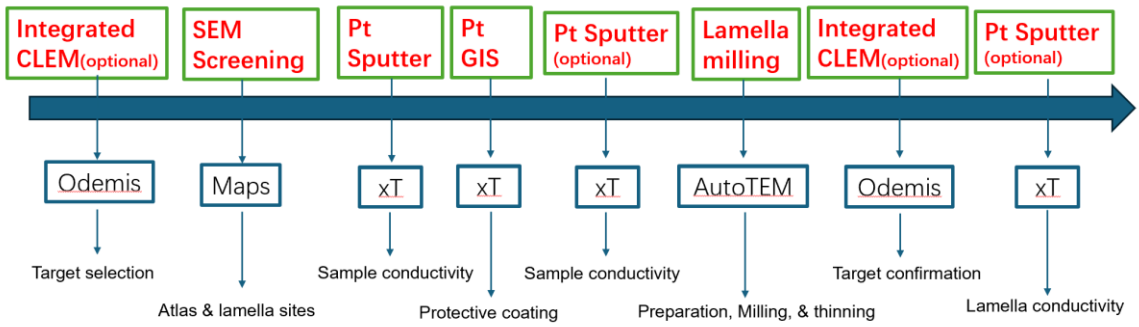
Aquilos 2 Cryo-FIB (AutoTEM)

Standard Operation Protocol

Content

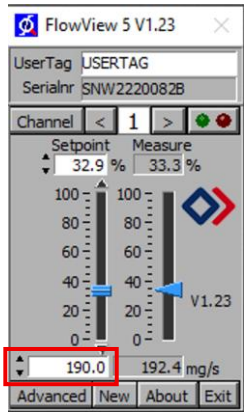
Workflow



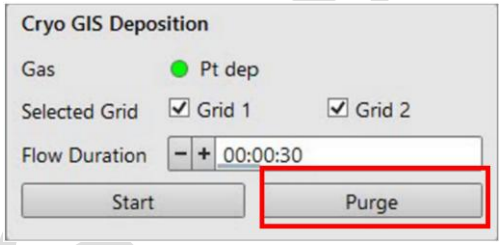
Preparation

1) Turn on the gas valve of high-pressure tanker in equipment room 1 (by staff).

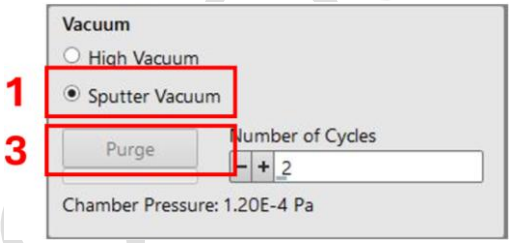
2) Set N_2 flow rate to 190mg/s to purge the cooling system at least 30min.

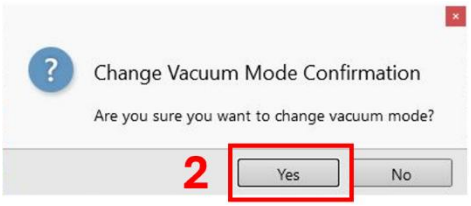


3) * During the cooling system purging period, you can purge GIS 30s meanwhile.

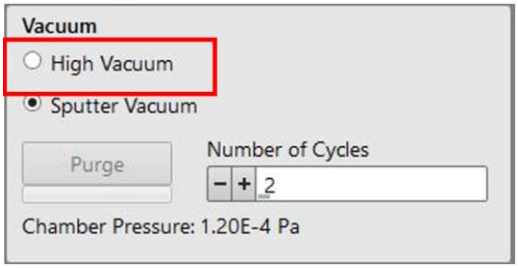


4) * And purge sputter coating Ar (0.05MPa) for 2 cycles.





5）After purge done， recover to High Vacuum mode

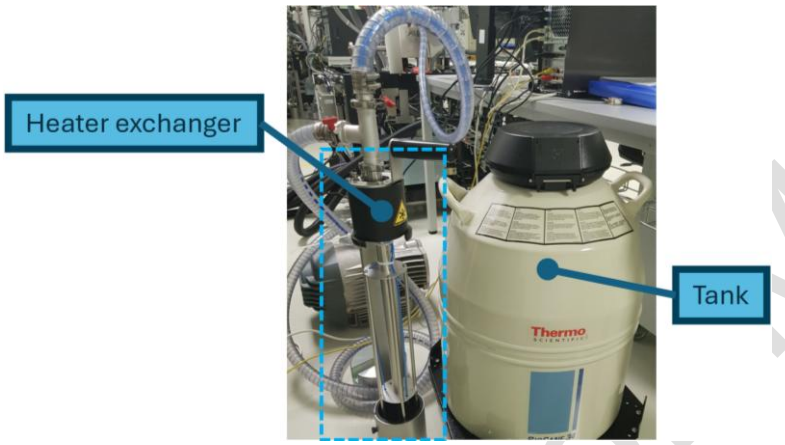


** The GIS purge and Sputter purge(step 3-5) can be performed anytime during the 30-minute cooling N2 Purge period

System cooling down and loading sample

1) Unload shuttle from microscope if any.

