

Aquilos 2 Cryo-FIB (Serial Lift-out) Standard Operation Protocol

CONTENT

Preparation.....	2
Sample Loading.....	3
Sputter Coating and GIS deposition.....	4
Sample Block Preparation.....	5
copper block preparation.....	7
Sample block Serial lift out.....	11
Automation by AutoTEM.....	20

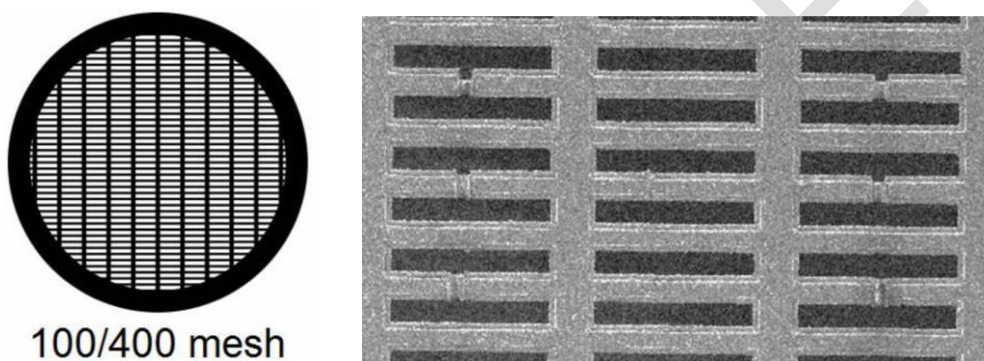
CPOS CryoEM

PREPARATION

- 1) HPF-Carrier Shuttle 3mm (P3/AG shuttle):



- 2) G100/400-C3 grid:



- 3) HPF sample:

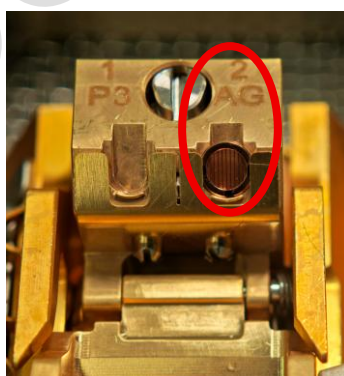
Prepare HPF samples in advance. Sample thickness should ideally be below $100\mu\text{m}$ to reduce the risk of incomplete freezing. The ideal area to be extracted should be located on the near surface of the HPF sample.

SAMPLE LOADING

- 1) At room temperature, clip G100/400-C3 grid with its bright side facing to the O-ring. Based on the position of the 100-mesh bars of the grid, mark three points at the corresponding O-ring edge position using two different marker pens (As shown below).



- 2) Place the clipped AutoGrid into the AG slot of the shuttle. Orient the grid so that the 100-mesh bars are aligned vertically within the shuttle (as shown below).



- 3) Add liquid nitrogen to cool down.
- 4) Place HPF (High pressure freezing) sample into the P3 slot of the shuttle.
- 5) Load the shuttle into SEM chamber.

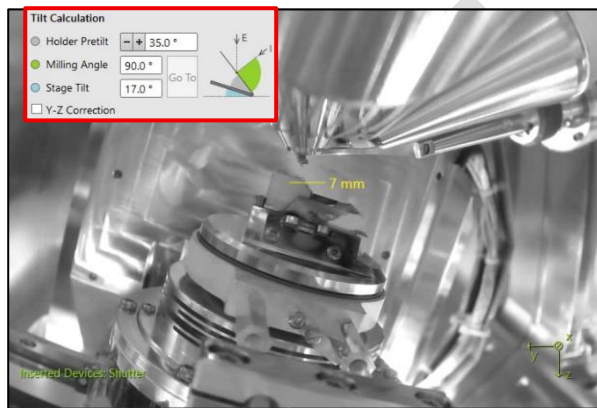
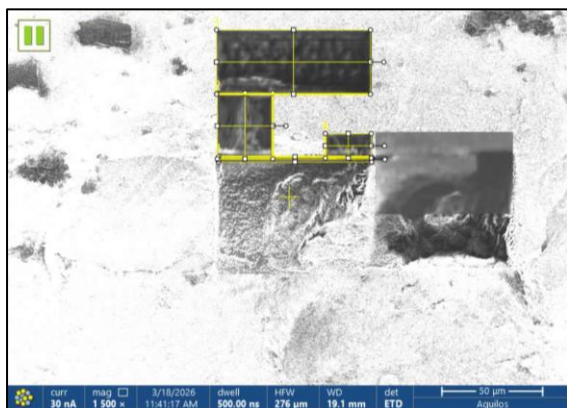
SPUTTER COATING AND GIS DEPOSITION

- 1) Perform sputter coating for 15 seconds.
- 2) followed by GIS deposition for 40 seconds
- 3) Perform sputter coating for 15 seconds again.

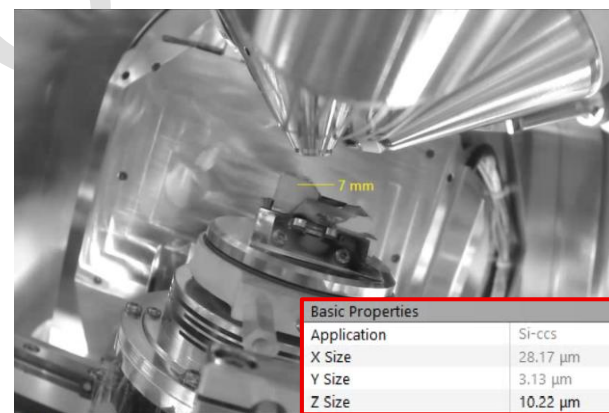
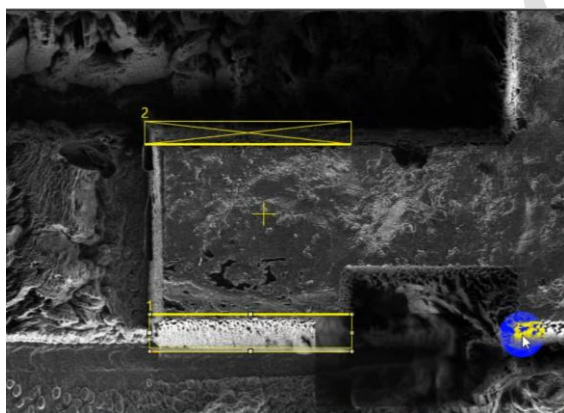
CPOS CryoEM

