Primo Micropatterning System
Standard Operation Protocol

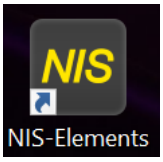
Contents

Turn on System

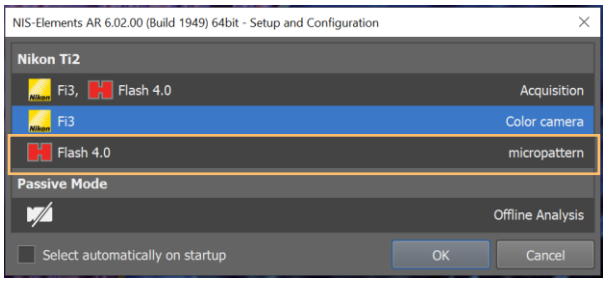
Please sign on the log sheet before switching on system.

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

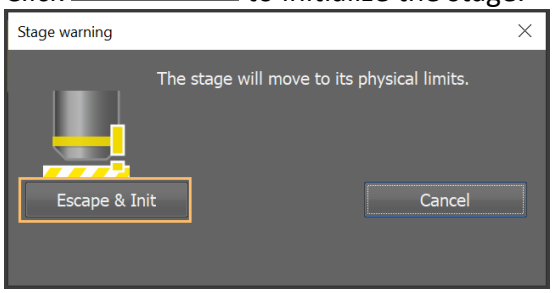
5. Click to log in **USER** account at the startup screen with the password attached on the screen bottom.
6. Log in your PPMS tracker
7. Double click NIS-Elements AR to start the NIS-elements software.



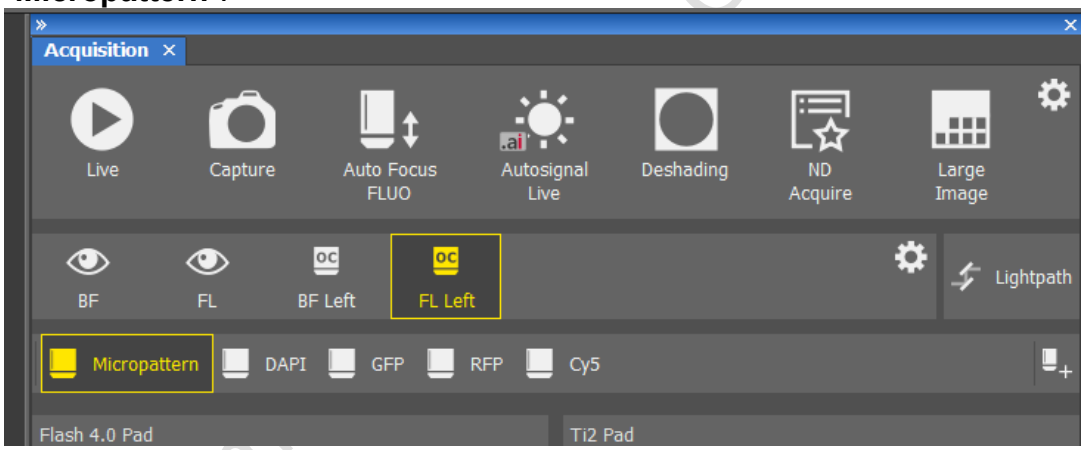
8. Choose Flash 4.0 camera, click “OK” on setup and configuration, DO NOT check “select automatically on startup”.



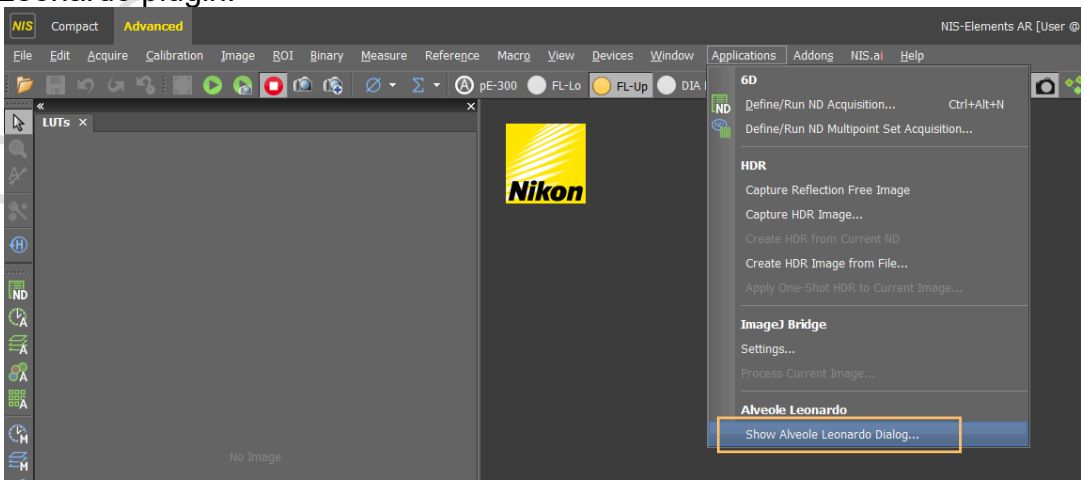
9. Click Escape & Init to initialize the stage.



