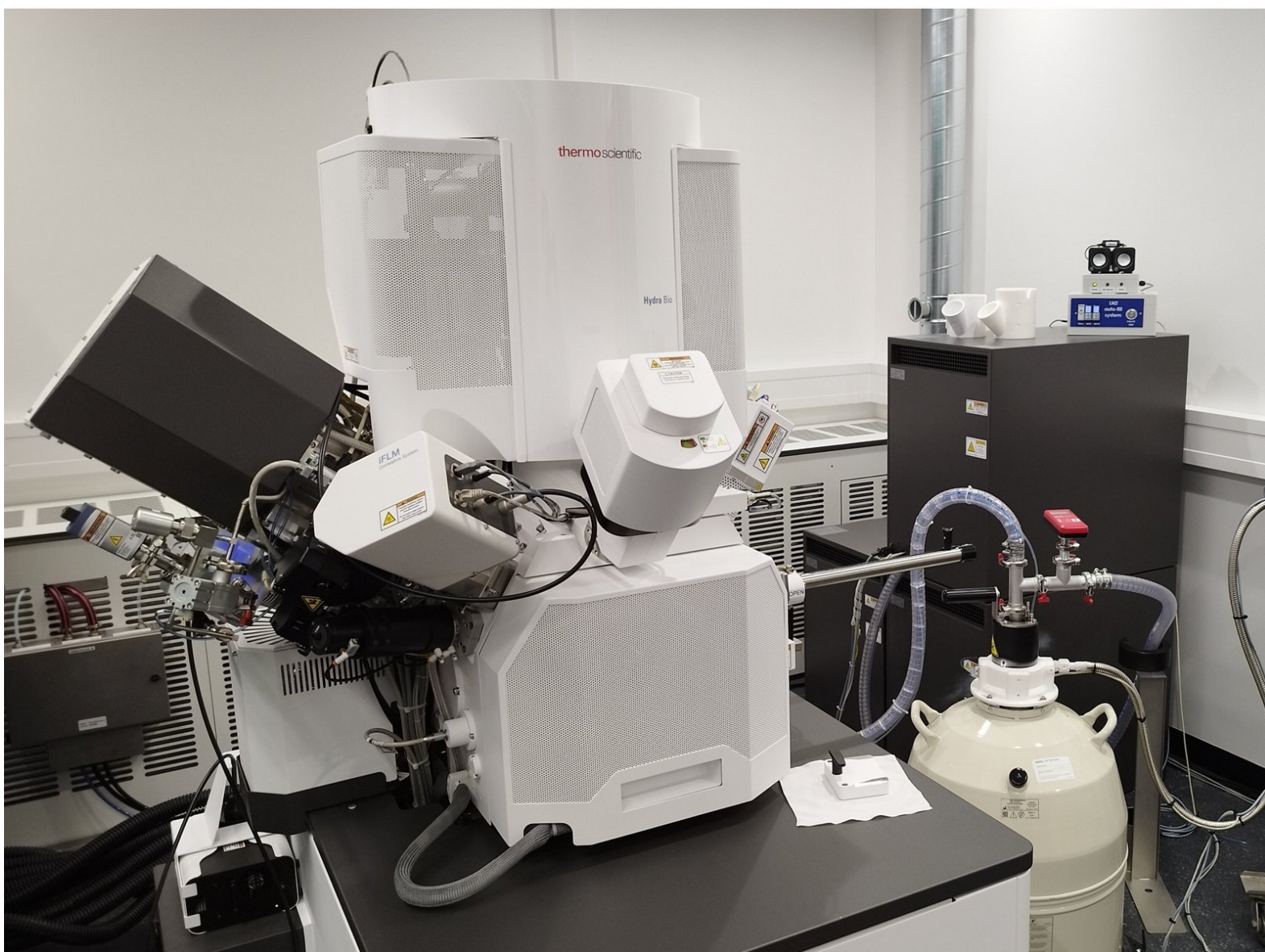
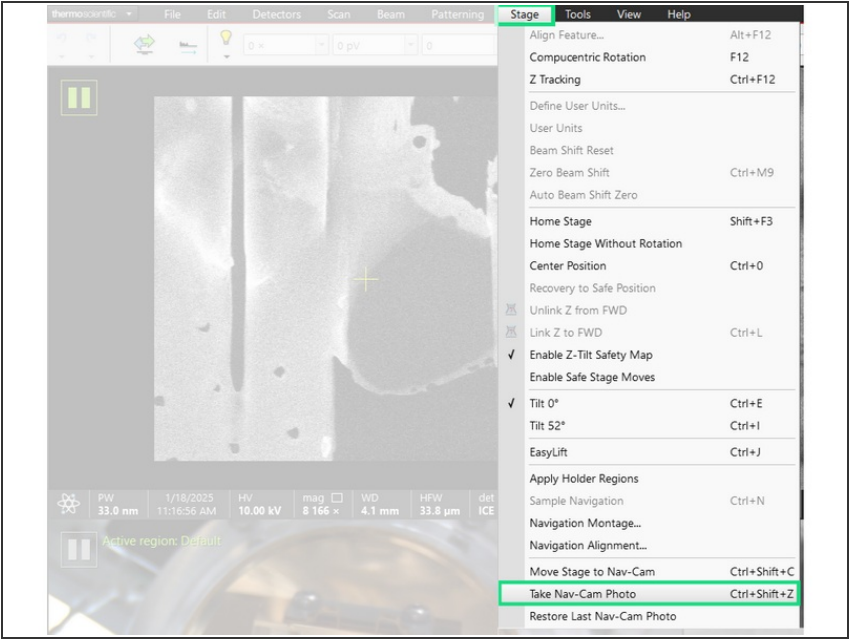


