Primo Micropatterning/Color Imaging System Standard Operation Protocol

Contents

Imaging and Flow Cytometry Core

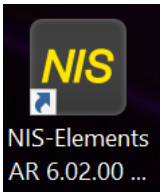
Micropatterning

Turn on System

Please sign on the log sheet before switching on system.

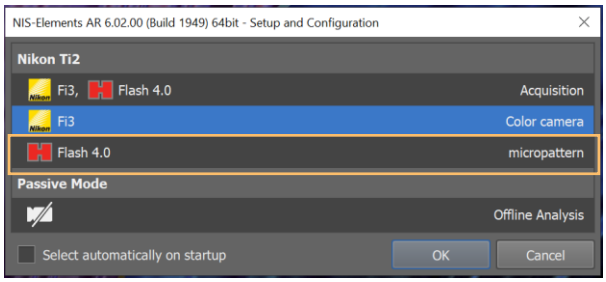
1. Switch on main power control ① wait for at least 5 sec before next step
2. Switch on microscope controller ② wait for at least 10 sec until the stage stop moving before next step
3. Turn on computer power ③
4. Turn on power switch ④, and turn on the Primo on/off button.

5. Click to log in **USER** account at the startup screen
6. Double click NIS-Elements AR to start the NIS-elements software.

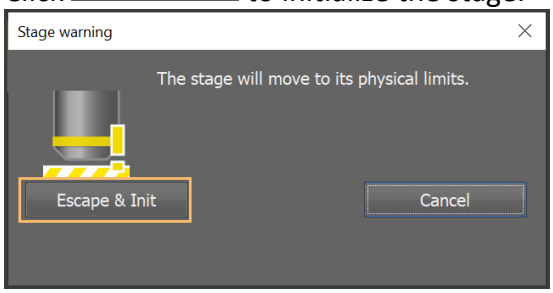


Imaging and Flow Cytometry Core

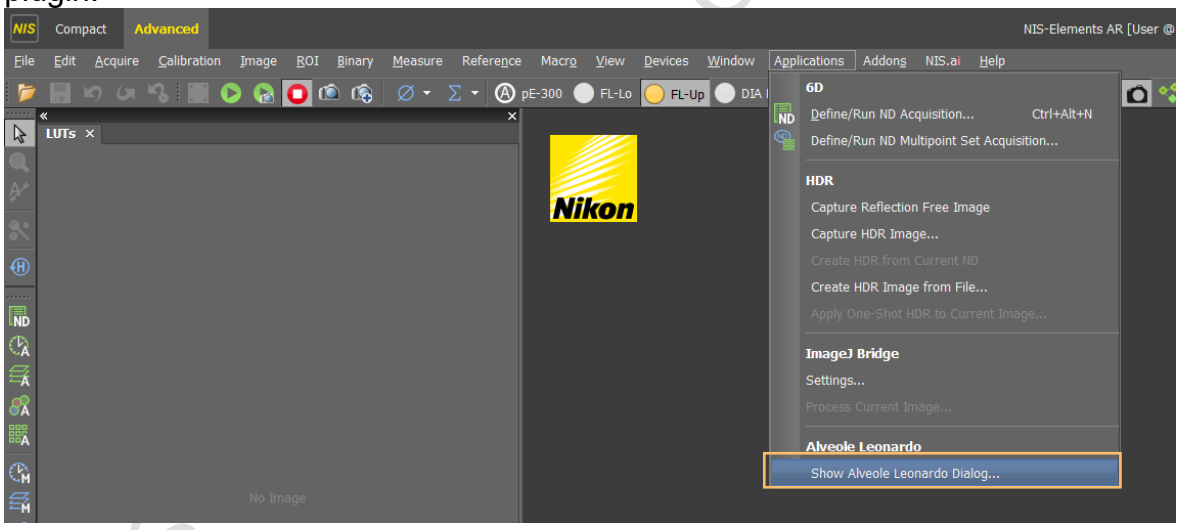
7. Choose Flash 4.0 camera, click “OK” on setup and configuration, DO NOT check “select automatically on startup”.



