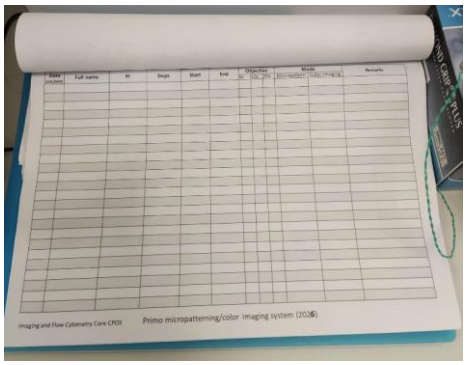
Primo Micropatterning System
Standard Operation Protocol

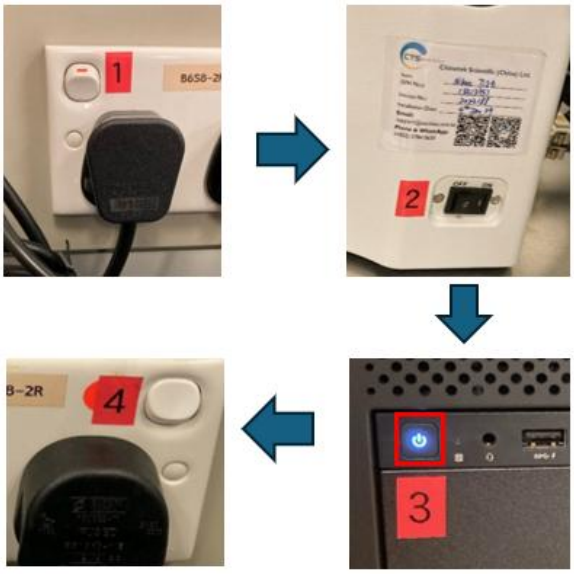
Contents

A. Turn on System

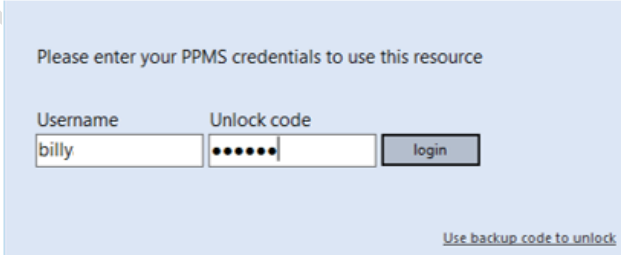
Please sign the log sheet before switching on the system.



1. Switch on main power control **1,** wait for at least 5 seconds before the next step.
2. Switch on the microscope controller **2,** wait for at least 10 seconds until the stage stops moving before the next step.
3. Turn on computer power **3.**
4. Turn on the power switch.



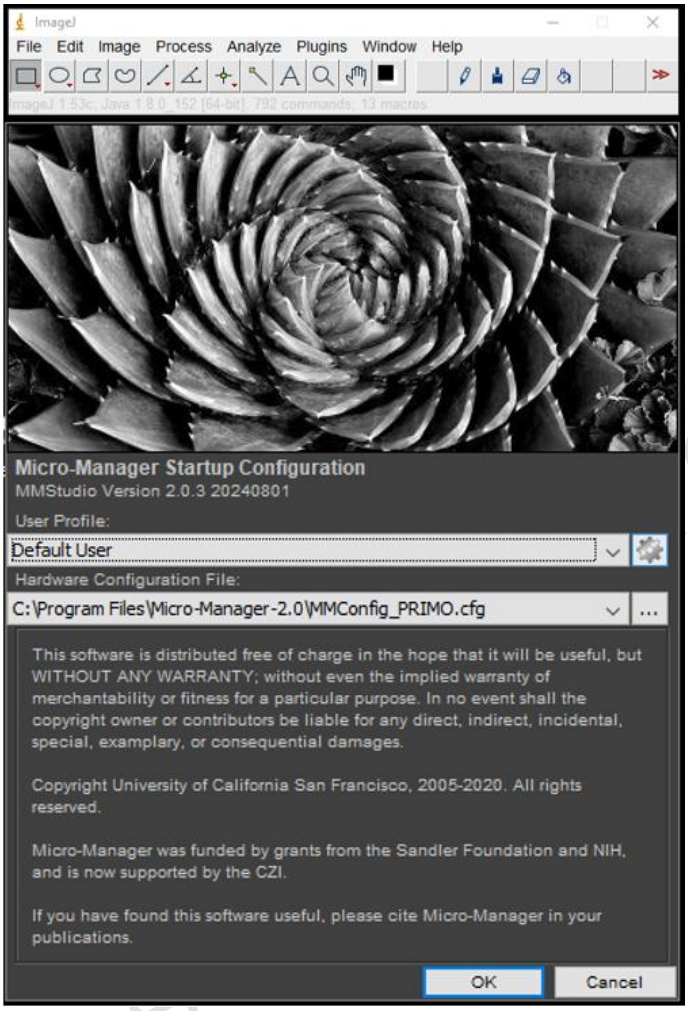
5. Click to log in to the "**Primo USER"** account at the startup screen with the password attached to the screen bottom.
6. Log in to your PPMS tracker.



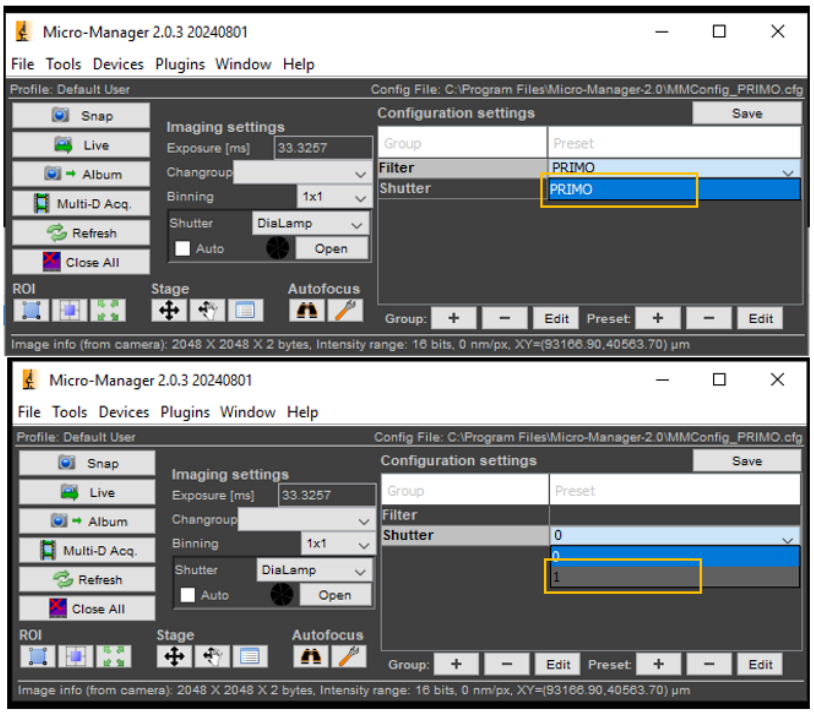
7. Double-click the Micro-Manager icon on the desktop 

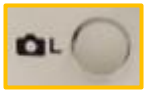
to start the software.

