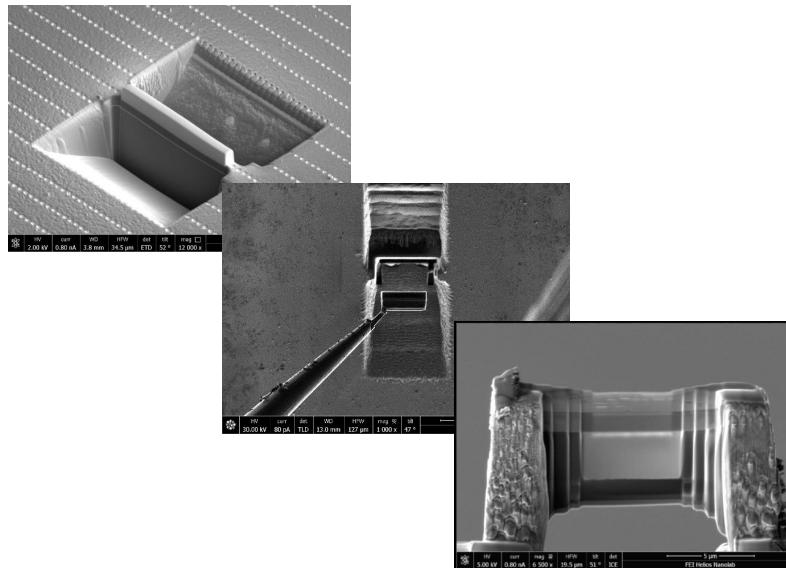


TEM Sample Manual Preparation Procedure



Yale WEST CAMPUS
Material Characterization Core
ywcmatsci.yale.edu

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

Manual TEM sample preparation procedures

1. Sample loading

- 1) Finish Z-link
- 2) Finish eucentric tilt correction.

2. E-beam Pt protection layer deposition:

Note: carbon layer is recommended for hard sample materials than silicon.

- 1) Keep the sample tilt at 0° .
- 2) Set e-beam to **2 kV** and **0.21 - 1.6nA**, and choose rectangle in Pattern Control panel. Typical size: **15 um (X) x 2um (Y) x 0.2um (Z: thickness)**. Dwell Time: **1 us**. Typical deposition time: **4 min**.
- 3) **If the Pt or C GIS source was not used weekly**, temporarily turn off (Not Pause) both electron and ion beam by clicking the **Beam On** button in Beam control when first clicking the ebeam/ion window. **Make sure** the orange background on the Beam On button disappears.
- 4) Click the **Cold** button next to Pt to warm up the Pt source. **Watch** closely the chamber pressure on the bottom left corner of the program window during Pt GIS warmup. If the pressure rises quickly into **10^{-3} Pa** range, **CONTACT** the core stuff immediately. Meanwhile click the same button again to switch the Pt back to Cold status.
- 5) **If the chamber pressure remains unchanged**, then turn on both e-beam and ion beam.
- 6) Insert the Pt GIS needle.
- 7) After the Pt GIS is warmed up, click the **Closed** button next to **Warm** to open the gas flow



first. Once the pressure starts to rise into high **10^{-4} Pa** range, click  on the top menu bar to start deposition/patterning.

- 8) **Alternately** if the sample surface is slightly charging, turn on reduced image area and change the scanning area to the same as deposition area. Keep the ebeam setting to **2 kV** and **0.2 up to 1.6 nA** and change the scanning time to 1 us, the same as the patterning dwell



time. Open the Pt gas flow and click  to start. To finish deposition, manually close Pt gas flow.

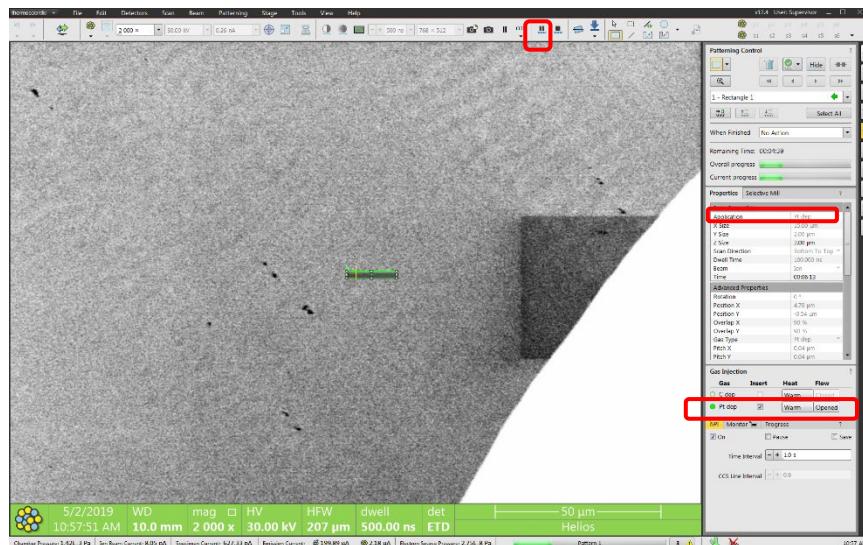
- 9) **Retract Pt needle** and switch ebeam setting back to imaging conditions, typical 5kV and 96pA.

3. Ion beam Pt protection layer deposition:

- 1) Single click the ion image window and check the Scan Rotation window below. **Make sure the ion beam rotation is set to 0°**.



- 2) Tilt stage to **52°** and adjust eucentric height to center the e-beam deposited Pt layer in electron image.
- 3) Grab a screenshot in ion image window at typical imaging condition: **30kV** and **26 or 41pA** and use beam shift on the control panel to center the Pt protection layer.
- 4) Draw a rectangle on top of the e-Pt layer with Typical size **15 um (X) x 2um (Y) x 2um (Z)**.
- 5) Click the **Cold** button next to Pt to warm up the Pt source if it was not used in e-beam deposition.
- 6) Choose **Pt dep** in Application and insert Pt GIS needle in highlighted areas below



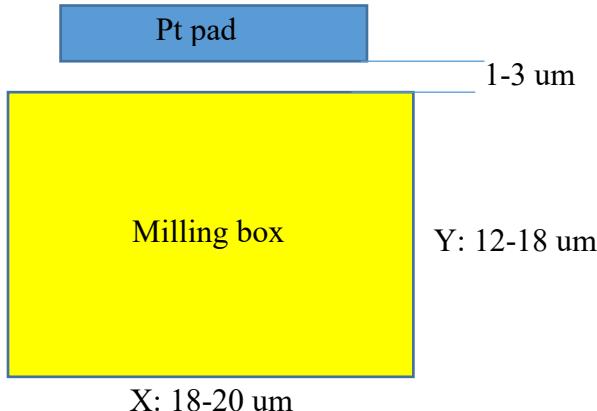
- 7) Shift e-beam to re-center the e-Pt layer in electron image as affected by Pt needle movement.
- 8) Choose typical **30 kV** and **90 pA** or up to **0.44 nA** on ion beam setting (smaller current favored for ion beam deposition).
- 9) If necessary, shift ion beam (use Shift key and left click to shift beam) outside e-Pt layer to adjust focus and stigma.
- 10) Open the Pt gas flow and monitor the chamber pressure increase.
- 11) Wait until chamber pressure rise gets slower.

-  12) Click  button to start deposition, typically takes ~ 8 mins.
- 13) After finish, **close** the Pt gas flow, **uncheck** the Insert box after deposition to retract GIS needle.