8. Click Escape & Init to initialize the stage.



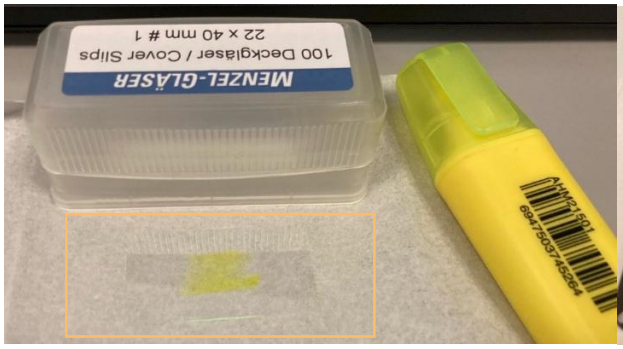
9. Click “**Show Alveole Leonardo Dialog…**” inside Application to initiate the Leonardo plugin.

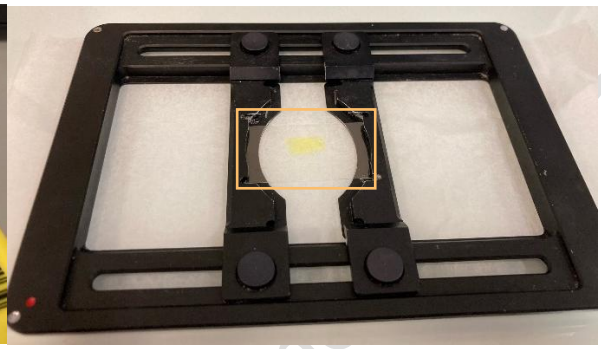


Imaging and Flow Cytometry Core

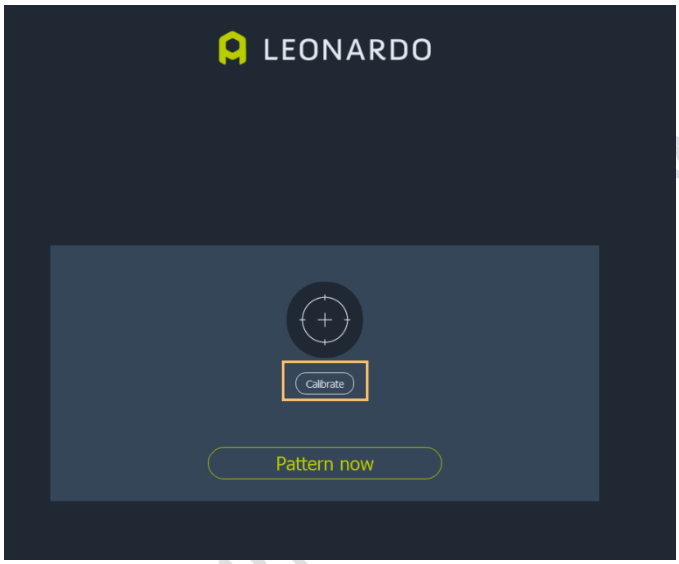
Calibration

Please perform calibration every time before the start of experiment with an identical sample carrier (e.g. coverslip or confocal dish from the same brand) as the experiment sample and paint a small area with yellow highlighter. Fix the sample carrier on the holder with highlighter mark facing upwards. Mount the holder on the stage.