SAMPLE BLOCK PREPARATION

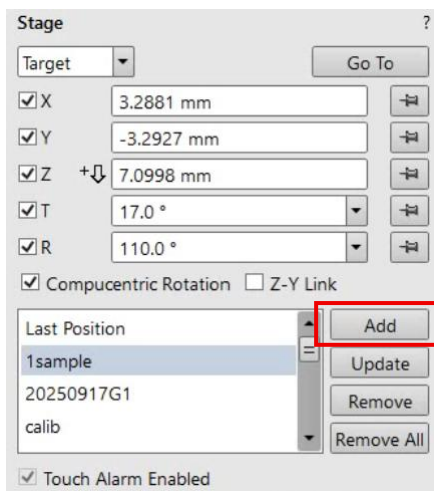
- 1) Set the rotation of electron and ion beam to 0 degrees.
- 2) Rotate stage 180° (absolute angle: **108°**) and tilt to **17°**, Perform rough trenching with the below pattern: **15nA or 30nA, RCS.**



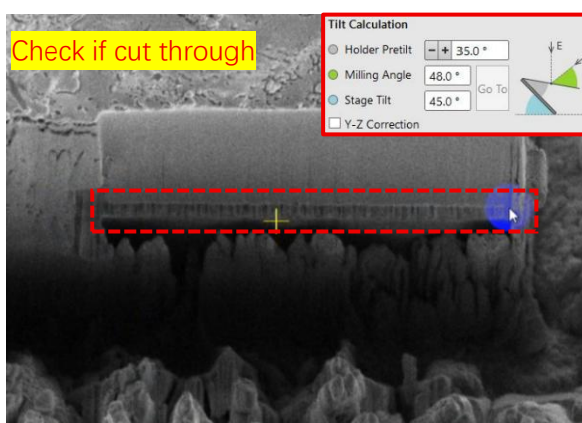
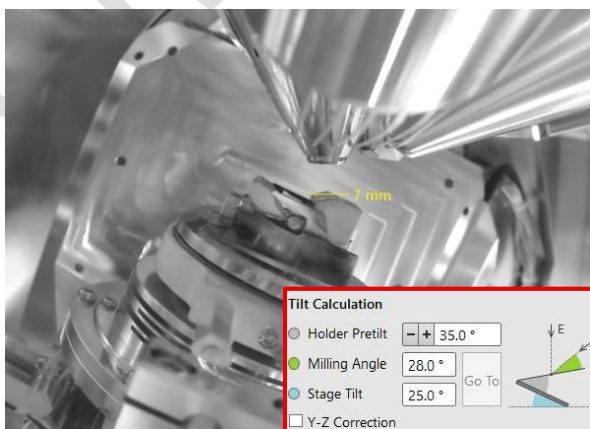
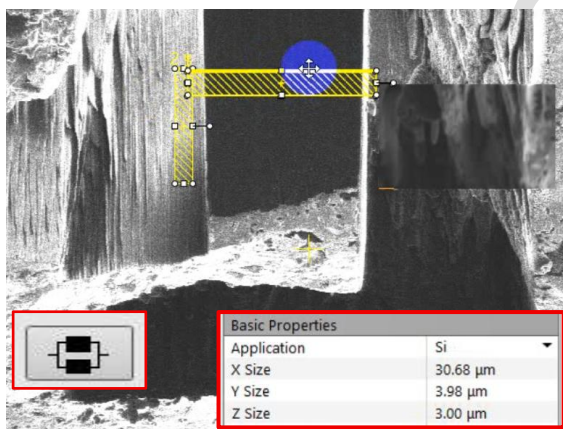
- 3) Trim top and bottom surfaces of the sample block using **3nA or 5nA, CCS.**



- 4) Add the sample position into the stage window.

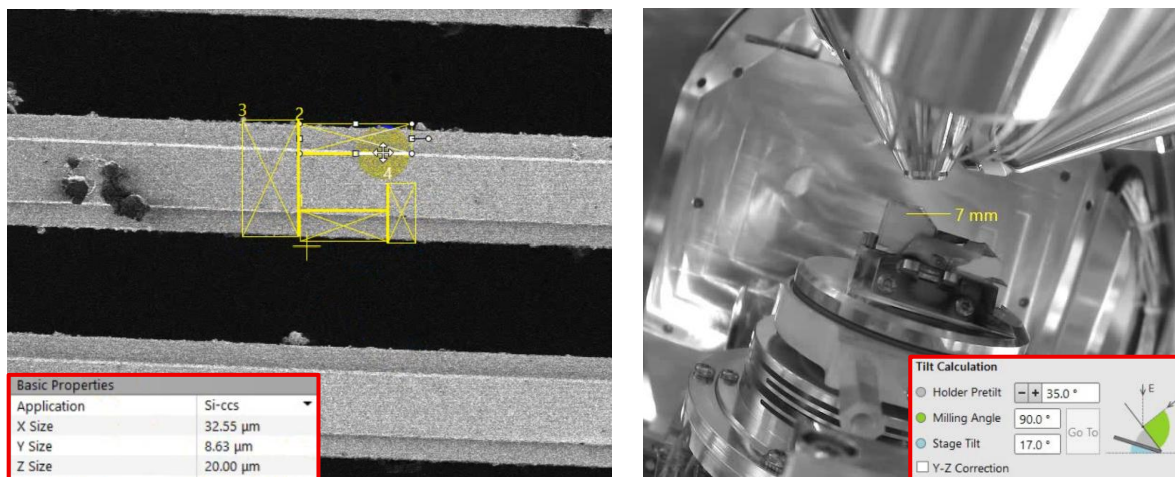


5) Rotate back the stage 180° (absolute angle: **-72°**) and tilt to **25° (20°-30°)**; Cut through the bottom and portions of the side area using **3nA or 5nA, Rectangle, Parallel**. Tilt to 45° and view the other side of the sample block by SEM image to confirm if it has been cut through.