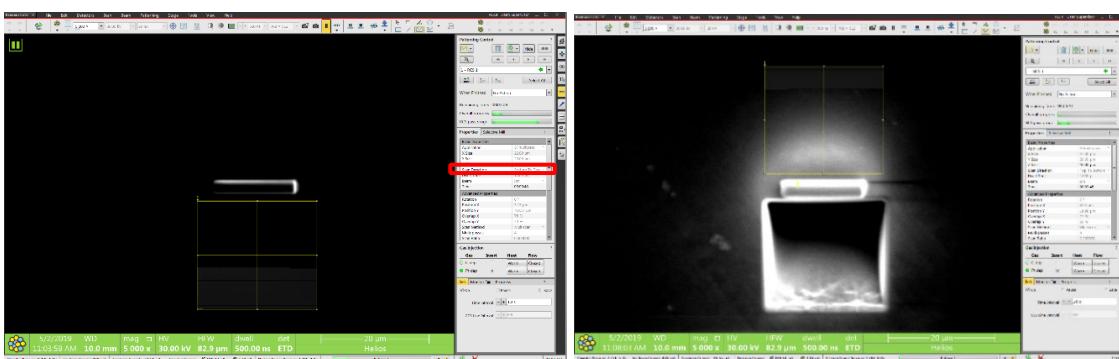
4. Bulk milling:

- 1) **Keep sample tilt at 52°.**

- 2) Use the  **Regular Cross Section** mode to define milling area below the Pt pad with a 1-3 um gap in between and the X slightly longer than Pt pad length, typical **X: 18-20 um**, **Z: 8-12 um** and the **Y: 12-18 um** (typically **1.5-2 times the Z**), e.g. 20x20x10 um.



- 3) Choose **Bottom to Top** in Scan Direction for the milling box below the Pt pad
- 4) Select **Si-multipass** in Application as highlighted.
- 5) Choose typical **30 kV** and **9.1 nA up to 20nA (for hard materials)** on ion beam setting to avoid over milling the Pt pad. **Never go above 20 nA** as it will damage the protection layer with increased ion beam size at high currents. The rough milling time for each box should take 5-15 mins.
- 6) Adjust ion beam focus and stigma off the milling site if necessary and shift the beam back.
- 7) Click Pattern Start button as highlighted to start cutting.
- 8) Bulk milling above the Pt pad: repeat steps 1) – 7) and choose **Top to Bottom** in Scan Direction.

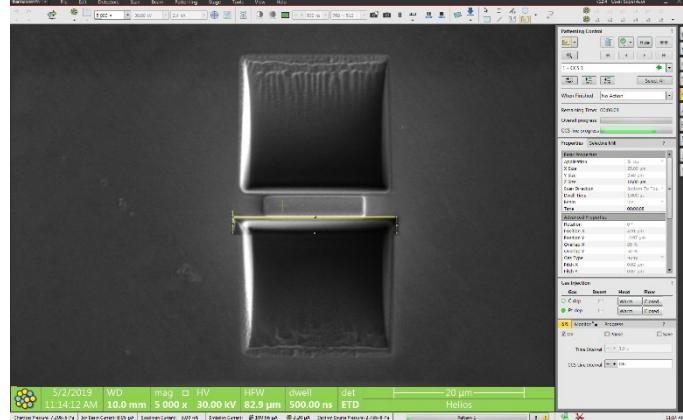


5. Intermediate milling

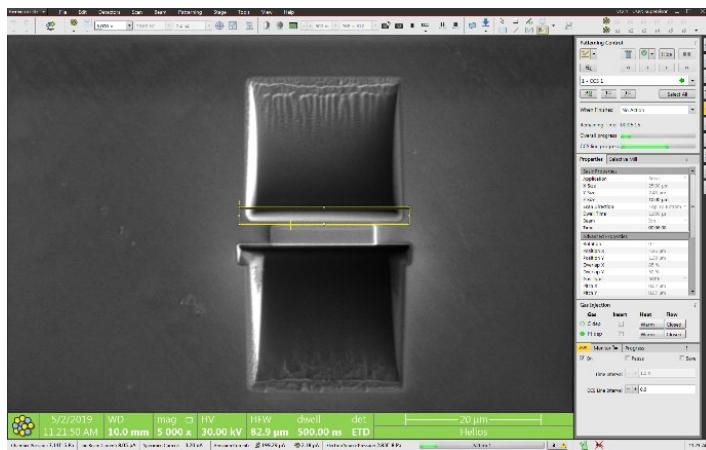
Note: this step is recommended to clean and correct the **overcut** from regular cross section milling so that the trench edge is perpendicular to the Pt pad edge.

- 1) **Tilt sample 53 – 53.5°** (typically 1-1.5° over tilt from 52°, e.g. +1.2°) to clean the **bottom edge** of the Pt pad.

- 2) Choose **Cleaning Cross Section** mode and draw a rectangle right underneath the Pt pad and inside the trench with a ~ 2 um gap from Pt pad as shown below. The X should be the as the milling box in **Section 4: Bulking Milling**, Y: ~2.5 um, **Z: 50%** of the bulk milling depth in **Section 4**, e.g. 4-5 um, e.g. 20 x 2.5 x 5 um and start cleaning as shown below.

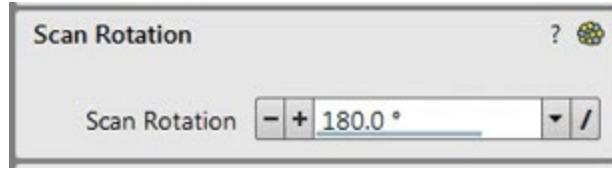


- 3) Choose typical **30 kV** and **2.4 nA** on ion beam setting. Can also choose smaller currents to avoid over milling the Pt pad.
 4) Start milling on **bottom edge** of Pt pad. Typical milling time should be ~2-5 mins.
 5) After finish, tilt sample **50.8°** (-1.2° from 52°) to clean the **top edge** of the Pt pad.
 6) draw another **cleaning cross section box right above** Pt pad as shown below and start milling.
 7) Cleaning **top edge** of the Pt pad: tilt sample **50.8°** (-1.2° from 52°) and choose **Cleaning Cross Section** mode. Draw a rectangle, similar size to Step 2) above and start cleaning. The lamella thickness should be **800nm – 1.5 um** after intermediate milling.

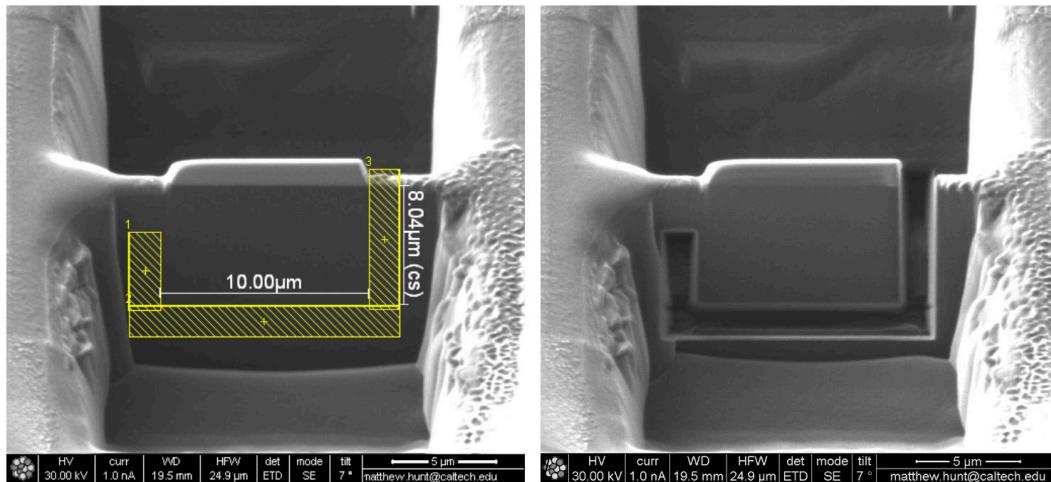


6. U shape-Cut

- 1) Tilt the stage back to **0°**.
- 2) Single click the ion image window and **change the ion beam rotation to 180°** as shown below. The ion image is the mirror of electron image after beam rotation and the Easylift probe will appear from the top of the ion image window once inserted and approach to the top right corner of the sample.



- 3) Center the sample in both electron and ion windows. May need to adjust Z in ion image window.
- 4) While the ion imaging is paused, adjust ion focus slightly and press F6 button on the keyboard to check the focus and quickly press F6 again to pause imaging. If beam stigma needs to be adjusted, move the imaging to a nearby area to avoid ion beam damage on the Pt pad.
- 5) Define three **U cut** boxes with typical z size of typical **1-3 um** (slightly larger than the lamella thickness) as shown below. Try not to put the bottom cutting box too deep in the trench to avoid Pt re-deposition and leading to the lamella stuck to the bottom.
- 6) Choose FIB scan direction: **Left to right** for side cuts and **Bottom to top** for bottom cut.
- 7) Ion beam setting: generally, **0.75-2.4 nA** at 30 kV is recommended.
- 8) Make sure **the application is Si** in the Patterning Control side panel.
- 9) Refresh ion image window briefly (F6) to adjust any drifts induced at different currents and click to start cutting.

