INSCOPER

User Guide

Inscoper I.S.

Interface version : 8.7

I.S

Table of contents

1. CONTACT & LEGAL

Disclaimer, copyright, information about certifications, contact information.

Thank you for purchasing the INSCOPER product.

Please read this manual carefully before using the product. For future reference, please keep it in a safe place.

While every effort has been made to ensure the accuracy of this manual, some errors may remain. Please contact us if any points are unclear.

1.1. Contact

If you have any questions regarding the use of this product, please contact us by e-mail at: support@inscoper.com.

Please specify the following information about your system:

- Product serial number,
- Contact details,
- Any problem(s) you may have.

1.2. Copyright

Changes will be made to the product on a periodic basis and these will be incorporated into new editions of user guides.

1.3. Disclaimer

The information contained in this manual is provided on an “as is” basis, without any warranties, conditions or representations of any kind, whether express, implied, statutory or otherwise, including, but not limited to, any warranties of merchantability, non-infringement or fitness for a particular purpose.

In no event shall Inscoper be liable for any loss or for any direct, indirect, special, incidental, consequential or other damages, regardless of the cause, whether arising in contract tort or in connection with the use of the information provided herein.

1.4. FCC/IC certification

Any changes or modifications to this equipment not expressly approved by INSCOPER may cause, harmful interference and void the FCC authorization to operate this equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

This device must be professionally installed.

2. SPECIFICATIONS

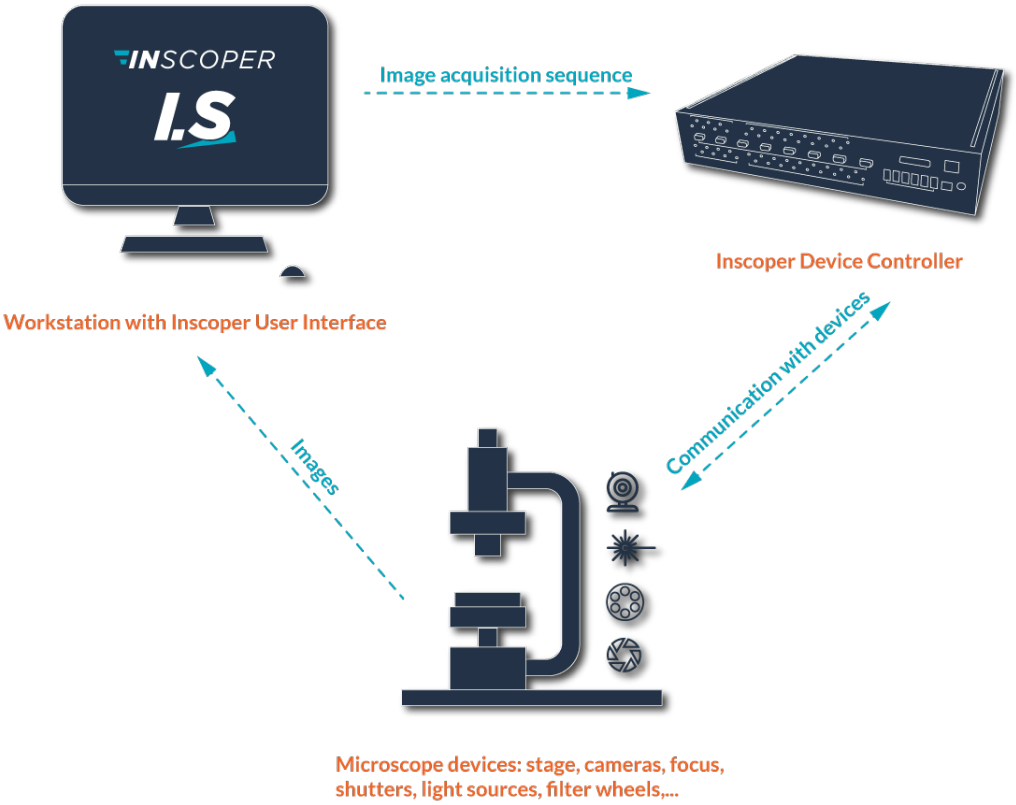
Operating specifications and parameters, input/output connexions, system requirements

2.1. Approach

Inscoper I.S. is a turnkey hardware solution that completely revolutionizes the way in which fluorescence microscopes are controlled in live cell imaging.

Inscoper's fundamental new approach involves dissociating the two functionalities managed by the acquisition software:

1. **User Interaction**: to configure the acquisition sequence, receive the acquired images, and display and save them;
2. **Device Control**: to communicate with the different devices in the microscopy system and run the acquisition sequence defined by the user.



This separation means that the Inscoper I.S. is free from hardware constraints. Therefore, regardless of the type of microscope, the Interface remains simple, easy to use and focused on user requirements rather than hardware issues.

2.2. Inscoper Device Controller

2.2.1. Warnings and cautions

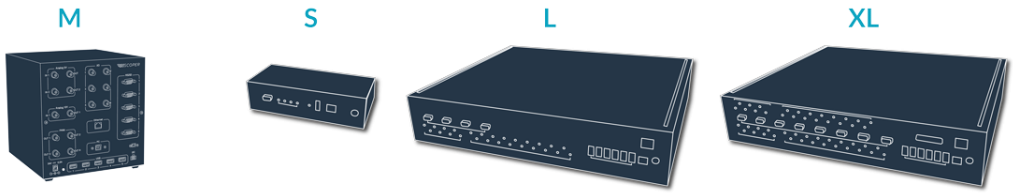
1. Always check that the Inscoper Device Controller is powered up before starting the computer. If in doubt, restart the computer.
2. The equipment can only be powered through Safety Extra Low Voltage that also complies with the limits of 6.3.1/6.3.2 of IEC 61010-1:2010.
3. Never use cables longer than 3 meters to connect devices (except for the Ethernet cable).
4. Please note that if the Inscoper Device Controller is used in a way that is not specified by INSCOPER, the protection provided by the device may be compromised.

2.2.2. Operating specifications

Parameter	Current Version	Next Versions		
Device Controller Model	M	S	L	XL
Weight	3.050 kg (6.724 lb.)	1.150 kg (2.20 lb.)	4.450 kg (8.82 lb.)	4.650 kg (8.88 lb.)
Dimensions (L*W*H)	230*230*230 mm (9.06*9.06*9.06 in.)	120*200*65 mm (4.72*7.87*2.55 in.)	420*434*84 mm (16.53*16.93*3.15 in.)	
Power input	24 VDC @ 2.5 A	24 VDC @ 3.75 A		
Operation temperature range	From 10 to 40 °C (from 50 to 104 °F)			
Altitude	Maximum 2000 m (6561,68 feet)			
Operating humidity range (non-condensing)	From 30 to 85 %			
Storage temperature range	From 0 to 50 °C (from 32 to 122 °F)			
Storage humidity range (non-condensing)	From 30 to 85 %			

NB: All there data are valuables for indoor use only.

2.2.3. Input / Output



<table>
<tr><td></td><td>Current Version</td><td colspan="3">Next Versions</td></tr>
<tr><td>TYPE</td><td>M</td><td>S</td><td>L</td><td>XL</td></tr>
<tr><td rowspan="2">ANALOG OUTPUTS</td><td>2x (0-5V)
1x (0-12V)</td><td>1</td><td>8</td><td>16</td></tr>
<tr><td>DAC resolution 12 bits
No Programmable Output range</td><td colspan="3">DAC resolution 14 bits
Sample rate 180 MS/s
Output range ±10 V, 0-5 V, +-5 V</td></tr>
<tr><td>ILDA</td><td>Via External Controller (MaxILDA)</td><td>-</td><td>-</td><td>1</td></tr>
<tr><td rowspan="2">ANALOG INPUTS</td><td>2x (0-5V)
1x (0-12V)</td><td>-</td><td>-</td><td>16</td></tr>
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No Programmable Input range</td><td colspan="3">ADC resolution 16 bits
Sample rate 1 MS/s
Input range ±2.5 V, ±5 V, ±10 V, ±12.5 V</td></tr>
<tr><td>I/O</td><td>6</td><td>4</td><td>18</td><td>18</td></tr>
<tr><td>SERIAL PORTS</td><td>5</td><td>1</td><td>4</td><td>8</td></tr>
<tr><td>USB host</td><td>5</td><td>1</td><td>6</td><td>6</td></tr>
<tr><td rowspan="2">Computer</td><td colspan="4">Windows 7/10/11</td></tr>
<tr><td colspan="4">1920 x 1080 px</td></tr>
</table>

2.3. System requirements

	Minimum requirements	Optimum configuration
Operating system	Windows 7 / 10 32 bit / 64 bit MAC OSX 10.5	Windows 10 64 bit MAC OSX 10.5
RAM	4 Go	16 Go
Hard disk drive	4 Go	128 GB SSD drive for fast image saving
Processor	Pentium 2 266 MHz	Core i5 3.2 GHz
Graphics card		NVidia GeForce 8 and 100 series or higher ATI Radeon HD 2400, 3000, 4000, 5000 and 6000 series Intel GMA 4500 and GMA HD
Screen	Resolution 1920 x 1080	2 screens highly recommended

2.4. Installation

The Inscoper I.S. should only be installed by INSCOPER staff or appointed representatives. The customer and/or user can be involved in the installation process provided explicit consent has been given by an INSCOPER representative. In the event of intervention on the microscopy system with INSCOPER equipment or software without INSCOPER's consent, the company declines all responsibility for any consequences resulting from this intervention.

To **request installation** of Inscoper I.S. on a microscope, the following three steps are necessary:

1. Send a list of all your devices connected to the microscope to contact@inscoper.com or via the dedicated form available at www.inscoper.com.
2. Give INSCOPER team **three-day access** to install the microscope.
3. **Test out** the microscope with your team: acquire images of your research samples and compare them with previous ones.

3. IMAGING SOLUTIONS

From core solution to custom techniques

- Inscoper I.S.
- Inscoper scanFRAP
- Inscoper fastFLIM
- Inscoper liveRATIO
- MAICO I.S.

NB: The Inscoper I.S. section covers all the general information on calibrating devices, common interface elements, dimensions setup and visualization tools. Specific information for each product is presented in its respective guide.

3.1. Inscoper I.S.

Incorporating a specially-designed device, Inscoper I.S. provides a new user experience with improved technical performance, system integration and ease-of-use.

Inscoper I.S. is a user-friendly solution that gives microscope users a very efficient interface which interacts with their system:

- Configure acquisition sequences,
- Control the camera, receive, display and save the acquired images,
- Follow the state of the microscope in real-time,
- Save or load projects,
- Edit acquired images with some basic operations.

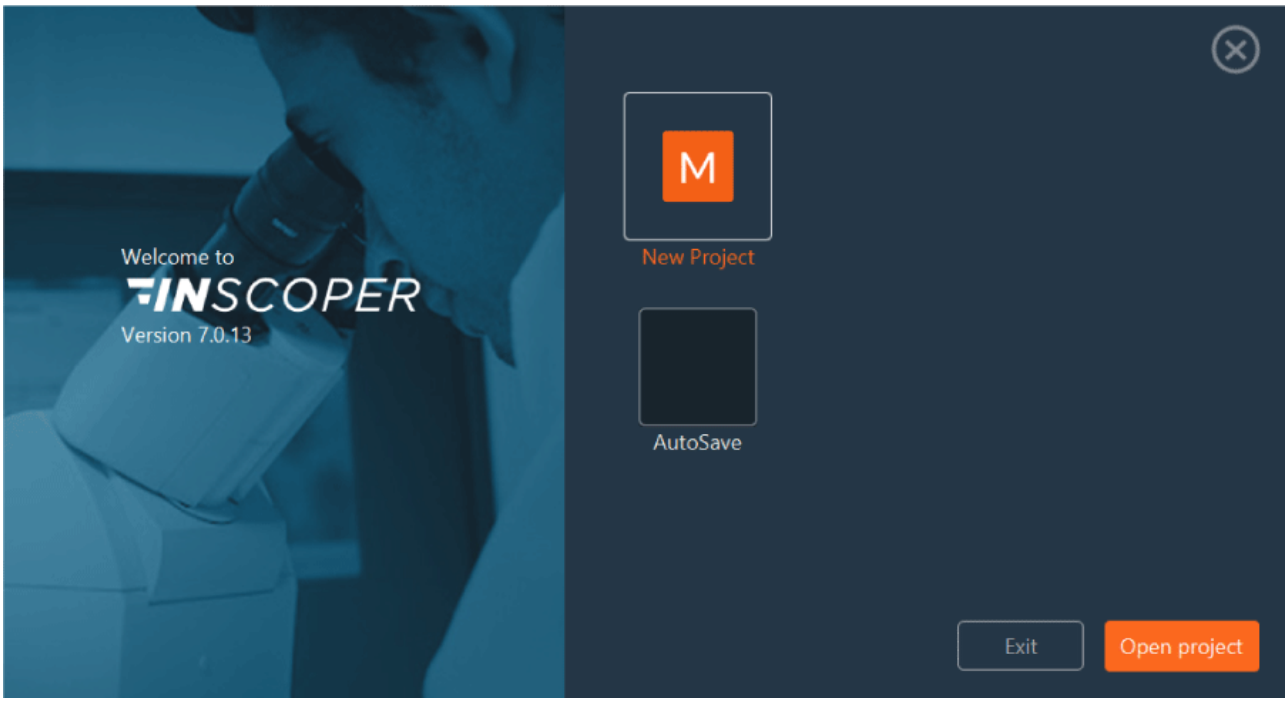
3.1.1. User journey

The Inscoper I.S. interface was designed as a user journey to help new users get accustomed to it and make it enjoyable for frequent use by advanced users.

The user journey has 3 successive stages:

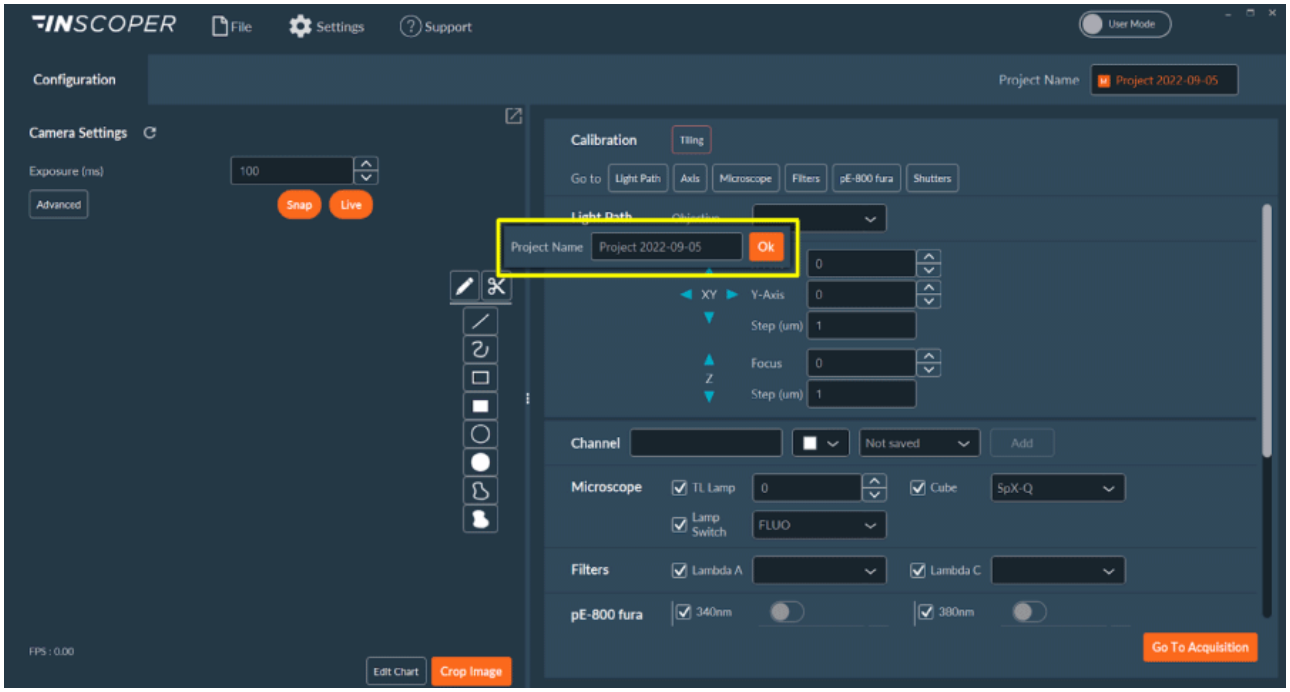
1. Configure the microscope's channels and general settings (Configuration Tab).
2. Define specific settings for the required image acquisition sequence. These settings are displayed as "dimensions" that can be combined and interlinked. Run the image acquisition sequence. Stop/Pause is possible.
3. View and/or manipulate acquisition results as raw images and graphics.

3.1.2. Getting started



When you launch the Inscoper I.S. software, the start window will appear. You will have access to the software version number and can choose from several options to begin your user journey:

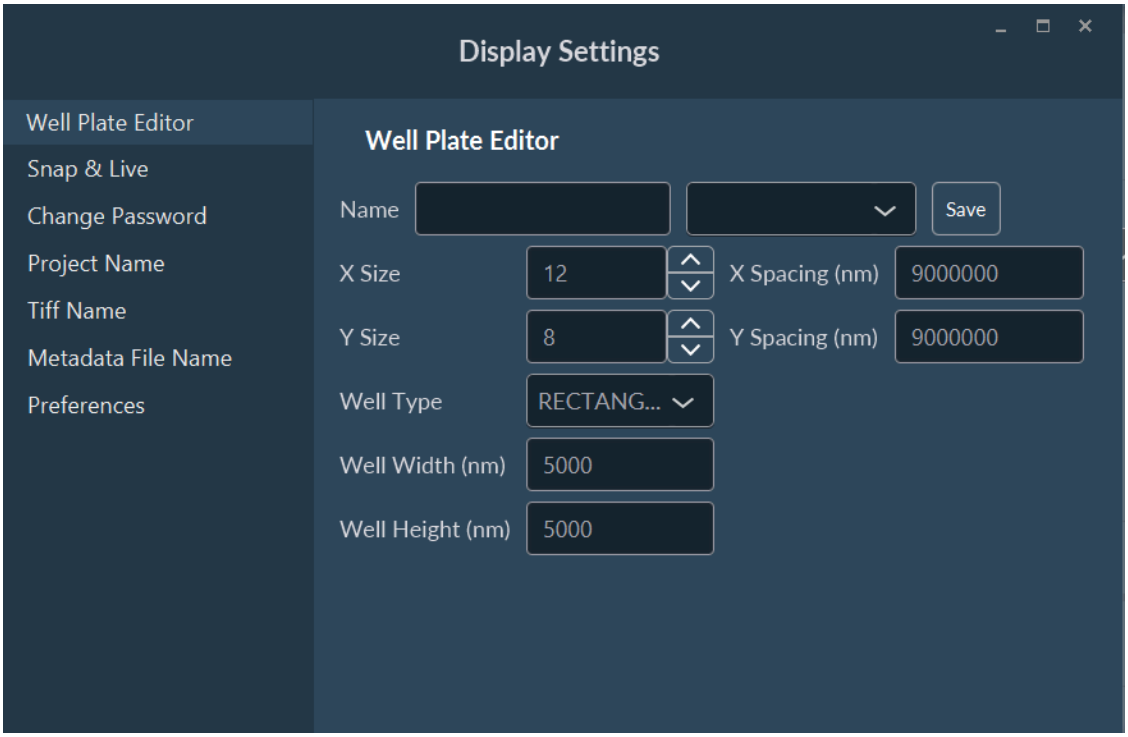
New Project	To create a new acquisition sequence, click on **New Project**. A loading screen will appear while the interface recognizes the devices of the microscope. Wait until it disappears before starting your project.
AutoSave	The **AutoSave** button opens the most recently created project, even if it has not been saved. If the configuration settings have been saved, you can resume from where you left off.
Open Project	Click on **Open Project** to open an old/existing project. Select your file (.cbf) in the tree structure, then click on **Open**. Your project will be recalled with the settings that you saved.



You can customize the name of your various projects. If you don't, the default project name is the current date.

3.1.3. Display settings

To access the interface settings, click on **Settings** > **Display Settings**.

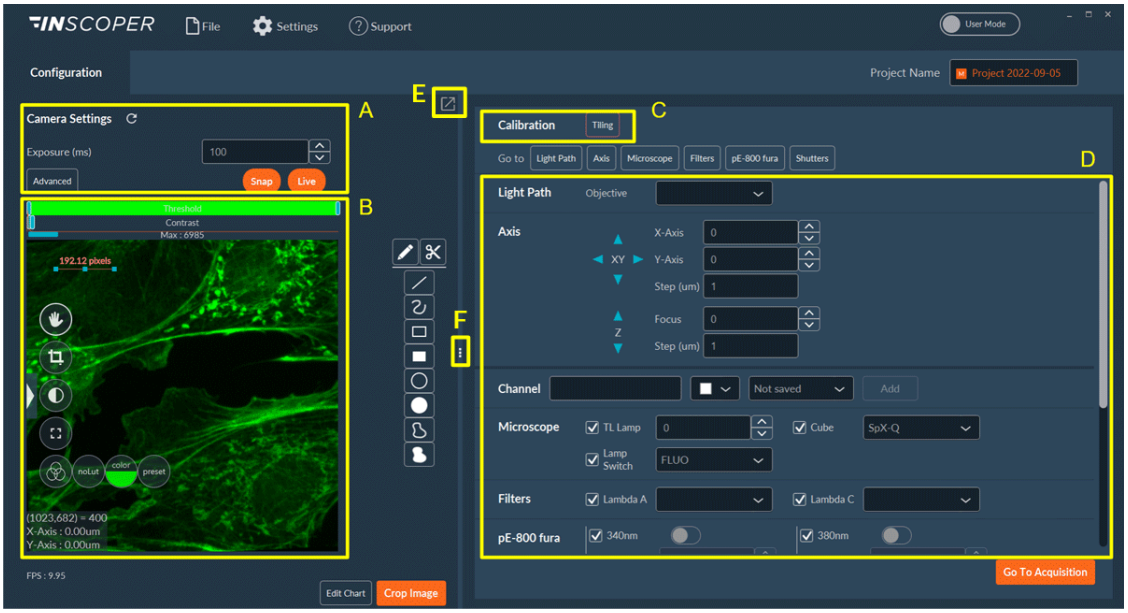


This section allows you to make some settings, such as:

1. Edit your well plate.
2. Set options for the snap.
3. Set or change the password to switch from user mode to expert mode.
4. Set the default name of your project.
5. Change the default name of the images saved during the acquisition.
6. Select the units of measurement and choose the default data processor for your acquisition, activate some warnings (if your interval between 2 time points is not enough or if there is not enough space on the disk for the storage).

3.1.4. Configuration

You can use this tab to control the state of each motorized device of the microscope in order to find the working focus plan and select the optimum parameters of the camera being used.



The screen can be divided into 4 parts:

- A - camera settings,
- B - live image viewing,
- C - calibration protocols,
- D - access to all the motorized devices of the microscope and the creation of channels and the saving of them for all your acquisitions.

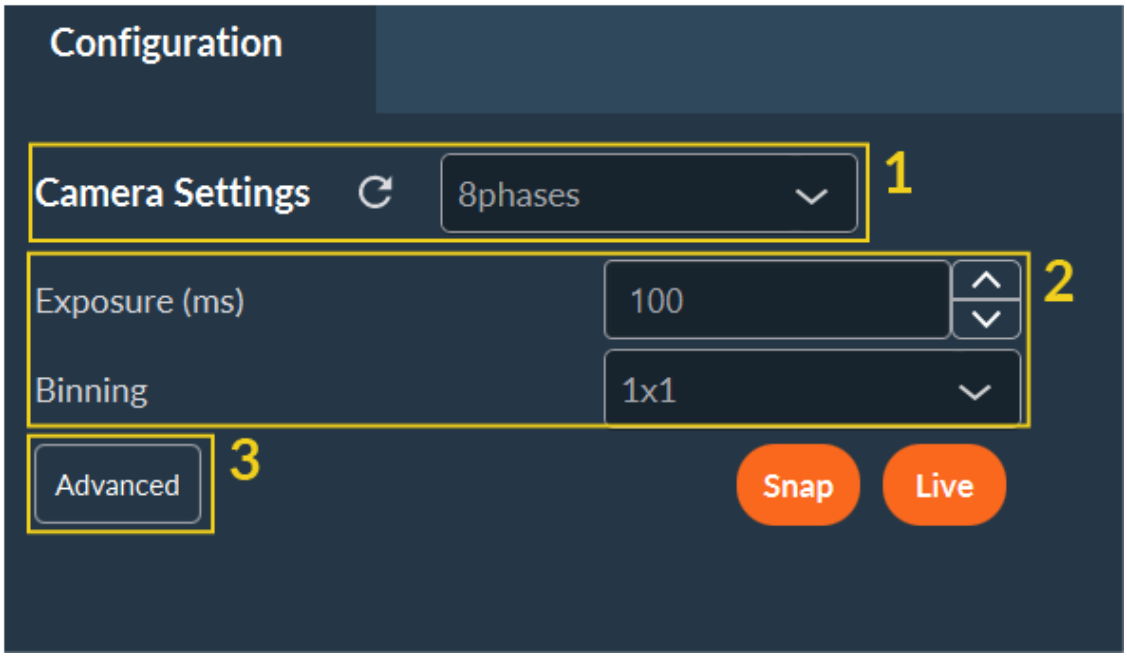
- E - If you have two screens, you can split the interface in two by clicking on this button. This way you can display the camera view on one of the screens (the image will be much larger), while the parameters will remain on another screen.
- F - If necessary, you can move the dividing bar between sections to enlarge one or other section (camera view or settings area).

You can modify the name of the project in the top-right corner of the screen. The default file name can be set later in the settings.