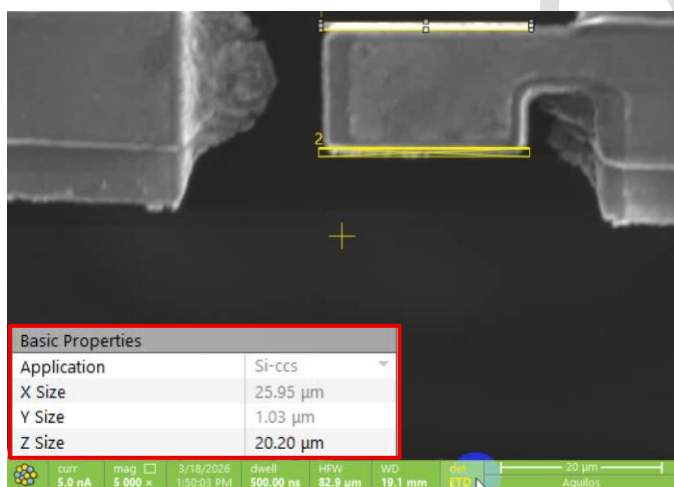


COPPER BLOCK PREPARATION

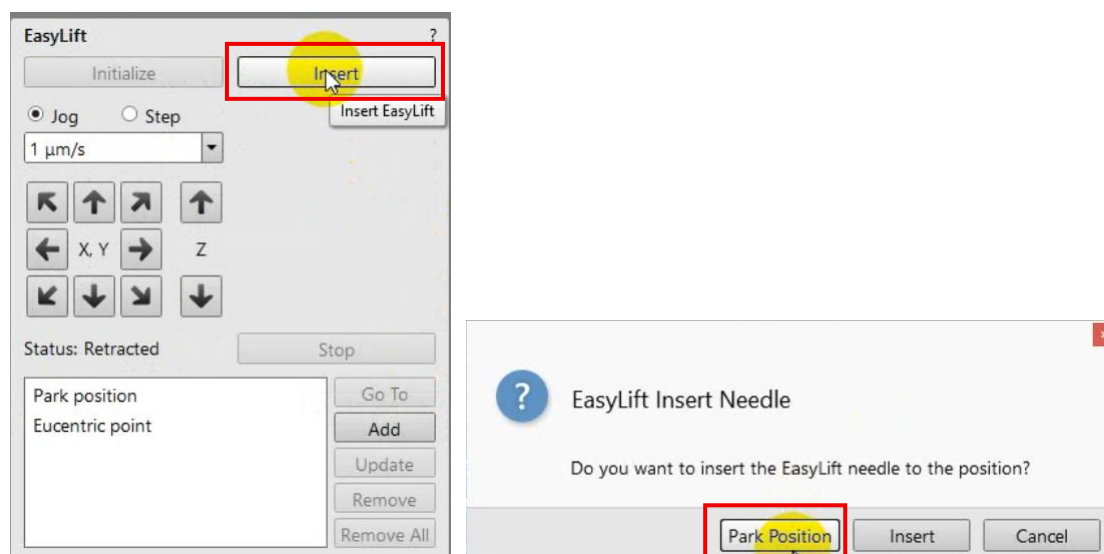
- 1) Rotate stage 180° (absolute angle: **108°**) and tilt to **17°**. Using **65nA, CCS**, cut the copper block for sample block attachment: x: 8 μm , y: 15 μm , z: 20 μm .



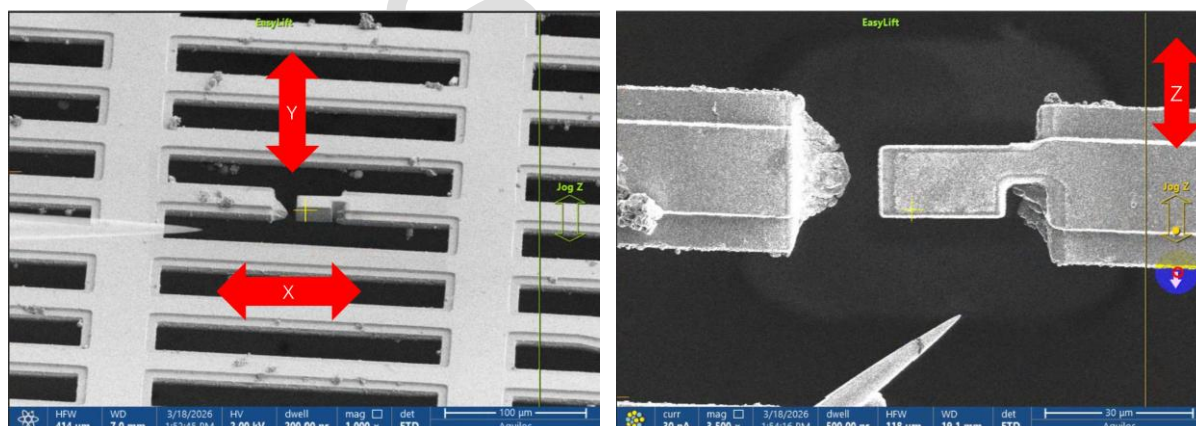
- 2) Trim the top and bottom surfaces of the copper block using **5nA, CCS**.



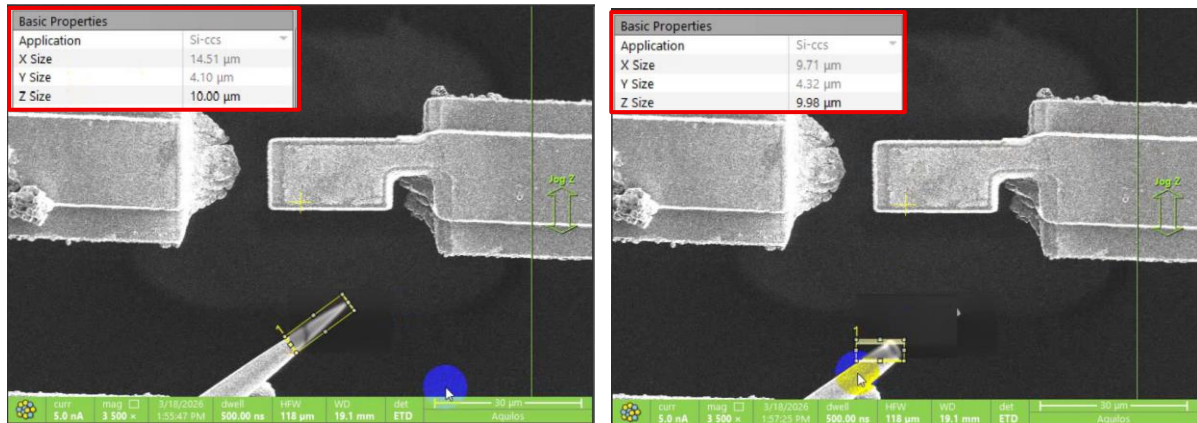
- 3) Insert the EasyLift needle and click “Park Position” in the pop-up window.