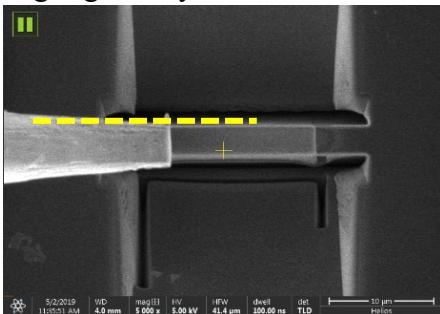


- 10) Check lamella clearance from substrate before lift-out:
 - a) Check if the U-cut area is clean and no materials left in the gap. without any
 - b) Tilt stage to **30°** and check in electron image quad if a clean cut was made,
 - c) Tilt back to **0°** and make a compucentric **180°** rotation, and tilt back to **52°** again to check the reverse side. Repetitive bottom cut in the front and back may be needed sometimes. Make sure the lamella is cleared from the substrate and only attached to substrate through the micro-bridge before lift-out.

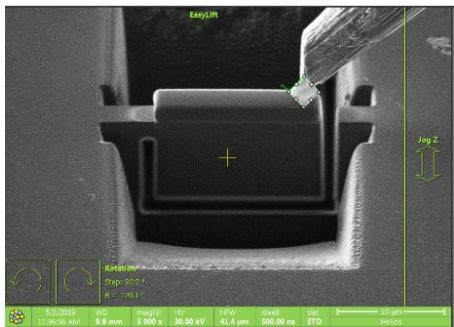
7. Sample lift-out:

- 1) **Keep the stage to **0°**.**

- 2) Center the sample in ion image window. Check and make sure the ion beam **Scan Rotation** is kept at **180°**. The micro-bridge should be on the left side of the image.
- 3) Adjust electron image resolution to **768x512**. Center the sample in electron image using beam shift, **do not** double click on the image to move the stage. The micro-bridge should be on the **right** side of the electron image with the e-beam rotation kept at **0°**.
- 4) In **Pattern Control > Properties**, select Pt Weld.
- 5) In **Pattern Control > Gas Injection**, check and make sure the **Pt gas is at warm** status.
- 6) In **Pattern Control > Gas Injection**, check **Insert** box to insert **Pt GIS needle**.
- 7) Zoom out both electron and ion images and unpause the electron image window. In **Probe Control** panel set the **EasyLift** probe speed to **10um/s** and click **Insert** button. **The probe should be inserted to the Park position**.
- 8) Use the **Jog Z** in ion image window to drag the probe slowly downward. Check if the probe end is clean when it is close to the sample surface. Use **narrow rectangle** milling patterns to mill/sharpen the probe if needed before attaching to lamella. EasyLift probe may need calibration if not used for months.
- 9) Use **Jog Z** to move down the probe slowly at **10um/s** and align the upper edge of the probe to the upper edge of the lamella by using X and Y control buttons in the EasyLift control window as shown below. The welding Pt pad will be deposited on the same side highlighted by the dash line.

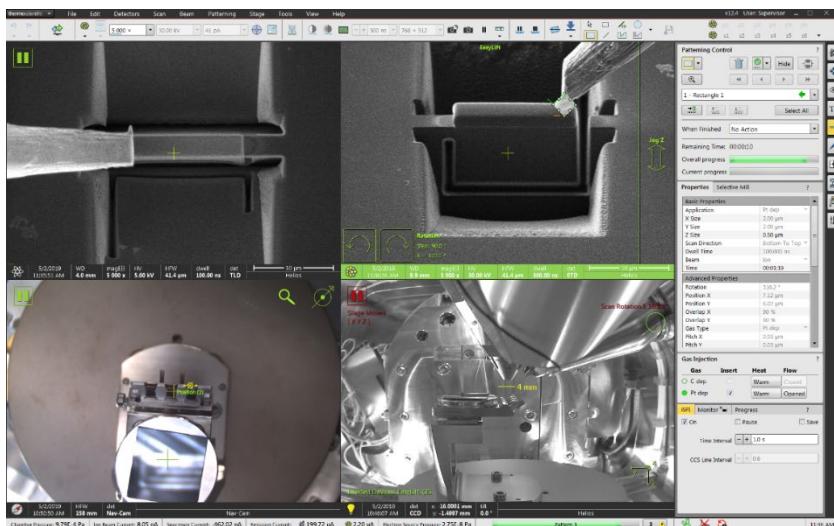


- 10) Activate **ion** image window, slow the speed to **1um/s** to **0.5** or **0.1um/s** and use the **Jog Z** in the ion image window to drag the probe slowly down to the top right corner of the lamella as shown below. **Stop** approaching if a slight drop and bending of the lamella are noticed, indicating the probe is touching lamella, and then pull back the probe slightly by clicking Z up buttons to release the stress on the lamella.



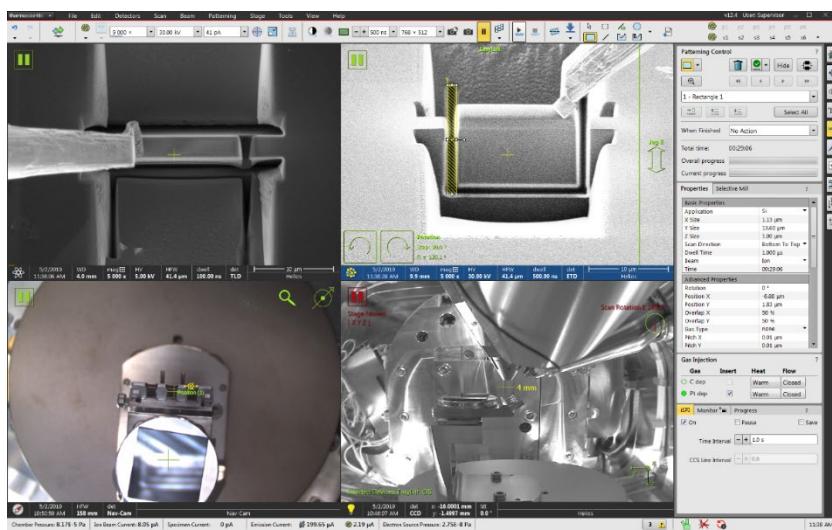
11) Define the Pt welding pad at the top right corner of the sample as shown below in ion image window. Rotate the Pt pad **45°**. Typical Pt pad size: **2x2x0.5 um** or **0.1 to 0.2 um**. Ion beam setting: **30kV, 41pA**. Choose Pt dep in Application and open the Pt GIS flow. Wait to see

the chamber pressure rises and click  button to start depositing.

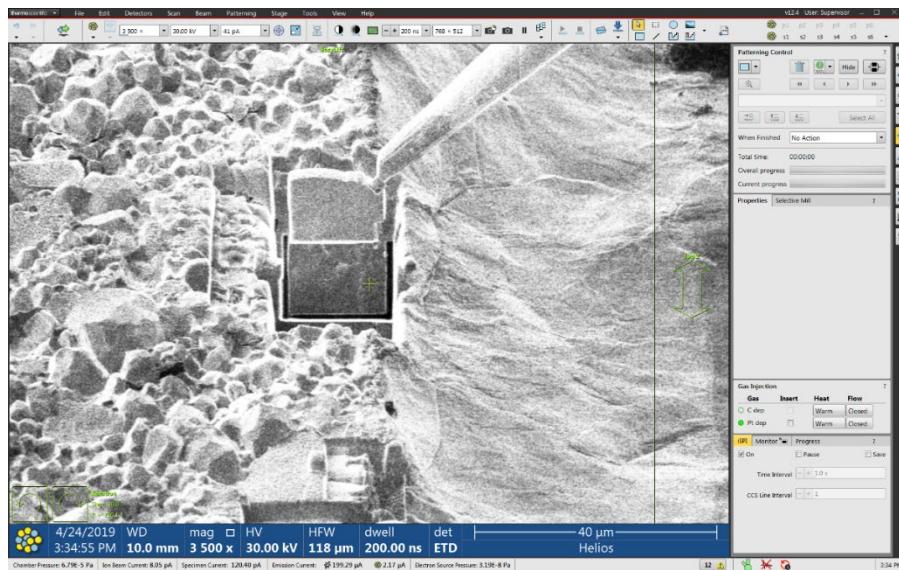


12) If necessary and depending on the EasyLift probe shape, can try to weld the top part of the probe afterwards with e-beam deposition. Typical e-beam setting: **2kV** and **0.21nA** and **1us** for dwell time.

