User Manual
LEONARDO

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1   Introduction

1.1   Aim of the User Manual

This User Manual has no contractual value and in no case can Alvéole be held responsible on the basis of the information contained in this Manual.

This User Manual details all the required knowledge for the use of LEONARDO software. Thus, after careful reading, the operator will be able to use the software in combination with the PRIMO system to project images and perform different experimental scenarios of patterning of proteins.

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1.2   Symbols

This symbol means: LASER RISK

Instructions preceded by this symbol may cause personal injury or damage to the eyes or skin of the user and his immediate surroundings if they are not respected or carried out.

This symbol means: WARNING

Instructions, preceded by this symbol, may cause a personal injury or damage to the PRIMO system and the installations if they are not properly observed or carried out.

This symbol means: INFORMATION

Additional information which does not affect the use of the device.

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1.4   Targeted version

This manual describes the functionalities of LEONARDO V4.6 software, incorporating a graphic chart specific to Alvéole. This manual applies to software version 4.6

LEONARDO V4.6 software is designed as a plugin to Micromanager v1.4.22 and should be compatible with any configurations of the patterning setup made of devices compatible with Micromanager v1.4.22

1.5   Specific documentation

LEONARDO software is dedicated to the management of the PRIMO system for protein patterning experiments. This manual only describes aspects related to the operation of the software. For further information on the operation of the equipment and the experimental protocol, please refer to the following documents:

ALVEOLE – User Manual PRIMO

ALVEOLE – Protocol for protein patterning

2   Disclaimer

2.1   Laser Risk

⚠	The UV Laser of the Optical Box is a Class 3B Laser. Laser radiation: do not look in the beam neither with the naked eye nor with the aid of an optical instrument.

ℹ	The microscope referred to in the rest of this manual is a Nikon Ti-Eclipse inverted microscope but the explanations should be suitable for all compatible microscopes

3   Terms and definitions

DMD (Digital Micro Device): individually controllable micro-mirror array for projecting grayscale images.

GUI (Graphical User Interface): Main window of LEONARDO Software.

Layer: result of transformation of the pattern after loading into the software which will then be sent to the DMD or displayed on the GUI to access rotation and realignment functions. Only one layer can be sent at a time.

LEONARDO: software design as a plugin to Micromanager to allow control of the devices used during patterning experiments.

Motorized stage (MS): X/Y translation stage used to move the sample over the microscope objective.

Optical unit: PRIMO Optical module fixed to the microscope allowing to project UV images

Pattern: grayscale image (.tif or .pdf) than can be loaded into the software to be printed with or without further modifications (rotation, alignment or replication).

Power supply unit: Housing including all electronical connectors to power and control the optical unit.

ROI: A Region Of Interest is an area you can define to create marks on the "Map".

Subpicture: result of the decomposition of large image by the software. Each subpicture has the dimension of the DMD.

4   Installation and Startup

4.1   Installing LEONARDO

The software is installed by an authorized technician from Alvéole when the PRIMO system is commissioned. All software upgrades are delivered to the user via the following dropox folder: Leonardo Delivery - software patterning. The user downloads the necessary file (s) available on the dropbox and then proceeds with the installation. A release note indicates the installation procedure.

4.2   How to switch on the setup

Turn on the various Patterning setup items in the following order:

1. Epifluorescence lamp (if required)
2. Camera
3. Bright Field light
4. Microscope
5. PRIMO
6. Computer

4.3   How to start LEONARDO

After installation of LEONARDO, the procedure for launching it is as follow:

a. Make sure that all devices of the setup dedicated to PRIMO are switched on and connected to the computer
b. Launch Micromanager
c. Launch LEONARDO in the Plugins menu
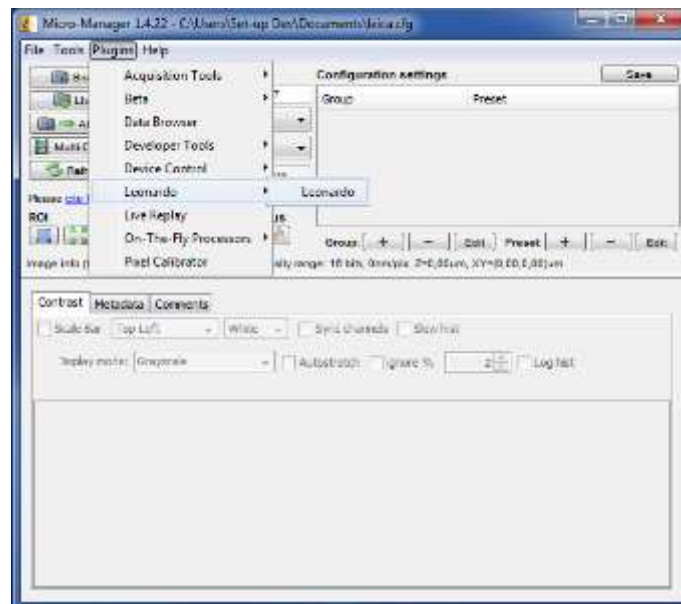
LEONARDO in the Plugins menu of Micromanager

d. LEONARDO automatically opens on the calibration wizard. Follow the steps to use all the functionalities (see Calibration procedure)

4.4   General description of the graphical interface

4.4.1   Homepage

Once launched the Homepage is displayed allowing the user to access the main functionalities of Leonardo.
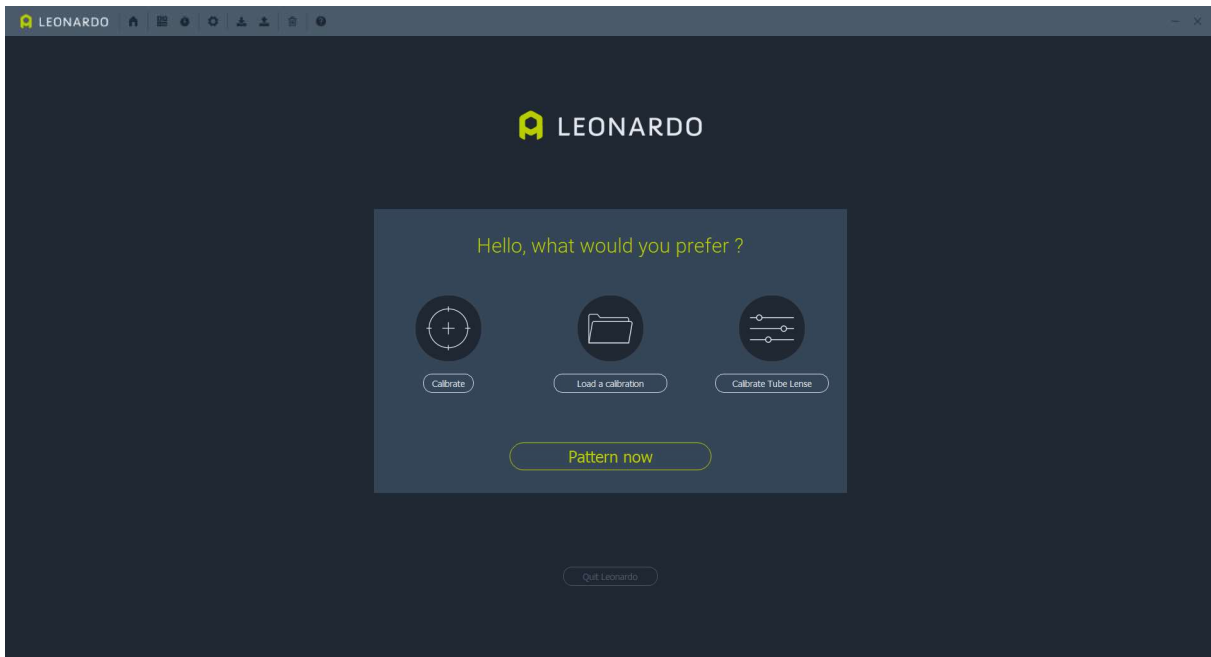