FIB-SEM - TFS Hydra Bio - Automated lamella milling

Written By: z mbstaff

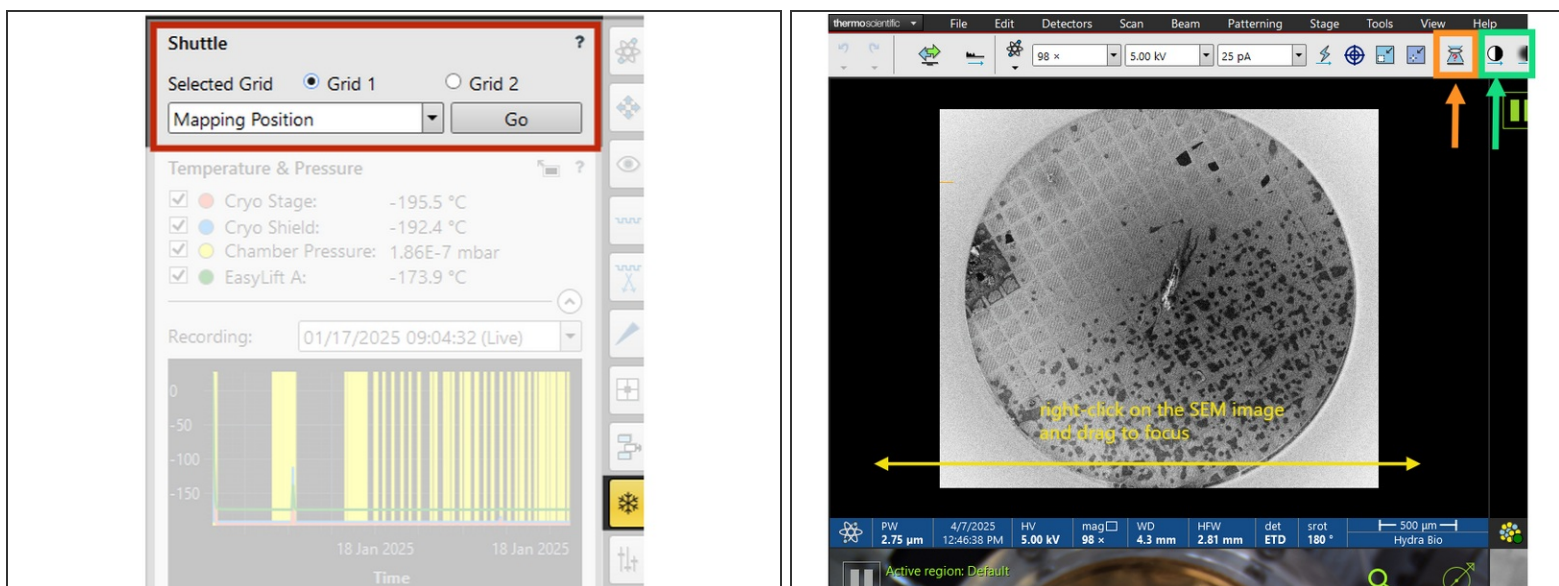


Step 1 — Take Nav-Cam image (optional)