13) Cut the micro-bridge (typical ion beam: **30kV, 41pA** or **1-3nA**): define a long and narrow rectangle (especially for flag type mounting) close to the left side of lamella. Typical Z depth is 1-3 um to cut through the entire thickness. Be cautious to choose high thickness above 5 um that could lead to sample redeposition to fill the undercut gaps at the bottom. Make sure the cutting edge is straight and smooth for flag type mounting. To protect lamella and Pt pad readjusting beam focus may be needed before cutting.



- 14) Lift out: click and hold on **Jog Z** in ion image window and slowly pull the lamella **just above** the substrate plane as shown below:

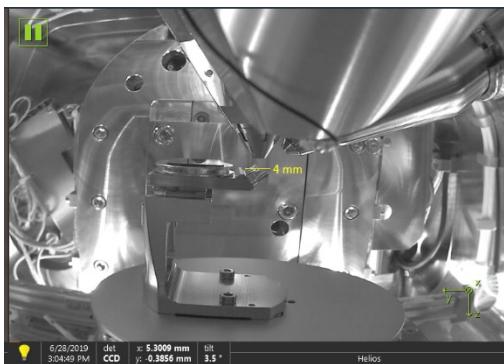


- 15) **Retract** Pt GIS needle.
16) **Fully retract** the EasyLift probe with the lamella.

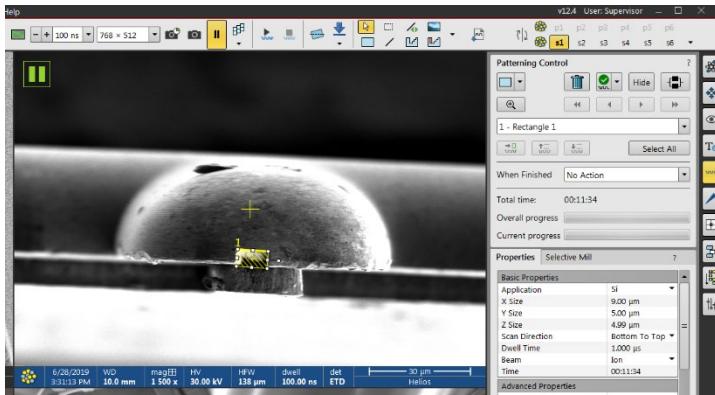
8. Lamella top mounting on grid post

The top mounting is highly recommended to reduce the lamella bending or twisting during thinning. It is also a preferred orientation for EDS in TEM system.

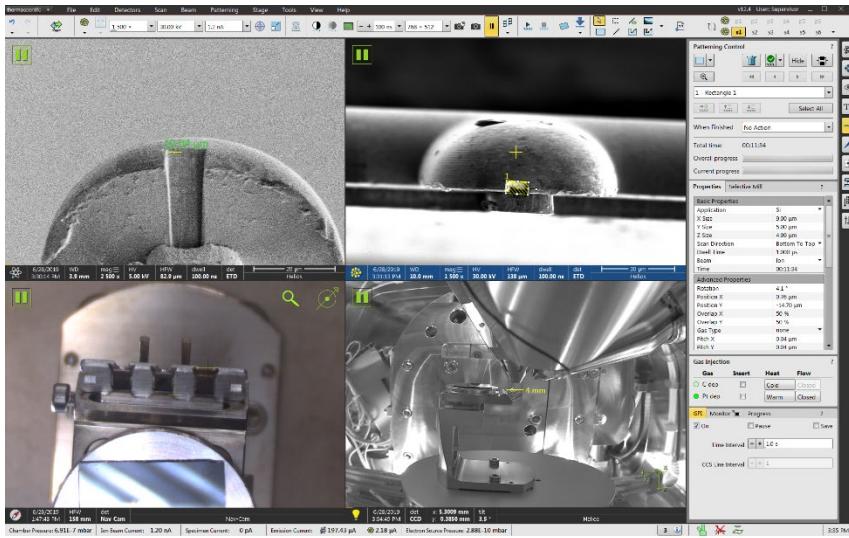
- 1) Adjust eucentric height alignment on intended TEM grid post.
- 2) For round top grid post, flat the top:
 - a. Tilt the stage to **0°** first, then click the **180° Rotate** key on the keyboard to rotate the stage. The **38°** tilted grid should be **on the left side of the sample**.
 - b. Tilt the stage to **14°** so that the front face of the grid post is normal to the ion beam.
 - c. Choose rectangle in the Patterning tab window and etch the top of the post away using large ion emission current (i.g. 26 nA or higher) to create a flat surface slightly larger than the lamella length for mounting.
- 3) Click the **180° Rotate** key to rotate the stage. The grid (**38°** tilted from sample holder) post should be parallel to ion beam and on the right side in CCD window as shown below.



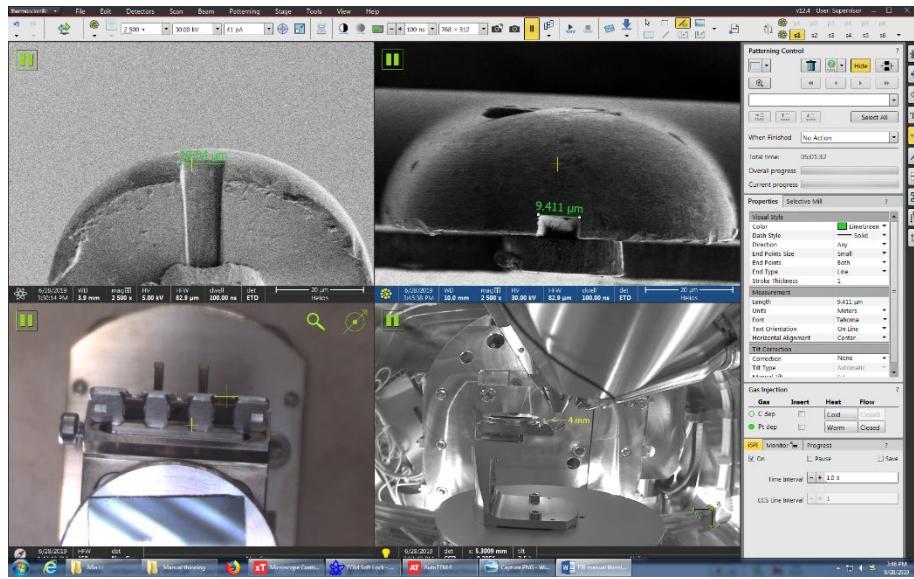
- 4) Align the post with ion beam carefully by adjusting tilt angle so the flat surface is parallel to the ion beam.