Figure 1: LEONARDO Homepage

Main actions:

- Calibrate: Launch the calibration wizard
- Load a calibration: Allows you to load a previously saved calibration and open Main View
- Calibrate Tube Lense: Launch the calibration wizard with the possibility to choose a personalized image
- Pattern now: Automatically loads the last valid calibration and open Main View
- Quit Leonardo: Close the application (Micro-Manager will NOT be closed)

Menu items:

Icon	Function
🏠	Home: Display the Homepage
🧮	Calculator: Open Windows Calculator
⏱	Timer: Open the Timer window
⚙	Preferences: Open the Preferences window
⬇	Save: Save the current session
⬆	Load: Load a previously saved session
🗑	Clean: Erase the content of the current session
❓	About: Display the "About" splash screen

4.4.2   Main View

Once the calibration procedure has been successfully completed or a calibration file loaded, the user can enter LEONARDO GUI. This GUI is structured in three areas:

- A "Positioning" area on the left which contains information about the current session (Calibration Data, Action and ROI lists, …)
- A "Visualize" area which is a virtual representation of the stage. It allows the user to visualize all the created actions/ROIs and to adjust display options. (Zoom, Image threshold, …) It should be considered as a Map (and will be named as such in the rest of the document) as it contains markers and can display saved images from the camera.
- A "Creation" area which is composed of buttons allowing the creation and configuration of LEONARDO's functionalities.
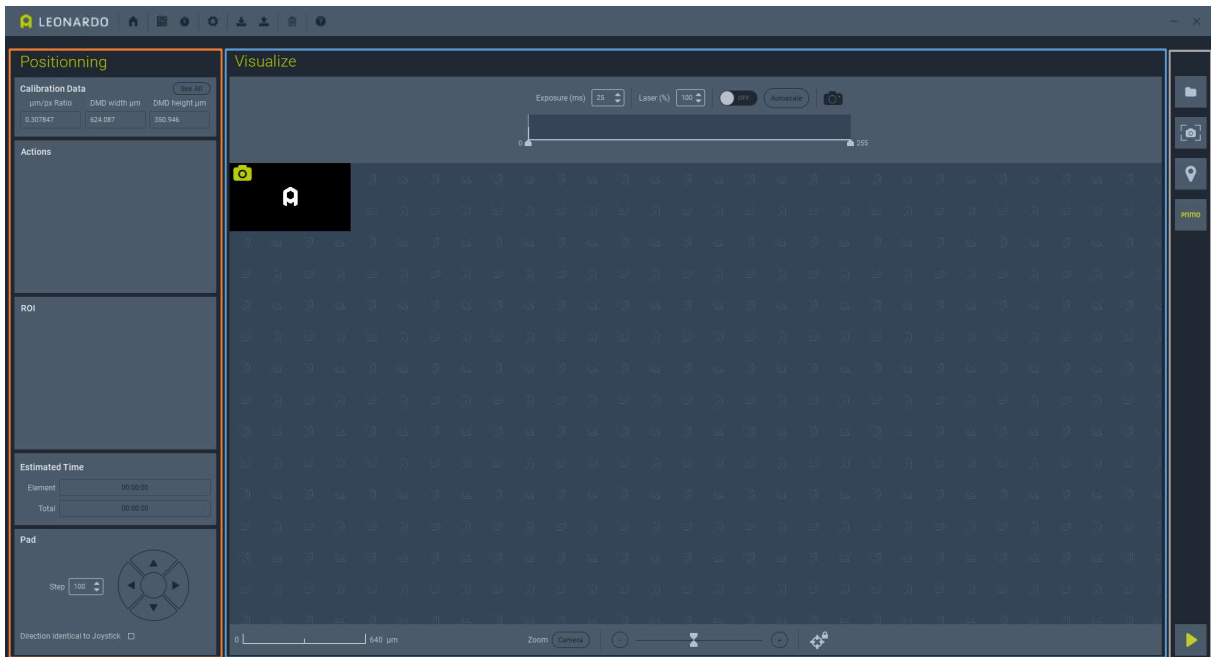
Figure 2: LEONARDO Main View

4.4.2.1   Positioning Area

The positioning area sums-up all the information about the actions performed by the user and allows him to move the stage without using an external joystick.

Five specific panels can be found in this section:

Figure 3: Calibration Data Panel

- ❖ The Calibration Data panel contains the data computed during the Calibration process. Only the ratio and the DMD insolation size are displayed but the complete data can be accessed by clicking the "See All" button. (See Calibration Data Window)

Figure 4: Action List Panel

❖ The Action List panel contains all the actions created by the user. They can be grouped using the Group Action (see Group Panel) as shown in Figure 4. Below is a description of all the functionalities available from this panel. The "See All" buttons allows you to Show/Hide the already performed patterning (which are hidden by default) If displayed they will be shown in green
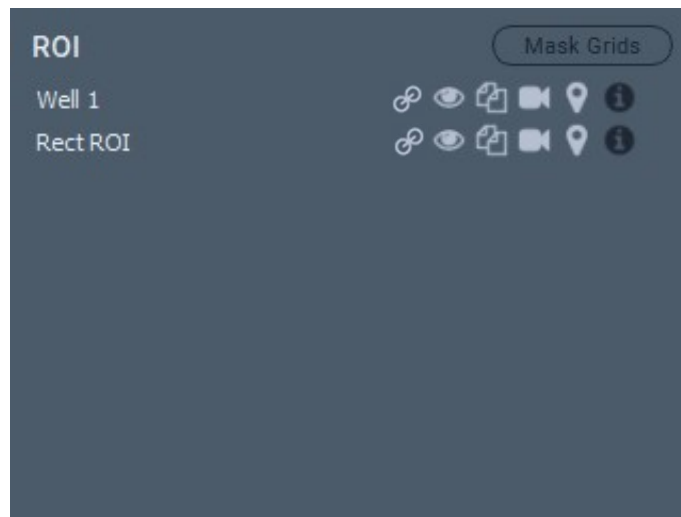
Figure 5: ROI List Panel

❖ The ROI List panel contains all the ROI created by the user. Below is a description of all the functionalities available from this panel. The "Mask Grids" button allows you to Show/Hide the DMD grids in ROIs (displayed by default)

List items:

Icon	Function
	Checked/Unchecked: Perform/Ignore this action for next Launch (Action Only)
	Grouped/Separated: Consider the elements inside the ROI as part/not part of it.
	Show/Hide: Show/Hide the corresponding element in the Map
	Duplicate: Create a new element with the same parameters
	Center Stage: Move the stage so that the camera is centered on the element

📍	Center View: Center the Map on the element
▲ ▼	Move Up/Down: Change the order of actions (Action Only)
❶	Info: Display the panel with the element information
➕ ➕	Collapse/Expand: Show/Hide the content of a group (Group Only)