10. Make sure the Light path is selected to “**FL left**” and the filter was selected as “**Micropattern**”.



11. Click “**Show Alveole Leonardo Dialog…**” inside Application to initiate the Leonardo plugin.

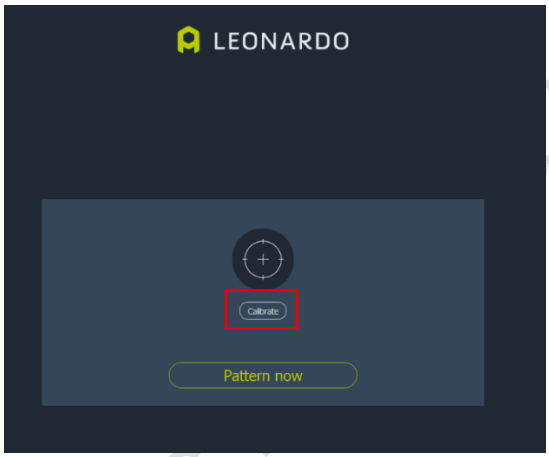


Calibration

Calibration should be performed prior to the start of each experiment using an identical sample carrier (e.g. a coverslip or confocal dish from the same manufacturer as the experimental sample).

1. A small area of the carrier should be marked with a yellow highlighter for calibration purposes.
2. Fix the sample carrier on the holder with highlighter mark facing upwards.
3. Mount the holder on the stage.

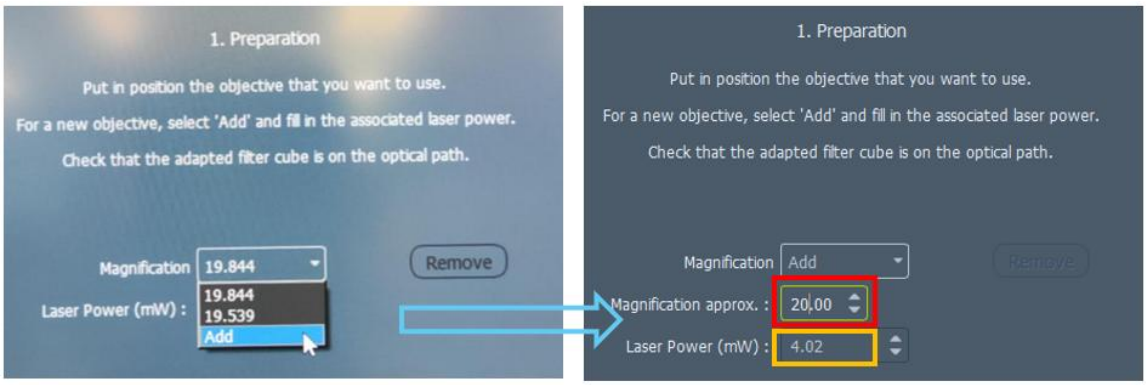
4. Click "Calibrate".

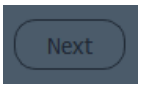


5. Choose "Add", then input the "Magnification" and corresponding "Laser Power (mW)" list in the below table.

***20x objective lens is recommended for micropatterning on TEM grid or glass.

Objective	Value (Magnification)	Laser Power (mW)
4x	3.5-4.5	4.71
10x	9.5-10.5	3.59
20x	19-21	4.02



6. Click "**Next**" 

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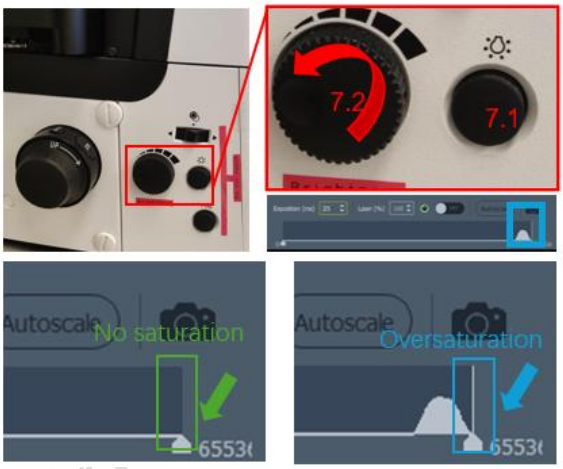
7. Focus Adjustment