8. Click “OK” to initiate the Micro-Manager.

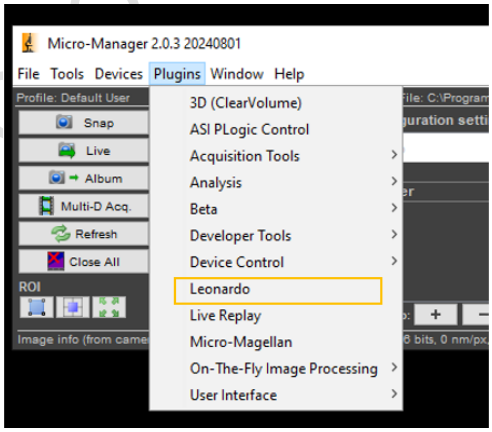


9. Select the filter as "**PRIMO**" and Shutter as "**1**"

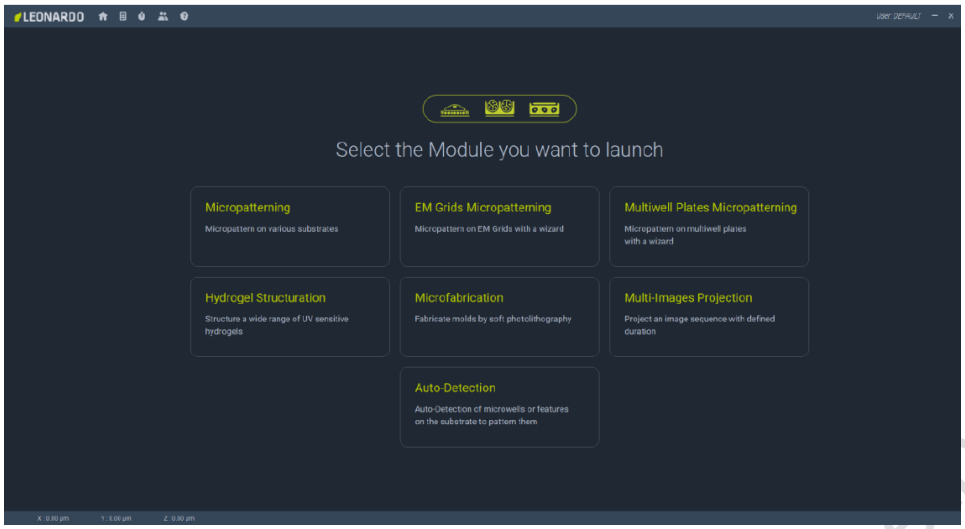


10. Press the  button on the front side of the microscope body to ensure the light path is selected for the left camera.

11. Click **Plugins** in **Micro-Manager** window and select "**Leonardo**" in the dropdown list.



12. The software Leonardo is initialized as follows.



B. Micropatterning Module

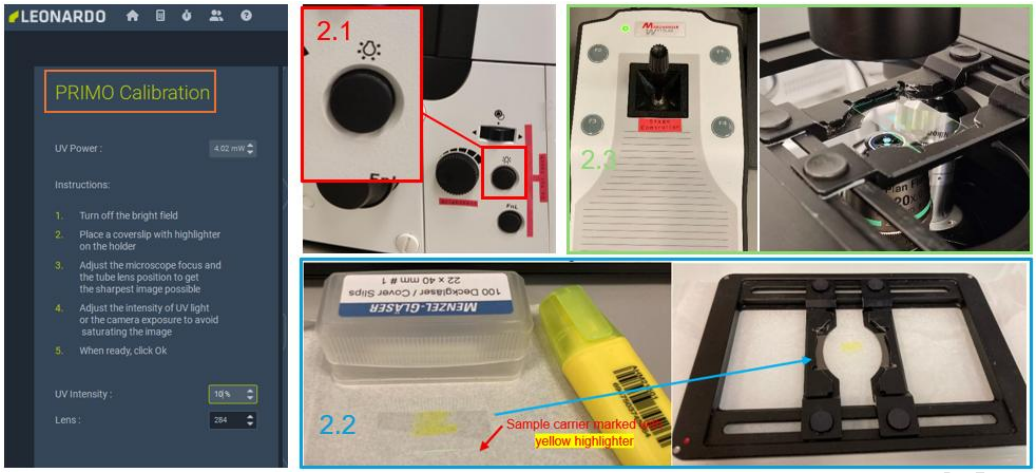
Click "**Micropatterning**", and the Calibration Wizard is displayed.

B1. Calibration

1. In the Calibration Wizard, under the "**Select Objective**", choose the objective to be used. Click  to perform **PRIMO CALIBRATION**.

Notes:
*** A 20x objective lens is recommended for micropatterning on a TEM grid or glass. ***
PRIMO CALIBRATION should be performed prior to each experiment using an identical sample carrier (e.g., a coverslip or confocal dish from the same manufacturer as the experimental sample).
CAMERA CALIBRATION calibrates the angle between the camera and the stage, should only be performed if the camera, stage, or microscope body is changed. Do not perform CAMERA CALIBRATION unless instructed. If you accidentally performed camera calibration, please contact Imaging staff for verification.

2. Follow the "**PRIMO Calibration**" instructions to do the calibration:
   2.1 Turn off the brightfield by pressing the button on the left side of the microscope.
   2.2 Mark a small area with a yellow highlighter on the carrier for calibration purposes.
   (**Notes:** the calibration sample carrier should be from the same manufacturer as the experimental sample.)
   Fix the sample carrier on the holder with highlighter marks facing upwards. Mount the holder on the stage.
   2.3 Move the stage with the joystick to move the yellow highlighter area directly above the objective lens.