Figure 6: Estimated Time Panel

❖ The Estimated Time panel gives an approximation of both the time needed to perform the current action and all the selected actions. During an element creation, the corresponding time is indicated in "Element" and updated according to the parameters modifications and bars are not filled. When actions are performed, the bars are progressively filled in green. If an action is selected/deselected in the Action List the Total Estimated Time will be updated.
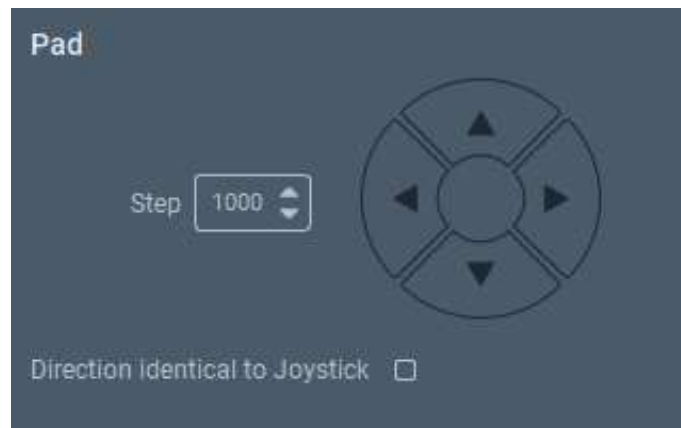
Figure 7: Pad Panel

❖ The Pad panel allows you to move the stage (if connected) without using an external joystick. The Step parameter can be used to modify the movement length in µm.
Please note that stage movement will result in an opposed camera movement on the Map. You can use the checkbox to revert the stage movement so that it corresponds to the Camera point of view.

4.4.2.2   Visualize Area

The Visualize area allows you to have a visual representation of your experiments and to manage part of the peripherals (laser, camera). It is separated in 3 panels described below:
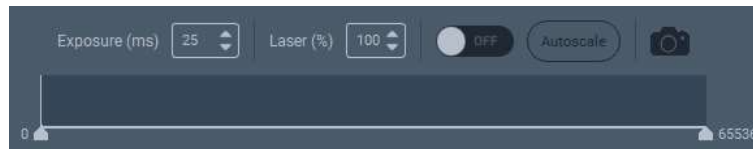
Figure 8: Camera (and Laser) Panel

❖ The Camera (and Laser) panel allows you to manage both peripherals and the rendering of the camera image. It contains:
  ▪ An Exposure Field to change the Camera exposure time in milliseconds (default is 25)
  ▪ A Laser Field to change the Laser power as a percentage of its maximum power (default is 100)
  ▪ An On/Off button to force the activation/deactivation of the Laser
  ▪ An Autoscale button (2 states) that allows you to activate/deactivate the image Autoscaling
  ▪ A Snapshot button that will save on disk the current image from the camera
  ▪ A histogram that allows you to manually define the min and max threshold of the camera image displayed on the Map
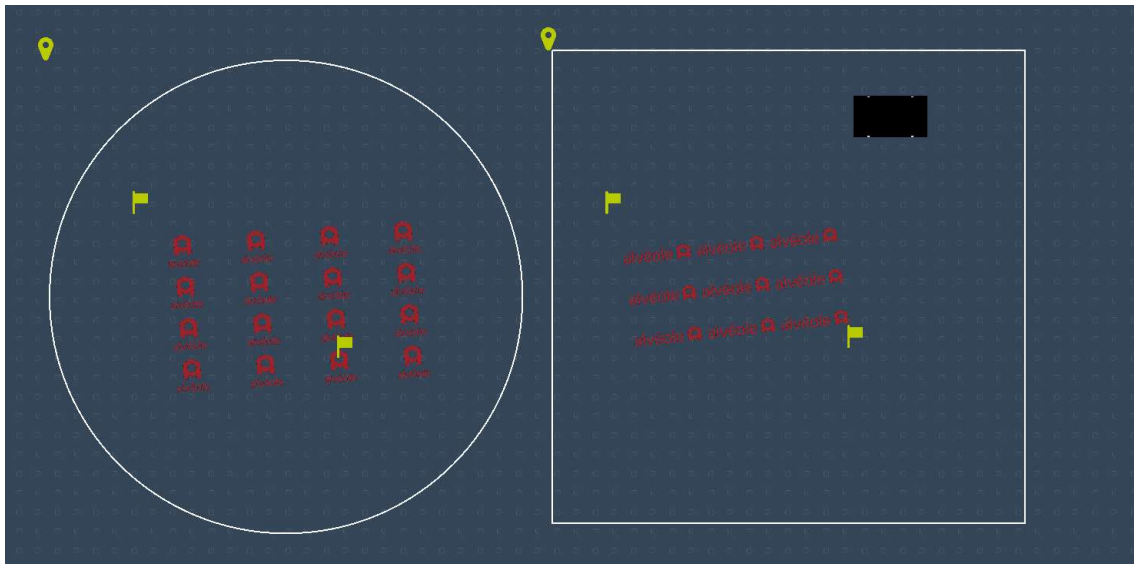
Figure 9: Map Panel

❖ The Map panel is a virtual representation of the Stage. All the elements and actions created will be added and visible on it. Markers are also added to indicates the position of each element. When outside the visible area (due to zoom for example) elements' direction is indicated by a grey marker near the side of the Map where the element is. Below is a list of the markers and their meaning.

Marker items:

Icon	Function
	ROI Marker (On/Off screen): Indicates an ROI
	Action Marker (On/Off screen): Indicates either a PRIMO or a NOMOS action
	Camera Marker (Off screen only): Indicates the Camera (Stage) direction when not visible in the current view
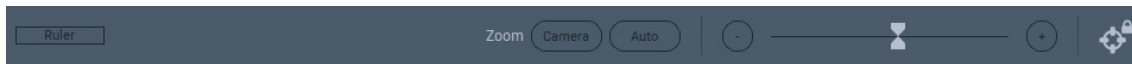
Figure 10: Zoom Panel

❖ The Zoom panel contains the following elements:
  ▪ A Ruler 2-states button allowing you to measure distances directly on the Map using the mouse when clicked
  ▪ A "Camera" Zoom button that sets the zoom level at camera size
  ▪ A "Auto" Zoom button that defines the most appropriate zoom level to see all the actions and ROIs currently on the Map
  ▪ A Slider with +/- buttons to change the zoom level. Each level multiply/divide the zoom ratio by 2
  ▪ A Lock/Unlock Camera Centered button that allows you to keep the Camera centered (visible) on the Map when moving the stage

4.4.2.3   Action Area

The Action area is the list of buttons used to create/define all the parameters of you experiment and to run it. The list of available buttons/functionalities is given here but more details can be found in each dedicated chapter.

Creation items:

Icon	Function
	Group: Create a new Group (see Group Panel)
	Snapshot: Save the current camera image at its current position on the Map
	ROI: Create a new ROI (see ROI Panel)
PRIMO	PRIMO: Create a new Patterning command (see PRIMO Panel)
	Launch: Execute all the commands selected in the Action list (see Software workflows)

4.4.3   Group Panel

Figure 11: Group Panel

The Group Panel can be used to group Actions that are not currently in another group.

It should be noted that:

- A new Group can be created only if at least one Action is available
- An action within a group must be removed from it before it can be deleted

The panel is composed of:

- A title (default is "Group + *id*")
- The list of Available actions that can be added to the Group
- "Add" and "Remove" Buttons to switch actions between the two lists
- The list of currently Selected actions

When the "OK" button is pressed, the panel is closed and the Group created.

