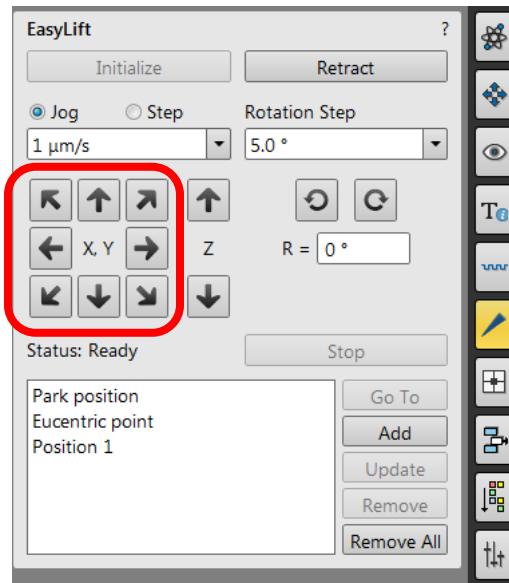
- > EasyLift needle edge alignment in ion imaging over sample: place green cursor over to the most top right-visible edge of the tip as instructed and click **▶ CONTINUE** in AutoTEM window after finish.



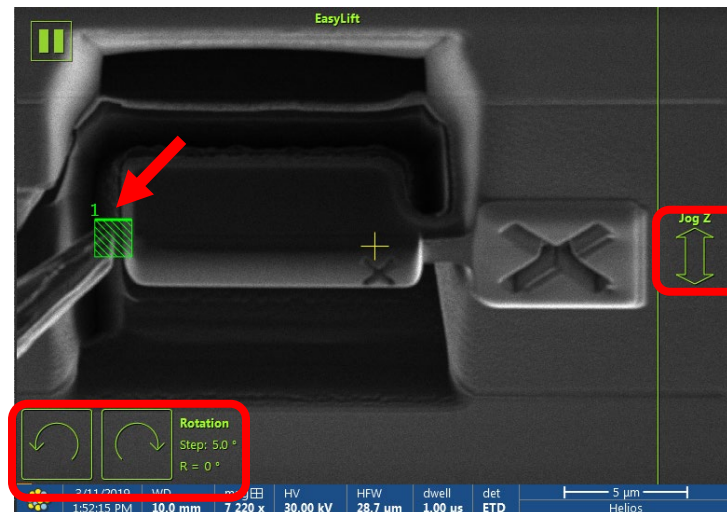
- > EasyLift needle welding position alignment in electron imaging over sample: in AutoTEM window check if the top right corner of the needle tip aligns with the top left corner of the lamella in highlighted area below.



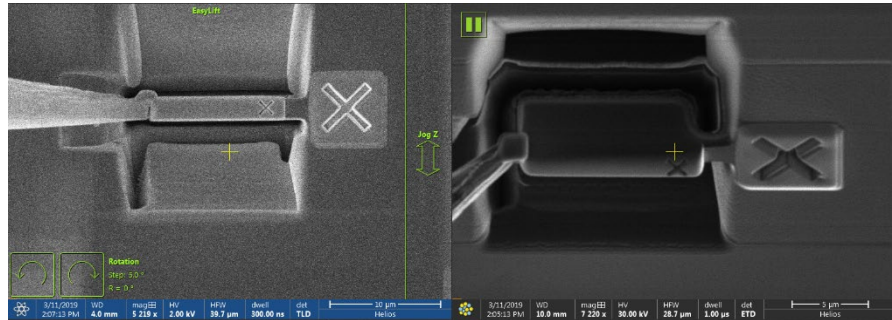
- > If alignment needs to be done, unpause ELECTRON electron window, go to **XT > EasyLift** control panel below. **Adjust X and Y only** in highlighted area and watch needle movement in electron image window. Click **▶ CONTINUE** in AutoTEM window after finish.



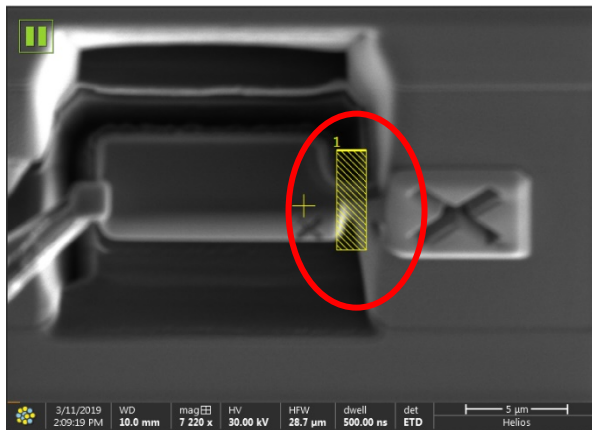
- > Drive to ChunkEasyLift needle welding position alignment in ion imaging: check and make sure the green hatched box (pointed by arrow below) is at the right position using AutoTEM guidance image. Make adjustments using **Jog Z** and **Rotation** control inside ion image window if necessary. Click **▶ CONTINUE** in AutoTEM window after finish.



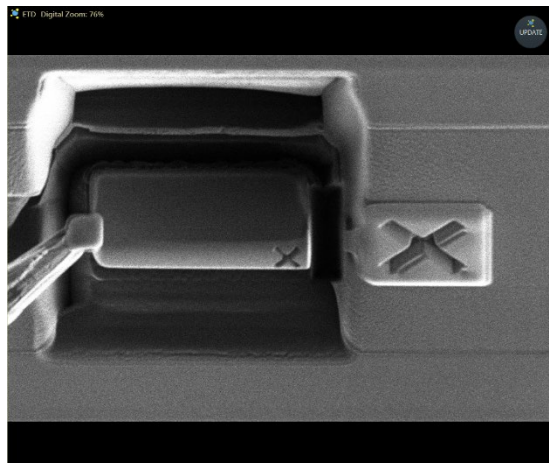
- > Attach needle: check if welding block is properly attached to the needle in both electron image (left) and ion image (right) below. Click **▶ CONTINUE** in AutoTEM window after finish.