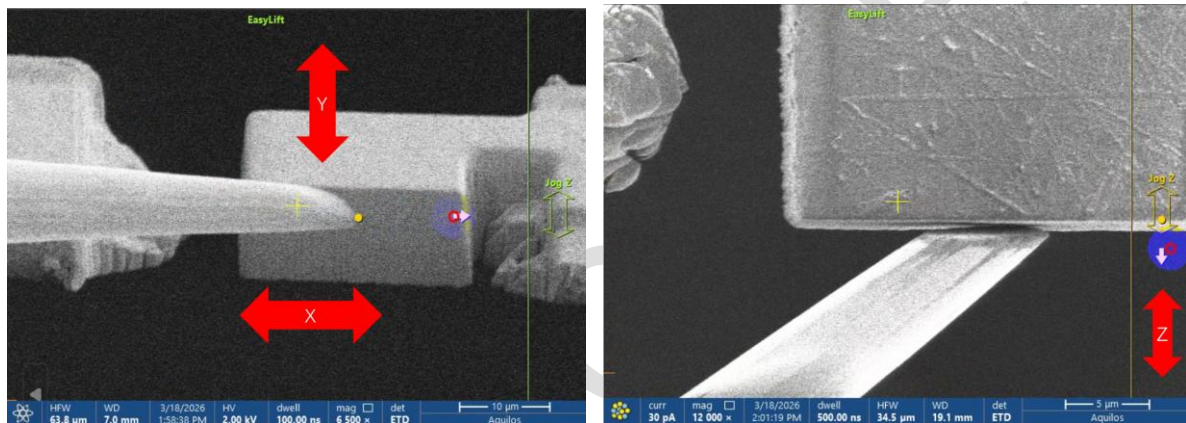
- 4) Don't move the SEM stage. In the SEM window, move the needle along the X and Y directions to bring it closer to the copper block. In the FIB window, move the needle along the Z direction until it closes to the copper block. Keep the needle moving continuously—avoid intermittent movement to prevent needle sticking.



- 5) Trim the needle tip using the ion beam: **5nA, CCS**. Shape its tip into a plane parallel to the copper block.



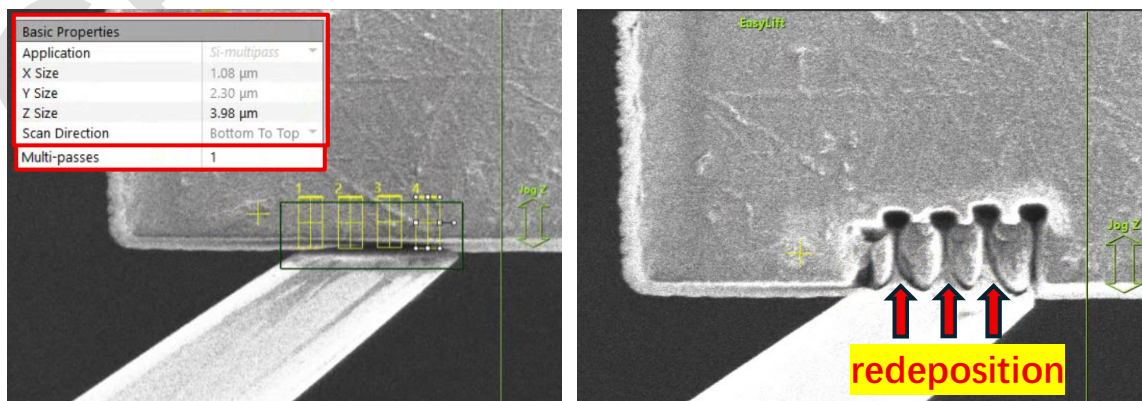
6) Move the probe along the XYZ direction until it attaches the copper block.



7) Redeposition on the bottom surface of the copper block use **RCS, Multipasses:**

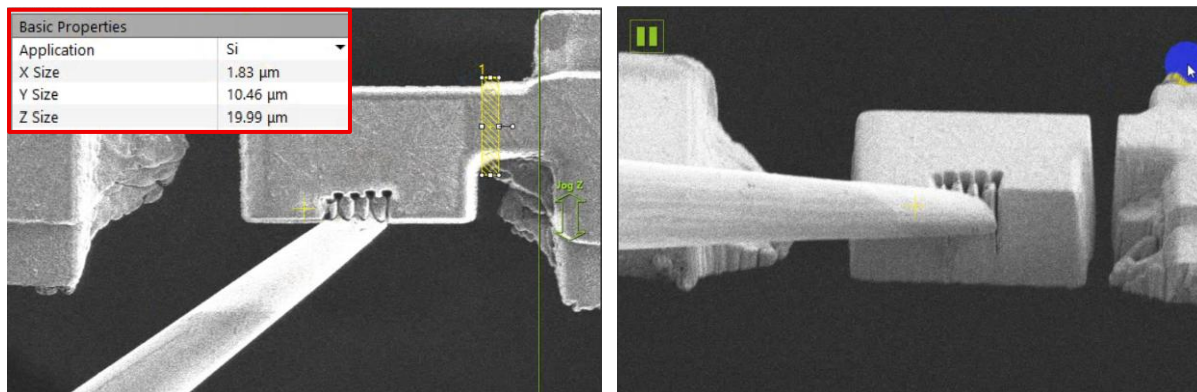
1, 1nA or 3nA, bottom to top. Z: 4 μm (3nA) or 5 μm (1nA). If the redeposition

is clearly visible in the gaps, it indicates that it has adhered firmly.



8) Cut through the copper block using the parameters: **5nA, rectangle pattern.**

Check if it has been cut through by SEM image.

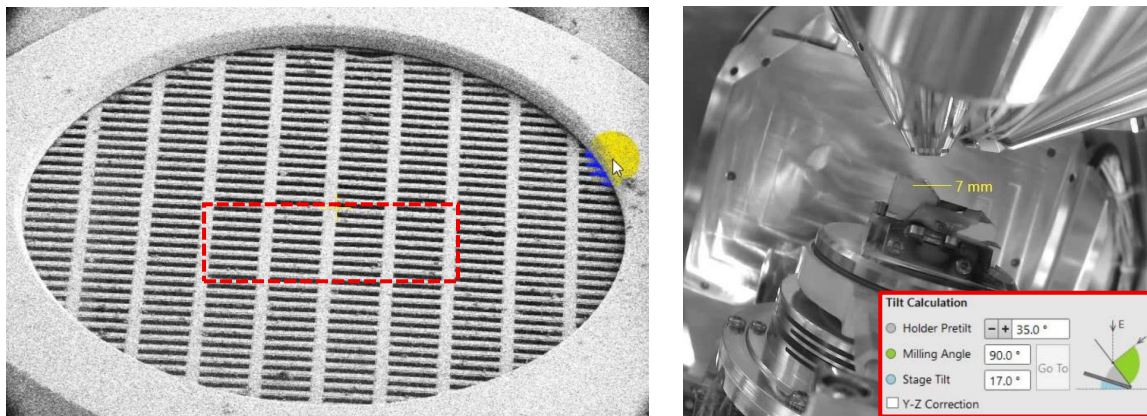


9) After cutting off, first move the needle upward in Z direction, then retract the needle.