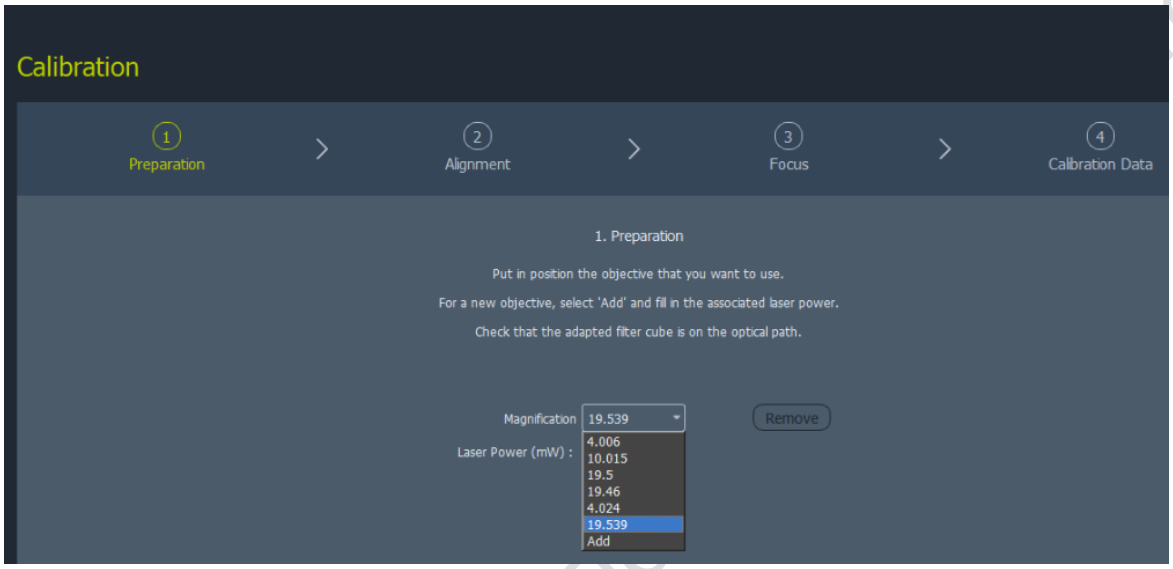


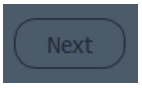
1. Click "**Calibrate**".



2. Choose the magnification of the objective lens that you are going to use from the drop-down list. The exact value may not be available as the drop-down list recorded the magnification calculated by the software from last calibration performed by CPOS staff. Therefore please choose the closest value to the respective objective lens.
   ***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



3. Click "**Next**" 

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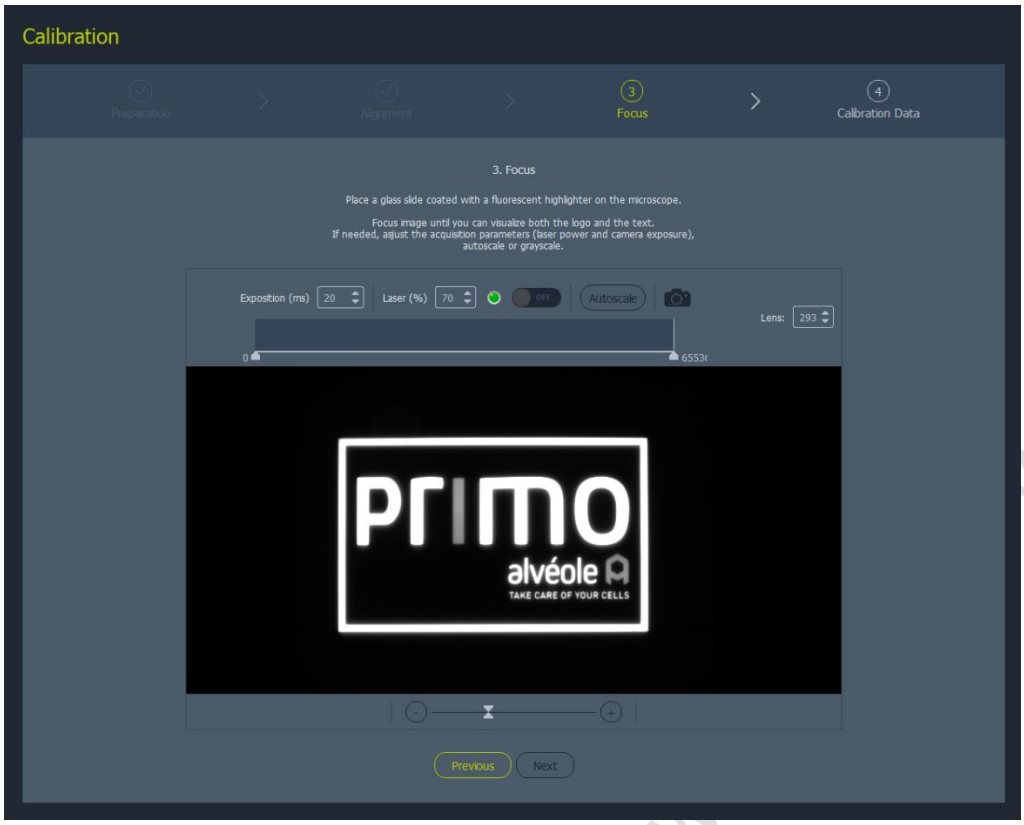
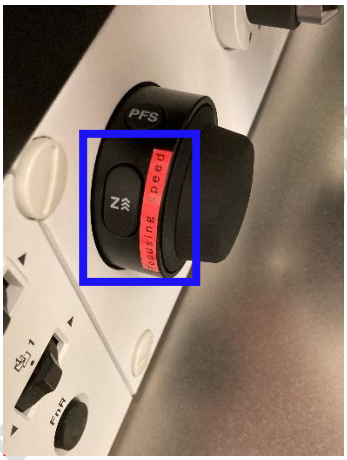
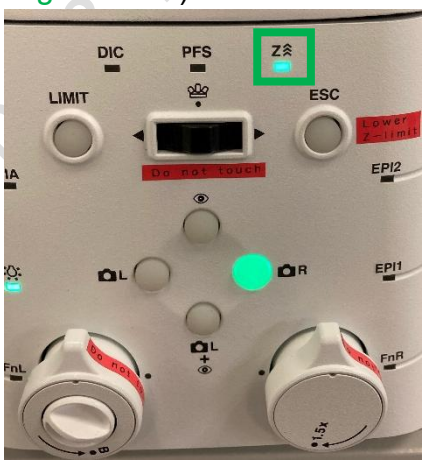
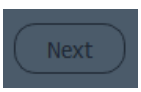
4. Adjust the focus manually with the focusing knob on the microscope until the four corners of the Pirmo image are clearly seen.