2) Fill the heat exchanger tank with liquid nitrogen.



3) Put heat exchanger into LN_2 dewar (cryo shield and stage temperature can be cooled down below 180C in 30min)

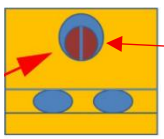
4) Put the autogrid shuttle into the preparation pot.

5) Pour clean LN2 into the preparation pot and wait till theLN2 equilibrates.

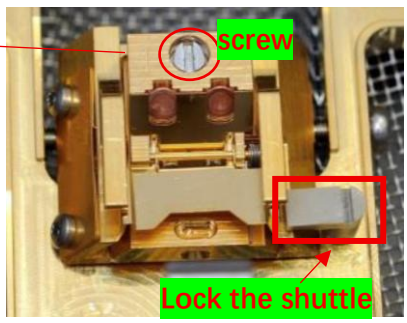
Preparation Pot

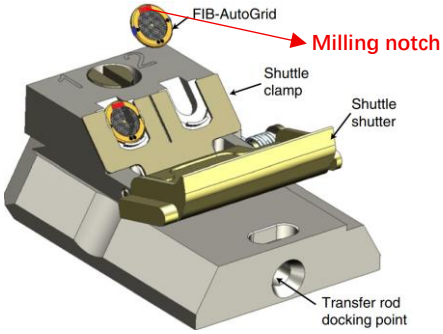
6) Turn on the main switch of the Nitrogen cylinder which tied to the preparation desk.

7) Rotate the screw clockwise to open the shuttle. Put your grids into the shuttle with Autogrid Tweezer. **Sample side (cells) is facing us and milling notch is facing up.**

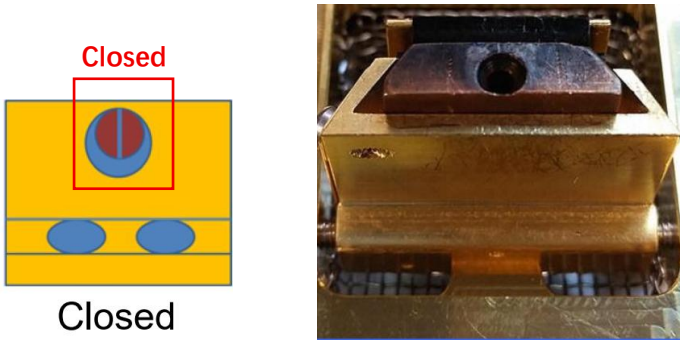


Open



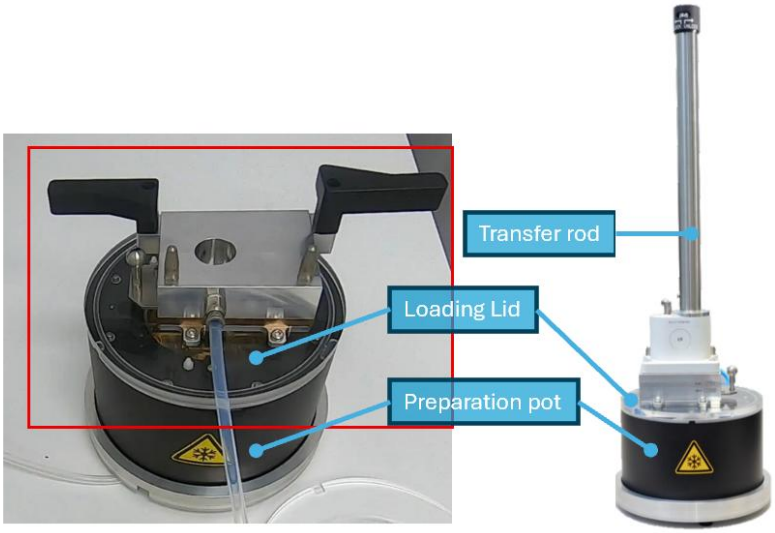


8) Rotate the screw anticlockwise to securely close the shuttle.



9) Unlock and place the shuttle vertically. Add a small amount of LN2 to maintain temperature.

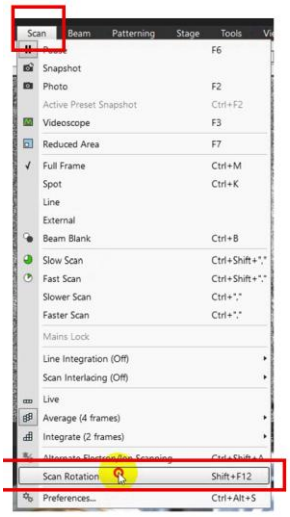
10) Put the loading lid and the transfer rod on the preparation pot. Transfer the shuttle into the transfer rod follow the training steps.