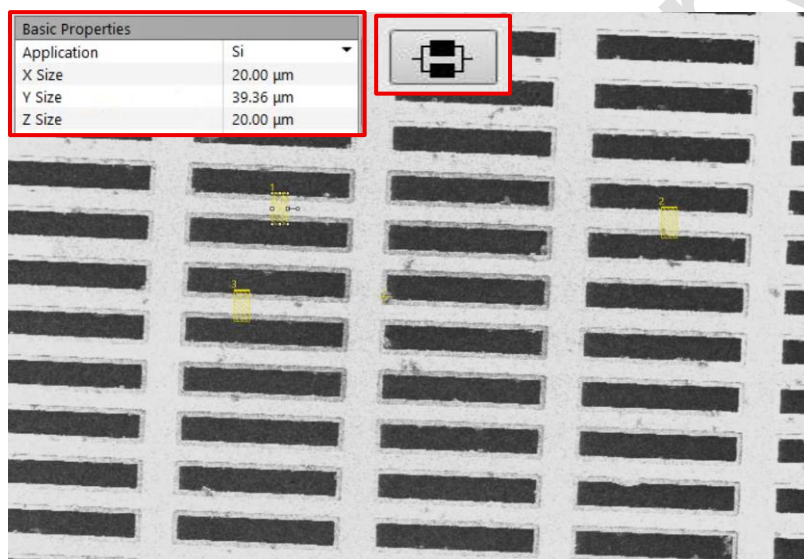


SAMPLE BLOCK SERIAL LIFT OUT

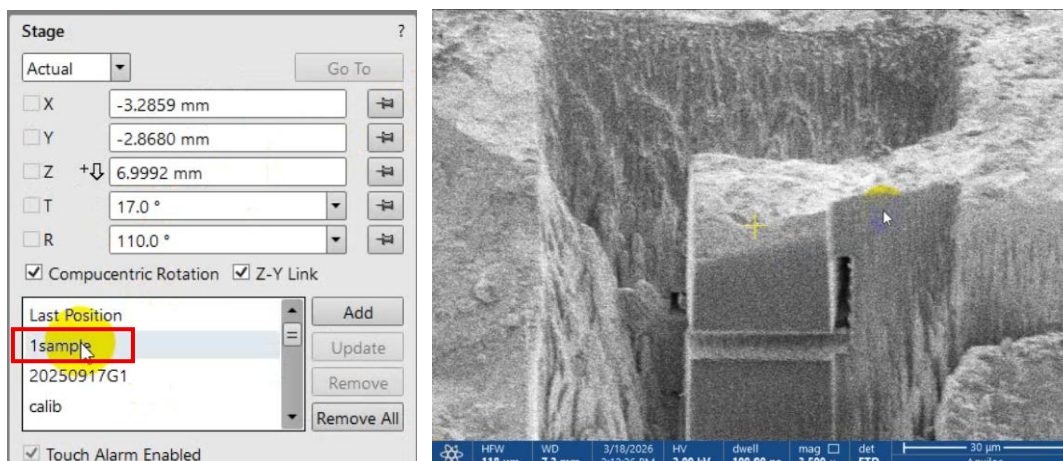
- 1) Locate the center of the 100/400 mesh grid and select some groups of adjacent and parallel 400-mesh bars.



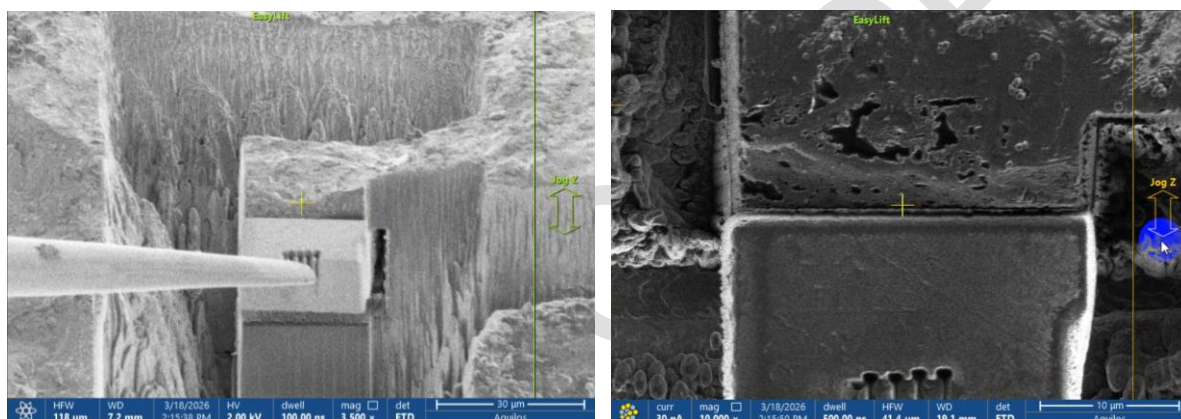
- 2) Trench milling of the grid bars using the parameters: **65nA, Rectangle**



- 3) Double click the sample item at the stage window to move stage back to the sample block position.

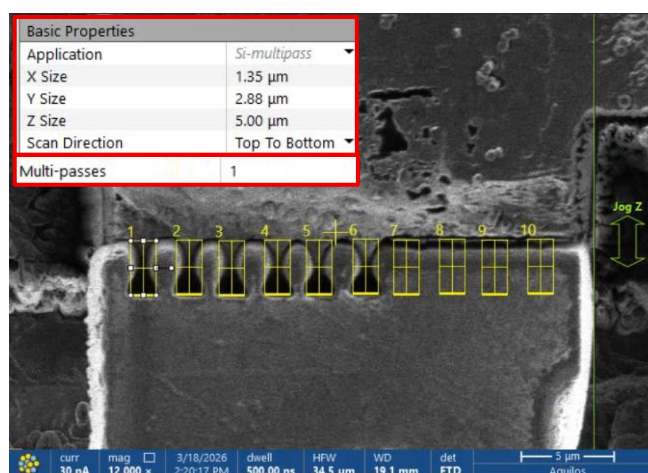


4) Insert the needle and move XYZ to attach the copper block to the sample block.

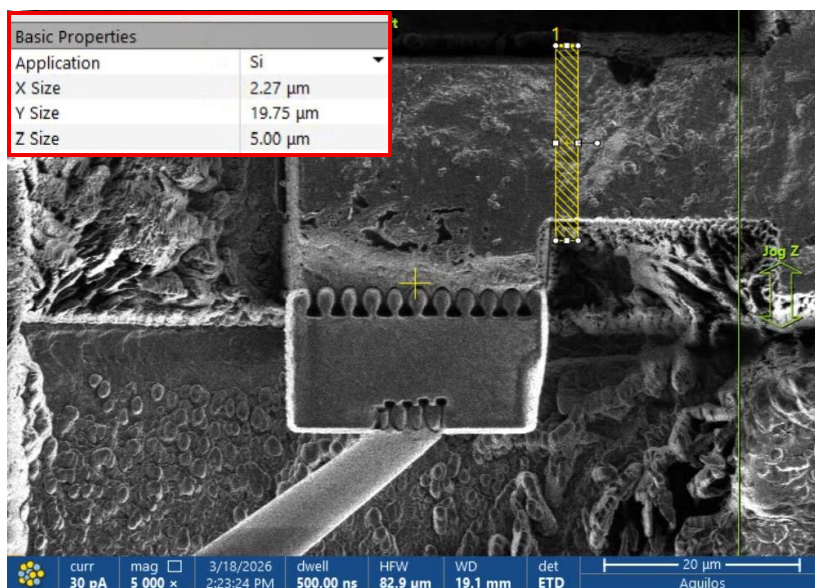


5) Redeposition on the interface between the copper block and sample block use

RCS, 1nA or 3nA, top to bottom. x: 1.5 μm, y: 3 μm, Z: 5 μm; multipass: 1.