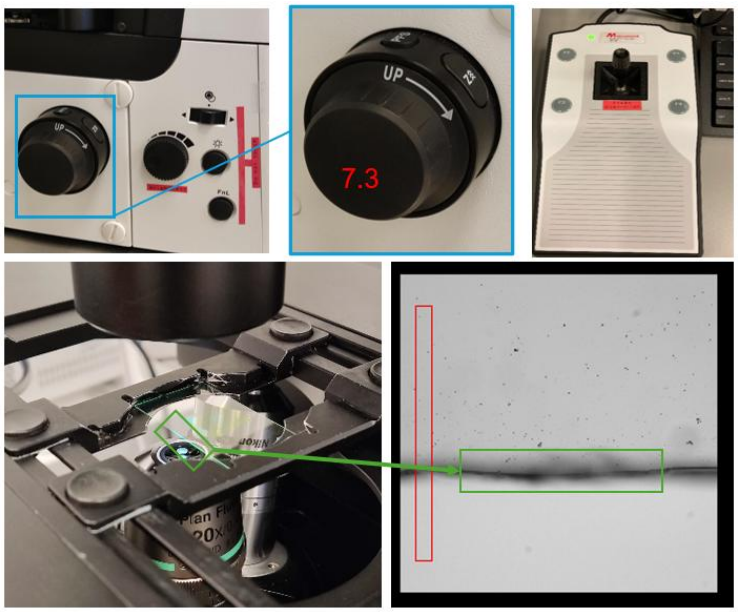
7.1 Press to turn on the BF light source button on the left side of the microscope body.

7.2 Adjust the light intensity by rotating the knob until no oversaturation. If there is an oversaturation, there will be a vertical line at 65535 in the intensity range window.

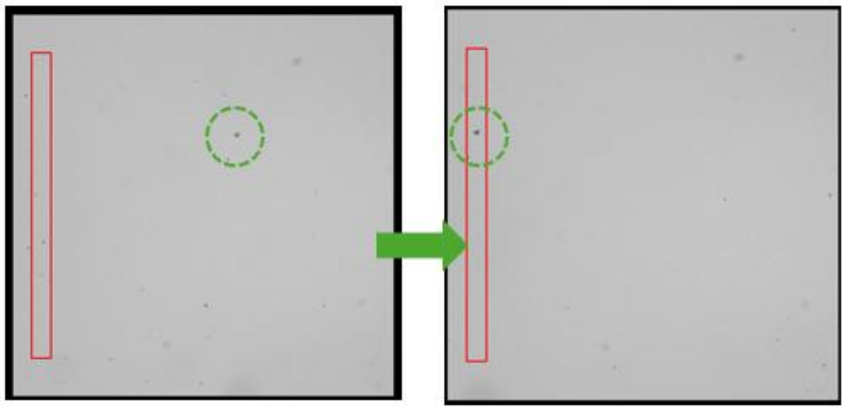
7.3 Focus by rotating the focus knob until the sample carrier surface comes into



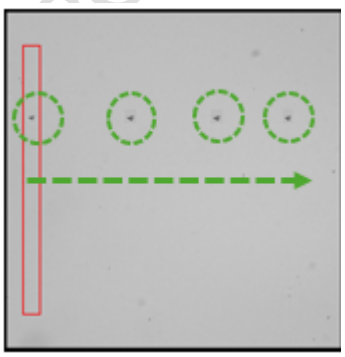
focus. If it is difficult to focus on the sample carrier initially, use the joystick to move to the edge of the coverslip—focusing on the edge is easier. Then, move to your highlighted area and look for a dust particle to fine-tune the focus.



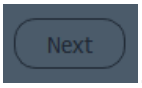
7.4 Find a dust particle and move it to the red rectangle on the left side of the live window using microscope joystick.



7.5 Double-click the dust to start calibration. During calibration,
the dust will move automatically to the right in a horizontal direction.
(The calibrated angle represents the accumulated angle between the camera and the stage, as well as between the camera and the Primo LED pattern.)



Note: The measured angle should normally be within ±1°. If the angle exceeds ±1°, please contact Imaging staff.

8.8.1 Click "**Next**" 

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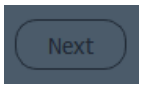
8.2 Tun off the BF light source.