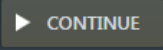


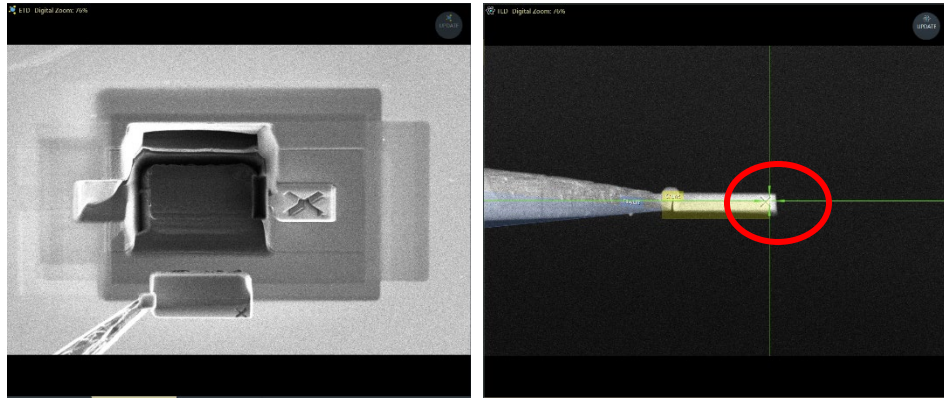
- > Check in XT ion image window if the yellow box (cutoff area) is correctly positioned. Click **▶ CONTINUE** in AutoTEM window if yes to start cutting micro-bridge.

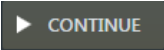


- > Check in AutoTEM if the cutoff is done properly and click **▶ CONTINUE** if yes to start chunk extraction.



- > The extracted chunk on the needle (left image below) will be driven to grid. Follow AutoTEM instruction to place the green cross to the top right corner of the chunk (right image below) in electron image in AutoTEM window. Click  after finish.



- > Follow AutoTEM instruction to place the green cross to the top right corner of the chunk in ion image in AutoTEM window. Click  after finish.



- > Driven to grid: place the green cross to the grid finger left edge in electron image in AutoTEM window. Click  after finish.



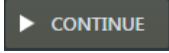
- > Driven to grid: move the yellow box to intended chunk mounting place on left edge of the finger in electron image in AutoTEM window. Click **▶ CONTINUE** after finish.

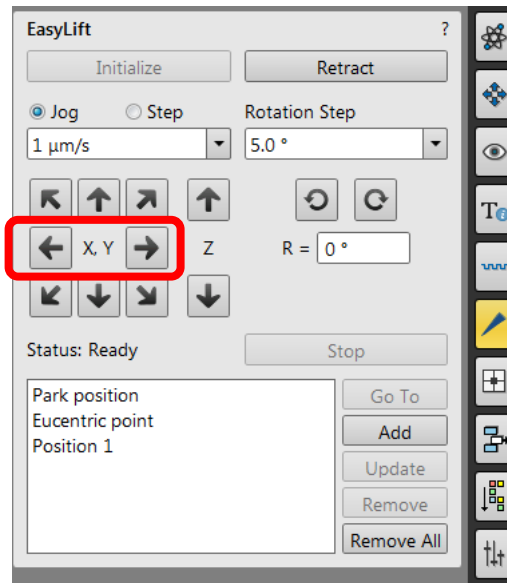


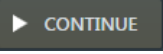
- > Driven to grid: check if the chunk top edge aligns with the finger top edge.

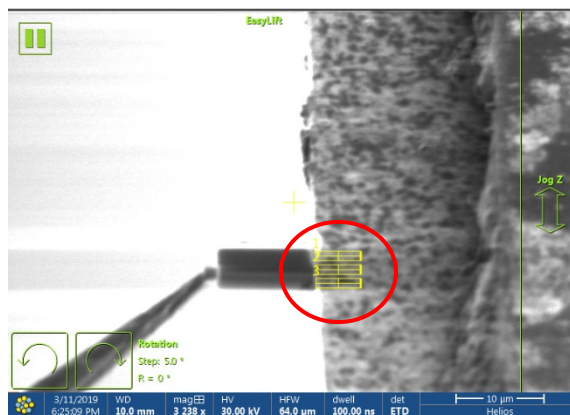


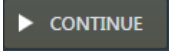
- > If alignment needs to be done, unpause electron image window, go to **XT > EasyLift** control panel below. **Adjust Y only** in highlighted area and watch

needle movement in electron image window. Click  in AutoTEM window after finish.



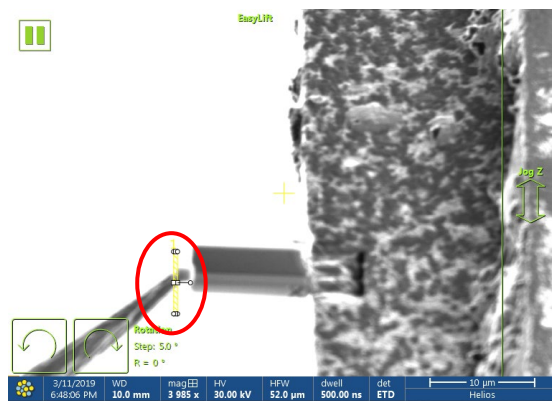
- > Grid cleaning: wait for the grid cleaning at mounting area to finish.
- > Check ion image in XT window if the mounting position needs to be adjusted in highlighted area below. Click  in AutoTEM window after finish.



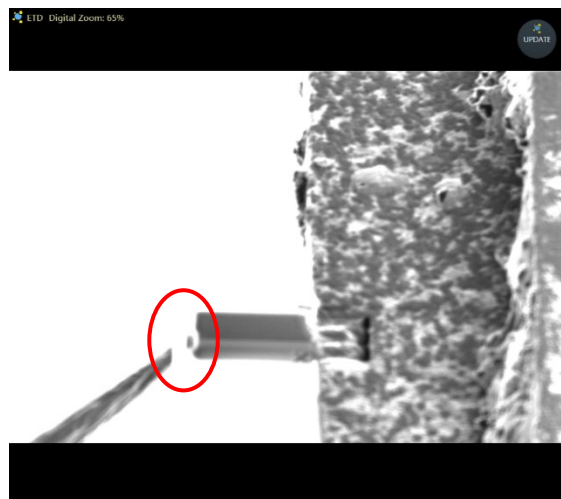
- > Chunk welding using copper sputtering: check in both electron and ion image window if the chunk is correctly welded to the grid. If yes, click  in AutoTEM window to continue.