When the "Delete" button is pressed, all the actions are removed from the Group (but are not deleted !!) and the Group is suppressed.

4.4.4   ROI Panel

Figure 12: ROI Panel
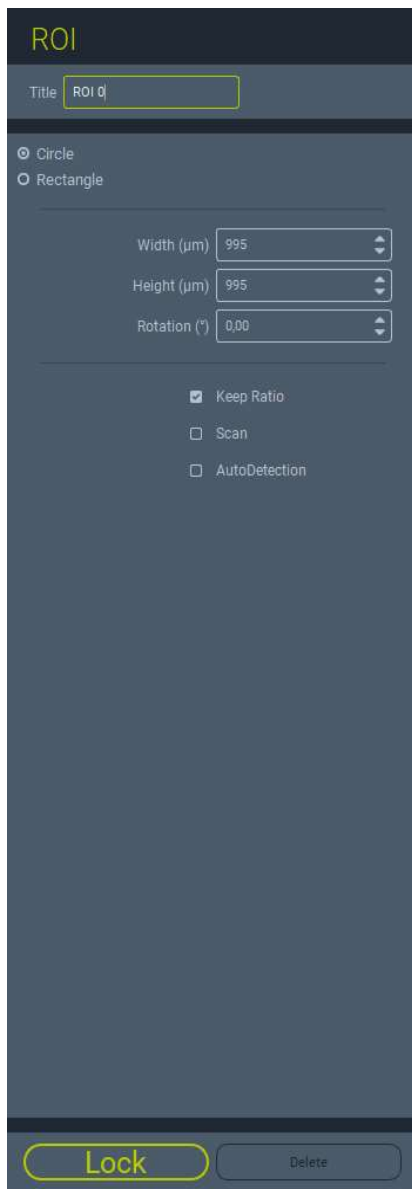
The ROI Panel creates a virtual representation on the Map. It can be used to represent wells or just to place markers at strategic points.

The created ROIs can be either circles or rectangles.

The panel is composed of:
- A title (default is "ROI + *id*")
- A radio button "Circle"
- A radio button "Rectangle"
- "Width" and "Height" fields in µm
- A "Rotation" field in °
- A "Keep Ratio" checkbox: If it is checked (default) changing one the size fields will automatically set the same value to the other
- A "Scan" checkbox
- A "Auto-Detection" checkbox (see Auto-Detection Window)

Before "Locking" the ROI, you can place it precisely by dragging-and-dropping it on the Map.

When the "Lock" button is pressed, the panel is closed and the ROI can't be moved anymore.

If the "Scan" checkbox is checked, Leonardo will do a camera mapping of the area covered by the ROI.

Note that for large areas, this process can take some time.

When the "Delete" button is pressed, the ROI is suppressed.

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4.4.5   PRIMO Panel

Figure 13: PRIMO Panel

The PRIMO Panel creates both a virtual representation of a patterning on the Map and an Action that can be performed using the "Play" button.

The panel is composed of:

- A title (default is "Print + *id*")
- A "Show/Hide" button that allows you to manage the layer displayed on the Map to adjust the position
- A "Preview" button which displays a miniature of the patterning that will be displayed on the Map. This button can also be used to modify the pattern file. Below is a field indicating the path of the current pattern file.

- The "Replication" set of fields is used to manage the number of replications horizontally and vertically and the spacing between the replications in µm.
- The "Sides" and "Centers" buttons allow you to indicates between which part of the replications the spacing has to be considered.
- A "Dose" field to choose the quantity of UV that will be delivered

- The "Final Size" - "Width" and "Height" fields indicate the total size of the item in microns

- For advanced users, the "Expert" area contains fields to tune your patterning more accurately by defining:
    - A "Rotation" (used for alignment on existing printing or plots)
    - If you want to apply "Stitching" or not
    - The maximum power the laser should use in percent. Used with the "Dose" it allows to perform a longer but smoother patterning.

Before "Locking" the Patterning, you can place it precisely by dragging-and-dropping it on the Map.

When the "Lock" button is pressed, the panel is closed and the Pattern's parameters can't be changed anymore.

When the "Delete" button is pressed, the Pattern is suppressed.

4.4.6   Calibration Data Window

Clicking on "See All" in Calibration Data Panel or "Check all Data" at the last step of Calibration wizard will open the following window:

Figure 14: Calibration Data Window

- A "Magnification" field with the value entered for the Calibration
- A "μm/px Ratio" field which correspond to the ratio between the computed DMD apparent size (based on the magnification value) and its size in pixels (basically : **"DMD Height" / 1140**)
- "DMD Width"/"Height" fields which correspond to the computed DMD apparent size in μm (based on the magnification value)
- A "Angle" field which correspond to the computed rotation between the DMD and the camera
- "Horizontal"/"Vertical Shift" fields which correspond to the shift of the DMD to the camera center
- "Horizontal"/"Vertical Ratio" fields which correspond to the X,Y deformation of the image

4.4.7   Preferences Window

Clicking on "Preferences" menu displays a window with the tabs described below. Its purpose is to let you choose the default values you wish to use for most of the parameters. A click on "Save" will apply these modifications in any further use of Leonardo. A click on "Cancel" will ignore the modifications you set.

- **General** :
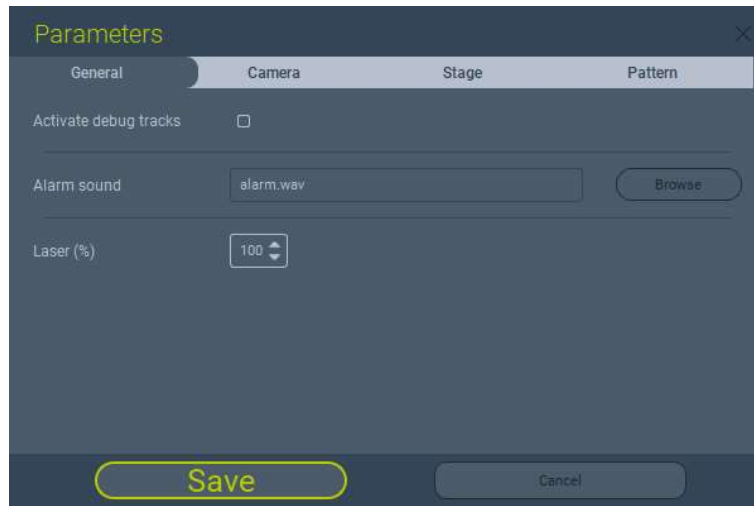
Figure 15: General Tab

- ▪ A checkbox allows to activate debug traces
- ▪ A field (with a Search button) allows to change the sound file played at the end of the timer
- ▪ A field allows to change the default power of the Laser (default is 100%)