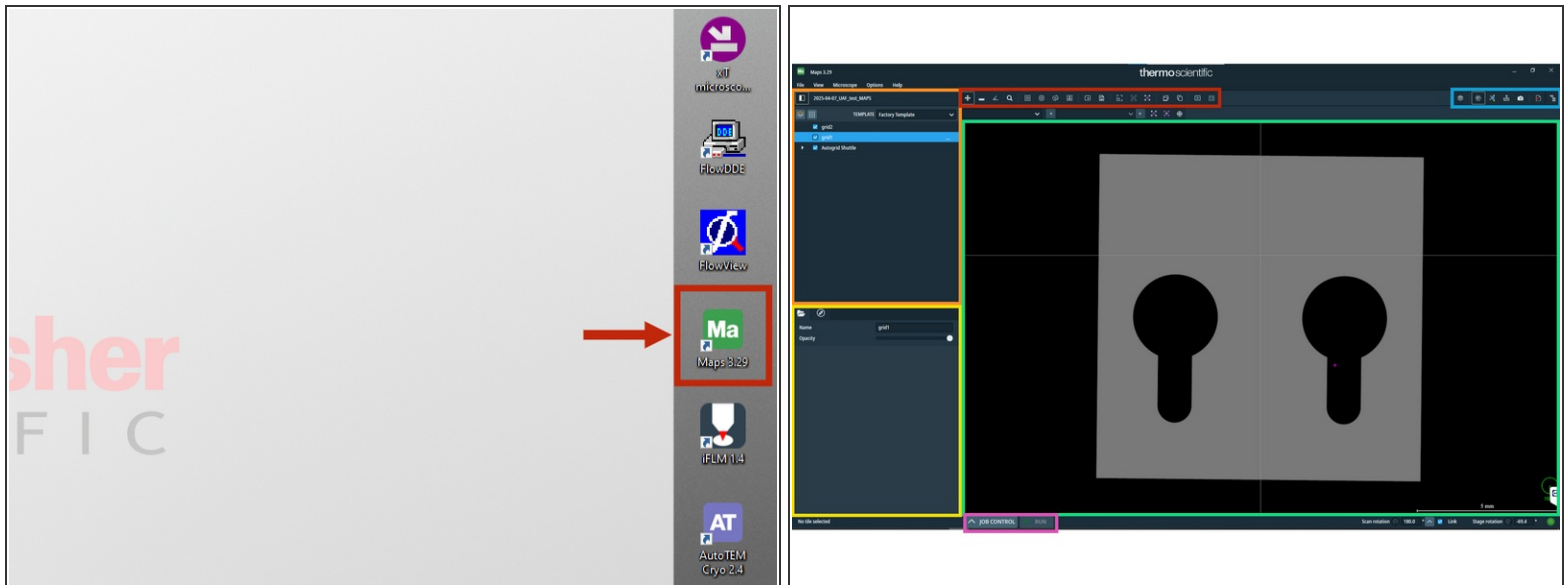
- You can take an image of the shuttle inside the chamber with the Nav-Cam. This can help you to recognize your samples on the CCD, SEM or FIB images, but it is not necessary for the AutoGrid shuttle.
- In the xTUI window select **Stage > Take Nac-Cam Photo**.
- The microscope will automatically move to the Nav-Cam position, acquire the image and move back to the initial position.