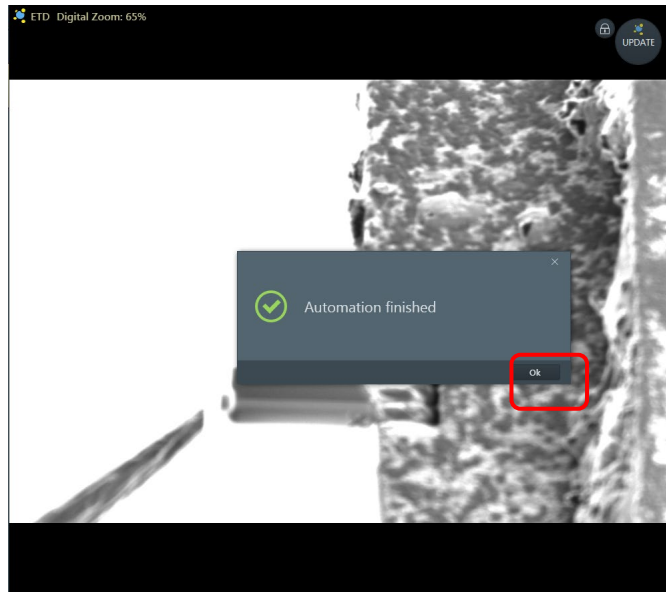
- > Slicing needle: check slicing yellow box in highlighted area below if the position is correct. Adjust if needed by dragging the box in XT ion image window. Click **▶ CONTINUE** in AutoTEM window to continue.

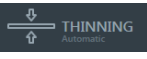
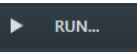
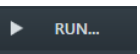


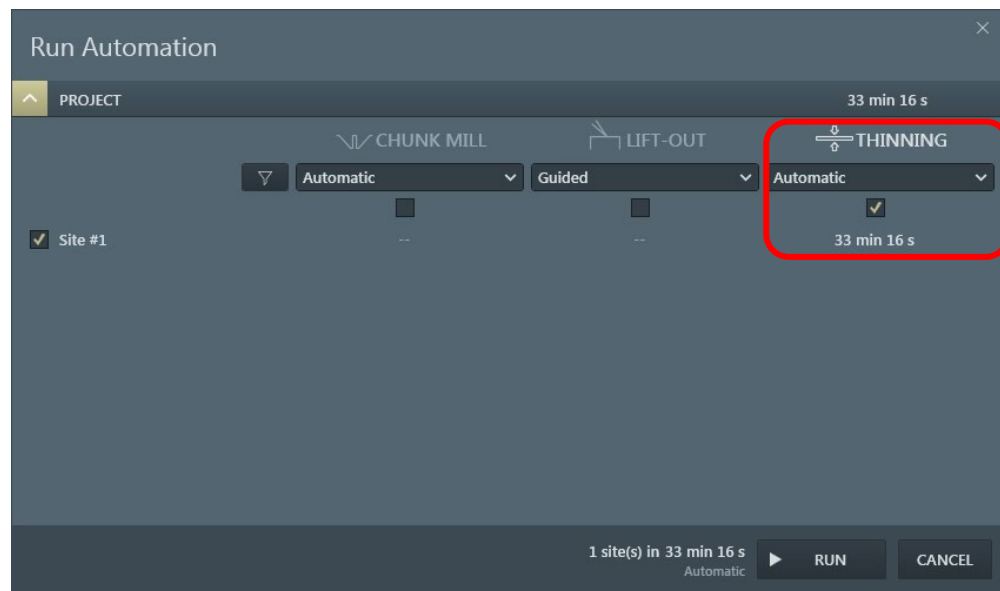
- > Slicing needle: check if the chunk is completely cut off in highlighted area. If not follow instructions in AutoTEM to repeat. Click **▶ CONTINUE** to continue.



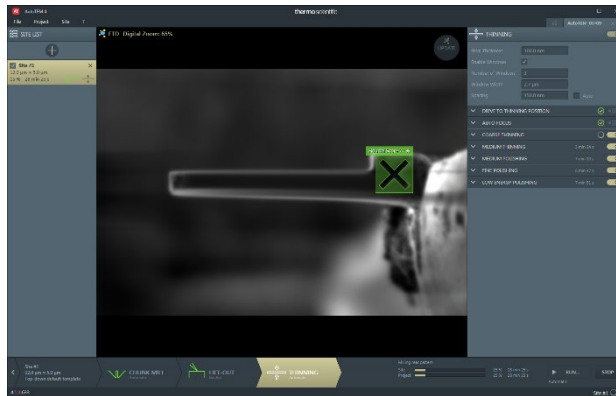
- > Click **Ok** on the window below to finish needle cutoff.



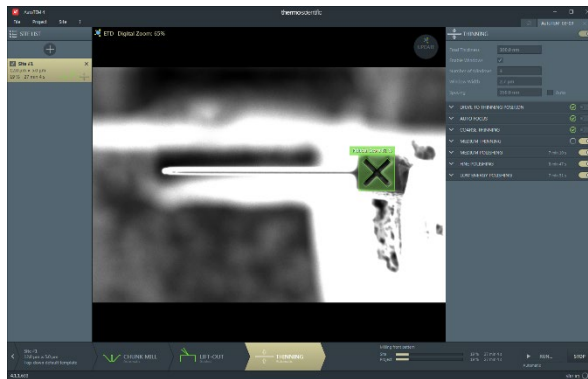
- d) Thinning: click  button on the bottom of the AutoTEM window and click the  to start guided chunk thinning.
- e) In Run Automation window, select THINNING process as highlighted below and click the  to start.