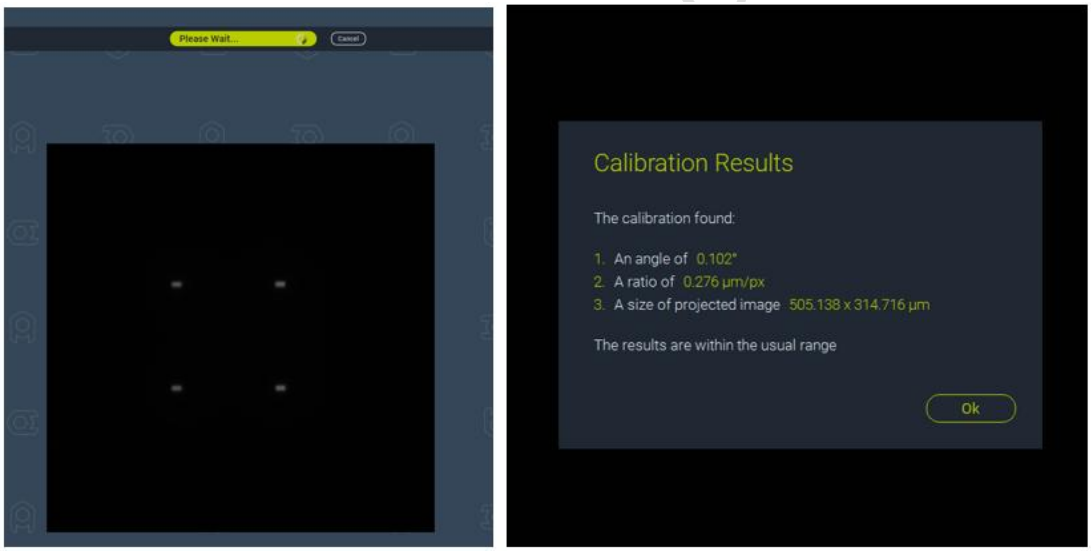
3. Reduce the **UV intensity** from 100% to 20%. Check the correction ring on the 20X lens to ensure it matches the thickness of your sample carrier. For example, if your sample is mounted on a No.1.5 coverslip, set the correction ring to 0.17mm. Adjust the focus knob on the left side of the microscope body until the "**TAKE CARE OF YOUR CELLS**" text is sharp and in focus.

(**Notes:** If the "TAKE CARE OF YOUR CELLS" text is not sharp after adjusting the focus knob and correction ring, please contact Imaging staff for assistance.)

4. Further adjust the UV intensity and camera exposure to avoid image saturation.

(**Notes: Don't adjust the LENS, which will be adjusted automatically after primo calibration is finished.**)

5. When ready, click **OK**. The software will do Primo Calibration automatically, and the calibration results will be displayed. Click "**Ok**" to apply the calibration.



B2. Set ROI and Pattern

1. **Set ROI**

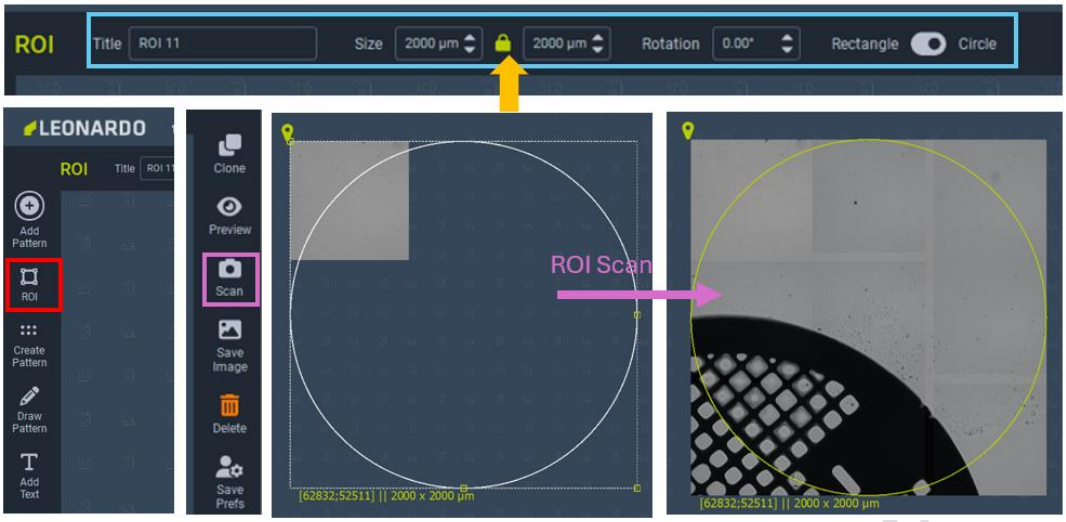
After the calibration, the micropatterning interface will be displayed.

1.1 Turn on the brightfield by pressing the button on the left side of the microscope.

1.2 Click the "**ROI**" icon to set an ROI.

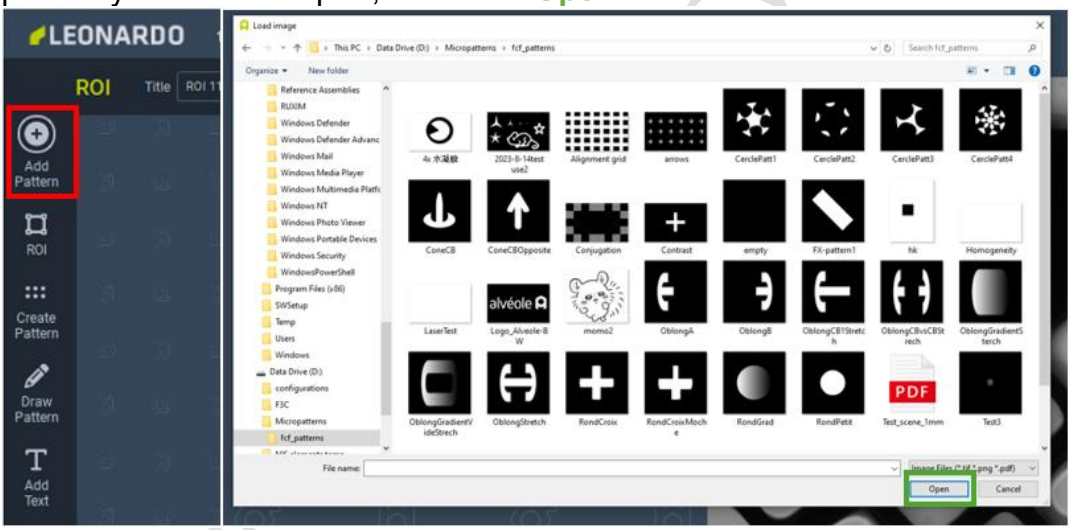
1.3 The **name**, **size**, **rotation,** and **shape** of the ROI could be edited. If the **Lock icon** is on, the horizontal and vertical dimensions of the circle or rectangle are locked to stay proportional. If unlocked, its horizontal and vertical

dimensions can be adjusted independently. Click **Scan** to have the preview.