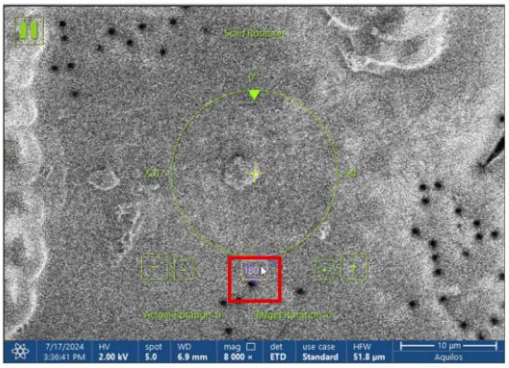


11) Transfer the rod into the SEM airlock and insert the shuttle into the microscope stage follow the training steps.

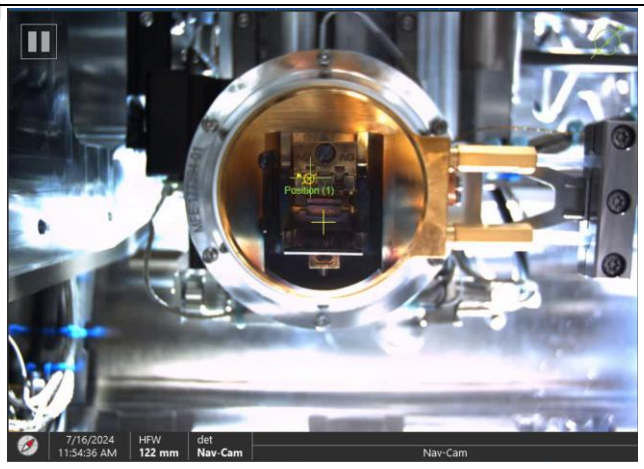
Microscope setup

1) If CLEM is required, please follow the steps of Delmic METEOR SOP.
2) Set *Scan* menu / *Scan Rotation* to 180° for both beams (or press Shift+F12).

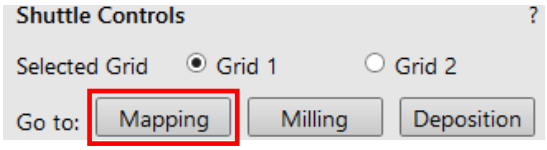




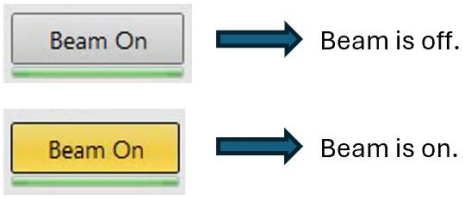
3) In the *Sample Exchange Window*  , Holder selection: AutoGrid Shuttle 35°.

(Optional) Click *Stage* menu / *Take Nav-Cam Photo*. Then it will automatically capture a navigation image of the whole shuttle and show in the third window.	

4) In the *Cryo* tab, select a grid and go to *Mapping position*.

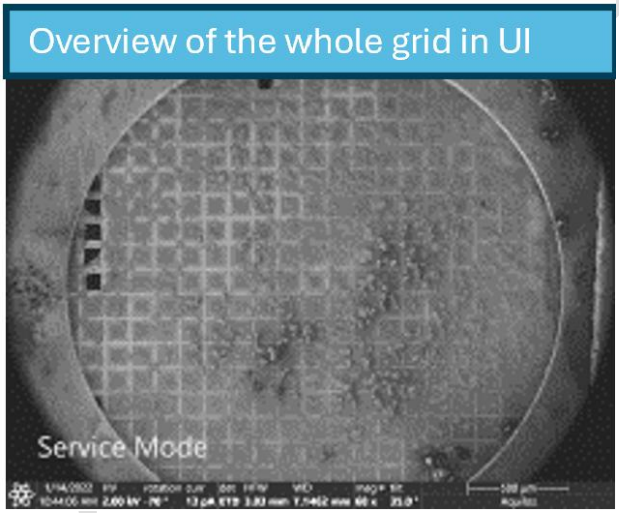


5) Click on the *Beam On* button to start the electron beam to observe the overall state of the sample.



6) Find some area to focus on, e.g. some ice particles. Run the *Lens Alignment*, focus and correct astigmatism. Click *Link Z to FWD*.

7) Zoom out so you can see the whole grid. Now the grid is prepared for taking Maps overview.