3.1.4.1. Process images

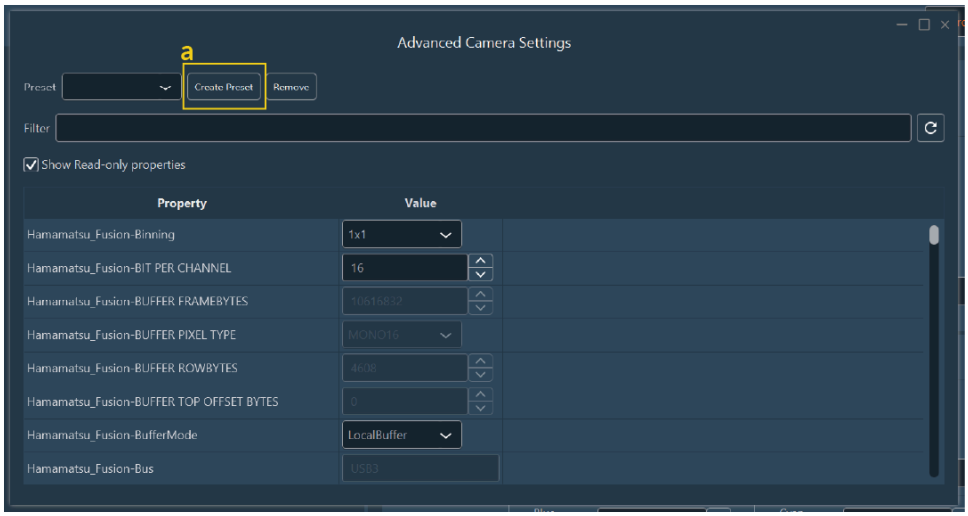
3.1.4.1.1. Camera settings

You can have up to 4 cameras on your system, and you can create presets for each of them.



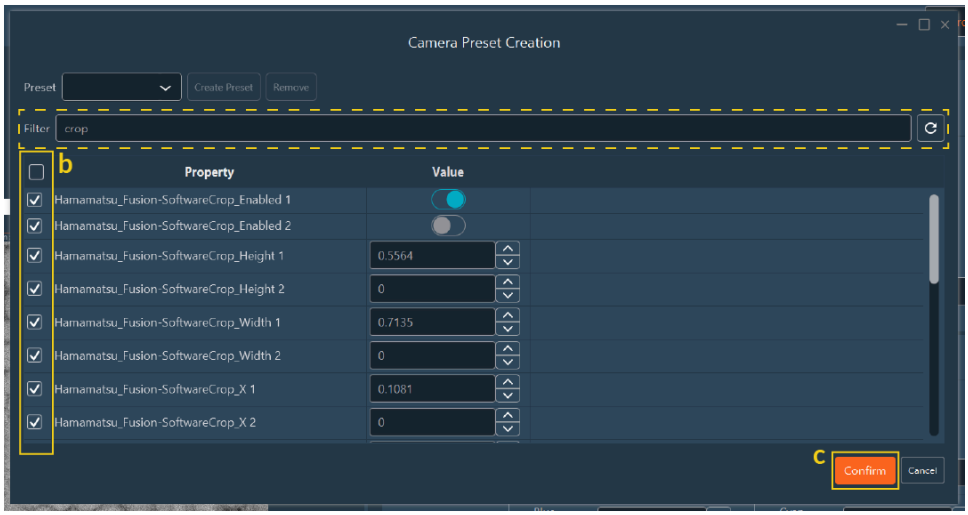
1. If you want to use the camera preset, you can select it in the drop-down list.
2. Set the exposure time and binning.
3. You can also have access to all **advanced settings** of the camera and create a camera preset:

a. Click on **Create Preset** :

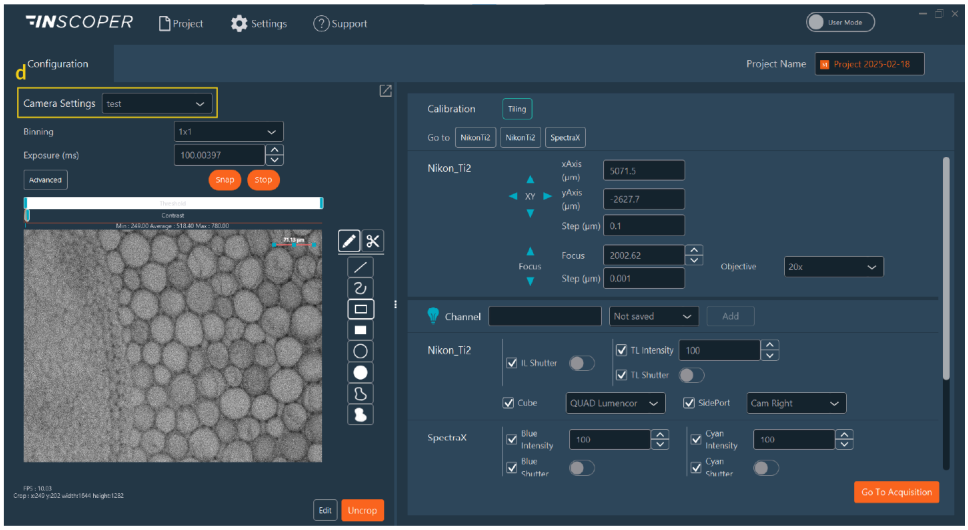


b. Select the parameters which will be saved in the camera preset by checking boxes. You can filter the parameters by writing in the search bar.

c. Then **Confirm**:

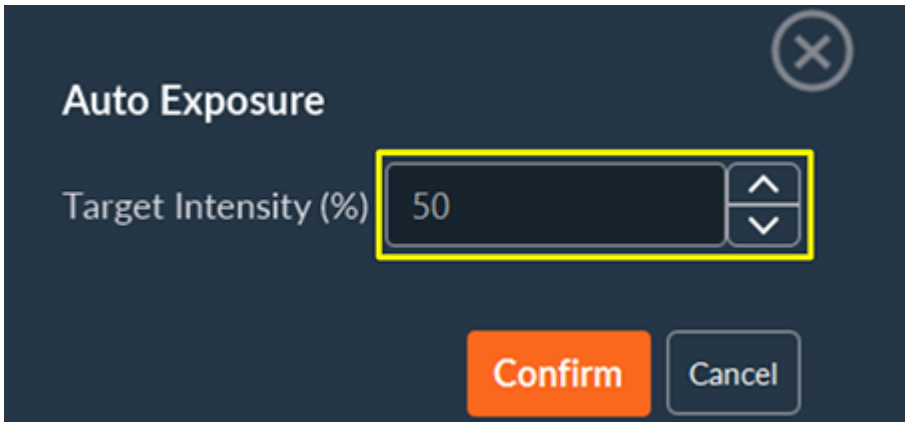


d. Choose the name and location for your camera preset:

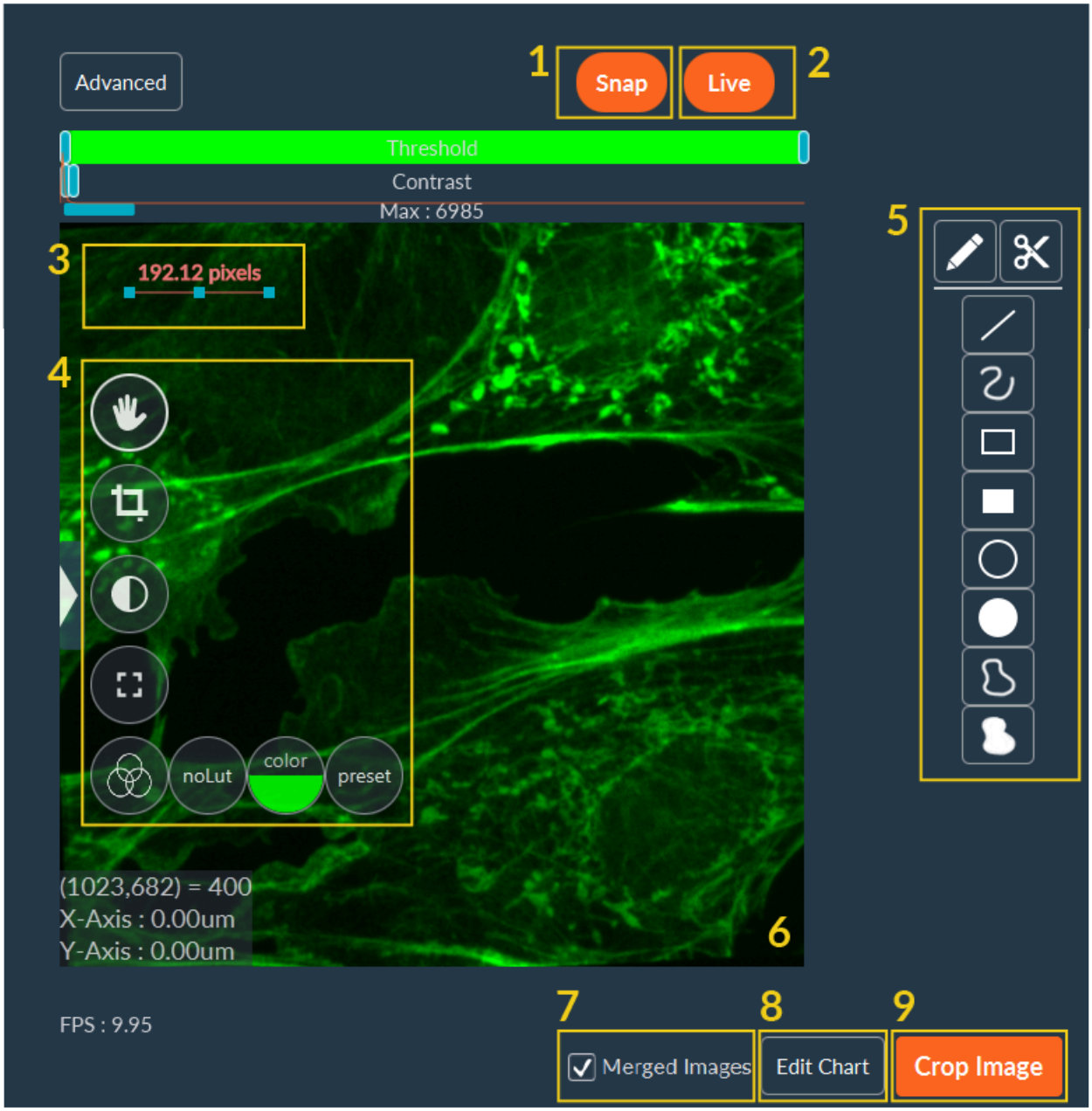


NB: If you have two or more cameras, you can view all cameras simultaneously in **Live**.

Right-click on the live image to access the **Auto Exposure** mode. This mode is useful for automatically adjusting the exposure time of the camera to avoid pixel overload. First, this option automatically calculates the intensity of the pixels in the image in real time. The contrast is then adjusted to the value you that you have requested.



3.1.4.1.2. Interaction with the live image



1. **Snap**: to take a snap of the current image.
2. **Live**: start/stop the camera using the button.
3. **Scale bar**: double click on it to modify orientation and length.
4. Tools to interact with the image (see more here).
5. **ROI tools**: draw some shapes on the image to perform ROI or crop.
6. **Numerical zoom**: turn the mouse wheel over the image.
7. **Merged images**: If you have more than one camera, you can merge the images from all the cameras in Live.
8. **Edit Chart**: possibility to see a real-time graph at the bottom of the image.
9. **Camera crop**: software or hardware crop.

3.1.4.1.2.1. Tools to interact with the image

	You can move inside the live image by drag-and-drop.
	You can select this option to add some ROI or make some crops on live images.
	You can choose this option to set the contrast automatically or manually. If it is manually, adjust the blue sliders on top of the camera view.
	Press this button to switch to full screen mode. To close this mode, press this button again or click on the cross in the top-right corner.
	You can change the LookUp Table (LUT) in real time using this option. You have 3 options: - no LUT; - LUT with one color; - Preset LUT.

NB: Some LUT are presetted like:

- **Pixel indicator** showing in red the overloaded pixels
- **Inscoper ratiometric** dedicated to ratiometric images visualization
- Conventional multicolor LUT as “fire”, “physics”, etc...

3.1.4.1.2.2. ROI creation

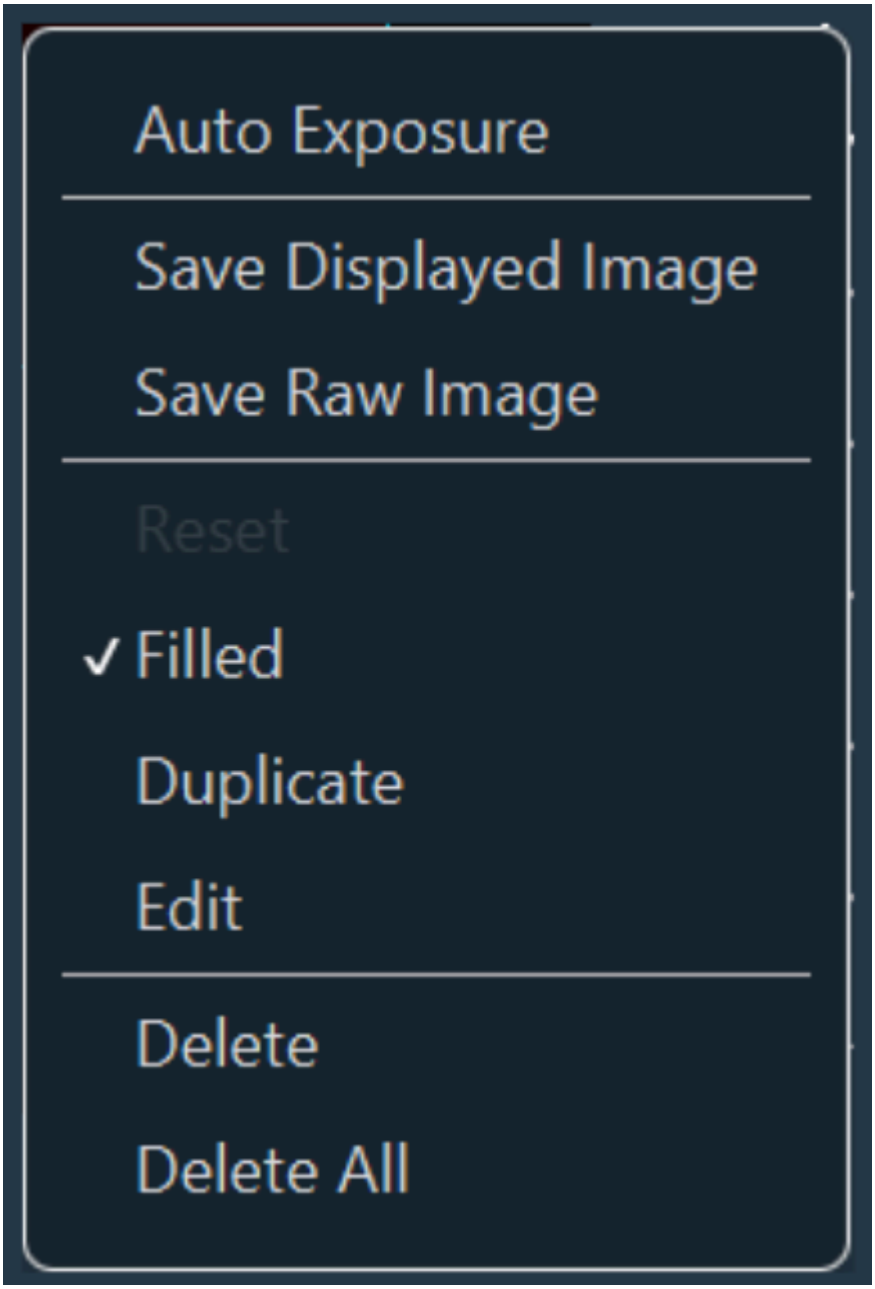
To create a ROI on the image, you can choose between two modes:

	The **pen** mode allows you to add one or multiple ROI.
	The **scissors** mode allows you to cut (remove) into a full shape while retaining the surrounding selected area.

Then, use the shape tools to draw desired shapes:

	Draw a straight line.
	Draw a freehand line.
	Draw the edge of a rectangle.
	Draw a filled rectangle.
	Draw the edge of a circle.
	Draw a filled circle.
	Draw a free form edge.
	Draw a filled free form.

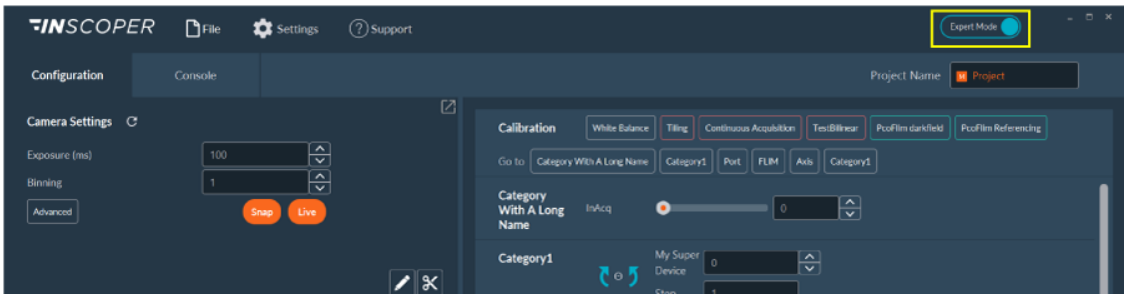
3.1.4.1.2.3. ROI manipulation

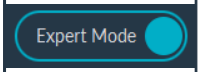
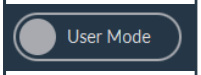


When you make a right click on a ROI, you have access to a new window dedicated to the manipulation of all these areas. You can fill some areas, duplicate and/or edit ROI. By editing the ROI, you can manually give dimensions of this form and center it in the camera field of view. You can also remove one or all of them.

3.1.4.2. User/Expert display modes

Two accreditation levels are available in Inscoper I.S., according to the user expertise in the microscopy field.

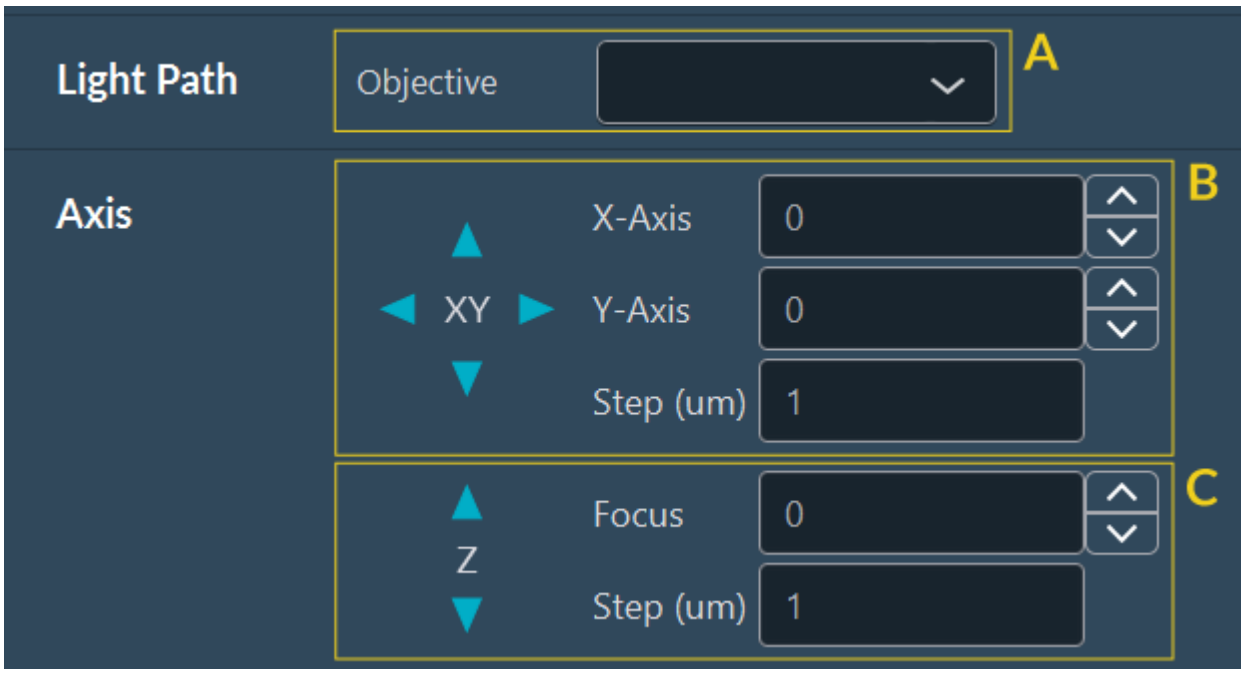


	**Expert Mode** allows access to all settings and parameters of the system, without any restriction. This mode is basically dedicated to microscope facility managers or researchers familiar with microscopy. The “Expert” users have to prepare and save some protocol that will be reused by basic users.
	**User Mode** allows a restricted access to some settings and parameters. The restrictions are fully customizable, from basic channel configuration to most advanced settings of the camera(s) or any other devices. This mode is dedicated to biologists that are not familiar with microscopy. Here, they just have to load some protocol already prepared and apply them on their samples.

NB: Switching from **User Mode** to **Expert Mode** is possible at any time. A password can be set to access the **Expert Mode**. These accreditation levels are optional, according to the use of the system.

3.1.4.3. Control of motorized devices

3.1.4.3.1. Interaction with motorized devices

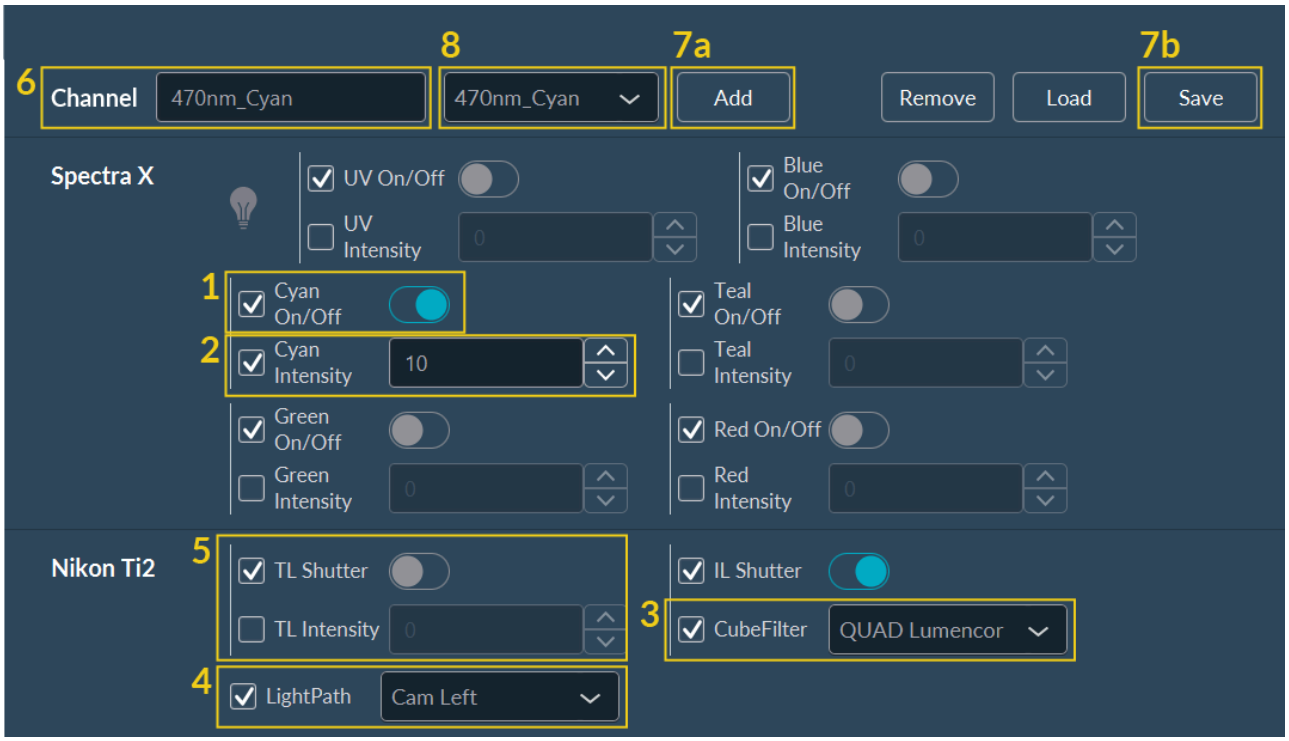


You have access to all devices controlled by Inscoper I.S., including microscope, stages, shutter, light source, wheel filters, piezo, microfluidic element, …

With this panel, objectives (A) can be automatically changed.

You also have access to a virtual joystick to move the XY stage (B) and the Z-focus (C) of the microscope.

3.1.4.3.2. Create/Load a channel



The expert users (if this option is activated) have access to all optical motorized elements of the microscope, like wheel filter, dichroic cube and light source. To manually create a fluorescent channel, you should follow the steps below:

1. Select the right excitation source.
2. Set its intensity.
3. Adjust filter cube.
4. Select the LightPath.
5. If necessary, select brightfield light instead of or in addition to fluorescence.
6. Name your channel.
7. Save it:
    a. temporarily by clicking on **Add** (User Mode)
    b. permanently by clicking on **Save** (Expert Mode).
8. The created channel will be available in the list.

NB: Some systems have more than one camera port. It is necessary to select the right one before imaging.

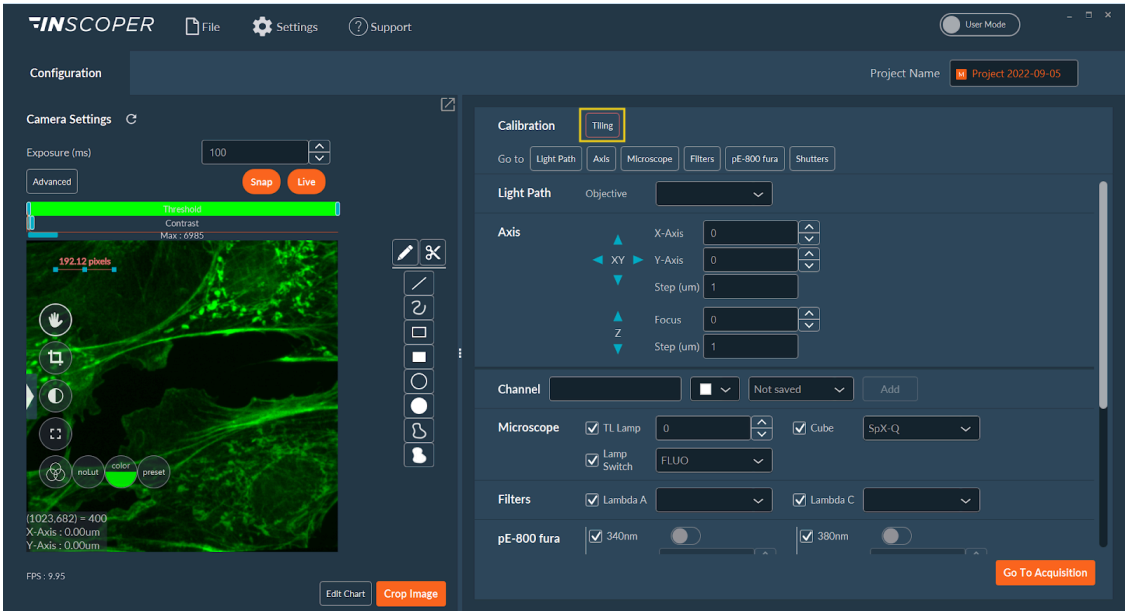
3.1.4.4. Calibration protocols

3.1.4.4.1. Tiling calibration

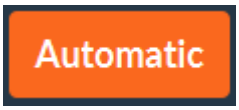
The tiling tool is used to image large samples. The whole final image is divided into several images, called "tiles", acquired one by one and "stitched" afterwards to constitute the whole sample.