- **Camera** :
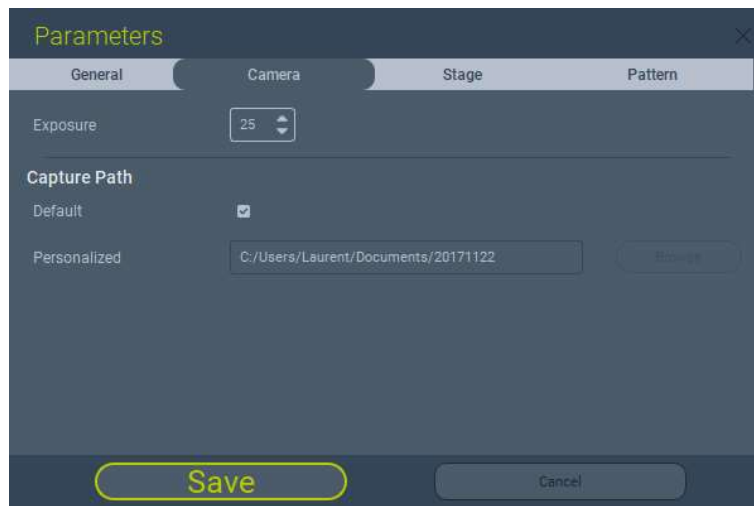
Figure 16: Camera Tab

- ▪ A field allows to change the default exposure of the Camera (default is 25 ms)
- ▪ A checkbox allows to change the image capture directory
  (if checked C:\Users*UserName*\Documents*YYYYMMDD*)
- ▪ If not, a field (with a Search button) allows to select your own directory

- **Stage** :

Figure 17: Stage Tab

- A field allows to change the default movement step (default is 100 µm)
- A field allows to change the default ROI width (default is 1000 µm)
- A field allows to change the default ROI height (default is 1000 µm)

- **Primo** :

Figure 18: Pattern Tab

- A field allows to change the default print angle (default is 0°)
- A checkbox allows to set the Stitching by default for patterns (default is no)
- A field allows to change the default gradient method (default is none)
- A field allows to change the default number of rows replications/spacing (default is 1/0)
- A field allows to change the default number of columns replications/spacing (default is 1/0)
- A field allows to change the default dose emitted at each insolation (default is 30 mj/mm²)
- A checkbox allows to display all the images for each patterning (default is no : Only the surrounding images of the patterns will be displayed)

4.4.8   Auto-Detection Window

When an ROI is created, if the AutoDetection checkbox is checked, LEONARDO will perform a scan (the corresponding checkbox will automatically be checked). After the scan, the dialog box shown below will be displayed.

It contains:

- The image of the scanned area with all found elements surrounded in orange.
- A Slider with +/- buttons to change the zoom level. Each level multiply/divide the zoom ratio by 2
- The collection of patterns that can be used to pattern the elements found
- A "Dose" field to define the dose used to pattern
- A Slider to define the minimum size of elements to detect
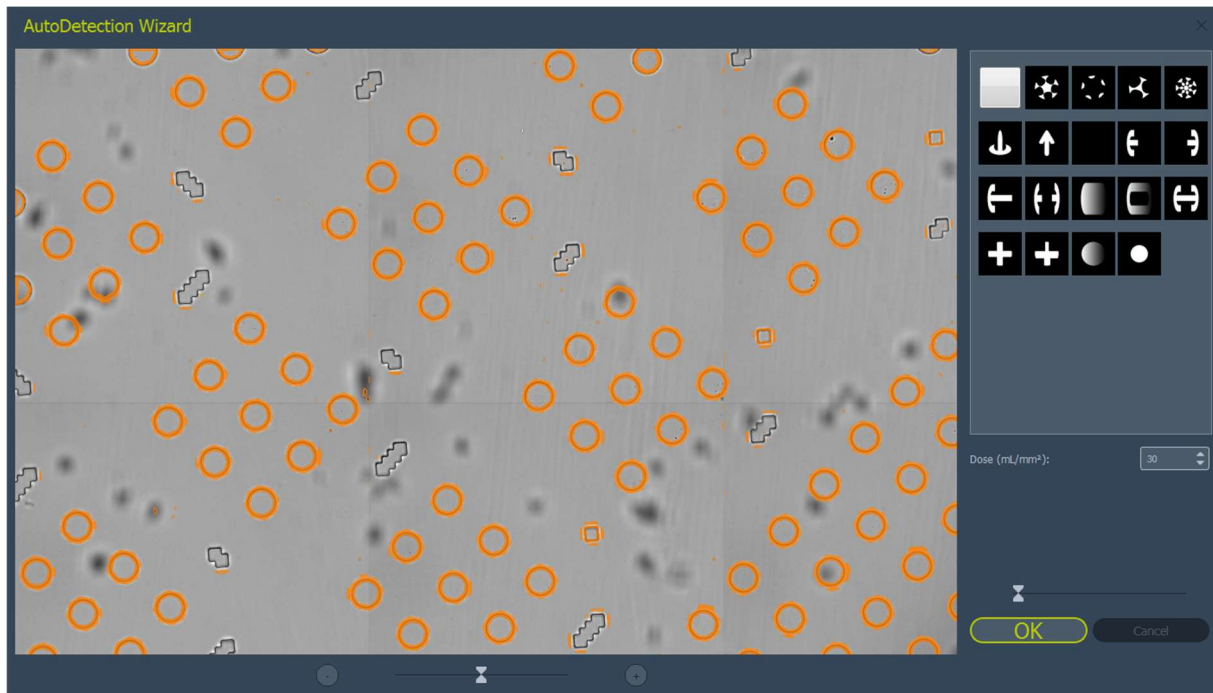
The list of images available is retrieved from the "C:\Programs\Micro Manager 1-4\membwell" directory. If you want to add new pattern templates, you must:

- Create a 200x200px ".tif" file and draw the pattern you want based on the below example
- Save it in the "patterns" directory
- Start LEONARDO, the new templates will automatically appear in the list

As shown in the drawing below, the file(s) created (in black) will be used as follow:

- The pattern (in blue) will be centered on the detected form (in orange)
- The size of the file will be adapted so that the 100x100px center of the file will fit the form
- On each side 50px are available if needed to pattern in-between forms

In conclusion, all the files you'll create should be 200x200px but the pattern drawn inside should be 100x100px. In some cases, depending on the drawing you're doing (the image shown below for example) if you want to be sure to properly pattern the sides of the form, you may need to draw it a little larger than 100x100px