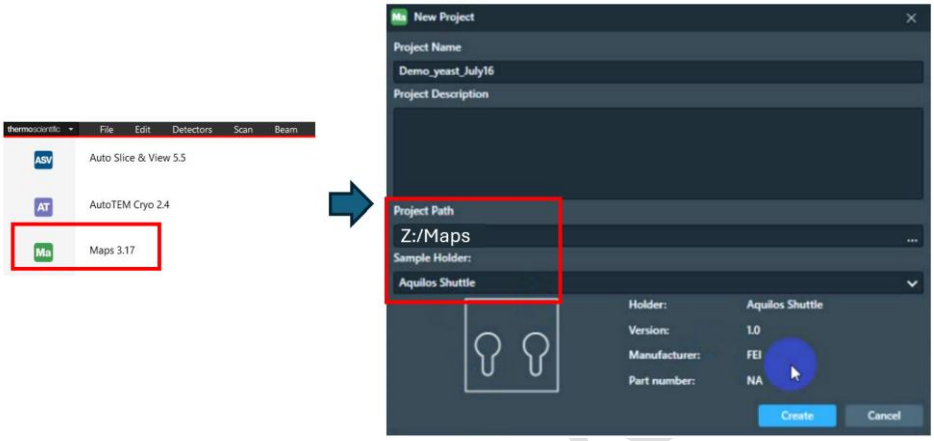


Maps project setup

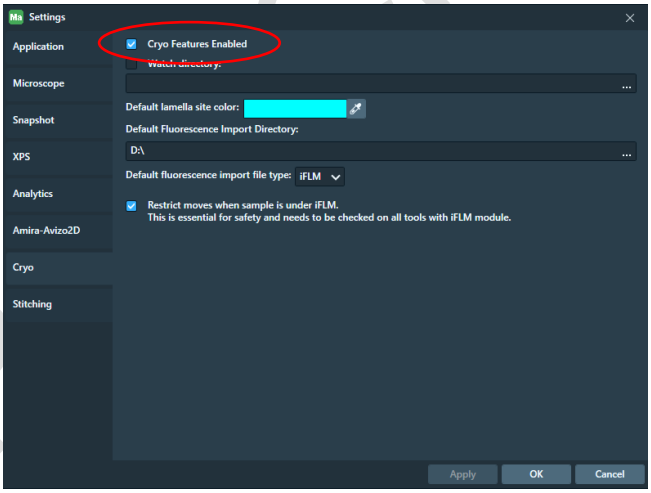
1) Open the Maps application and create a new project:

   a. Project path: Z:/Maps

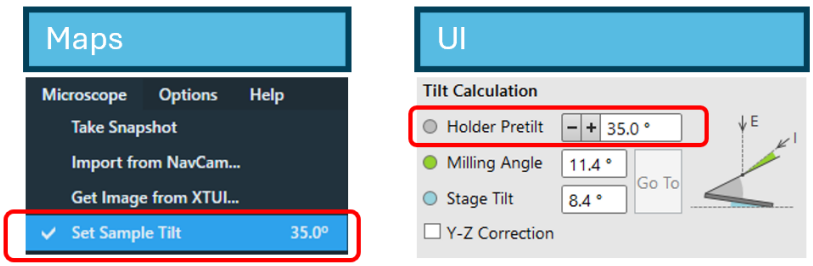
   b. sample holder: Aquilos Shuttle



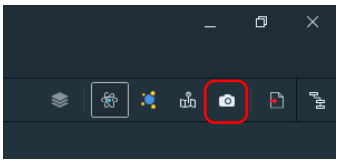
2) In Maps top panel, select the *Options* tab /*Settings* / *Cryo* and make sure *Cryo Features Enabled* are ticked.



3) In the *Microscope* tab, make sure the sample tilt is the same as in UI and the check mark is visible.

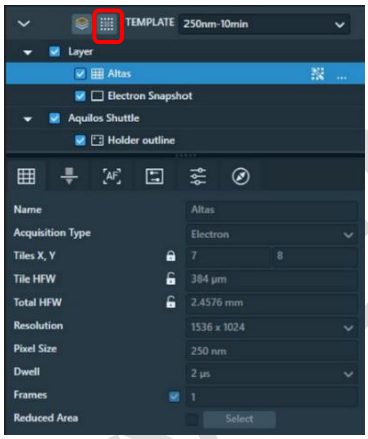


4) Take a snapshot of your grid. This will take a low-resolution image of your grid.

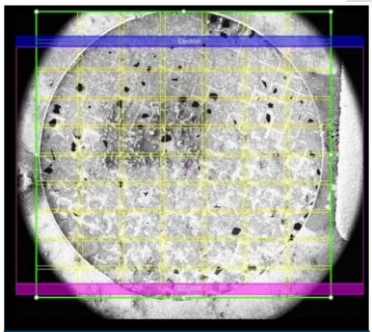


5) Right-click in the Maps Electron view window and press *Add Tiles Here*. Or click the below icon to *Add Tiles*. You can also adjust the parameters for the tile set. We recommend using:

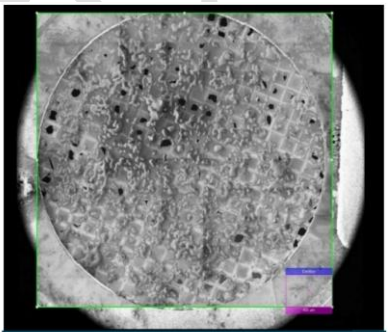
Pixel size: 250 nm;
Resolution: 1536 × 1024;
Dwell time: 2 µs



6) Adjust the tiles so they cover your grid. Click *Run*.