In order to use the tiling tool as efficiently as possible, it is necessary that the orientation of the camera is the same as the orientation of the stage. The calibration phase is used to make this adjustment.

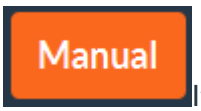
Click on **Tiling** in the Calibration section (ff not yet calibrated, the button will be colored orange).



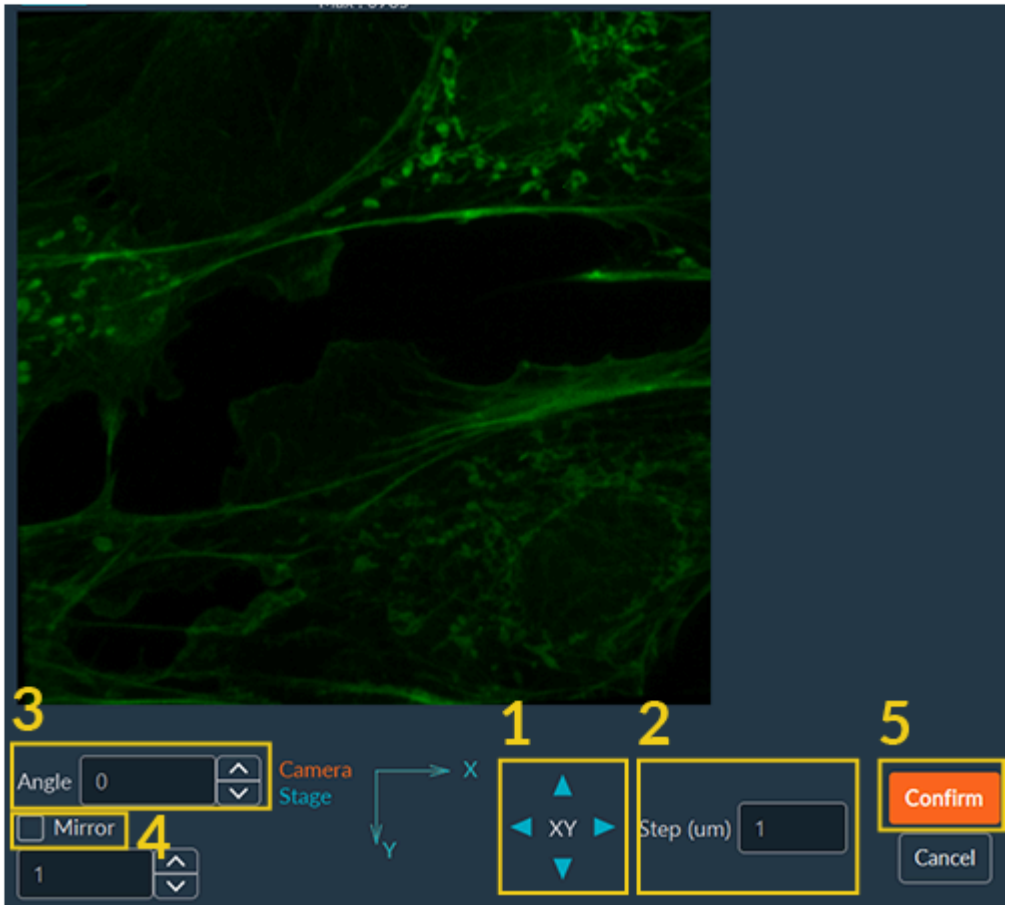
Calibration protocol can be performed according to two different approaches: **Automatic** or **Manual**.



This mode is a fully-automated calibration protocol. You have nothing to do, except click on **Automatic** and validate the calibration at the end.



It is a semi-automated mode. Here, you have to test and validate the orientation of both camera and stage using the software.

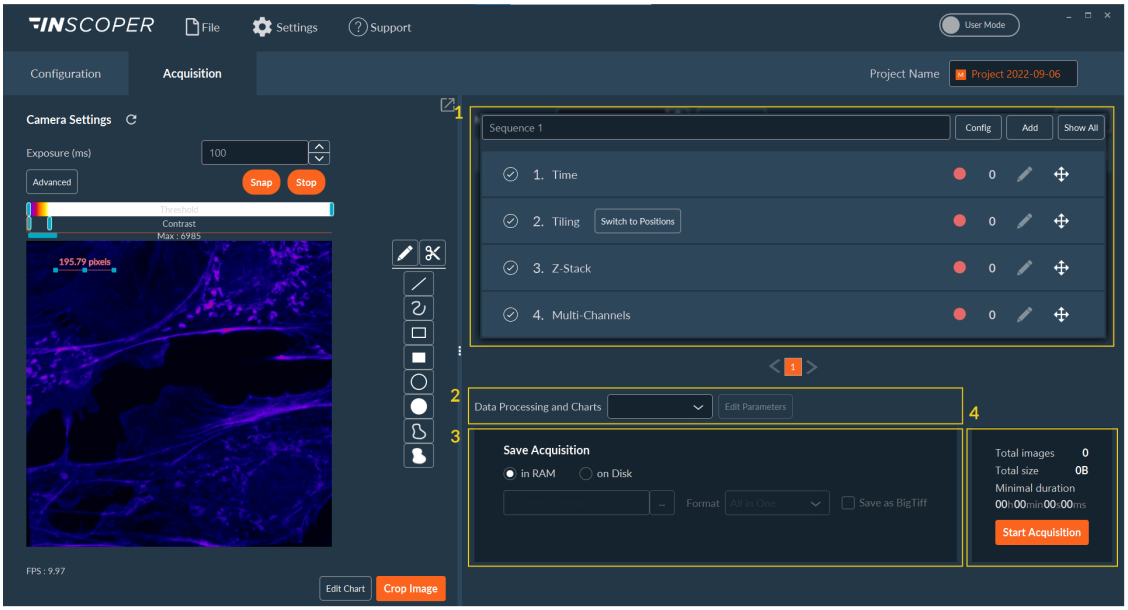


NB: First, be sure that the camera is switched on **Live**.

1. Use the virtual joystick to move the X and Y and observe whether the joystick and the camera have the same orientation.
2. Adjust the step if necessary.
3. If the orientation is not the same, you can add a rotation.
4. You can add a mirror effect on the image if necessary.
5. Click on **Confirm**.
   Once the calibration finished, the Tiling button turns green.

3.1.5. Acquisition

This tab allows you to set the entire acquisition sequence, using all the system's dimension modules.



This tab can be used to prepare a **multi-dimensional acquisition**.

1. Choose the dimension(s) from a large panel.
2. Select the right data processor for your acquisition.
3. Set the path for the backup files.
4. Launch your acquisition.

The interface may be different (especially the dimension's list) depending on the type of equipment in your microscope system.

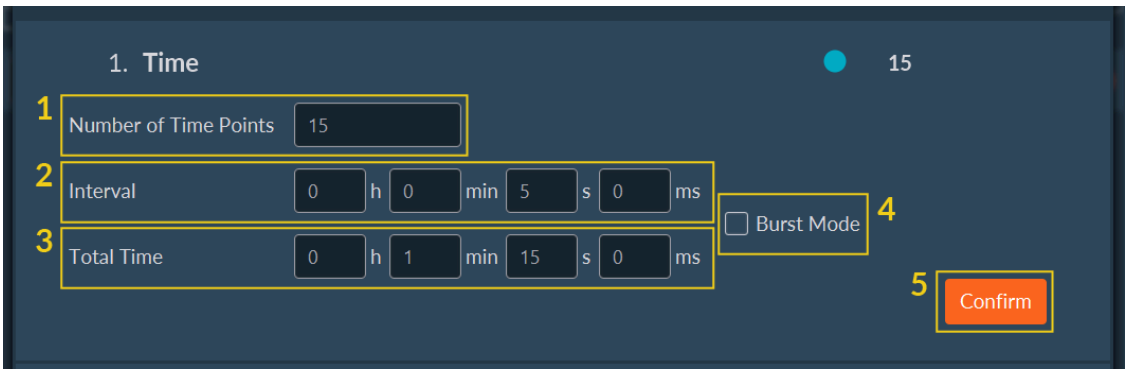
3.1.5.1. Dimensions

All available dimensions are displayed in the same way. The list of UI controls for the dimensions set up is as follows.

	The dimension is not activated and won't be included in the acquisition sequence. Click on to activate it.
	The dimension is activated and will be included in the acquisition sequence. Click to de-activate.
	Dimension parameter set.
	Click to set up the dimension according to your application.
	Drag and drop to change the order of all dimensions to suit your needs.

3.1.5.1.1. Time

The **Time** dimension is used to run time-lapse and image cells over a long period of time.



1. Determine the required **number of time points** for the whole acquisition.
2. Set an **Interval** between each time point.
3. The **Total Time** value is set automatically after the number of time points and the interval between them have been set.
4. Activate the **Burst Mode** checkbox if you want to take pictures as quickly as possible.
5. When you have finished editing the dimension, click **Confirm**.

3.1.5.1.2. Multichannels

This dimension is useful to select two (or more) channels and add them in the acquisition sequences.

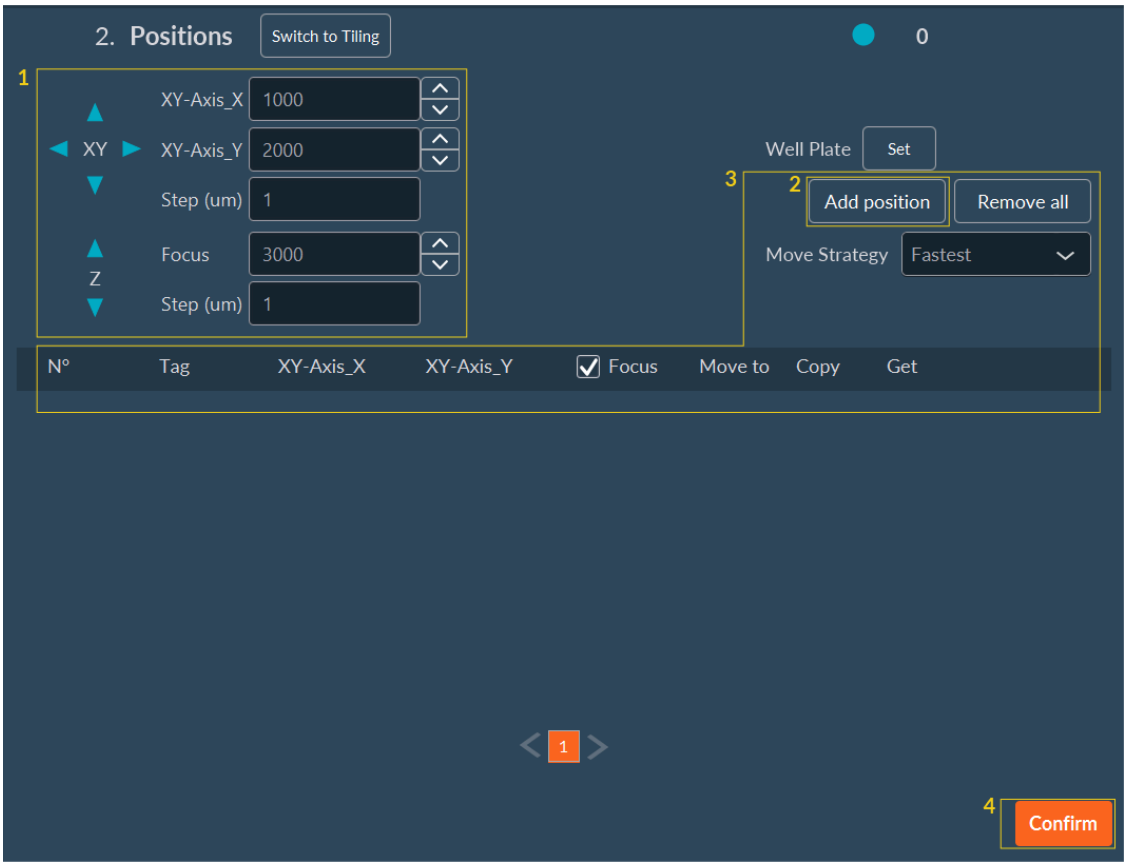
1. Click on **Add Channel**.
2. Select it in the list of the pre-configured wanted channel and customize their settings according to the sample.
3. For each channel, you can apply some additional parameters:
    a. **Z-stack**: apply z-stack dimension;
    b. **Z-Offset**: apply a different offset if the focus varies;
    c. **Shutter Blink**: optimize shutter control to limit sample exposure and prevent photobleaching;
    d. **Camera**: select the one that you would like to use;
    e. **Exposure(ms)**: adjust exposure time;
    f. **Intensity:** adjust the excitation light (depending of the light source of your system);
4. Click on **Live channel** to have a preview of your settings.
5. Once you have finished editing the dimension, click on **Confirm**.

> NB: After customizing the exposure time of the light intensity, click again on **Live channel** to update the image.

> NB: You can drag and drop channels to change their order.

3.1.5.1.3. Positions

This dimension is dedicated to image different XYZ positions from a sample. You can switch from this dimension to the **Tiling** dimension by the button **Switch to Tiling**.



To use the **Positions** dimension:

1. Move the stage and the focus to the desired position.
2. Click on **Add position** to add the XYZ coordinates in the position list.
3. Make other adjustments or manipulations if necessary:
    a. **Add position**: add a new position/s in the position list;
    b. **Remove all**: delete all position from the position list;
    c. **Move Strategy**: When performing a multiposition acquisition with a significant distance between two positions, two options are available for the stage moving between these two positions:
        - **Fastest:** Move directly from position A to position B;
        - **XY Split:** Move between 2 positions with small steps. Indicate the step by filing the Step (µm);
    d. **Tag**: Users can add names to the selected positions;
    e. **X-Axis/Y-Axis**: change/adjust position if necessary;
    f. **Focus**: Use the focus value used for the selection of the positions. If the checkbox is not selected, the current focus value of the microscope will be used.
    g. **Move to**: move the stage to this position;

h. **Get**: update coordinate of the position by the actual XYZ position of the system;

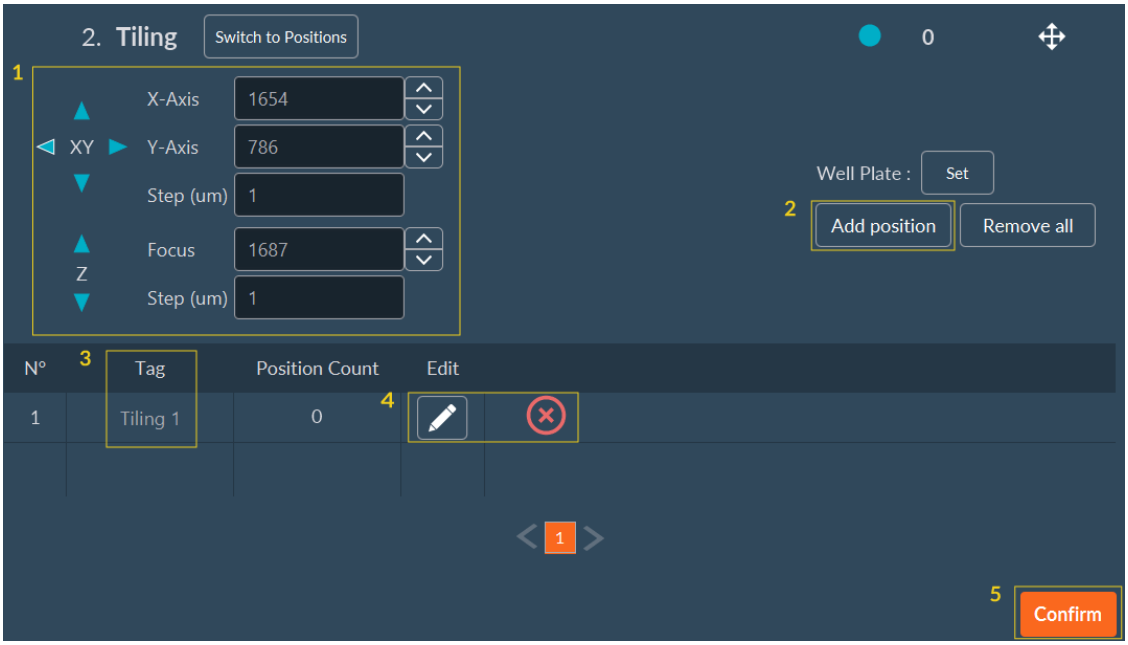
i.  : delete this position from the position list.

4. When all positions are set up, click on **Confirm**.

3.1.5.1.4. Tiling

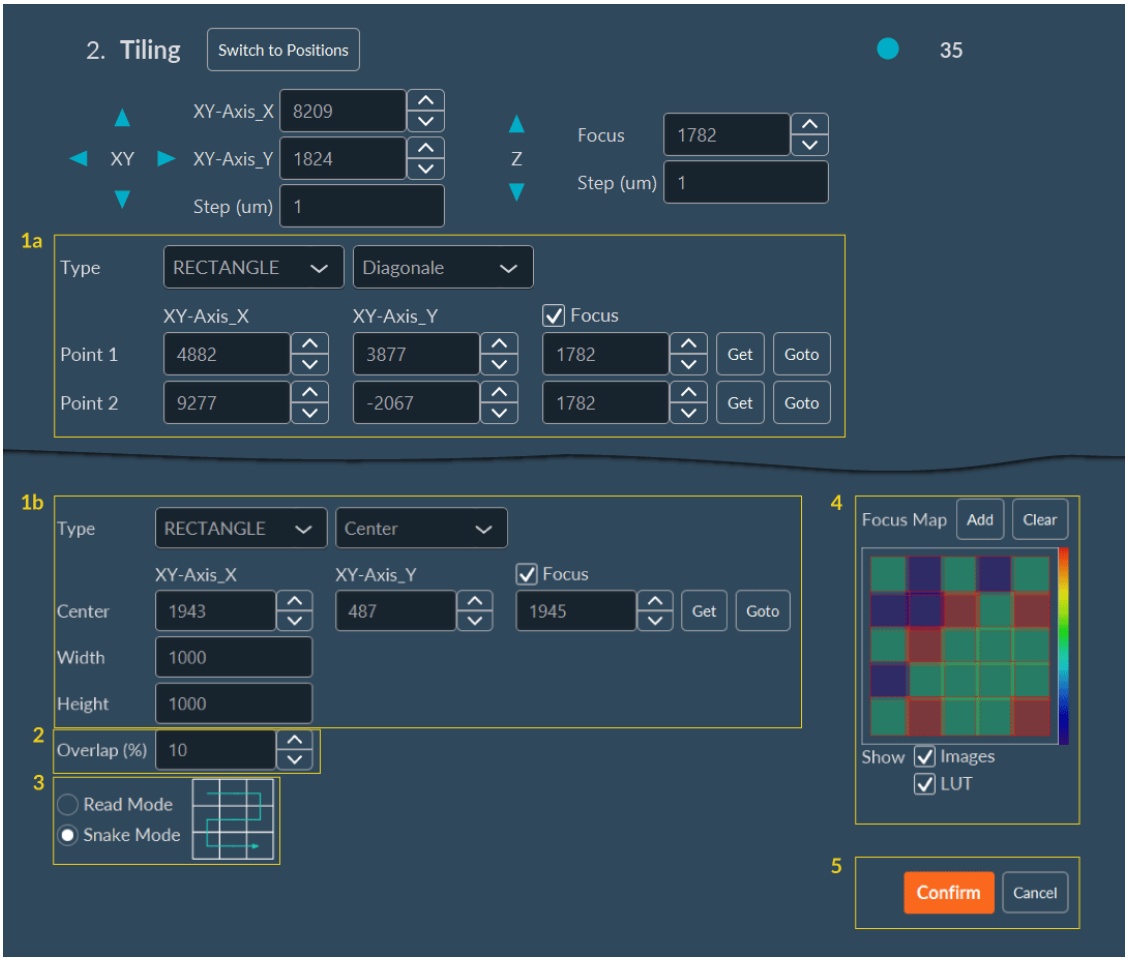
3.1.5.1.4.1. Tiling creation

The tiling tool is used to image large samples. You can switch from this dimension to the Positions dimension by the top button **Switch to Positions**.



1. Move the stage and the focus to the desired position.
2. Click on **Add position** to add a tiling in the tiling list.
3. You can name each tiling in the **Tag** column.
4. Click on  to edit/customize the tiling (see the Tiling edition) or on  to delete it from the list.
5. When all tilings are set up, click on **Confirm**.

3.1.5.1.4.2. Tiling edition



1. Choose the way to generate your tiling and set its specific parameters:
    a. For the **Diagonale** setup:
        - Move the stage to the top left corner of the structure of interest and click on **Get** for the first position.
        - Move the stage to the bottom right corner of the structure of interest and click on **Get** for the second position.
    b. For the **Center** setup:
        - Set the position of the center of your tiling.
        - Set the width and the height of your tiling.
2. Select an appropriate overlap pourcentage (10% is usually enough for final stitching for example).
3. Select the acquisition order of tiles.
4. Adjust the **Focus map** if you need to use it.
5. Click on **Confirm**.

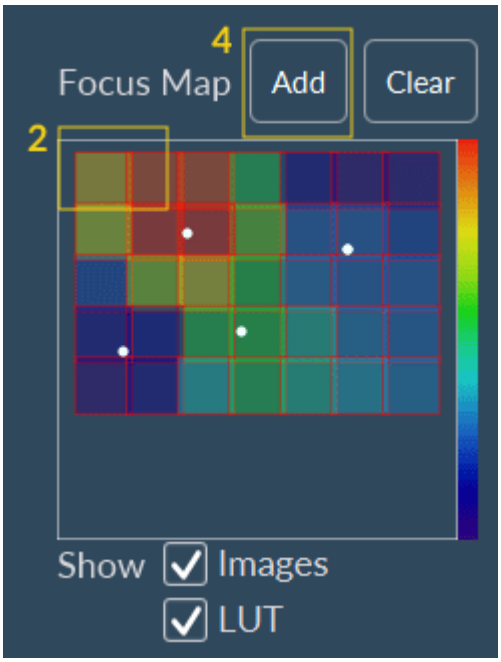
NB: **Snake mode** is recommended for the screening mode because of its speed. This mode is optimized when the acquisition needs to be done fastly.

NB: Users can remove the **Focus** checkbox if they don't want to consider the Z coordinate used for the tiling creation.

NB: The **GoTo** button can be used to move the stage to the previously saved position.

3.1.5.1.4.3. Focus Map

The **Focus Map** is a feature dedicated to adapt the focus during the acquisition when imaging samples that are not flat.



To use the **Focus Map**:

1. Edit a tiling.
2. Click on a random tile to move the stage to this position.
3. Adjust the focus.
4. Click on **Add**.
5. Repeat theses steps as long as needed.

NB: A LUT will help you to appreciate the focus variability of your samples.

NB: The button **Clear** can be used to remove all points on the Focus Map.

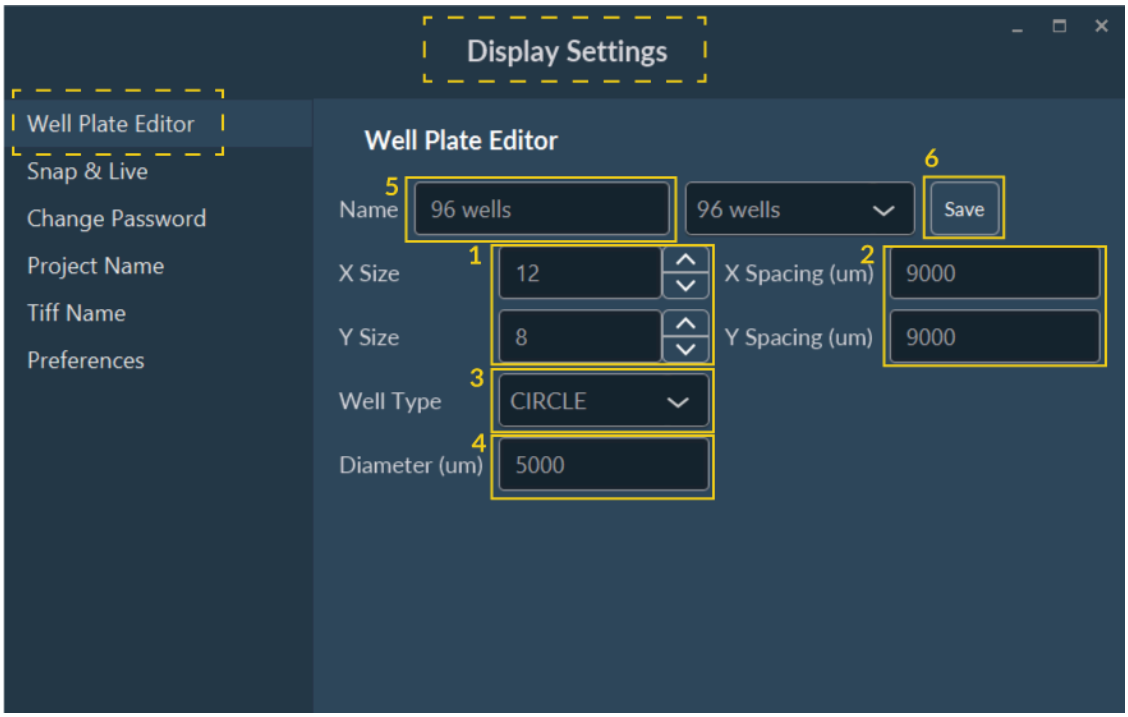
3.1.5.1.5. Multiwell plate mode

The Inscoper software has a strong feature for imaging multiwell plates and for some applications including High Content Imaging.