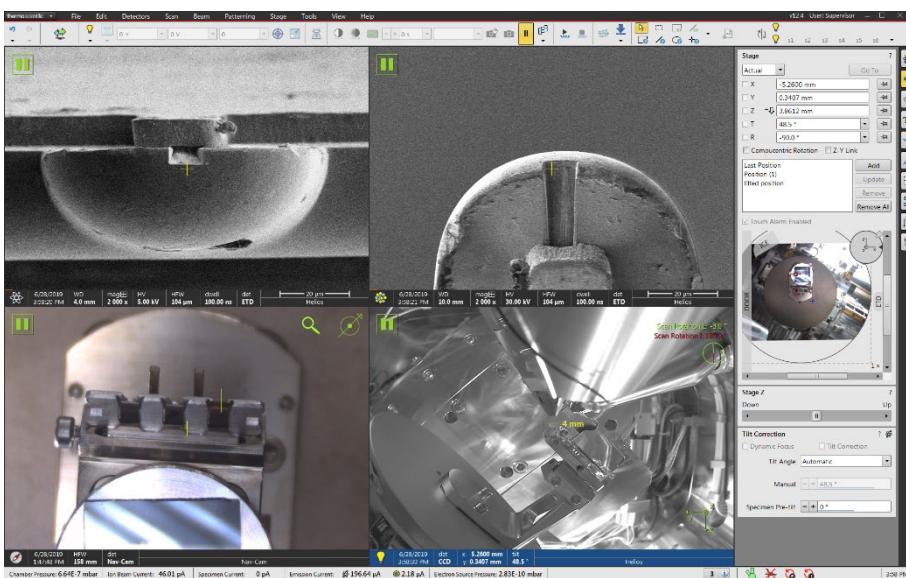
- 5) Check and make sure the Scan Rotation in the ion beam setting is **set to 0°**.
 6) Cut a trench on the grid post top with the width slightly smaller than lamella width, e.g. choose X size: 9 um for 12 um wide lamella, Y: 5um and depth (Z): 10um. Ion beam: 30kV and 1.2 – 20nA. Typical mill time: 10 min.



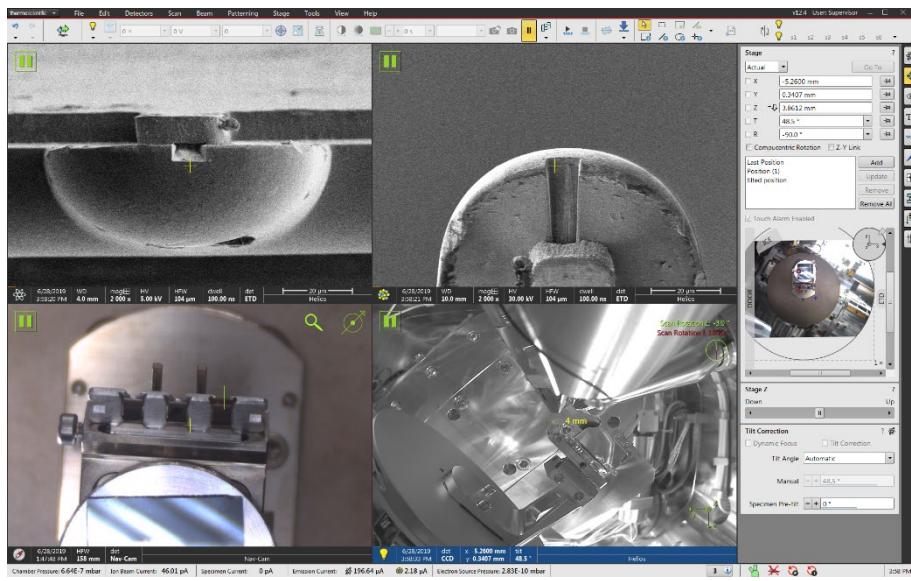
- 7) Check the trench in both electron and ion image windows. Keep milling if needed. The final trench is slightly larger than set value, in this case 9.4 um vs 9 um (set value).



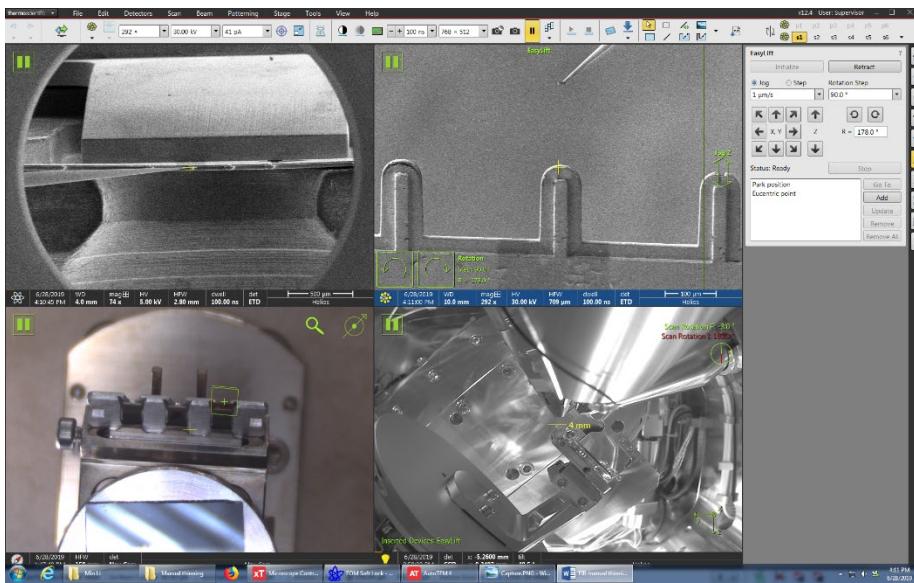
- 8) Click the **180° Rotate** key to rotate the stage and tilt the stage to **52°**. The grid post should be on the left side of sample holder in CCD window as shown below:



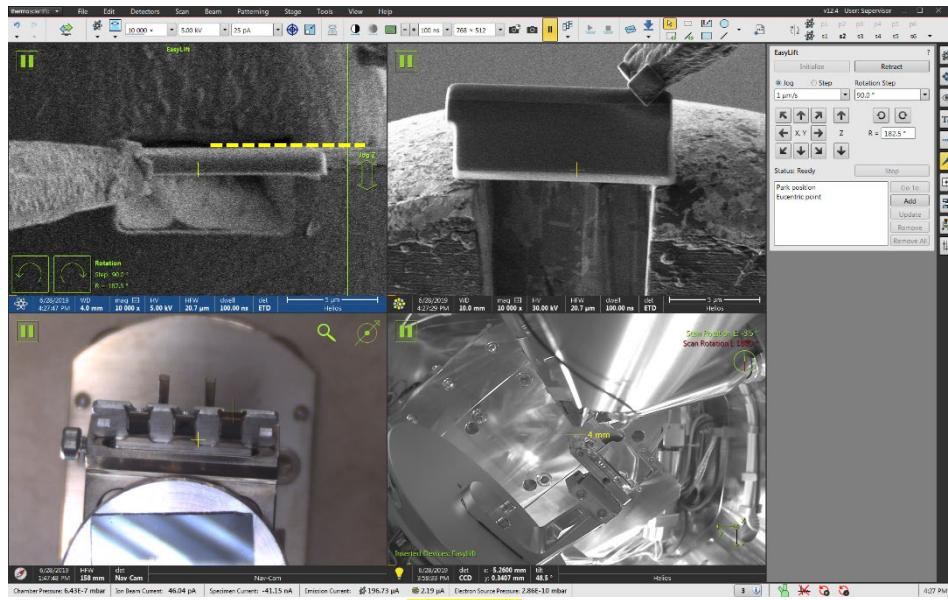
- 9) Slightly adjust the tilt to align the trench surface with electron image (see below). Click to activate ion image window and change the **Scan Rotation to 180°** so the post is pointing upward in ion image window as shown below:



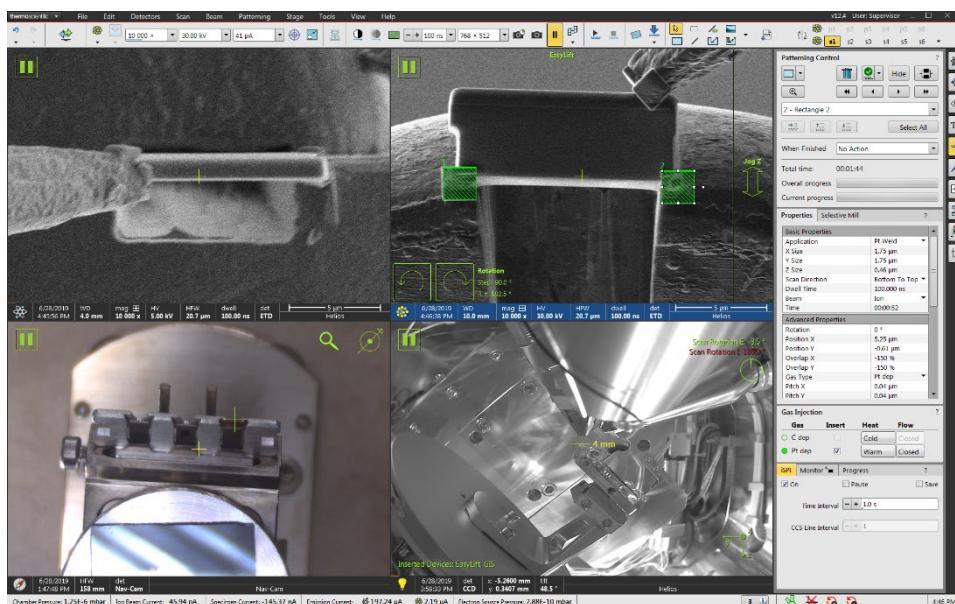
- 10) Insert Pt GIS needle and keep the gas warm before EasyLift probe approaching.
 11) **Zoom all the way out** in both electron and ion image windows and insert the EasyLift probe to **Park** position:



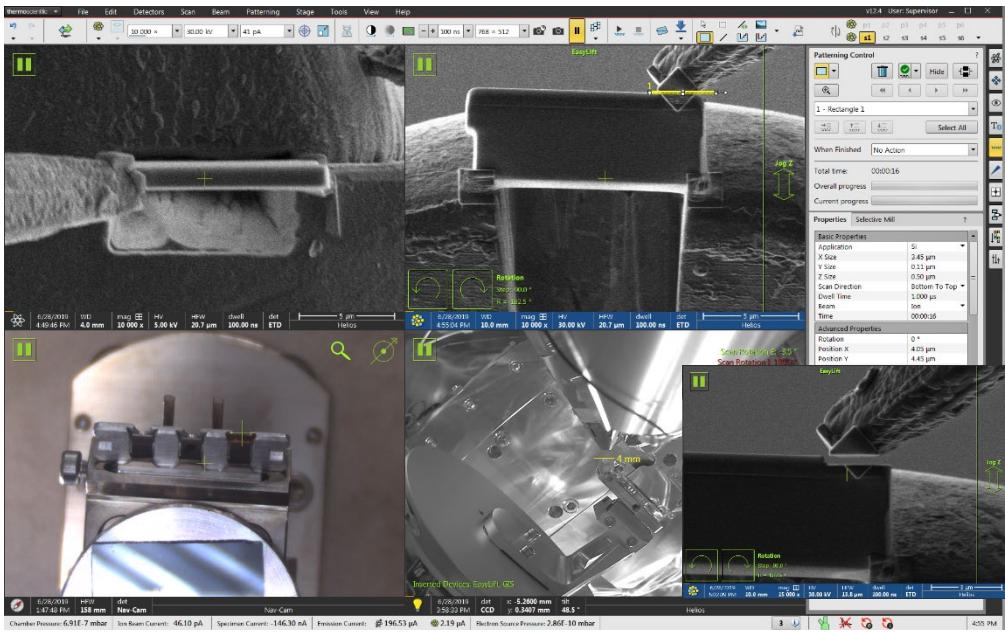
- 12) Activate ion image window, slowly approach the EasyLift probe with the lamella attached to the trench on top of the post.
- Align the lamella X and Y in both electron and ion image windows before touching the post. **Align the lamella upper edge with the trench edge** as highlighted by the dashed line below.



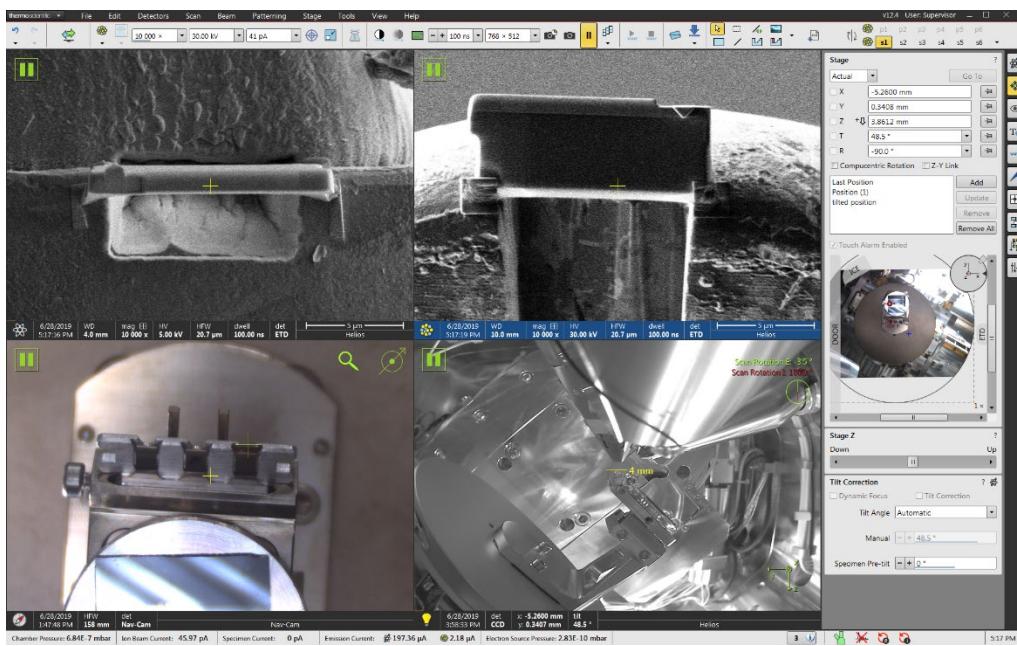
- b. Clicking Z down button at **200nm/s** and stop if the lamella starts to move laterally, indicating it is touching the post. Move Z upward with one or two clicks on the Z upward button to release the stress on the lamella.
- 13) Weld the lamella by applying two **Serial** small square pad Pt deposition (**Z~500 nm**) on two corners at the bottom. Slice the probe after welding. The ion beam setting: **30kV** and **10-41pA**. Typical time ~2 min.



- 14) Check both electron and ion image window to make sure the lamella was welded with Pa pads onto the post. Slice the probe along the top edge of the lamella and slowly retract the probe out just above post.



- 15) Retract the Pt GIS needle and **Cold** the gas.
- 16) Clean probe tip for next user.
- 17) Fully retract the probe.
- 18) Check to make sure the lamella was mounted safely on top of the post as shown below.



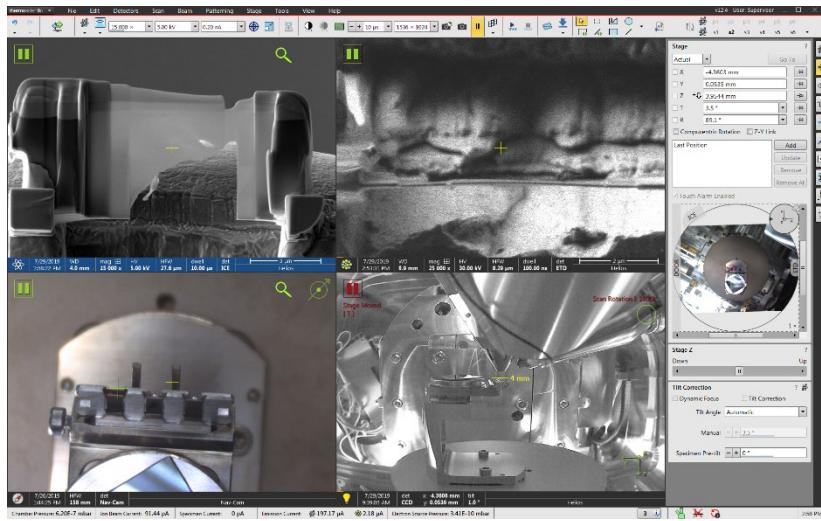
9. Lamella thinning

- 1) Change the ion beam **Scan Rotation** back to **0°**.
- 2) **Tilt the stage back to 0°**.
- 3) **Rotate the stage 180°** so the grid holder is on the **right side** in CCD window as shown below.