(Focusing speed can be adjusted by the Z ^^^ button. Maximum focusing speed is reached when the indicator light is ON.)

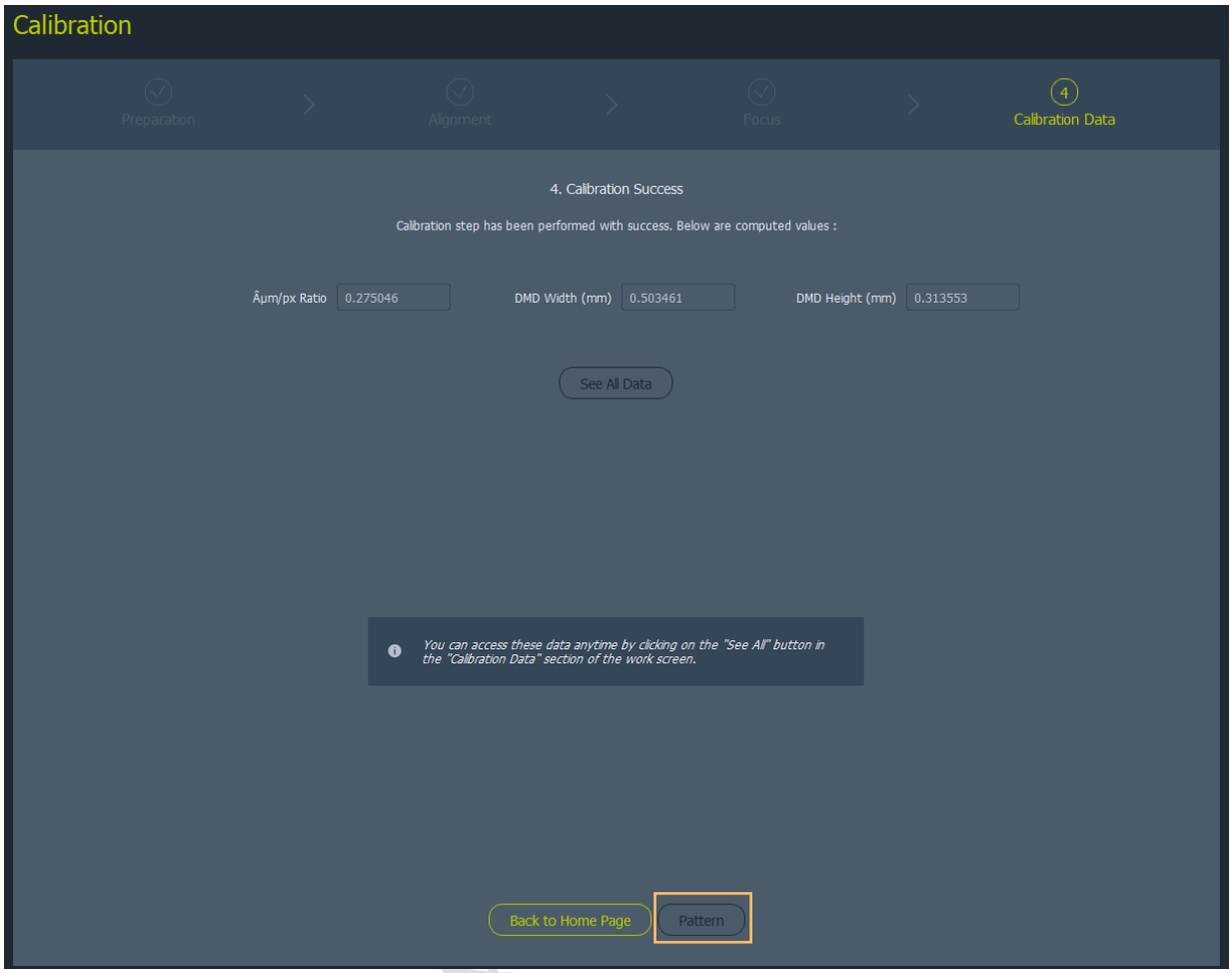