8.3 Reduce the "**Exposition**" or "**Laser (%)**" if there is oversatuation.

8.4 Adjust the zoom slider until the small text reading "**TAKE CARE OF YOUR CELLS**" becomes visible.

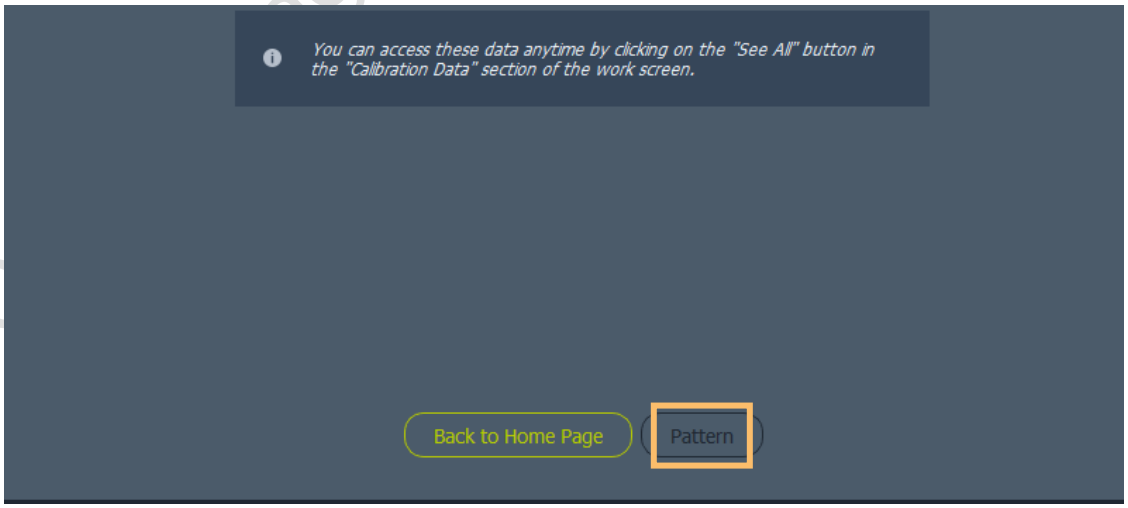
8.5 Check the correction ring on the 20X lens to ensure it matches the thickness of your sample carrier. For example, if your sample is mounted on a No.1.5 coverslip, set the correction ring to 0.17mm.

8.6 Manually adjust the focus manually with the microscope's focusing knob until the "**TAKE CARE OF YOUR CELLS**" text is sharp and in focus.

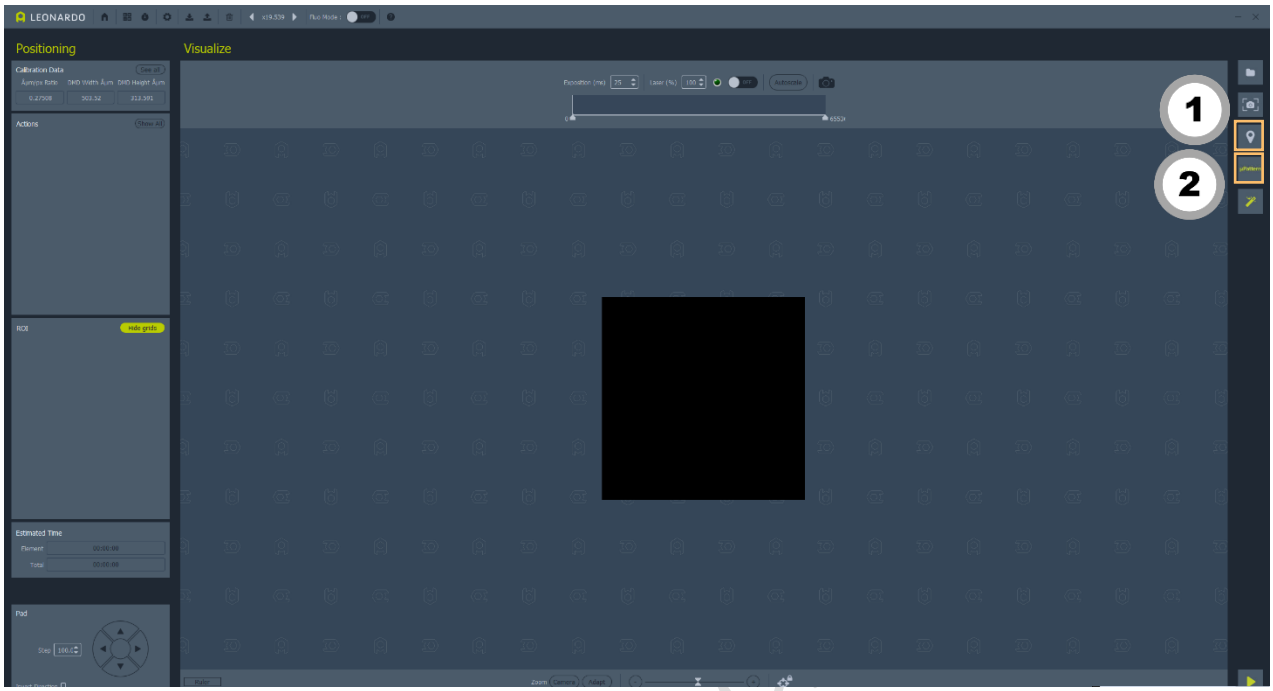
9. Click "**Next**" 

. Wait for at least1 minute while the software performs the calibration.