3.1.5.1.5.1. Well plate editor

The Multiwell plate mode of the Inscoper I.S. is compatible with all commercial or homemade supports (multi-well, multi-slide, etc.). An option **Well Plate Editor** allows you to simply add a large panel of supports.

This following window is accessible by clicking on **Settings** then **Display settings** at the top of the window.



To add a new well plate:

1. Enter the number of horizontal and vertical wells.
2. Enter the horizontal and vertical spacing between the wells.
3. Enter the well type (circle, square, rectangle, ...).
4. Add the dimensions of the wells.
5. Name the support.
6. Save this new well plate.

> NB: You can edit the saved well plate by directly selecting it in the drop-down menu, make corrections and save these new settings.

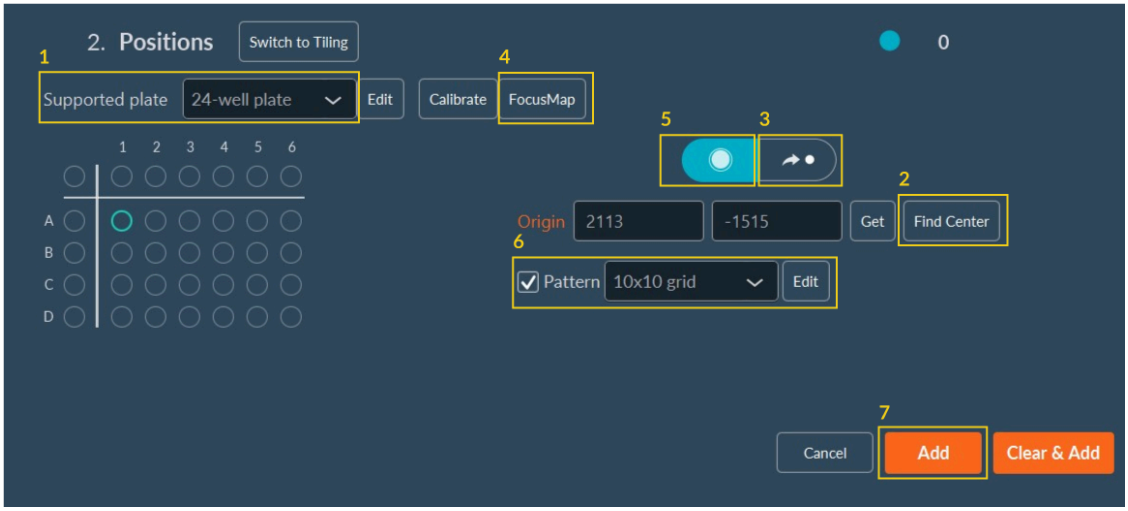
> NB: You can use this feature to add a multi-slide holder, considering the slide as rectangular wells.

3.1.5.1.5.2. Multiwell plate feature

This option is accessible from the Positions or Tiling dimension using the **Set** button.

	You can select the wells you wish to image by clicking on this button
	After calibration, you can automatically move the XY stage to a selected well when this button is activated
	Unselected well
	Selected well
	Current position of the objective

3.1.5.1.5.2.1. In Positions dimension



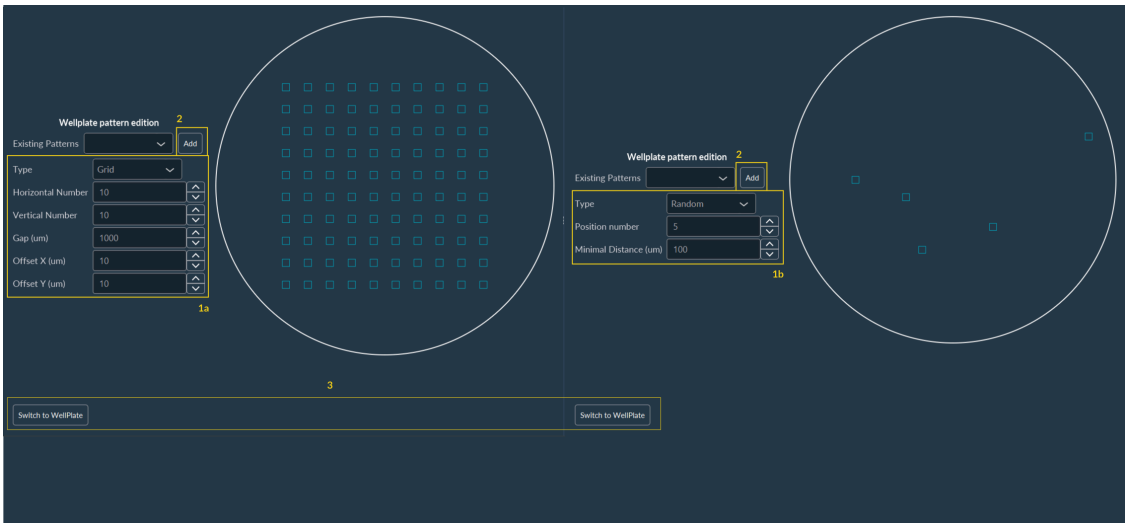
1. Use the **Supported plate** dropdown list to select the support you need.
2. Detect the well plate using the Find Center.
3. Automatically move the stage to some wells.
4. Create a Focus Map for some wells.
5. Select the wells that you want to image.
6. By checking the box **Pattern**, you can generate random points in each selected well by entering the number of desired points and the minimal distance between them.
7. Validate the settings by clicking on **Add**.

> NB: In the virtual well plate map, you can select a whole line by clicking on the letter indicationg that line. Similar features are available for the selection of a whole line, or a whole plate

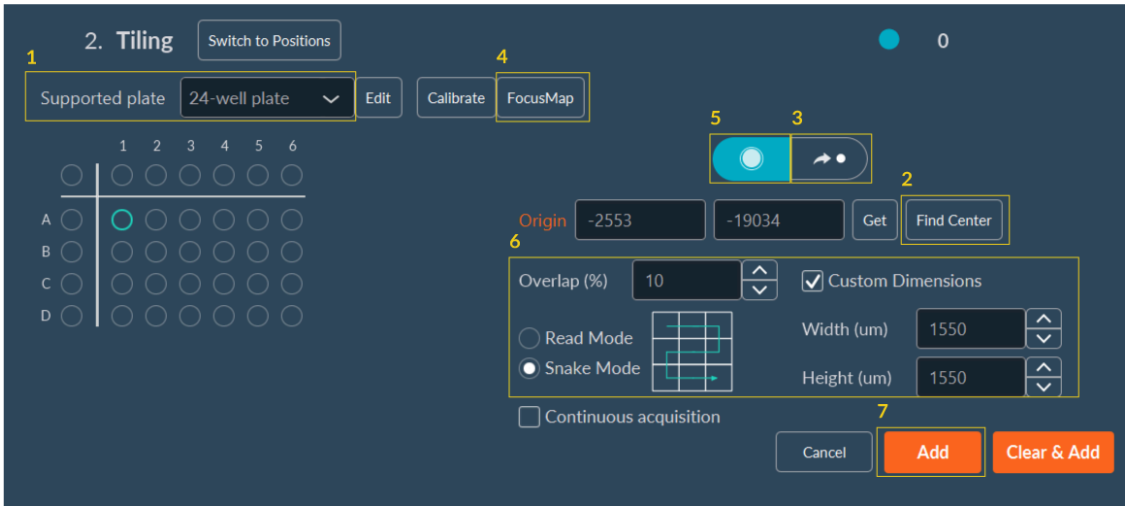
> NB: You can select multiple wells at once by holding down the mouse and moving the mouse over different wells.

To edit pattern, click on **Edit**.

1. Choose which pattern you want to create: Grid or Random.
   a. If Grid, set:
      - the number of image you want to acquire horizontally and vertically;
      - the distance between 2 positions;
      - the offset X and Y (allows you to move your pattern in the well).
   b. If Random, indicate the position number and the minimal distance between positions.
2. Save your pattern by click on **Add**.
3. Come back to the dimension by clicking on **Switch to WellPlate**.

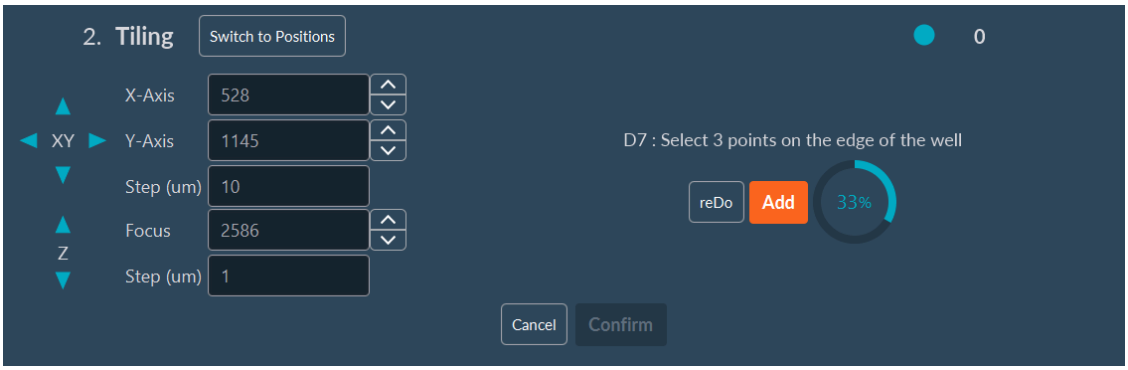


3.1.5.1.5.2.2. In Tiling dimension



1. Use the **Supported plate** dropdown list to select the support you need.
2. Detect the well plate using the Find Center.
3. Automatically move the stage to some wells.
4. Create a Focus Map for some wells
5. Select the wells that you want to image.
6. By checking the box **Custom Dimentions**, you can choose the size of your tiling which will be applicated in each selected well, the overlap of your tiles and the screening mode.
7. Validate the settings by clicking on **Add**.

3.1.5.1.5.2.3. Multiwell plate calibration



This step is needed to get precise positions of the well, and then to optimize the XY stage movements. To use the well plate feature:

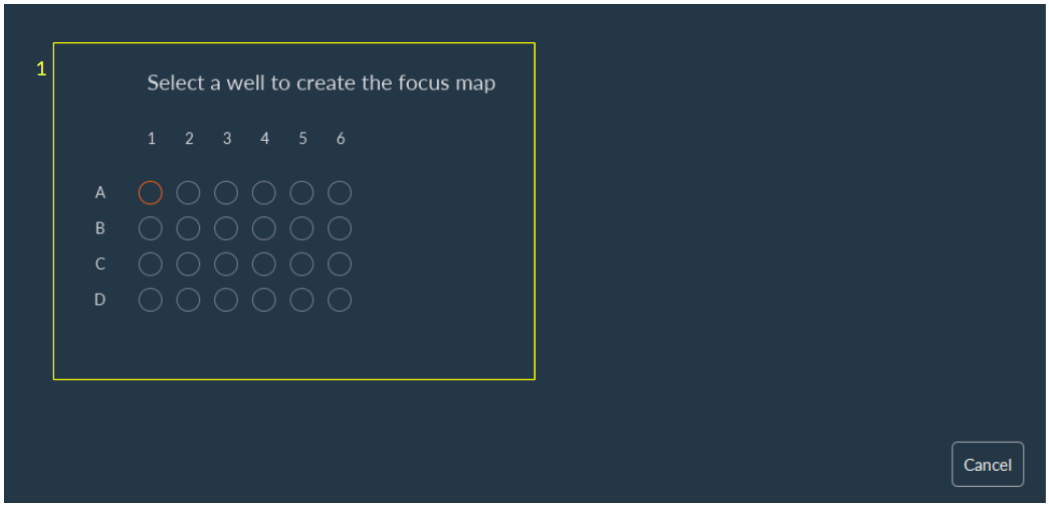
1. Click on **Find Center** to start the semi-automated protocol.
2. Select the well to be used for well plate calibration.
3. Move manually the stage to this position if it was not previously done.

4. Fit the edge of the well with the virtual cross by moving the xy stage on the live image and click on **Add**
5. Repeat twice on different sides of the well.
6. Click on **Confirm** to finish.

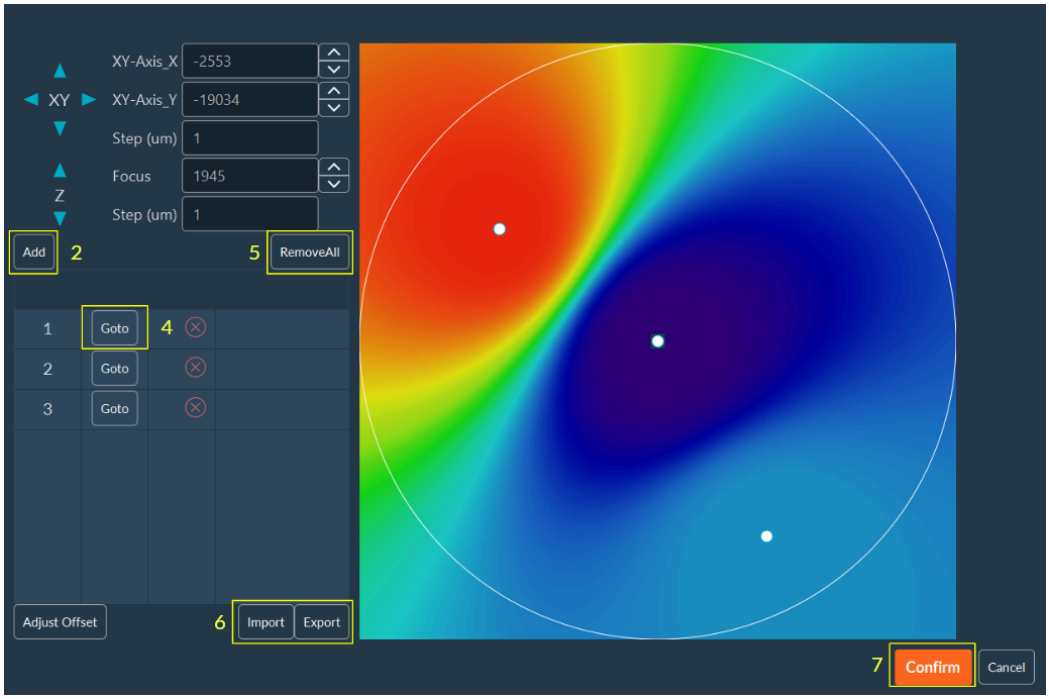
   When these steps are finished, you can move to each well automatically.

3.1.5.1.5.2.4. Focus Map creation

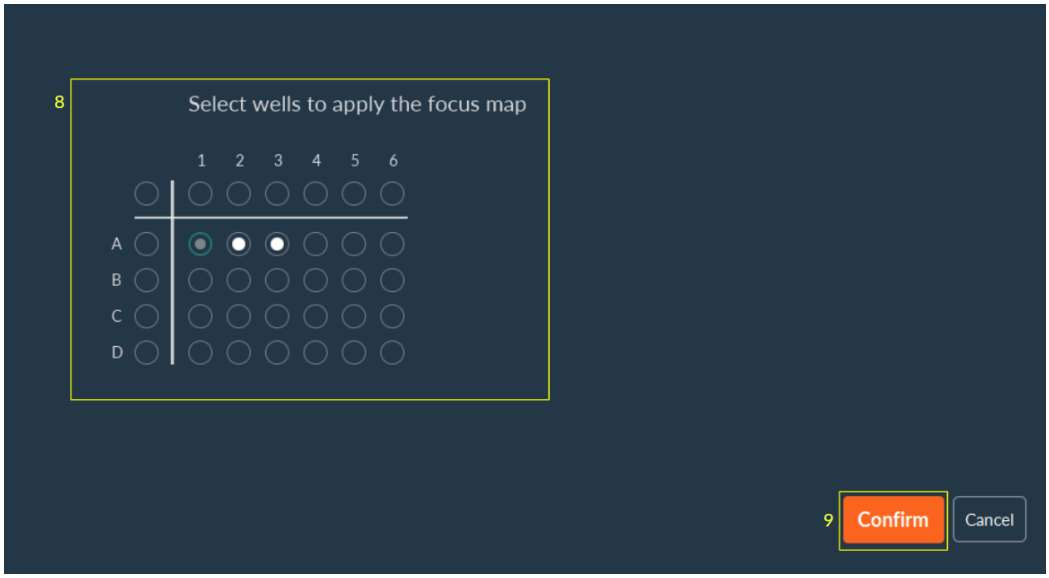
1. Select the well to create the Focus Map.



2. Choose the position in the well where you want to make the focus and click on **Add**.
3. Repeat this process until you are ok with the focus of your well.
4. Click on **Go to**, to move to the position.
5. Click on **Remove all** to delete all the points of your focus map if necessary.
6. You can import or export a Focus Map.
7. Click on **Confirm** to validate your Focus Map.



8. Select the wells where you want to apply the focus map.
9. Click on **Confirm** to validate your settings.

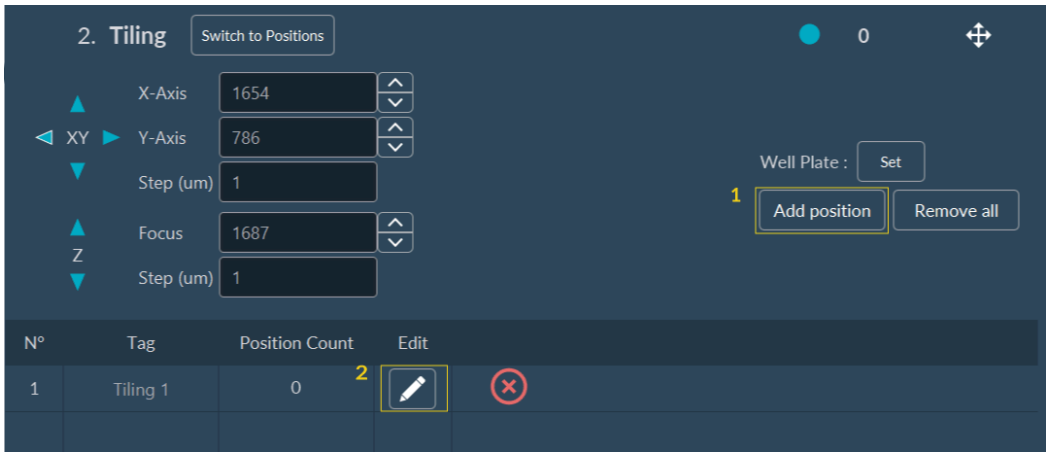


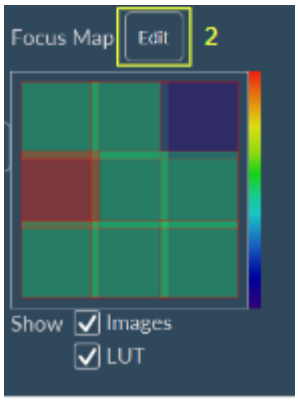
3.1.5.1.6. Automated Focus Map

The Automated Focus Map is a software optional feature of the Inscoper I.S. that automatically generates a relief map of the biological sample when performing tiling imaging.

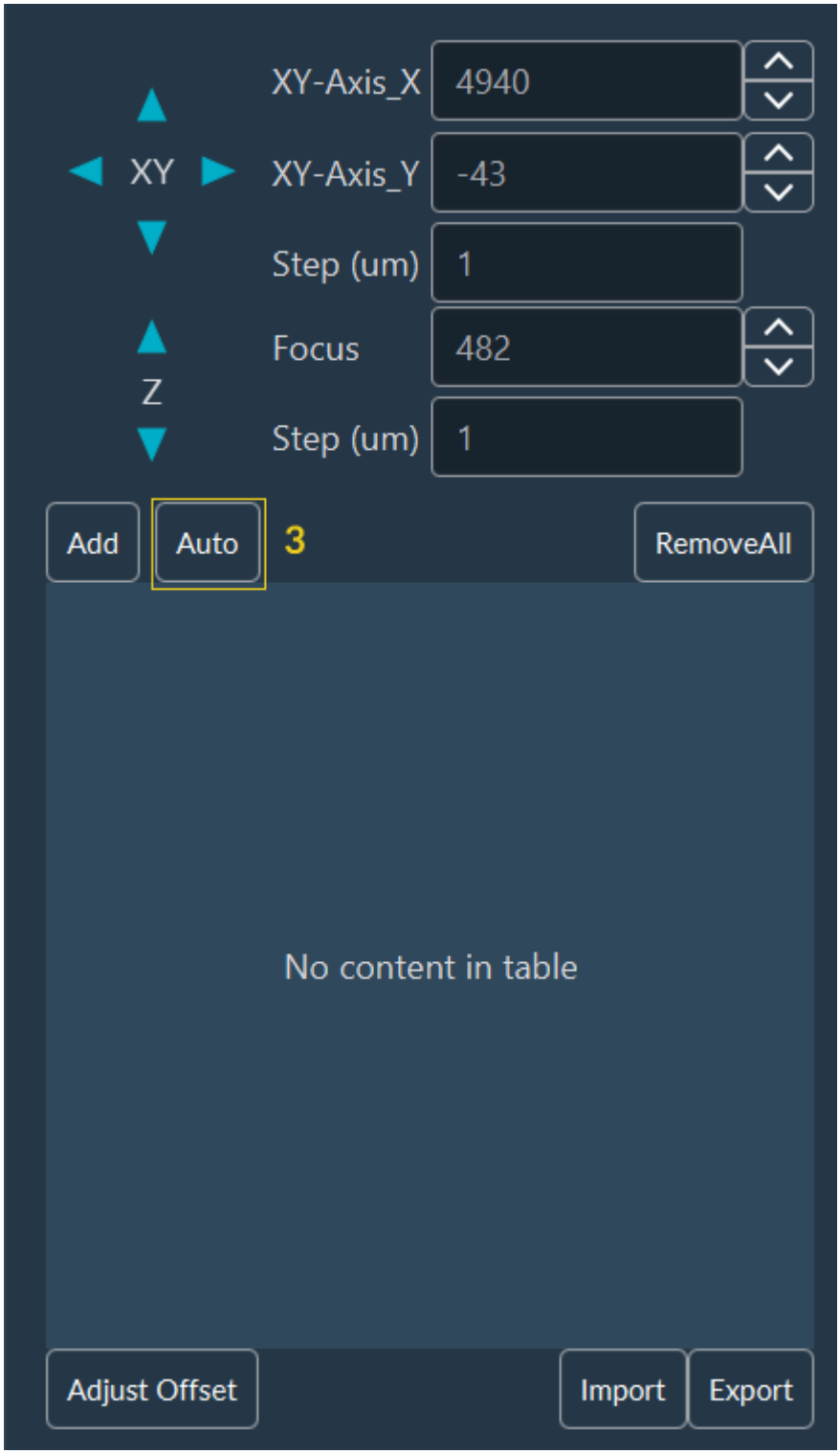
3.1.5.1.6.1. Automated Focus Map - Simple tiling

1. In the tiling dimension, click on **Add position** to create the tiling.
2. Click on **Edit** to customize the tiling and adjust the Focus Map.

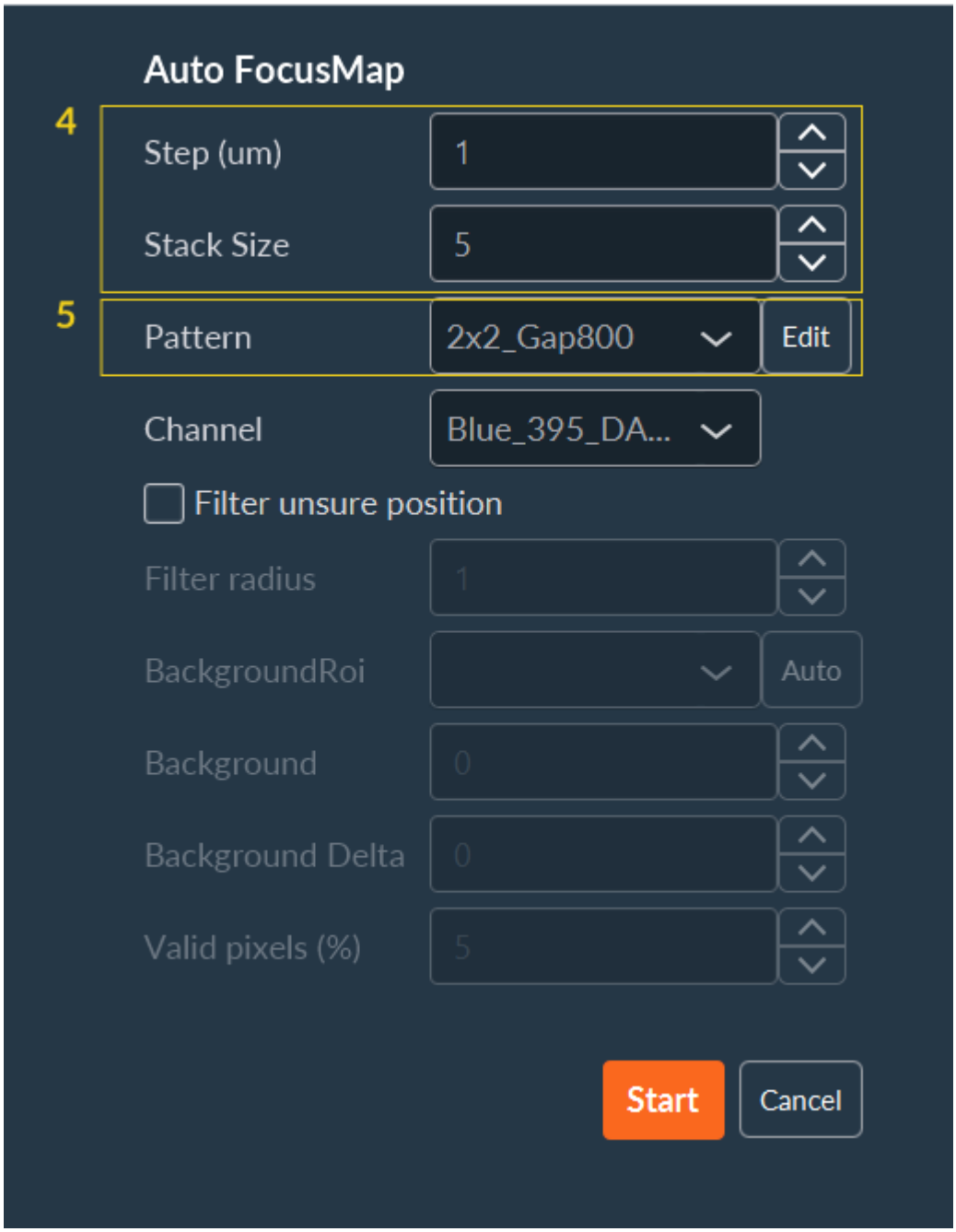




3. Click on **Auto** to configure the Focus Map.



4. Choose the **step** and **size** of the stack.
5. You can select an existing pattern from the drop-down list (after that go to the step 9) **OR** you can create a new pattern by clicking on **Edit**.