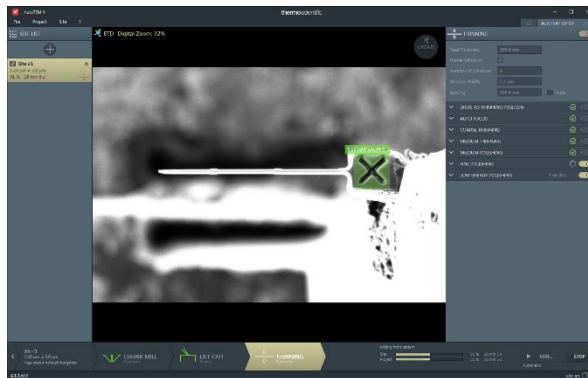
- f) Driving to thinning position
 g) Auto focus
 h) Coarse thinning:

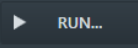


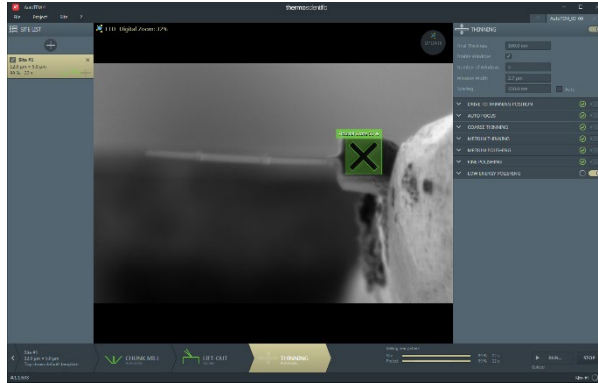
i) Medium thinning starts:



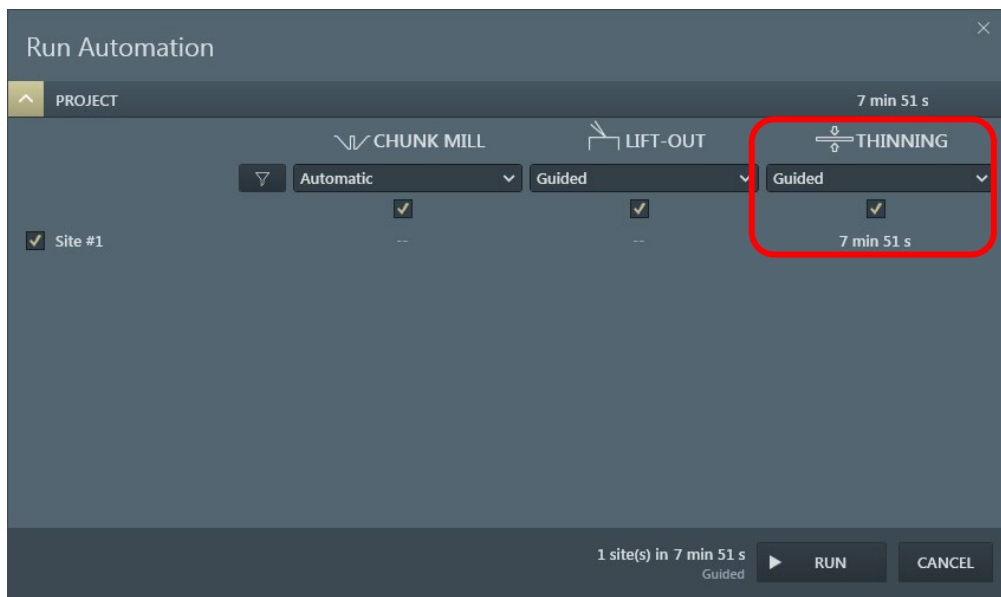
j) Medium polishing:



k) Fine polishing. If polishing fails due to low fiducial score, stop the processing and click the  to restart.

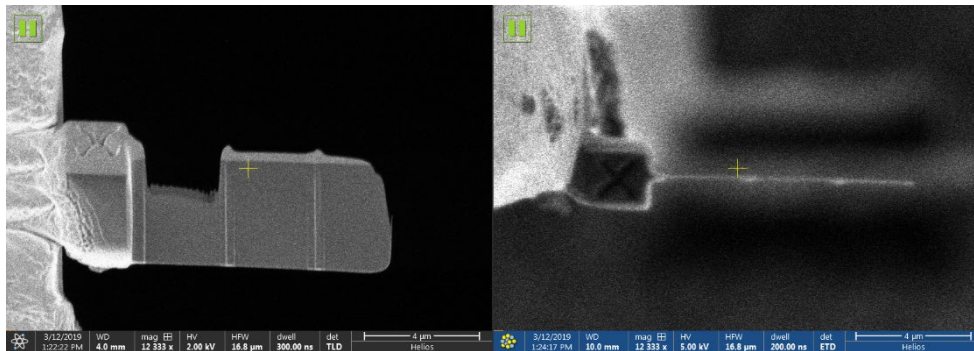


l) In Run Automation window, select THINNING, guided process as highlighted below and click the **RUN...** to start.





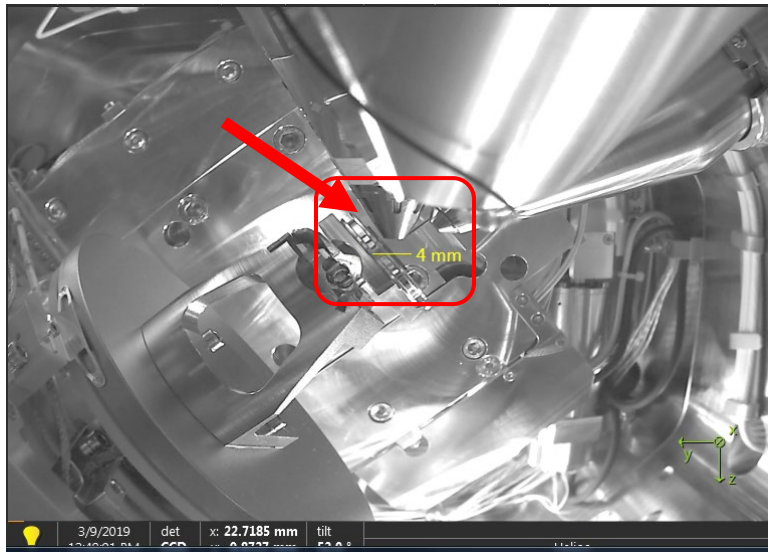
m) Click on **Ok** on AutoTEM to finish the process.