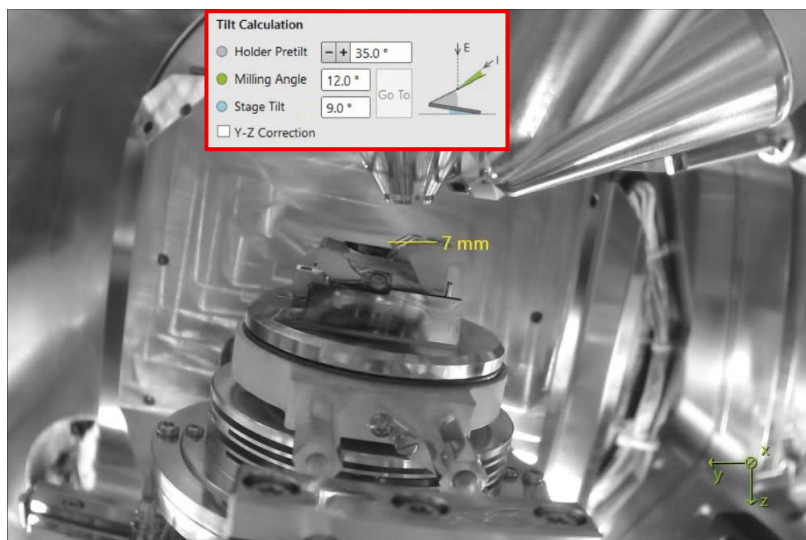
6) After welding is complete, cut off the side area of the sample block using **5nA**, **rectangle pattern**.



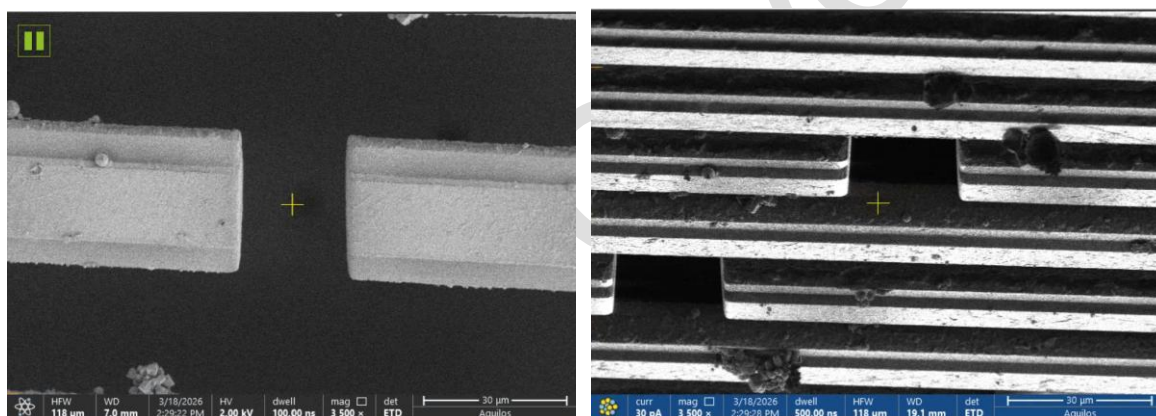
7) After cutting off, first move the needle upward in Z direction, then retract the needle.



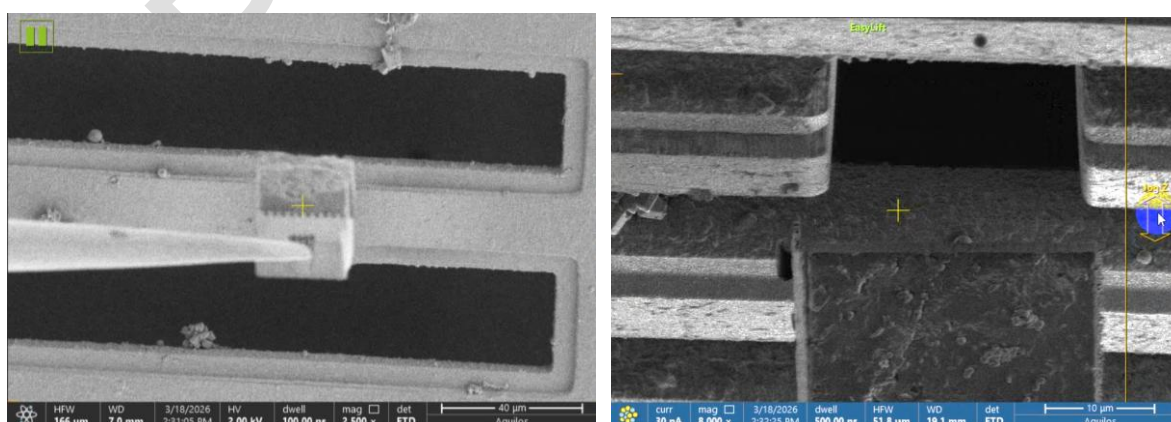
8) Rotate the stage back to -72° (absolute angle), milling angle 12° .



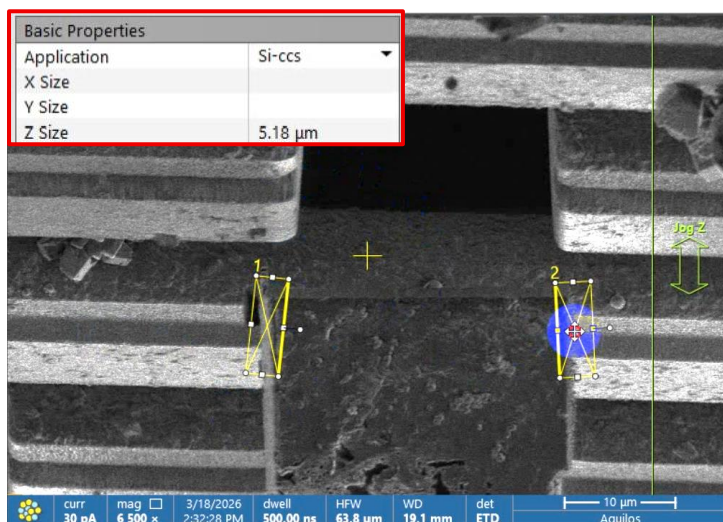
9) Move to the trench of the copper grid and adjust the eucentric height. After that, add the stage position of the trench in the stage window.