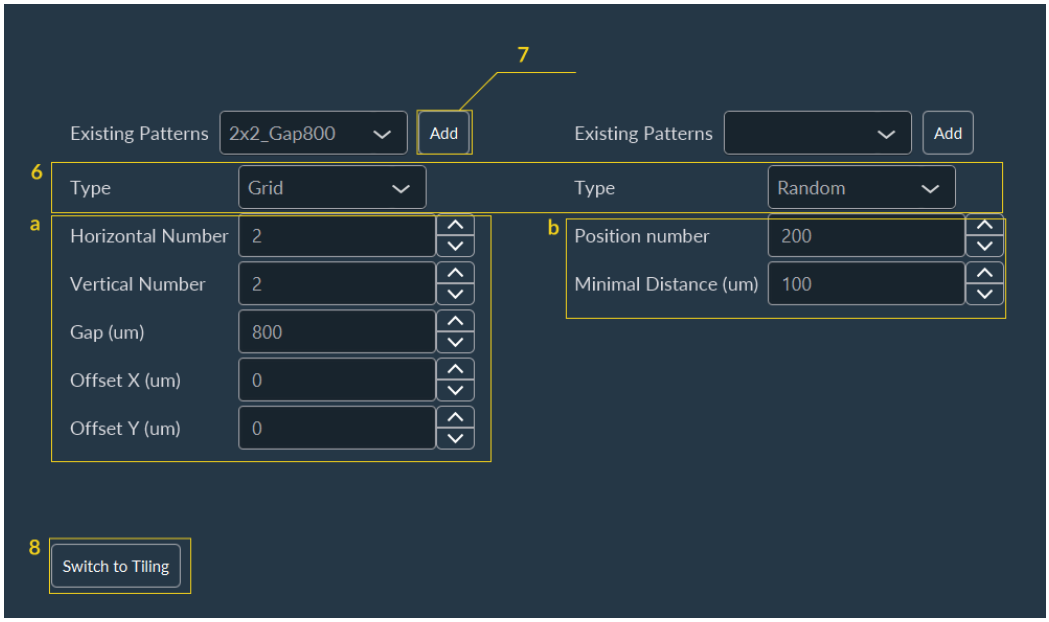


6. To create a new pattern, click **Edit** choose the pattern you want: **Grid** or **Random**:

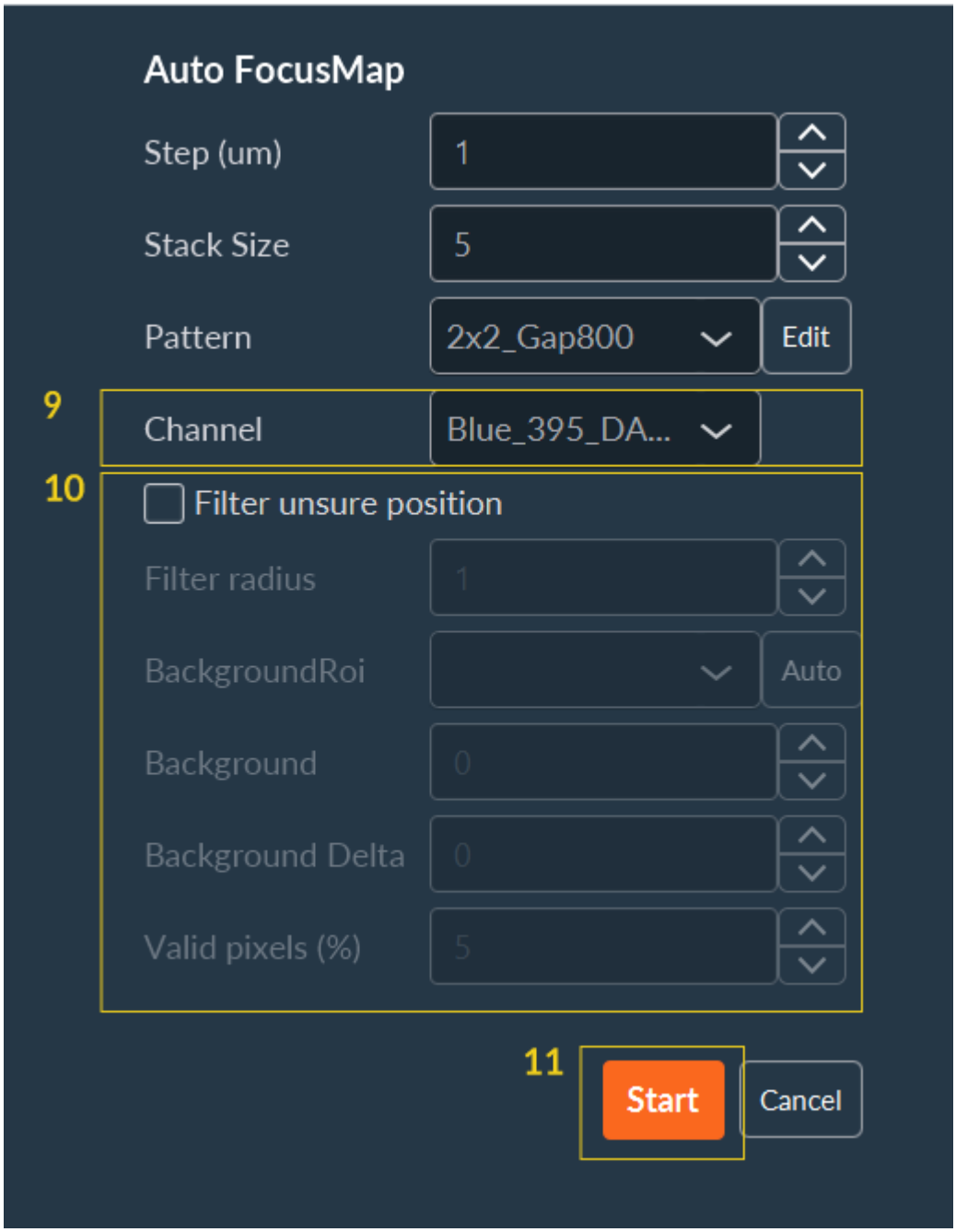
    a. For **Grid**, indicate the number of image you want to acquire horizontally and vertically, and the Gap (distance between 2 images).
    The offset X and Y allows you to move your pattern in the well.

    b. If you choose a **Random**, indicate the position number and the minimal distance between positions.

7. Save your pattern by clicking on **Add**.
8. Come back to the dimension by clicking on **Switch to Tiling**.

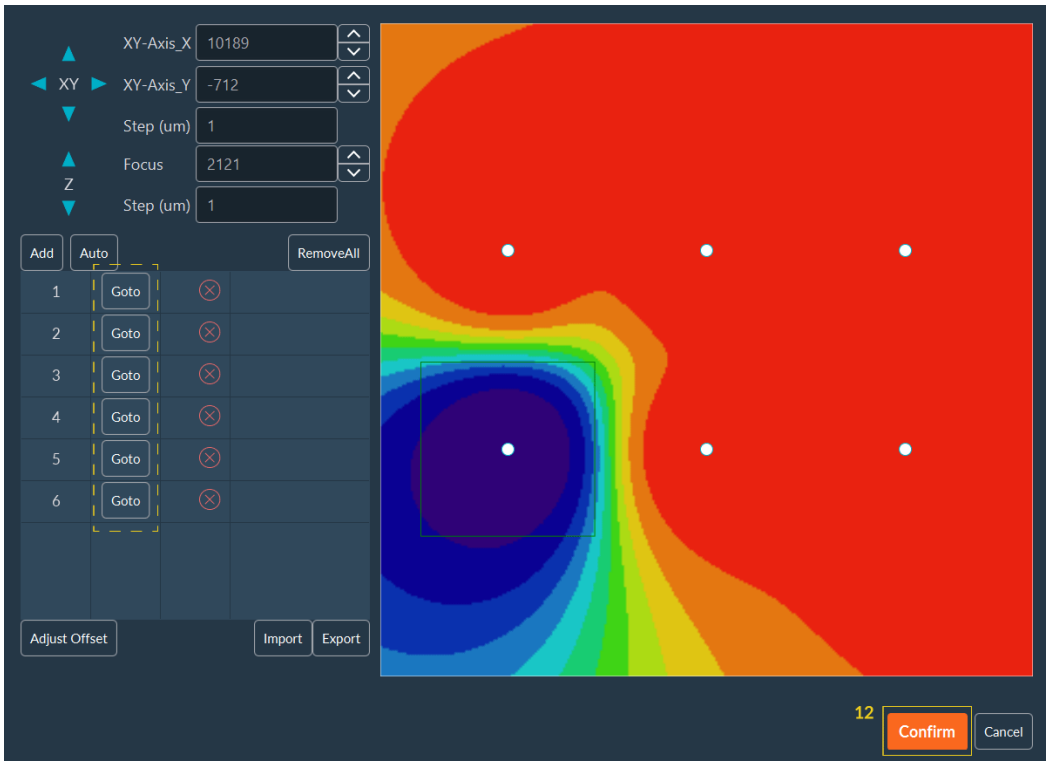


9. Choose the **channel** to acquire the stack.
10. You can apply some filters by checking the box **Filter unsure position**:
    - Filter Radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on 5x5 square around the pixel, …).
    - BackgroundRoi: automatic calculation of Background and BackgroundDelta values by drawing an ROI. Background will be the mean value of the ROI and BackgroundDelta will be the double of the standard deviation.
    - Background: background mean value.
    - Background Delta: amplitude of the noise.
    - Valid pixels: percent of pixel that should be far of the background to validate the position
11. Click on **Start**.



System will perform all points to create the Focus Map and you will obtain a list of points. By clicking on **Goto** or on the image, you can check the focus.

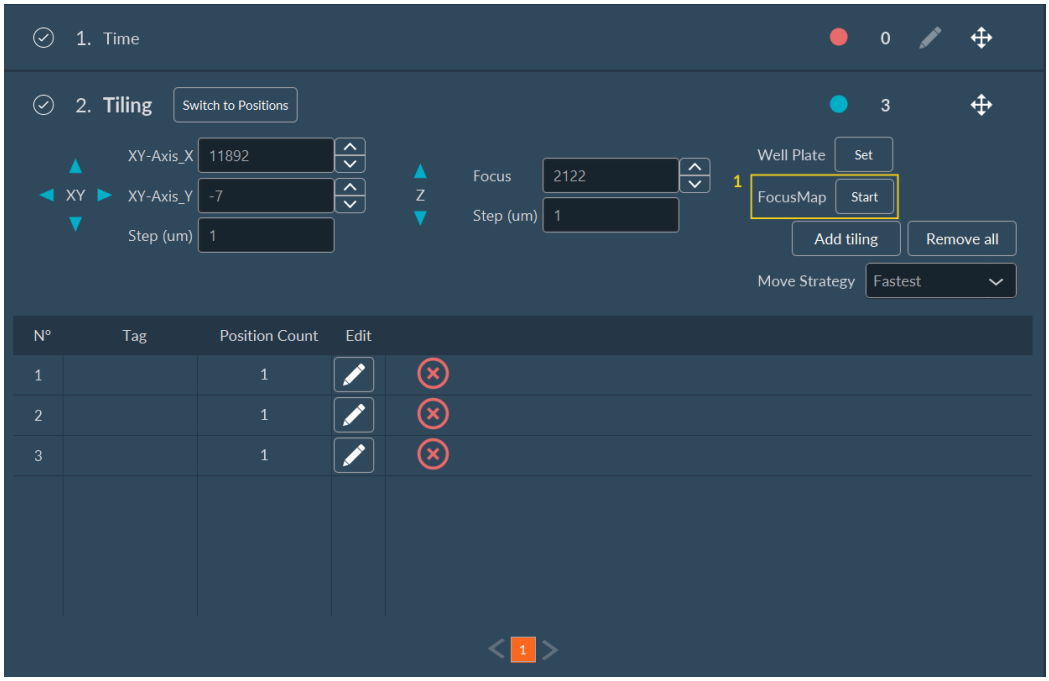
12. Click on the **Confirm**, the **Focus Map** is ready to use. You can continue to configure your acquisition sequence.



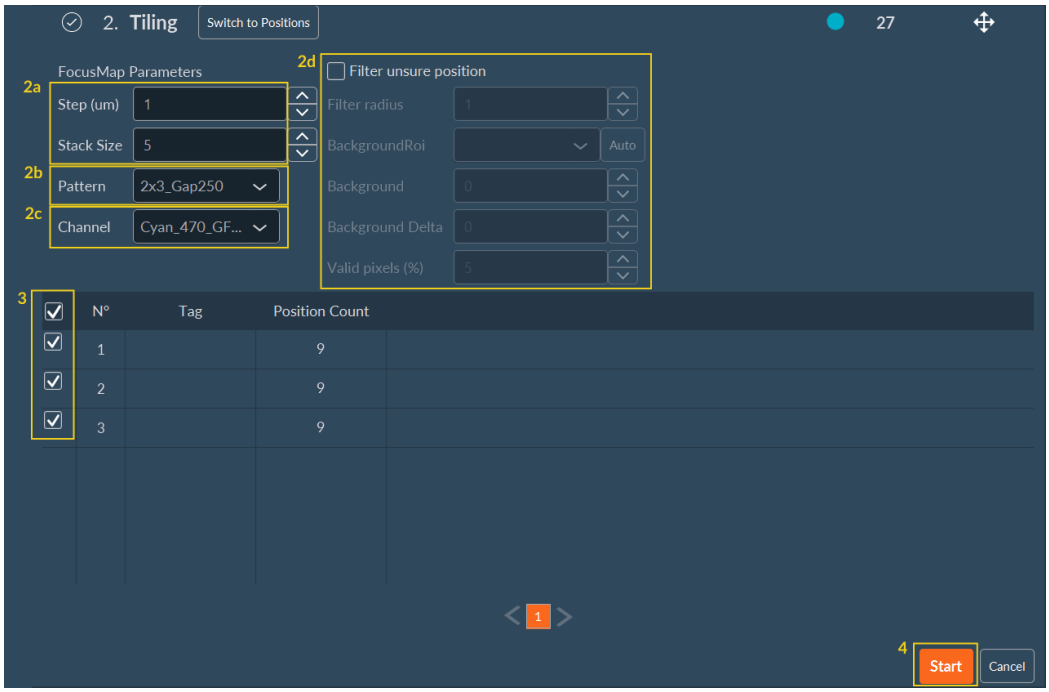
3.1.5.1.6.1.1. Automated Focus Map - Multiple area tiling

In **Tiling dimension**, after the configuration of all your tiling, you can do a FocusMap for all the positions.

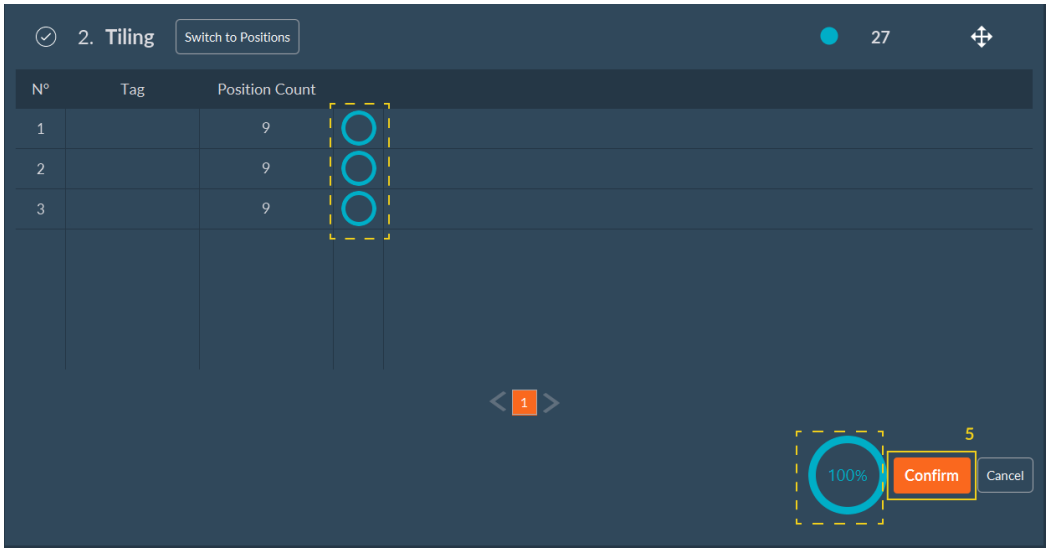
1. Click on **Start**.



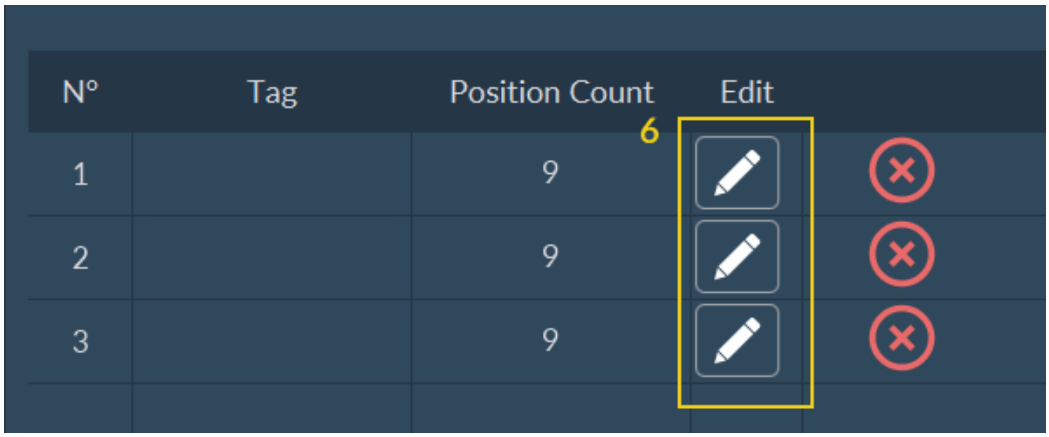
2. Proceed to the Focus Map Automation:
    a. Choose the step and size to the stack.
    b. Choose the pattern.
    c. Choose the channel to acquire the stack.
    d. You can apply some filters by checking the box “Filter unsure position”
        - Filter radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on 5x5 square around the pixel, …)
        - BackgroundRoi: automatic calculation of Background and BackgroundDelta values by drawing ROI. Background will be the mean value of the ROI and BackgroundDelta will be the double of the standard deviation.
        - Background: background mean value
        - Background Delta: Amplitude of the noise
        - Valid pixels: percent of pixel that should be far of the background to validate the position
3. Select the positions where the Focus Map will be applied by checking the boxes on the left.
4. Click **Start**.



5. When the focus map is ready, a blue circle will appear on the right side of each position and at the bottom right (near the “Confirm” button).You will return automatically to the Tiling dimension. Once the blue circle indicates 100%, the focus map is finished and you need to click on **Confirm**.



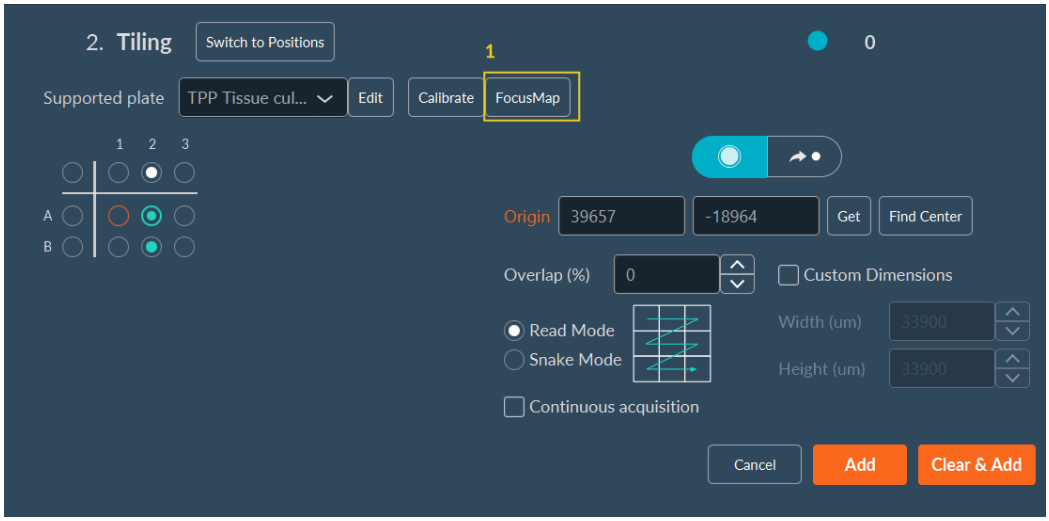
6. You can verify the focus map for each tiling by clicking on **Edit**. Then you can continue to configure your acquisition sequence.



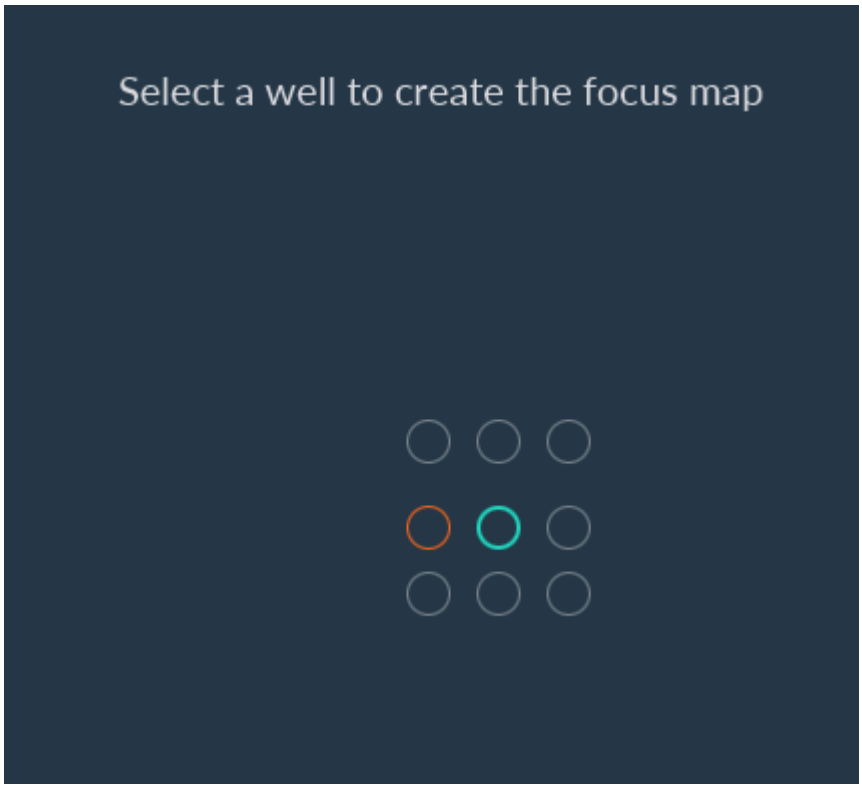
3.1.5.1.6.1.2. Automated Focus Map - Multiwell plate

This option is accessible from the **Positions** or **Tiling** dimension using the **Set** button.

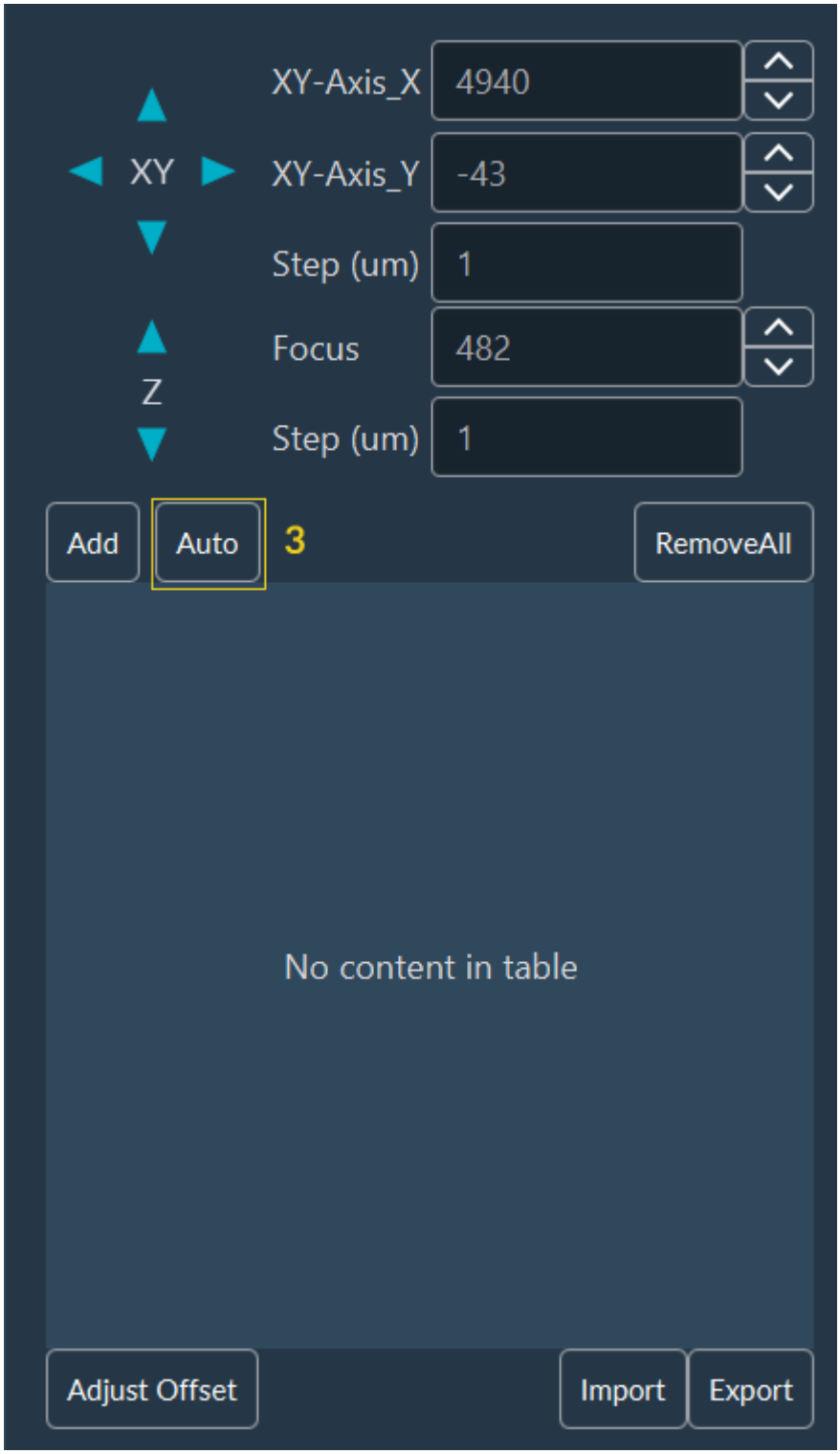
1. Click on **Focus Map** to configure it.