200 px

Marge

Well detected

Marge

100 px
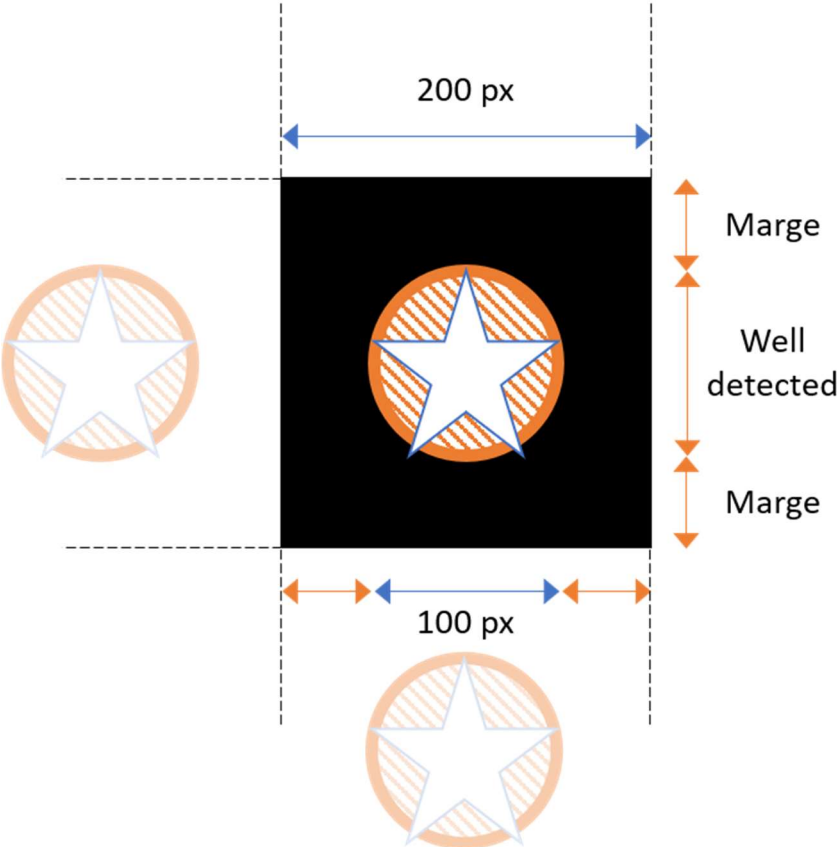
4.5   Software workflows

4.5.1   Course of action

In its new version, LEONARDO is now designed to allow you to prepare your whole experiment (or even many) before launching all the actions in a row. In this way, you can either continue to perform "One-Shot" patterning or define all you want to do first and launch it, allowing you to gain time.

There are as many ways to use LEONARDO as experiments protocols now allows even more freedom and possibilities. Nevertheless, the basics of LEONARDO usage will always be the same. This chapter aims to give you an overview of the main workflows of LEONARDO. Further you'll find links to tutorials for specific usages.

4.5.2   Define your experiment "Map"

If you just need to perform a "One-Shot" pattern, this step can be ignored.

If your experiment contains wells or specific area, you should first consider to represent these areas on LEONARDO's Map using the ROI button (see ROI Panel). It will then allow you to quickly see and spot any action of your experiment.

All the ROIs you define will appear in the ROI List on the left part of the screen (see *Positioning Area*).

4.5.3   Select/Organize your actions

Whatever your experiment is, you'll need to select at least one action to perform. Note that contrary to previous versions of Leonardo, when you create an action, it won't be performed directly but only added to the Actions List (see *Positioning Area*)

After creating all the actions you want, you'll be able to both:
- Reorder them using the Up/Down buttons of the Action List
- Group them using the Group Button
- Deactivate/Reactivate them using the checkboxes of the Action List

These options will allow you to have a more comprehensive report of your experiment(s).

4.5.4   Use the Auto-Detection Wizard

If you want to pattern onto micro-structures you can use the Auto-Detection Wizard. It will avoid you to make all the appropriate image files by yourself and also to align them on the structures. Moreover, it allows you to choose between a group of existing patterns. Of course, you'll be able to add your own patterns to the list.

To use the wizard, set the focus and the light of the microscope in order to clearly see the contours of the structures (see Auto-Detection Window for an example) then click on the "ROI" button, set the size of the area you want to pattern, check the "Auto-Detection" checkbox and then click on "Lock".

Note: The area for Auto-Detection shouldn't be larger than 3x3mm in order to have quick detections.

Once the scanning is finished, the Auto-Detection dialog box appears. By default, all the detected contours will be selected and thus patterned on the edges of the detected structures. To select or deselect elements, perform a right-click drag & drop in the Scan View.

When elements are selected, they are highlighted in green. You can then:

- Change the image to pattern on these elements by selecting another one on the right
- Change the UV Dose to apply on these elements

Click on the "OK" button once you are satisfied with your preparation. LEONARDO will then create and automatically place all the patterns in the view. You can then directly launch the patterning or scan another area.

4.5.5   Launch the process

Once everything is prepared, you are now ready to let PRIMO do its work. To do so just click the "Launch" button at the bottom-right corner of Leonardo. Here is a description of what will happen when you do so:

For each selected action:

- The corresponding configuration panel will appear on the right of the screen allowing you to see what is the current action performed. On this panel, only the "Abort" button will be available.
- Simultaneously, the Estimated Time for the current action will be displayed on the left above the total remaining time.
- By default, the Map view should also be coordinated with the action. If it is a patterning, every time an insolation has been done, the corresponding layer is displayed in blue instead of green.
- When an action is over, its title in the Action List will become green

While the actions are performed, you won't be able to:

- Create, remove or change the order or configuration of the actions because it could impact the behavior of the process
- Use the functionalities of the lists, because their modification could impact the behavior of the process
- Use the Pad Panel (and you **MUST NOT** use the joystick), because moving the stage during PRIMO/NOMOS actions could result in degraded result and broken hardware. (NOMOS can be a few µm from the stage and moving it during process may break its fiber and/or valves)

While the actions are performed, you will still be able to:

- Change the camera exposure or the image threshold value to have a better view of what's going on
- Move onto the Map to see where is the current action happening
- Let Leonardo manage your patterning and gain time by doing any other task that requires your skills.

As soon as an action is performed, it is marked as such (Title in green) and is not editable anymore. You cannot change it, but you can still see the associated information, duplicate it and so on.

4.5.6   Optional: Stop the process

If, for any reason, you want to abort the current course of action, just press the "Abort" button of the currently loaded action (bottom-right). As soon as possible (described below), Leonardo will interrupt its work and gives you back full control of the software. While the right panel is still open and the left panels are greyed, please consider the task as "going on". **Keep the same use restrictions as during action and DO NOT move the stage**.

If you press "Abort", the moment when Leonardo will be able to take your request into account is:

- For PRIMO, at the end of the currently printed DMD

When "Abort" is pressed, it will result in:

- The current action being cancelled BUT marked as performed (not editable anymore)
- If it is a Patterning with multiple shots, the performed shots will be displayed blue on the Map, while the others will stay red
- ALL the following actions being cancelled AND NOT marked as performed

5   Calibration procedure

5.1   Calibration method used

In order to obtain correct patterning results, the software must have access to the following parameters:

- Size ratio between the pixels of the DMD and the pixels of the camera
- Angle between the DMD and the camera (for calculating the positions of the stage during the replication and patterning of large images)
- The X and Y offset between the center of the DMD and the center of the camera
- The magnification of the microscope objective used
- The angle between the Camera and the Stage

These parameters are calculated during the calibration procedure. The calibration method is separated in two parts and based on the following principle:

1st part (Camera/Stage angle & Magnification)

- Turn the microscope light "On" and find a dust on the glass slide
- Make sure that the dust is on the glass side (not on the objective) and that it has a dark and distinct shape to avoid false detection on other similar elements
- By moving the stage, LEONARDO will measure the angle and the magnification used.

2nd part (Camera/DMD angle & DMD position relative to Camera)

- Using a highlighter coated glass slide (the highlighter is UV-autofluorescent)
- Projection of a reference image containing 4 rectangles whose size and position in the image are known
- Acquiring an image of these rectangles with the camera
- Calculation   from   the   coordinate   image   and   the   size   of   the   4   rectangles:
  Measuring the angle between DMD and camera
  Measurement of center-to-center distances between X and Y rectangles
  DMD / camera pixel size ratio and X / Y offset between the center of the DMD and the center of the camera

5.2   Calibration wizard

To complete calibration procedure, follow the different steps proposed by LEONARDO (see figures below):

a. In the "Preparation Page", indicate the approximate value of the objective magnification and the laser power then click "Next" to continue
b. In the "Alignment Page" find a dust, place on the left of the image and double-click in it middle. Wait for LEONARDO to compute the angle/magnification and click "Next" to continue.
c. In the "Focus Page" adjust focus (on the microscope) and visualization parameters (Laser intensity in % and camera exposure time in ms) to obtain a sharp, contrasting image on the camera. Then click on the Calibrate button to launch the calibration processing.
d. The "Calibration Data Page" acknowledges that the calibration is successful. This window will only appear if LEONARDO managed to compute all calibration parameters, otherwise you'll be sent back to the "Focus Page" to update the parameters in order to have an image with a better quality.