5. Click “**Next**” 

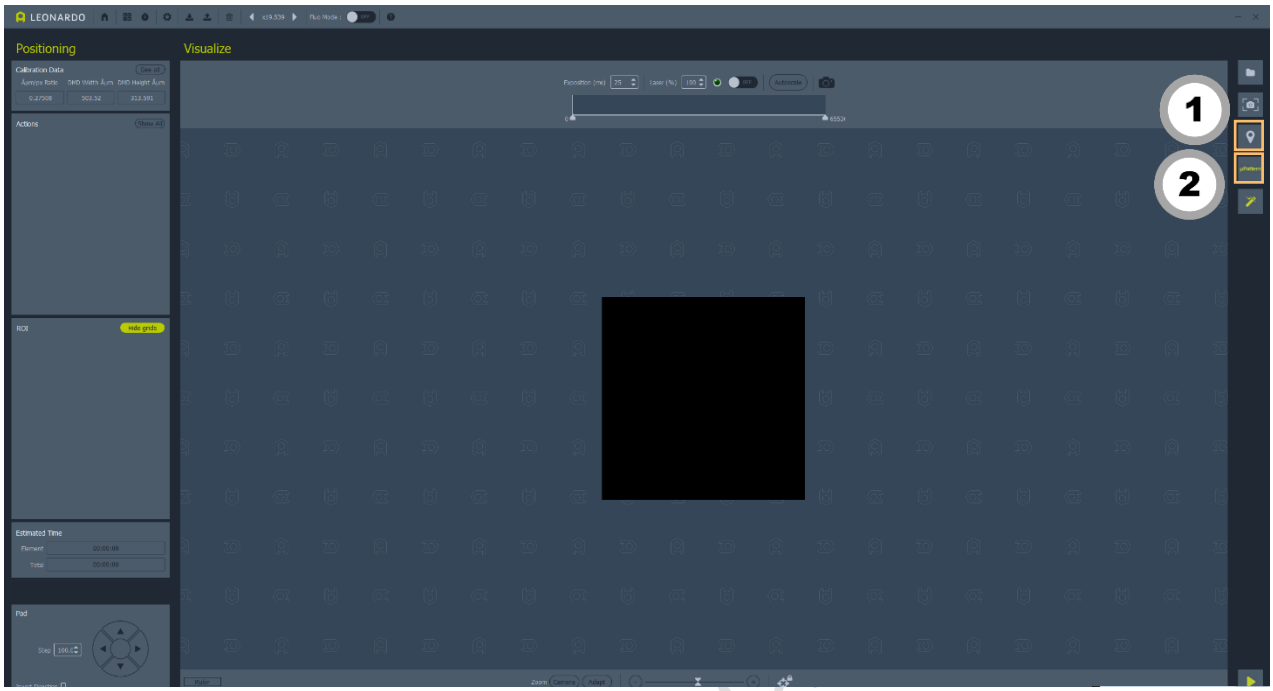
. Wait for around 1 minute for the software to run the calibration.