- 4) In electron image window, choose Field Free mode and select ICE detector, and choose **3-5 kV, 0.2 nA** to monitor the lamella transparency during thinning.
- 5) Adjust the electron image focus on the lamella and **click the Z-link** button.
- 6) Change the stage Z height to **4mm** as the TEM grid is lower than the sample surface.
- 7) **Readjust eucentric height** on the grid.
- 8) Adjust ion beam focus quickly by alternating between focusing and double-clicking the F6 (pause) key everytime if new beam voltage/current is chosen before milling.
- 9) **30 kV Cleaning Cross-Sections**, tilt **1-2°** away from ion beam (~0° for tilted grid holder and ~52° for vertical grid holder):
 - a) At relatively high current (e.g. **0.26-0.79 nA**), set **Z depth** just enough to mill all the way through the bottom of the lamella on both sides (e.g. 1.5° for front side, -1.5° for back side, or for vertical grid holder 53.5° for front side, 50.5° for back side). Aim for **500-1000 nm** thickness when done with this step.
 - b) Select scan direction: Bottom to Top at +1.5° tilt and Top to Bottom at -1.5°.
 - c) At relatively low current (e.g. **0.23 or 0.43 nA**), set **slightly lower Z depth** (e.g. 0.25 – 0.50 um less) to leave some material at the bottom of the lamella on both sides, which will add mechanical stability to the lamella. Aim for **150-250 nm** thickness when done with this step.

- d) At even low current (e.g. **41** or **80 pA**), set **slightly lower Z depth** (e.g. 0.3 um less). Aim for **80-100 nm** thickness when done with this step. Mill until uniform contrast in electron image.

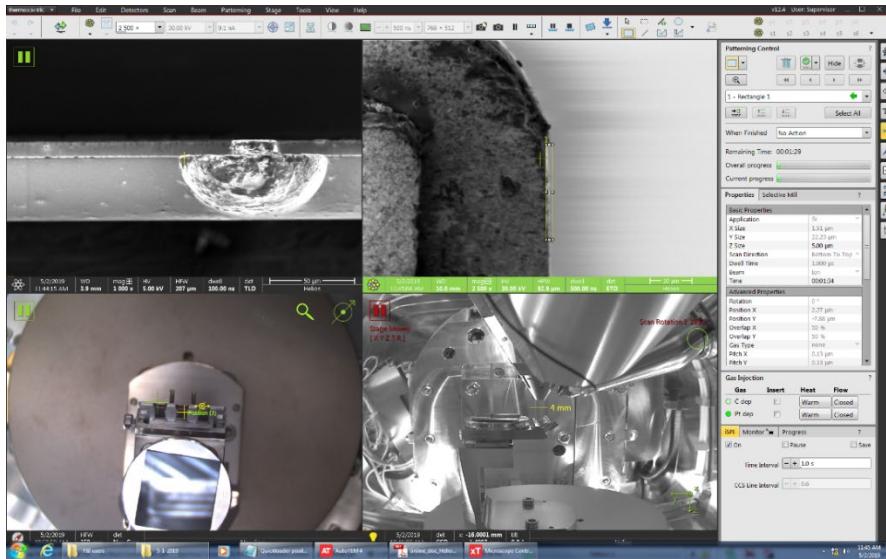


10) Low kV polishing:

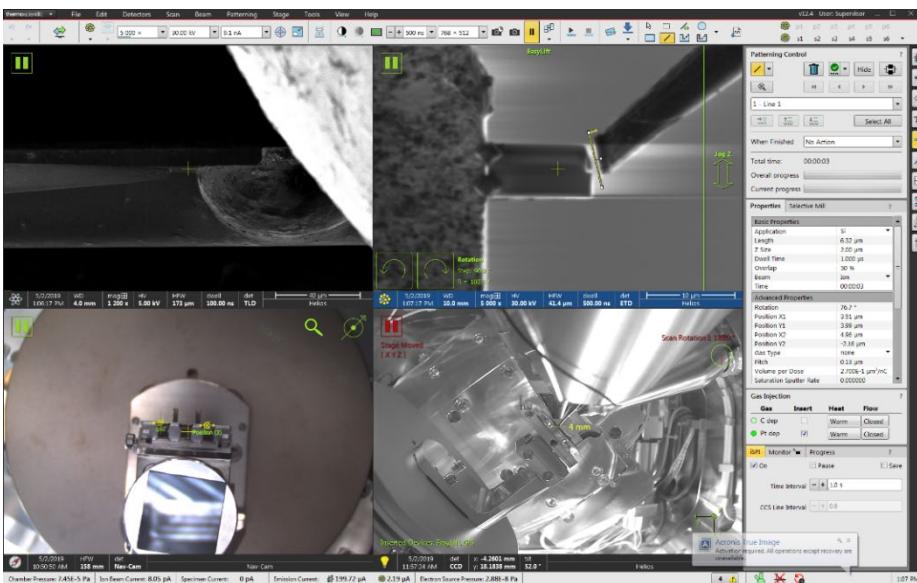
- 5 kV**, 70-100pA or even smaller currents, tilt $\pm 4\text{-}5^\circ$ away from ion beam to start the lower/upper side of the lamella. Please check the company training slides prepared separately.
- Adjust ion beam focus quickly by alternating between focusing and double-clicking the F6 (pause) key everytime if new beam voltage/current is chosen before milling.
- Turn on **iSPI** in the **Patterning** window and monitor the lamella thickness changes during thinning, noting thickness of Pt protection layer. Allow Pt to reduce to \sim half the thickness it had before you started this step, then manually stop it.
- Repeat 2kV, 1kV and 500 kV cycles following the company training slides. **It is strongly recommended to finish the 500 kV polishing** to remove the 30kV thinning induced amorphous layers (\sim 20-30 nm thick on each side of the lamella) as much as possible to improve the TEM resolution. Spent at least 20-30 seconds, controlled by current and polishing rectangle sizes, on each side of the lamella at each lower voltage.

10. Lamella side mounting on grid post

- Define grid position: rotate the stage **180°** and tilt to **52 °**. The grids should appear in the side view in electron image as shown below:



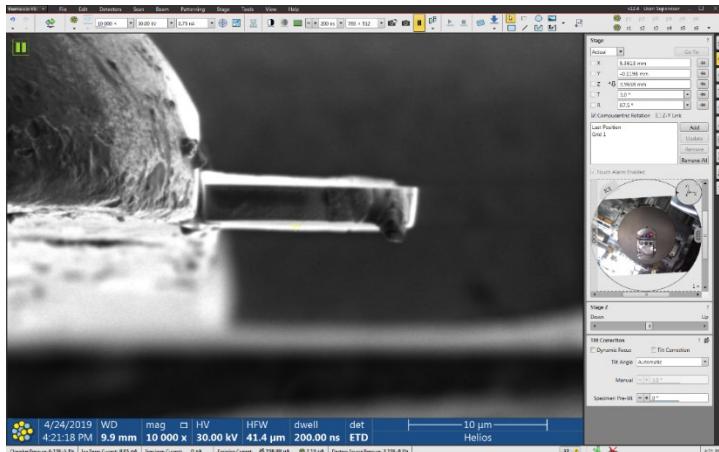
- 2) Cleaning mounting area on the grid post as shown in above ion image window. Use rectangle mode and typical Z of **5 um**.
- 3) Approach the EasyLift probe slowly at < **5um/s** and align the probe with sample in both electron and ion image windows as show below. Weld the lamella onto the grid post using two square Pt pad and use the **line** cut mode to separate the probe. Try to cut the probe on sample side to save probe material.



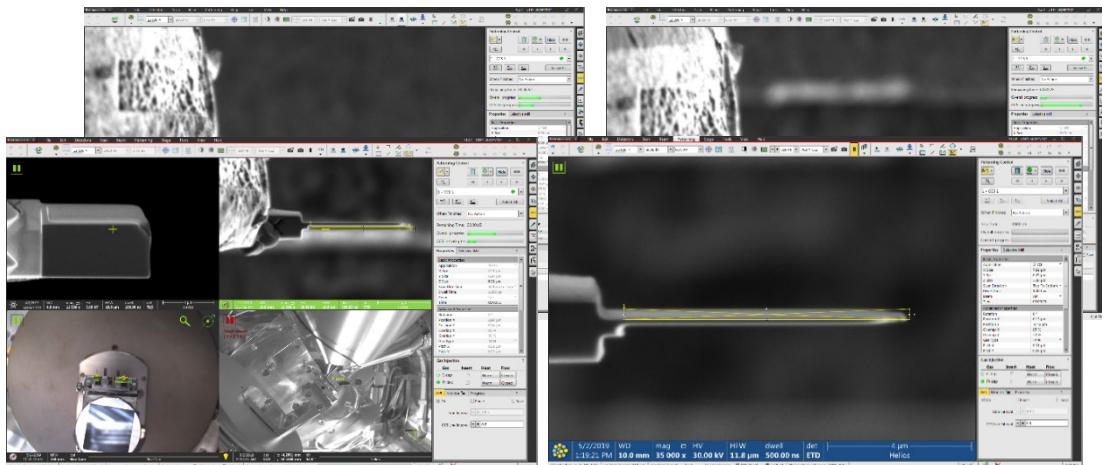
- 4) Retract both Pt GIS needle and EasyLift probe.