n) Check electron (left) and ion (right) image of the lamella in XT below:

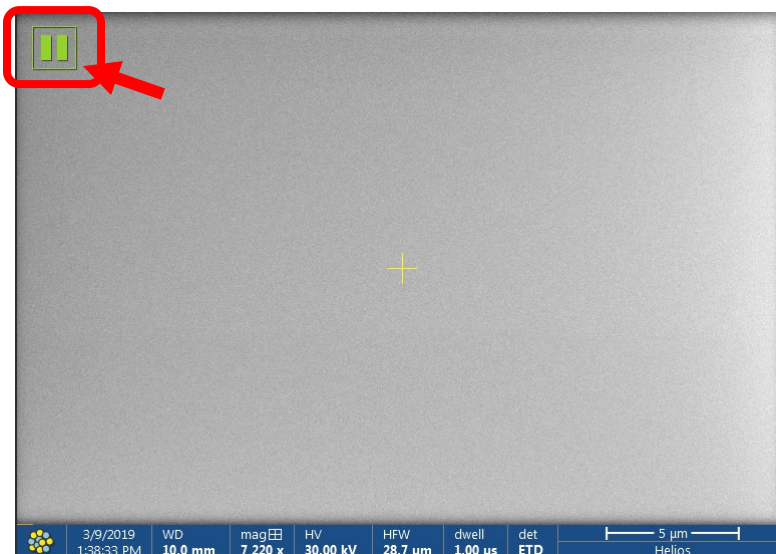


8.2 Cross section

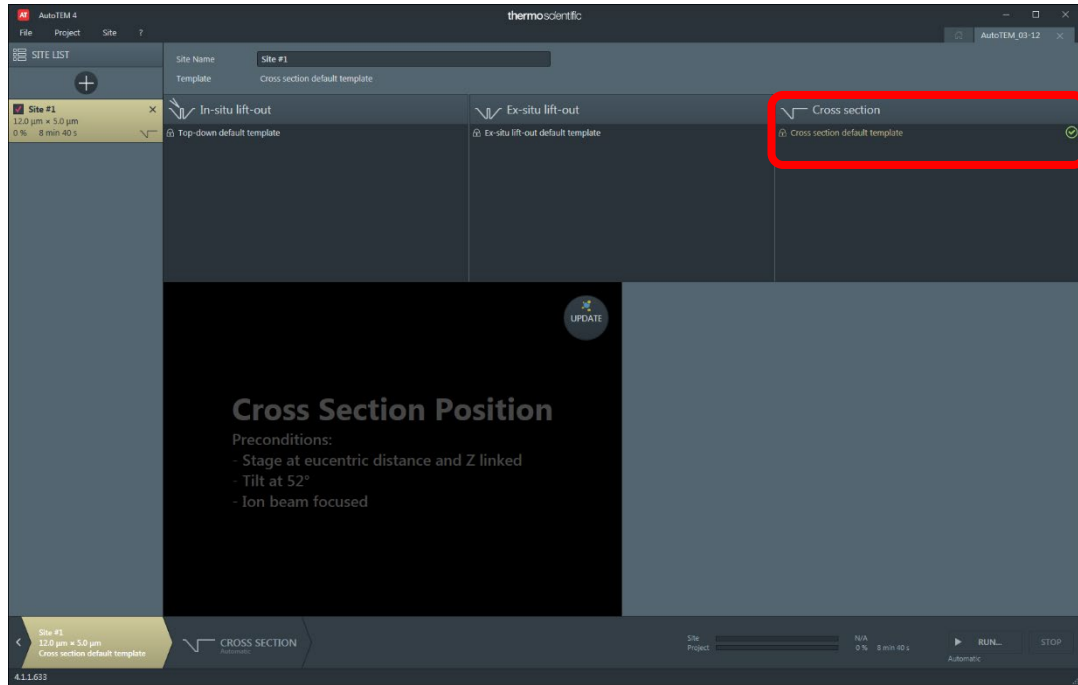
- 1) Click  button on the top left corner of the **XT** window and click to open **AutoTEM 4.1**.
- 2) Inside **AutoTEM 4** window, click  to create a new project.
- 3) Sample registration in AutoTEM:
 - a) Inside ELECTRON window, choose an area on the sample surface for the project. **Do NOT** move too far from reference feature to **hit** electron column which is less




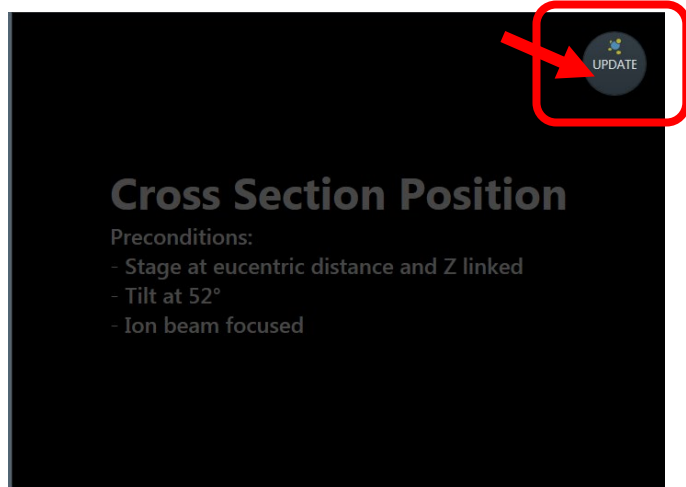
- b) Click on ion imaging window on the right side of ELECTRON IMAGE window and unpause the ion beam by clicking the  sign on its top left corner. Adjust contrast and focus if necessary.