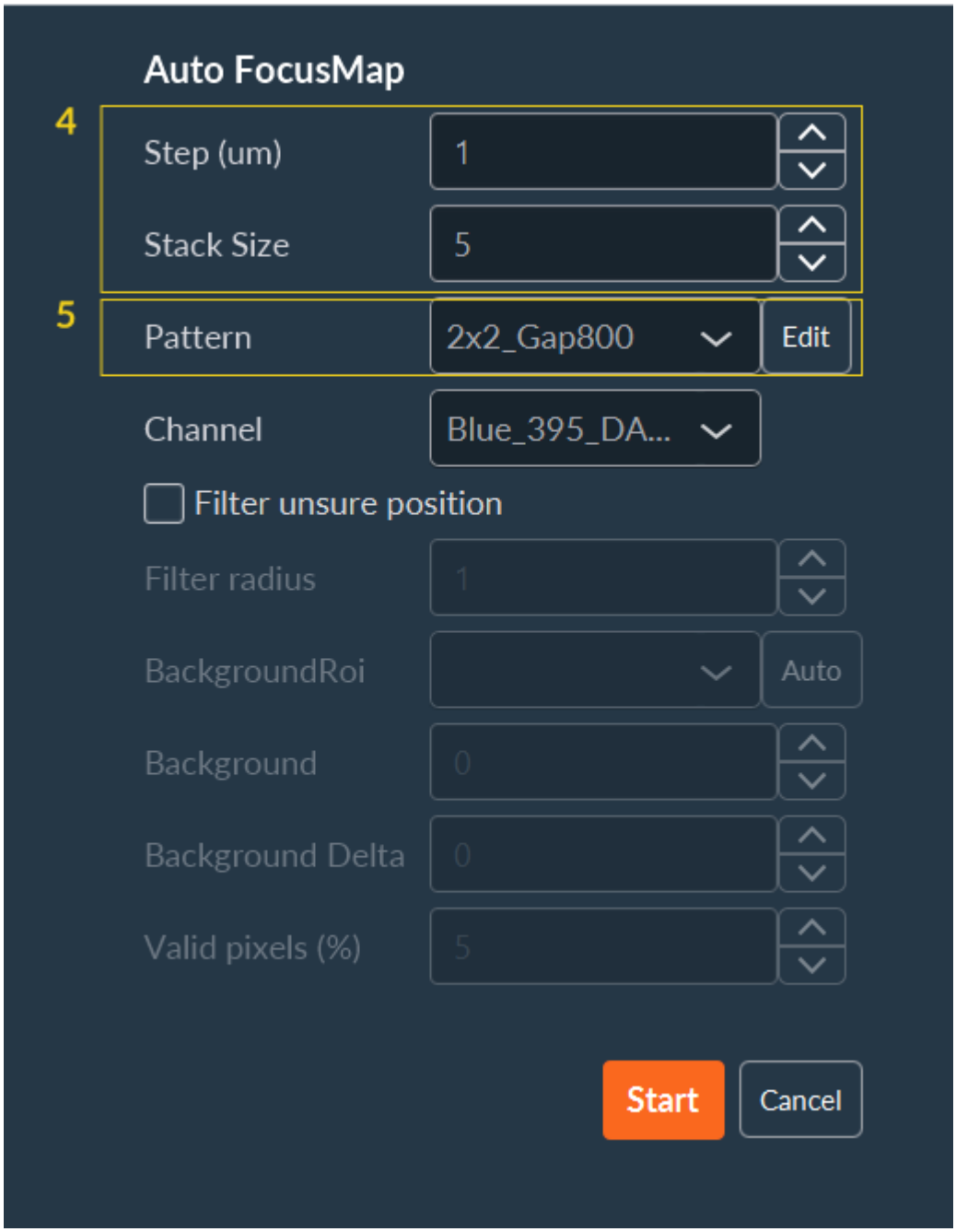
2. Select your well where you would like to create your **Focus Map** then click on **Confirm**.



3. Click on **Auto** to configure the Focus Map.



4. Choose the **step** and **size** of the stack.
5. You can select an existing pattern from the drop-down list (after that go directly to the step 9) OR you can create a new pattern by clicking on **Edit**.

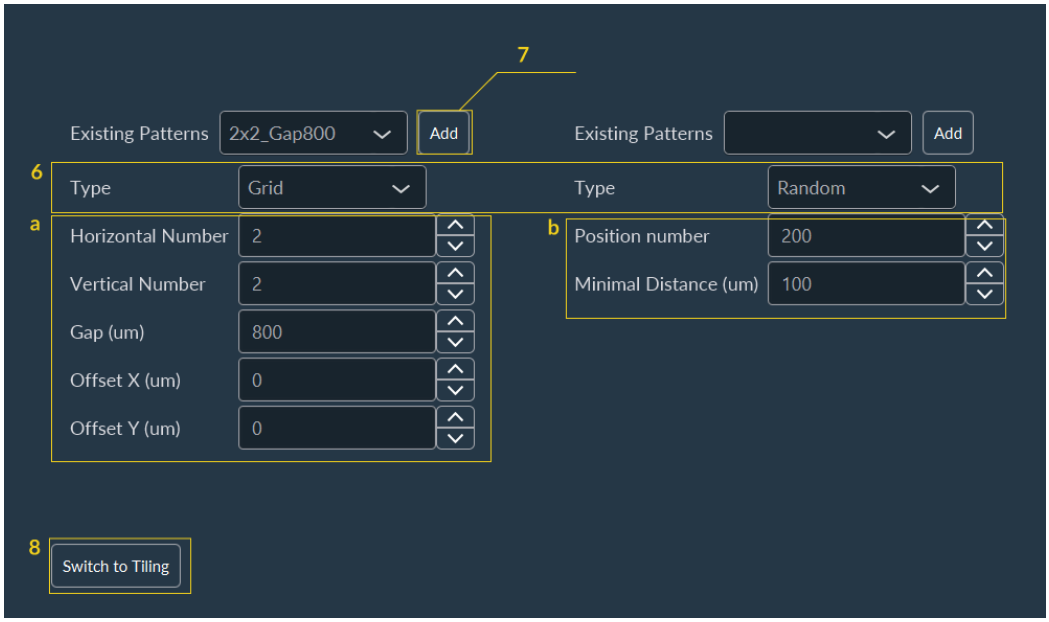


6. Select the pattern you want: **Grid** or **Random**:

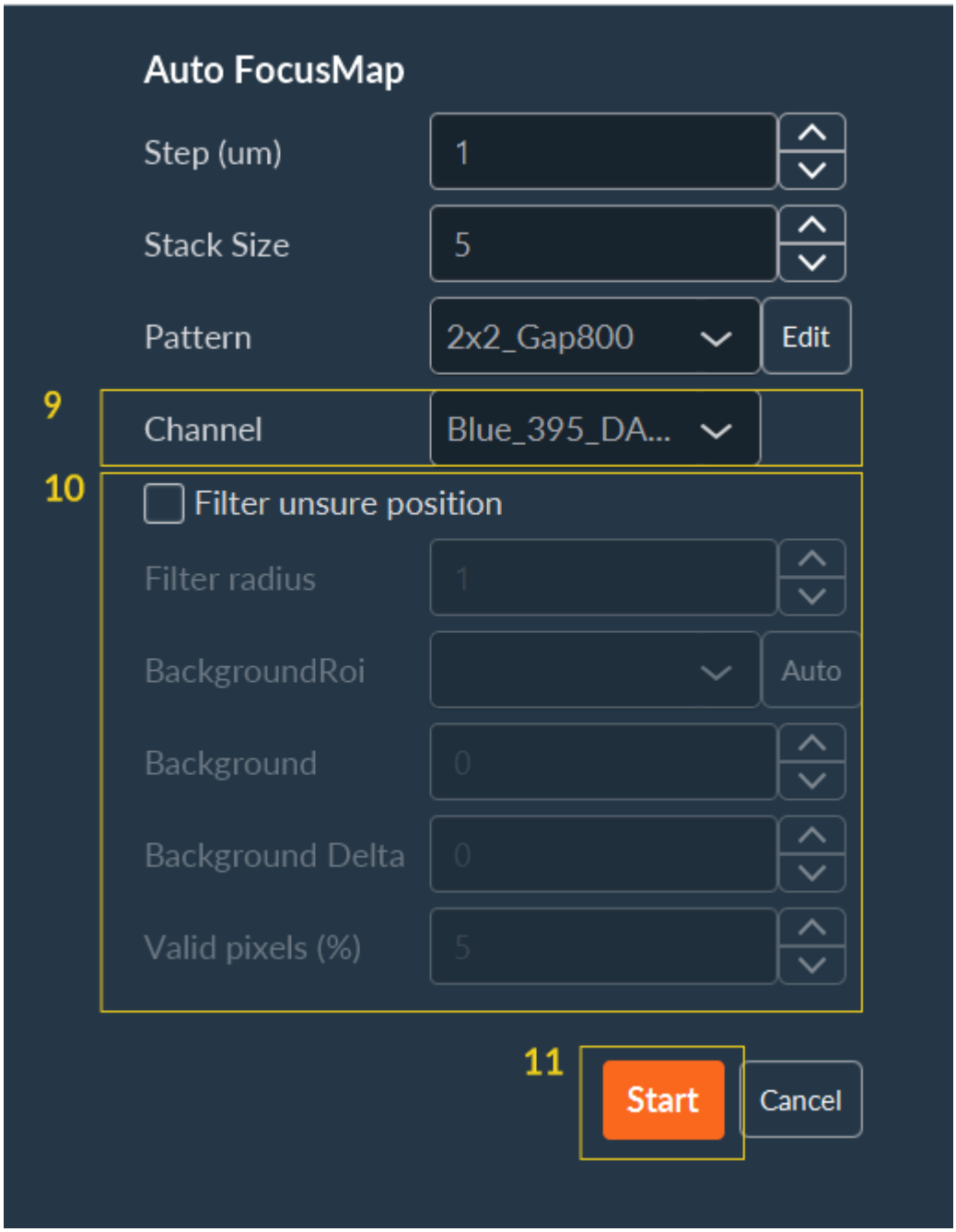
    a. For **Grid**, indicate the number of image you want to acquire horizontally and vertically, and the Gap (distance between 2 images).
    The offset X and Y allows you to move all your patterns in the well.

    b. If you choose a **Random**, indicate the position number and the minimal distance between positions.

7. Save your pattern by clicking on **Add**.
8. Come back to the dimension by clicking on **Switch to Tiling**.

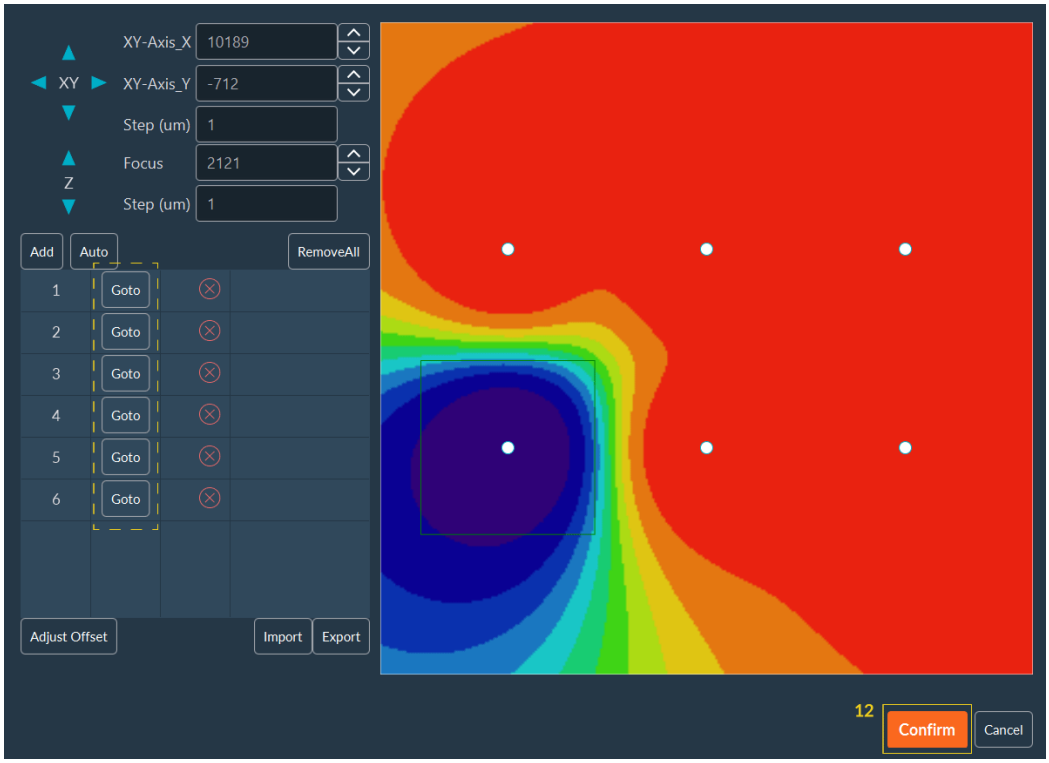


9. Choose the **channel** to acquire the stack.
10. You can apply some filters by checking the box **Filter unsure position**:
    - Filter Radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on 5x5 square around the pixel, …).
    - BackgroundRoi: automatic calculation of Background and BackgroundDelta values by drawing an ROI. Background will be the mean value of the ROI and BackgroundDelta will be the double of the standard deviation.
    - Background: background mean value.
    - Background Delta: amplitude of the noise.
    - Valid pixels: percent of pixel that should be far of the background to validate the position
11. Click on **Start**.

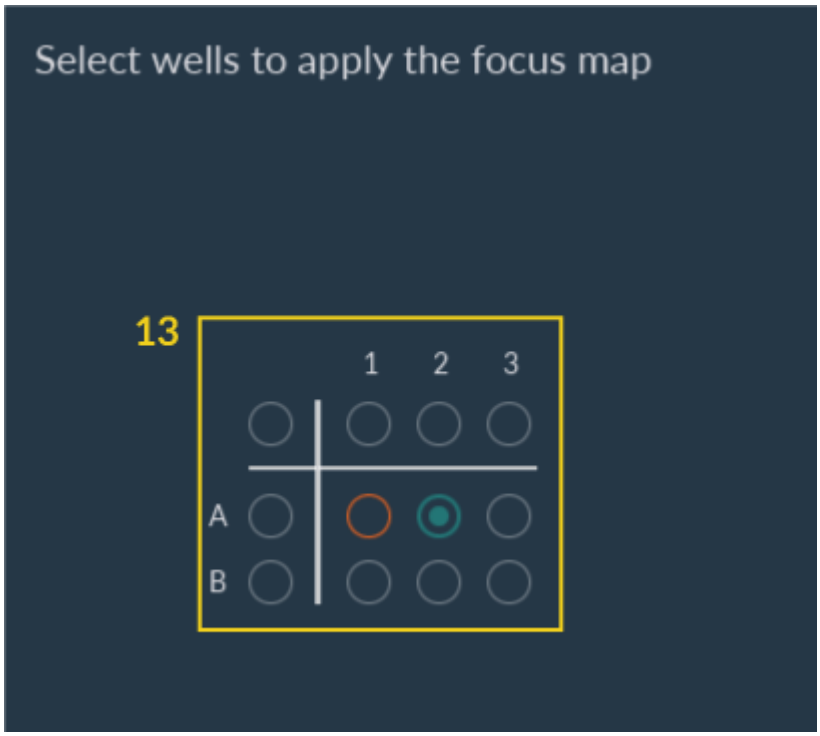


System will perform all points to create the Focus Map and you will obtain a list of points. By clicking on **Goto** or on the image, you can check the focus.

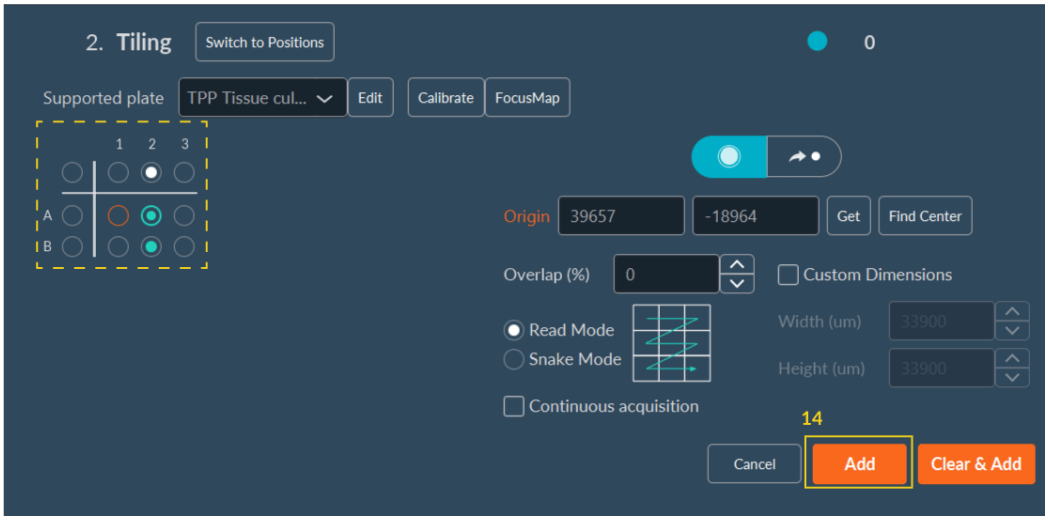
12. Click on the **Confirm**, the **Focus Map** is ready to use.



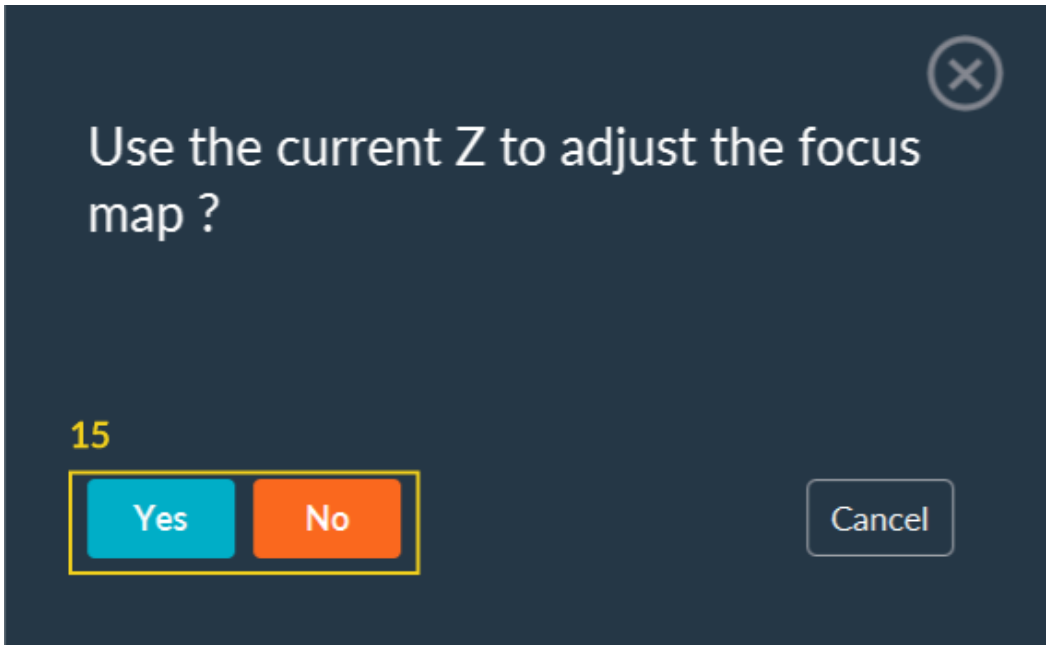
13. Select the wells where you want to apply the Focus Map then click on **Confirm**.



14. Select the wells that you want to acquire and click on **Add**.



15. Select if you want to use or not the current Z to adjust the FocusMap.

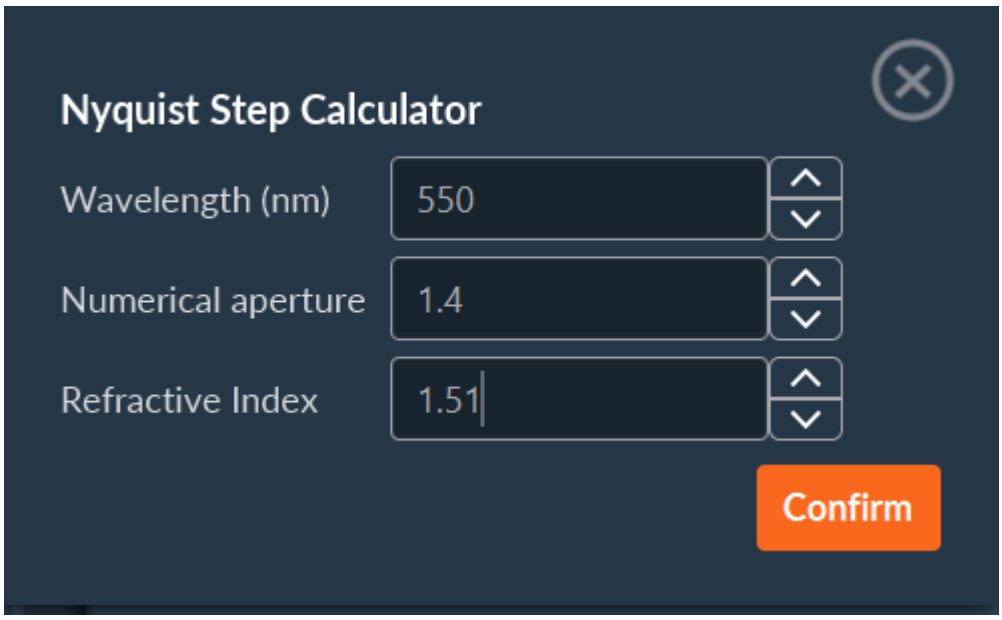


After this step, you can continue to configure your acquisition sequence.

3.1.5.1.7. Z-stack

The volumetric imaging with Inscoper I.S. can be performed using the Z-Stack dimension.

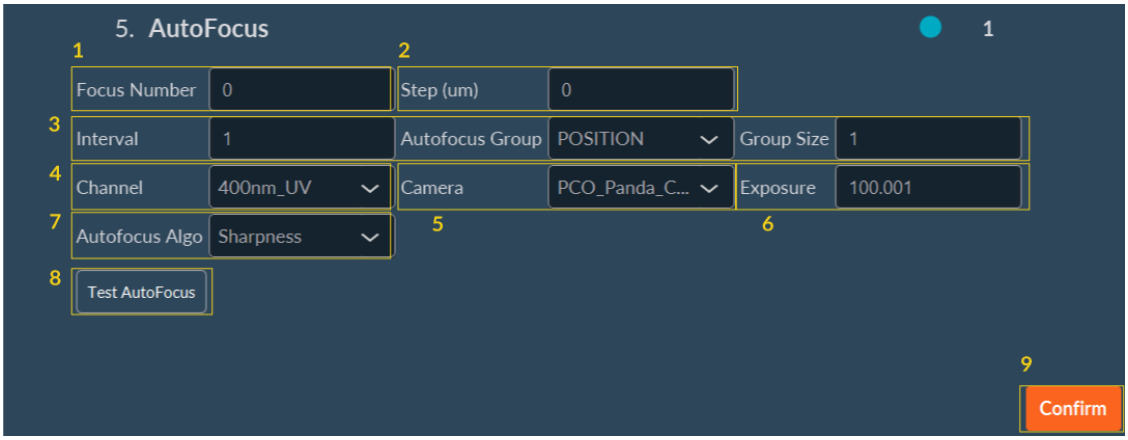
1. Select the mode you want to use: **Min/Max Plane** (limits of your stack) or **Center Plane** (middle of your stack).
2. Select your reference plane clicking on **Get**.
3. Adjust the step (Interval between each plane of volume) and the size (number of steps). For the step size you can help you with the Nyquist Step Calculator:
   Indicate the emission wavelength, the numerical aperture of your objective and the refractive index of your objective immersion medium. Once all information is set up, click on **Confirm** to validate your settings. The step size will be automatically calculated.



4. Once you have finished editing the dimension, click on the **Confirm**.

3.1.5.1.8. Autofocus

The Inscoper I.S. software has an auto-focus mode to keep samples at the perfect focus throughout the acquisition sequence.