Step 2 — Link Z-height to Working Distance



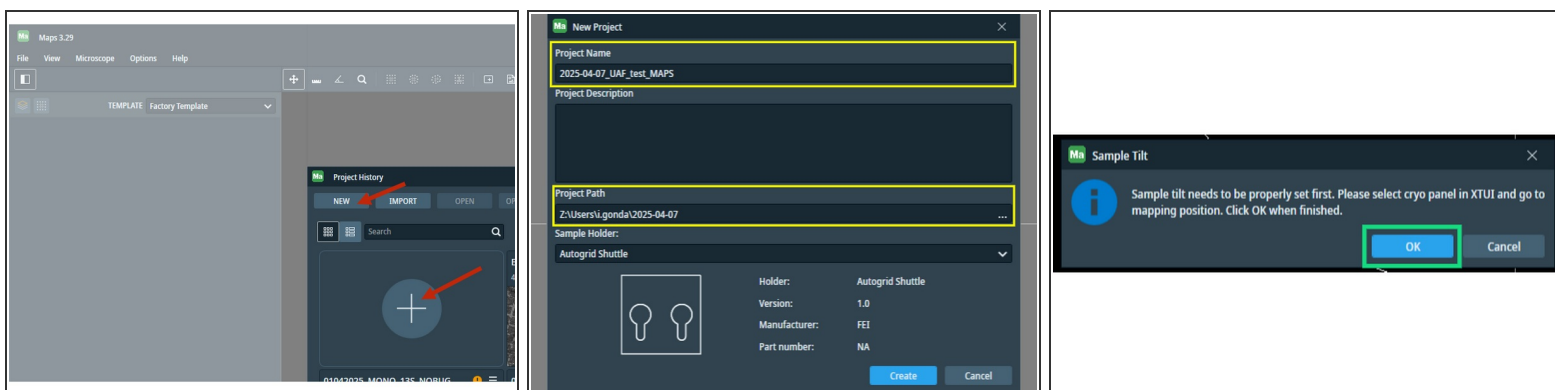
- Go to **Mapping Position** on **Grid 1** or **2**. This will move the respective grid perpendicular and centered to the electron beam.
- **Zoom in and focus** on the surface of the grid **either** with the **auto-functions**...
- ... **or manually** by right-clicking on the SEM image and dragging the mouse vertically.
- Link the current Z height to the working distance.
- ① *Cross-check if the stage Z position is ca. 4 mm.*

Step 3 — Overview of Maps software



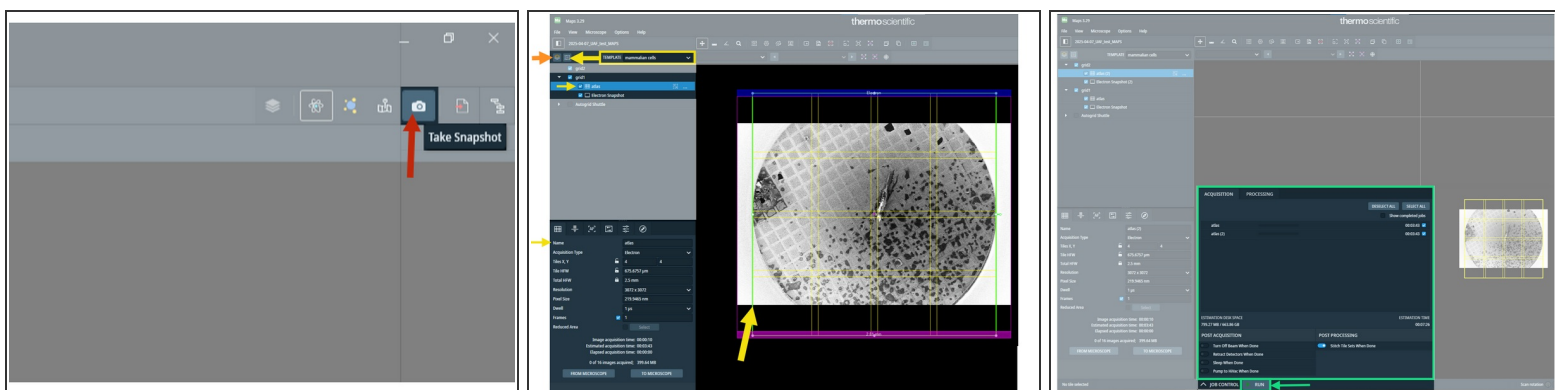
- ★ The icons of all necessary software can be found on the right side of the right-most display.
- ① Open Maps and familiarize yourself with the overview:
 - Layer management
 - Details and modification of currently selected layer element
 - Image window
 - Image acquisition buttons
 - Job Control and Run scheduled jobs
 - Actions on the image window (measurement, place tileset, export snapshot)