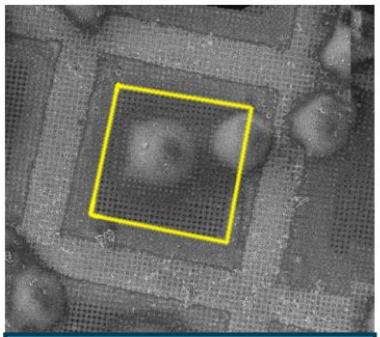


(A) Snapshot with overlayed tile set

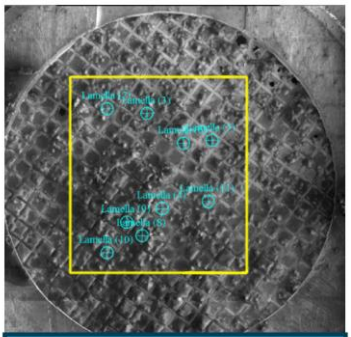


(B) Finished tile set

7) Choose your lamella sites by finding a suitable cell, right-click on the cell and select "Add Lamella Site Here".



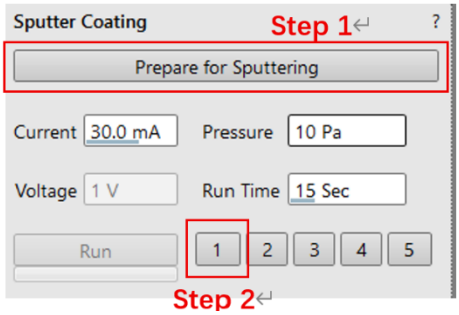
(A) Good square



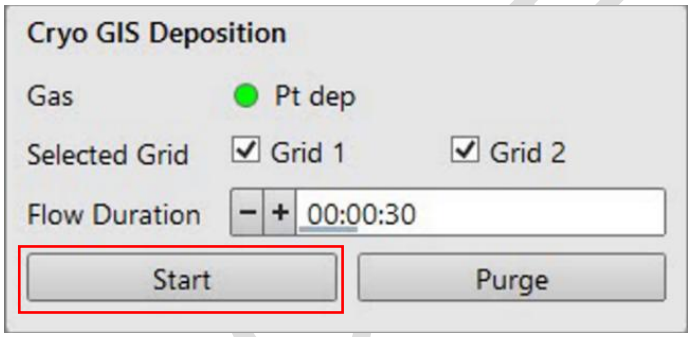
(B) Usable areas for lamella site selection on grid

GIS coating

1) Before GIS, you can optionally do sputter coating to enhance conductive.



2) The *Flow Duration Time* is **30s**.
3) Start GIS coating.

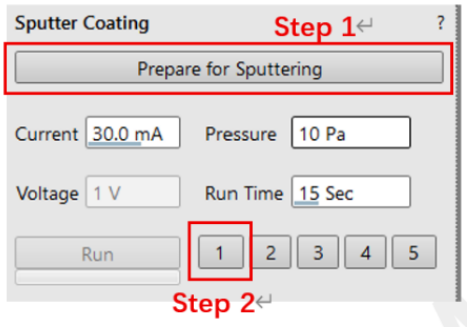


4) Second Sputter Coating after GIS (optional)

Current: 30 mA

Pressure: 10 Pa

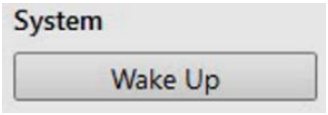
Run Time: 15 s



AutoTEM Cryo lamella milling

Wake up the ion beam before running AutoTEM. Please follow these steps at xT software.

i: Click the ***Wake Up*** button and wait until fully woken up.

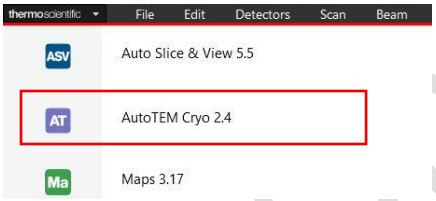


ii: Set the electron beam and ion beam.

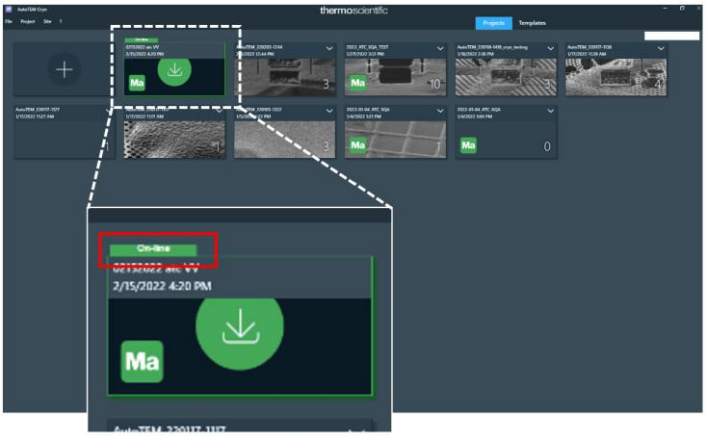
	Voltage	Current
E beam	2.00 kV	13 pA
Ion beam	30 kV	10 pA