2. **Set Pattern**

2.1 Click "**Add Pattern**", browse the folder **D:\Micropatten\fcf_patterns**, select the pattern you want to import, and click "**Open**".

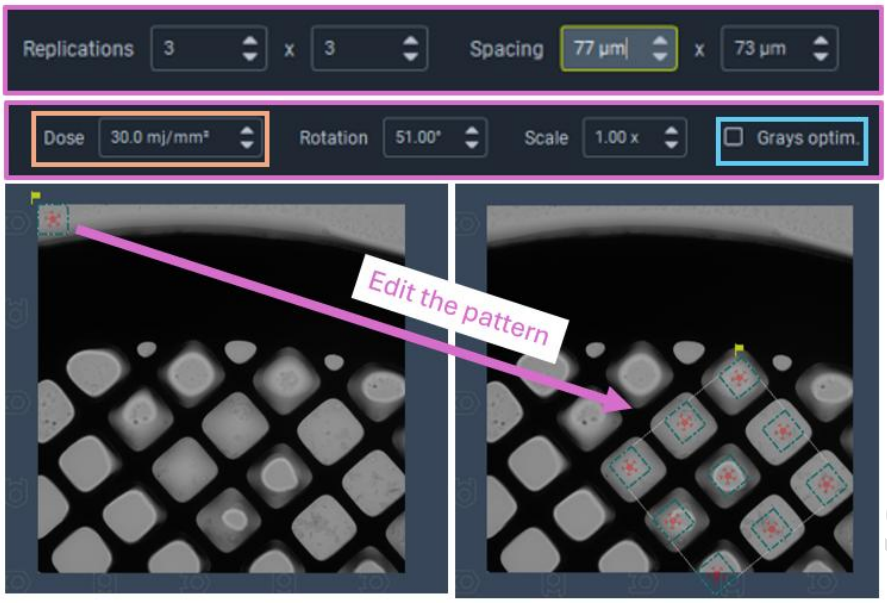


(***Notes: Other PNG, TIFF, or PDF images can also be imported for patterning.)***

Pattern size	Format
< 1.5cm	PNG, TIFF
> 1.5cm	PDF

2.2 Edit the pattern you imported. The **rotation**, **scale,** and **replications** could be edited and applied to the pattern. The **Dose** will depend on the passivation protocol, photo initiator, and substrate you selected. Please refer to your primo sample preparation protocol. A **Grays optim.** checkbox: If checked, the intensity of the patterning will be adjusted in order to optimize the patterning of gradients. If there are no gray pixels in the current image, the box cannot be checked. If the image

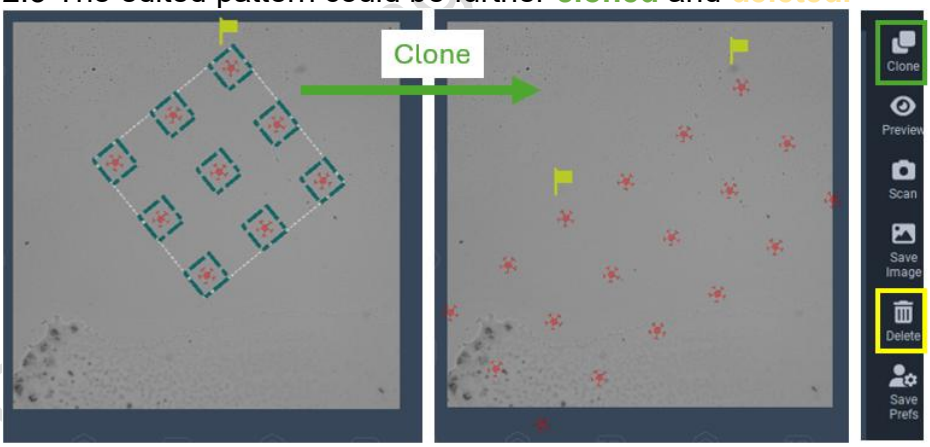
implies stitching (larger than one DMD), the box will be forcefully checked.



(Notes: Typical UV dose ranges are listed below. Dose settings are dependent on the PLPP or PLPP Gel concentration, substrate type, and sample preparation conditions. Users are required to apply dose settings as specified in the corresponding validated protocol.)

Photoinitiator	Laser power	Dose
PLPP	100%	1200-1800
PLPP gel	100%	30-60

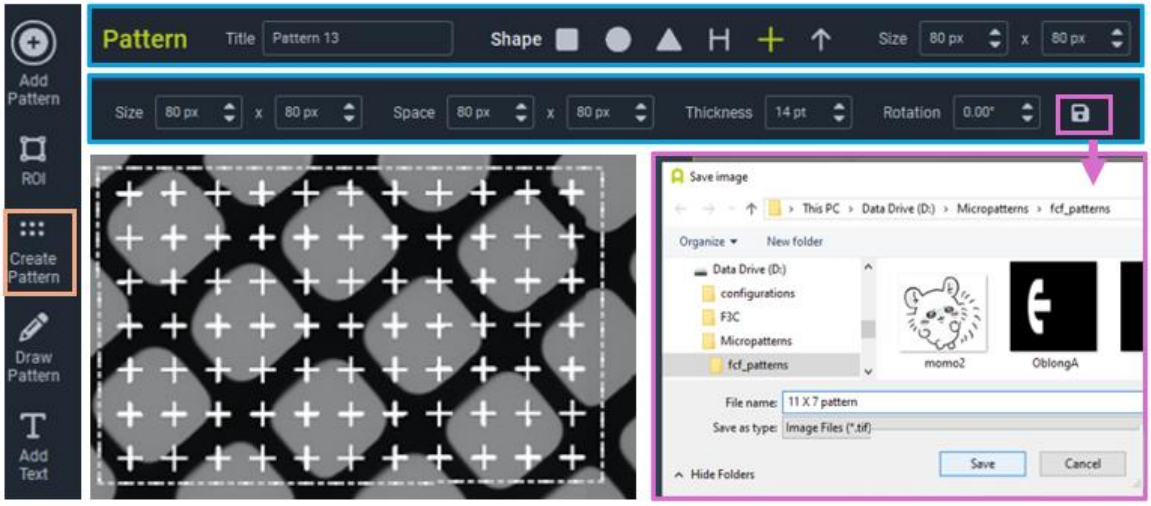
2.3 The edited pattern could be further **cloned** and **deleted.**