11. For side mount lamella:

- 1) Align sample with ion beam: adjust stage tilt so that the white edges on both sides of the lamella (in dark) appears symmetric in ion image window. Typical $1\text{--}3^\circ$ tilting might be needed for adjustment.



- 2) Large mass cutting: choose **Cleaning Cross Section** mode and define cutting box on both sides of the sample to start cutting alternately. Typical beam settings: **30kV** and **0.75 - 1nA**. Tilt the sample **1.2° up** for bottom side cutting and tilt **1.2° (-1.2 °)** to cut the upper side. Choose **Bottom to Top** in **Scan Direction** for bottom side cutting and **Top to Bottom** to cut the upper side.
- 3) Initial thinning: choose **Cleaning Cross Section** mode and define cutting boxes on both sides of the sample to start thinning alternately. Typical beam settings: **30kV** and **0.75nA**. Tilt the sample **1.2° up** for bottom side cutting and tilt **1.2° (-1.2 °)** to cut the upper side.
- 4) **5kV thinning**: use **5kV** and **0.21nA** for ion beam. Choose **Cleaning Cross Section** mode



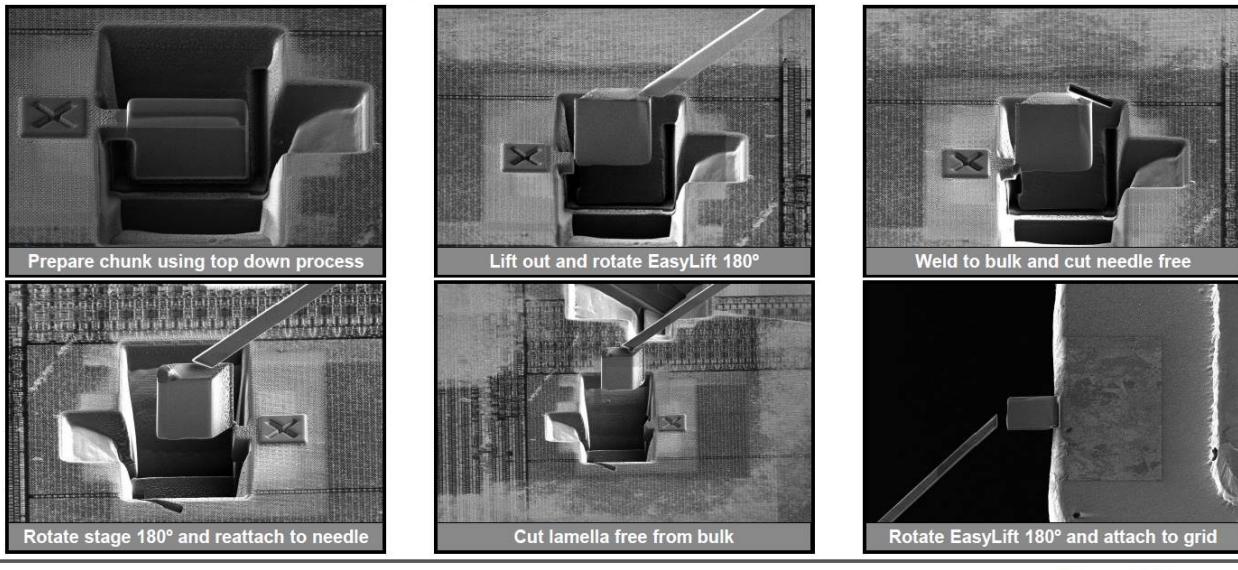
and define cutting boxes on both sides of the sample to start thinning alternately. Typical Tilt the sample **1.0 ° up** for bottom side cutting and tilt **1.0 ° (-1.0 °)** down to cut the upper side.

- 5) **2kV cleaning:** use 2kV and **0.15nA** for ion beam. Choose **Cleaning Cross Section** mode and define cutting boxes on both sides of the sample to start thinning alternately. Typical Tilt the sample **1.0 ° up** for bottom side cutting and tilt **1.0 ° (-1.0 °)** down to cut the upper side.

12. Inverted lift out and side mounting:

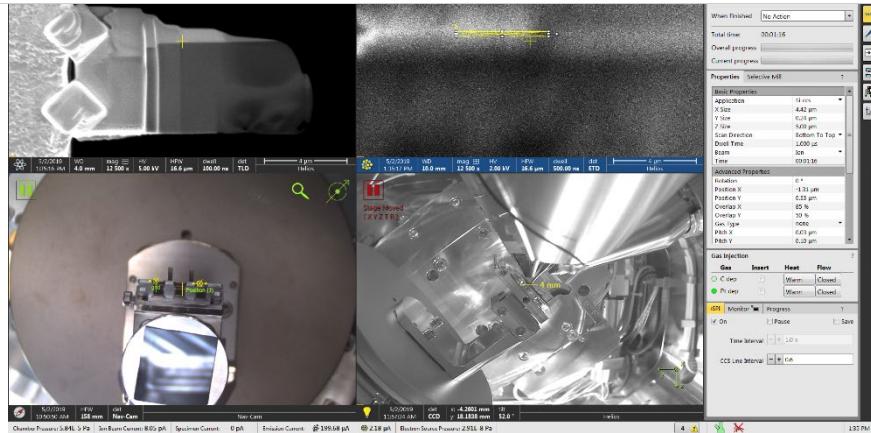
Inverted Lift Out

Double attach method using EasyLift



69 Proprietary & Confidential

ThermoFisher
SCIENTIFIC

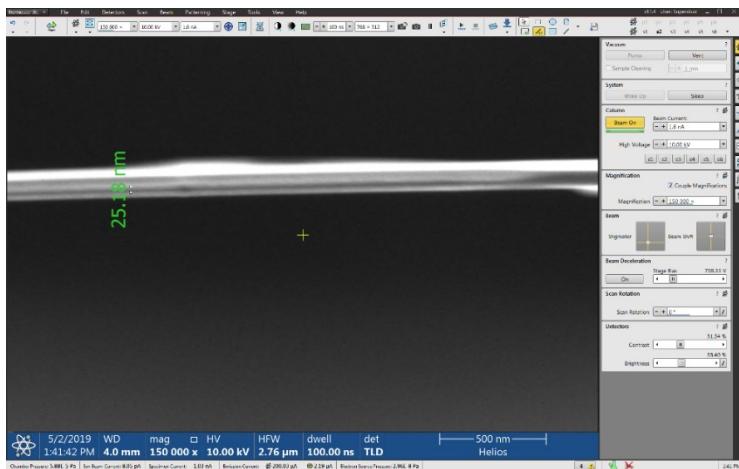


13. Tips to prevent over amorphization:

- 1) In TEM, check with EELS for sample thickness if it is too thick or too thin,
- 2) In TEM, check with SAED if the sample was not completely amorphized,
- 3) Back to FIB, to avoid over amorphization or excess damage, use 16 keV to 300 nm thick, 5 kV to 100 nm, 2 kV to almost as thin as you can go. Finally sputter the entire surface at 500 V.
- 4) For hard samples such as STO supported materials, try 3-5 large tilt angle for thinning.

14. Thickness measurement

- 1) Align the sample with electron beam by tilting stage to ~52°. The dark region in the middle should be the sample cross section as shown below:



- 11) The side view of final sample in electron image window: the thinnest part should be close to the interface between protection layer and sample. The stepped cutting should produce a step like cross section with the area close to mounting area the thickest.