If no image appears during the calibration step (c) check that:

- PRIMO is switched on, and the Laser is turned on (key-switch on ON)
- you put a glass slide coated with fluorescent highlighter
- the microscope sends the images to the camera (not through the eye-pieces)
- the filter cube for PRIMO is on the optical path
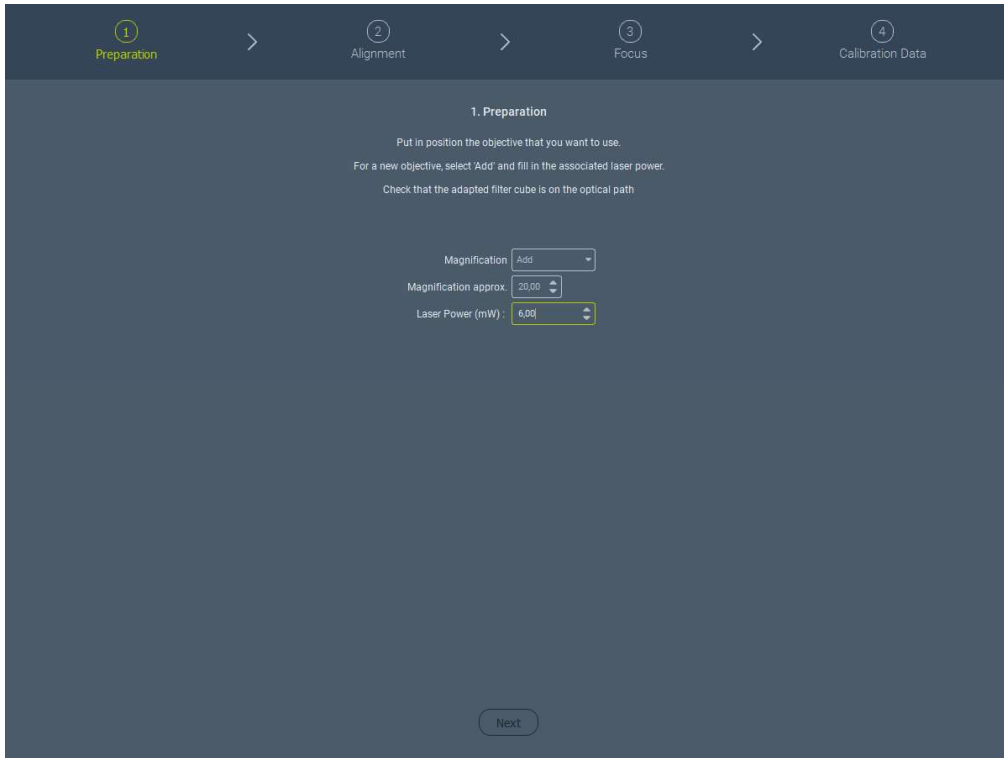- no imaging filter cube is on the epi-fluorescence path
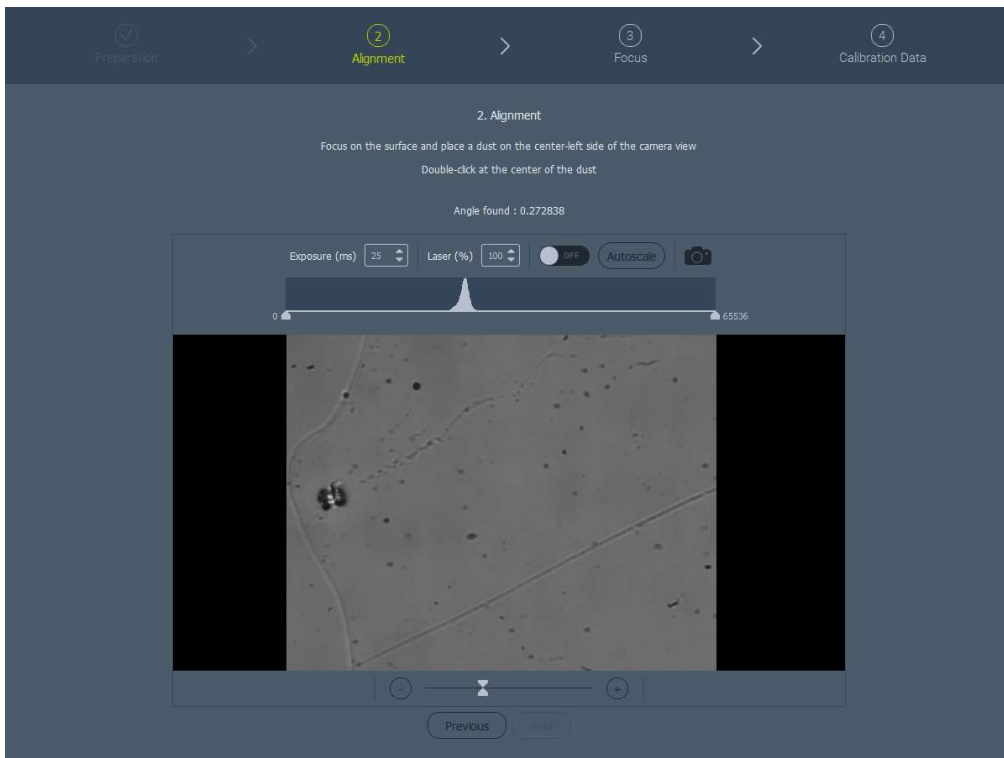

Figure 19: Preparation Page (Calibration Wizard)
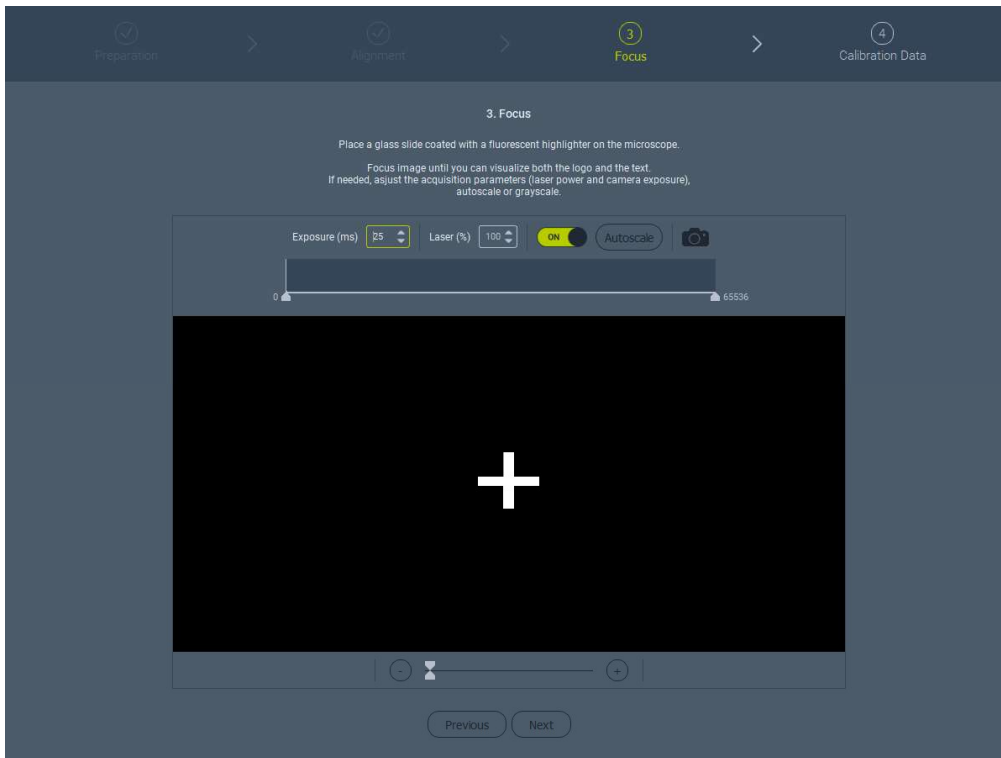