Step 4 — Create Maps project



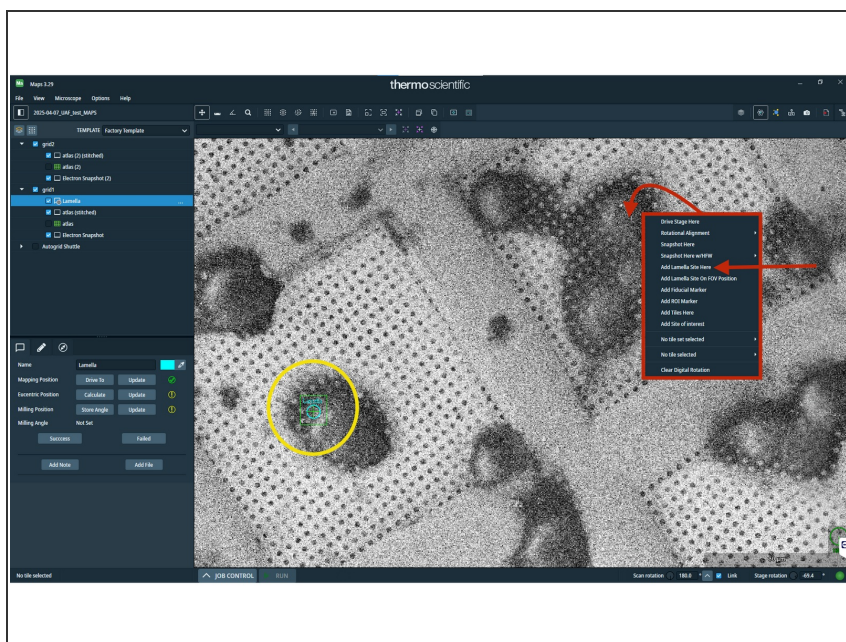
- Upon opening of Maps, create a new project for your session.
- Give a meaningful name to your project and set the right folder path.
 - ✧ **Project name:** *Date_ProjectID_SampleID_Maps* and **Project Path:** *Z:\Users\i.gonda\Date*
 - ⚠ Please save your data in the mounted folder **Z:\Users\i.gonda** . This disc is physically the Support PC D:\ drive and your data can be easily transferred from here.
- Make sure your shuttle is in mapping position, perpendicular to the electron beam.

Step 5 — Acquire Atlas overview of your grids



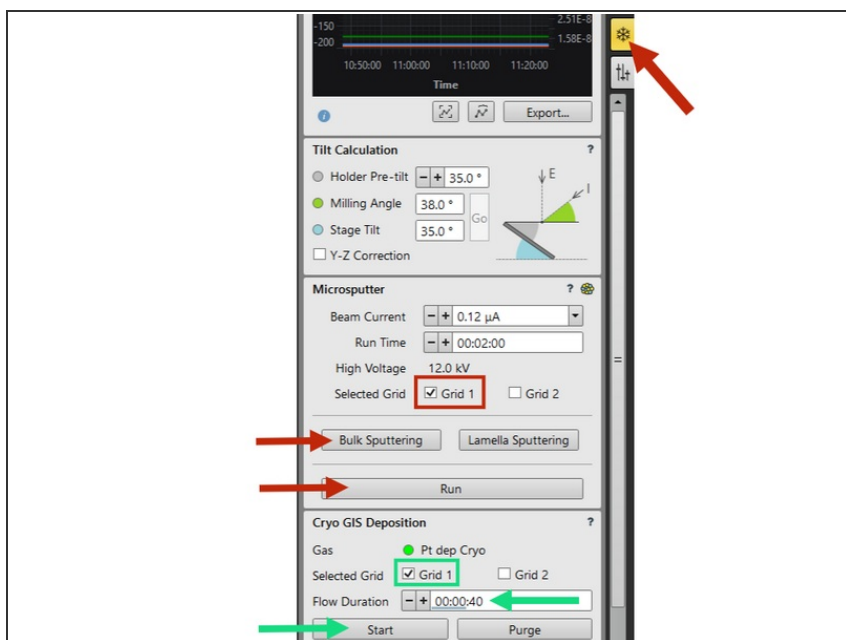
- Take a SEM snapshot of your grid, that will guide the placement of the Atlas tile-set.
- Add new layer for each grid.
- **Select "mammalian cells" template** from the drop-down list and **add tile-set** on your grid. Then **adjust tile-set position** on the previously taken SEM snapshot.
- ① Repeat these tasks for Grid 2 as well: change mapping position to grid 2, acquire SEM snapshot and add Atlas tile-set.
- Check in **Job Control** the scheduled tasks and hit **Run**.

Step 6 — Select lamella positions in Maps



- **Right-click** at positions, where you would like to mill lamellae.
- Select **Add Lamella Site Here**.
- Added lamella positions will show up with a turquoise marker.

Step 7 — Coat your sample with a protective organometallic layer.