10. Click "**Pattern**".



Patterning on Glass



1. Click  to locate the ROI for patterning with brightfield light source ON. Adjust the shape, size, orientation and localization of the ROI. Check the box "**Scan**" to perform a preview scan. Click 
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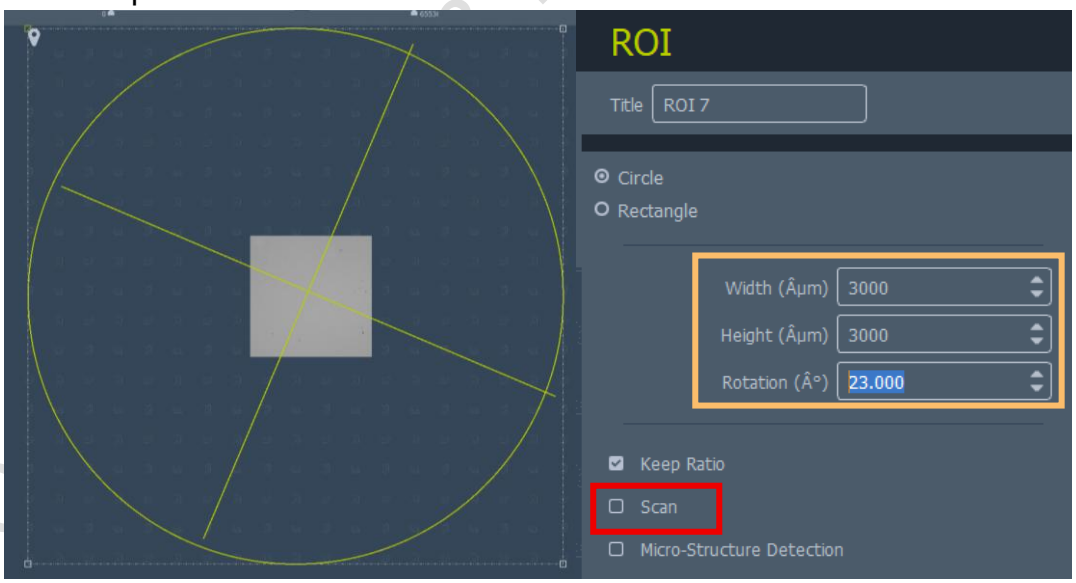