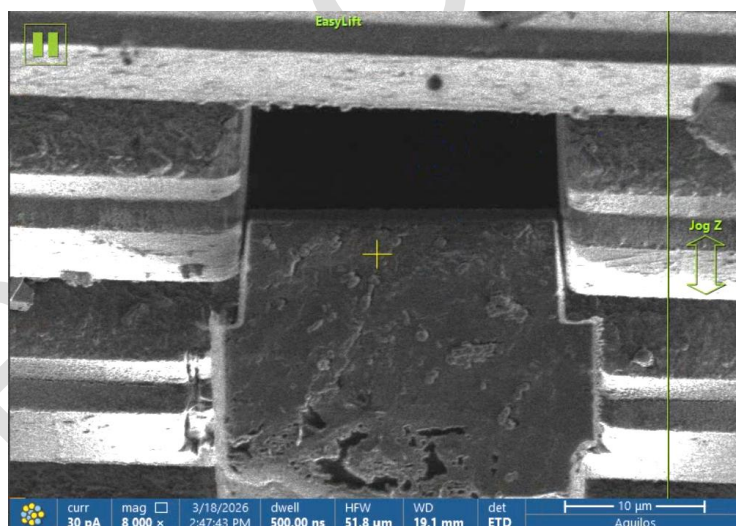
10) Insert Easylift needle and move it to the nearby position of the trench.



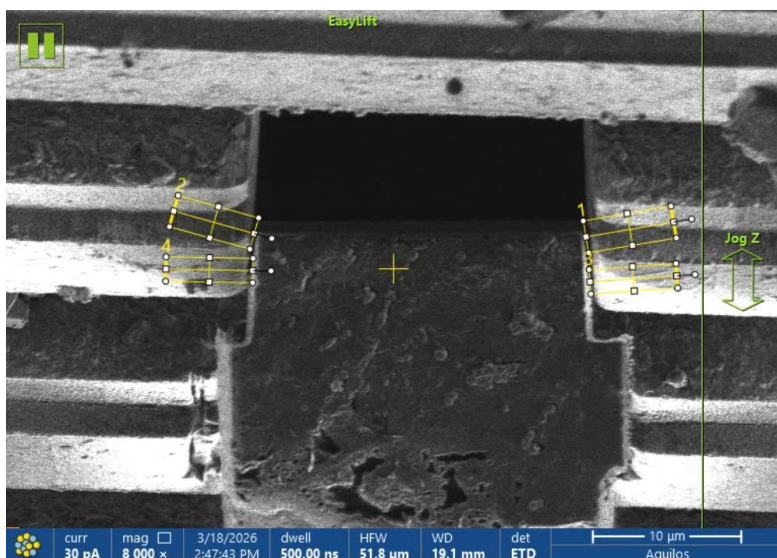
- 11) Trim the front end of the sample block along the extension line of the trench to match the width of the trench. **1nA, CCS, Z: 5 μ m.**



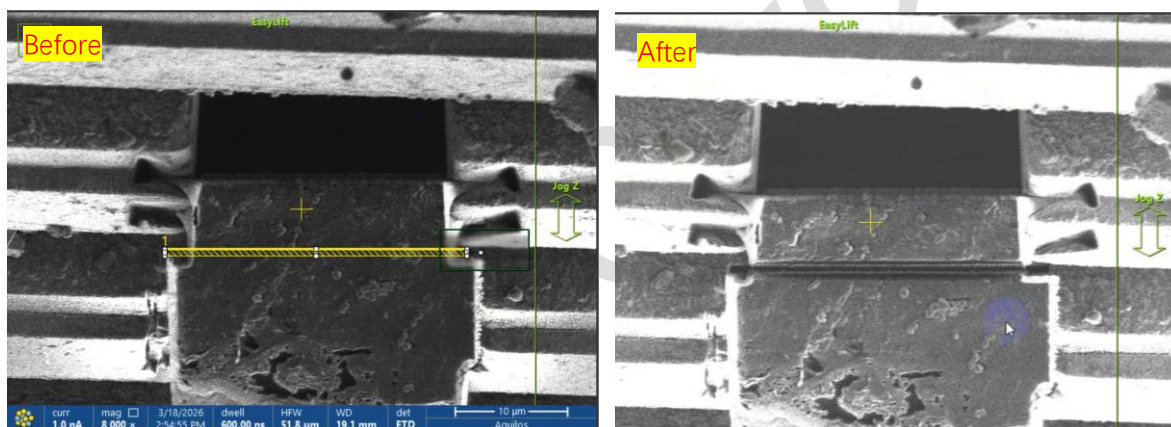
- 12) After trimming, move the sample block into the trench. If it does not align well, it needs to fine adjust xyz position of the sample block before insertion.



- 13) Weld the sample piece to the grid on both sides. **RCS, 1nA, Multipasses: 1, Z: 5 μ m.**



14) Cut off the sample block using **rectangle pattern, 1nA, Z: 5 μm**.



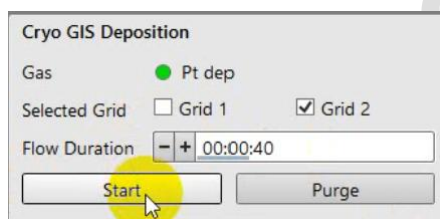
15) Slightly move EasyLift needle in X direction to confirm that it has been cut off.

Then move it in Z direction to make it far away from the grid. Press the shortcut keys Ctrl+J to exit the control page of EasyLift. Now you can double-click the image to move stage to another trench position for sample piece attachment.

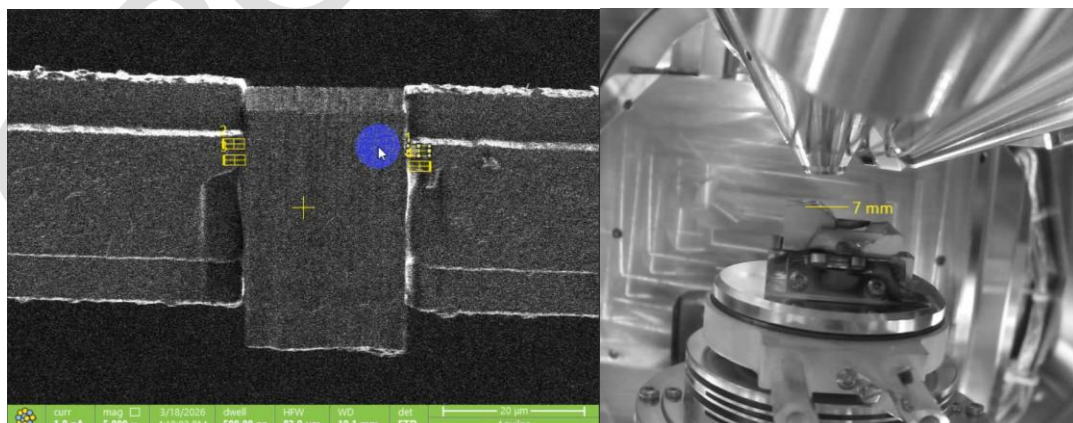


16) After all sample pieces are welded, perform fluorescence to check.