3. Create Pattern (Optional)

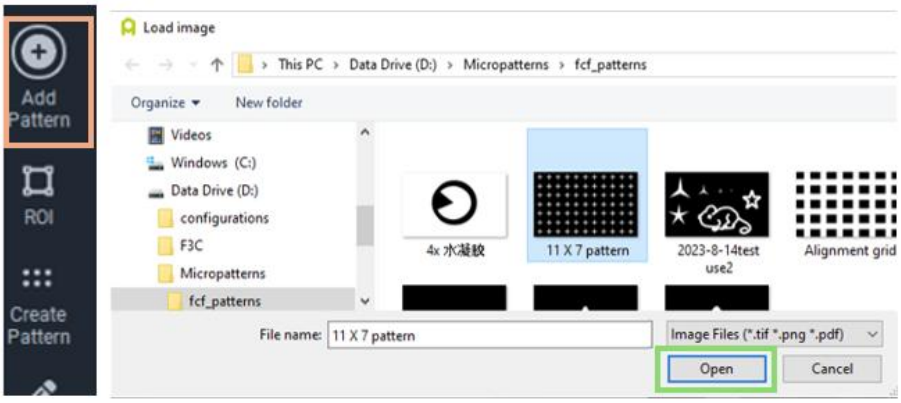
3.1 Arrays of basic shapes can be created by clicking "**Create Pattern**". Select the shape, then set the size, spacing, thickness, and rotation. After editing, click the

"Save" button to save the pattern.



(Notes: The Create Pattern button helps create arrays of basic shapes the size of the DMD directly within Leonardo, avoiding the need to use a third-party drawing software to create patterns. The shapes will be automatically adjusted to fit as many as possible within the DMD-sized area. A margin equivalent to half of the spacing will be put on the edges. Once the pattern has been saved, it can then be reused like a normal pattern.)

3.2 The saved pattern could be reused like a normal pattern. Click "Add pattern", select the pattern you saved, and click "Open". The pattern will be imported to micropatterning software. The following procedures are the same as part "2 **Set Pattern**" in this SOP.



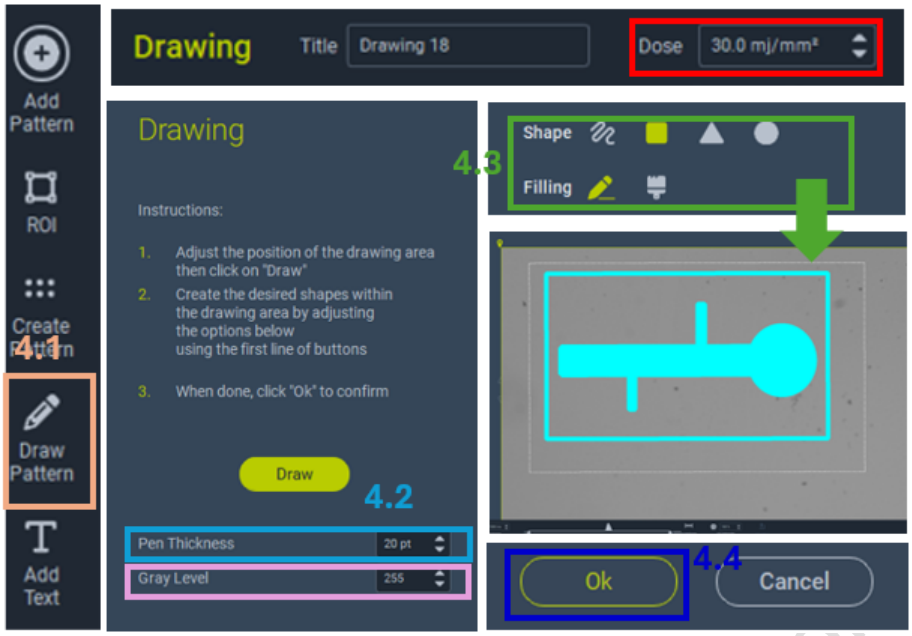
4. Draw Pattern (Optional)

4.1 Click "Draw Pattern" to open the "Drawing Wizard". In the Drawing Panel, you could rename the title and **Dose**. Refer to part "**B2 – 2 - 2.2**" in this SOP to set the Dose.

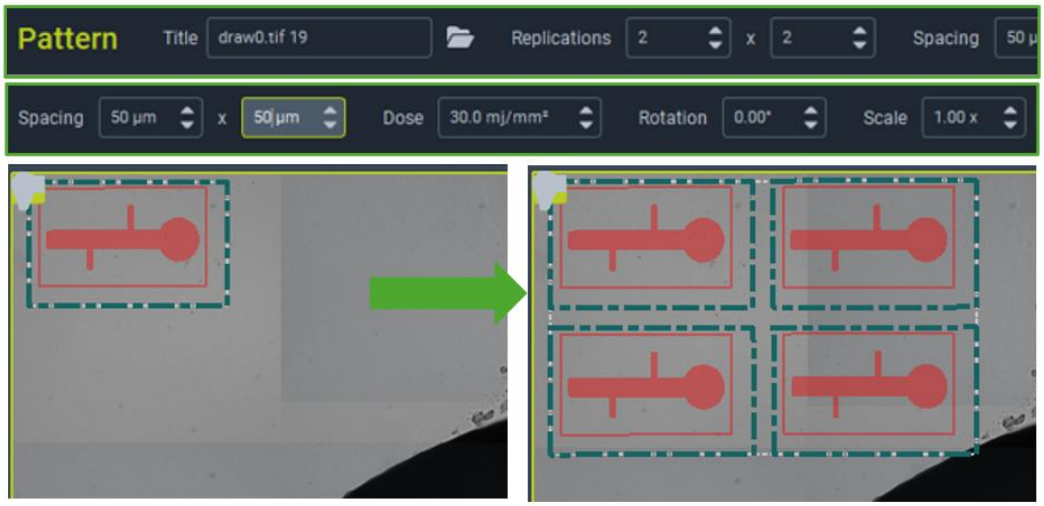
4.2 In the Drawing Wizard, set **Pen Thickness** to change the size of the line drawn. Set the Gray Level as the default, which only changes exposure and does not affect the Dose.