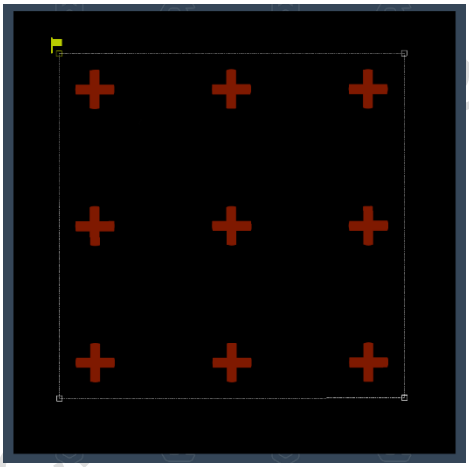
2.Click 

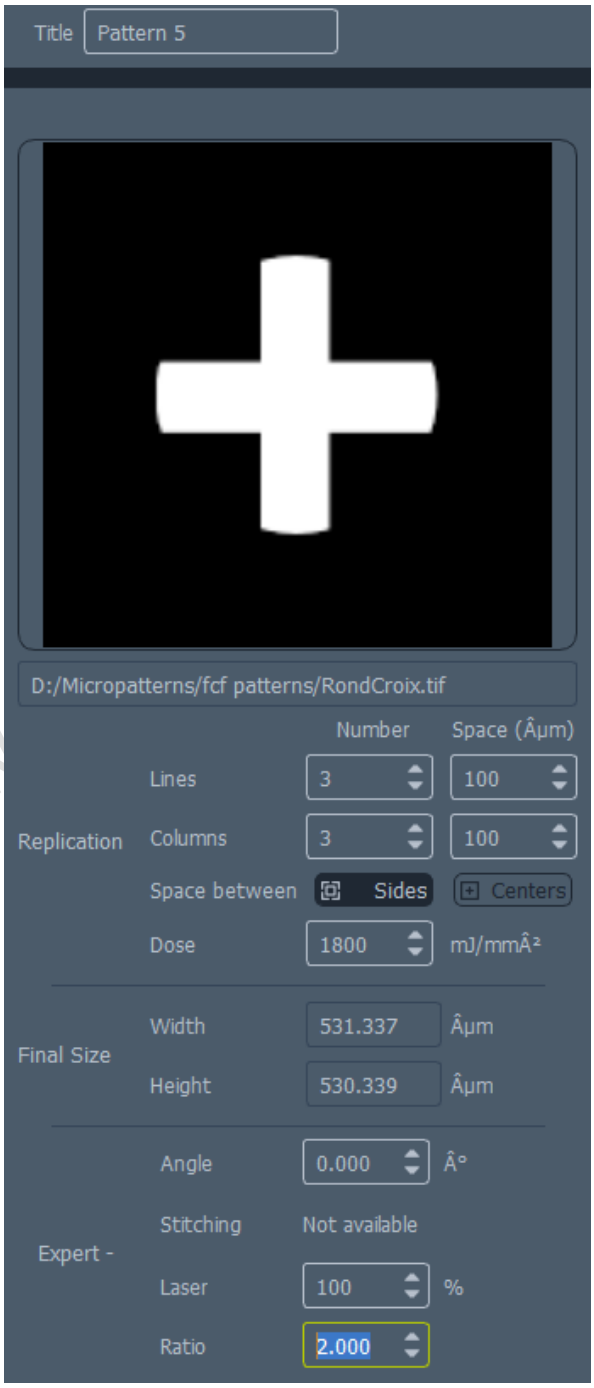
to add in the patterns.
Load the image from **D:\Micropatterns\fcf patterns**. Other PNG, TIFF or PDF images can also be imported for patterning.

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

Adjust repetition, size and ratio of the patterns. ***Recommended laser power for patterning with different photoinitiator.*** The typical UV doses are listed below. It might depend on the density of PLPP or PLPP Gel, substrate, etc… dose adjustment might be needed.

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





3.Click 

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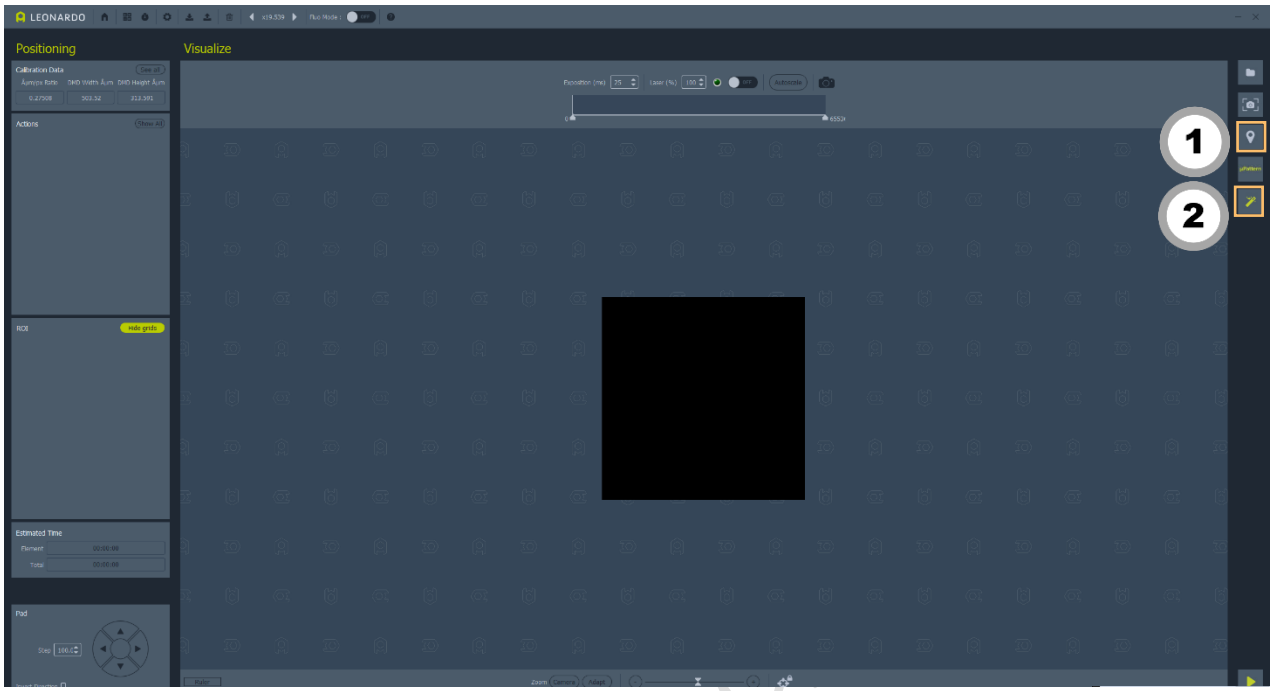
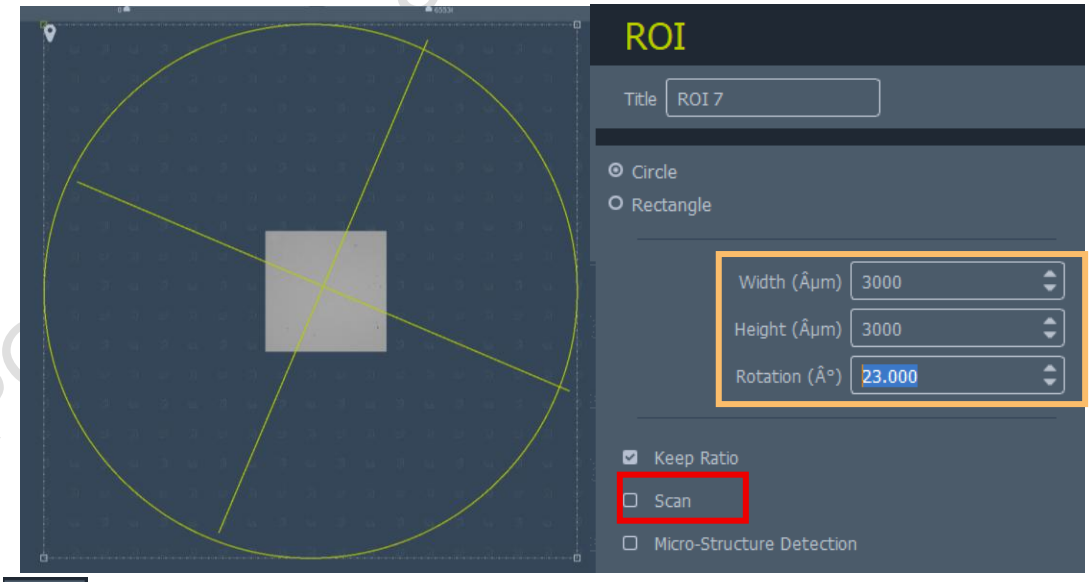
4.Disable the brightfield light source and click  to start patterning.