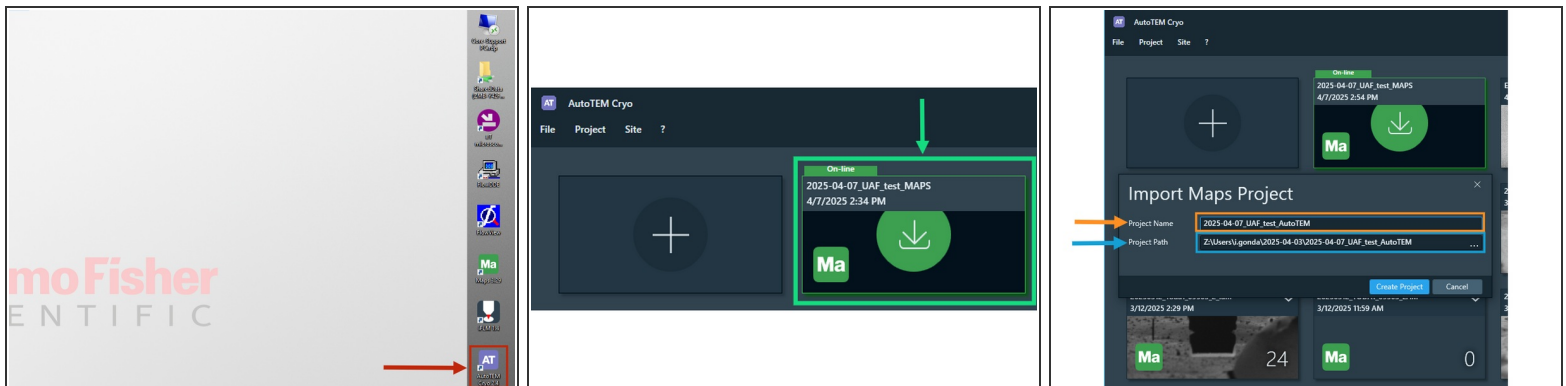


- ⚠ The GIS needle will not insert if the Z-height and Working Distance are not linked. If you missed this step, please visit [Step 2](#).
- First you need to create a thin layer of platinum over the sample by microsputtering. In the **Cryo** tab, **select the grid(s)** you wish to coat, choose **Bulk Sputtering** parameters and hit **Run**.

① After the Pt layer, you need to deposit a organometallic layer, that will protect the front of your lamella. This gaseous compound in precipitated on the cold surface from the Gas Injection System (GIS). Optimal times vary between devices and the sample; on this device it is typically 20-40 seconds.

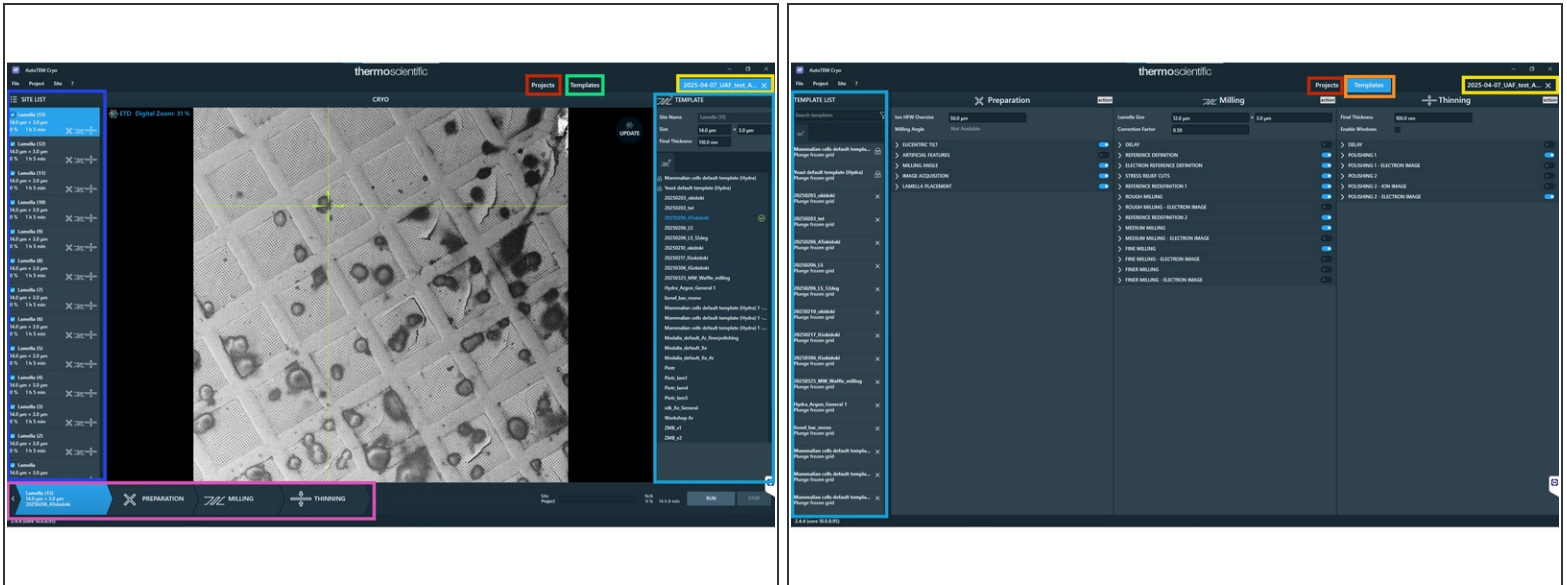
- **Select the grid(s)** you wish to coat, choose an appropriate **flow duration** and hit **Start**.
- Finally, coat your grid again with a thin layer of Pt using the **microsputter tab** (red instructions). This will reduce surface charging.