Open AutoTEM software

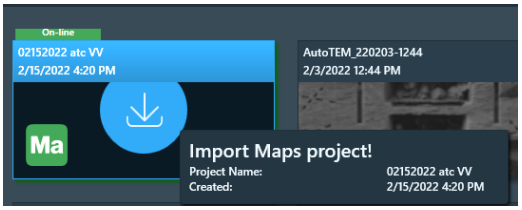
1) Open the **AutoTEM Cryo**



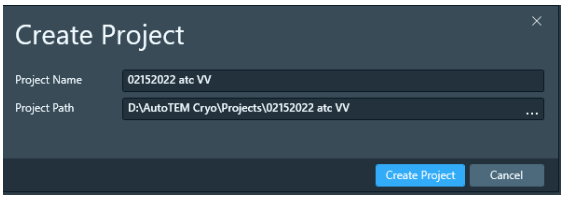
2) Your current project linked to **Maps** is marked green with the "***On-line***".



3) If you hover above the project, ***Import Maps project!*** text will appear. Click the project once.



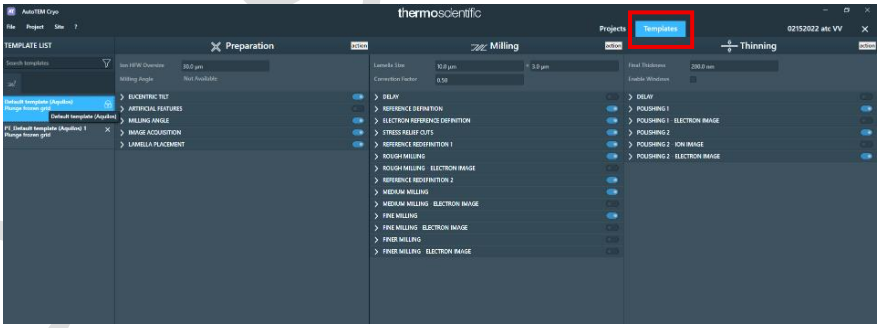
4) Create the new project, fill in the *Project Name* and select the ***Project Path***.



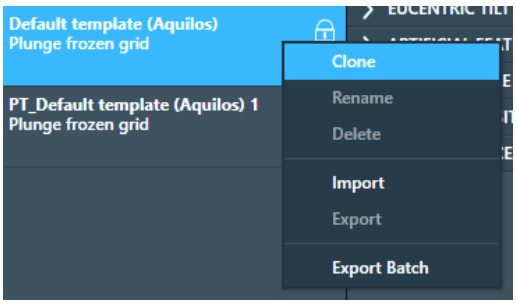
5) **AutoTEM Cryo** will load your lamella sites from Maps. These sites will appear in the ***Site list*** panel on the left.

AutoTEM Cryo template

1) Open the ***Templates*** tab on the top panel.



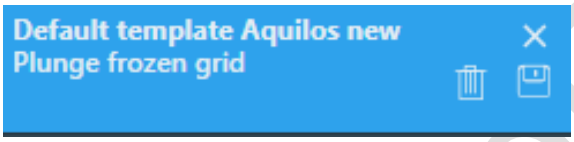
2) The ***Default Template*** is locked. To be able to adjust the parameters, a new template must be created: right-click on the default template and click ***Clone***.



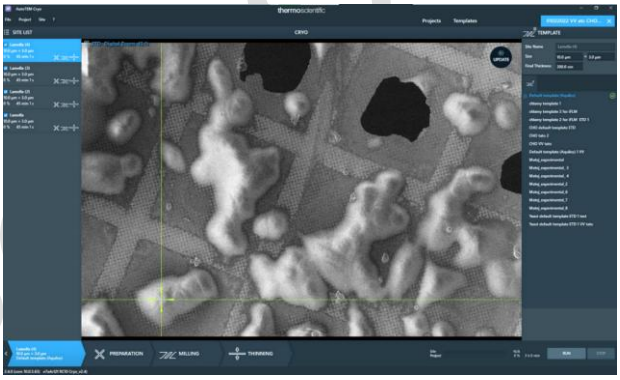
3) The *Create template* window will appear. Write your new template name and click ***Create Template***

The new template will appear in the list and after you select it you can adjust the parameters as you wish.

4) After the template adjustments are finished, click on the ***Save*** icon near the template name. For deleting a template, click the ***Trash*** icon.