Figure 20: Alignment Page (Calibration Wizard)
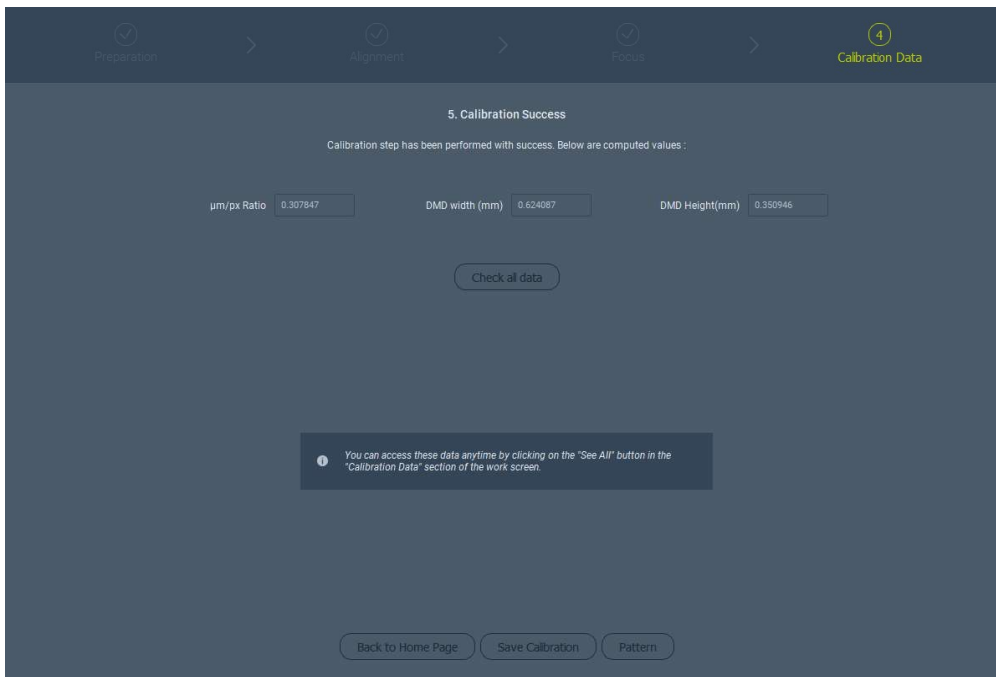
Figure 21: Focus Page (Calibration Wizard)

Figure 22: Calibration Data Page (Calibration Wizard)

6   LEONARDO features and options

6.1   Group creation

A Group allows you to regroup a number of actions under the same name. It mainly helps you organize your experiments.

As shown in the video "Groups.mp4":

- To create a group, just click on the "Group" Action Button on the left of the window.
- Then decide which of the existing actions you want to add to the group by clicking on them and the "Add" button. (Clicking on the "Remove" button will remove the selected action from the group)
- Once you selected all the actions you wanted click on "OK"
- You can expand or coalesce the group in the action list by clicking on the "+" button

6.2   Center stage on element

At any time, you can move the stage back to any element on the view.

As shown in "Center_Stage.mp4", just click on the "Camera" icon next to the element you want to go to in the list.

Note that for ROI, the stage will move to the center of the ROI.

6.3   Center view on element

When the view is not automatically centered on the camera, you can also center the view on an element without moving the stage.

As shown in "Center_View.mp4":

- First, to stop the camera pursuit, click on the "Camera Lock" button at the bottom of the window
- If you now move the stage, it won't be centered automatically anymore
- To center the view on an element, just click on the "Marker" icon next to the element you want to go to in the list

6.4   Reset the session

It is possible to clean the whole view by deleting all the actions and ROIs.

To do so, just click on the "Garbage" icon as shown in "Reset.mp4"

6.5   Save the session

Leonardo allows you to save your session either to remind you of your experiments or to reload it later.

As shown in "Save_session.mp4":

- When you want to save just click on the "Save" button at the top of the window.
- Select the path and the name of the file you want to save to
- Wait for the "Saving" popup to close (depending on the number of elements, the session saving may take a while)

Note that you can save your session either before or after the actions have been performed.

6.6   Load the session

After a session has been saved, you may want to reload it to perform the same experiment again for example.

As shown in "Load_session.mp4", just click on the "Load" button at the top of the window and select the file you want to load.

Note that in all cases, when loading a file, all the actions will be marked as not performed.

7   Feedbacks on LEONARDO

Feedbacks are always welcome! Please do not hesitate to contact us for any issue you may encounter or to suggest improvements. Or just to let us know you enjoy it !

7.1   In case of malfunction

If a software malfunction occurs while using Leonardo, please report it to Alvéole by email to laurent.siquier@alveolelab.com. We will study your problem as fast as possible and work with you to find solutions while correcting LEONARDO. Please when contacting us, follow these recommendations:

- Give a legible and descriptive title to the observed anomaly
- Describe precisely the experimental steps which led to the appearance of the anomaly and allow it to be reproduced
- Give as many details on our setup as possible

Failure to follow these simple recommendations will slow the overall resolution process.

7.2   Request for evolution

We are always willing to improve LEONARDO software to help our users achieve their goals as easily and pleasantly. For any request of evolution address Alvéole by email laurent.siquier@alveolelab.com describing as precisely as possible the experimental scenario you wish to achieve. Also indicate what prevents you from doing it with the current version of the software and what you think can be done to improve LEONARDO. Alvéole will study your request and get back to you as soon as possible.

8   Troubleshooting

Issue	Potential cause	Solutions
Micro-Manager crashes upon start-up or when Leonardo is launched.	Conflict with the Dell Backup & Recovery software.	Uninstall Dell Backup & Recovery: - Open Windows Parameters - Open Application Manager - Find "Dell Backup & Recovery" and uninstall it Note that deleting this software will remove some functionality from your computer. Contact Alvéole if you need some advice.
During calibration or patterning, the projected image suddenly disappears or changes	If you have an NVIDIA graphic card, it is probably a refreshing issue from the NVIDIA drivers	Uninstall the NVIDIA drivers: - Open Windows Parameters - Open Application Manager - Find "NVIDIA Drivers" and uninstall it Note that you'll need generic drivers installed to replace the NVIDIA drivers. Please contact Alvéole if you have doubt on how to install generic graphical card drivers.
	If you don't have an NVIDIA graphic card, it can be due to a software that modifies windows background.	Contact Alvéole so that we can find which software is disturbing LEONARDO.

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