4.3 Press the "**Draw**" button. Select the **type of shape** you will draw and whether the shape is **filled or not**. Using the mouse, you can then directly draw a pattern.

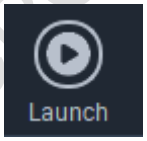
4.4 After the pattern is drawn, click "OK".



4.5 The drawn pattern could be used as a normal pattern. Following procedures the same as part "**B2 – 2 - 2.2 & 2.3**" in this SOP.



B3. Launch the patterning

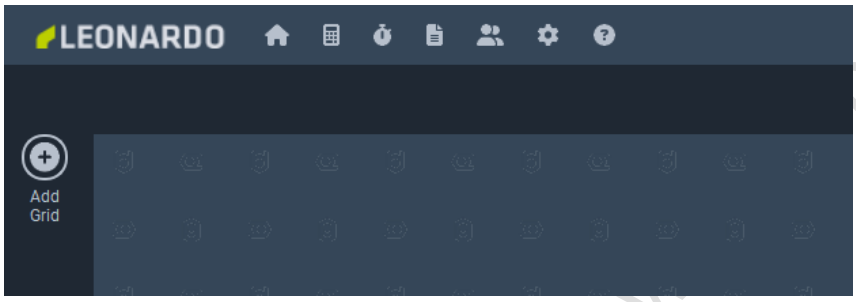


Click to start the patterning. Turn off the brightfield (BF) illumination lamp by pressing the button on the left side of the microscope body. While patterning is in progress, do not disturb the anti-vibration table or switch to the desktop or other interfaces.

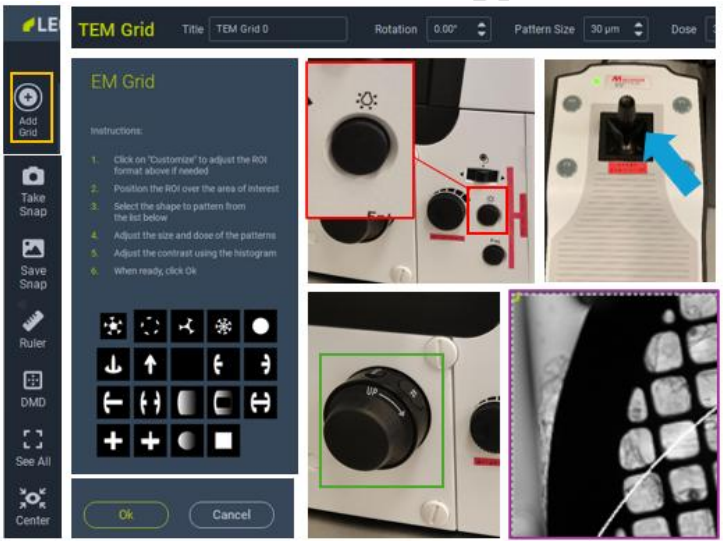
C. Patterning on EM Grid

1. Click "**EM Grids Micropatterning**" and the Calibration Wizard is displayed

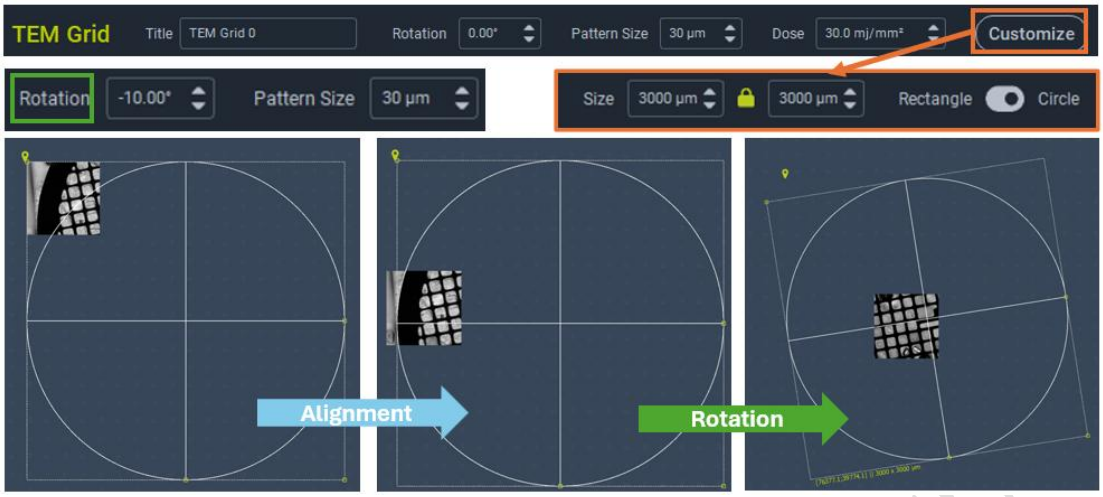
2. Follow the **B1** part in this SOP to finish the Primo Calibration.
3. After calibration is finished, it will launch the "**EM Grids Micropatterning"** interface.