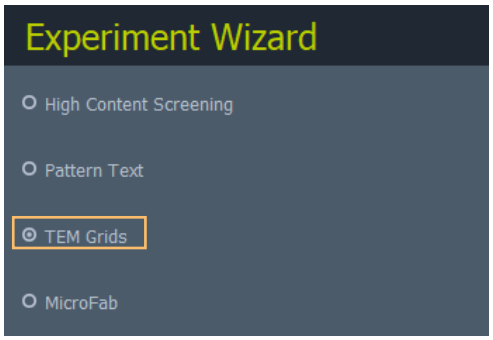
Patterning on TEM Grid



1. Click  to locate the ROI for patterning with brightfield light source ON. Adjust the size, orientation and localization of the Circle ROI. (For the standard TEM grid, usually the width and height are both 3000 (Aμm). Check the box "**Scan**" to perform a preview scan. Click Lock.



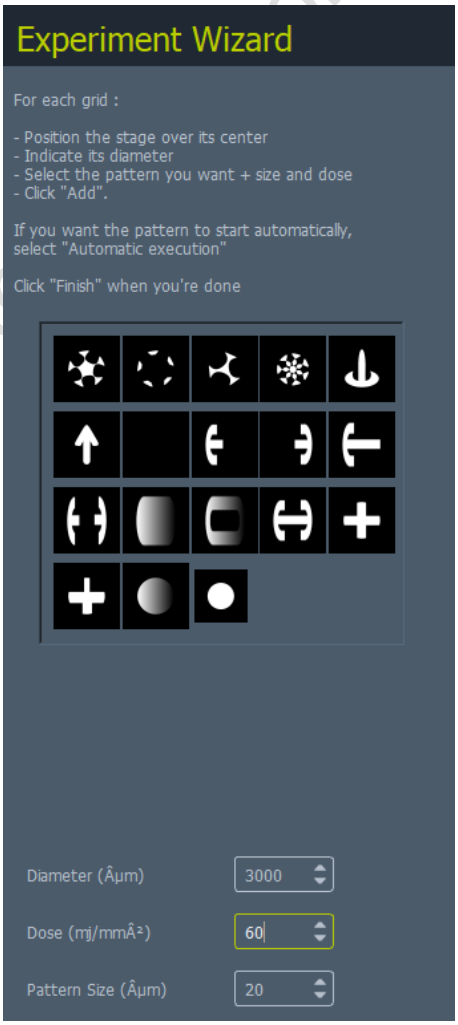
2. Click  to start the experiment wizard. Choose "TEM Grids". Click Next.