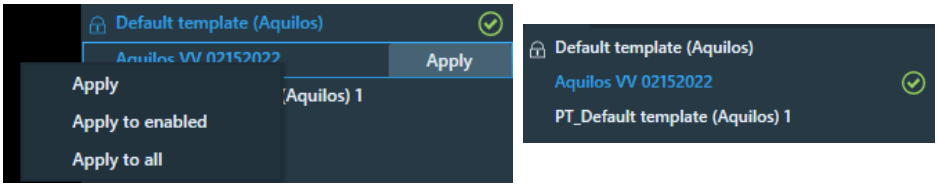


5) Return to your project click on its name in the right top corner.
6) There are four main tabs at the bottom panel from left to right: ***Site information***, ***Preparation***, ***Milling*** and ***Thinning***.
7) Click ***Site information***. The list of the templates will be shown at right panel.



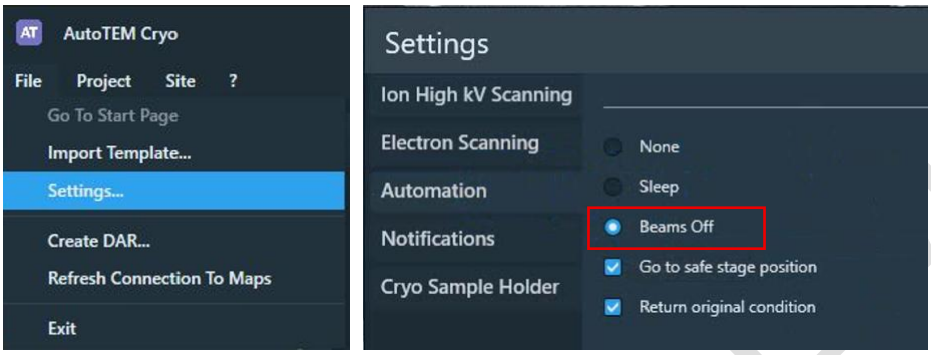
8) To select the template you'd like to use, right-click on its name, and select:
   a) ***Apply*** – the template will be applied for the lamella currently selected.
   b) ***Apply to all enabled*** – the template will be applied to all selected lamellae.
   c) ***Apply to all*** – the template will be applied to all lamellae: selected, and unselected.

After the template is applied, the green checkmark will appear next to the name.

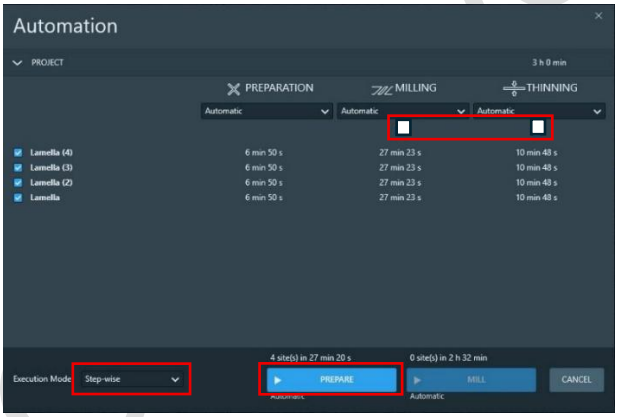


Auto lamella milling

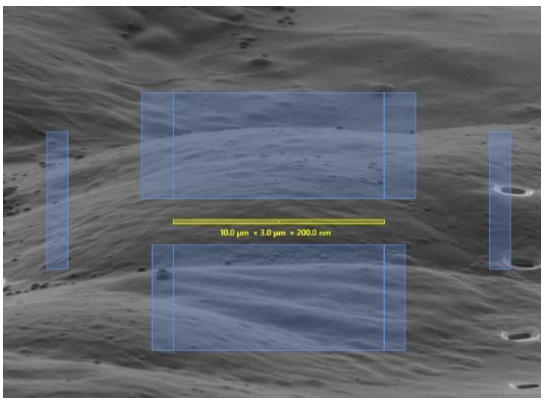
1) If you plan to mill overnight and will continue to use ion beam on the next morning, check "Beam off".
   If there are very few targets on your sample and milling will be finished before midnight, in this case you should choose "Sleep".



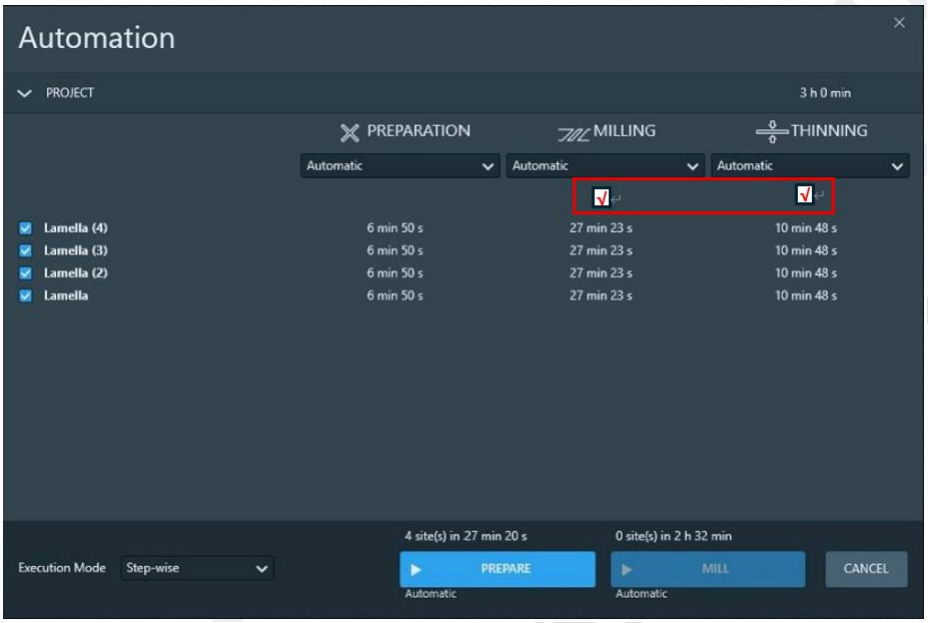
2) Click ***Run*** in the bottom right corner. The *Automation* window will open. Use "step-wise", uncheck milling and thinning.