6. Click “**Pattern**”.

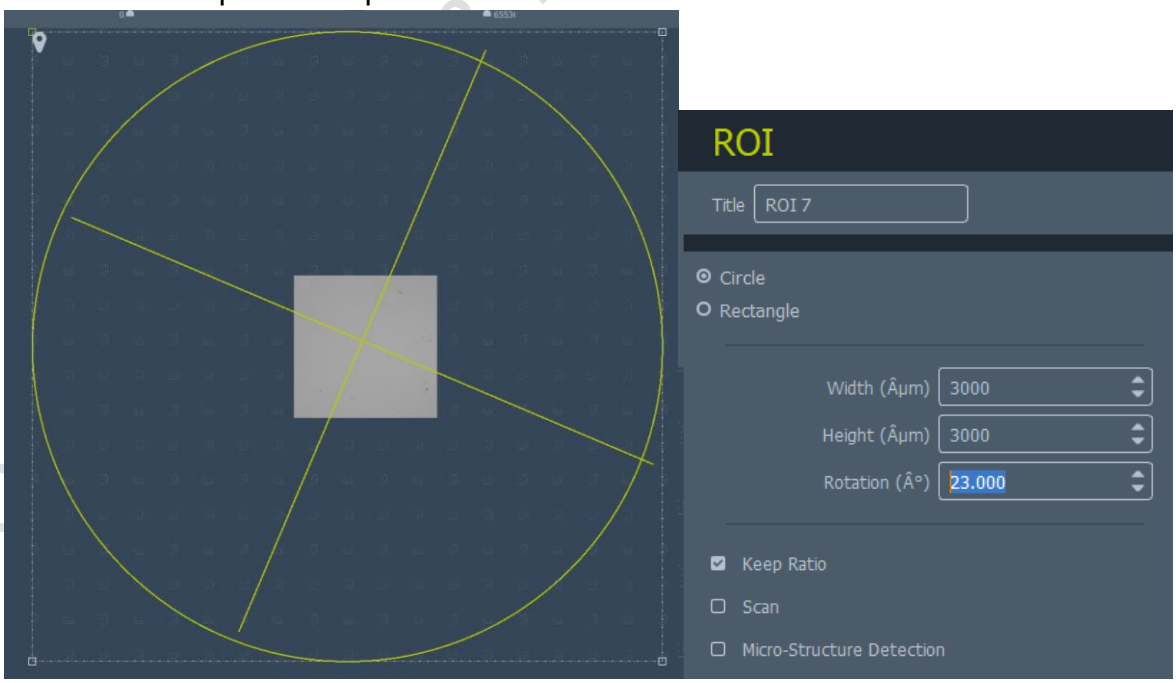


Imaging and Flow Cytometry Core

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 Lock.



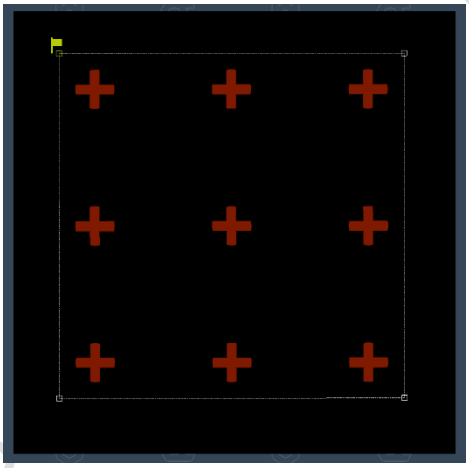
2. Click 

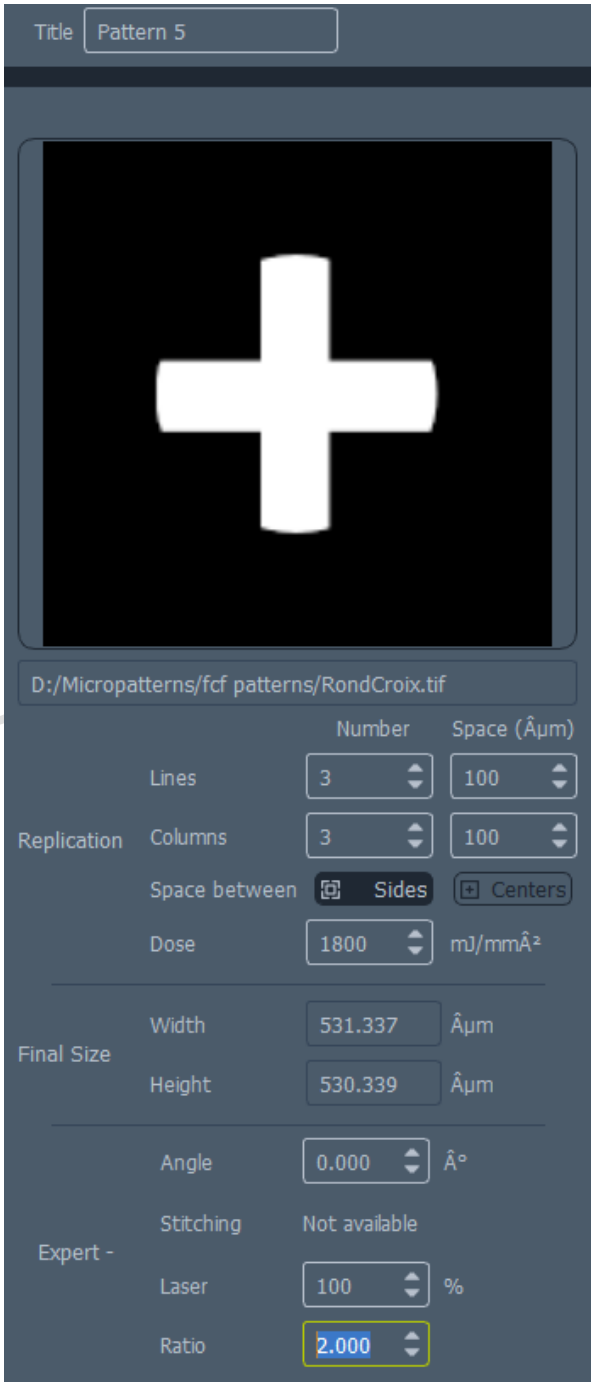
to add in the patterns.
Load the image from **D:\Micropatterns\fcf patterns**. Other PNG, TIFF or PDF images can also be imported for patterning.