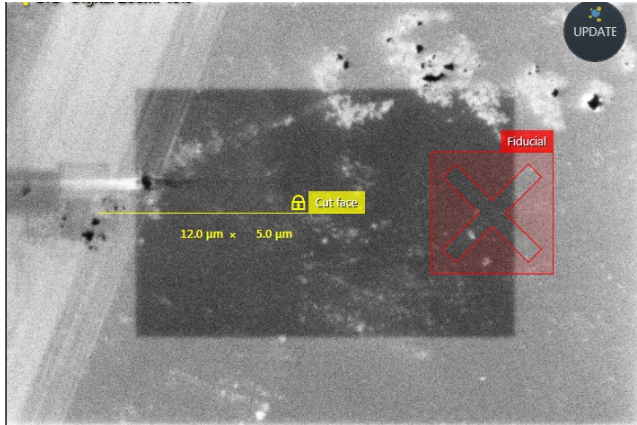
- c) Go back to **AutoTEM 4** window and click highlighted area below to activated cross section process.



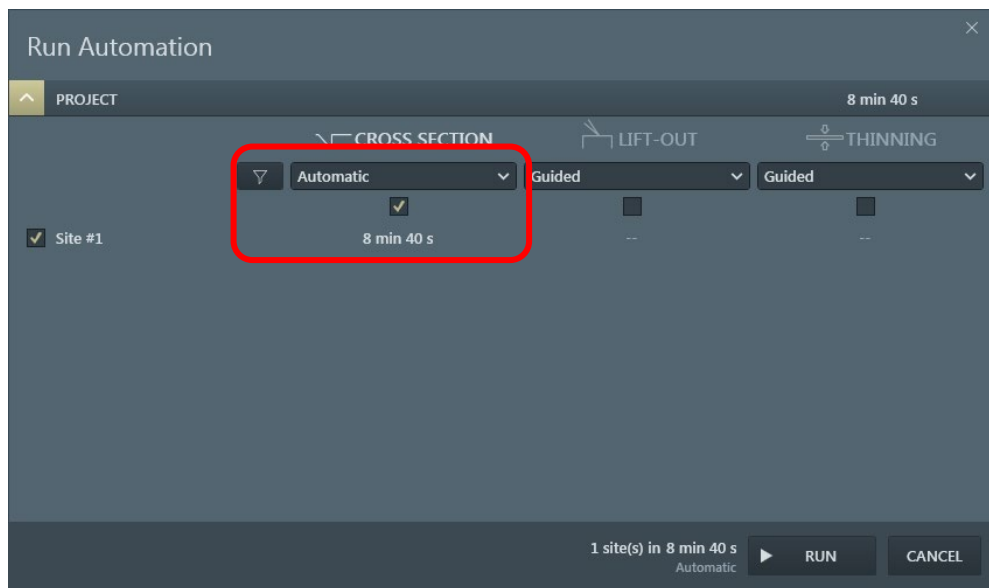
- d) Go back to **AutoTEM 4** window and click  in the **Cross Section Position** window to register project working area on sample in AutoTEM.



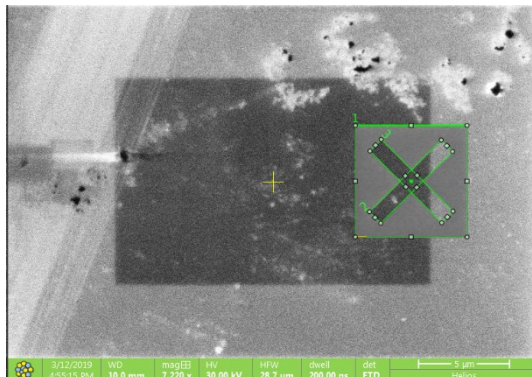
- e) The **cut face** and **Fiducial** marks will appear on selected surface area below. Click  in the window to start cross section cut.



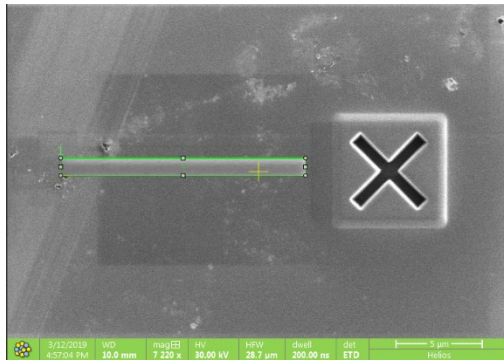
- o) In Run Automation window, select CROSS SECTION process as highlighted below and click the **RUN...** to start.



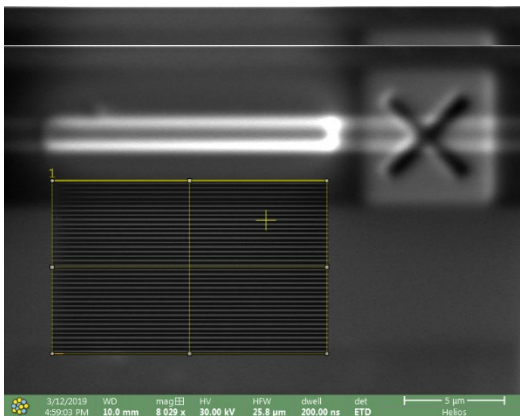
- p) Create fiducial in ion image window:



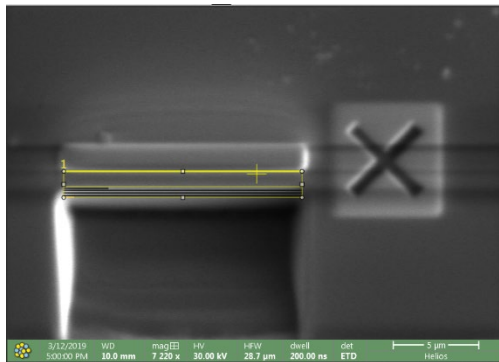
q) Add protective layer in ion image window:



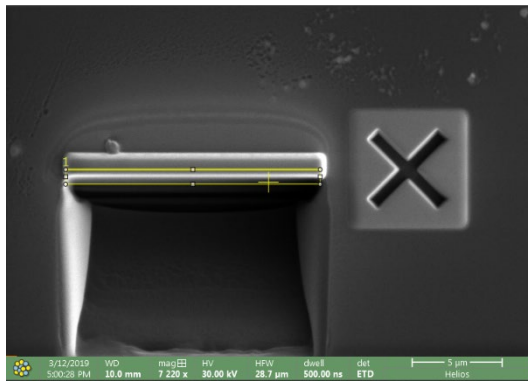
r) Rough milling starts in ion image window:



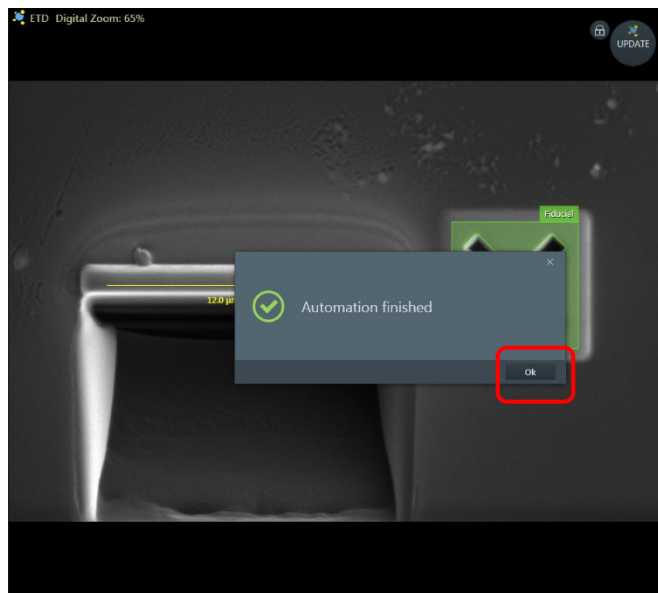
s) Medium milling starts in ion image window:



t) Fine milling starts in ion image window:

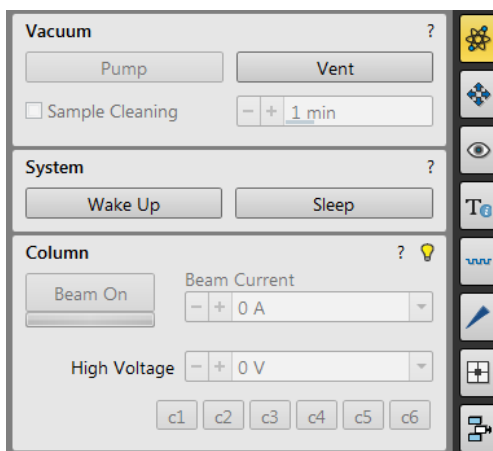


u) Click **Ok** on the window below to finish cross section cut.

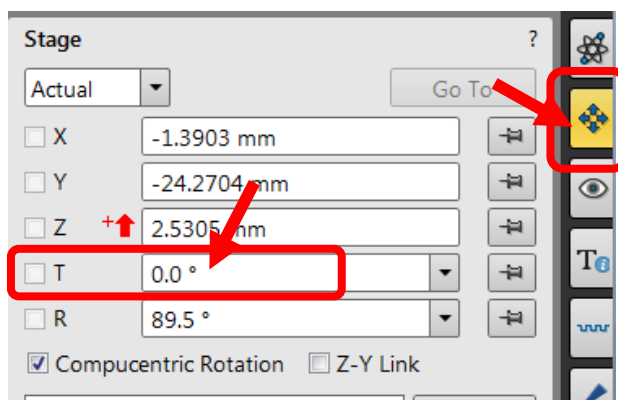


8 Closing measurement

- 1) Click in **Beam Control** > **System** panel below to **turn both electron and ion beam off**.

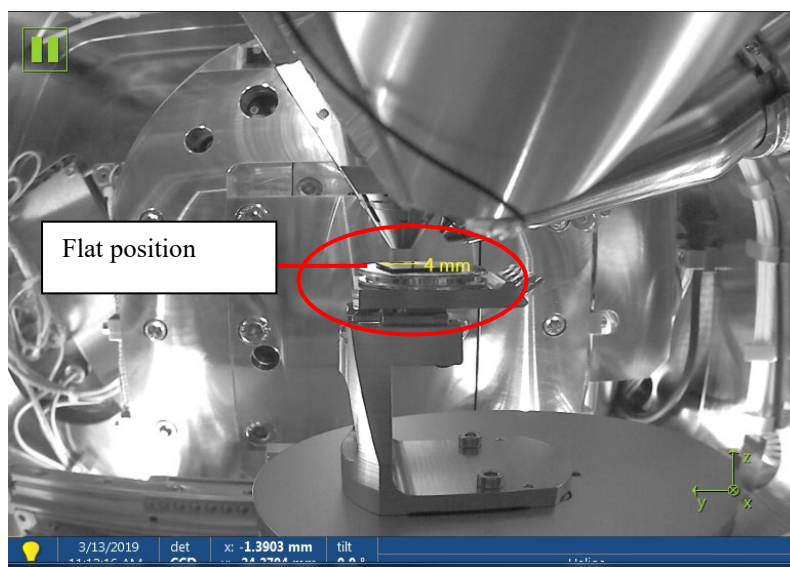



- 2) Tilt the sample stage down to 0° in **Navigation** > **Stage** control panel in the highlighted area below:

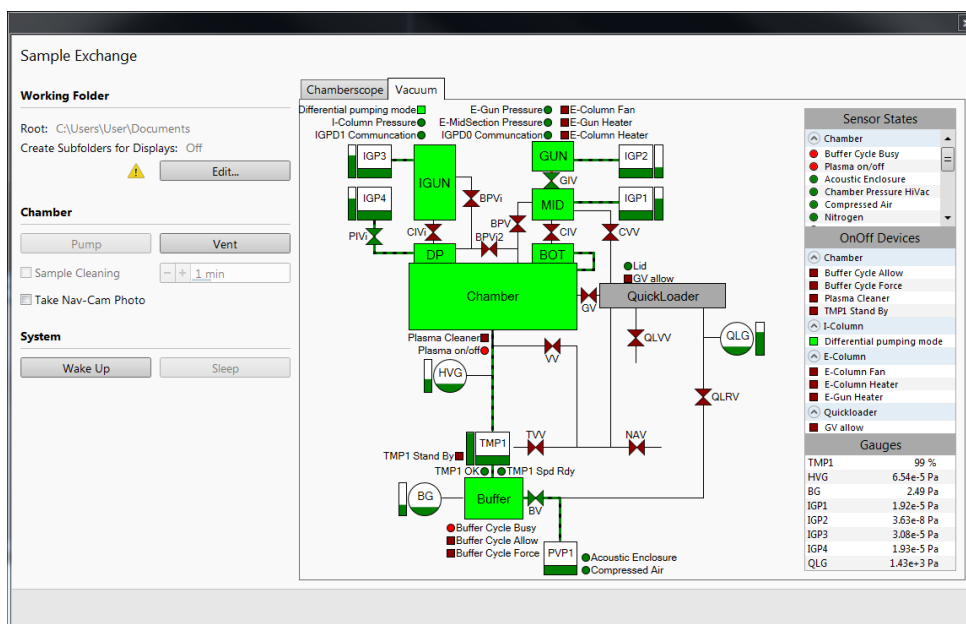


- 3) Activate **CCD** window and check the stage has been tilt back to flat position as show below:

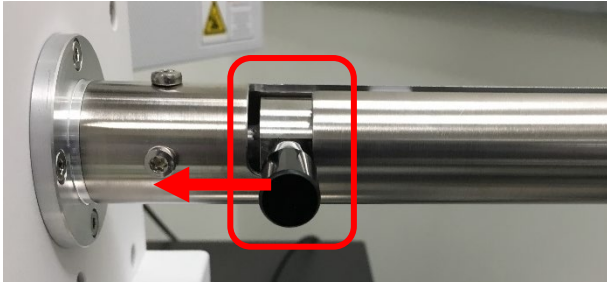
Note: the **CCD** window was set to **OFF mode in every 10 minutes** to protect EDS and EBSD detector.



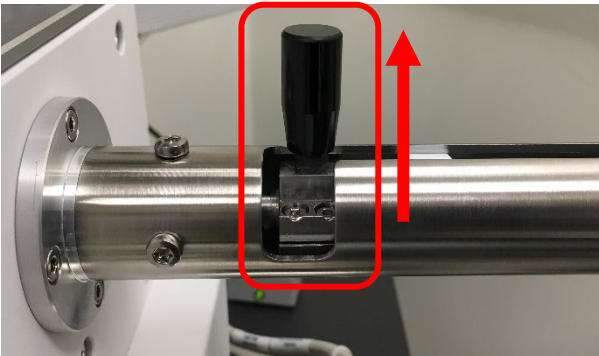
- 4) Click  button on tool bar to open the **Sample Exchange** window below:



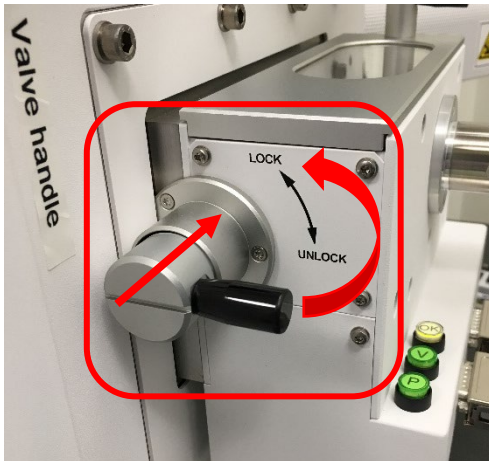
- 5) Repeat **Step 12) – 17)** in **Section 5 Sample Loading** to push the transfer rod into the sample holder.
- 6) When the transfer rod handle is turned down to **Unlock** position, **VERY SLOWLY** push the handle **forward** in the slot as shown below:



- 7) **Hold** the handle and **SLOWLY** turn **upward** as shown below and pull the transfer rod all the way to the end and turn **upward** to the **LOCK** position.

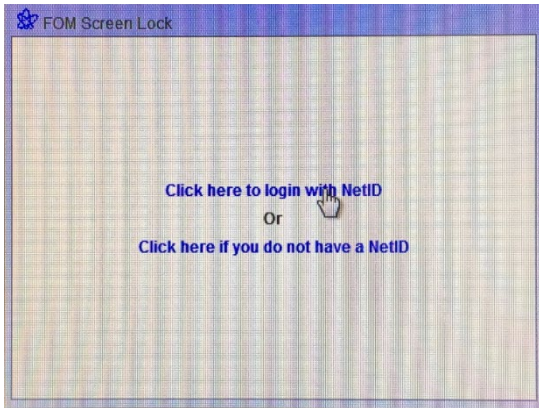


- 24) Turn the **Valve handle** downward to **Unlock** position and push the valve into sealing position as shown below, and turn the handle upward to **Lock** position.



- 8) **Wait** the **Chamber Pressure** to reach below **1.5E-4 Pa** before log off **FOM**.
- 9) **DO NOT** close or minimize **XT** program
- 10) Use **ONLY** the Core USB flash drive for data transfer.
- 11) **Log off FOM** from **Support PC** computer on the left side or user's **FOM SEM calendar**.
Make sure the following FOM Screen Lock window with dark background appear on the monitor to stop being continuously charged.

Note: if reporting issues, please check “**Something wrong**” in FOM logoff window and describe the issues in the empty box below.



- 12) **Sign off** logbook.
- 13) **Remove ONLY** samples from the stub on the specimen holder and **clean** the holder with Kimwipes using **IPA**. **Do Not disassemble the specimen holder**.
- 14) **Store** the specimen holder in assigned organizer box.
- 15) **Clear** the FIB work bench.

Warning: failed to follow checklist twice in one month will lead to temporary **account suspension**.