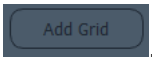


3. Choose the pattern. Adjust the diameter based on the inner diameter size of the TEM grid “2600(Aμm)”. The typical UV doses are listed below. The dose adjustment might be needed, which might depend on the density of PLPP or PLPP Gel, substrate, etc…. Please refer to your sample preparation protocol.

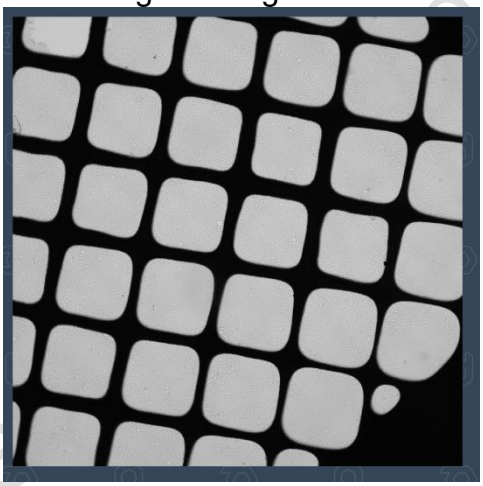
*** Recommended dose ***

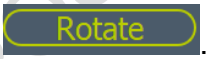
PLPP	1200-1500
PLPP Gel	60



4. Click “**Add Grid**” 

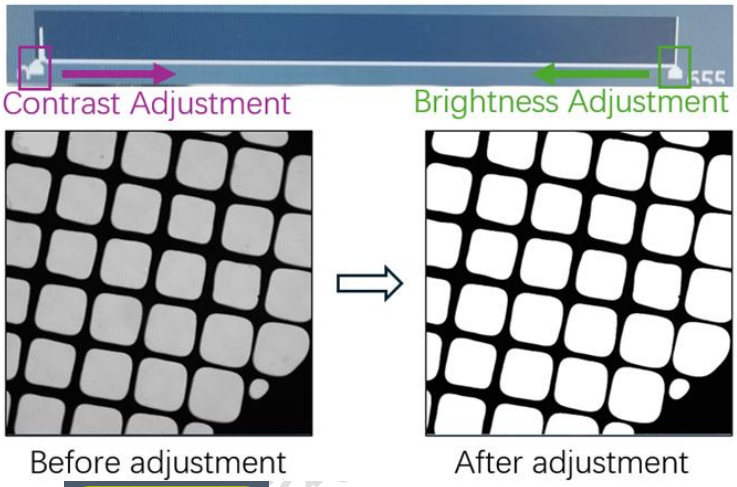
. Drag and pull the ROI to fit on the grid with brightfield light source on.



5. Click 

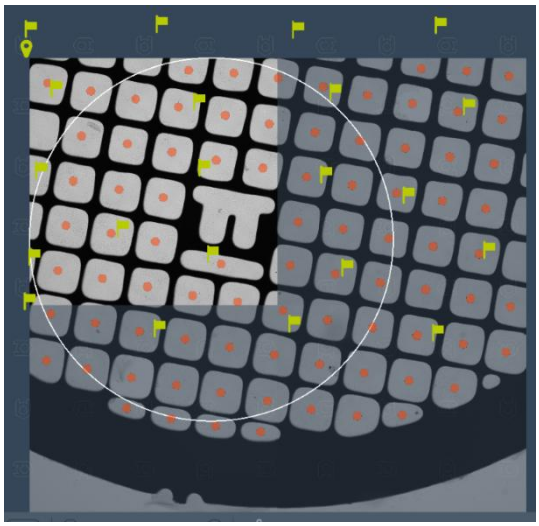
. Adjust the orientation of the ROI and don’t check **“Scan”**

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. Click 

. Click “Finish”. The software will scan the ROI and fit the patterns in the middle of the grids.



8. Disable the brightfield light source and click  to start patterning.

Turn off System

Please check timetable to see if the equipment will be used by other users.
Please switch off system if no one books equipment over 1h after you.

1. Switch objective to lowest magnification (4x) in the software and press "**ESC**" to reach the Lower Z-limit.
2. Exit Leonardo and NIS-elements software.
3. Shut down power switch ④.
4. Shut down the computer ③, wait until the PC is completely off.
5. Switch off microscope controller ②, wait for 5 seconds.
6. Switch off main power control ①.