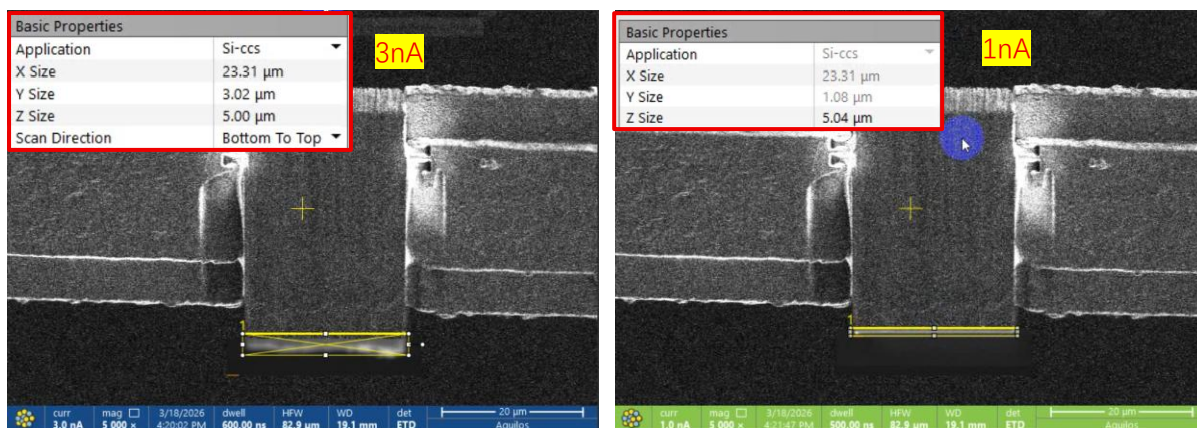
17) GIS 40s to the sample pieces.



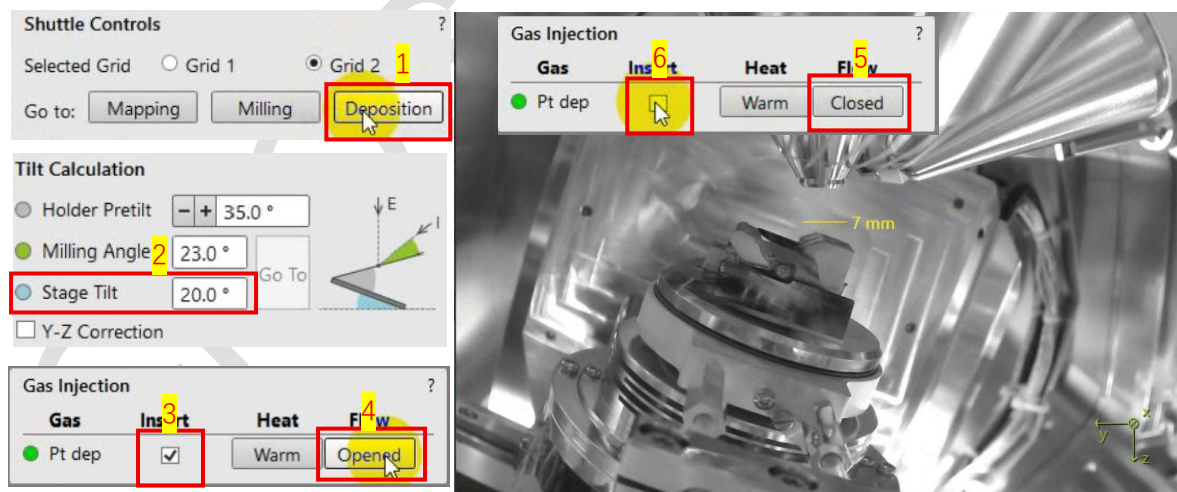
18) Go to sample piece position by double-clicking the name. Rotate the stage 180° (absolute angle: 108°), stage tilt angle 5°. Reinforce the weld between sample and grid on the top surface. RCS, 1nA, Multipasses: 1, Z: 5um.



19) Trim GIS-coated surface. First use 3nA, CCS, then 1nA, CCS. The angle between the sample block and the copper grid is 12°. When trimming the first GIS-coated surface, set stage tilt to 5° with rotation at 180°.

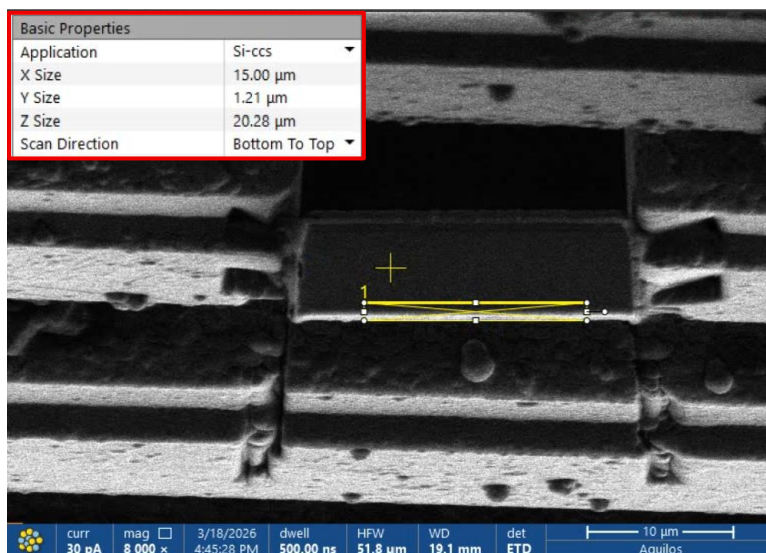


20) Click “Deposition” and set the stage tilt 20°. Click “Insert” and “Closed” button to perform GIS deposition manually for 2minutes. After that, close flow and retract the GIS needle.



21) Perform sputter coating 15s.

- 22) Double click 1# sample position to go here. Now begin manual mill the sample surface by using the parameters. **3nA, CCS, Z: 20um**. And then **1nA, CCS, Z: 20um**.



- 23) Move to the next sample site and mill the surface with 3nA and 1nA as the previous step.

AUTOMATION BY AUTOTEM

1) Once all sample sites are ready, open the AutoTEM software to process them.