1. Select the number of focus to perform to each position.
2. Select the step (µm) between each focus.
3. Specify the frequency of autofocus in the acquisition sequence:

    a. Interval: number of images taken between each autofocus.

    b. Autofocus group:
        - GLOBAL - applying autofocus on all images
        - POSITION - applying autofocus on a set of groupe size (XY) positions
        - TILING - applying autofocus on all tiling groups

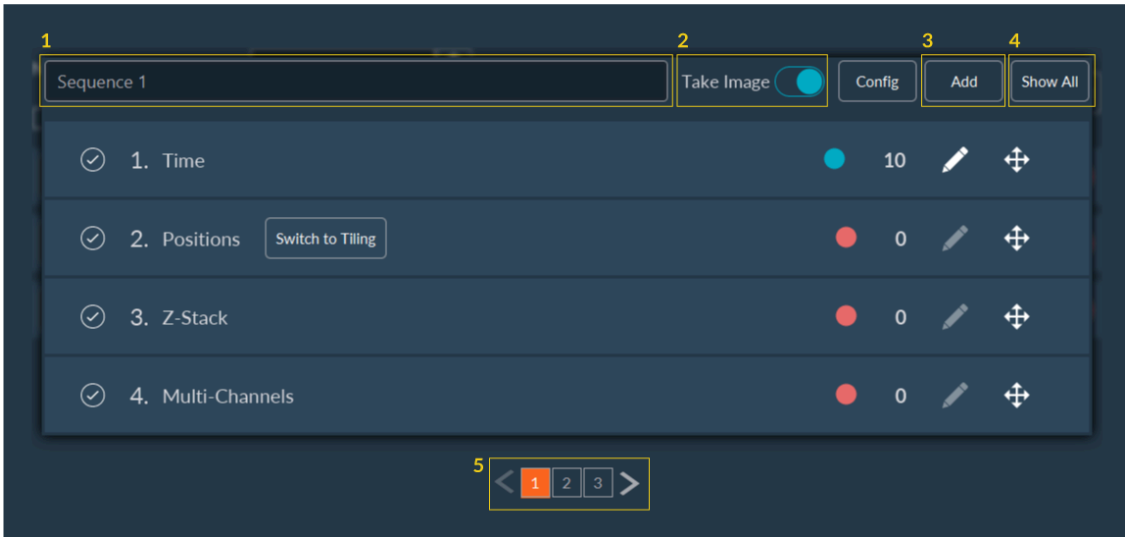
    c. Group size: number of positions to which the found focus value is applied.

4. Select the channel to use.
5. Select the camera to use and its exposure time.
6. Select the autofocus algorithms to be applied : maximal intesity or sharpness (the best focus plane will be the sharpest).
7. Test the autofocus protocol.
8. Click on **Confirm**.

> NB: The Autofocus dimension must be executed after the active dimension during an acquisition sequence.

3.1.5.1.9. Multi-sequence acquisition

When an acquisition sequence is ready, it is possible to launch it or to add others to create a multi-sequence acquisition.



Each sequence has several options for setting up:

1. You can assign a name to a sequence to make it easier to identify.
2. Disable the image acquisition for a specific sequence by deactivating the "Take Image" button.
3. Add a new sequence to the multi-sequence acquisition.
4. You can have a synthetic view of the different sequences already prepared.
5. You can seamlessly navigate between sequences. The selected sequence is highlighted in orange.

In the summary tab below you have the following options:

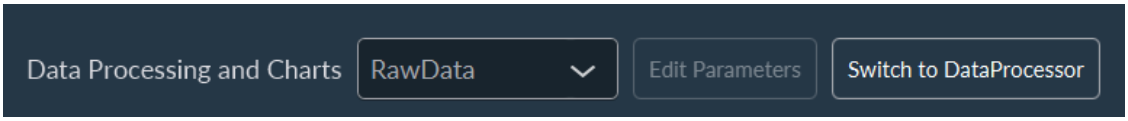
1. Adjust the loop number to determine how many times the entire acquisition sequence will be repeated.
2. Make intermittent sequences by checking the checkbox and specifying the dimension to consider.
3. Choose to take image (or not) during each sequence.
4. Activate/deactivate a sequence by clicking on the coloured button (changing red to blue when activated and inversely).
5. Edit the acquisition sequence by clicking on **Pen**.
6. Duplicate the current sequence by clicking on **Copy**.

7. Delete the current sequence by clicking on **Delete**.

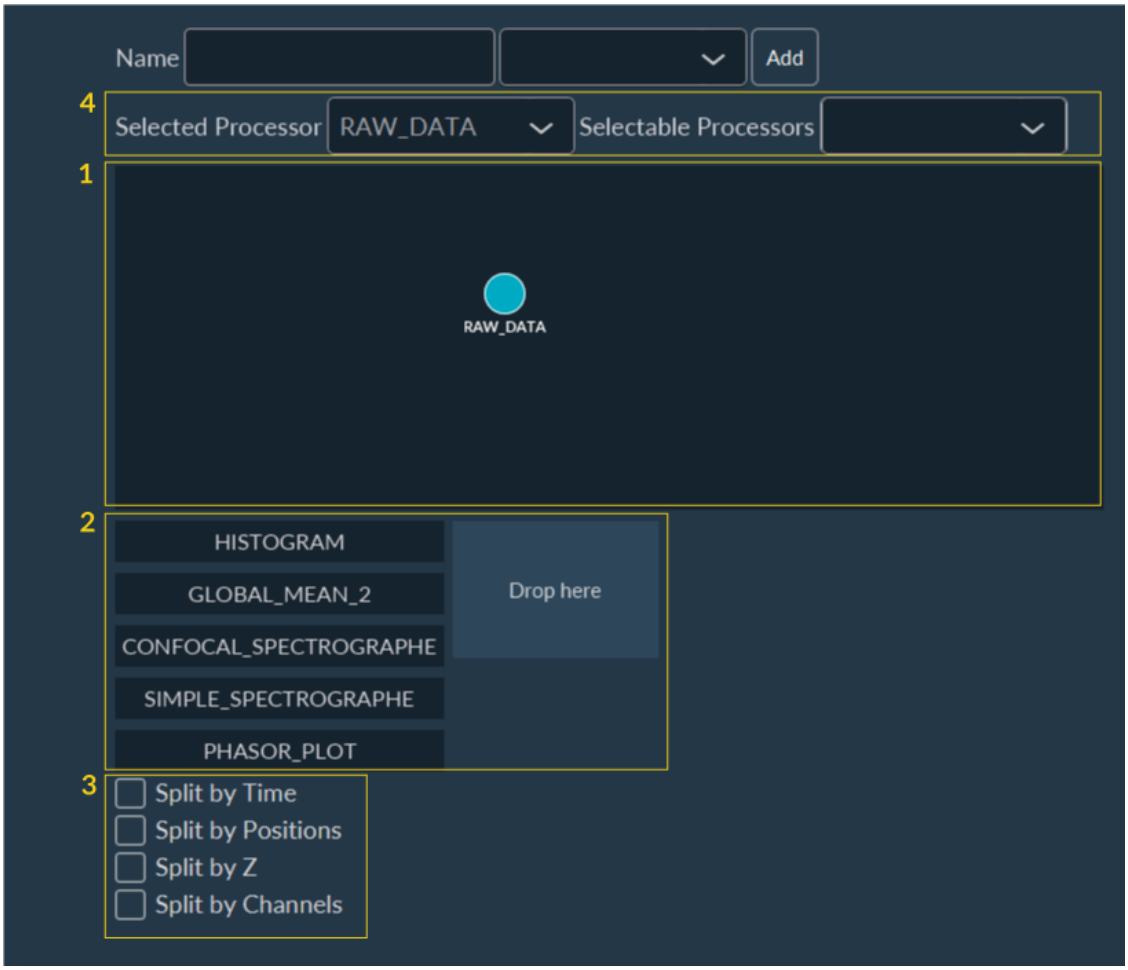
3.1.5.1.10. Data Processor

3.1.5.1.10.1. Interface description

The Data Processor feature allows you to fully customize the visualization of your data during and after acquisition. For example, you can visualize raw images only, visualize graphs of fluorescence intensity, apply algorithms to post-process or analyze your data in real time.



In **User Mode**, you have access to the pre-configured data processing. You can select it from the drop-down menu and start the acquisition. However, the **Expert mode** gives access to the button called **Switch to Data Processor**, dedicated to the setup of this data processing.



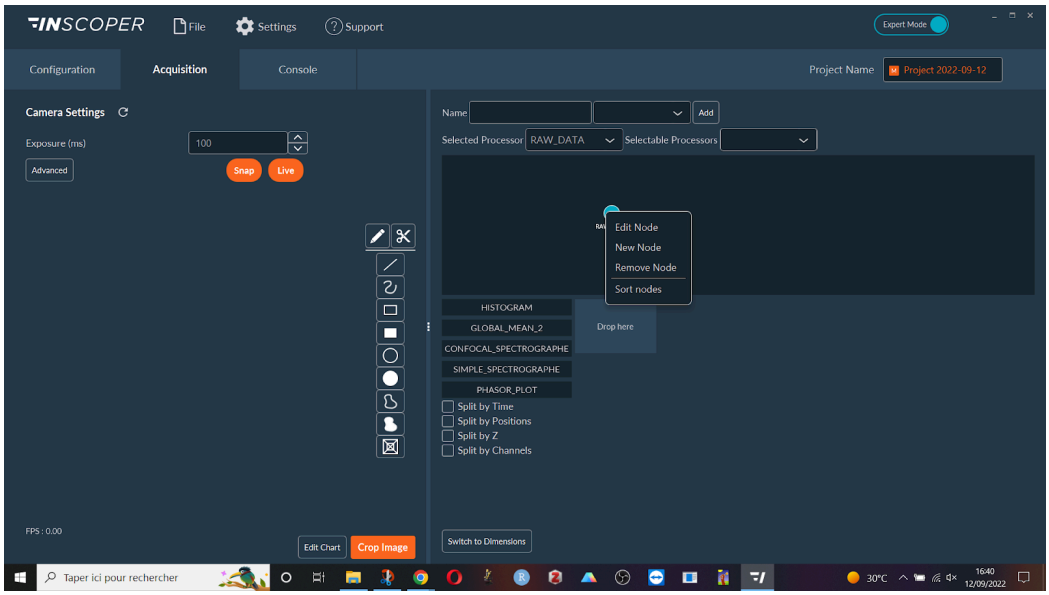
1. Space for creating data processing workflow.
2. Data visualization customizing tools.
3. Tools for analyzing data after splitting by dimension.
4. Tools to customize the image visualization after pre-treatments:

> NB: The list of available treatments depends on the system.

   a. Selected Processor: Select the image of the workflow that you wish to view during acquisition.
   b. Selectable Processor: Select all the images that you wish to see after acquisition in the visualization tab. In principle, all steps are selected.

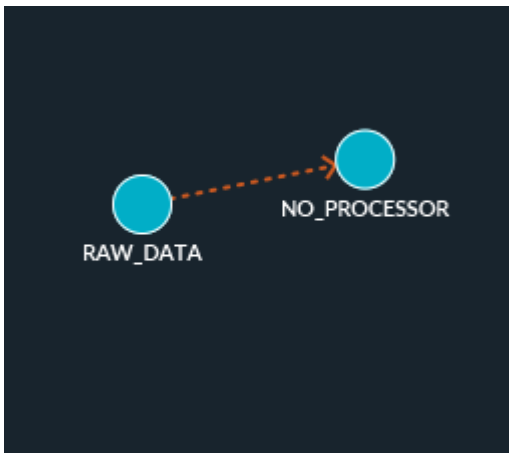
3.1.5.1.10.2. Create a new data processing workflow

1. The starting point of the workflow are the raw images. They are represented by a node named RawData. To apply a treatment to these images, you need to create a new node by right-clicking on RawData and selecting **New Node**.



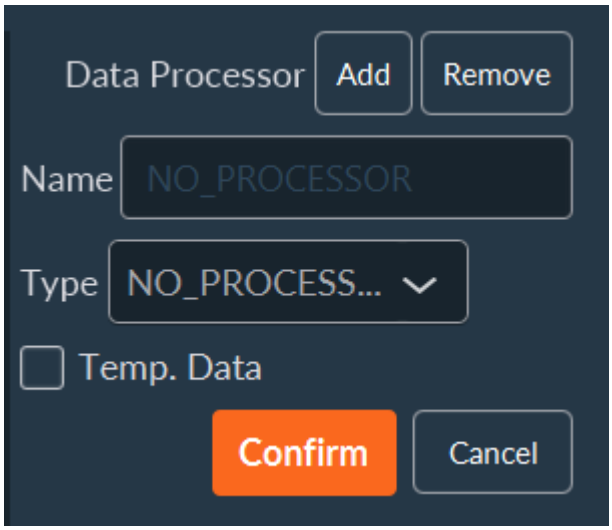
2. A new node will appear on the screen. At the moment there is no treatment associated with this node. To add one, double click on it or right click and select **Edit Node**.

> NB: For better ergonomics, users can right-click in the area and select **Sort Nodes**. This option will organize all nodes to make them easily visible. Nodes can also be moved by dragging and dropping.



3. In the current window, the **Type** drop-down menu allows you to select the treatment to be applied from the list of available algorithms (stitching, shading correction, maximum projection, background subtraction, etc.). The **Temp. Data** check-box can be unchecked if you don't want to

save this step to disk. This can be useful to reduce the duration and the total size of the data on the computer/server.



The list of all applicable processes is detailed in the following table.

NO_PROCESSOR	No effect.
SIMPLE_TILING	Position each image at its theoretical position on a global tiling.
STITCH_TILING	Stitch adjacent images of a tiling, taking into account the overlap (requires SIMPLE_TILING before).
STANDARD_DEVIATION_ON_FLY	Calculates the standard deviation of the intensity pixel by pixel of an image stack.
SHADING_CORRECTION	Removes unwanted signals from the image (misalignment, dust in the optical system, ...) Given an image of the background, the following calculation is made for each pixel: (current intensity of the pixel / initial intensity of the pixel on the background) x average intensity of the background.
FILTER	Noise reduction by removing anomalous pixels from an image (despeckle). For each pixel, the intensity values of the surrounding pixels (according to a radius value) are recovered. It is then possible to apply a median or average filter to each pixel from these values. The pixels with an intensity value lower than this calculated value are then ignored (value equal to 0) thanks to a median or average filter (the median filter is recommended).
TIME_MAX	Keep the maximum value for each pixel of a stack from the “Time” dimension.
FOCUS_MAX	Keep the maximum value for each pixel of a stack from the “Z-stack” dimension.
TIME_AVERAGE	Keep the average value for each pixel of a stack from the "Time" dimension.
FOCUS_AVERAGE	Keep the average value for each pixel of a stack from the "Z-stack" dimension.
CHANNEL_MULTICOLOR	Merge images from different channels. The maximum intensity values for each pixel are retrieved and a blend of LUTs is performed.
SUBTRACT_BACKGROUND	Removes background from an image to improve contrast.
CHANNEL_RATIO	Rationalize the intensity of a single pixel using several different channels. The "SUBSTRACT_BACKGROUND" is directly present in "CHANNEL_RATIO" to optimize the output.
MULTI_CHANNEL_MERGE	Merges channels. Mainly used for SPIM with two excitation beams.

NB: All these elements can be performed after the acquisition or in real-time. Data will be actualized and implemented in the final output during the acquisition.

3.1.5.1.10.3. Data visualization personnalization

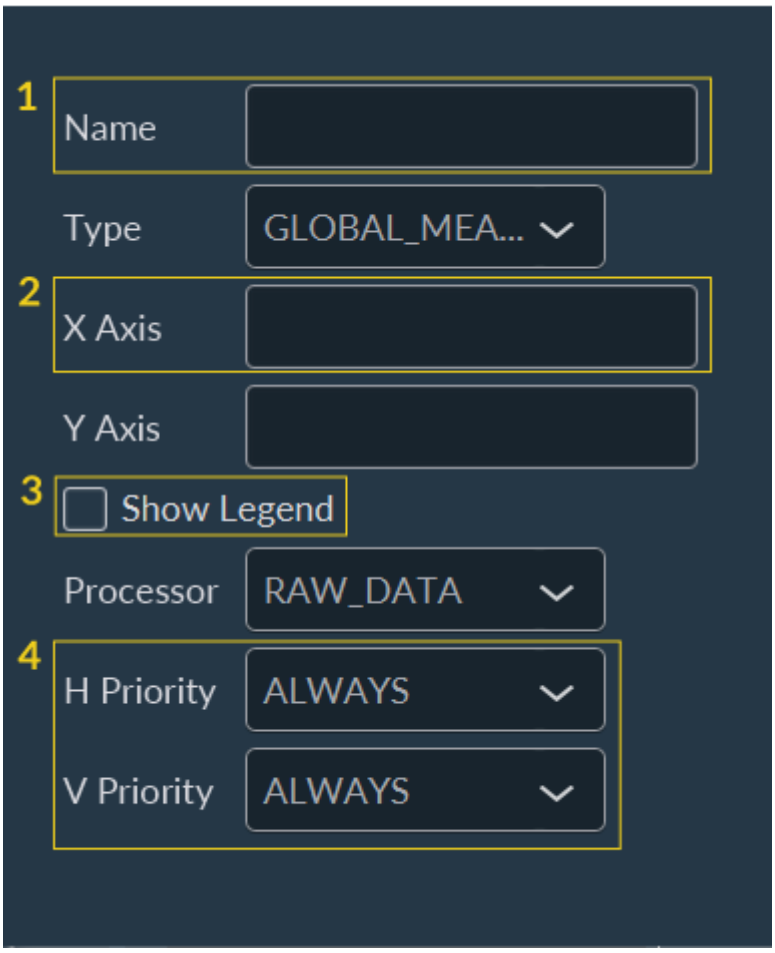
You can customize the display of the data during the acquisition, such as the evolution of the fluorescence intensity for example.

To customize the data visualization window:

1. Select a type of data to be displayed on the screen during acquisition:
    - Histogram: element used to monitor the evolution of the intensity distribution.
    - Global mean: element used to monitor the evolution of the average intensity of an acquisition sequence on a frame-by-frame basis or organized by a dedicated dimension.
    - Global statistic: similar to “Global mean”, but have some statistical elements added directly in the graphics, giving a similar result to boxplots.
    - Device values: element used to periodically retrieve information/values from devices, using "get" functions.
2. Drag and drop it on the central square. The data type is resumed here. You can modify it using the drop-down menu.
3. Select the data to consider for the visualization.
4. Graphs can be customized to add some elements like the title of the axes or curves name.
5. If you want to see more than one data during the acquisition. You have to repeat this procedure from the beginning by drag and drop a data type on another empty square.

To customize the graphics:

1. Add a name to the graph.
2. Add a title to both X and Y axis.
3. Select to see or hide the legend of each curve.
4. Select “Always” to ensure that graphics will always be visible.



NB: This customization step is optional, dedicated to helping researchers interpret their results during acquisitions.

3.1.5.1.11. Saving images and metadata

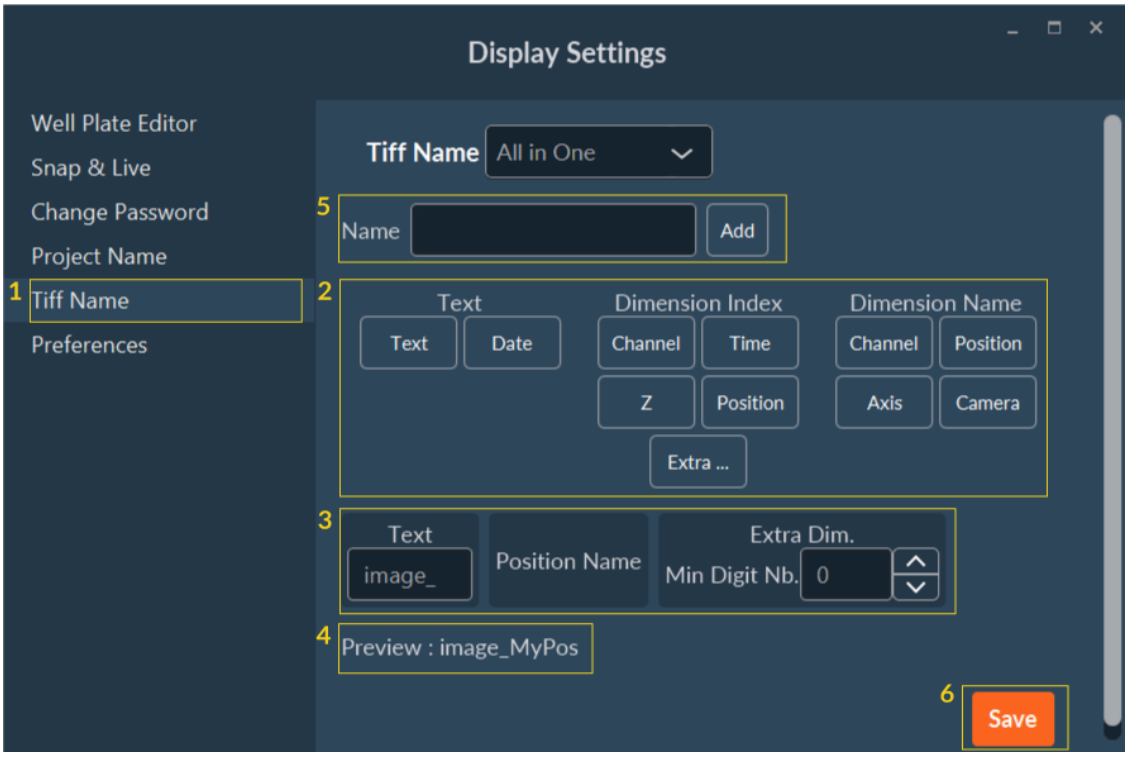
3.1.5.1.11.1. Interface presentation

To save the acquired images and metadata:

1. Select the type of data storage you want:
    - in the RAM of the computer (not recommended: all data stored in the RAM of the computer are not saved and will be lost when the program is closed)
    - in the computer/hard disk, SSD or servers (recommended).
2. Select the path to save your data.
3. Select the format to save all images:
    - all in one file;
    - one tiff per image.
4. It is also possible to create a .bigTiff file (recommended for long and heavy acquisitions)

3.1.5.1.11.2. Store name customization

This feature is dedicated to standardize the name of all saved files. This feature is accessible by clicking on **Settings**, on the top of the window, and then on **Display Settings**.



To standardize the name of all saved files:

1. Select the **Tiff Name** tab.
2. Here are all the elements that can be automatically added to the standardized name of saved files. Add them by dragging and dropping them to the name construction tools below. Generally the name composition is the text + dimension name + dimension index. For example: if you have 3 positions, the name of your files will be image_pos1, image_pos2, image_pos3.
3. Name construction tool that shows schematically the name of the future saved file.
4. Name preview.
5. Enter a name and click on the **Add** to save this new default name.
6. When you like the name structure, click **Save**.

3.1.5.2. Data export

3.1.5.2.1. Video exportation

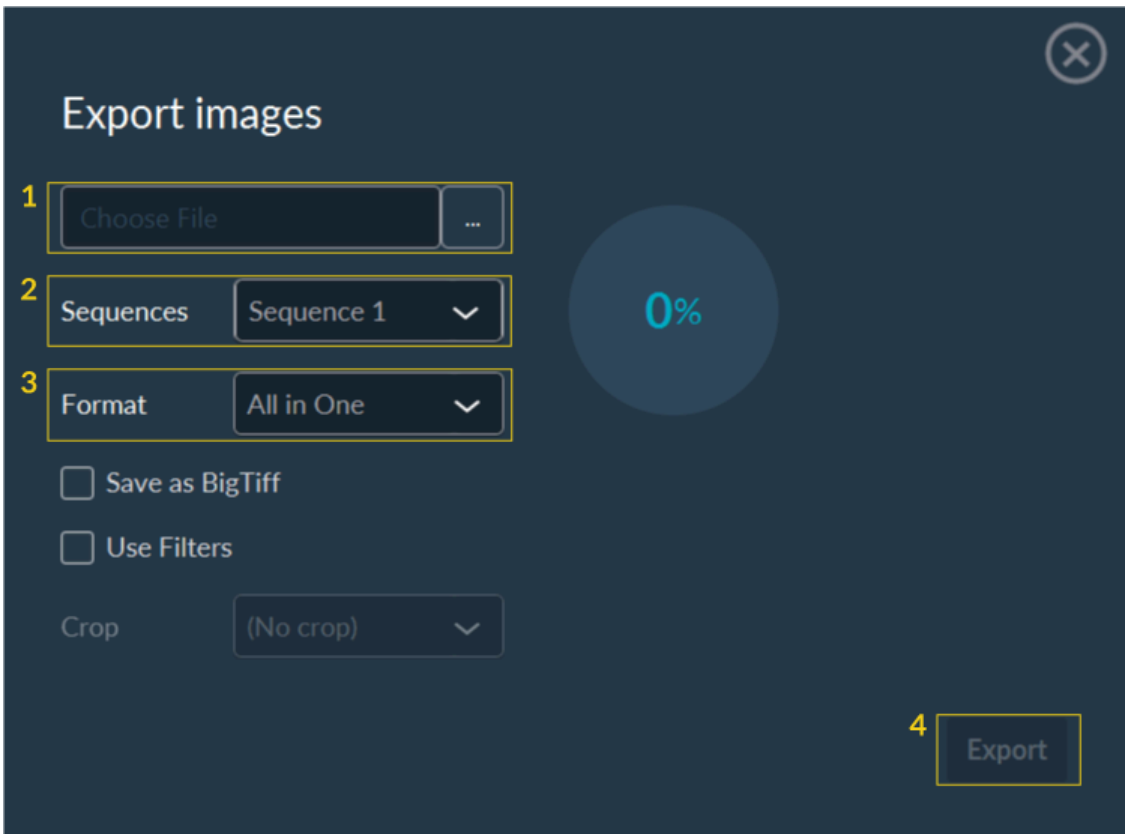
It is possible to export a video from the Inscoper I.S.. Select with filters all images to put together and click on **Video** of the "Export" list.

To export a video:

1. Select the path.
2. Select the sequence to export.
3. Select the format to use.
4. Validate by clicking on **Export**.

3.1.5.2.2. Stack exportation

It is possible to export a stack of images from the Inscoper I.S.. Use the filters to select all the images to be packed and click on **Stack** of the "Export" list.