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.

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





3. Click 

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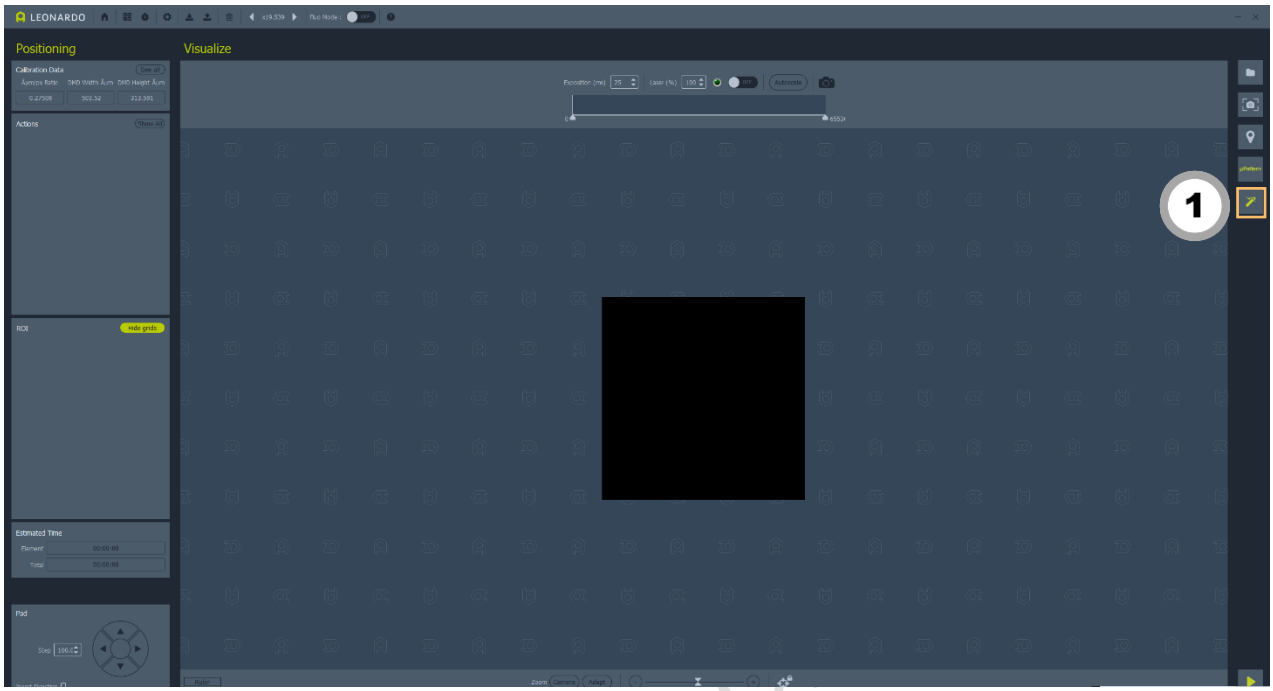
4. Disable the brightfield light source and click  to start patterning.