Step 8 — Open AutoTEM and create new project



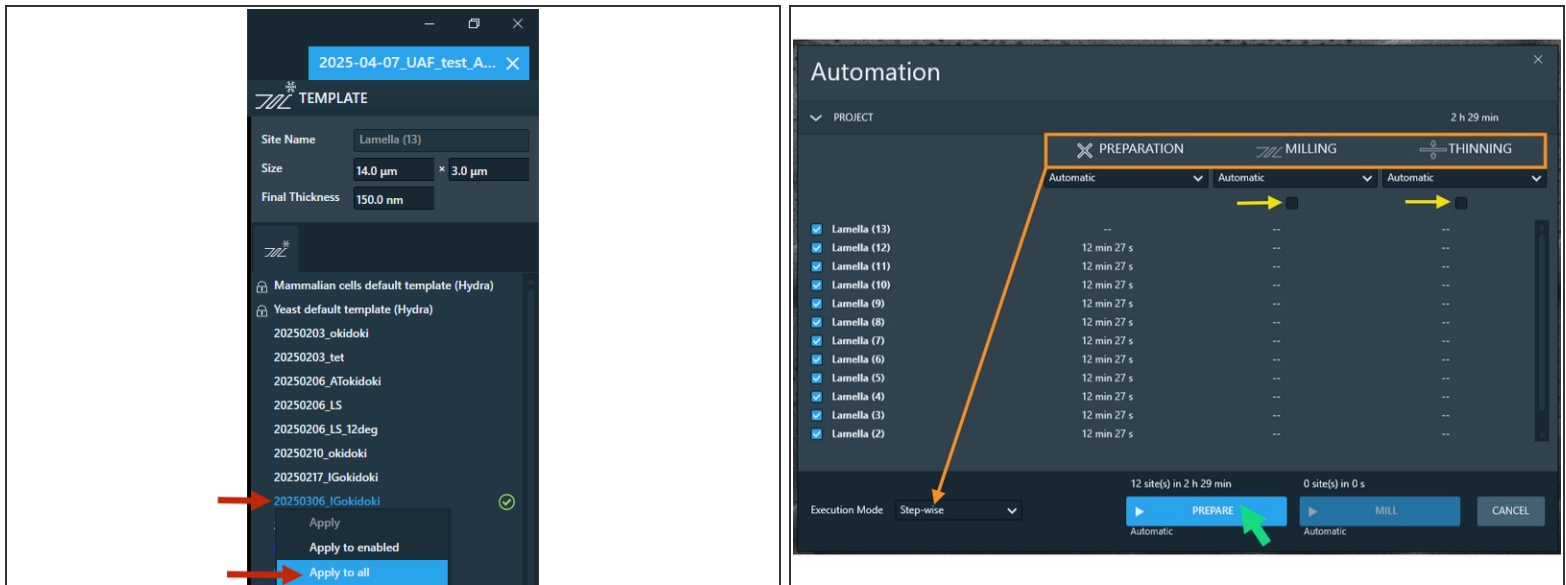
- Open AutoTEM. You find the shortcut on the right side of the right-most display.
- AutoTEM recognizes the currently **on-line** (open) **Maps project** and offers to import it and create a AutoTEM project from that.
- A good convention is to change the *"_Maps"* ending of the project to *"_AutoTEM"*.
- Make sure the AutoTem project is also saved in the same folder as your Maps project.