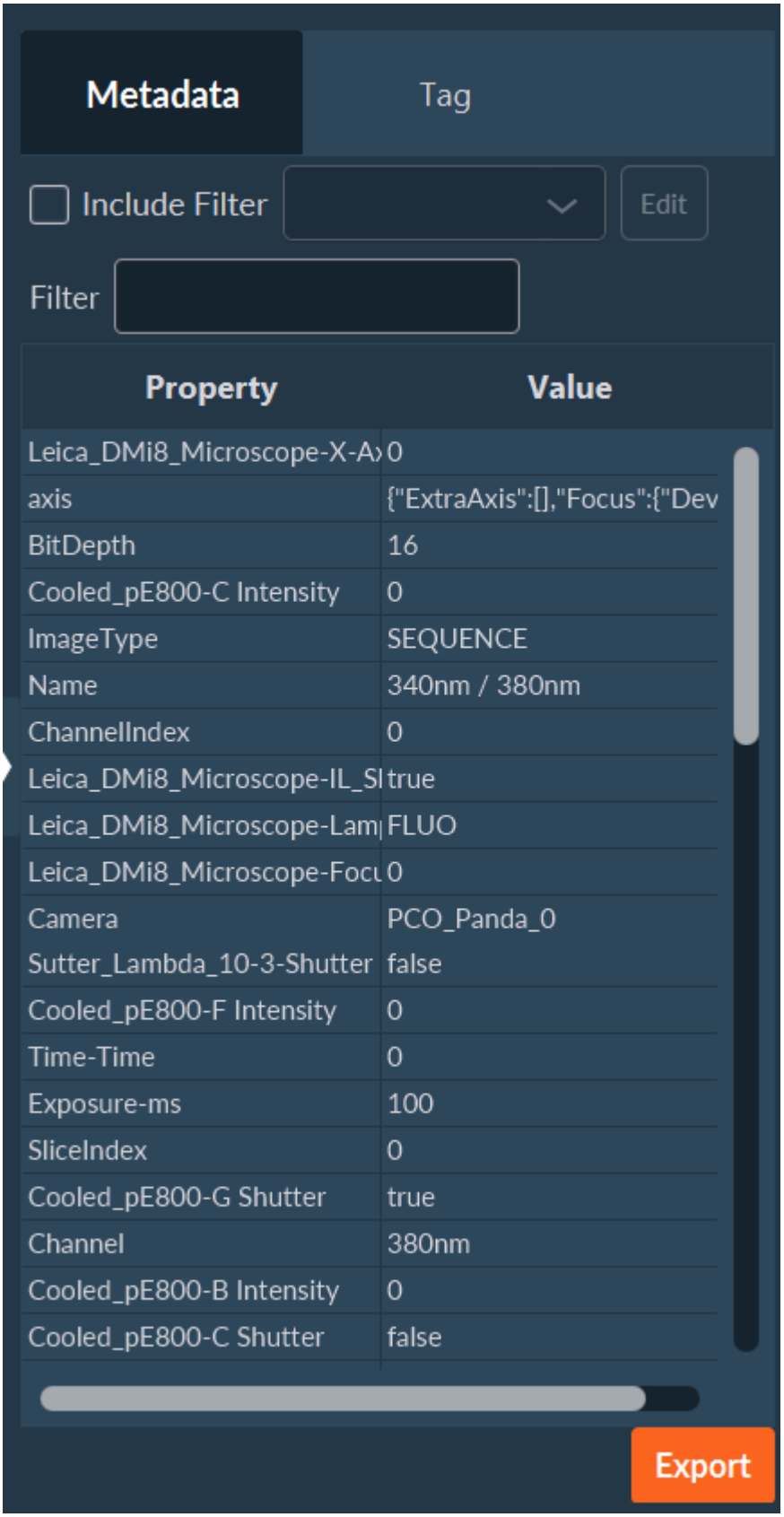


To export a stack:

1. Select the path.
2. Select the sequence to export.
3. Select the format to use.
4. If necessary, you can check the **Save as BigTiff** box.
5. If necessary, you can check the **Use Filters** box to select the dimensions you are interested in.
6. Validate by clicking on **Export**.

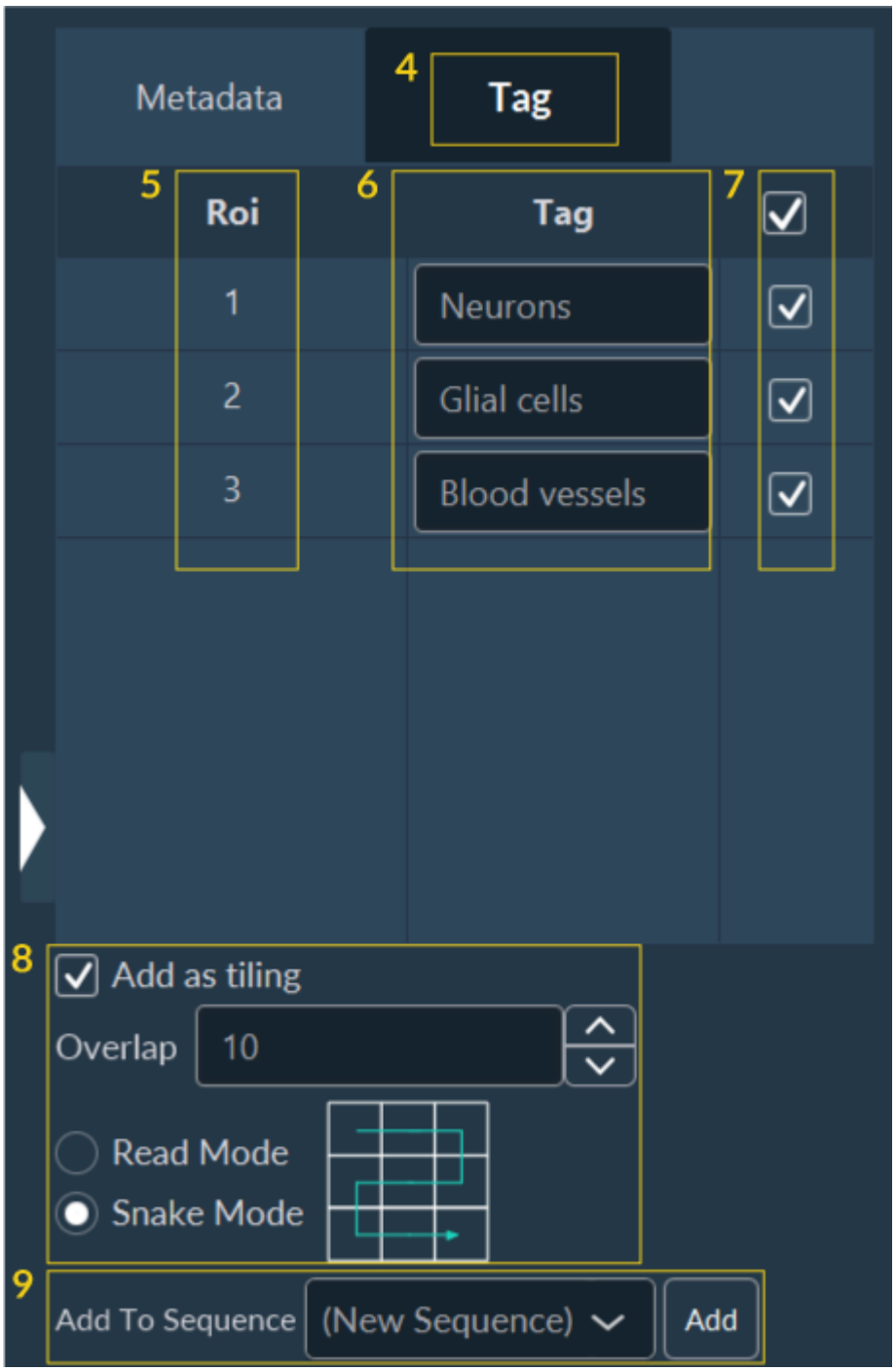
3.1.5.2.3. Metadata access

On the right side of the window there is a white triangle. You can click on it to access all the metadata. In this tab, you can access all the metadata, including the camera, light source or microscope settings; a search bar and some filters are available to facilitate the search for some specific parameters. This list can also be exported, if necessary, by clicking on the **Export** button located in the lower right part of the screen. All metadata are bio-format compatible.


Metadata
Tag
Include Filter
Edit
Filter
Property
Value
Leica_DMi8_Microscope-X-A
0
axis
{"ExtraAxis":[],"Focus":{"Dev
BitDepth
16
Cooled_pE800-C Intensity
0
ImageType
SEQUENCE
Name
340nm / 380nm
ChannelIndex
0
Leica_DMi8_Microscope-IL_Sl
true
Leica_DMi8_Microscope-Lam
FLUO
Leica_DMi8_Microscope-Focu
0
Camera
PCO_Panda_0
Sutter_Lambda_10-3-Shutter
false
Cooled_pE800-F Intensity
0
Time-Time
0
Exposure-ms
100
SliceIndex
0
Cooled_pE800-G Shutter
true
Channel
380nm
Cooled_pE800-B Intensity
0
Cooled_pE800-C Shutter
false
Export

3.1.5.3. Semi-automated feedback microscopy feature

The Inscoper I.S. can be used to perform some feedback microscopy experiments. For example, you can image a large sample using the tiling dimension. Then you can select a few areas of interest on this large image and automatically import them into a new sequence.



To use this feature:

1. Realize a large image using the Tiling dimension.
2. Add some ROI to structures of interest.

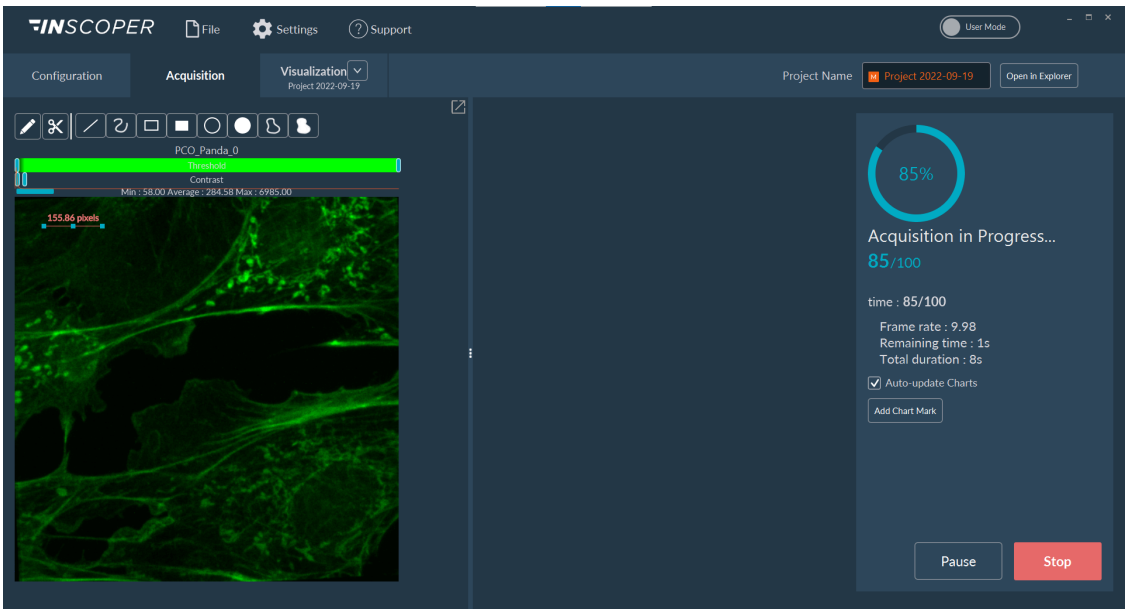
3. In the **Visualization** tab, click on the white triangle on the right of the window to open the **Metadata** tab.
4. Select the **Tag** tab.
5. Here all ROI are presented. You can add, edit or remove ROI according to your needs.
6. Add some tag name to each ROI if necessary.
7. Select ROI of interest. Positions of these ROI will be re-sent to the **Acquisition** tab later.
8. If tilings are needed, adjust here all the settings.
9. Select where all new positions will be stored (new sequence of pre-existing one).

NB: This feature can be used to (1) fastly scan a sample with a low magnification objective and then (2) rescan it with a higher magnification.

NB: Similarly, it can be used to (1) prescan a sample with a brightfield light and then to (2) use fluorescence only in structures of interest to prevent phototoxicity

3.1.6. Visualization

3.1.6.1. Visualization during acquisition

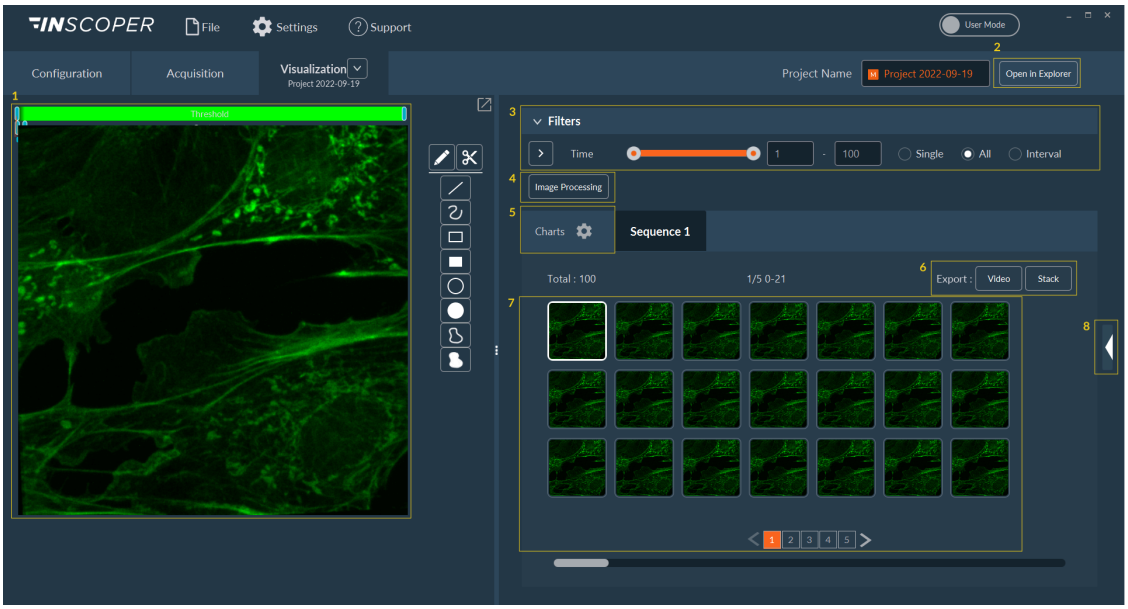


You can monitor the acquisition sequence while it is running. The left part of the window shows the current images of the sequence. The middle part is reserved for graphics if needed (not here). And in the right part of the window, you can follow the progress of the acquisition, pause or stop the sequence.

NB: For large image acquisitions, it is possible to see the total tiling with the different images implemented during the acquisition.

3.1.6.2. Visualization after acquisition

3.1.6.2.1. Interface presentation



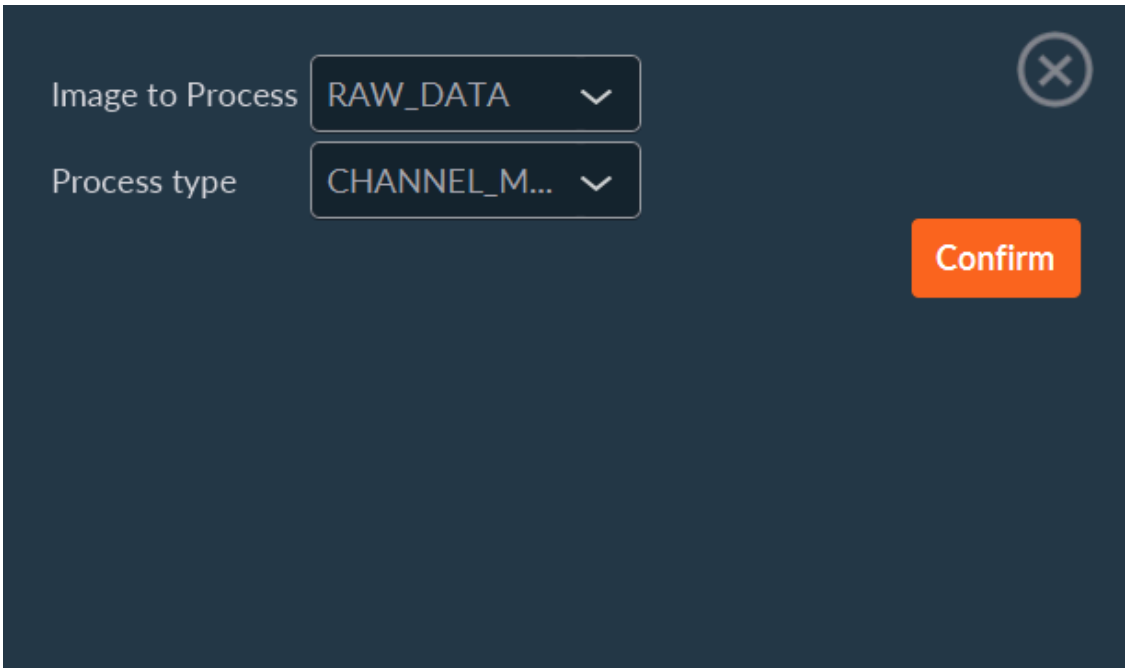
1. As in the rest of the software, this part of the screen is reserved for viewing images.
2. Opens the location where images are stored. Only works if you save your data to disk.
3. Filters to select images by dimensions.
4. Data processor access.
5. Button to switch to graphics visualization.
6. Options to export the current acquisition sequence by video or stack.
7. All acquired images.
8. Metadata access.

3.1.6.2.2. Interacting with filters

The Inscoper I.S. offers various tools to facilitate navigation between images, such as filters for example. In the **Filters** section, you can select a single image, a group or all images. The **Play** button next to the dimension name allows you to replay the selected sequence.

3.1.6.2.3. Image Processing

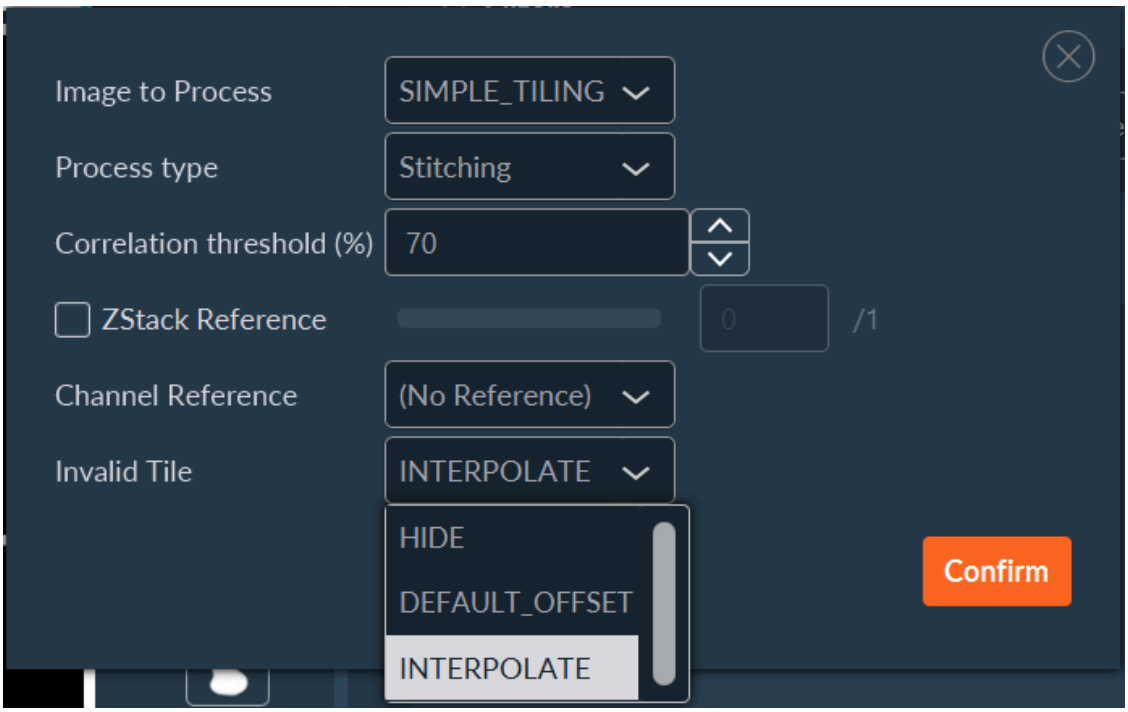
In this window you can process images acquired with processors of your choice.



1. Select the images to be processed in the drop-down list.
2. Select the processor you wish to apply.
3. Then click on **Confirm** to finalize processing.

NB: The variety of processor types depends on your acquisition workflow.

Here is an example with tiling:



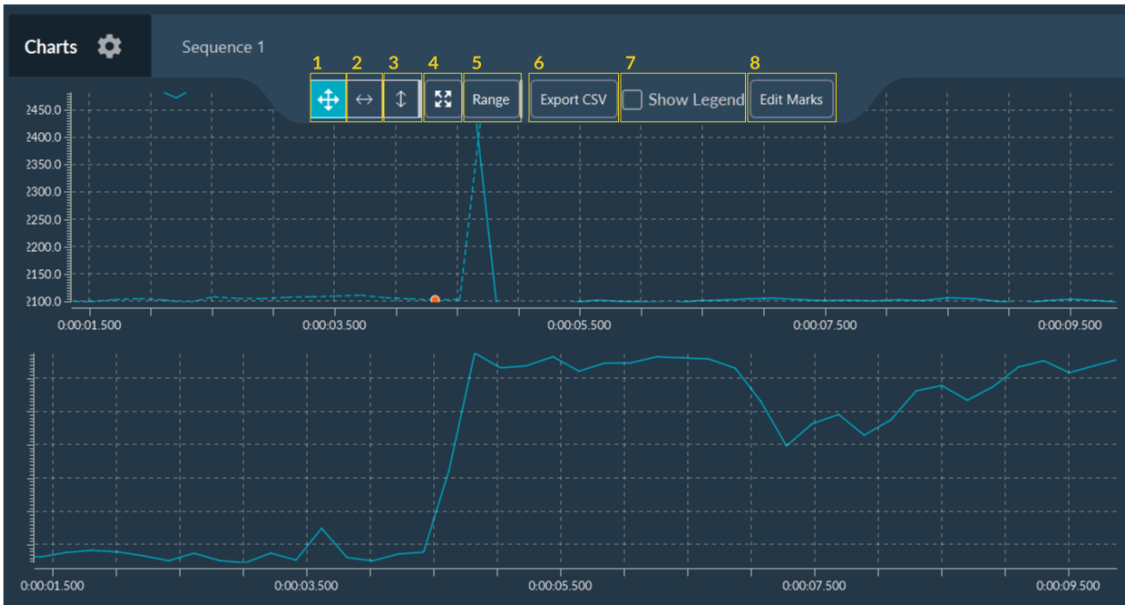
1. Select the image to process.
2. Select the process type.
3. Indicate the correlation threshold (%).
4. Check if you want to apply ZStack reference and select the good plane to calculate the stitching.
5. Select the Channel Reference if needed.
6. Select the action to apply to the invalid tile:
    - **HIDE:** ignore this tile
    - **DEFAULT_OFFSET:** use the offset of this tile in the non stitched tiling
    - **INTERPOLATE:** create a linear model of all valid relative offsets, and use it to generate a valid offset for the tile
7. Click on **Confirm**.

3.1.6.2.4. Interacting with graphics

You can interact with charts by changing their appearance, editing them, adding time markers, or exporting them. Simply hover over a graph to open a new tab.

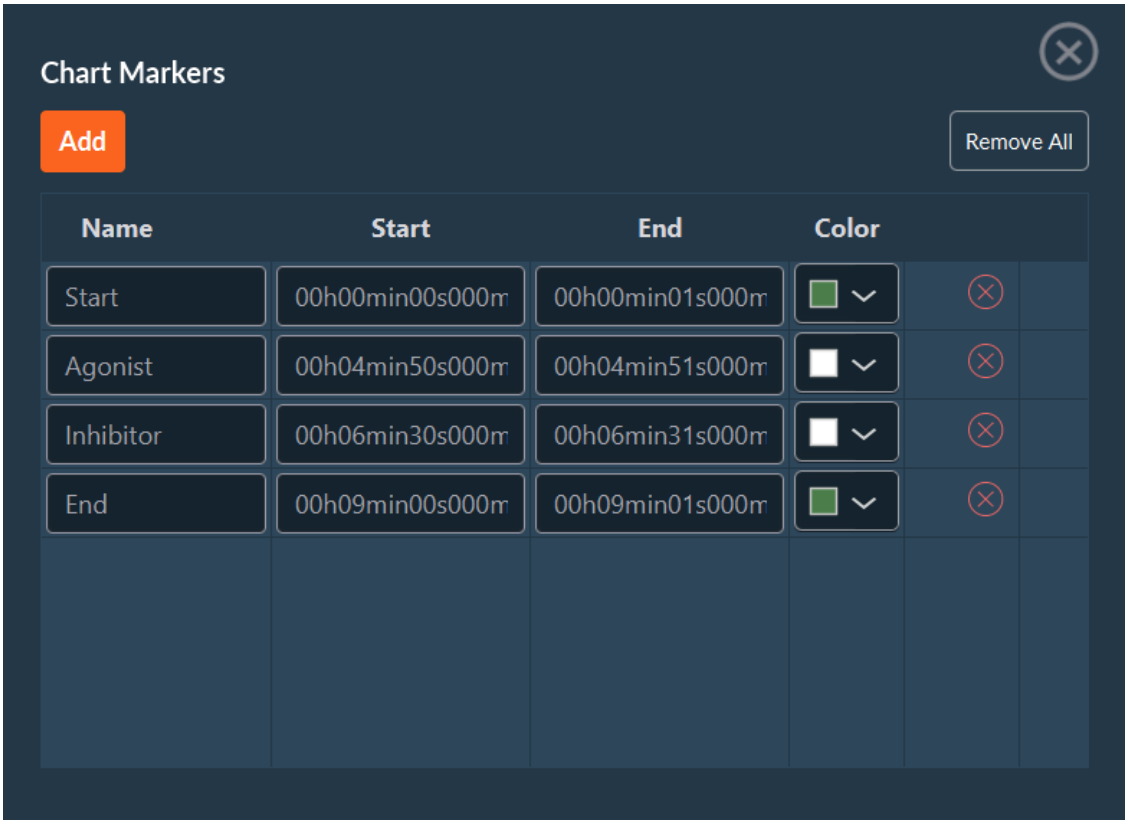
It is possible to:

- move inside a graph by holding down the mouse wheel click;
- zoom in and out using the mouse wheel;
- select an area with the mouse to zoom in;
- view a specific image by left-clicking directly on the graph;
- and (v) return to the initial view by right-clicking.



1. Enable XY zoom mode.
2. Enable X zoom mode.
3. Enable Y zoom mode.
4. Zoom to origin and enables auto-ranging
5. Modify the range of XY axes values for the graph visualization.
6. Export all data to a .csv file.
7. Show/hide curves legends
8. Tool to add some markers to the graph (see below).

If more than one graph is displayed, they are synchronized (movement, zoom in/out, ...) to facilitate the analysis of the curves.



In this window you can add information about your experiment as markers. These events, which are fully customizable, can be associated with the acquisition itself (start, pause, end), external events (addition of an inhibitor, medium supplementation), or others. These markers can be saved in a .csv file and reused at any time.