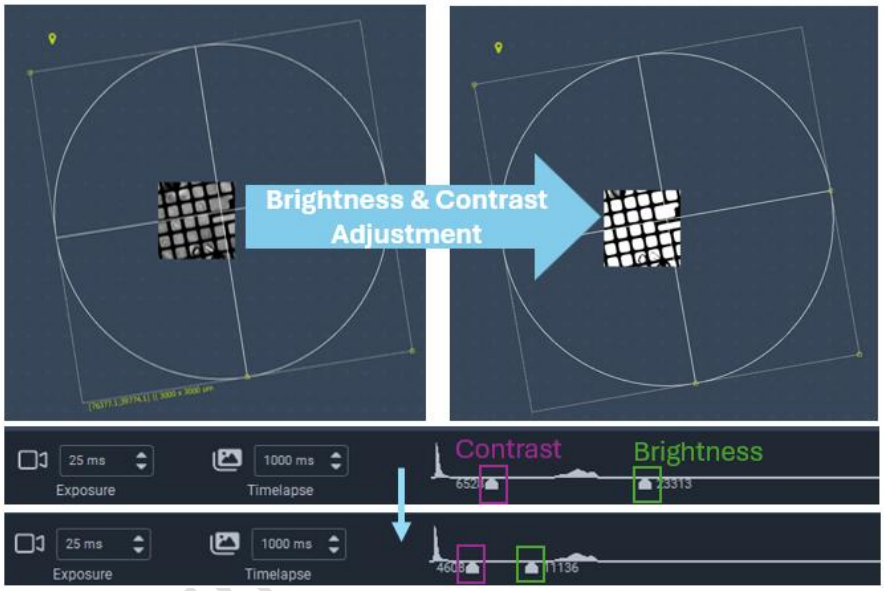
4. Click "Add Grid". The EM Grid Wizard launches, and the live view appears. Turn on the brightfield illumination using the button on the left side of the microscope. Use the joystick to move the stage until the edge of the EM grid is visible in the live view. Adjust the focus knob until the focus is sharp.



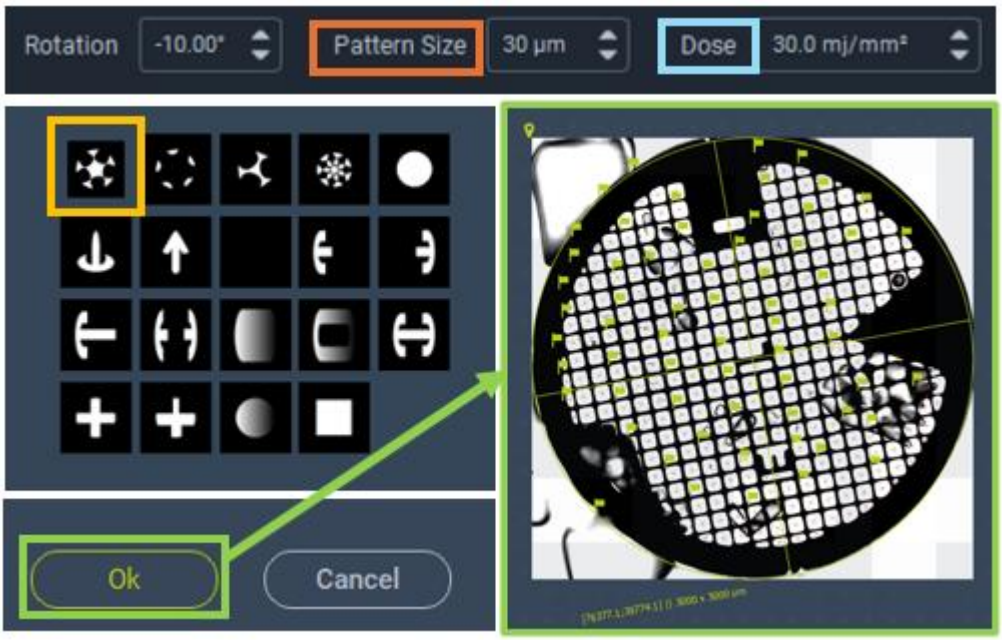
5. At the top of the TEM Grid Wizard, click **Customize** to adjust the ROI size and shape. After making the adjustments, use the mouse to drag the ROI so that it aligns with the edge of the TEM grid in the live view. Once aligned, move the live view to the center of the grid, then rotate the ROI to modify the orientation, typically to align it with the grid bars.



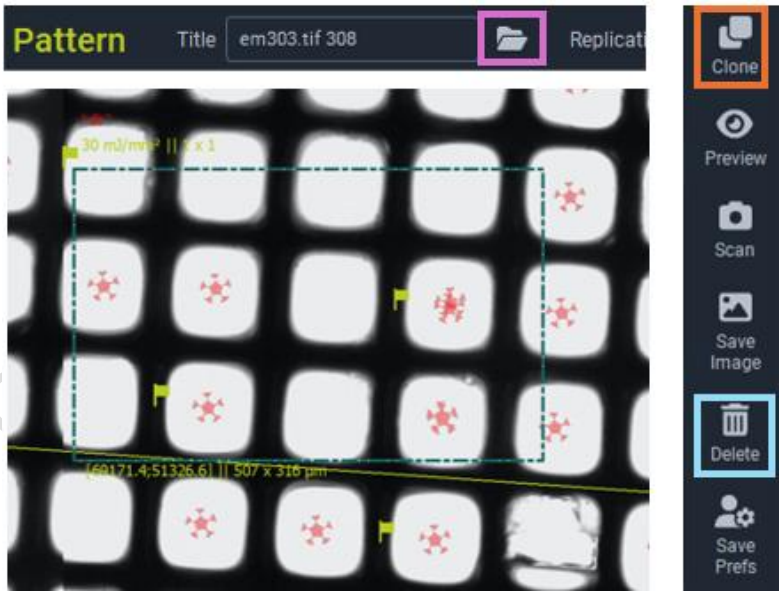
6. Adjust the brightness and contrast based on the live window view until the bar is sharp and clearly visible and the grid square is clean, free from obscuring dust or debris. This ensures optimal pattern assignment in the next step.



7. Select the desired **pattern** and define the **Pattern Size**. For the dose setting, refer to step B2.2 in this SOP. Click “**OK**" at the bottom of the TEM Wizard. The ROI scanning will then be processed, and the pattern will be automatically assigned within the scanned ROI.