Imaging and Flow Cytometry Core

Patterning on TEM Grid



1. Click  to start the experiment wizard. Choose “TEM Grids”. Click 

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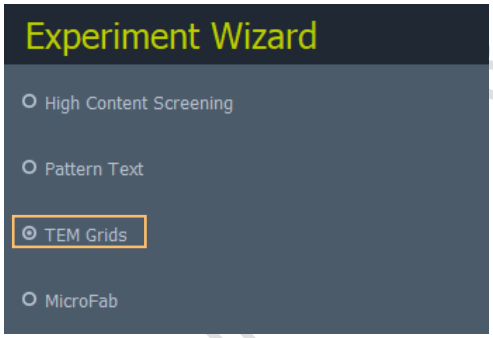
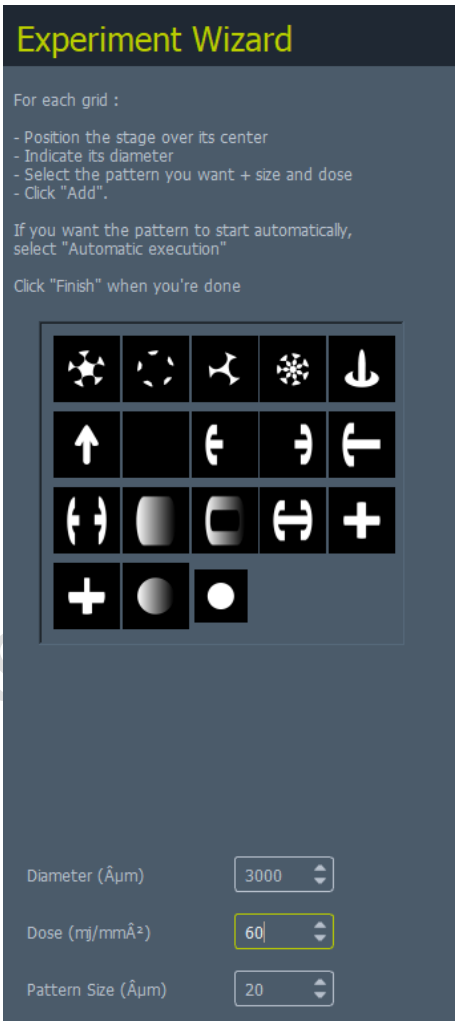
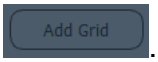


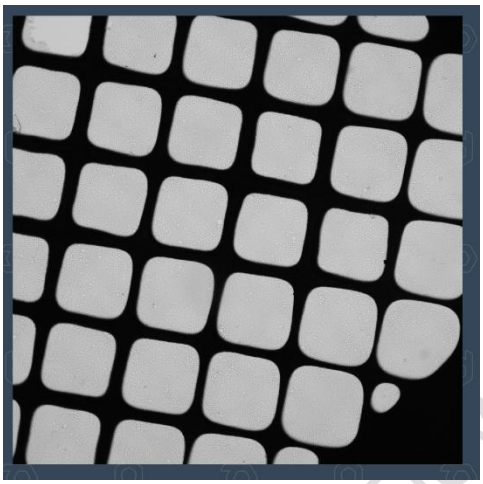
2. Choose the pattern. Adjust the diameter depends on the size of the grid and the size of the pattern.
*** Recommended dose ***

PLPP	1200-1500 mJ
PLPP Gel	60 mJ



3. Click "**Add Grid**" 

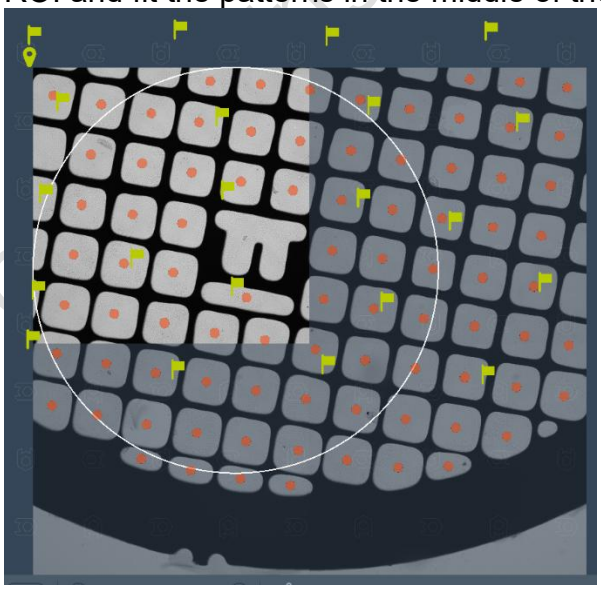
. Drag and pull the ROI to fit on the grid with brightfield light source on.



4. Click 

. Adjust the orientation of the ROI (if needed).
5. Click 

. The software will scan the ROI and fit the patterns in the middle of the grids.



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

Imaging and Flow Cytometry Core

Turn off System

Please check if the equipment will be used by other users. Please switch off system if no one books equipment over two sessions (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. Transfer data to Imaging & Flow Cytometry Core storage server.
4. Turn off the Primo on/off button, shut down power switch ④.
5. Shut down the computer ③, wait until the PC is completely off.
6. Switch off microscope controller ②, wait for 5 seconds.
7. Switch off main power control ①.