3) Click ***Prepare*** at the bottom and the preparation will start.
4) After the *Image Acquisition* step is finished, **AutoTEM Cryo** will prompt the user to place the lamella graphics.



5) After the *Preparation* is finished, click ***Run*** in the bottom right corner. The *Automation* window will open. Select the lamella sites you'd like to mill. Mill the lamella to around 180nm thick.



Manual fine thinning

You can further mill the lamella via manual operation.

For each lamella:

(1) In AutoTEM, right click on the lamella target and "Go to thinning position".

(2) Center the lamella on both SEM and Ion view windows.

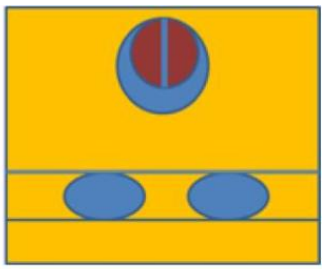
(3) Use as high as possible magnification to see the whole lamella at ion beam window.

(4) Use **CCS pattern** (), **voltage: 30kV, beam current: 10pA or 30pA**, optionally 0.1 to 0.3 over-tilt, to fine thin the lamellae and follow the operation steps. During the fine thinning, keep monitoring the SEM image by pressing F4 shortcut. Once the GIS layer is almost consumed, or the lamellae has a little crack, stop thinning immediately.

After experiment

(1) Prepare the LN2 and cool down the preparation pot.

(2) Unload your samples, transfer it to the preparation pot and then to your storage dewar. After taking out your sample from the shuttle, counterclockwise the screw to close the shuttle and put the shuttle on the heat plate of controller station. Put the preparation pot into oven.



Closed

(3) Put the transfer rod back to SEM airlock, lock the push-pull rod and open the valve of transfer rod (as shown in the image below). This will keep the transfer rod free of moisture.

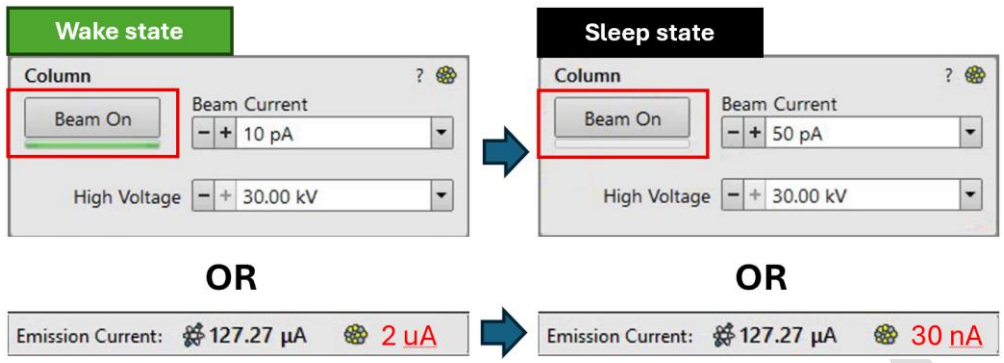
(4) Take out the heater exchanger and set N2 flow rate.

a) If the experiment is finished before 5:30pm, keep the gas flow at **190sccm** for 0.5 hour. CryoEM staff will turn off the gas flow before leaving.

b) If the experiment is finished after 5:30pm, set the gas flow **50sccm** overnight. CryoEM staff will turn off the gas flow on the next day.

(5) **Make sure the Ion beam is in SLEEP status. If not, click the sleep at xT software. If you forget to make the Ion beam sleep, we will charge you extra for the Ion beam overtime usage time.**

Tips: how to check if the ion beam is in sleep state?



(6) Turn off the main switch of the nitrogen gas near the preparation pot.

(7) Turn off the power of the controller station which connects to the preparation pot

(8) Fill in logbook.

(9) Pour off the remaining liquid nitrogen of 4L dewar and place back the dewar into the sample preparation room.

(10) Clear the FIB table.