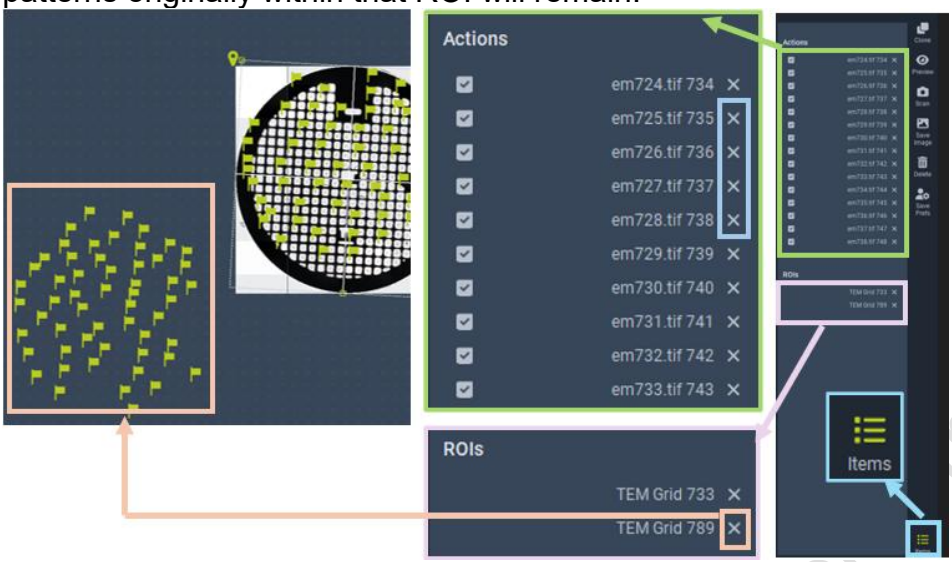


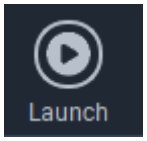
8. Repeat steps 4 - 7 for each additional grid until patterns have been assigned to all of your grids
9. For any unsuccessfully assigned square, use one of the following methods to assign the pattern:
   Method 1: Select a pattern in the image, click **Clone**, and drag the clone to the failed region.
   Method 2: At the top of the Pattern Wizard, open the folder and follow step **B2.2** to add new patterns.
   To remove automatically duplicated patterns, select the pattern and click **Delete**.



10. To batch modify patterns, click "**Items**" in the lower-right corner of the wizard. A list of all patterns and ROIs will be displayed. Undesired patterns can be deleted

directly from this list. If an ROI is deleted, the ROI overview will be removed, but any patterns originally within that ROI will remain.



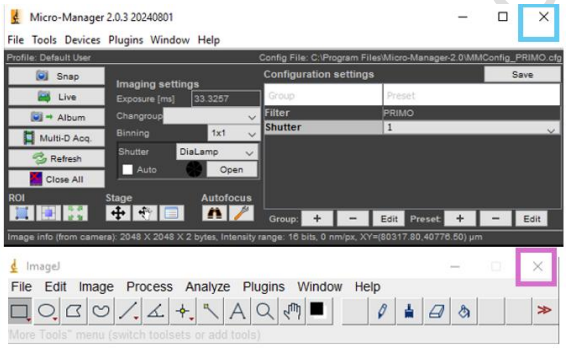
11. After the patterns have been optimized in steps 9 and 10, click 

to start the patterning. Turn off the brightfield (BF) illumination lamp by pressing the button on the left side of the microscope body. While patterning is in progress, do not disturb the anti-vibration table or switch to the desktop or other interfaces.

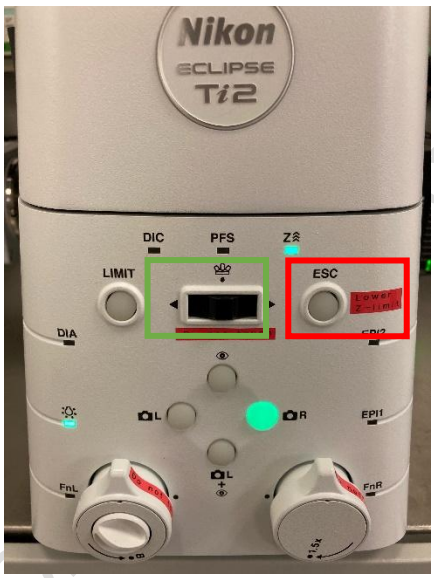
D. Turn off System

Check the calendar/**Timetable** to see if the equipment is booked by another user within 1 hour after your session.
Yes → Perform steps 1–4.
No → Perform steps 1–8 to switch off the whole system.

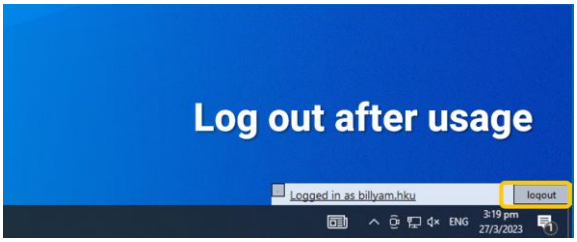
1. Click  (located at the top left of the Leonardo software) to return to the homepage. Then exit Leonardo, Micromanager, and ImageJ.



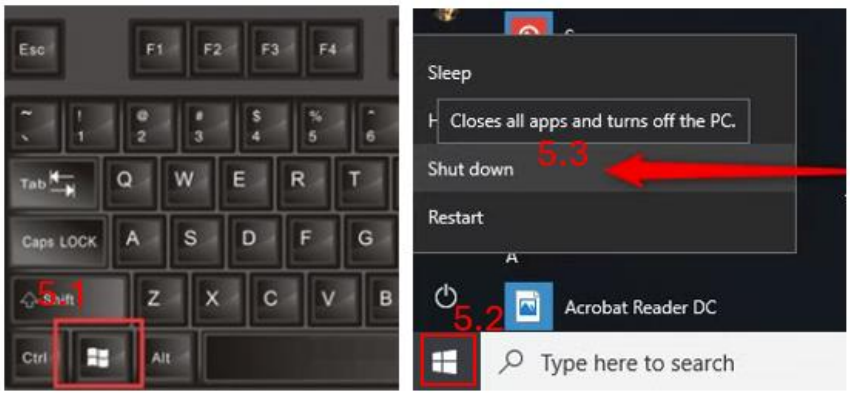
2. Switch the objective to the lowest magnification (4x) by turning the toggle button on the front of the microscope body, then press "ESC" to reach the Lower Z-limit.



3. Shut down the power switch **4**
4. Logout PPMS Tracker.



5. Press the **Win** key on the keyboard, click the **Windows** icon, then click **Shut down** to turn off the computer. Wait until the PC is completely off.



6. Switch off the microscope controller **2**, wait for 5 seconds.
7. Switch off the main power control **1**.
8. Cover the microscope body with the protective cover and complete the logbook entry.