Step 9 — Overview of AutoTEM interface



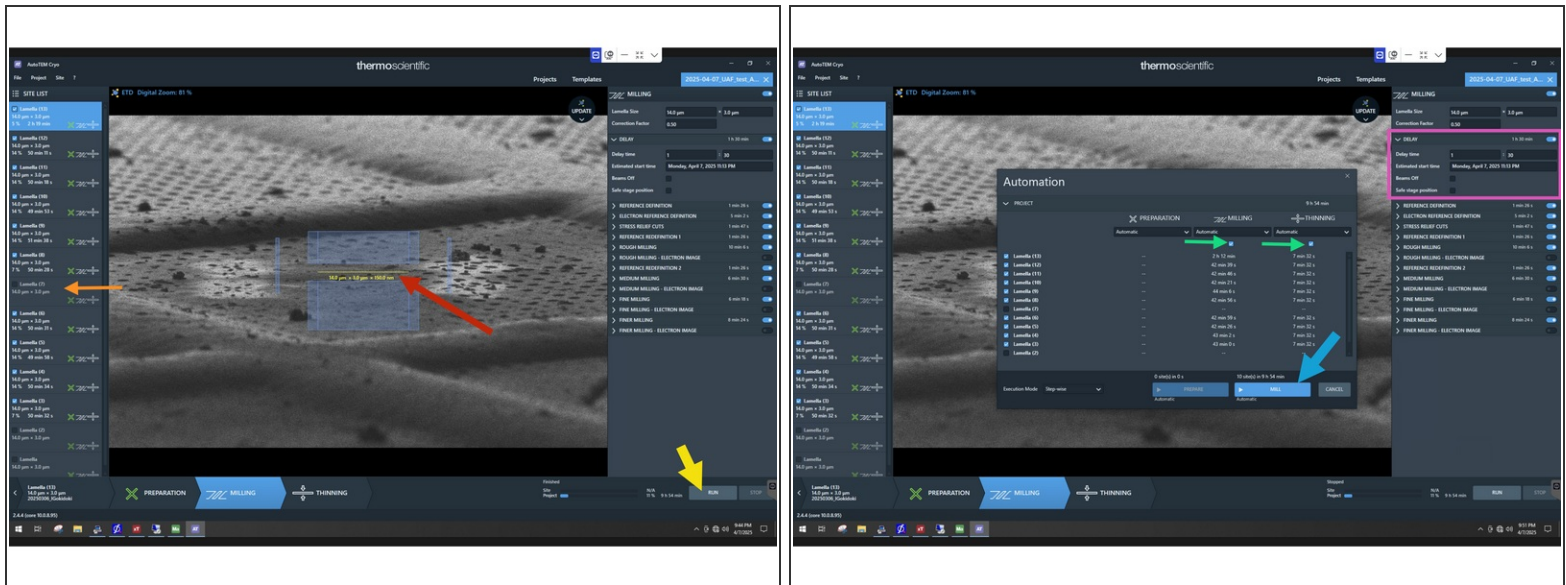
- Top bar: Project overview
- Top bar: Template editor
- Top bar: Access currently open project
- Bottom bar: Lamella preparation workflow
- Left sidebar: List of lamella sites (project view) or List of templates (template editor)
- Right sidebar: Available templates or Template editor

Step 10 — Prepare lamella sites for milling



- Apply your carefully adjusted template for the lamella sites. **Right click** on your template (right-side panel) and select **Apply to all**.
- Lamella preparation times are listed in 3 columns for the three steps: **Preparation**, **Milling** and **Thinning**. When setting up lamella make sure to execute **Step-wise** (NO Site-wise).
- For lamella site preparation, **untick** the Milling and Thinning steps. You will need to manually adjust and confirm the position of each lamella.
- Start site preparation with the **Prepare** button. It takes ca. 6-8 mins per lamella.

Step 11 — Finish site preparation and start automated milling



- **Adjust lamella position** and nominal lamella **thickness**. A nominal 110nm thickness corresponds to ca. 180nm thick lamellas)
- You may **untick** lamella sites, that you **don't want to mill** (due to time restrictions or bad grid square).
- Click Run to start milling.
- Tick on Milling and Thinning steps.
- **Adjust delay** on the first lamella on the top of the list. Ideally, you want to unload just after the milling has finished.
- ⚠ **Untick Beams Off and Safe stage position**. Otherwise, the milling starts immediately. (This is a software bug, guide will be updated, if bug is fixed.)
- Hit **Mill** to start milling.